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Physiology at high altitude: understanding mechanisms and identifying countermeasures

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Physiology at high altitude: understanding mechanisms and identifying countermeasures

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Supervisors Dr. Jamie H. Macdonald *and* Dr. Samuel J. Oliver *and* Dr. Paul G. Mullins

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Statement of Originality

The work presented in this thesis is entirely from the studies of the individual student, except where otherwise stated. Where derivations are presented and the origin of the work is either wholly or in part from other sources, then full reference is given to the original author. This work has not been presented previously for any degree, nor is it at present under consideration by any other degree awarding body.

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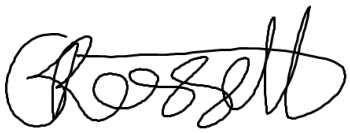

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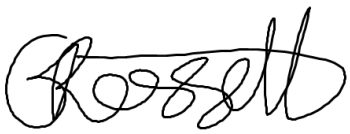

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Thesis Format

The PhD was designed so that I could gain experience of, and develop expertise in, a variety of techniques and experimental designs. The thesis is presented in a paper format. Chapter 2 and Chapter 3 are peer-reviewed articles, and have been included as published, with the exception of conversion to British English in the interest of consistency through the thesis. Chapter 4 has been prepared for submission to the journal *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, and has been included according to the journal's formatting requirements.

A literature review (Chapter 1) provides a brief background and outlines the scope and aims of the research presented in the thesis.

The thesis consists of three independent experimental studies. The first study was a field-based study conducted on an expedition to the Nepal Himalaya in 2015 (Chapter 2). This study principally investigated the effect of sea-level fitness on health and exercise responses on a high-altitude trekking expedition. The second experiment was a lab-based study which investigated the effect of dietary nitrate on AMS and exercise performance in hypoxia (Chapter 3). Finally, the third study was a lab-based study which utilized magnetic resonance imaging techniques to investigate the effect of hypoxia on regional brain blood flow at rest and during cognitive tasks (Chapter 4).

A general discussion (Chapter 5) contains a summary and critical analysis of the main findings of the research, and highlights the scientific and applied implications.

List of Publications

Publications arising from work presented within this thesis

Rossetti, G. M. K., Macdonald, J. H., Wylie, L. J., Little, S. J., Newton, V., Wood, B., Hawkins, K. A., Beddoe, R., Davies, H. E. & Oliver, S. J. (2017). Dietary nitrate supplementation increases acute mountain sickness severity and sense of effort during hypoxic exercise. *Journal of Applied Physiology.* 123(4): 983-992. doi: jap.00293.2017.

Rossetti, G. M. K., Macdonald, J. H., Smith, M., Jackson, A. R., Callender, N., Newcombe, H. K., Storey, H. M., Willis, S., van den Beukel, J., Woodward, J., Pollard, J., Wood, B., Newton, V., Virian, J., Haswell, O., & Oliver, S. J. (2017). MEDEX2015: Greater sea-level fitness is associated with lower sense of effort during Himalayan trekking without worse acute mountain sickness. *High Altitude Medicine & Biology.* 18(2): 152-162. doi: 10.1089/ham.2016.0088.

Conference proceedings

Rossetti, G. M. K. (2018). Acute hypoxia decreases microvascular endothelium-dependent function in healthy men. **Poster presentation at Europhysiology 2018**. London, UK, September 2018.

Rossetti, G. M. K. (2017). Dietary nitrate supplementation increases Acute Mountain Sickness (AMS) symptoms and sense of effort during submaximal

exercise in hypoxia. **Verbal presentation at Pan-Wales Postgraduate Conference in Sport and Exercise Sciences.** Swansea, Wales, UK. April 2017.

Rossetti, G. M. K. (2017). Dietary nitrate supplementation increases acute mountain sickness severity and sense of effort during submaximal exercise in hypoxia. **Verbal and poster presentations at Hypoxia 2017: The 20th international hypoxia symposium.** Lake Louise, Alberta, Canada. February 2017. Early Career Researcher Presentation Award: 2nd place.

Rossetti, G. M. K. (2016). The effects of chronic dietary nitrate supplementation on AMS and exercise capacity in hypoxia: Preliminary Results. **Verbal presentation at BMRES: Altitude Research Conference**. Birmingham, England, UK. November 2016.

Rossetti, G. M. K. (2016). MEDEX 2015: Is fitness important for an individual's performance and health at altitude? **Poster presentation at Physiology 2016: Joint meeting of the American Physiological Society and The Physiological Society**. Dublin, Ireland, July 2016.

Summary of Findings

The overall aim of this thesis was to increase understanding of physiological responses to altitude and identify modifiable factors that could enhance health and exercise performance at altitude.

With this in mind, the specific aims of the first study (Chapter 2) were to determine the relationship between sea-level fitness and submaximal exercise responses and AMS during chronic altitude exposure, and to determine the utility of sea-level fitness and hypoxic exercise testing before an expedition. Greater sea-level fitness ($\dot{V}O_{2max}$) was associated with, and predicted, lower sense of effort (RPE_{ascent} r = -0.43; p < 0.001; RPE_{fixed}; r = -0.69; p < 0.001) and higher step rate ($STEP_{RPE35}$; r = 0.62; p < 0.01), but not worse AMS (r = 0.13; p = 0.4) or arterial oxygen desaturation (r = 0.07; p = 0.7). Lower RPE_{ascent} was also associated with better mood, including less fatigue (r = 0.57; p < 0.001) on a high-altitude expedition. Hypoxic sensitivity was not associated with, and did not add to the prediction of, submaximal exercise responses or AMS. This study concluded that greater sea-level fitness is related to lower sense of effort during submaximal exercise and better mood (less fatigue, tension, and confusion) at altitude.

The second study (Chapter 3) aimed to determine the efficacy of chronic dietary nitrate supplementation as an AMS prophylactic and ergogenic aid at altitude. Five days nitrate supplementation (6.4 mmol nitrate daily) increased plasma NO metabolites compared to placebo but did not reduce AMS or improve exercise performance. After 4 h hypoxia, nitrate increased AMS (Acute Cerebral Mountain Sickness score; AMS-C) and headache severity (visual analogue scale (VAS); whole sample △10[1,20] mm; p = 0.03) compared to placebo. In addition, after 5 h hypoxia, nitrate increased sense of effort during submaximal exercise (△7 [-1,14]; p = 0.07). This study concluded that dietary

nitrate increases AMS and sense of effort during exercise, particularly in AMS-susceptible individuals. Dietary nitrate is therefore not recommended as an AMS prophylactic or ergogenic aid non-acclimatized individuals at altitude.

Finally, the third study (Chapter 4) aimed to increase understanding of cerebral physiology in hypoxia, specifically to characterise the anatomical distribution of regional changes in resting cerebral blood flow (CBF) and neurovascular activity during cognitive tasks in hypoxia. Acute hypoxia induced reductions in regional CBF (rCBF) to default mode network (DMN) regions including the posterior cingulate cortex (PCC) and right angular gyrus (AG). These reductions persisted during a DMN-dependent memory task where we observed an inversion of the haemodynamic response to neural activity, despite no impairment in memory task performance. In contrast, hypoxia induced increases in visual search-related activations in visual attention network (VAN) regions including the middle temporal areas (MT), frontal eye fields (FEF), and left intraparietal sulcus (IPS). This increase in activation, coupled with no change in task performance, indicates the maintenance of oxygen delivery in hypoxia despite reduced blood oxygenation. This study concluded that acute hypoxia induces region-dependent alterations in neurovascular responses.

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Chapter 1

Literature Review

Hundreds of millions of people travel to high altitude every year, with many more living at altitude. Almost all high-altitude travel requires the completion of exercise, whether for trekking, military deployment, high-altitude mining, or religious pilgrimage. A key factor of the high-altitude environment is low oxygen availability (hypoxia). Hypoxia affects many aspects of physiology with implications for exercise performance, high-altitude health conditions including acute mountain sickness (AMS), and cognitive performance. This thesis seeks to understand physiological responses to hypoxia and identify methods to enhance health and performance at altitude. In addition, improving understanding of physiological responses to hypoxia has potential application to numerous clinical conditions characterised by hypoxia. Such conditions include, but are not limited to, obstructive sleep apnoea, pulmonary edema, heart conditions, and anaemia.

1.1 The high-altitude environment: hypoxia and human physiology

For the purposes of this thesis, "high altitude" is defined as altitudes $\geq$ 2500 m, or an equivalent fraction of inspired oxygen ($F_IO_2 \leq 0.158$). An overview of altitude definitions and some fundamental associated physiological effects is depicted in Figure 1.1.

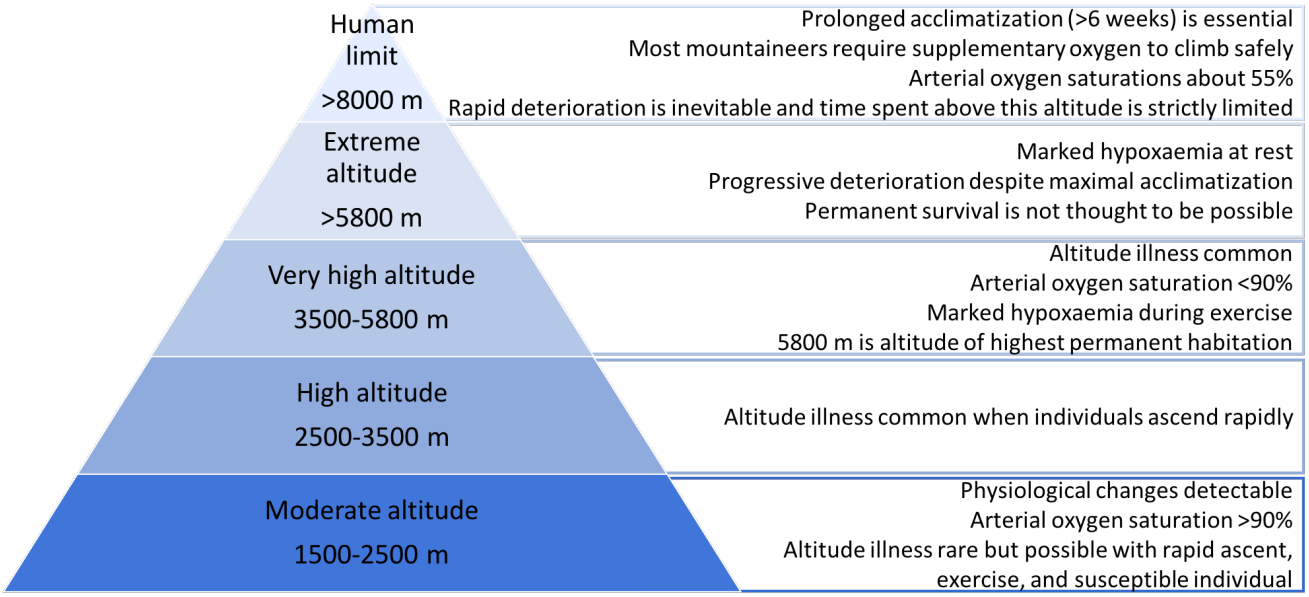


Figure 1.1: Definitions of altitude and associated physiological changes. Adapted from Imray et al., 2011.

1.1.1 Hypoxia, from the atmosphere to the capillaries

One of the most notable physical aspects of high-altitude environment is the low oxygen availability. The partial pressure of oxygen (PO_2) is reduced as a consequence of lower atmospheric barometric pressure (hypobaria). Reduced PO_2, whether hypobaric ($F_IO_2 = 0.209$) or normobaric (reduced F_IO_2), results in reduced PO_2 within the lung, and consequently reduced blood oxygen content (hypoxaemia; West, 1982). This occurs because reduced alveolar PO_2 reduces the driving pressure of oxygen into the pulmonary capillaries, slowing the passive process of oxygen diffusion. Under normoxic conditions, such as at sea level, diffusion is sufficiently rapid for the PO_2 of pulmonary capillary blood to be equal to alveolar PO_2 after 0.2 - 0.3 seconds transit along the capillary. In hypoxia, since the concentration of oxygen in the alveolar is reduced, the concentration gradient between the alveolar and capillary is diminished, slowing diffusion. The consequence of this is most pronounced at extreme altitudes where the process is slowed to such an extent that alveolar and capillary PO_2 do not equalize, even at the end of capillary transit (West et al., 1983).

There are many ways in which the human body attempts to protect oxygen availability against a decline in atmospheric oxygen. It is important to have

an understanding of the key protective responses at each stage of the oxygen cascade before more complex mechanisms and interventions can be investigated. This first section of the literature review seeks to provide such an overview, as outlined in Figure 1.2.

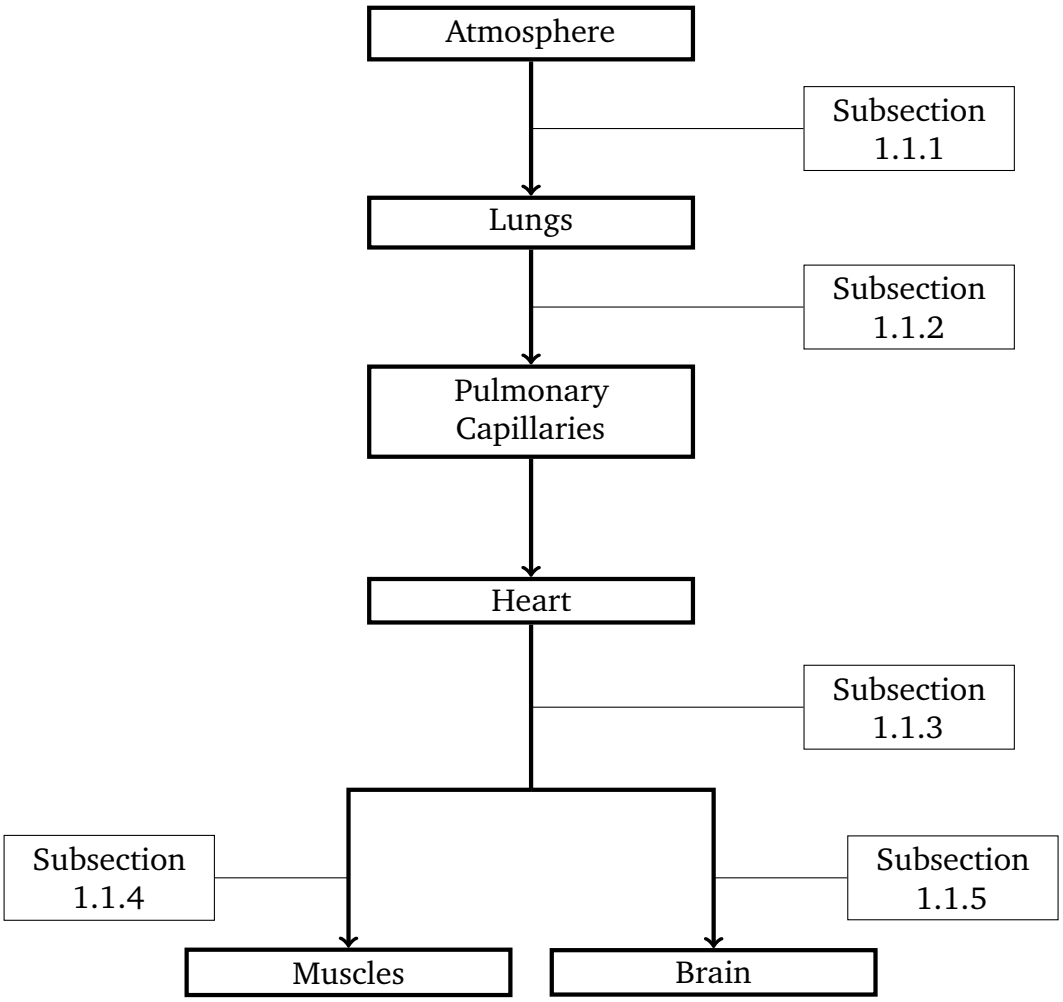


Figure 1.2: The oxygen cascade: transport of oxygen through the body. Call-outs define relevant subsections of this chapter.

1.1.2 The ventilatory response to hypoxia

The acute hypoxic ventilatory response (HVR)

The lungs are responsible for transporting oxygen from the atmosphere into the body, making them the site of one of the principle physiological responses to atmospheric hypoxia; a rise in ventilation in an attempt to maintain inspired PO_2 against the fall in atmospheric oxygen. In acute hypoxia, ventilation is increased

through combined increases in tidal volume and respiratory frequency (Pamenter and Powell, 2016). This occurs since chemoreceptors in the glomulus cells (type I) of the carotid body respond to hypoxaemia by closing potassium channels to induce membrane depolarization and the opening of calcium channels. As a consequence, neurotransmitters acetylcholine and adenosine triphosphate (ATP) are released and excite the carotid sinus nerve and cranial nerve IX, leading to the respiratory centres in the brain (Teppema and Dahan, 2010).

This increase in ventilation, termed the hypoxic ventilatory response (HVR), occurs almost immediately with a hypoxic exposure and typically peaks after three to ten minutes. However, the HVR is variable between individuals, and it has been suggested that an elevated HVR is predictive of physiological success at altitude. Schoene *et al.* (1984) observed those with a higher HVR at sea level (before an expedition) experienced minimal hypoxaemia during exercise at 6,300 m and achieved the highest sleeping heights on an ascent of Mt Everest (8,848 m). In addition, a blunted HVR is a key physiological risk factor for severe high-altitude illnesses, including high-altitude cerebral oedema (HACE) and high-altitude pulmonary oedema (HAPE; Richalet et al., 2012).

Hypoxic ventilatory decline (HVD)

Hypoxia-induced hyperventilation will cause a reduction in the partial pressure of arterial carbon dioxide (P_aCO_2), termed hypocapnia (Steinback and Poulin, 2007; West et al., 2012), unless P_aCO_2 is experimentally controlled. After 20-30 min hypoxia, hyperventilation-induced hypocapnia suppresses ventilation to restore normocapnia. However, this hypoxic ventilatory decline (HVD) is not solely caused by hypocapnia since it occurs (although to a lesser extent) in isocapnic hypoxia. Isocapnic hypoxia refers to the experimental clamping of P_aCO_2 by the adjustment of inspired CO_2 in response to hypoxia-induced alterations in end-tidal CO_2. The physiological cause of HVD is not completely clear, but it may be due to a decline in oxygen sensitivity, or gamma-Aminobutyric acid-ergic (GABAergic) inhibition in the respiratory control circuit (Pamenter and Powell, 2016).

Ventilatory acclimatisation

Over approximately the first two weeks at altitude, individuals experience a gradual rise in ventilation, starting from the cessation of HVD after ∼30 min exposure. This hyperventilation results in an increase in alveolar and arterial PO_2, with an accompanying fall in P_aCO_2 (Ainslie et al., 2013). This is achieved through an increase in sensitivity to oxygen of the carotid body glomus cells, and an accompanying increase in central nervous system (CNS) responsiveness to afferent input from the carotid body. As such, there is a shift away from carbon dioxide, towards oxygen, as the primary stimulus for ventilation. In one study, chemosensitivity to hypoxia was shown to double over 12 days at altitude (3810 m; Sato et al., 1994).

The magnitude of ventilatory acclimatisation, measured by the change in hypoxic chemosensitivity (HVR), is more closely related to an individual's altitude tolerance, rather than HVR as measured at sea level. Bärtsch *et al.* (2002) observed that pre-expedition HVR had no effect on AMS incidence, but those who experienced either mild or no AMS had increased HVR at altitude, compared to their pre-expedition HVR, while those with moderate to severe AMS presented with decreased HVR compared to sea level.

Alternatively, it has been suggested that ventilation is reduced at extreme altitude specifically to reduce the oxygen cost of breathing (W_B; West, 1982), and that a reduced ventilatory response to hypoxia is associated with greater ventilatory reserve, greater ventilatory efficiency, and summit success at extreme altitudes (Bernardi et al., 2006). Indeed, though maximal aerobic capacity ($\dot{V}O_{2max}$) increases with acclimatisation to altitude, approximately 30% of the increase in $\dot{V}O_{2max}$ is consumed by the respiratory muscles, in order to meet the elevated respiratory demand (Wilhite et al., 2013). This increase in W_B is associated with respiratory fatigue, and reduced exercise capacity (Amann, 2012; Amann et al., 2007a). However, the relationship between hypoxia, ventilation, and W_B, is affected by the nature of hypoxia (hypobaric or normobaric), as hypobaria reduces W_B since the air density is lower. In addition, the rate of diffusion

from the alveolar to the pulmonary capillary may be altered by the reduced barometric pressure (Loeppky et al., 1997).

1.1.3 The cardiovascular response to hypoxia

The cardiac response to acute hypoxia

In acute hypoxia, heart rate increases to maintain oxygen delivery despite hypoxaemia. Hypoxia $\leq F_IO_2$ 0.15 (equivalent $\sim$2,750 m), induces only mild hypoxaemia (oxygen saturation $>$ 92%), but is sufficient to stimulate vagal withdrawal and sympathetic activation, resulting in tachycardia (Iwasaki et al., 2006). Stroke volume is unchanged with acute hypoxia, therefore the rise in heart rate is proportional to the increase in cardiac output (Siebenmann and Lundby, 2015). At rest this increase in cardiac output matches the degree of hypoxaemia such that oxygen delivery to the tissues is unchanged. Although this may not be the case at extreme altitude (Lador et al., 2008), or during high intensity exercise (Naeije et al., 1982; discussed in detail in Section 1.1.4). The increase in cardiac output results in reduced transit time through the pulmonary capillary, but any harmful effect of this is outweighed by the increase in oxygen delivery for a given oxygen saturation (West, 1982).

The cardiac response to chronic hypoxia

The relationship between heart rate and cardiac output is disrupted with more chronic hypoxic exposure (multiple days), since stroke volume is reduced (Stembridge et al., 2016). This reduction in stroke volume is caused by hypovolaemia-induced reduction in cardiac filling (preload), and an increase in vascular resistance (afterload), while contractility and ejection fraction are maintained (Boussuges et al., 2000). Impaired stroke volume means a greater heart rate is required for a given cardiac output, causing elevated cardiovascular strain (Gonzalez-Alonso et al., 2000). The combined effect of these two opposing determinates of cardiac output (tachycardia and reduced stroke volume) are

such that cardiac output at rest returns to its sea level volume after 2-3 days hypoxic exposure (Klausen, 1966; Naeije, 2010; Figure 1.3). In contrast, since maximal heart rate cannot be increased, this reduction in stroke volume means maximal cardiac output is reduced.

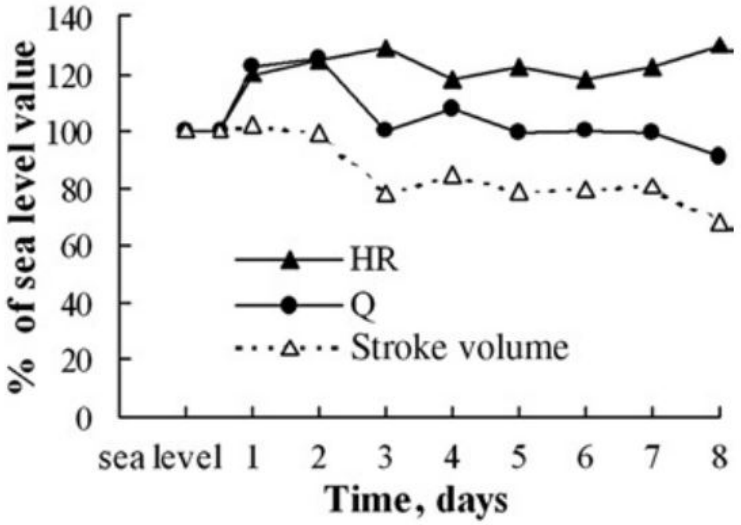


Figure 1.3: Temporal changes in cardiac output (Q), heart rate (HR), and stroke volume over eight days at altitude (3,800 m). Figure taken from Naeije (2010) and data from Klausen (1966).

1.1.4 Oxygen delivery to the muscles in hypoxia

Given the increase in cardiac output, muscle vascular conductance (vasodilation) is increased in hypoxia to compensate for hypoxaemia and maintain oxygen delivery (Joyner et al., 2014). This compensatory vasodilation occurs despite a conflicting increase in vasoconstrictive drive (Hanada et al., 2003). The mechanism of compensatory vasodilation involves nitric oxide (NO), adenosine, prostaglandins, and endothelium-derived hyperpolarizing factor (Spilk et al., 2013), though the relative contributions of these different factors is not certain. At least half of the compensatory vasodilation response in hypoxia is achieved through the action of the vasodilator NO (Joyner et al., 2014). Evidence to support this comes from studies that have used pharmacological interventions to block the production of NO. In the endothelium, L-arginine is converted into NO by endothelial nitric oxide sythase (eNOS; Stamler and Meissner, 2001), a pathway that can be blocked by the use of N^G-monomethyl-L-arginine (L-NMMA). Casey *et al.* (2011; 2010) used L-NMMA to block this NO production

pathway and observed a substantial reduction in the vasodilatory response to hypoxia.

The relative contributions of these mechanistic pathways, perhaps even the fundamental mechanism, is dependent on exercise intensity. At rest, it appears adenosine released from the endothelium is primarily responsible for NO-mediated vasodilation (Marshall, 2000), but adenosine is not required for compensatory hyperaemia during exercise in hypoxia (Casey et al., 2009). Similarly, at rest and low exercise intensity, β-adrenegic receptor stimulation through systemic epinephrine contributes to NO-mediated vasodilation, but does not contribute to compensatory vasodilation at higher intensities (Casey et al., 2011; Wilkins et al., 2008).

In addition, the L-arginine-NO pathway is downregulated in hypoxia, while nitrite (NO_2^-) reduction (the nitrate$\rightarrow$nitrite$\rightarrow$NO pathway) is upregulated, providing an alternative source for NO-mediated vasodilation in hypoxia (Kim-Shapiro et al., 2006). Further, deoxyhaemoglobin may act as an upstream regulator, stimulating the release of ATP from red blood cells and contributing to NO stimulation in hypoxia (Stamler et al., 1997). Finally, endothelium-derived prostacyclin (PGI_2) may also contribute to the hyperaemic response during exercise in hypoxia (Joyner et al., 2014). The proposed intensity-dependent mechanisms of vasodilation during exercise in hypoxia are outlined in Figure 1.4.

Not only intensity-dependent, the hypoxia-induced hyperaemic response is also dependent on the type of exercise. During whole-body exercise, competing blood flow demands outside the working muscle influence oxygen delivery to the working muscle and contribute to locomotor muscle fatigue (Amann et al., 2007a). As described above (Section 1.1.2), ventilation is substantially increased in hypoxia, in order to protect alveolar PO_2 from the decline in atmospheric PO_2 (Pamenter and Powell, 2016). During exercise, where the oxygen demand is increased from rest, ventilation increases to a greater extent, requiring elevated inspiratory muscle work (Cibella et al., 1999). In one study, inspiratory muscle work during fixed workload exercise was 36% higher in hypoxia compared

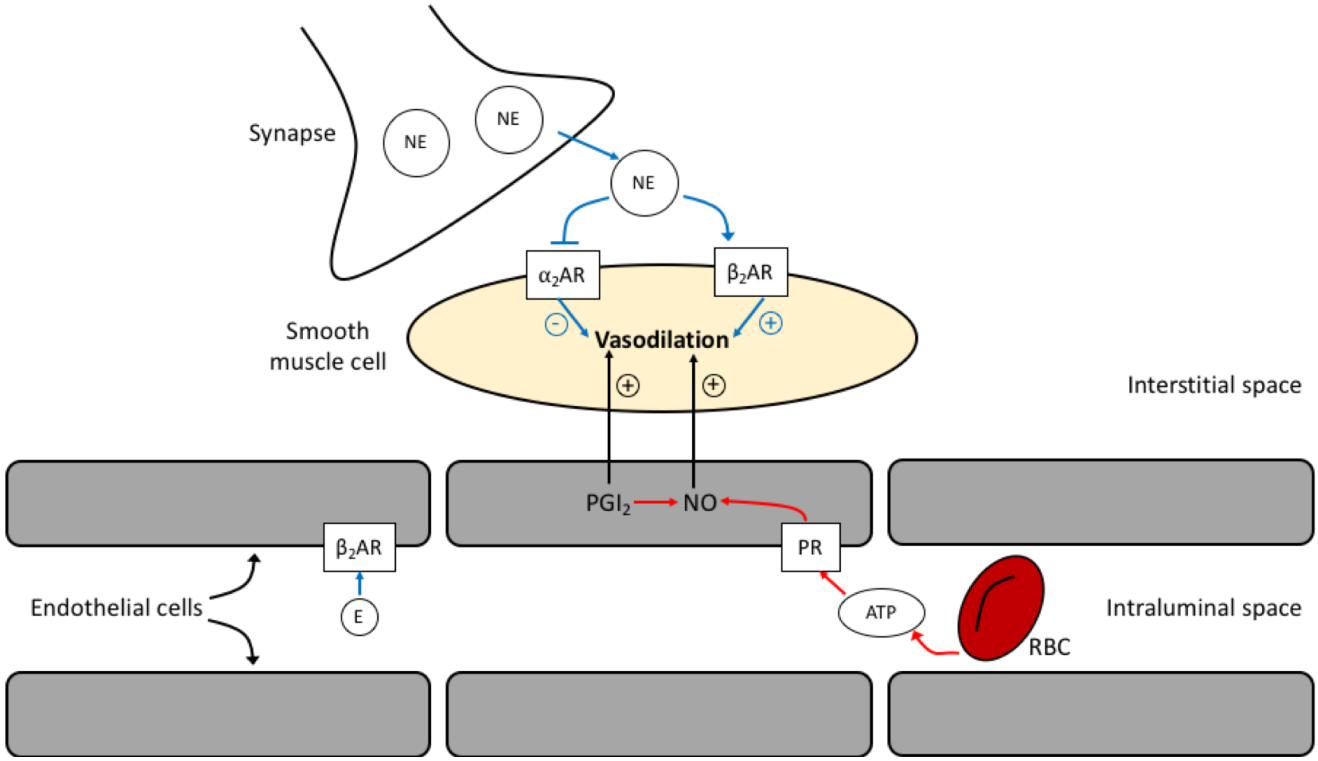


Figure 1.4: Proposed mechanisms of hypoxia-induced vasodilation during exercise. Mechanisms predominantly responsible for vasodilation during low-intensity exercise are indicated by blue arrows, mechanisms predominantly responsible for vasodilation during high-intensity exercise are indicated by red arrows. Nitric oxide (NO) is the final common pathway for the hypoxic compensatory dilator response. At lower exercise intensities, systemic epinephrine (E) and norepinephrine (NE) release contribute to NO-mediated vasodilation via β_2-adrenergic receptors (β_2AR), while simultaneously causing functional sympatholysis (reduced sympathetic vasoconstriction) via inhibition of α_2-adrenergic receptors. β_2-adrenergic contribution decreases with increasing exercise intensity. At higher exercise intensities, adenosine triphosphate (ATP) released from red blood cells (RBC) and/or endothelium-derived prostacyclin (PGI_2) may be responsible for stimulating NO. Figure adapted from Joyner (2014).

to normoxia, which accounted for a 16% reduction in exercise performance (Amann et al., 2007a). In contrast to whole-body exercise, when exercise is isolated to a specific muscular unit (e.g. quadriceps), muscle blood flow can increase sufficiently to maintain oxygen delivery equal to normoxia, even in extreme hypoxia ($F_IO_2 = 0.10$) at maximal workloads (Rowell et al., 1986). For this type of exercise, maximal power output is still reduced in hypoxia, but caused by a reduction in oxygen extraction, not oxygen delivery. Indeed, even for whole-body exercise, maintenance of oxygen extraction may determine extent of $\dot{V}O_{2max}$ impairment between individuals with a similar baseline capacity (Ponsot et al., 2010).

1.1.5 Oxygen delivery to the brain in hypoxia

Global cerebral blood flow (gCBF)

Although the human brain constitutes only 2% of body mass, it accounts for 20% of an individual's oxygen consumption at rest (Raichle and Gusnard, 2002). Oxygen delivery is closely matched to oxygen demand through adjustments in cerebral blood flow (CBF; Hoiland et al., 2016). The following equations govern the relationships between arterial oxygenation, gCBF, global cerebral oxygen delivery ($gCDO_2$), and cerebral oxygen extraction fraction (cOEF).

Arterial oxygen content:

$$C_aO_2(ml \cdot dl^{-1}) = [Hb] \times 1.36(\%S_aO_2 \div 100) + 0.003P_aO_2 \quad (1.1)$$

Global cerebral oxygen delivery:

$$gCDO_2(ml \cdot min^{-1}) = gCBF(ml \cdot min^{-1}) \times C_aO_2(ml \cdot dl^{-1}) \div 100 \quad (1.2)$$

Cerebral oxygen extraction fraction:

$$cOEF(\%) = [(C_aO_2(ml \cdot dl^{-1}) - C_jVO_2(ml \cdot dl^{-1})) \div C_aO_2(ml \cdot dl^{-1})] \times 100 \quad (1.3)$$

Where gCBF, global cerebral blood flow; C_aO_2, arterial oxygen content; [Hb], arterial haemoglobin concentration, P_aO_2, partial pressure of oxygen in arterial blood; cOEF, cerebral oxygen extraction fraction; C_jVO_2, jugular venous content of oxygen; 1.36 represents the affinity of oxygen for haemoglobin; and 0.003 represents the solubility of oxygen dissolved in blood.

At sea level, typical $gCDO_2$ is approximately 10 ml $O_2 \cdot 100g$ brain tissue$^{-1} \cdot min^{-1}$ (Kety and Schmidt, 1948). cOEF refers to the proportion of delivered oxygen that is consumed by the brain, and is approximately 35% at rest. The product of

CDO_2 and cOEF is equal to cerebral oxygen consumption ($CMRO_2$), so when metabolism is unchanged CDO_2 is inversely proportional to cOEF (Ainslie et al., 2016). Therefore, $CMRO_2$ can be maintained through alterations in CBF (and consequently CDO_2) or cOEF.

In hypoxia, gCBF increases to maintain $gCDO_2$ in the face of reduced blood oxygenation (Ainslie and Ogoh, 2010; Ainslie et al., 2014; Ainslie and Subudhi, 2014; Lucas et al., 2011). The increase is achieved through dilation of the pial vessels and a reduction in cerebral vascular resistance (Cohen et al., 1967), and is proportional to the degree of hypoxaemia (Willie et al., 2014). Below arterial PO_2 50 mmHg, for each 1% reduction in arterial oxygen saturation (S_aO_2), gCBF increases by 0.5-2.5% (Hoiland et al., 2016). This increase in gCBF to maintain CDO_2 occurs without alteration in cOEF since $CMRO_2$ is unaltered in hypoxia (Ainslie et al., 2014). The fall in C_aO_2 is greatest with acute hypoxia, and partially recovered over the course of acclimatisation to a given altitude ($\sim$2 weeks). Consequently, the inverse is true for gCBF (at least at rest) which returns towards baseline as acclimatisation recovers C_aO_2 (Ainslie and Subudhi, 2014; Lucas et al., 2011).

Although the hypoxia-induced increase in gCBF has been extensively observed, the mechanisms by which hypoxia triggers this compensatory response, are less certain. Studies have compared hypoxaemic hypoxia with hypoxia induced through experimentally lowered haemoglobin concentration by haemodilution. A reduction in C_aO_2 induced by haemodilution elicits a smaller increase in gCBF compared to the same change in C_aO_2 induced by hypoxaemic hypoxia, such that CDO_2 is not maintained (Hoiland et al., 2016). This blunted CBF response to reduced C_aO_2 despite maintained S_aO_2 indicates deoxyhaemoglobin as the primary regulator for CBF changes in response to hypoxia. Increased CBF is most likely achieved through deoxyhaemoglobin-mediated release of NO and ATP, and the reduction of NO_2^- to NO by deoxyhaemoglobin (Hoiland et al., 2016).

Even during maximal exercise at altitude, CDO_2 is maintained through marked elevation in CBF (Ainslie et al., 2016). CDO_2 during exercise at a given relative

intensity (% $\dot{V}O_{2max}$) was no different between sea level and high altitude (5,050 m), and elevated for a given absolute workload due to a reduction in $\dot{V}O_{2max}$ at altitude (Smith et al., 2014). However, the capacity to maintain CDO_2 during high intensity exercise may be dependent on the degree of hypoxaemia. In normoxia to moderate hypoxia ($F_IO_2 > 0.12$; $S_aO_2 > 75$ %; equivalent 0 - 5,000 m) peripheral (skeletal muscle) fatigue is the dominant limiting factor for exercise tolerance, but evidence indicates a shift towards central fatigue as the dominant limiting factor under more extreme hypoxia ($F_IO_2 < 0.12$; $S_aO_2 < 75\%$; equivalent > 5000 m; Goodall et al., 2014a). For example, Amann *et al.* (2007) observed significantly less peripheral fatigue determined by potentiated quadriceps twitchforce at task failure in extreme hypoxia ($F_IO_2 = 0.10$) compared to normoxia, or moderate hypoxia ($F_IO_2 = 0.15$). Further, Goodall *et al*. (2014) observed a 19% reduction in CDO_2 (compared to normoxia) in the final minute of fixed workload exercise in hypoxia, that was accompanied by elevated central fatigue. Both the reduction in CDO_2, and elevation in central fatigue, were attenuated after 14 days acclimatisation, attributed to a two-fold increase in corticospinal excitability.

Influence of carbon dioxide (CO_2)

The CBF response to hypoxia is not only determined by hypoxia in isolation, but is also affected by carbon dioxide status and sensitivity, particularly given the hyperventilation-induced hypocapnia that occurs in hypoxia (as described in Section 1.1.2). Carbon dioxide tightly regulates CBF; elevated carbon dioxide (hypercapnia) increases CBF, while hypocapnia results in cerebro-vasoconstriction and reduced CBF (Fox et al., 1992; Reivich, 1964). Indeed, carbon dioxide controls CBF over and above any effects of oxygen.

Due to the conflicting and confounding effect of hypocapnia, many studies have investigated the effects of hypoxia on cerebral parameters (gCBF, CDO_2, cOEF) under isocapnic conditions. CBF is greater in hypoxia when the vasconstrictive stimulus of hypocapnia is removed, compared to poikilocapnic conditions (where individuals are allowed to become hypocapnic; Ainslie and Poulin, 2004). In

addition, Van Dorp *et al.* (2007) observed the poikilocapnic hypoxia-induced reduction in brain oxygenation was somewhat ameliorated with isocapnia, associated with a similar recovery in cognitive performance. Poikilocapnic studies are able to give a real-world picture of the cerebrovascular response to the hypoxic environment, while isocapnic hypoxia provides a useful tool to determine the underlying mechanisms of this physiological response. Both experimental conditions have merit, but particular attention should be paid to whether the exposure is isocapnic or poikilocapnic when drawing conclusions from any study.

Regional cerebral blood flow (rCBF)

The majority of studies have focused on CBF alterations at a global level and much less investigation has been conducted regarding the effect of hypoxia on regional cerebral blood flow (rCBF). The few studies that have investigated rCBF in hypoxia have demonstrated that the increase in blood flow is not uniform across brain regions. One study used positron emission tomography (PET) imaging to determine regional responses to 30 min isocapnic hypoxia (Binks et al., 2008). Phylogenetically older areas of the brain (including the nucleus accumbens, pallidum, putamen, and thalamus), exhibited the greatest increases in CBF, a finding the authors attributed to their necessity for homeostatic regulation. Homeostatic regulation is not only vital for survival, but the complex ventilatory (Section 1.1.2) and cardiovascular (Section 1.1.3) responses required to tolerate hypoxia are likely to contribute to the exaggerated elevation in CBF in these regions. This finding is consistent with evidence from ultrasound studies which observed a greater increase in flow in the vertebral arteries (VA) compared to internal carotid arteries (ICA), suggesting CBF increases more to the brain stem than to middle or anterior brain regions (Lewis et al., 2014; Ogoh et al., 2013; Willie et al., 2012). In contrast, a recent study used used 4D flow magnetic resonance imaging (MRI) to provide a high spatial resolution rCBF measurements. This study reported similar relative changes in CBF in the VA compared to the ICA, but smaller CBF changes in the posterior cerebral arteries (PCA; Kellawan et al., 2017). However, the hypoxic exposure was

very acute, lasting only ∼5 min, and the authors only investigated CBF in the macrovasculature.

Differentiation between macrovasculature and microvasculature CBF alterations is essential for interpretation of regional cerebral haemodynamic responses (Krings et al., 1999; Zirak et al., 2014). The only study to investigate rCBF of the microvessels with prolonged (> 2 h) exposure to poikilocapnic hypoxia observed increased blood flow in cortical grey matter that was accompanied by reduced blood flow to the default mode network (DMN) with 2-10 h exposure to hypoxia (Lawley et al., 2017). The reduction in blood flow was most prevalent in the posterior cingulate cortex (PCC), which is largely responsible for long-term memory function (Raichle, 2015). This is the only study to observe reductions in blood flow to some brain regions in hypoxia. This unusual finding may relate to the use of poikilocapnic, rather than isocapnic hypoxia, the duration of exposure, or the use of an accurate and precise measure of rCBF (arterial spin labeling MRI). The cause and consequences of the observed reductions in rCBF are unknown, and warrant further investigation.

1.2 The high-altitude environment: how hypoxia affects exercise performance

1.2.1 Functionally-relevant exercise performance

Exercise performance is most commonly understood in terms of ability to complete a given distance in the least amount of time (time trial), or ability to tolerate a given intensity for the longest duration (time to exhaustion). Indeed, such assessments of exercise performance are commonly used to assess exercise performance in hypoxia, and provide insight to the effect of the hypoxic environment on exercise capacity. However, typical assessments of endurance performance (e.g. 5 km running time trial) have limited functional relevance for high-altitude exercise performance, as they do not represent the types of

activity commonly undertaken at altitude (e.g. trekking and mountaineering). To define functionally-relevant altitude performance, one must first identify the task that must be completed. The task for the mountaineer is arguably successful ascent of a mountain. However, summit success, as with any field-based test, is confounded by external factors such as weather, and partner failure (Wagner et al., 2008).

In the absence of a functionally-relevant, internally-valid, measure of altitude exercise performance, much of the altitude literature has used $\dot{V}O_{2max}$ as a proxy for endurance performance, but this is inappropriate since the constructs of fitness and performance are conceptually distinct. This concept is illustrated by the non-linear relationship between $\dot{V}O_{2max}$ decrement and submaximal exercise performance decrement (described in Section 1.2.4). The following section outlines the effect of hypoxia on exercise performance in reference to the available literature, despite the limitations of the assessments used.

1.2.2 Impaired maximal aerobic capacity ($\dot{V}O_{2max}$)

At maximal exercise, the combined effects of reduced arterial oxygen content and reduced maximal cardiac output impair oxygen delivery, resulting in reduced maximal aerobic capacity ($\dot{V}O_{2max}$). The relationship between altitude and $\dot{V}O_{2max}$ decline is not linear across all altitudes, since $\dot{V}O_{2max}$ is minimally affected at low altitude but declines rapidly (and intolerably) at the most extreme altitudes (Fulco et al., 1998). However, a relatively linear and undisputed relationship exists between altitude and $\dot{V}O_{2max}$ decline for the intermediate altitudes, covering all heights between "moderate" and "very high" altitudes (according to altitude definitions in Figure 1.1). Fulco *et al.* (1998) examined the decline in $\dot{V}O_{2max}$ across hundreds of studies and thousands of participants (Figure 1.5). Between 700 and 6300 m, $\dot{V}O_{2max}$ declines by 8% with every 1000 m of altitude (Fulco et al., 1998; Grover et al., 1986).

Although the relationship between altitude and $\dot{V}O_{2max}$ decline is well-established at a population level, certain inter-individual factors may moderate

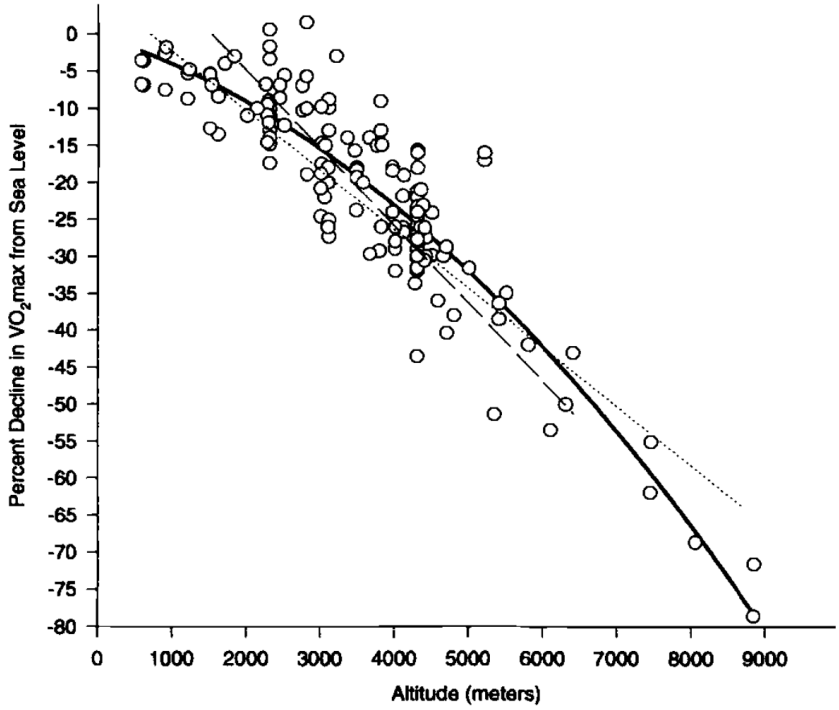


Figure 1.5: Percent $\dot{V}O_{2max}$ decline with increasing elevation. Each point on the graph represents a mean value from a previous study. The solid regression line is derived from these points, the dashed line represents the regression line of Buskirk et al., 1967, and the dotted line represents the regression line of Grover et al., 1986. Figure taken from Fulco (1998).

the relationship. Masschelein *et al.* (2015) identified 12 genetic variations (single nucleotide polymorphisms) that combined accounted for 23% of the variance in hypoxia-induced $\dot{V}O_{2max}$ decline. In addition, superior sea level $\dot{V}O_{2max}$ is associated with an elevated decline in $\dot{V}O_{2max}$ at altitude (Chapman, 2013). This phenomenon is commonly explained through pulmonary gas exchange limitations at high cardiac outputs and is discussed in detail in Section 1.5.

1.2.3 Relative and absolute intensities

Since maximal exercise capacity is decreased at altitude, submaximal exercise intensity defined relative to altitude-specific maximum (%$\dot{V}O_{2max}$) is proportionally decreased (Noordhof et al., 2013). Accordingly, submaximal exercise responses for a given relative workload are largely unchanged. However, under conditions of hypoxia, greater ventilation and cardiac output are required

to meet a given oxygen demand. Therefore, since the oxygen cost ($\dot{V}O_2$) for a given absolute workload (e.g. running 10 km·h^{-1} at 1% gradient) is largely unchanged at altitude (Knuttgen and Saltin, 1973), the physiological response required for an absolute workload is increased. Taken together, this means that a given absolute workload corresponds to a higher relative intensity at altitude. Almost all exercise activities completed at altitude require sustained submaximal exercise lasting many hours. Such prolonged work cannot usually be sustained at intensities above 60-70% $\dot{V}O_{2max}$ (Garber et al., 2011), therefore this increase in the relative intensity for a given workload is of particular concern for those who travel to high altitude.

1.2.4 Quantifying the effect on exercise performance

The effect of hypoxia on submaximal exercise performance is not linear across different exercise intensities (Peronnet et al., 1991). The effect on performance in running, cycling, and swimming races is dependent on the duration of event (and therefore intensity). Fulco *et al.* (1998) defined the effect on submaximal performance according to event durations < 2 min, 2-5 min, 20-30 min, and 2-3 h. Altitude had the most profound effect on performance with event duration 2-3 h, since these events require the largest proportional oxygen contribution. For these long duration events, the performance decrement exceeds the $\dot{V}O_{2max}$ decrement. In contrast, for all other durations the contribution of anaerobic energy sources result in a performance decrement that is proportionally lower than the $\dot{V}O_{2max}$ decrement. For example, a 15% reduction in $\dot{V}O_{2max}$ at 2300 m, would accompany a 17% reduction in an event lasting 2-3 h, but only a 2% reduction in performance on an event lasting < 2 min. In fact, the reduced air density, and thererefore reduced aerodynamic drag, at altitude can result in improved performance for short-duration running and cycling events (Bassett et al., 1999; Wilber, 2004).

1.2.5 Other measures of fitness may be important for determining exercise performance at altitude

As discussed above, the effect of altitude on $\dot{V}O_{2max}$ has been extensively studied. However, $\dot{V}O_{2max}$, is not the only important measure of fitness for determining long-duration submaximal exercise capacity, and other measures of fitness, such as fractional utilization of $\dot{V}O_{2max}$ (e.g. ventilatory threshold) and economy, are also important for endurance performance (Bassett and Howley, 2000; Joyner and Coyle, 2008). Anaerobic threshold refers to the relative intensity that an individual is able to maintain through predominantly aerobic energy sources, and represents the exercise intensity that can be maintained for prolonged periods (Gaskill et al., 2001). As mentioned above, for the average individual this is 60-70%, but in sedentary individuals it can be as low as 40%, and in some trained individuals it can be as high as 85% (Åstrand and Rodahl, 1970). Individuals with a higher anaerobic threshold are able to maintain high intensity exercise for longer, even compared to individuals with the same $\dot{V}O_{2max}$ (Coyle et al., 1988). Therefore individuals with a higher anaerobic threshold are likely better able to accommodate the increase in relative intensity for the sustained maximal exercise at altitude.

Economy refers to the $\dot{V}O_2$ for a given absolute workload; superior economy refers to the ability to maintain an absolute workload with a lower $\dot{V}O_2$, and is closely associated with superior exercise performance (Conley and Krahenbuhl, 1980). Therefore an individual with superior economy would be able to maintain higher absolute workloads despite the limited oxygen availability at altitude.

1.2.6 Predicting exercise performance at altitude

As has been shown in Sections 1.2.2 and 1.2.5, maximal aerobic capacity and other measures of fitness are insufficient to predict exercise performance at altitude. Consequently, other methods to predict exercise performance at altitude have naturally been sought. One study was able to significantly predict

percent increase from sea level in time trial duration on the basis of body mass index (BMI) classification and altitude, accounting for 47% of the variability (Beidleman et al., 2015). The model equation is outlined below.

Percent increase in time trial duration:

$$\triangle TT(\%) = 100 + exp[-1.517 + (1.323ALT) + (3.124BMI_{class}) - (0.769 \times ALT \times BMI_{class})] \quad (1.4)$$

Where BMI_{class}, $<$ 25 kg/m = 0 (normal weight) and $\geq$ 25 kg/m = 1 (overweight); ALT, altitude (km).

Using oxygen saturations to predict exercise performance

S_aO_2 has received a great deal of attention in regard to predicting altitude exercise performance, since hypoxaemia reflects the degree of hypoxia within an organism, and S_aO_2 is easily and accurately assessed by pulse oximetry (S_pO_2; Basnyat, 2014). One study found S_aO_2 during ascent to base camp correlated with maximal altitude reached on an expedition to Broad Peak (8051 m; Tannheimer et al., 2002). However, other studies have repeatedly shown that S_aO_2 measured at rest before a summit attempt does not differentiate between those who successfully summit and those who are unable to summit (Davies et al., 2009; Wagner et al., 2012). This may be due to S_aO_2 being assessed at rest in these studies, since S_aO_2 at rest may not be related to S_aO_2 during exercise, and has been shown to have poor predictive power compared to S_aO_2 during exercise (Richalet et al., 2012).

Lazio *et al.* (2010), reported significantly higher post-exercise S_aO_2 in summiters compared to non-summiters. The authors concluded S_aO_2 on completion of the 6-minute walk test predicted summit success and proposed its use as a screening tool. However, this conclusion was made on the basis that post-exercise $S_aO_2 \geq$ 75% had 97% sensitivity for determining summit success, despite poor specificity (32%) for this criterion. This specificity score means that the cut-off criterion will wrongly predict summit success in 68% of individuals who fail to reach

the summit, therefore the practical usefulness of this criterion is limited. In addition, Daniels (2012) attempted to replicate the study by Lazio *et al.*, but found no difference in post-exercise S_aO_2 from the 6-minute walk test according to maximum altitude reached.

Further, there is also controversy over the extent the change in S_aO_2 determines changes in exercise performance, even when S_aO_2 is assessed during the exercise performance itself. Chapman *et al.* (2011) found that those with the greatest reduction in S_aO_2 had significantly greater performance decrement during 3 km time trial in hypoxia compared to normoxia. In contrast, Saugy *et al.* (2015) found no correlation between change in S_aO_2 and change in time trial performance from normoxia to hypoxia in endurance-trained males.

A possible explanation for these contradictory findings is that although S_aO_2 is closely related to severity of hypoxic exposure, it may not provide useful insight to differentiate between individuals subjected to the same (or at least similar) hypoxic exposure. Due to its close relationship with the severity of hypoxic exposure, when considered over all hypoxic exposures (including passive and active, moderate to extreme altitude, acute to chronic, and even medicated versus non-mediated), degree of hypoxaemia is closely related to performance impairment. However, it fails to account for inter-individual differences in physiological compensation for hypoxaemia. Physiological components that affect the relationship between S_aO_2 and exercise performance include elevated cardiac output to maintain oxygen delivery, enhanced oxygen extraction at the muscle, and improved economy.

1.3 The high-altitude environment: how hypoxia causes acute mountain sickness (AMS)

1.3.1 AMS definition, measurement, and prevalence

AMS is a self-limiting condition that occurs with recent altitude ascent (> 2,500 m) or hypoxic exposure, and is characterized by the presence of headache accompanied by other symptoms including nausea (or poor appetite), fatigue (or weakness), dizziness (or light-headedness), and difficulty sleeping (Roach et al., 1993). The most common assessment of AMS is the Lake Louise Questionnaire (LLQ) score, a self-report scale where individuals report severity of the five symptoms from 0 (none), to 3 (severe, incapacitating). However, a recent meeting of the Lake Louise AMS Score Consensus Committee concluded difficulty sleeping should be removed from AMS assessment since it is likely a separate, unrelated consequence of hypoxia *per se*, rather than a symptom of the condition (Roach et al., 2018).

The LLQ is not the only available assessment for AMS. The Environmental Symptoms Questionnaire (ESQ) is designed to measure subjective reactions to extreme environments including, but not limited to, high altitude (Sampson et al., 1994). The full questionnaire has 67 items where individuals report symptom severity from 0 (not at all) to 5 (extreme) and each item is weighted according to a factor loading. A shortened 11-item version exists to assess AMS only, separated into the cerebral (AMS-C), and respiratory (AMS-R) components of the condition. Individuals can be classified as AMS present or absent on the basis of an AMS-C score of ≥ 0.7 (Sampson and Kobrick, 1980).

The ease of use (only four or five items) makes the LLQ ideal for use in the field, and for monitoring symptoms in an applied setting, while the factor weighting of the ESQ accounts for relative importance of different symptoms. Both measures are valid and useful assessments for AMS; therefore the decision over which

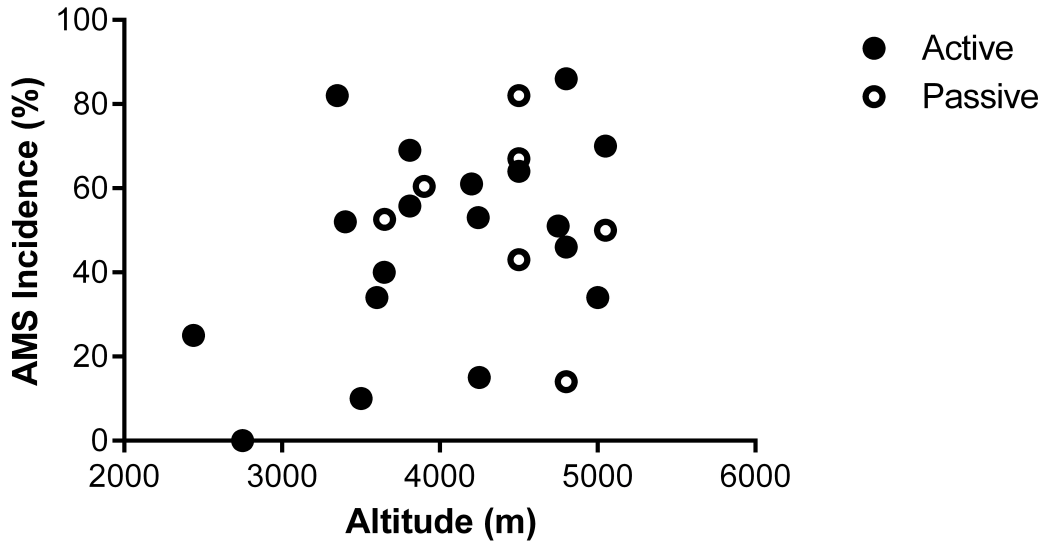


Figure 1.6: Acute mountain sickness (AMS) prevalence according to altitude. Each individual point represents the group incidence derived from a previous study (Anderson et al., 2011; Broessner et al., 2016; Hackett et al., 1976; Honigman et al., 1993; Hsu et al., 2015; Kanaan et al., 2015; Lawley et al., 2014a; Lipman et al., 2012; Maggiorini et al., 1990; Mairer et al., 2012; Mandolesi et al., 2014; Ren et al., 2015; Roach et al., 2000; Schommer et al., 2012; Tsianos et al., 2006; Vardy et al., 2006; Zheng et al., 2014; Ziaee et al., 2003). Data delimited according to active (filled circles) or passive (open circles) exposure, with all mountaineering or trekking studies counted as active.

scale should be used should be based on how the specific situation matches to the relative merits of the two scales.

Approximately half of those who visit high altitude suffer from AMS (Bärtsch and Swenson, 2013). The prevalence of AMS varies according to ascent rate, and pre-exposure (altitude history), since adequate exposure to a given altitude allows acclimatisation to occur. The preventative effects of slow ascent rate and pre-exposure are discussed in detail in Section 1.3.3. It is widely believed that altitude is positively related to AMS, since the severity of the hypoxic stimulus is greater, although above ∼3000 m, studies at higher altitudes do not generally report greater incidence of AMS (Figure 1.6). Similarly, although exercise may exacerbate AMS through elevated cardiac output exacerbating hypoxaemia (Roach et al., 2000), experimental studies provide conflicting results regarding the effect of exercise on AMS (Mairer et al., 2013; Sandoval et al., 2001). Indeed, AMS prevalence cannot be differentiated according to active versus passive exposures (Figure 1.6; filled versus open circles). It is possible

that altitude and exercise may increase the severity of AMS, without affecting prevalence.

Other demographic factors are related to increased AMS prevalence, including female sex, age younger than 46 years, and obesity (Bärtsch and Swenson, 2013; Imray et al., 2011; Ri-Li et al., 2003). However, evidence for these associations is almost exclusively from cross-sectional field-studies, which do not account for behavioural differences such as ascent rate, pre-exposure or degree of exertion. Therefore, it is not appropriate to assume a mechanistic, causative link exists between these demographics and AMS prevalence.

1.3.2 AMS pathophysiology

The precise pathophysiology of AMS is not certain, but a growing body of data supports the hypothesis that high-altitude headache occurs as a result of arterial dilation causing elevated cerebral blood volume (CBV), intracranial pressure (ICP) and trigeminovascular sensitization, driving the accompanying symptoms (Lawley et al., 2016). As described previously, gCBF is increased in hypoxia such that CDO_2 is maintained despite hypoxaemia (Section 1.1.5). This increase in CBF results in a concomitant elevation of CBV (Willie et al., 2012), and ICP (Lawley et al., 2014a), so long as sagittal sinus pressure is also increased (Ursino and Lodi, 1997). However, the degree of ICP elevation is not consistent across individuals (Wilson and Milledge, 2008), and may be greatest in those who develop AMS (Lawley et al., 2014a). In particular, elevated CBV may cause a transient rise in ICP in all individuals (Wright et al., 1995), that is recovered through spatial compensation by cerebral spinal fluid (CSF) outflow in those who do not develop AMS (Lawley et al., 2016). It is individual variance in spatial compensation capacity and craniospinal elasticity, summarized as "cerebral-spinal compliance", that may determine whether an individual develops AMS.

Bailey *et al.* (2009) propose an alternative model which places the production of free radicals at the centre of AMS pathophysiology. Hypoxia catalyses the

production of the hydroxyl radical (OH), which may cause sodium-potassium pump failure resulting in astrocyte swelling, further oxidative stress, oxygen diffusion limitation, and increased production of NO. The production of NO, combined with free radical-mediated lipid peroxidation, membrane permeability, and inflammation, results in direct stimulation of the trigeminovascular system, causing high-altitude headache and AMS. However, this model relies on elevated free-radical production in individuals suffering from AMS compared to their healthy counterparts, a hypothesis not supported by empirical data (Bailey et al., 2009a; Bailey et al., 2009b).

1.3.3 Interventions to prevent AMS

Non-pharmacological prophylaxis

The most effective preventions for AMS are non-pharmocological, and behavioural. Slow ascent allows for adequate acclimatisation, preventing the development of AMS at all but the most extreme altitudes. In AMS-susceptible individuals (determined by AMS history in regular mountaineers), AMS incidence was reduced from 58% to 33% on ascent to Cappanna Margherita (4,559 m) when ascent was more than 3 days, compared to faster ascents (Schneider et al., 2002). In the same study, pre-exposure to altitude (> 4 days above 3000 m in the 2 months prior) was similarly effective in reducing AMS incidence, and the combination of slow ascent and pre-exposure reduced AMS incidence to 7%. Current guidelines recommend limiting ascents to 300-500 m a day above 2500 m, with a rest day every 3-4 days (Luks, 2012). These behavioural interventions of slow ascent and pre-exposure are particularly beneficial since they are free from side effects. However, these interventions are costly, logistically difficult, and therefore not always possible for individuals to complete; consequently, alternative strategies to enable fast ascent without AMS have been sought.

Alternative therapies such as the radical scavenger ginkgo biloba have been investigated, although the evidence for this an effective prophylaxis is weak. In

particular, multiple studies have compared gingko biloba to the pharmacological intervention acetazolamide, and placebo, finding that AMS incidence was significantly lower with acetazolamide, but no different between placebo and gingko biloba (Chow et al., 2005; Gertsch et al., 2004). This finding is consistent with studies that have compared the effect of these two interventions on hypoxic pulmonary vasoconstriction (Ke et al., 2013). Further, one multi-study article reported gingko biloba significantly reduced AMS incidence in the first study, but had no preventative effect in the second study (Leadbetter et al., 2009). The authors cite differences in the source and composition of the gingko biloba used as the cause of the discrepancies across the two studies. Inconsistencies in preparation and content of commercially available gingko biloba prevents confident interpretation of its effectiveness from the current literature (Tissot van Patot et al., 2009).

Pharmacological prophylaxis

The most common pharmacological prophylactic for AMS is the carbonic anhydrase inhibitor acetazolamide (Bärtsch and Swenson, 2013). Acetazolamide prophylaxis is associated with a 50% relative reduction in AMS (Ritchie et al., 2012), and the effectiveness of acetazolamide prophylaxis is increased when the risk of AMS is high, for example with fast ascent rates (Kayser et al., 2012). Acetazolamide protects against AMS through inhibition of peripheral chemoreceptors, and enhanced central chemoreceptor output achieved through tissue and metabolic acidosis. This enables hyperventilation despite low P_aCO_2, increasing S_aO_2. Acetazolamide may increase ventilation by up to 50% at altitude, achieved primarily through an increase in tidal volume (not breathing frequency; Tojima et al., 1986). In addition, acetazolamide attenuates the increase in gCBF, and improves dynamic cerebral autoregulation in hypoxia, although these effects may be independent of AMS development (Subudhi et al., 2011).

A minimum dose of 250 mg daily is required for a protective effect, but no greater benefit is observed with higher doses (Ritchie et al., 2012). This should

be particularly considered in light of the associated side effects which are increased with higher doses. Side effects include paraesthesia, polyuria, rash, dysgeusia, and micturition (Low et al., 2012; Smedley and Grocott, 2013). Further, acetazolamide use is associated with reduced cognitive performance (Wang et al., 2013), and impaired exercise tolerance (Bradwell et al., 2014; Hackett et al., 1985) possibly related to it inducing hypohydration (Brechue and Stager, 1990).

The second most common pharmacological prophylactic for AMS is the use of glucocorticoids, such as dexamethazone. Dexamethazone has similar efficacy to acetazolamide in preventing AMS ($\sim$50%; Tang et al., 2014). Dexamethazone attenuates cytokine and inflammatory responses to hypoxia, reducing capillary permeability (Johnson et al., 1984). However, the negative side effects of dexamethazone are such that it is not recommended for use except when acetazolamide is contraindicated, or extreme ascents are necessary (Imray et al., 2011). These side effects include acute psychosis, depression, and glucose intolerance (Ellsworth et al., 1991).

1.3.4 Interventions to alleviate AMS

AMS is a self-limiting condition, meaning it will resolve on its own with no long-term harm to the individual. As such, for mild to moderate AMS, treatment is not necessary, merely the abstention from activities known to exacerbate the condition is required; physical exertion and continued ascent. If AMS persists after a day or two of rest, it can be treated easily, since the cause is known and self-inflicted (altitude); a descent of 500-1000 m is advised (Bärtsch and Swenson, 2013). If evacuation or descent is not possible, the hypoxic stimulus can be removed through the use of supplemental oxygen (Imray et al., 2011) or simulated descent by a portable hyperbaric chamber (Bärtsch et al., 1993). Even when descent is possible, individuals are often reluctant to cease ascent (or to descend) given the financial commitments and time constraints associated with high-altitude travel in those who travel for leisure, and the necessity of time-dependent travel in those who travel for work (e.g. military

deployments). Therefore alternative interventions to treat the condition, whilst allowing continued activity or ascent, must be considered.

Analgesics (paracetamol and non-steroidal anti-inflammatory drugs; NSAIDs) are commonly used to reduce AMS symptoms (Broome et al., 1994; Gertsch et al., 2012), but evidence that these treat the condition (rather than symptom management through pain relief) is lacking (Gertsch et al., 2010; Zafren, 2012). Indeed, ibuprofen may actually blunt acclimatisation to altitude, evidenced by a reduced isocapnic HVR at altitude with ibuprofen compared to placebo (Basaran et al., 2016). It is far more likely these analgesics mask symptoms, which could lead to continued ascent with AMS and further complications from more severe illnesses such as HACE and HAPE.

In individuals who treat AMS pharmacologically, the interventions used as pharmacological prophylaxis are also effective at alleviating AMS. The Wilderness Medical Society recommend 250 mg of acetazolamide every 12 h until symptoms resolve (Luks et al., 2014). In one study, acetazolamide significantly reduced AMS score compared to placebo, and returned 83% of AMS sufferers to a healthy (non-AMS) state (Grissom et al., 1992). In addition, acetazolamide use improved physiological markers of altitude tolerance, including alveolar and arterial PO_2 and pulmonary oxygen diffusion, indicating acetazolamide was effective in treating the condition, not merely symptoms. The administration of acetazolamide accompanied by dexamethasone is also more effective than treatment with acetazolamide alone (Bernhard et al., 1998).

1.3.5 Inter-relationship between AMS and exercise performance at high altitude

The relationship between AMS and exercise performance is controversial. Low AMS was associated with a faster ascent rate averaged over an expedition, but had no impact on summit success, when participants were allowed to select their own ascent rate (Pesce et al., 2005). However, when participants were randomly allocated to fast and slow ascent groups, those selected to ascend

slowly had a lower incidence of AMS, but a decreased chance of summit success (Bloch et al., 2009). These studies were limited by using total ascent over the expedition which is unlikely to predict summit success on long expeditions (Pesce et al., 2005), rather than walking pace, which is a more appropriate measure of exercise performance. More recent studies have found no relationship between AMS scores and summit success (Lazio et al., 2010; Wagner et al., 2008; Wiseman et al., 2006). However, these studies are unable to accurately determine the relationship between AMS and mountaineering performance as they all report AMS only when individuals felt worst, and therefore do not provide a comprehensive assessment of AMS over the expedition (for example during a summit attempt).

In contrast, Hazlerigg *et al.* (2016) examined the sense of effort during exercise and AMS score, on each day of an ascent to ∼5000 m. Although not a direct measure of exercise performance, sense of effort determined by rating of perceived exertion (RPE) during submaximal exercise is closely related to exercise capacity, and a well-established predictor of exercise performance (Eston, 2012). RPE during exercise was higher in individuals with AMS, and in those who went on to develop AMS the following day. Further support exists for the proposed relationship between greater exercise tolerance (assessed by lower RPE) and protection from AMS. Burtscher *et al.* (2011) found high RPE was a risk factor for high-altitude headache in mountaineers, when RPE was assessed using a 4-point scale (very low, low, moderate, heavy). One further study observed increased RPE for a day's trekking was associated with an elevated AMS score, and a lower S_aO_2 at the end of the day and the following morning (Mellor et al., 2014).

Taken together these studies suggest that those who choose to ascend faster, and succeed in ascending further, put themselves under greater hypoxic stress and increase their risk of experiencing AMS, while those who experience AMS at low altitude are unable to succeed in reaching the summit. To date, no study has measured AMS scores and altitude performance on the same day to assess the relationship between the two.

1.4 The high-altitude environment: how hypoxia affects cognitive performance

Cognitive impairment is the most common reported symptom of hypoxic events in military pilots (Cable, 2003), and is one of the most common symptoms experienced by fatalities on Mt Everest (Firth et al., 2008). Therefore cognitive impairment is an important aspect of human performance in hypoxia.

1.4.1 Acute hypoxia and cognition

Numerous aspects of cognitive function are affected by hypoxia, including simple and choice reaction time (Phillips et al., 2015), working memory (Asmaro et al., 2013), and memory recognition and emotion identification (Lefferts et al., 2016). Indeed, a recent meta-analysis found that in acute hypoxia, cognition was impaired equally across all neurocognitive domains (McMorris et al., 2017).

However, this finding is not consistent across all levels of hypoxia. It appears some aspects of cognition are robust to hypoxia, particularly low to moderate hypoxia. Pilmanis *et al.* (2016) investigated the effect of 20-100 min low-moderate hypoxia (equivalent ∼1,500-3,700 m) on a battery of cognitive tasks. The hypoxia stimulus did not affect performance on the majority of tasks, including mathematical and spatial processing, and choice reaction time. Similarly, a 2 h simulated ascent to 4500 m did not alter word fluency, word association, or lateralized lexical decision performances (Pavlicek et al., 2005). However, some cognitive functions are impaired even by low to moderate hypoxia. Accuracy and reaction time were significantly impaired on the Continuous Performance Task from only 1524 m (Pilmanis et al., 2016). The Continuous Performance Task is an assessment of executive and attentional functions; it requires participants to view consecutive stimuli and identify whether each stimulus matches the one shown previously (Roebuck et al., 2016). The task activates frontal, parietal and occipital regions belonging to the

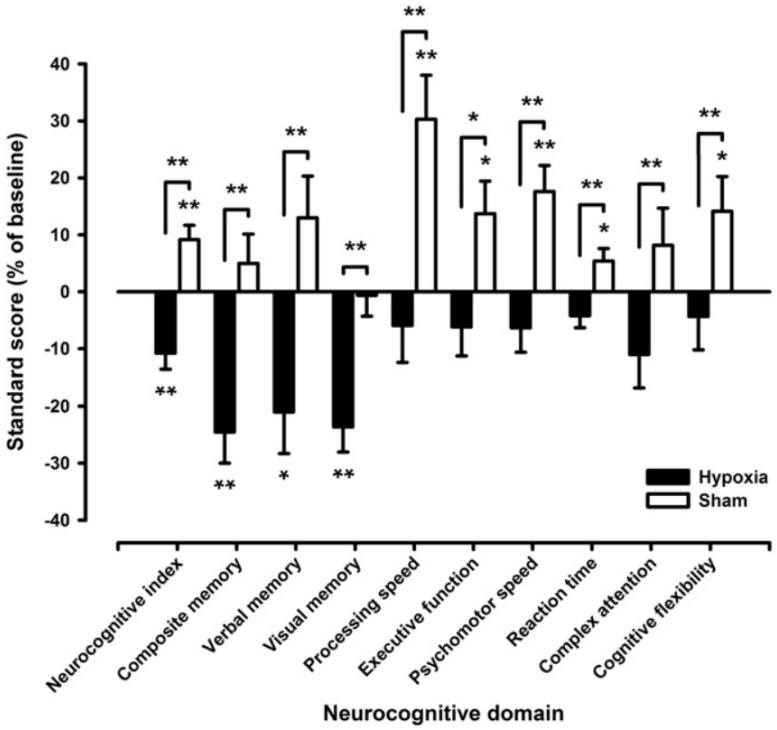


Figure 1.7: Effects of acute hypoxia on neurocognitive domains. Change in neurocognitive domain standard scores collected during hypoxia (black fill) and sham-hypoxia (white fill) normalised to the baseline condition. Significance labelling above individual bars show one sample comparisons relative to baseline. Significance labelling with comparator bars shows independent sample comparison of hypoxia to sham interventions. Bars show mean change in standard score from baseline ± SEM; *p < 0.05, **p < 0.001. Figure taken from Turner et al., 2015.

salience network (SN; Bartés-Serrallonga et al., 2014; Qiao et al., 2018). Studies using progressive hypoxic exposures have demonstrated a linear relationship between the severity of the hypoxic exposure (and hypoxaemia), and the decline in performance on executive functioning and working memory tasks (Asmaro et al., 2013; McMorris et al., 2017; Ochi et al., 2018). Ochi *et al.* (2018), observed significantly greater interference on a Stroop task with four progressive hypoxic exposures, with a weak but significant correlation between individual hypoxaemia and stroop interference across the four exposures.

With extreme hypoxia, the cognitive effects can remain even after individuals return to normoxia. Phillips *et al.* (2015) exposed healthy adults to extreme hypoxia (F_IO_2 = 0.10) for 30 min, and observed impaired simple and choice reaction time for up to 120 min post.

The effect of hypoxia on cognitive performance may be underestimated when simply compared to baseline performance, since a reduction in performance must be considered in the context of expected learning effects. For example, acute exposure to extreme hypoxia ($F_IO_2 = 0.10$) elicits a greater reduction in memory performance than executive functioning when both are expressed as comparisons to baseline scores. However, when compared to a sham-hypoxia group completing the same cognitive test protocol while exposed to normoxia ($F_IO_2 = 0.21$) the impairment seems similar across the tasks, since the sham group demonstrate a greater learning effect on the executive functioning task (Turner et al., 2015; Figure 1.7).

1.4.2 Chronic hypoxia and cognition

Studies investigating the effect of prolonged hypoxic exposures on cognitive outcomes in lowlanders have typically been field studies with small sample sizes. For example, a recent field study observed no impairment in working memory over a four-week ascent to 8043 m (Malle et al., 2016). The authors primarily attributed this null-finding to adequate acclimatisation since participants completed a two-week pre-acclimatisation trek, and no AMS was observed except on the final ascent. However, since the study was conducted on just four participants, it is likely the study was simply underpowered to detect an effect. Similarly, Bonnon *et al.* (1999) observed up to 30 d altitude exposure had no effect on cognition, and attributed this to adequate acclimatisation, despite no justification of their sample size of just seven. Since chronic exposure studies are typically completed in the field (with limited control of confounding variables such as hydration, sleep, and nutrition), and are characterised by small sample sizes, it is difficult to interpret these null-effects with any confidence. That they observe no cognitive impairment could be a result of adequate acclimatisation (as most conclude) or type II statistical errors (as a result of insufficient sample size).

The few rigorous studies to investigate the effects of chronic hypoxia on cognition do not support the findings of these poorly-controlled field studies. The “Everest-

Comex 97" study used a 31 d simulated Mt Everest (8,848 m) summit in a decompression chamber to observe the effects of chronic and extreme hypoxia on cognitive function. The study observed significant impairments in psychomotor ability (determined by a pegboard task), and mental efficiency (determined by a number ordination task) from 8000 m (Abraini et al., 1998). Interestingly, the cognitive deficits observed in low-landers are exacerbated in those who reside at high altitude for extended durations (Gao et al., 2015; Rimoldi et al., 2016). Taken together, this suggests hypoxia-induced cognitive impairments are not attenuated with acclimatisation.

1.4.3 Understanding underlying cerebral physiology is necessary for cognitive performance interventions

Cognitive impairment in hypoxia has logically been attributed to reduced C_aO_2, resulting in impaired CDO_2. However, this assumption is in direct contradiction to the wealth of evidence that CDO_2 is maintained at rest, and even during moderate exercise, in hypoxia (described in Section 1.1.5). Further, what limited data there are regarding regional differences in CBF responses to hypoxia were collected at rest, and are therefore unable to elucidate domain-specific differences in cognitive impairment (Lawley et al., 2017). As such, the mechanisms governing cognitive performance deficits at altitude are unclear. Given the central role of CO_2 in the governance of CBF, it is likely hyperventilation-induced hypocapnia contributes to cognitive impairment. Van Dorp *et al.* (2007) found that the addition of CO_2 to return participants to normocapnia ameliorated, but did not reverse, hypoxia-induced impairments in cognitive function. Although a contributing factor, hypocapnia only accounted for $\sim$50% of performance decrements. In addition, hypocapnia cannot explain regional differences in CBF, since known regional differences in sensitivity to CO_2 do not correlate with the observed rCBF responses (Ito et al., 2000; Lawley et al., 2017), or the brain regions responsible for the most severely affected neurocognitive domains. Elucidating mechanisms of cerebral physiology during

cognitive tasks in hypoxia warrants further investigation and may provide insight to potential targets for future interventions.

1.5 Cardiovascular fitness is a modifiable factor affecting health and performance at high altitude

1.5.1 Cardiovascular fitness and hypoxic sensitivity

Cardiovascular fitness affects physiological responses to hypoxia, and greater cardiovascular fitness is associated with a reduced HVR. Endurance athletes display a blunted HVR compared to healthy controls, with a negative correlation observed between $\dot{V}O_{2max}$ and HVR (Byrne-Quinn et al., 1971). Interestingly, this is in contrast to the elevated HVR present in experienced mountaineers (Schoene, 1982). Highly-trained individuals hypoventilate at sea level, both at rest and during exercise, which reduces W_B and enables greater physiological economy (Milledge et al., 1983; Schoene, 1982), but may prevent effective acclimatisation at altitude.

Endurance athletes experience a large arterial oxygen desaturation with altitude (Chapman, 2013; Gore et al., 1996; Mollard et al., 2007; Woorons et al., 2005), partly due to their blunted HVR. This exacerbated hypoxaemia is most prevelent in trained individuals during exercise, even with fixed workload exercise that should be more tolerable in trained compared to untrained individuals (Lhuissier et al., 2012). Endurance athletes perform on the steep part of the oxygen-haemoglobin dissociation curve at sea level (Ferretti et al., 1997; Wehrlin and Hallén, 2006), causing a greater reduction in S_aO_2 for the same reduction in PO_2. Endurance-trained athletes suffer exercise-induced hypoxaemia (EIH) even at sea level, caused by reduced erythrocyte transit time through alveolar capillary preventing full oxygen saturation of haemoglobin (Powers et al., 1988). This is an effect of the increased cardiac output for the same total blood volume

in physically-fit individuals. This exaggerated loss of S_aO_2 for a given PO_2 contributes to the greater reduction in $\dot{V}O_{2max}$ experienced by individuals with a high sea-level fitness (compared to their low-fit counterparts). Especially since the reduction in $\dot{V}O_{2max}$ is related to the change in S_aO_2 in elite endurance athletes, but not in recreationally-active individuals (Faiss et al., 2014).

However, Lhuissier *et al.* (2012) observed a non-significant trend for elevated cardiac response to exercise in hypoxia in trained compared to untrained individuals. Considered in combination with their already-elevated cardiac output, this suggests that although the S_aO_2 is reduced in trained athletes, the actual total oxygen delivery is maintained through higher cardiac output (West, 1982).

1.5.2 Cardiovascular fitness and high-altitude exercise performance

Napoli *et al.* (2009) found no association between self-reported exercise per week and summit success on an ascent to 4322 m, but reported no measure of fitness. In contrast, other studies suggest that greater physical fitness is associated with improved mountaineering performance. High $\dot{V}O_{2max}$ was associated with summit success on expeditions to Mount Cho-Oyu (8,201 m; Horii et al., 1994), and Muztagh Ata (7,546 m; Bloch et al., 2009). Richalet *et al.*, (1988) observed a significant ($p < 0.001$), moderate ($r = 0.47$), positive correlation between $\dot{V}O_{2max}$ and maximum altitude reached in mountaineers. Other proxy measures of physical fitness, such as a lower heart rate for a fixed absolute workload (Tsianos et al., 2006; Wagner et al., 2012), and greater sea level training volume (Wagner et al., 2008), have also been associated with summit success.

Maximal aerobic capacity declines linearly with increasing altitude (Wehrlin and Hallén, 2006), and to a greater extent in endurance athletes (Faiss et al., 2014; Mollard et al., 2007; Woorons et al., 2005). Much of the existing literature has misinterpreted this association to suggest trained athletes perform worse

at altitude compared to healthy controls. Despite a greater decrement from normoxic $\dot{V}O_{2max}$ in the elite athletes, Faiss *et al.* (2014) observed a significant difference in hypoxic (F_IO_2 = 14.6) $\dot{V}O_{2max}$ between performance groups of ski mountaineers, with 52 ml·kg^{-1}·min^{-1} in elite and 42 ml·kg^{-1}·min^{-1} in recreational. Similarly, in a study in sea-level athletes, Mollard *et al.* (2007) found that while trained men experienced a greater absolute decrease in $\dot{V}O_{2max}$ from sea level to 4500 m compared to untrained men, no difference was observed when change in $\dot{V}O_{2max}$ was assessed proportionally, and trained subjects had significantly greater $\dot{V}O_{2max}$ at all altitudes compared to controls. The same relationship was found for performance, with a greater power output at maximal exertion in the trained subjects at all altitudes, despite a greater altitude-induced decrement.

Higher maximal capacity will also enable well-trained individuals to perform greater submaximal workloads for the same RPE (Ekkekakis and Petruzzello, 1999). Previous authors have suggested that although individuals with a higher sea-level $\dot{V}O_{2max}$ experience a greater decrement on arrival at altitude, they may be able to recover from this decrease more quickly than their unfit counterparts during acclimatisation (Richalet et al., 1988). This suggests physical capacity is greater in fitter individuals over the duration of an expedition and highlights a limitation associated with laboratory studies where evidence is obtained from an acute hypoxic exposure. There is therefore a the need for studies examining the importance of sea-level fitness for exercise capacity over a prolonged period of hypoxic exposure (e.g. field studies).

1.5.3 Cardiovascular fitness and high-altitude illnesses

Given the association with exacerbated hypoxaemia, some have proposed high cardiovascular fitness may be a risk factor for high-altitude illnesses (such as AMS). Regular physical activity has been found to significantly predict severe high-altitude illness risk (Richalet and Canouï-Poitrine, 2014; Richalet et al., 2012), a finding the authors attributed to greater arterial desaturation. Further, it has been suggested that greater muscular oxygen extraction of highly-trained

individuals (Saltin, 1969), contributes to the development of hypoxaemia, and disposes such individuals to AMS (Van Thienen and Hespel, 2016).

Evidence regarding this proposed association between fitness and AMS is mixed. Karinen *et al.* (2010) observed that individuals who experienced AMS on an expedition had a higher aerobic capacity compared to those who did not suffer AMS. However, the relevance of these associations has been questioned, with some authors suggesting that there is a statistical relationship, but not a clinically meaningful one (Bärtsch, 2014). Further, the relationship remains controversial, with many studies finding no difference in fitness or physical activity between those with and without AMS (Burtscher et al., 2011; Honigman et al., 1995; Schneider et al., 2002; Wu et al., 2015), or no relationship between fitness and AMS severity (Milledge et al., 1991). Indeed, the suggestion that greater sea-level fitness is associated with worse AMS is contradictory to the well-established positive relationship between AMS and obesity (Hackett et al., 1976; Hirata et al., 1989; Yang et al., 2015).

An alternative hypothesis is that fitter individuals complete more exercise when trekking and ascend at a faster rate, causing an increase in AMS risk that is not caused by fitness *per se*, as physical exercise has been found to exacerbate AMS (Roach et al., 2000), and faster ascent rate limits an individual's capacity for acclimatisation (Luks, 2012; Windsor and Rodway, 2007). As described above (Section 1.3.5), higher RPE during exercise at altitude is associated with an increased risk of experiencing AMS the following day. Fitness is inversely related to RPE for a given workload (Eston, 2012), suggesting that when activity and ascent profile are controlled, fitter individuals may even have a reduced risk of AMS.

1.6 Diet is a modifiable factor affecting health and performance at high altitude

1.6.1 Macro- and micro-nutrients contribute to health and performance at high altitude

Diet has a profound effect on human health and performance (Thomas et al., 2016). Therefore nutritional interventions have unsurprisingly been sought to optimise health and performance at high altitude. Increased workload demands and basal metabolic rate, coupled with loss of appetite and malabsorption, result in a negative energy balance and weight loss at altitude, providing an additional nutritional concern (Kasprzak et al., 2015; Macdonald et al., 2009). The magnitude of this loss is influenced by the extent of altitude exposure and physical demands of the expedition. Macdonald *et al.* (2009) reported a body mass loss of 2.4 kg (3.5%), composed of 45% fat, 20% residual mass, and 35% total body water, over a three-week commercial trek to 5,100 m. In contrast, Benso *et al.* (2007) reported a much larger body mass loss of 5 kg (7%) in climbers following a seven-week expedition to Mt Everest (8,848 m). This energy deficit provides an additional ergolytic effect over the effect of hypoxia *per se*. An acute comparable energy deficit (energy intake restriction resulting in 3.5% loss of body mass) resulted in a 10% reduction in 30 min time trial performance in normoxia (Oliver et al., 2007).

It is recommended macro-nutrient proportions at high altitude consist of 60% carbohydrate, 22-25% fat, and 12-15% protein (Hill et al., 2011). In particular, the importance of ensuring adequate carbohydrate contribution at high altitude is well-established. Carbohydrate supplementation has been shown to improve exercise tolerance and performance in both laboratory and field investigations (Fulco et al., 2005; Oliver et al., 2012b). Acute carbohydrate ingestion improves oxygen delivery during exercise since it elevates carbon dioxide production and acts as a respiratory stimulant (Golja et al., 2008). However, despite alleviating hypoxaemia, carbohydrate supplementation does not protect against

AMS (Gray and Milne, 1986; Swenson et al., 1997). In addition to macro-nutrient contributions, numerous micro-nutrients have been investigated for potential beneficial effects in hypoxia including, but not limited to, iron (Ren et al., 2015; Talbot et al., 2011), caffeine (Berglund and Hemmingsson, 1982; Fulco et al., 1994; Hackett, 2010; Stadheim et al., 2015), and dietary nitrate.

1.6.2 Dietary nitrate

Dietary nitrate supplementation, particularly in the form of beetroot juice, has been shown to effectively increase the bioavailability of NO (Wylie et al., 2013). At sea level, this increase in NO bioavailability has been associated with numerous physiological effects including a reduction in resting blood pressure (Siervo and Lara, 2013), increased skeletal muscle blood flow (Ferguson et al., 2013) resulting in increased oxygen delivery (Umbrello et al., 2013), and notably an increase in oxygen economy (Larsen et al., 2007). NO modulates energy metabolism through a reduction in proton leakage over the inner mitochondrial membrane, improving oxygen economy at submaximal work rates (Larsen et al., 2007). As described in Section 1.2.5, economy refers to the oxygen cost of a given exercise workload and is a key determinant of exercise performance (Joyner and Coyle, 2008). Hoon *et al.* (2013) completed a meta-analysis of 17 studies investigating the effects of dietary nitrate supplementation on exercise performance. Dietary nitrate moderately improved performance on time to exhaustion tests, but did not alter performance when assessed by time trial or graded exercise tests.

Dietary nitrate and exercise performance in hypoxia

Since the proposed mechanisms of dietary nitrate's performance benefits relate to improved oxygen economy combined with increased skeletal muscle blood flow, and therefore improved oxygen delivery to the muscles, it has been proposed any performance benefit is likely increased in hypoxia (Clements et al., 2014). In particular, NO plays a central role in hypoxia-induced vasodilation during

exercise (Casey et al., 2010, Section 1.1.4). In addition, the reduction of nitrate (NO_3^-) to NO_2^-, and NO, is enhanced under hypoxic conditions (Kim-Shapiro et al., 2006). To date, eight studies have investigated the effect of beetroot juice supplementation on exercise performance in hypoxia, with mixed results. Of the eight studies completed, four observed a significant improvement in exercise performance with nitrate compared to placebo, with the other four finding no difference. These studies are summarised in Table 1.1.

Dietary nitrate has been shown to improve economy during steady-state exercise in hypoxia (Kelly et al., 2014; Masschelein et al., 2012; Muggeridge et al., 2014), resulting in enhanced muscle oxygenation, and improvements in exercise performance in these studies. Dietary nitrate may also protect against the hypoxia-induced exacerbated metabolic cost of exercise. Dietary nitrate supplementation returned depletion of phosphocreatine, and production of fatigue-related metabolites adenosine diphosphate (ADP) and inorganic phosphate (P_i) to equal normoxia (Vanhatalo et al., 2011), and lowered blood lactate accumulation (Masschelein et al., 2012) following hypoxic exercise.

However, these conclusions are not consistent across the literature. Although Masschelein *et al.* (2012) observed improved muscle tissue oxygenation when assessed by near-infrared spectroscopy (NIRS), this was not confirmed when magnetic resonance (MR) techniques were utilised (Vanhatalo et al., 2014). Indeed, Vanhatalo *et al.* (2014) even observed a reduction in tissue oxygenation determined by T2*relaxation, a finding replicated by Bourdillon *et al.* (2015), accompanied by no improvement in exercise performance. Further, acute supplementation protocols in trained runners and cyclists have failed to show any improvement in oxygen economy, arterial oxygen saturation, or exercise performance in hypoxia (Arnold et al., 2015; MacLeod et al., 2015). Puype *et al.* (2015) sought to determine the potential for beetroot juice as a training aid in hypoxia. Chronic dietary nitrate supplementation did not improve the effectiveness of a six-week high-intensity exercise training intervention completed under hypoxic conditions. It is possible that by reducing the hypoxic stress during training, dietary nitrate actually reduced the training stimulus, countering any beneficial effects it could have otherwise had.

	Author, Year	Population	N (Pla|Nit)	Dosage (mmol/day)	Altitude equivalent	Exercise	Performance test	Change	Conclusion
1	Arnold et al., 2015	$\dot{V}O_{2max}$ 66(7)	10|10	1 × 7.0	4000 m	Running	TTE (GXT)	+9 s	⟷
					2500 m		TT (10 km)	-12 s	⟷
2	Bourdillon et al., 2015	Trained cyclists	12|12	3 × 7.5	5000 m	Cycling	TT (15 km)	+150 s	⟷
3	MacLeod et al., 2015	$\dot{V}O_{2max}$ 68(6)	11|11	1 × 6.5	2500 m	Cycling	TT (10 km)	-5 s	⟷
4	Kelly et al., 2014	$\dot{V}O_{2max}$ 58(6)	12|12	3 × 8.4	3700 m	Cycling	TTE (75%)	+17 s	↑
5	Muggeridge et al., 2014	$\dot{V}O_{2max}$ 52(6)	9|9	1 × 7.0	2500 m	Cycling	TT (16.1 km)	-38 s	↑
6	Puype et al., 2015	$\dot{V}O_{2max}$ 61(2)	11|11	46 × 5.0	4000 m	Cycling	TT (30 min)	+17 W	⟷
							TTE (GXT)	+43 s	⟷
7	Masschelein et al., 2012	$\dot{V}O_{2max}$ 62(2)	15|15	6 × 5.0	5000 m	Cycling	TTE (GXT)	+29 s	↑
8	Vanhatalo et al., 2011	Active	9|9	1 × 9.3	3000 m	Knee-extensor	TTE	+84 s	↑

Table 1.1: Previous studies investigating the effect of dietary nitrate supplementation on exercise performance in hypoxia. $\dot{V}O_{2max}$, Maximal aerobic capacity; TT, Time trial; TTE, Time to exhaustion; GXT, Graded exercise test. Studies completed up to the commencement of the study reported in Chapter 4 of this thesis are included. ↑ indicates nitrate improved exercise performance; ⟷ indicates nitrate had no effect on exercise performance.

In conclusion, although there is some limited support for an ergogenic effect of nitrate on exercise performance in hypoxia, the data do not support any enhancement in effectiveness compared to nitrate use in normoxia. Of practical and applied relevance, all previous studies are limited by their use of very brief exposures to hypoxia (< 2 h). The effect of dietary nitrate supplementation on exercise responses with a more prolonged exposure to hypoxia warrants investigation.

Dietary nitrate and AMS

The observed physiological benefits of dietary nitrate supplementation may also have implications for AMS. In particular, the vasodilatory effects of dietary nitrate may not only affect the active muscles, but may also aid oxygen diffusion in the pulmonary capillary. Pulmonary arterial hypertension is in-part associated with reduced bioavailability of NO (Duplain et al., 2000), and consequently NO, NO_3^-, and NO_2^- therapies have the potential to treat pulmonary arterial hypertension (Baliga et al., 2012; Bueno et al., 2013). Higher levels of endogenous exhaled NO is associated with lower systolic pulmonary artery pressure at altitude, and protection against HAPE (Schoene, 2008). In addition, inhalation of NO is able to reduce pulmonary artery pressure and pulmonary vascular resistance, resulting in improved alveolar PO_2, S_aO_2, and pulmonary oxygen diffusion in HAPE sufferers (Anand et al., 1998). However, Bourdillon *et al.* (2015) found three days of dietary nitrate supplementation had no effect on pulmonary vasoconstriction in healthy individuals during exercise in hypoxia. Pulmonary vasoconstriction was determined by echocardiography to measure systolic right ventricle to right atrium pressure gradient (RV-RA gradient) as an index of pulmonary artery pressure during steady-state exercise. The authors observed an 8 mmHg increase in RV-RA gradient with hypoxia, that was not altered with nitrate supplementation. One study has investigated the effect of dietary nitrate supplementation on macrovascular endothelial function in hypoxia. Bakker *et al.* (2015) found an acute dose of beetroot juice taken at high altitude was able to restore macro-vascular function, assessed by flow-mediated dilatation (FMD), to normoxic levels. Taken together, this suggests that beetroot juice

supplementation may improve peripheral vascular function at altitude, but may not benefit the pulmonary vasculature.

However, it must be considered that dietary nitrate supplementation may have a deleterious effect on AMS. Not only involved in peripheral vasodilation, NO mediates hypoxia-induced cerebral vasodilation (Van Mil et al., 2002), increasing CBF and potentially contributing to elevations in ICP. In addition, NO directly stimulates the trigeminovascular system (Ashina et al., 1999), responsible for headache pain sensation, and possibly further contributing to AMS pathophysiology.

At the commencement of the study reported in Chapter 4 of this thesis, only two studies had investigated the effect of chronic dietary nitrate supplementation on AMS, both with poorly controlled study designs and inconclusive results. Hennis *et al.* (2016) reported dietary nitrate supplementation had no effect on AMS symptoms over a trekking expedition to Mt Everest Base Camp (5,380 m). However, the authors did not perform any biochemical confirmation of the nitrate supplementation, and therefore it was not possible to determine whether the nitrate supplementation was effective. This is of particular concern since the authors reported poor compliance to the intervention. Furthermore, as AMS is determined by subjective responses, the use of a non-taste-matched placebo means that placebo or nocebo effects cannot be dismissed. Masschelein *et al.* (2012) found six days dietary nitrate supplementation had no effect on AMS incidence or symptom score. However, the study assessed AMS after only 2 h exposure to hypoxia, and after a maximal exercise test where the exercise completed was greater in the nitrate trial. Given the possible relationship between exercise and AMS (Roach et al., 2000), this represents a fundamental confound in the study that prevents interpretation of the findings.

1.7 Thesis Aims

The overall aims of this thesis were to increase understanding of physiological responses to high altitude, and to develop and test practical strategies that could be used by those travelling to high altitude.

With this in mind, the specific aims of the first study (Chapter 2) were to determine the relationship between sea-level fitness and submaximal exercise responses and AMS during chronic altitude exposure, and to determine the utility of sea-level fitness and hypoxic exercise testing before an expedition.

The second study (Chapter 3) aimed to determine the efficacy of chronic dietary nitrate supplementation as an AMS prophylactic and ergogenic aid at altitude.

Finally, the third study (Chapter 4) aimed to increase understanding of cerebral physiology in hypoxia, specifically to characterise the anatomical distribution of hypoxia-induced alterations in CBF, and to relate CBF alterations to neurovascular activity during cognitive tasks in hypoxia.

Chapter 2

MEDEX2015: Is fitness important for individual's readiness to perform at altitude?

2.1 Abstract

This study examined the complex relationships of fitness and hypoxic sensitivity with submaximal exercise responses and acute mountain sickness (AMS) at altitude. Determining these relationships is necessary before fitness or hypoxic sensitivity tests can be recommended to appraise individuals' readiness for altitude. Forty-four trekkers (26 men; 18 women; 20-67 years) completed a loaded walking test and a fitness questionnaire in normoxia to measure and estimate sea-level maximal aerobic capacity ($\dot{V}O_{2max}$), respectively. Participants also completed a hypoxic exercise test to determine hypoxic sensitivity (cardiac, ventilatory, and arterial oxygen saturation responses to acute hypoxia, F_IO_2=0.112). One month later, all participants completed a three-week trek to 5085m with the same ascent profile. On ascent to 5085m, ratings of perceived exertion (RPE_{ascent}), fatigue by Brunel Mood Scale, and AMS were recorded daily. At 5085m, RPE during a fixed workload step test (RPE_{fixed}) and step rate during perceptually-regulated exercise ($STEP_{RPE35}$) were recorded. Greater sea-level $\dot{V}O_{2max}$ was associated with, and predicted, lower sense of effort (RPE_{ascent} r = -0.43; $p < 0.001$; RPE_{fixed}; r = -0.69; $p < 0.001$) and higher step rate ($STEP_{RPE35}$ r = 0.62; $p < 0.01$), but not worse AMS (r = 0.13; p = 0.4) or arterial oxygen desaturation (r = 0.07; p = 0.7). Lower RPE_{ascent} was also associated with better

mood, including less fatigue ($r = 0.57$; $p < 0.001$). Hypoxic sensitivity was not associated with, and did not add to the prediction of, submaximal exercise responses or AMS. In conclusion, participants with greater sea-level fitness reported less effort during simulated and actual trekking activities, had better mood (less fatigue), and chose a higher step rate during perceptually-regulated exercise, but did not suffer from worse AMS or arterial oxygen desaturation. Simple sea-level fitness tests may be used to aid preparation for high-altitude travel by enabling better aerobic exercise prescription and identifying those people who might benefit most from the aerobic training.

2.2 Introduction

Many people travel to altitude for work and leisure including trekkers, military personnel, and miners (Government of Nepal, 2013). As well as high-altitude illness, fatigue presents a major psychophysiological risk factor for summit failure, injury, and fatality at altitude (Firth et al., 2008; Oliver et al., 2012a). Recent commentaries in this and other journals highlight the potential importance of adequate sea-level fitness to reduce fatigue and therefore enhance altitude exercise performance, including trekking times and summit success (Bärtsch and Swenson, 2013; Burtscher et al., 2015). However, the relationships between sea-level fitness, submaximal exercise responses at altitude, and acute mountain sickness (AMS) are complex (MacInnis et al., 2015), and as yet unknown.

Numerous studies indicate that individuals with high sea-level maximal aerobic capacity ($\dot{V}O_{2max}$) have high altitude $\dot{V}O_{2max}$ (Fulco et al., 1998). Yet there is evidence that the absolute loss of $\dot{V}O_{2max}$ in high-fit individuals is greater at high altitude than their less-fit counterparts (Ferretti et al., 1997; Marconi et al., 2004; Mollard et al., 2007). In fact, the decline in very high-fit individuals is so great at high altitude that their $\dot{V}O_{2max}$ is no different *or even lower* than their less-fit counterparts (MacInnis et al., 2015). Furthermore, it is often assumed that individuals with high sea-level $\dot{V}O_{2max}$ have greater exercise performance.

However, $\dot{V}O_{2max}$ is not the only determinant of long-duration submaximal exercise responses, and other measures of fitness, such as fractional utilization of $\dot{V}O_{2max}$ (e.g. ventilatory threshold) and economy, are potentially as important (Bassett and Howley, 2000; Coyle et al., 1988). For trekking activities, which are typically submaximal, sense of effort during exercise (most often assessed by rating of perceived exertion; RPE) is also functionally important because it appraises the individual's comfort level. Sense of effort is also an essential component of general fatigue (Enoka and Stuart, 1992). Despite the well-documented relationship between fitness and exercise performance at sea level, the relationship between sea-level fitness and sense of effort during submaximal exercise at altitude is unclear.

Even if high sea-level fitness is associated with greater exercise capacity and reduced sense of effort, this may be at the cost of exacerbating AMS. Indeed, regular endurance training has been identified as a risk factor for altitude illness (Karinen et al., 2010; Richalet et al., 2012). A possible explanation for this is that fitter individuals experience greater arterial desaturation with acute hypoxia even during submaximal exercise (Lhuissier et al., 2012), which is likely a result of greater cardiac output (Richalet and Lhuissier, 2015), or an indirect effect of greater oxygen extraction in the muscle (Van Thienen and Hespel, 2016). Alternatively, worse AMS may occur because fitter individuals exercise at a greater intensity at altitude and/or gain altitude quicker. These arguments provide possible reasons for the common anecdotal field observation of poorer-than-expected exercise performance and AMS in high-fit persons at high altitude. Despite the anecdotes and plausible physiological responses, evidence is lacking to explain the complex relationship between sea-level fitness, exercise, and AMS.

Some authors further advocate that hypoxic sensitivity is an important physiological factor determining altitude exercise performance (Schoene et al., 1984) and illness risk (Richalet and Canouï-Poitrine, 2014). This has led to the development of various resting and exercising hypoxic sensitivity tests to predict altitude exercise performance and illness susceptibility (Lazio et al., 2010; Rathat et al., 1992). However, these are not routinely implemented, perhaps due to a

lack of clinically-relevant discrimination at an individual level (Bärtsch, 2014), or due to their complexity and requirement for specialist equipment including a method to simulate a high-altitude environment.

In summary, the relationships of fitness and hypoxic sensitivity with sense of effort during submaximal exercise and AMS at altitude are complex and unknown. Determining these relationships is necessary before fitness or hypoxic sensitivity tests can be recommended to appraise individuals' readiness for altitude. Therefore, the first aim of this study was to explain the relationship of sea-level fitness with submaximal exercise responses (sense of effort during submaximal exercise and step rate during perceptually-regulated exercise) and AMS during chronic altitude exposure. The second aim was to determine the utility of sea-level fitness (as assessed by $\dot{V}O_{2max}$, ventilatory threshold, economy, and a simple questionnaire-based estimation of $\dot{V}O_{2max}$) and hypoxic exercise testing to predict submaximal exercise responses and AMS at altitude. Finally, we aimed to determine whether physiological responses to hypoxia could explain the relationship between fitness and submaximal exercise responses. To this end, we assessed sea-level fitness and acute physiological responses to hypoxia ($F_IO_2 = 0.112$; equivalent 5000 m) one month before a three-week trek to the Manaslu Circuit in the Nepal Himalaya. On the trek, sense of effort during submaximal exercise was assessed during simulated and actual trekking activities and physiological responses to chronic hypoxia were assessed at Base Camp (5058 m). AMS was assessed daily. We hypothesised that high sea-level fitness would be associated with submaximal exercise responses (lower sense of effort during submaximal exercise and higher step rate during perceptually-regulated exercise) at altitude, without increased AMS. Second, we hypothesised that sea-level and hypoxic exercise tests would be significant predictors of submaximal exercise responses. Third, we hypothesised that hypoxic exercise tests would be significant predictors of AMS at altitude.

2.3 Methods

2.3.1 Participants and study design

Forty-four trekkers, 26 men and 18 women (mean (SD): age 39 (14) years, body mass 69.0 (14.5) kg, height 172 (10) cm) from the MEDEX Manaslu trek volunteered for this observational cohort study. All participants were lowlanders, with an altitude of residence below 500 m. Forty-one participants (93%) had previously travelled to high altitude (> 1500 m), and of these 41 participants, 32 (78%) reported previous AMS, one (2%) had a history of HACE, and none (0%) had a history of HAPE. Nine (20%) participants had a history of migraine (confirmed by a physician), three (7%) were smokers, and average alcohol consumption was 81.0 (63.4) $g \cdot week^{-1}$. Self-report physical activity was assessed on a scale developed by Jackson *et al.* (1990), which ranged from 0 - Avoids walking or exercise (e.g. always uses elevators, drives whenever possible instead of walking), to 7 - Runs more than 10 miles per week or spends more than 3 hours per week in comparable physical activity. Self-report physical activity ranged from 1-7, with mean of 5 (2), and $\dot{V}O_{2max}$, ranged from 29 to 62 with mean 45 (8) $mL \cdot min^{-1} \cdot kg^{-1}$. The study received ethical approval from the North West Wales Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki 2008. All volunteers provided written informed consent. Data were collected between February and April 2015. An overview of the study is depicted in Figure 2.1.

2.3.2 Pre-trek experimental procedures

One month before the trek, participants completed assessments of sea-level fitness and hypoxic sensitivity. Participants were asked to refrain from exhaustive exercise, caffeine and alcohol for twelve hours before all tests.

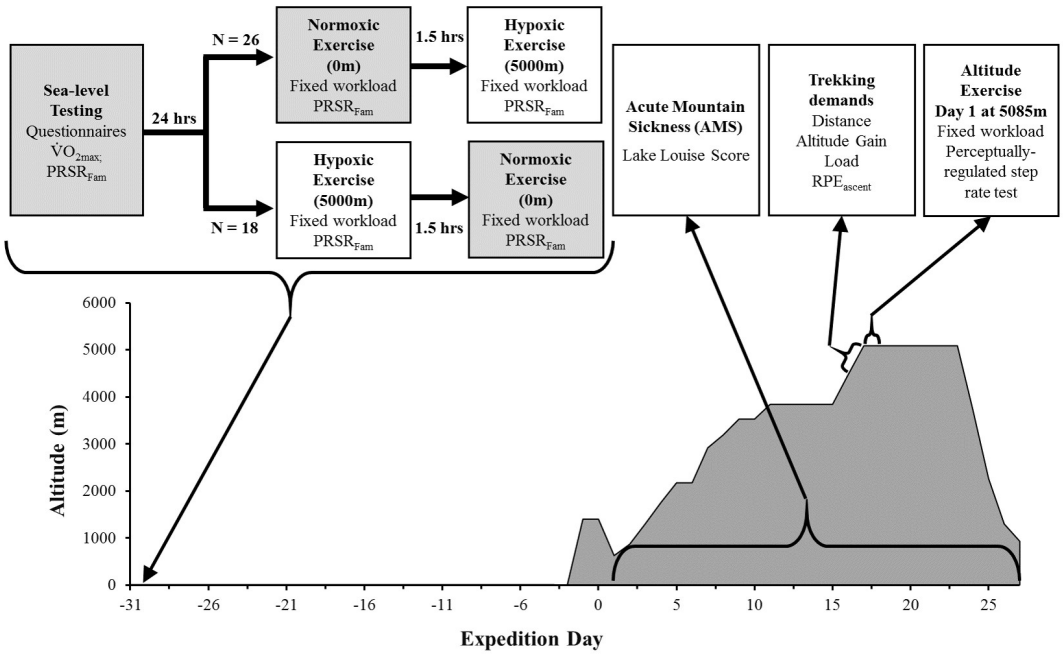


Figure 2.1: Schematic representation of study protocol. Grey boxes indicate procedures undertaken in normoxia, white boxes indicate procedures undertaken in hypoxia. LLS, Lake Louise Score; Load, external load for the trekking session (kg); $PRSR_{Fam}$, perceptually regulated step rate test familiarization; RPE_{ascent}, rating of perceived exertion on ascent to Base Camp.

Sea-level fitness ($\dot{V}O_{2max}$, ventilatory threshold, and economy) was determined during a walking test to exhaustion on a motorized treadmill (H-P-Cosmos, Sports & Medical GmbH; Nussdorf) with simultaneous gas analysis (Cortex Metalyzer, Biophysik GmbH; Leipzig). Participants wore a weighted rucksack (15 kg for men and 12.5 kg for women). The test consisted of 5 $k{\cdot}h^{-1}$ walking with a ramped increase in gradient from 5% to 25% over 18 minutes ($1.11\%{\cdot}min^{-1}$), followed by a ramped increase in speed ($0.67\ km{\cdot}h^{-1}{\cdot}min^{-1}$) thereafter. Rating of perceived exertion (RPE) was recorded each minute of the test using the Borg CR100 (Borg and Borg, 2001). $\dot{V}O_{2max}$ was identified by two or more of the following criteria (Pescatello et al., 2013): volitional fatigue, a plateau in $\dot{V}O_2$ despite an increase in workload, respiratory exchange ratio ≥ 1.15, heart rate $\geq$ 95% age-predicted heart rate maximum (220-age). $\dot{V}O_{2max}$ was also predicted using the equation provided in Matthews *et al.* (1999). Ventilatory threshold was determined using the method outlined by Gaskill *et al.* (2001) and economy as $\dot{V}O_2$ ($ml{\cdot}kg^{-1}{\cdot}min^{-1}$) at a gradient of 6%.

Hypoxic sensitivity was determined using a modified version of the Richalet test (Canoui-Poitrine et al., 2014; Richalet et al., 2012), with the exercise modality changed from cycling on an ergometer to stepping in time to a metronome, and the F_IO_2 chosen to match the specific demands of the expedition. Participants completed fixed-workload step tests in normoxia and hypoxia (F_IO_2 = 0.112; 5000 m). Step tests were conducted in an environmental chamber (Hypoxico Inc; NY), separated by 1.5 to 3 hours. Each step test included: 4 min 30 s of seated rest and 4 min 30 s of exercise. During the exercise, participants wore a 7 kg rucksack whilst stepping at 24 steps·min^{-1} on a 21 cm step. Ventilation ($\dot{V}$E) was determined by collection of expired gases (Douglas bag system, Cranlea Ltd; Birmingham) for the final minute of exercise, and oxygen saturation (S_pO_2) and heart rate were measured by a pulse oximeter (9550 OnyxII, Nonin Medical Inc; Minnesota) and a heart rate monitor (RS800CX; Polar UK; Warwick), and recorded in the final 30 seconds of exercise. RPE was recorded in the final 30 seconds for familiarisation.

Hypoxic sensitivity was determined using equations described previously (Canoui-Poitrine et al., 2014; Richalet et al., 2012):

Desaturation during exercise:

$$\triangle\, SpO_2e(\%) = SpO_{2EH} - SpO_{2EN} \tag{2.1}$$

Hypoxic cardiac response:

$$HCRe(bpm \cdot \%^{-1}) = (HR_{EH} - HR_{EN}) \div \triangle SpO_{2e} \tag{2.2}$$

Hypoxic ventilatory response:

$$HVRe(L \cdot min^{-1} \cdot kg^{-1}) = (\dot{V}E_{EH} - \dot{V}E_{EN}) \div \triangle SpO_2e \div BM \times 100 \tag{2.3}$$

Where S_pO_2, oxygen saturation; HR, heart rate; $\dot{V}$E, minute ventilation (L·min^{-1}); EH, exercise in hypoxia (baseline); EN, exercise in normoxia (baseline); and BM, body mass (kg).

2.3.3 Trek experimental procedures

Participants arrived in Kathmandu (1300 m) and were transported to Arughat (518 m) by bus to begin the trek. The 44 participants travelled in five groups of mixed age, sex, and sea-level fitness. Each group completed the Manaslu trekking itinerary and, therefore, the same altitude profile, an ascent profile that is typical of other high-altitude treks (e.g., Dhaulagiri circuit). The ascent profile included 4 days trekking above 3000 m, with 2 days of ∼300 m ascent per day, and 2 days of ∼600 m ascent per day. They all completed the ascent to Base Camp (5085 m) in 15–17 days trekking. This variation in ascent was due to limited overnight accommodation at some locations. Participants abstained from prophylactic medication, and all other medications taken were recorded, but not restricted.

Trekking demands

On each day of the trek physical and physiological demands were assessed, but for the benefit of clarity only data from the final day of trekking on ascent to Base Camp are presented. To assess physical demands, after breakfast, body mass was assessed by weighing participants in base layers using mechanical scales (Salter Housewares, Kent; UK); loaded weight was assessed by weighing participants in full trekking attire including boots and rucksack; and external weight was calculated by subtracting the body mass from the loaded mass. Participants were able to walk at their chosen pace and the start and end times of each individual's trekking day was recorded. The trekking route was tracked using a global satellite positioning system (GPS; inReach SE, Delorme, Yarmouth; ME). Energy expenditure (EE) was then calculated using an equation validated previously (Pandolf et al., 1977):

$$EE = 1.5BM + 2.0(BM + L)(L/BM)^2 + \eta(BM + L)[1.5V^2 + 0.35VG] \quad (2.4)$$

Where EE, energy expenditure; BM, body mass; L, external load; V, walking speed; G, gradient; η, terrain coefficient (specified in Appendix A). Relative trekking intensity for the ascent to Base Camp was calculated as:

Relative trekking intensity:

$$Intensity_{Rel} = RPE_{ascent} \div EE(kJ \cdot min^{-1}) \quad (2.5)$$

Where RPE_{ascent}, session RPE (Fanchini et al., 2016) recorded 30 minutes after trekkers completed 6.3 km walking exercise from 4472 to 5085 m; EE, energy expenditure (calculated from the equation provided in (Pandolf et al., 1977).

To assess physiological demands, participants wore heart rate monitors (RS800CX, Polar, Warwick; UK) throughout the day's trek, and heart rate was averaged for the trekking session.

Sense of effort during submaximal exercise

To determine the relationship between sea-level fitness and sense of effort during submaximal exercise at altitude, we assessed sense of effort during submaximal exercise by recording RPE. RPE was recorded using the Borg CR100 (Borg and Borg, 2001) which asks participants to rate the intensity of the exercise sensation using numbers from 0-100 and verbal descriptors (e.g. "moderate", equivalent to 25). Extensive evidence supports the use of RPE as a valid and appropriate method to record sense of effort and perceptual responses to exercise (Eston, 2012). Sense of effort was determined from session RPE (Fanchini et al., 2016) recorded 30 minutes after trekkers completed 6.3 km walking exercise from 4472 to 5085 m (RPE_{ascent}). Session RPE has been validated as a quantitative

measure of exercise load (Foster et al., 2001). Participants also completed the Brunel Mood Scale (BRUMS; Terry et al., 1999) on arrival at Base Camp to determine the psychological effects of the exercise, including self-reported fatigue. To further determine sense of effort during submaximal exercise at altitude, all participants completed the fixed-workload step test the day after arriving at 5085 m (day 16-18 of the expedition), breathing altitude ambient air (549 (1) mbar) but otherwise using the same protocol as completed at sea level. Specifically, participants wore a 7 kg rucksack whilst stepping at 24 steps·min^{-1} on a 21 cm step. The primary outcome variable for this test was RPE at 4 min 30 s of stepping (RPE_{fixed}). In addition, S_pO_2, heart rate, and minute ventilation ($\dot{V}$E) were determined using methods as described for the sea-level step tests. Exercise ventilation reserve and ventilatory efficiency were calculated using equations adapted from Bernardi *et al.* (2006):

Exercise ventilation reserve:

$$EVR(\%) = ((\dot{V}E_{max} - \dot{V}E_{alt}) \div \dot{V}E_{max} \times 100) \tag{2.6}$$

Ventilatory efficiency:

$$\dot{V}_{Eff}(\% \cdot L^{-1} \cdot min^{-1}) = SpO_2 \div \dot{V}E_{alt} \tag{2.7}$$

Chronic change in heart rate:

$$\triangle HR(bpm) = HR_{EN} - HR_{EA} \tag{2.8}$$

Where $\dot{V}E_{max}$, maximal exercising ventilation from sea-level $\dot{V}O_{2max}$ test; $\dot{V}E_{alt}$, exercising ventilation during fixed-workload step test at altitude; S_pO_2, oxygen saturation during fixed-workload step test at altitude; HR_{EN}, heart rate during fixed-workload exercise in normoxia (baseline); HR_{EA}, heart rate during fixed-workloads step test at altitude (Base Camp, 5085 m).

Immediately after the fixed-workload step test, submaximal exercise capacity was determined by assessing step rate during perceptually-regulated exercise ($STEP_{RPE35}$). This perceptually-regulated step rate test provided assessment of exercise production at a relative workload. Clamping RPE to produce self-paced exercise in this manner is a validated tool for determining functional and endurance exercise capacity (Coquart et al., 2016; Eston, 2012). Each participant was asked to complete stepping exercise for 4 min at a step rate that was equivalent to an RPE of 35 (described on the RPE scale as "somewhat strong"). An RPE of 35 was chosen because it has been previously reported as the typical sensed effort of mountain walkers and workers (Ainslie et al., 2002; Callender et al., 2012). During this exercise, participants were free to alter their step rate. In the final minute step rate ($STEP_{RPE35}$), HR, and S_pO_2 were recorded. For the purpose of familiarisation, participants completed three practice trials (two in normoxia, one in acute hypoxia) that included familiarisation with the CR100 scale and completing the entire stepping exercise. In a separate pilot study (n = 6), we showed that with three practice sessions this perceptually-regulated step rate test has good reliability, with intraclass correlation coefficient of 0.94, coefficient of variation of 2.4%, and limits of agreement bias and 95% confidence intervals (lower limit; upper limit) of 1.0 (-1.5; 3.5) steps·min^{-1}. The perceptually-regulated step rate test also has good face validity, with trekkers and expedition leaders reporting that it was representative of their normal trekking pace.

Both step tests were repeated two days later (on the third day at Base Camp), in a sub-sample of 21 participants. The sub-sample was representative of whole study sample, with no difference in age, height, body mass, $\dot{V}O_{2max}$, or sex ratio (all $p \geq 0.5$).

Acute mountain sickness (AMS)

Each morning on the trek, participants recorded AMS symptoms using the Lake Louise Score (LLS; Roach et al., 1993) under the supervision of a researcher. From these symptoms clinically-defined AMS was identified when the participant

was higher than 2500 m, LLS total exceeded three or more, and headache with at least one other symptom was present. An individual with AMS at any point over the expedition was classified as AMS susceptible (AMS+); individuals without AMS over the expedition were classified as AMS resistant (AMS-). Percentage of days with AMS and peak LLS were also calculated.

2.3.4 Statistical analysis

The primary independent variable of fitness was sea-level $\dot{V}O_{2max}$ (extensive exploratory analyses revealed no additional benefit of the fitness variables ventilatory threshold or economy). The primary outcome variable of sense of effort during submaximal exercise was RPE recorded during the fixed workload test performed at high altitude (RPE_{fixed}).

To determine the relationships between sea-level fitness with i) submaximal exercise responses at altitude (RPE_{fixed}, RPE_{ascent}, and $STEP_{RPE35}$); ii) acute physiological responses to hypoxia (HVRe, HCRe, $\triangle S_pO_2e$); iii) chronic physiological responses to hypoxia (exercise ventilation reserve, ventilatory efficiency, chronic change in heart rate, and S_pO_2 at altitude); iv) the percentage of trekking days with AMS, and peak AMS score, Pearson's correlations were used. For all correlational analyses, the strength of a relationship was determined by the r value (trivial < 0.1, small 0.1 - 0.3, moderate 0.3 - 0.5, large/strong 0.5 - 0.7, very large/strong > 0.7; Hopkins et al., 2009).

To determine whether hypoxic exercise testing significantly adds to sea-level fitness testing to predict sense of effort during submaximal exercise at altitude and AMS, hierarchical regression was used and r^2change was reported. To determine the utility of $\dot{V}O_{2max}$ and hypoxic sensitivity for predicting AMS susceptibility, Receiver Operating Characteristic curves were calculated and comparison of area under the curves (AUC) was completed.

To investigate whether classical physiological responses to hypoxia mechanistically explain the relationship between fitness and sense of effort

during submaximal exercise at altitude, ventilatory and cardiac responses to acute normobaric hypoxia (HVRe, HCRe) and chronic high-altitude exposure (chronic change in heart rate, exercise ventilation reserve, ventilatory efficiency), were investigated using a mediation analysis. Analysis was completed using the SPSS macro PROCESS (Hayes, 2013) with 5000 bootstrap samples. An indirect effect (evidence of a mechanistic explanation) was deemed significant if the upper and lower 95% confidence interval limits of the size of the indirect path did not include zero.

A sample size estimation for the correlation between sea-level $\dot{V}O_{2max}$ and RPE_{fixed} indicated that 37 participants would be needed to produce a 90% chance of obtaining statistical significance at the 0.05 level for a minimum important effect size of r = 0.5 (Bland, 2015).

Diagnostic accuracy analyses were completed using MedCalc version 15.8 (MedCalc Software, Ostend; Belgium), all other analyses were completed using SPSS version 22 (IBM Corp, Armonk; NY). Statistical significance was set at $p < 0.05$ for all analyses.

2.4 Results

2.4.1 Trekking demands

Physiological and perceptual responses to the submaximal step tests are shown in Table 2.1. Physical, physiological, and perceptual demands of the final day's trek into Base Camp are shown in Table 2.2. The trekkers took 262 (52) min to complete the 6.3 km trek with 613 m altitude gain from 4472 to 5085 m.

	Fixed-workload step test			Perceptually-regulated step test		
	Sea level	Acute normobaric hypoxia[a]	Chronic high altitude	Sea level	Acute normobaric hypoxia[a]	Chronic high altitude
RPE	20 (7)	30 (11)	46 (23)	35[b]	35[b]	35[b]
Step rate	24[b]	24[b]	24[b]	36 (7)	30 (5)	27 (6)
HR (bpm)	116 (6)	143 (19)	132 (19)	145 (23)	157 (20)	135 (15)
S_pO_2 (%)	97 (3)	70 (5)	75 (5)	96 (5)	70 (4)	72 (4)
$\dot{V}E$ (L·min^{-1})	33 (7)	47 (11)	40 (10)	-	-	-

Table 2.1: Physiological and perceptual responses to step tests. N = 44. Values are mean (SD). HR, Heart Rate; $\dot{V}E$, Ventilation. [a]Conducted as familiarization trials, but data included here for completeness; [b]By design, values are the same in all participants for this variable.

2.4.2 Sea-level fitness and submaximal exercise responses at altitude

Greater sea-level fitness was associated with lower sense of effort (RPE_{fixed} and RPE_{ascent}) and higher step rate ($STEP_{RPE35}$) at altitude (Figure 2.2). Ascent time to Base Camp was not related to fitness (r = -0.11; p = 0.48; Table 2.2). Therefore, fitter persons ascended with less sensed effort (lower RPE) but a similar walking speed compared to their less-fit counterparts. Lower RPE_{ascent} was also associated with less negative mood (total mood disturbance; r = 0.50; p = 0.001), specifically less fatigue (r = 0.57; p < 0.001), tension (r = 0.40; p = 0.01), and confusion (r = 0.35; p = 0.03). Lower RPE_{ascent} was also associated with increased vigour, although only a small correlation (r = -0.28; p = 0.09). Lower sense of effort and higher step rate in fitter individuals did not come at the cost of worse arterial oxygen saturation: sea-level $\dot{V}O_{2max}$ was not related to S_pO_2 during the fixed-workload (r = 0.07; p = 0.67) or perceptually-regulated (r = 0.16; p = 0.33) step tests at altitude.

2.4.3 Acute mountain sickness (AMS)

Twenty-five participants (61%) had clinically-defined AMS at least once during the expedition. Of those with AMS, it lasted 4 (2) days. The highest incidence

Trekking Variable	Ascent to Base Camp	Relationship to $\dot{V}O_{2max}$	
		r	p
Walking speed ($km \cdot h^{-1}$)	1.5 (0.3)	0.22	0.17
External load (kg)	11.1 (2.4)	0.15	0.37
Energy expenditure (kJ)	2298 (584)	-0.21	0.20
Heart rate (bpm)	126 (14)	-0.03	0.86
Session RPE	51 (20)	-0.43**	0.005
Relative exertion ($RPE \cdot kJ^{-1} \cdot min^{-1}$)	5.9 (2.6)	-0.35*	0.03

Table 2.2: Summary of trekking demands and relationship to sea level $\dot{V}O_{2max}$. N = 44. Values are mean (SD). *p < 0.05; **p < 0.01.

of AMS for a given day was 47%, occurring on day one at Base Camp (5085 m). AMS was not related to any sea-level assessment variables. None of sea-level fitness, hypoxic sensitivity, or physiological responses to chronic hypoxia was related to AMS susceptibility, percentage of days with AMS, or peak LLS (r = 0.05 to 0.26; p = 0.12 to 0.91; Figure 2.3). AUC were all below 0.70, indicating poor diagnostic accuracy for all methods. Two (5%) participants took acetazolamide in the treatment of AMS for one and eight days each, while 30 (68%) participants took some form of analgesic medication, with 2.2 (1.9) days spent on analgesics across the whole sample. There was no relationship between fitness and number of days on acetazolamide (r = -0.08; p = 0.60) or analgesic medications (r = 0.20; p = 0.22).

2.4.4 Acclimatisation, sea-level fitness, and submaximal exercise responses at altitude

In a sub-sample, the step tests were repeated on day three after participants' AMS symptoms had reduced (LLS decreased from 3.3 (2.5) to 2.1 (2.1); p = 0.06), and sense of effort during submaximal exercise had decreased across the sub-sample (RPE_{fixed} decreased from 57 (31) on day one to 44 (19) on day three; p < 0.01). Submaximal step rate during perceptually-regulated exercise increased ($STEP_{RPE35}$ increased from 26 (6) on day one to 28 (5) on day three; p < 0.01). These adaptive changes (representative of enhanced acclimatisation) did not affect the relationship between fitness and submaximal exercise responses, which were consistent with those observed on day one.

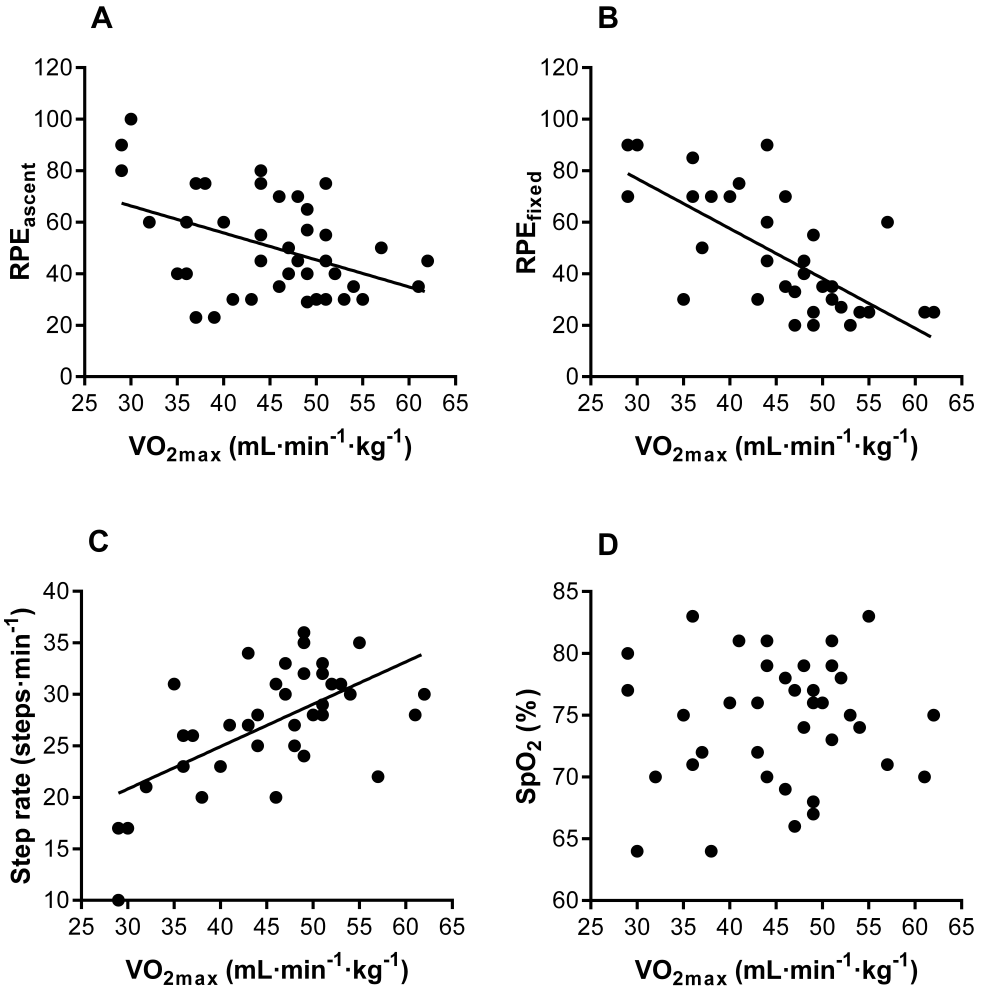


Figure 2.2: Relationship between sea-level fitness ($\dot{V}O_{2max}$) and submaximal exercise at altitude. Greater sea-level fitness was associated with (A) reduced session RPE from ascent to Base Camp (RPE_{ascent}; r = -0.43; p = 0.005), (B) reduced RPE at a fixed workload (RPE_{fixed}; r = -0.69; p < 0.001), and (C) greater step rate during perceptually-regulated exercise ($STEP_{RPE35}$; r = 0.62; p < 0.001). Sea-level fitness was not related to (D) oxygen saturation during fixed-workload step test at altitude (S_pO_2; r = 0.07; p = 0.67).

Greater sea-level fitness was associated with lower RPE_{fixed} on day three (r = -0.75; p < 0.001), and greater $STEP_{RPE35}$ on day three (r = 0.70; p = 0.001), and was not related to S_pO_2 during the fixed-workload (r = 0.26; p = 0.28). Greater sea-level fitness tended to be associated with greater S_pO_2 during the perceptually-regulated step test (r = 0.43; p = 0.058), despite participants producing a higher absolute workload.

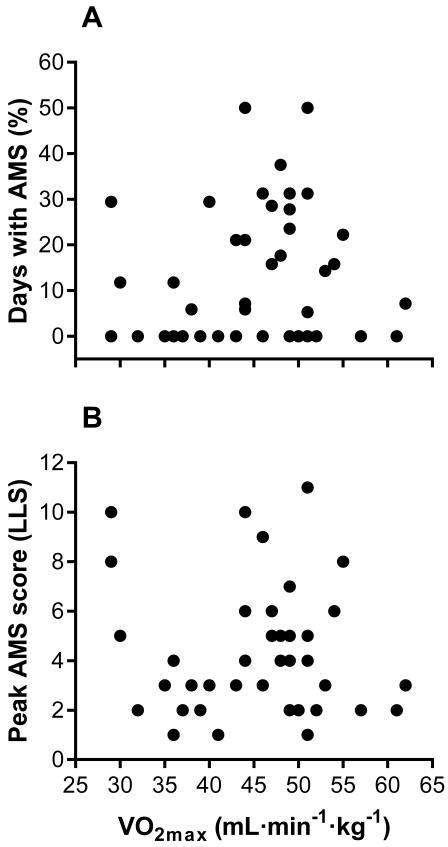


Figure 2.3: Relationship between sea-level fitness ($\dot{V}O_{2max}$) and acute mountain sickness (AMS). Sea-level fitness was not related to (A) percent of trekking days with clinically-defined AMS (r = 0.13; p = 0.41), or (B) peak AMS score (r = -0.05; p = 0.74).

2.4.5 Physiological mechanisms

Hypoxic sensitivity and submaximal exercise responses at altitude

Individuals with lower HVRe (Figure 2.4) and higher HCRe (Figure 3B) had lower sense of effort compared to their counterparts. HVRe was positively related to RPE_{fixed} (r = 0.38; p = 0.02), and negatively related to $STEP_{RPE35}$ (r = -0.39; p = 0.02). There was a moderate negative relationship between HCRe and RPE_{fixed} , (r = -0.31; p = 0.07), but HCRe was not related to $STEP_{RPE35}$ (r = 0.19; p = 0.26). $\triangle S_pO_2e$ was not related to any measure of sense of effort at altitude (r = 0.23 to 0.25; p = 0.15 to 0.17).

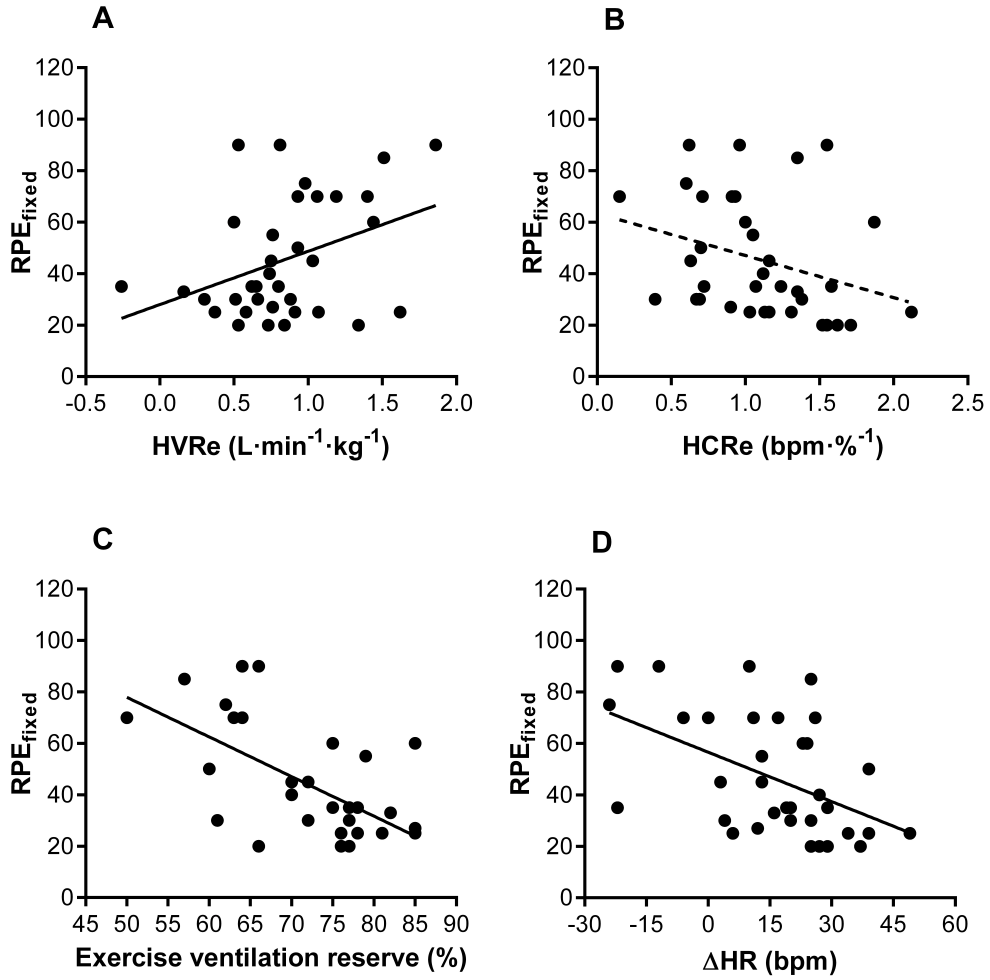


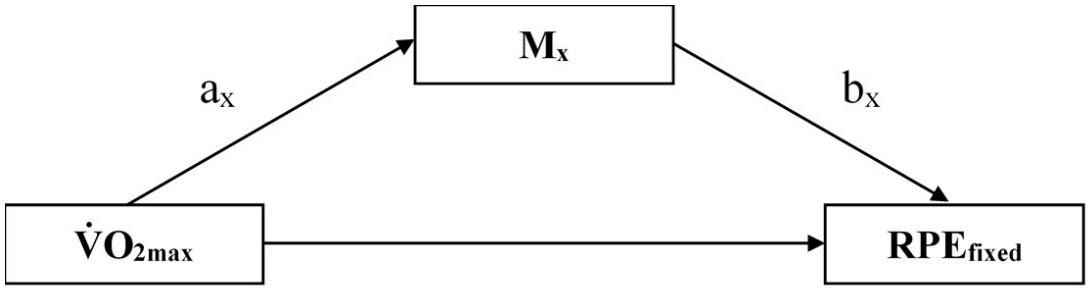
Figure 2.4: Relationships between ventilatory and cardiac responses to acute and chronic high altitude with sense of effort at altitude (RPE_{fixed}). Reduced RPE_{fixed} was associated with (A) reduced hypoxic ventilatory response (HVRe; r = 0.38; p = 0.02), (B) elevated hypoxic cardiac response (HCRe; r = -0.31; p = 0.07), (C) elevated exercise ventilation reserve at altitude (r = -0.60; p < 0.001), and (D) elevated chronic change in heart rate (r = -0.49; p = 0.003).

Physiological responses to chronic high altitude

Individuals with less ventilatory stress at altitude and a greater cardiac response to chronic high altitude had lower sense of effort compared to their counterparts (Figure 2.4). Greater exercise ventilation reserve was associated with lower RPE_{fixed} (r = -0.61; p <0.001), and superior $STEP_{RPE35}$ (r = 0.44; p = 0.01). Greater ventilatory efficiency was associated with lower RPE_{fixed} (r = -0.44; p = 0.01), and superior $STEP_{RPE35}$ (r = 0.44; p < 0.001). A larger chronic change in heart rate was associated with lower RPE_{fixed} (r = -0.49; p < 0.01), and superior $STEP_{RPE35}$ (r = 0.41; p = 0.01).

Mediation analysis

Cardiac parameters tended to explain (*negatively* mediate) the relationship between sea-level fitness and submaximal exercise sense of effort at altitude (Table 2.3). HVR_e also tended to explain (*positively* mediate) the relationship between sea-level fitness and submaximal exercise sense of effort at altitude. In contrast, exercise ventilation reserve and ventilatory efficiency did not mediate the relationship between sea-level fitness and sense of effort during submaximal exercise at altitude.



Variable (M_x)	a_x ($\dot{V}O_{2max} \rightarrow M_x$)	b_x ($M_x \rightarrow RPE_{fixed}$)	Indirect effect (ab_x)
Acute normobaric hypoxia			
HVRe	-0.27 (-0.58; 0.04)	0.21 (-0.07; 0.49)	-0.06 (-0.25; 0.01)
HCRe	0.37 (0.06; 0.69)*	-0.05 (-0.34; 0.23)	-0.02 (-0.18; 0.08)
Chronic high altitude exposure			
EVR	0.85 (0.60; 1.10)**	-0.29 (-0.72; 0.13)	-0.25 (-0.55; 0.33)
$\dot{V}E_{Eff}$	0.59 (0.25; 0.93)**	-0.14 (-0.46; 0.18)	-0.08 (-0.28; 0.11)
$\triangle$HR	0.42 (0.12; 0.72)*	-0.23 (-0.51; 0.05)	-0.10 (-0.32; 0.00)

Table 2.3: Mediation analysis summary for acute normobaric hypoxia and chronic high altitude cardiac and ventilatory parameters. If a variable M_x explains (mediates) the relationship between $\dot{V}O_{2max}$ and RPE_{fixed}, the indirect effect (ab_x) should not span zero. The values suggest that HCRe and chronic change in heart rate tended or did significantly explain (*positively* mediate) the relationship between $\dot{V}O_{2max}$ and RPE_{fixed}. In contrast HVRe tended to explain (*negatively* mediate) the relationship between $\dot{V}O_{2max}$ and RPE_{fixed}. Values are standardized regression coefficients and 95% confidence intervals (lower limit; upper limit) for direct effects of $\dot{V}O_{2max}$ on mediators (a_x), direct effects of mediators on RPE_{fixed} (b_x), and indirect effects of $\dot{V}O_{2max}$ on RPE_{fixed} through mediators (ab_x). *$p < 0.05$; **$p < 0.01$.

2.4.6 Utility of variables to predict submaximal exercise responses at altitude

The equation of Matthews *et al.* (1999) was used to calculate a simple questionnaire-based estimation of $\dot{V}O_{2max}$. The predicted values were very strongly related to the measured values from the maximal exercise test ($r = 0.80$; $p < 0.001$). Furthermore, this simple fitness assessment negatively predicted sense of effort during submaximal exercise at altitude (RPE_{fixed}), although the prediction was significantly improved with the addition of laboratory-assessed $\dot{V}O_{2max}$ (see Table 2.4, *Analysis 1*).

$\dot{V}O_{2max}$ alone was sufficient to predict submaximal exercise responses at altitude, with hypoxic exercise testing providing no additional benefit. Specifically, hypoxic sensitivity did not account for any additional variance than laboratory $\dot{V}O_{2max}$ when predicting sense of effort for RPE_{fixed} ($r^2change = 0.07$; $p = 0.22$; see Table 2.4, *Analysis 2*), RPE_{ascent} ($r^2change = 0.05$; $p = 0.52$), or $STEP_{RPE35}$ ($r^2change = 0.06$; $p = 0.33$). In addition, hypoxic sensitivity did not account for any additional variance than questionnaire-based estimation of $\dot{V}O_{2max}$ when predicting submaximal exercise responses for RPE_{fixed} ($r^2change = 0.09$; $p = 0.18$), RPE_{ascent} ($r^2change = 0.06$; $p = 0.48$), or $STEP_{RPE35}$ ($r^2change = 0.09$; $p = 0.26$).

2.5 Discussion

The primary findings of this study were that greater sea-level fitness is associated with lower sense of effort and higher step rate during perceptually-regulated exercise, but not worse AMS or arterial desaturation. We were able to demonstrate that these relationships are robust, and are not affected by acclimatisation. Consequently, simple sea-level fitness tests predicted sense of effort during submaximal exercise at altitude, and no additional screening information was gained from hypoxic sensitivity testing.

Model	Variable	B	SE B	β	r^2	p	r^2change	p
				Analysis 1				
1	Questionnaire $\dot{V}O_{2max}$	-1.85	0.40	-0.62**	0.39	< 0.001		
2	Questionnaire $\dot{V}O_{2max}$	-0.61	0.59	-0.21	0.47	< 0.001		
	Laboratory $\dot{V}O_{2max}$	-1.49	0.56	-0.53*			0.11	0.01
				Analysis 2				
1	Laboratory $\dot{V}O_{2max}$	-1.91	0.35	-0.69**	0.47	< 0.001		
2	Laboratory $\dot{V}O_{2max}$	-1.55	0.41	-0.56*	0.54	< 0.001		
	HVRe	9.81	8.00	0.18			0.07	0.22
	HCRe	-8.80	7.64	-0.17				
	$\triangle S_pO_2e$	-0.67	0.57	-0.16				

Table 2.4: Summary of hierarchical regression analyses for variables predicting sense of effort during submaximal exercise at altitude (RPE_{fixed}). In *Analysis 1*, model 1 shows the utility of questionnaire-based estimation of $\dot{V}O_{2max}$, whilst model 2 shows the additional utility of laboratory-assessed $\dot{V}O_{2max}$ (note the significant r^2 in model 1 and r^2change value in model 2). In *Analysis 2*, model 1 shows the utility of laboratory-assessed $\dot{V}O_{2max}$, whilst model 2 shows the lack of benefit of additional hypoxic exercise testing (note the significant r^2 in model 1 but insignificant r^2change value in model 2). B, unstandardized beta coefficient (the magnitude of the effect in raw units); SE B, standard error of B; β, standardized beta coefficient (the magnitude of the effect in standardized units, allowing comparison between variables). *p < 0.05; **p < 0.001.

Greater sea-level fitness was associated with lower sense of effort during an arduous trekking day, lower sense of effort during submaximal exercise, and a superior step rate during perceptually-regulated exercise (at the typical chosen effort of mountain walkers and workers) at altitude. Importantly, lower sense of effort during trekking was also associated with better mood, including less fatigue, tension and confusion. High fitness may therefore also protect against the major risk factors of musculoskeletal pain (Jakobsen et al., 2015), injury (Burtscher et al., 2015), and mortality (Firth et al., 2008), and enhance productivity in those travelling to altitude for work and leisure. Lower sense of effort and better mood indicates trekkers more comfortably met the demands of the trek, suggesting high fitness may also protect against summit failure and improve expedition enjoyment. Consequently, this study provides the first empirical evidence that simple sea-level fitness assessments may be useful to aid preparations for high-altitude travel. Further, it provides preliminary evidence to support the recommendation that individuals should complete cardiorespiratory training to improve aerobic fitness before high-altitude travel (Bärtsch and Swenson, 2013). Aerobic training can improve $\dot{V}O_{2max}$ by 20%, although the response varies between 0-50%, depending on genetics, age, initial fitness, and

the exact training type (Bacon et al., 2013; Bouchard et al., 2011; Milanovic et al., 2015). Aerobic fitness is therefore a factor that can be modified to the substantial benefit of those that travel to altitude for work or leisure. As higher fitness was not associated with greater AMS or arterial desaturation, we recommend increasing fitness as much as possible before altitude travel. But of course increased fitness should not be used to ascend more quickly than current guidelines, which would increase altitude illness risk.

The most useful variable to predict sense of effort during submaximal exercise at altitude was $\dot{V}O_{2max}$ as determined by laboratory maximal exercise testing. Even sea-level fitness ($\dot{V}O_{2max}$) estimated by a short questionnaire collecting simple demographic information also provided a strong prediction of sense of effort at altitude. Since the addition of hypoxic sensitivity variables did not improve the prediction of sense of effort during submaximal exercise at altitude, it must be concluded that technically demanding hypoxic exercise testing has no additional benefit beyond simple fitness testing for screening individuals' readiness to perform at altitude. It must be acknowledged that this study used a modified version of Richalet's proposed test. However, research by Richalet's group showed exercise intensity and F_IO_2 do not affect HVR or HCR obtained from the test (Lhuissier et al., 2012). Given the ease of administration (no arduous exercise or specialist equipment required), this simple questionnaire-based assessment of sea-level fitness provides medical and outdoor practitioners with a useful tool to help patients and clients prepare for altitude travel.

Importantly, lower sense of effort during submaximal exercise in fitter individuals did not come at the cost of worse altitude illness. Increased sea-level fitness was not a risk factor for AMS at altitude when ascent rate and trekking energy expenditure were similar in individuals. Additionally, in this study, all participants followed the same ascent profile. In contrast, previous studies to show a positive relationship between fitness and AMS have measured across multiple expeditions without accounting for differences in ascent rate (Karinen et al., 2010; Richalet et al., 2012). This suggests that any observed relationship between fitness and AMS is likely an artefact of behavioural differences. That is, fitter individuals likely ascend faster than their less-fit counterparts and it is

this increased ascent rate that is responsible for their increased AMS (Schneider et al., 2002).

Physiological responses provided some explanation for the lower sense of effort during submaximal exercise in fitter individuals at altitude. Contrary to previous studies with acute hypoxic exposures, high-fit individuals had similar or better S_pO_2 than less-fit individuals during exercise tests completed at the high-altitude Base Camp on day one and three, respectively. This was accompanied by an elevated heart rate response and a lower ventilatory response to acute hypoxia and chronic altitude exposure. Thus, at steady state submaximal exercise (typical of that required during trekking), the lung was able to accommodate the increased cardiac output without compromising pulmonary gas exchange. It is not clear whether the lower ventilatory response in high-fit individuals is due to decreased chemosensitivity or a more efficient ventilatory system, but whatever the cause this response can be considered adaptive as it was associated with lower sense of effort during submaximal exercise. In support of this interpretation, hyperventilation is associated with increased work of breathing and dyspnea (Amann et al., 2007a; Babb et al., 2008), which is a major determinant of RPE (Bernhadt et al., 2013). Increased work of breathing is particularly detrimental at altitude as it elevates peripheral and central fatigue (Ainslie and Ogoh, 2010; Amann, 2012) by reducing locomotor and cerebral blood flow that occur to maintain respiratory muscle demands (Amann et al., 2007a).

2.5.1 Limitations

This study included no altitude measure of maximal exercise capacity, such as $\dot{V}O_{2max}$, time to exhaustion, or time trial tests. However, RPE during submaximal exercise is closely related to maximal exercise capacity (Coquart et al., 2014; Coquart et al., 2016; Eston, 2012). In addition, the assessment of maximal exercise capacity has limited functional relevance to the assessment of trekking and other submaximal work and exercise commonly performed at altitude. Due to their crucial role in fatigue (a major risk factor for mortality on high-altitude

treks), we believe sense of effort and perceptually-regulated exercise are the best methods available to assess trekking exercise. This study provides preliminary evidence of the physiological mechanisms likely to explain the relationship between sea-level fitness and sense of effort during submaximal exercise at altitude. Future studies that experimentally manipulate fitness through training or other methods are required to confirm the importance of cardiorespiratory adaptations for submaximal exercise and fatigue at high altitude.

2.6 Conclusion

Understanding the determinants of exercise and illness at altitude is important to better prepare those who travel to high altitude (Puthon et al., 2015). This study indicates that greater sea-level fitness is related to lower sense of effort during submaximal exercise at altitude and better mood (less fatigue, tension and confusion). Importantly, the lower sense of effort during submaximal exercise in high-fit individuals did not come at the cost of worse AMS or greater arterial oxygen destauration. This study provides the first empirical evidence to support recent recommendations that people might complete sea-level aerobic fitness training before high-altitude travel (Bärtsch and Swenson, 2013; Burtscher et al., 2015). Indeed, our data suggest low-fit persons may improve their trekking experience by increasing sea-level fitness because it is associated with less effort and better mood during trekking at altitude. The study also indicates that a sea-level fitness assessment could be used to aid preparation for high-altitude travel by enabling better aerobic exercise prescription and identifying those people who might benefit most from the aerobic training. Given that fatigue and confusion are major risk factors for injury and fatality at altitude, sea-level fitness assessment and exercise training should be considered as part of preparations for high-altitude travel.

2.7 Author contributions

For this research study, G.M.K.Rossetti contributed to conception and design, pilot testing, data collection, data analysis, interpretation of results, preparation of figures, manuscript drafting, and manuscript revisions. A full list of authorship contributions for this study is provided in Appendix C (Table C.1).

Chapter 3

Dietary nitrate supplementation increases acute mountain sickness severity and sense of effort during hypoxic exercise

3.1 Abstract

Dietary nitrate supplementation enhances sea-level performance and may ameliorate hypoxaemia at high altitude. However, nitrate may exacerbate acute mountain sickness (AMS), specifically headache. This study investigated the effect of nitrate supplementation on AMS symptoms and exercise responses with 6 h hypoxia. Twenty recreationally-active men (mean (SD): age 22 (4) years, $\dot{V}O_{2max}$ 51 (6) mL·min^{-1}·kg^{-1}) completed this randomized double-blind placebo-controlled crossover study. Twelve participants were classified as AMS- based on Environmental Symptom Questionnaire (AMS-C) score < 0.7 in both trials, and five participants were classified as AMS+ based on AMS-C score ≥ 0.7 on placebo. Five days nitrate supplementation (70 mL beetroot juice containing 6.4 mmol nitrate daily) increased plasma NO metabolites by 182 μM compared to placebo but did not reduce AMS or improve exercise performance. After 4 h hypoxia ($F_IO_2 = 0.124$) nitrate increased AMS-C and headache severity (visual analogue scale (VAS); whole sample $\triangle$10[1,20] mm; $p = 0.03$) compared to placebo. In addition, after 5 h hypoxia, nitrate increased sense of effort during submaximal exercise ($\triangle$7 [-1,14]; $p = 0.07$). In AMS- nitrate did

not alter headache or sense of effort. In contrast, in AMS+ nitrate increased headache severity (△26 [-3,56] mm; p = 0.07), sense of effort (△14 [1,28]; p = 0.04), oxygen consumption, ventilation, and mean arterial pressure during submaximal exercise. On the next day, in a separate acute hypoxic exercise test (F_IO_2 = 0.141), nitrate did not improve time to exhaustion at 80% hypoxic $\dot{V}O_{2max}$. In conclusion, dietary nitrate increased AMS and sense of effort during exercise, particularly in those who experienced AMS. Dietary nitrate is therefore not recommended as an AMS prophylactic or ergogenic aid nonacclimatised individuals at altitude.

3.2 New & noteworthy

This is the first study to identify that the popular dietary nitrate supplement (beetroot) does not reduce acute mountain sickness (AMS) or improve exercise performance during 6 h hypoxia. The consumption of nitrate in those susceptible to AMS exacerbates AMS symptoms (headache) and sense of effort and raises oxygen cost, ventilation, and blood pressure during walking exercise in 6 h hypoxia. These data question the suitability of nitrate supplementation during altitude travel in nonacclimatised people.

3.3 Introduction

Many people engage in altitude travel for work and leisure, with 300 million overnight stays in alpine regions each year (Bartaletti and International Commission for the Protection of the Alps (CIPRA), 2008) and hundreds of thousands of individuals travelling to other high-altitude regions such as the Himalayas and Kilimanjaro (Government of Nepal, 2013). Approximately half of those that visit high altitude suffer from illnesses such as acute mountain sickness (AMS; Bärtsch and Swenson, 2013). AMS can lead to high-altitude cerebral oedema (HACE) which if left untreated can be fatal (Hackett and Roach, 2004). High-altitude exposure also reduces exercise capacity (Fulco et al., 1998).

Finding interventions to counteract hypoxaemia, the root cause of these negative effects, is therefore important.

Nitric oxide (NO) is a potent signalling molecule that modulates human physiological function via its role in the regulation of blood flow, muscle contractility and mitochondrial respiration (Stamler and Meissner, 2001). NO can be produced via the oxygen-dependent oxidation of L-arginine with the help of NO synthase (NOS) enzymes (Lundberg et al., 2011). However, it is now clear that NO can also be generated by the serial reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and then NO (Duncan et al., 1995). An increase in NO bioavailability has been observed after the consumption of dietary inorganic nitrate (Wylie et al., 2013) and has been associated at sea level with a reduction in resting blood pressure (Siervo and Lara, 2013), increased skeletal muscle blood flow (Ferguson et al., 2013), and enhanced exercise performance (Hoon et al., 2013). Importantly, the reduction of nitrite to NO is increased in environments of low oxygen tension, and therefore dietary nitrate consumption may be a particularly effective method to increase NO bioavailability at altitude. During acute exposure to hypoxia ($\leq$ 2 h), nitrate supplementation has been shown to improve peripheral and muscle oxygenation during rest and exercise (Masschelein et al., 2012), and improve muscle energetics (Vanhatalo et al., 2011), lower the oxygen cost of submaximal exercise (Kelly et al., 2014), and enhance exercise performance in some (e.g. Masschelein et al., 2012; Shannon et al., 2017) but not all (e.g. Arnold et al., 2015; Bourdillon et al., 2015) studies. NO may also improve diffusion capacity within the lung, and enhance oxygen delivery (Umbrello et al., 2013), potentially ameliorating the root cause of AMS (hypoxaemia). Because of these proposed beneficial physiological and exercise responses, some have recommended dietary nitrate for altitude travel (Bakker et al., 2015; Muggeridge et al., 2014), with a recent study concluding its use is safe and feasible at altitude (Hennis et al., 2016).

However, nitrate supplementation may be harmful during hypoxia. Dietary nitrate may increase AMS by elevating headache. Not only the cardinal symptom of AMS, it has been suggested that high-altitude headache contributes to the accompanying AMS symptoms of gastrointestinal symptoms, fatigue,

dizziness, lightheadedness, and poor sleep (Lawley et al., 2016). A growing body of data supports the proposed pathophysiology of high-altitude headache as a result of arterial dilation causing elevated cerebral blood volume and intracranial pressure. This leads to increased arterial and intracranial pressure transmission, trigeminovascular sensitization, and pain sensation (Lawley et al., 2016). Given that NO is implicated in hypoxia-induced vasodilation (Van Mil et al., 2002), and directly stimulates the trigeminovascular system (Ashina et al., 1999), supplementing with nitrate may exacerbate high-altitude headache pathophysiology. However, to date no laboratory studies have been completed to investigate the effects of dietary nitrate on AMS. Resolving the efficacy and safety of this dietary intervention, particularly in reference to the cerebral component of AMS, is of clinical and timely importance. In addition, the effect of dietary nitrate consumption on exercise responses during longer-duration hypoxic exposures (> 2 h) is unknown. Since the physiological stimulus of exercise may increase AMS severity in hypoxia (DiPasquale et al., 2015), the effects of nitrate on exercise responses, whether positive or negative, could potentially alter nitrate's effect on AMS symptoms. Given that individuals travelling to high altitude will engage in exercise, it is important to investigate the effects of nitrate under conditions of exercise in hypoxia. In addition, AMS is a condition that is greatly affected by individual susceptibility (Wilson et al., 2013). It is possible that nitrate may exacerbate AMS pathophysiology and symptoms in individuals who experience AMS, but have beneficial physiological and exercise performance effects in individuals who do not suffer AMS.

The primary aim of the present double-blind placebo-controlled crossover study was to determine the effect of five days of dietary nitrate supplementation on high-altitude headache and AMS symptom severity with a 6 h exposure to hypoxia. We hypothesised five days of dietary nitrate supplementation would decrease high-altitude headache and AMS symptom severity with a 6 h exposure to hypoxia. Secondary aims of the present study were to explore: the effect of five days dietary nitrate supplementation on submaximal exercise responses during 6 h hypoxia; and the interaction of AMS (AMS present or absent) and nitrate supplementation on high-altitude headache severity and submaximal exercise responses during 6 h hypoxia. In addition, due to equivocal findings in

the literature (e.g. Bourdillon et al., 2015; Masschelein et al., 2012), we also determined the effect of six days dietary nitrate supplementation on exercise performance in acute hypoxia.

3.4 Methods

3.4.1 Participants

Twenty recreationally-active men were recruited into the study (mean (SD); age, 22 (4) years; height, 180 (10) cm; body mass, 79 (11) kg; maximal oxygen consumption [$\dot{V}O_{2max}$], 51 (6) ml·kg^{-1}·min^{-1}). Eleven participants (45%) had previously travelled to high altitude ($\geq$ 2500 m), and of these 11 participants, 2 (18%) reported previous AMS, and none had a history of HAPE or HACE. Participants had not travelled to altitude ($\geq$ 1500 m) in the preceding six months, and had no medical contraindications to maximal exercise testing. All participants provided written informed consent. Ethical approval was granted by the Ethics Committee of the School of Sport, Health, and Exercise Sciences at Bangor University, and the study was registered on *www.clinicaltrials.gov* (trial ID: NCT03101904).

3.4.2 Study design

The study followed a double-blind placebo-controlled crossover design. Normoxic (F_IO_2 = 0.209; sea level) and hypoxic (F_IO_2 = 0.141; equivalent 3225 m) maximal exercise tests were conducted at baseline. Participants then completed two six-day supplementation periods, separated by a minimum ten-day washout. On day five of each supplementation period, a 6 h hypoxic exposure (F_IO_2 = 0.124; equivalent 4219 m) was conducted to assess AMS and responses to submaximal exercise. On day six, a time to exhaustion test was conducted at 80% $\dot{V}O_{2max}$ reserve in acute hypoxia (F_IO_2 = 0.141; equivalent

3225 m) to assess exercise performance. An overview of the protocol is depicted in Figure 3.1.

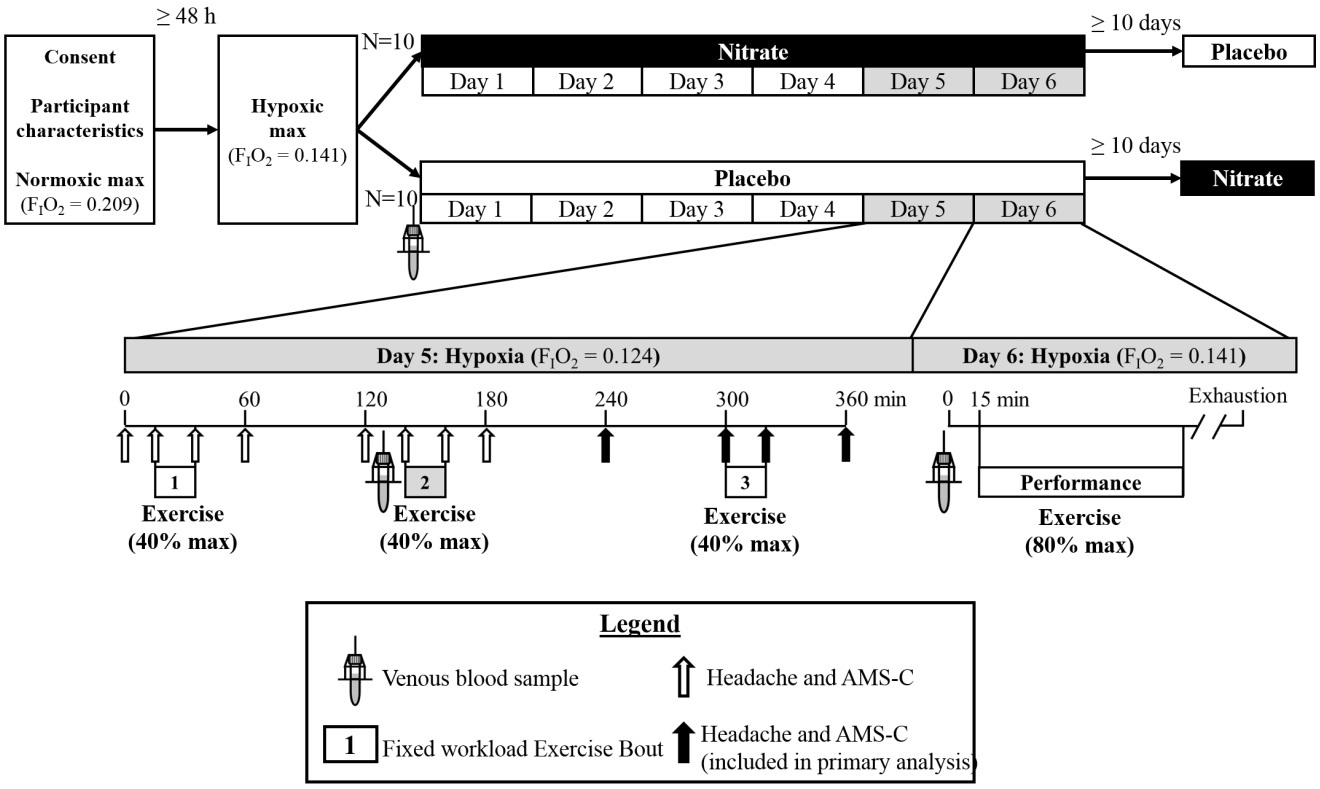


Figure 3.1: Schematic representation of study protocol. Supplementation was taken at the same time each day, 2.5 h before any exercise tests. Participants completed a 6 h exposure to hypoxia on day five, and an acute hypoxic exercise to exhaustion test on day six. All exercise intensities are set at a proportion of hypoxic $\dot{V}O_{2max}$ reserve. Additional physiological data was obtained by online gas analysis and a stress blood pressure system during *exercise bout 2*. Filled black arrows indicate headache and AMS data included in the primary analysis.

3.4.3 Supplementation

Participants were randomly assigned to receive six days of inorganic nitrate or placebo supplementation, separated by a minimum ten-day washout. Supplementation consisted of a daily nitrate-rich concentrated beetroot juice shot (nitrate; 70 mL/day containing 6.4 mmol NO_3^-; Beet-It Sport, James White drinks Ltd, Ipswich, UK) or a nitrate-depleted concentrated beetroot juice shot (placebo; 70 mL/day containing ~0.003 mmol NO_3^-; James White drinks Ltd, Ipswich, UK) that was identical in appearance, taste, and texture. Placebo shots were created by passing the NO_3^- rich concentrated beetroot juice through a Purolite A520E NO_3^- selective ion exchange resin before pasteurisation

(Lansley et al., 2011). Supplements were ingested 2.5 h before any experimental tests, and at the same time each day throughout both supplementation periods. Participants were phoned or texted to remind them to ingest the supplement and were asked to confirm ingestion by phone or return text.

To isolate nutritional effects of the intervention, participants avoided high NO_3^- food and drink throughout the study, and diet and physical activity was matched before each trial, confirmed by food and exercise diaries. Participants also abstained from using antibacterial mouthwashes as this has previously shown to lessen the reduction of NO_3^- to NO_2^- by commensal bacteria within the oral cavity (Govoni et al., 2008).

Before (day 0) and on day five (in hypoxia) and day six (pre-hypoxia) of each supplementation phase, venous blood samples were collected into lithium heparin-coated 6 mL vacutainers (BD Vacutainer tubes; Becton, Dickinson and Company: New Jersey), immediately centrifuged at 4000 rpm and 4°C for 7 min before the plasma was extracted and stored at -80°C for later analysis of plasma NO metabolites (nitrate + nitrite [NO_x]). To confirm participants were blind to the supplementation they were receiving and that the placebo was successful, a manipulation check was conducted after each supplementation was completed, by asking participants to guess what intervention they had received (possible responses were “beetroot”, “placebo”, or “don’t know”).

3.4.4 Procedures

Maximal aerobic capacity ($\dot{V}O_{2max}$)

All exercise tests consisted of loaded walking whilst carrying a 15 kg rucksack on a motorized treadmill (H-P-Cosmos, Sports & Medical GmbH; Nussdorf-Traunstein: Germany). At baseline, participants completed an incremental exercise test to exhaustion with simultaneous online pulmonary gas analysis (Cortex Metalyzer, Biophysik GmbH; Leipzig: Germany). The incremental exercise test protocol commenced at 5 $km \cdot h^{-1}$ and 1% gradient with a ramped

increase in gradient to 25% over 18 min. If 25% was reached, treadmill speed was increased by 0.66 km·h^{-1}·min^{-1}. Sense of effort during exercise (rating of perceived exertion; RPE) was recorded each minute of the test using the Borg CR100 (Borg and Borg, 2001). $\dot{V}O_{2max}$ was identified if all of the following criteria were met: volitional fatigue; a plateau in oxygen consumption ($\dot{V}O_2$) despite an increase in workload; respiratory exchange ratio (RER) $\geq$ 1.15. Participants also completed the same maximal exercise protocol in hypoxia (F_IO_2 = 0.141, equivalent 3225 m), with the two tests separated by a minimum of 48 h.

Six-hour hypoxic exposure

On day five of each supplementation period, participants completed a 6 h poikilocapnic hypoxic exposure in a normobaric hypoxic chamber (F_IO_2 = 0.124; equivalent 4219 m: Hypoxico, Inc. New York, NY). After 15 min seated rest, participants completed the first of three 20 min bouts of submaximal exercise at 40% hypoxic $\dot{V}O_{2max}$ reserve. The submaximal exercise protocol during the 6 h exposure consisted of *exercise bout 1*, completed between 15-35 min; *exercise bout 2*, 140-160 min; and *exercise bout 3*, 300-320 min. Blood pressure and heart rate measured by a stress BP system (Tango+; SunTech Medical, Inc., Morrisville, NC; USA), and oxygen saturation (S_pO_2) measured by pulse oximetry (9550 OnyxII; Nonin Medical Inc, Minnesota), were assessed every hour and pre- and post-each exercise bout throughout the 6 h exposure. Mean arterial blood pressure (MAP) was calculated to account for heart rate using the equation provided by Moran *et al.* (1995):

$$MAP = DBP + ((0.01 \times exp(4.14 - 40.74 \div HR)) \times (SBP - DBP)) \quad (3.1)$$

Where MAP, Mean arterial blood pressure; DBP, Diastolic blood pressure; HR, Heart rate; SBP, Systolic blood pressure.

High-altitude headache and AMS. High-altitude headache was assessed every hour and pre- and post-each exercise bout throughout the 6 h exposure on day

five of supplementation, using a 0-100 mm visual analogue scale (VAS; Kelly, 2001). Participants were asked to mark on the line the intensity/feeling that corresponded to their headache, where the beginning of the line indicated no perception/feeling at all and the end of the line indicated maximum perception/feeling. AMS was assessed by the Acute Cerebral Mountain Sickness (AMS-C) score calculated from the 11-item Environmental Symptoms Questionnaire (ESQ; Sampson et al., 1994). Participants rated the severity of each item from one to five, and the ratings were multiplied by their factorial loadings and summed. In order to investigate whether any effects of nitrate supplementation were affected by the presence or absence of AMS, participants were split into two groups depending on whether they had AMS on placebo. Participants with AMS-C < 0.7 in both trials were classified as AMS-, while participants with AMS-C ≥ 0.7 on placebo were classified as AMS+ (Sampson and Kobrick, 1980). Participants with AMS-C < 0.7 on placebo, but AMS-C ≥ 0.7 on nitrate were included in the whole sample analyses, but were not included in AMS+/- sub-analyses.

Submaximal exercise responses. Sense of effort during the three submaximal exercise bouts was assessed by RPE. RPE was recorded using the Borg CR100 (Borg and Borg, 2001) which asks participants to rate the intensity of the exercise sensation using numbers from 0-100+ and verbal descriptors (e.g. "moderate", equivalent to 25). Extensive evidence supports the use of RPE as a valid and appropriate method to record sense of effort and perceptual responses to exercise (Eston, 2012). RPE, heart rate and S_pO_2 were recorded at 2 min intervals during the three exercise bouts. In addition to these variables, the study design targeted *exercise bout 2* to examine extra physiological responses, including ventilatory parameters and exercising blood pressure, in an attempt to identify possible mechanisms of nitrate's effect on exercise and AMS before AMS was present. We did not collect these data during *exercise bout 1* because this bout occurred too early, when variable responses such as the hypoxic ventilatory response (West et al., 2012) would likely mask any mechanistic physiological changes. We did not collect these data during *exercise bout 3* because this bout occurred too late, when AMS was already present. To be consistent with a cause and effect relationship, any physiological changes must be observed before

the onset of AMS; at *exercise bout 3* it would not be possible to differentiate between cause and consequence of AMS. Thus, during *exercise bout 2* exercising blood pressure was recorded every 2 min and ventilation (VE), $\dot{V}O_2$, RER, and end-tidal carbon dioxide ($P_{ET}CO_2$) were measured using online gas analysis. To account for the hypoxic conditions, the gas analyser was calibrated using ambient air (12.4% O2; 0.03% CO_2) and a F_IO_2–specific calibration gas (8% O2; 5% CO_2; Industrial Gases, BOC ltd, Surrey, UK).

Exercise performance (time to exhaustion; TTE)

On day six of each supplementation period, participants completed a time to exhaustion (TTE) test at 5 km·h^{-1} and a gradient corresponding to 80% of their hypoxic $\dot{V}O_{2max}$ reserve in acute hypoxia (F_IO_2 = 0.141, equivalent 3225 m). Exercise performance was defined as TTE determined by the time from onset of test to task failure (volitional exhaustion or inability to maintain treadmill speed). During the exercise test S_pO_2, heart rate, and RPE were recorded each minute. Participants were blind to time elapsed and provided no verbal encouragement.

3.4.5 Plasma nitric oxide metabolites (NO_x)

All glassware, utensils, and surfaces were rinsed with deionised water to remove residue NO_3^- and NO_2^- before blood analysis. After thawing at room temperature, plasma samples were initially deproteinized using cold ethanol precipitation. Initially 0.5 mL of sample was placed in a chilled microcentrifuge tube, along with 1 mL of cold (0°C) ethanol; then the samples were vortexed and left to stand at 0°C for 30 min. Thereafter, samples were centrifuged at 14,000 rpm for 5 min, and the supernatant removed for subsequent analysis. NO_x (nitrate + nitrite) in the deproteinized plasma samples was reduced to NO in the presence of 0.8% (w/v) vanadium trichloride in 1 M hydrochloric acid. The production of NO was detected by a Sievers gas-phase chemiluminescence NO analyser (Sievers NOA 280i; Analytix, Duham, UK) and the NO_x concentration

was derived by plotting signal (mV) area against a calibration plot of 1-750 μM sodium NO_3^-.

3.4.6 Statistical analysis

Differences between nitrate and placebo were determined by confidence intervals relating to *a priori* meaningful differences (Hopkins and Batterham, 2016), supported by statistical differences testing by repeated measures analysis of variance (RM ANOVA) or t-tests as appropriate ($p < 0.05$). For the primary analysis, (to determine the effect of dietary nitrate supplementation on high-altitude headache) a 2 × 4 (Trial × Time) RM ANOVA was used to compare high-altitude headache severity by VAS from 4 to 6 h between nitrate and placebo trials. The time course of 4 to 6 h was chosen for the primary analysis based on the expected time that AMS would develop from previous data from our laboratory at a similar F_IO_2 (Lawley et al., 2017, 2013, 2014b) that showed no incidence of AMS before 4 h, but an AMS incidence of 50% after 6 h. A sample size estimation for the primary analysis indicated that 16 participants were needed to produce an 80% chance of obtaining statistical significance at the 0.05 level for a two-tailed design (Stevens, 2002), based on a minimum important difference of 10 mm (Kelly, 2001), a standard deviation of the difference of 18 mm, and an estimated average correlation of 0.4 (data from Lawley et al., 2013). To determine the influence of AMS, all analyses were repeated including a factor for AMS presence or absence (AMS+ or AMS-), and interpreted on the basis of significant interactions.

The effect of nitrate supplementation on exercise performance (TTE) was determined by paired samples t-test. A sample size estimation for this analysis indicated that 12 participants were needed to produce an 80% chance of obtaining statistical significance at the 0.05 level for a two-tailed design (Bland, 2015), based on a minimum important difference of 30 s, and a standard deviation of the difference of 33 s (data from Masschelein et al., 2012). To determine the effect of nitrate supplementation on i) RPE, ii) S_pO_2, and ii) heart rate, a 2 × 5 (Trial × Isotime) RM ANOVA was used to compare nitrate

and placebo trials at 0%, 25%, 50%, 75%, and 100% of isotime during the TTE test. Resting values taken immediately before commencing the TTE were recorded as 0% isotime. 100% isotime was defined as the last complete minute of the shortest TTE, and the corresponding minute in the longest TTE for each participant. The minute identified as 100% isotime was multiplied by 0.25, 0.50, and 0.75 and rounded to the nearest complete minute to give 25% isotime, 50% isotime, and 75% isotime, respectively.

Five participants did not complete the TTE on day six due to injury or technical reasons, e.g. cramp or tripping whilst on the treadmill, and one of these was also removed from analyses relating to RPE on day five due to failure to use the RPE scale consistently. All analyses were completed using SPSS version 23 (IBM Corp, Armonk; NY).

3.5 Results

Participants were sufficiently blind to the intervention since the manipulation check indicated participants guessed correctly in only 18% of trials, guessed incorrectly in 34% of trials, and were unable to distinguish between interventions in 48% of trials.

3.5.1 Plasma nitric oxide metabolites (NO_x)

Participant compliance with the supplementation protocol was 100%. This self-reported compliance measure was confirmed by plasma NO_x data. Plasma NO_x was similar between trials at baseline (nitrate = 27 (9); placebo = 28 (9); [-5, 3] μM; p = 0.7). The nitrate supplementation effectively altered plasma NO_x concentrations. Compared to placebo, nitrate supplementation increased plasma NO_x on day five (mean diff [95%CI]: $\triangle$182 [155, 208] μM; $p < 0.001$). Compared to placebo, nitrate supplementation also increased plasma NO_x on day six in the 15 participants that completed the time to exhaustion tests ($\triangle$244

[201, 286] μM; p < 0.001). By design, plasma NO_x was not altered compared to baseline, with five (△-3 [-8, 2] μM; p = 0.3) or six (△-3 [-10, 1] μM; p = 0.2) days of placebo supplementation.

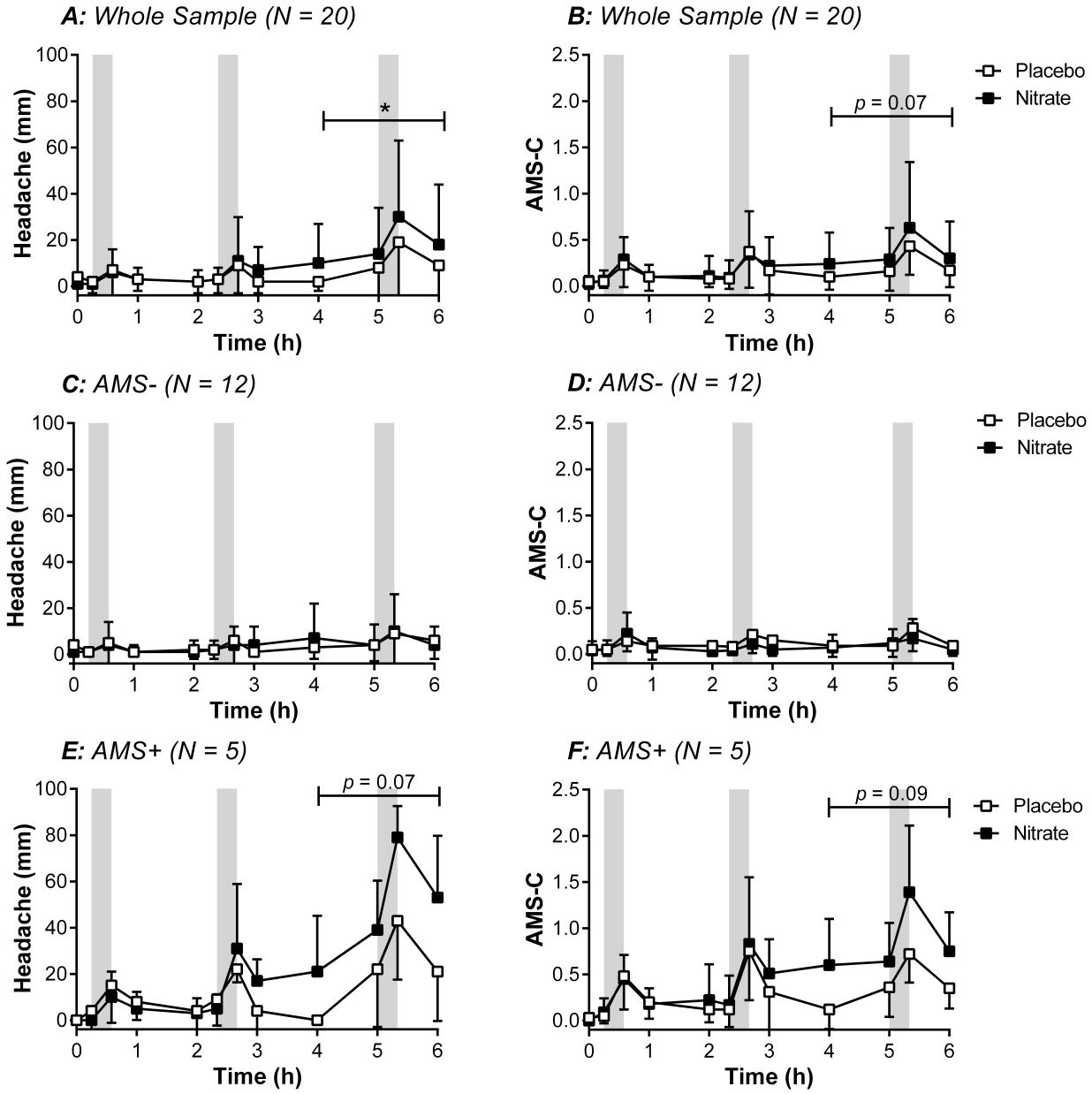


Figure 3.2: High-altitude headache and AMS during 6 h in hypoxia (FIO2 = 0.124) on day five. Grey shaded bars indicate submaximal exercise bouts (40% $\dot{V}O_{2max}$ reserve). From 4 to 6 h, in the whole sample, (A) nitrate increased headache by visual analogue scale (VAS), and (B) tended to increase AMS-C score calculated from the Environmental Symptoms Questionnaire. In AMS- (participants with AMS-C score < 0.7 in both trials) dietary nitrate had no effect on (C) headache, or (D) AMS-C. In AMS+ (participants with AMS-C ≥ 0.7 on placebo), nitrate increased (E) headache and (F) AMS-C. *Nitrate significantly higher than placebo (p < 0.05).

3.5.2 High-altitude headache and AMS

There was no headache at 0 h (pre-hypoxia) in either trial (nitrate = 1 (4); placebo = 4 (9); [-7, 2] mm; $p = 0.2$). Although headache tended to increase after *exercise bout 1* (△4 [-1, 9] mm; $p = 0.08$) and *exercise bout 2* (△8 [0, 15] mm; $p = 0.05$), there was no effect of nitrate (both $p \geq 0.4$; Figure 3.2A). As expected, AMS and headache increased in the latter part of the trial ($p < 0.05$). Unexpectedly this effect was exacerbated by nitrate. From 4 to 6 h, nitrate increased headache severity compared to placebo (△10 [1, 20] mm; $p = 0.03$) and tended to increase AMS-C compared to placebo (△0.15 [-0.01, 0.31]; $p = 0.07$; Figure 3.2B).

3.5.3 Physiological responses to 6 h hypoxia

Resting physiological responses (heart rate, S_pO_2, and blood pressure) changed throughout the 6 h exposure, but were not affected by nitrate. Heart rate increased over the hypoxic exposure (△16 [11, 21] bpm; $p < 0.001$), and after each exercise bout, but was not affected by nitrate (△-2 [-5, 2] bpm; $p = 0.4$). S_pO_2 decreased over the hypoxic exposure (△-14 [-17, -11] %; $p < 0.001$), and after each exercise bout, but was not affected by nitrate (△0 [-1, 1] %; $p = 0.8$). Nitrate supplementation had no effect on any measure of resting blood pressure, with no difference in SBP (△1 [-4, 5] mmHg; $p = 0.7$), DBP (△-1 [-4, 2] mmHg; $p = 0.4$), or MAP (△1 [-3, 4] mmHg; $p = 0.8$).

3.5.4 Submaximal exercise responses

Dietary nitrate increased sense of effort compared to placebo during submaximal exercise in 6 h hypoxia (Figure 3.3A). Nitrate elicited a small but significant increase in RPE for *exercise bout 1* (△4 [0, 8]; $p = 0.03$), and a larger increase for *exercise bout 3* (△7 [-1, 14]; $p = 0.07$), although had no effect on RPE for *exercise bout 2* (△2 [-5, 9]; $p = 0.5$). In the nitrate trial, headache at 5 h (pre-

	Nitrate	Placebo	95%CI diff LB	95%CI diff UB	p
Exercise bout 1					
Heart rate (bpm)	129 (21)	133 (17)	-12	2	0.2
S_pO_2 (%)	76 (3)	76 (3)	-1	1	0.8
Exercise bout 2					
Heart rate (bpm)	138 (20)	136 (20)	-6	10	0.6
S_pO_2 (%)	75 (4)	75 (4)	-1	1	0.7
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.70 (0.30)	1.69 (0.29)	-0.07	0.10	0.7
$\dot{V}_E$ ($L \cdot min^{-1}$)	59.3 (11.7)	59.9 (12.7)	-4.5	3.2	0.7
RER	0.97 (0.08)	0.97 (0.08)	-0.02	0.04	0.6
$P_{ET}CO_2$ (mmHg)	32.6 (3.3)	32.1 (2.8)	-0.3	1.3	0.2
SBP (mmHg)	179 (17)	166 (25)	-1	27	0.06
DBP (mmHg)	58 (9)	60 (11)	-9	5	0.5
MAP (mmHg)	113 (8)	110 (16)	-4	11	0.3
Exercise bout 3					
Heart rate (bpm)	139 (23)	140 (24)	-12	10	0.8
S_pO_2 (%)	76 (4)	75 (4)	-1	2	0.3

Table 3.1: Physiological responses to submaximal exercise in hypoxia. All data are end-exercise and presented as mean (SD). LB, lower bound; UB, upper bound; S_pO_2, oxygen saturation; $\dot{V}O_2$, oxygen consumption, $\dot{V}_E$, ventilation; RER, respiratory exchange ratio; $P_{ET}CO_2$, end-tidal carbon dioxide; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure. N = 20.

exercise bout 3) was positively correlated with change in RPE from *exercise bout 1* to *exercise bout 3* ($r = 0.67$; $p < 0.01$). When analysed across the whole sample, nitrate had no effect on any physiological response to the three submaximal exercise bouts (Table 3.1).

3.5.5 Influence of AMS presence or absence (AMS+/-)

Twelve participants were classified as AMS- and five were AMS+ (see Section 3.4.4). There were no differences in baseline characteristics or NO_x concentrations at any point between AMS+ and AMS- (all $p > 0.5$; data not shown).

In AMS-, nitrate had no effect on high-altitude headache (△2 [-5, 9] mm; $p = 0.6$; Figure 3.2C) or AMS-C (△-0.04 [-0.13, 0.06]; $p = 0.4$; Figure 3.2D). In AMS+, nitrate increased altitude illness severity from 4 h: in AMS+, high-altitude headache severity was more than doubled with nitrate compared to

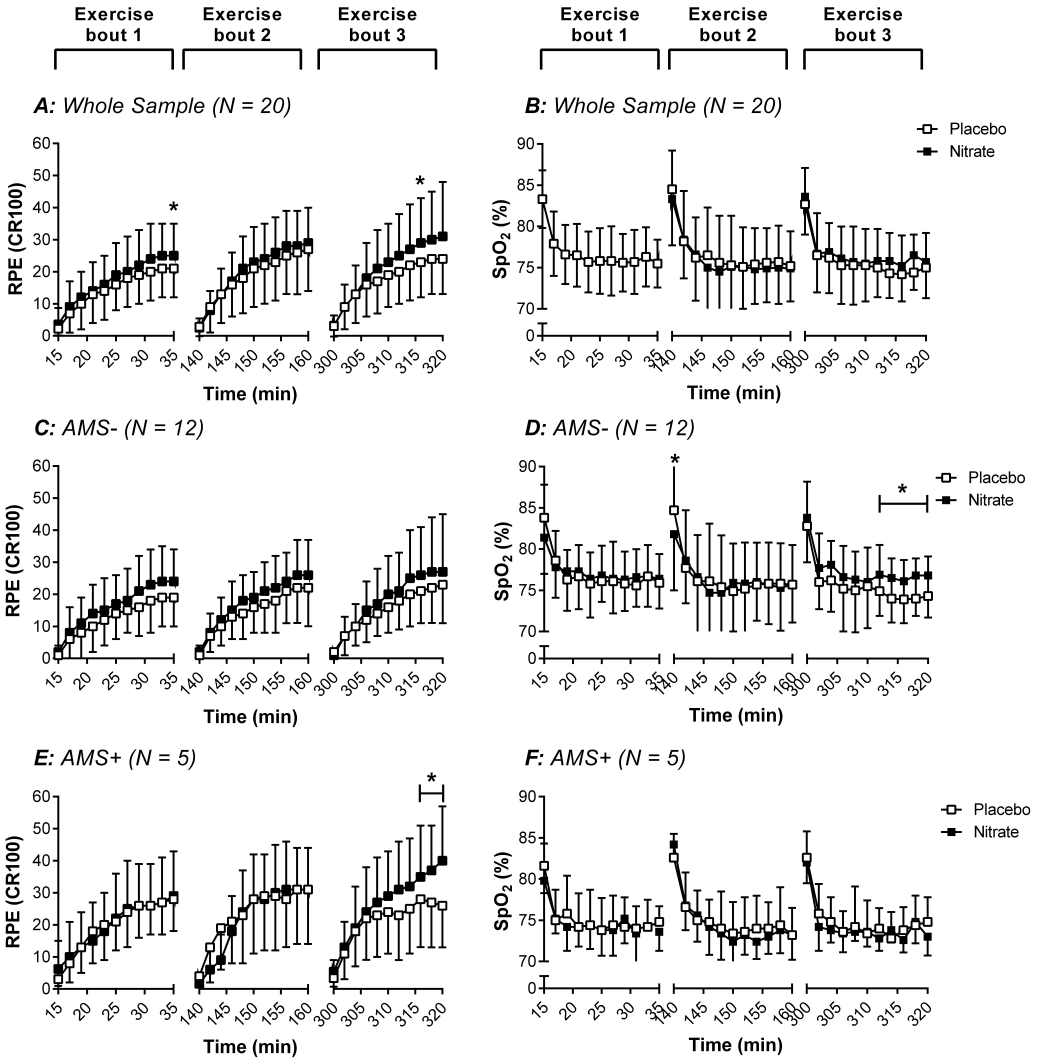


Figure 3.3: Sense of effort (RPE) and oxygen saturation (S_pO_2) during submaximal exercise. In the whole sample, dietary nitrate (A) increased RPE, but (B) had no effect on S_pO_2. In AMS- (participants with AMS-C score < 0.7 in both trials), dietary nitrate had (C) no effect on RPE, but (D) increased S_pO_2 during *exercise bout 3*. In AMS+ (participants with AMS-C ≥ 0.7 on placebo), dietary nitrate (E) increased RPE, but (F) had no effect on S_pO_2. *Nitrate significantly higher than placebo ($p < 0.05$).

placebo (△26 [-3, 56] mm; p = 0.07; Figure 3.2E) and AMS-C was similarly increased (Δ0.46 [-0.10, 1.02]; p = 0.09; Figure 3.2F). In AMS-, there was no difference in RPE between nitrate and placebo for any of the exercise bouts (Figure 3.3C). In AMS+, by the end of the exposure, dietary nitrate had increased sense of effort during submaximal exercise compared to placebo (△14 [1, 28]; p = 0.04; Figure 3.3E).

Depending on whether participants were AMS- or AMS+, nitrate had opposite effects on physiological responses to submaximal exercise during the hypoxic

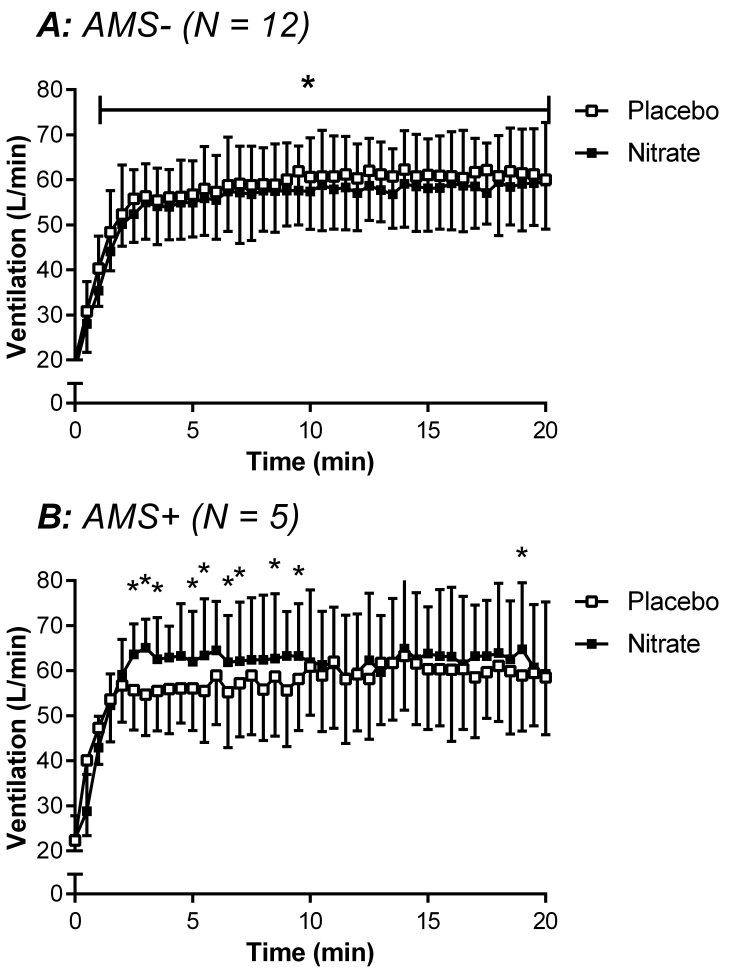


Figure 3.4: Ventilation during submaximal exercise (*exercise bout 2*). Dietary nitrate (A) decreased ventilation during *exercise bout 2* in AMS- (participants with AMS-C score < 0.7 in both trials), and (B) increased ventilation in AMS+ (participants with AMS-C ≥ 0.7 on placebo). *Nitrate significantly higher than placebo ($p < 0.05$).

exposure (determined by significant AMS-trial interactions). During *exercise bout 2,* in AMS-, nitrate decreased ventilation in comparison to placebo (-3.0 [-5.5, -0.4] L·min^{-1}; p = 0.03; Figure 3.4A), and had no effect on $\dot{V}O_2$ (△-0.02 [-0.13, 0.09] L·min^{-1}; p = 0.6; Figure 3.5A) or MAP (△-1 [-9, 6] mmHg; p = 0.7; Figure 3.6A).

In contrast, in AMS+, nitrate increased ventilation (△ 3.1 [-0.7, 7.0] L·min^{-1}; p = 0.1; Figure 3.4B), $\dot{V}O_2$ (△0.10 [0.03, 0.17] L·min^{-1}; p = 0.02; Figure 3.5B), and MAP (△7 [-3, 17] mmHg; p = 0.1; Figure 3.6B). During *exercise bout 3*, in AMS- nitrate increased S_pO_2 compared to placebo from 12 min onwards (△ 3 [0, 4] %; p = 0.03; Figure 3.3D). However, in AMS+, nitrate had no effect on S_pO_2 for any exercise bout (Figure 3.3F). The effect of nitrate on $P_{ET}CO_2$ was not

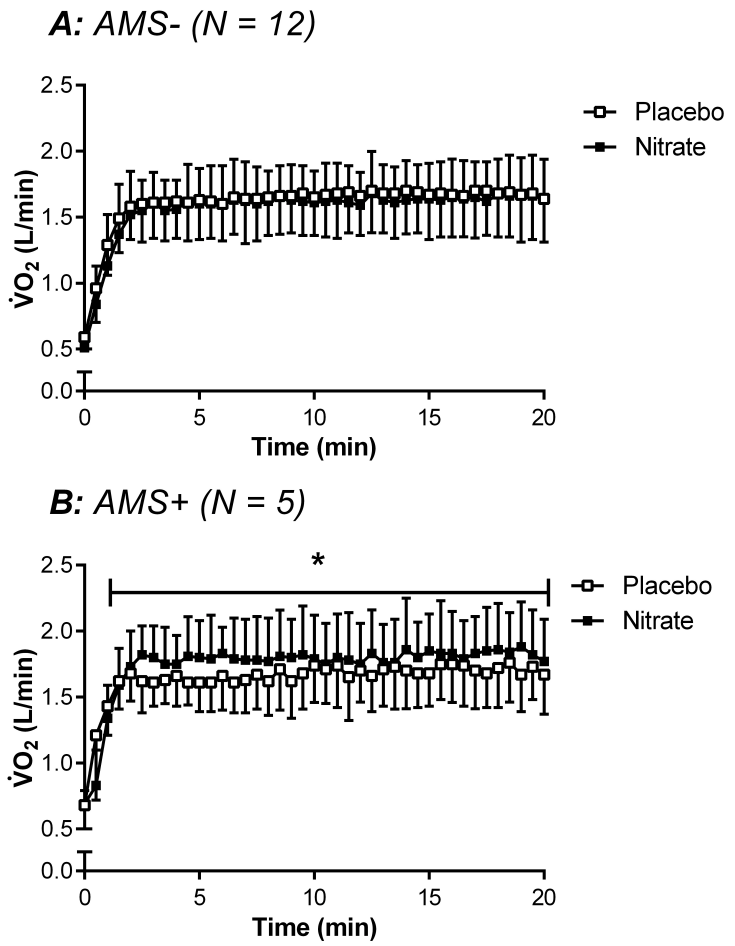


Figure 3.5: Oxygen cost ($\dot{V}O_2$) of submaximal exercise (*exercise bout 2*). Dietary nitrate (A) had no effect on $\dot{V}O_2$ during *exercise bout 2* in AMS- (participants with AMS-C score < 0.7 in both trials), but (B) increased $\dot{V}O_2$ in AMS+ (participants with AMS-C $\geq$ 0.7 on placebo). *Nitrate significantly higher than placebo ($p < 0.05$).

altered depending on the presence or absence of AMS (AMS-trial interaction; p = 0.2).

3.5.6 Exercise performance (time to exhaustion; TTE)

Six days dietary nitrate supplementation had no effect on TTE in hypoxia ($\triangle$10 [-103, 123] s; p = 0.9). Dietary nitrate supplementation had no effect on heart rate, S_pO_2, or RPE at any isotime, or at exhaustion (Table 3.2).

Whether participants experienced AMS did not influence the effect of nitrate on exercise performance (TTE; group-trial interaction; p = 0.9). Specifically,

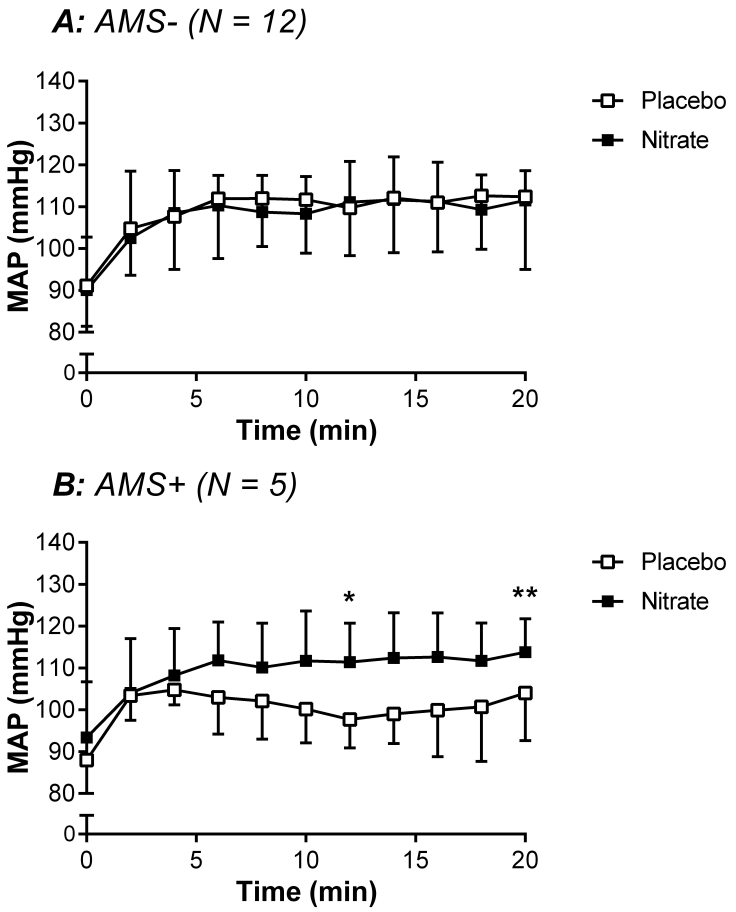


Figure 3.6: Mean arterial pressure (MAP) during submaximal exercise (*exercise bout 2*). Dietary nitrate (A) had no effect on MAP during *exercise bout 2* in AMS- (participants with AMS-C score < 0.7 in both trials), but (B) increased MAP in AMS+ (participants with AMS-C ≥ 0.7 on placebo). Nitrate significantly higher than placebo (*p < 0.05; **p < 0.01).

nitrate had no effect on TTE in AMS- (△16 [-115, 148] s; p = 0.8) or in AMS+ (△10 [-103, 123] s; p = 0.9).

3.6 Discussion

The primary findings of this study were that dietary nitrate supplementation did not reduce AMS severity, specifically high-altitude headache, or improve exercise performance in hypoxia. When assessed over the whole sample, nitrate had no effect on any physiological response to hypoxia. However, opposing effects of nitrate were observed in those with and without AMS. In participants who did not develop AMS (AMS-), nitrate decreased ventilation and improved S_pO_2

	Isotime					Exhaustion
	0%	25%	50%	75%	100%	
Heart rate (bpm)						
Placebo	84 (10)	145 (24)	153 (33)	161 (27)	172 (26)	168 (34)†
Nitrate	88 (11)	149 (17)	157 (27)	168 (18)	168 (24)	171 (22)†
S_pO_2 (%)						
Placebo	89 (3)	85 (5)	82 (4)	81 (3)	81 (4)	81 (4)†
Nitrate	89 (4)	84 (3)	81 (5)	81 (2)	81 (4)	82 (4)†
RPE (CR100)						
Placebo	2 (2)	24 (13)	49 (15)	76 (15)	96 (10)	102 (8)†
Nitrate	2 (2)	21 (11)	49 (15)	73 (15)	94 (13)	102 (11)†

Table 3.2: Heart rate, S_pO_2, and RPE during the time to exhaustion (TTE) tests. Data are presented as mean (SD). Dietary nitrate supplementation had no effect on heart rate, S_pO_2, or RPE at any isotime, or at exhaustion. S_pO_2, Oxygen saturation; RPE, Rating of perceived exertion. †Main effect for time ($p < 0.05$). N = 15.

during exercise but this did not translate into reduced AMS symptoms or an improvement in exercise performance. In contrast, in AMS+, nitrate increased ventilation and oxygen cost of exercise, headache and AMS severity, and sense of effort during submaximal exercise in 6 h hypoxia.

The increase in headache and AMS that occurred in the latter part of the trial was exacerbated by nitrate. It is clear this was not due to nitrate increasing exercise-induced headache, since there was no effect of nitrate on headache post *exercise bout 1* or *2* that occurred earlier in the hypoxic exposure, before AMS was present. In those that developed AMS, dietary nitrate induced a 26 mm increase in headache, which is of sufficient magnitude to have clinical relevance (Kelly, 2001). This finding is in agreement with a previous study that reported greater headache at altitude after L-arginine supplementation (Mansoor et al., 2005). However it contrasts the null finding reported by Hennis *et al.* in the field (2016); the only previous study to investigate the effects of chronic dietary nitrate supplementation on AMS. This difference in results is most likely due to differences in study design. Specifically, in the study by Hennis *et al.* (2016), biochemical confirmation of the nitrate supplementation was not performed and therefore it was not possible to determine if the nitrate supplementation was effective. This is of particular concern since the authors reported poor compliance. Further, as AMS is determined by subjective responses, the use of a non-taste matched placebo means placebo or nocebo effects cannot be

dismissed. In contrast, we completed a double-blind placebo-controlled study, and report 100% compliance to the supplementation protocol, with confirmation of successful blinding and increased plasma NOx in the nitrate trial.

When activated in the brain, NO directly stimulates the trigeminovascular system, responsible for headache pain sensation (Ashina et al., 1999). In addition, an increase in cerebral blood flow and resultant increase in intracranial pressure can also result in trigeminovascular system activation, and is suggested to cause high-altitude headache and AMS (Lawley et al., 2016). Therefore increasing the bioavailability of NO through dietary nitrate supplementation is likely to directly stimulate trigeminovascular afferents, and concurrently elevate hypoxia-induced cerebral vasodilation. This provides a possible mechanism for the increase in high-altitude headache and AMS observed herein. Further support for this explanation is provided by previous studies that have utilized NO-synthase inhibitors (e.g. L-NMMA; Ashina et al., 1999) and artificial vasoconstriction (King and Robinson, 1972) to successfully reverse headache.

Nitrate supplementation did not benefit exercise performance as assessed by time to exhaustion in acute hypoxia. This finding is in agreement with the only previous randomized controlled trial (RCT) to assess exercise performance in acute hypoxia (< 2 h) following six days dietary nitrate supplementation (Bourdillon et al., 2015). Bourdillon *et al.* (2015) found dietary nitrate supplementation did not alter hypoxic pulmonary vasoconstriction or 15 km time trial performance in acute hypoxia (F_IO_2 = 0.11). A further novel approach of the current study was to examine exercise responses during 6 h hypoxic exposure after dietary nitrate supplementation. Contrary to our hypothesis, nitrate did not improve submaximal exercise performance even in AMS- where nitrate improved S_pO_2 during exercise (3% after 5 h in hypoxia). Further, in those that developed AMS+, nitrate actually impaired exercise by increasing sense of effort; participants had to invest more effort to achieve the same exercise output in the nitrate trial, compared to placebo. In those that developed AMS (AMS+), nitrate also increased the oxygen cost of fixed workload exercise, which may have been driven by an increase in ventilation. Increased ventilation is associated with greater respiratory muscle demand and dyspnea (Amann et al.,

2007a), which are important contributing factors of sense of effort (Bernhadt et al., 2013). As exercising sense of effort, ventilation, and oxygen cost of exercise were increased and present before AMS, nitrate should be considered the cause of the negative effects observed in AMS+, rather than an effect of AMS symptoms. In addition, the elevated AMS symptoms may have contributed to the increase in sense of effort in the final bout of exercise, as the increase in sense of effort was proportional to headache severity immediately before exercise. By the end of the final exercise bout, sense of effort was increased by 54% in those who experienced AMS, equivalent to an entire verbal descriptor (from "moderate", to "somewhat strong"). Since the exercise typically completed at altitude is often long-duration and submaximal, this finding has great importance for those travelling to altitude for work and recreation as increased sense of effort is associated with poorer mood, and increased fatigue (Rossetti et al., 2017a), which is an important risk factor at altitude (Firth et al., 2008).

3.6.1 Limitations

This study is limited by the absence of a direct measure of cerebral blood flow to support the proposed mechanistic interpretation. However, the conclusion of cause and effect is supported by the use of a strong experimental design, and a theoretical explanation backed by a wealth of existing literature (Lawley et al., 2016). This study was also limited by the duration of exposure (6 h), and is therefore unable to conclude the effects of nitrate on physiological and perceptual responses with more chronic exposure to altitude, for example over many days or weeks. Another limitation of this study is that all participants were men, and thus the findings may not be applicable to women. In addition, the study design did not allow separation of the effects of nitrate in hypoxia *per se* from any interaction with exercise, although the effects of nitrate supplementation with exercise in normoxia have been studied in detail in previous literature (McMahon et al., 2017).

3.7 Conclusion

In conclusion, dietary nitrate increases AMS symptom severity, specifically headache, and sense of effort during submaximal exercise, particularly in those who experience AMS. Therefore, dietary nitrate is not recommended as an AMS prophylactic or ergogenic aid in nonacclimatised individuals at altitude.

3.8 Author contributions

For this research study, G.M.K.Rossetti contributed to conception and design, pilot testing, data collection, data analysis (excluding NO_x chemiluminescence), interpretation of results, preparation of figures, manuscript drafting, and manuscript revisions. A full list of authorship contributions is provided in Appendix C (Table C.2).

Chapter 4

Hypoxia modifies neurovascular coupling in the default mode network (DMN)

4.1 Abstract

Hypoxia is known to increase global resting cerebral blood flow (CBF). However, recent findings have indicated that CBF does not change uniformly across the cerebral cortex during hypoxia. Here we characterised the heterogenous regional neurovascular responses to hypoxia by determining hypoxia-induced changes in resting CBF and task-evoked blood oxygen level-dependent (BOLD) responses. In hypoxia, we observed significant reductions in resting CBF in the posterior cingulate cortex (PCC) and both left and right angular gyrus (AG) regions of the default mode network (DMN). This occurred concurrent with increases in CBF across most of the rest of the brain including regions of the visual attentional network (VAN). We then investigated task-evoked BOLD responses and behavioural performance during a paired associate memory task and a motion detection task under normoxic and hypoxic conditions. Hypoxia did not appreciably affect performance in either task, suggesting neural activity was not altered by hypoxia. As expected, during memory search in normoxia, regions in the DMN showed positive BOLD responses, while regions in the VAN showed negative responses. During visual search in normoxia, the opposite pattern of BOLD responses was observed in these same networks (negative BOLD in DMN, positive in VAN). However, in hypoxia the tasks evoked the opposite pattern of

BOLD responses in the DMN. In hypoxia, negative BOLD responses were evoked in the PCC and AG during memory search, while a positive BOLD response was seen in these areas during visual search. In conclusion, hypoxia induces region-dependent alterations in neurovascular coupling, and appears to induce an inversion of the hemodynamic response to DMN neural activity in healthy adults.

4.2 Significance

Oxygen and glucose delivery are critical to maintaining brain function, and decreased oxygen availability (hypoxia) induces a compensatory rise in cerebral blood flow (CBF). However, hypoxia-induced CBF changes are not uniform across the brain. This study provides novel evidence that regions of the default mode network (DMN) show hypoxia-induced hypoperfusion in concert with altered neurovascular coupling. The DMN may be more sensitive to factors that affect CBF responses, such as arterial oxygen and carbon dioxide content, and energetic load elsewhere in the brain. A finding of particular relevance since the DMN is central to neurodegeneration in Alzheimer's Disease. This surprising finding may reflect either uncoupling or a reversal of the neurovascular response, akin to that observed during neonatal development.

4.3 Introduction

Oxygen and glucose delivery are critical to maintaining brain function. In hypoxia, global cerebral blood flow (CBF) increases as arterial blood oxygen content diminishes (Ainslie et al., 2016), but the hypoxia-induced changes in CBF are not uniform across the brain (Binks et al., 2008). For example subcortical nuclei show large increases, while some cortical areas show decreases (Binks et al., 2008; Noth et al., 2008). In a recent study with limited brain coverage, we observed increased resting CBF following 2 and 10 h exposure to hypoxia in most cortical regions except the posterior cingulate cortex (PCC;

Lawley et al., 2017), where reductions were observed. The anatomical specificity of the affected region is particularly important since the PCC is responsible for memory function (Raichle, 2015), and is the origin of functional abnormalities in the progression of neurodegenerative diseases such as Alzheimer's (Girouard and Iadecola, 2006; Love and Miners, 2016).

However, the previous study could not establish the cause of the observed reductions in PCC regional CBF (rCBF). The reductions could reflect either diminished local neural activity, or a disruption in the coupling of the vascular response to neural activity (Barreto et al., 2017; Vingerhoets and Stroobant, 1999). The first hypothesis has some plausibility since the reduction in CBF was observed in the PCC, a region belonging to the default mode network (DMN), where decreases in neural and metabolic activity accompany a number of goal-directed processes (Raichle, 2015; Raichle et al., 2001). The alternative hypothesis is that these regions undergo changes in the coupling between local neural activity and the local vascular response during hypoxia. For example, hypoxia-induced disruptions in neurovascular coupling have been documented in the sensorimotor cortex of isoflurane-anaesthetized rats during forepaw stimulation (Sicard and Duong, 2005; Sumiyoshi et al., 2012). In contrast, a positron emission tomography (PET) study in humans found no appreciable effect of hypoxia on CBF changes in the visual cortex evoked by visual stimulation (Mintun et al., 2001), suggesting hypoxia does not affect neurovascular coupling in the human visual cortex. These findings were confirmed by a functional Magnetic Resonance Imaging (fMRI) study by Tuunanen *et al.* (2006) who also found no differences in the amplitude of CBF and blood oxygen level-dependent (BOLD) responses to visual stimulation during hypoxia. This evidence supports the idea that neurovascular coupling is not governed by a requirement to match oxygen delivery to neural activity-evoked increases in oxygen consumption ($CMRO_2$).

There is currently very limited evidence concerning the effect of hypoxia on task-evoked vascular responses outside visual cortex. This is an important limitation in the literature given the regional heterogeneity in CBF responses to hypoxia. Studies using transcranial Doppler ultrasonography to assess posterior

and anterior cerebral artery blood velocity have reported no effect of acute or chronic hypoxia on task-evoked blood velocity changes during visuo-spatial attention, verbal fluency, or central executive tasks (Caldwell et al., 2018; Caldwell et al., 2017). However, these studies had very limited anatomical specificity, since they measured blood flow in large intracranial arteries.

In this study we aimed to settle two issues. First, to characterise the anatomical distribution of rCBF changes during hypoxia across the entire cortical surface, we measured the effect of hypoxia on rCBF across the whole brain. The second aim was particularly relevant to an outstanding theoretical issue, namely whether regional heterogeneities in hypoxia-induced rCBF changes primarily reflect changes in neural activity or neurovascular coupling. We aimed to determine the relationship between hypoxia effects on resting rCBF and task-evoked BOLD responses, by assessing the effect of hypoxia on task-evoked BOLD responses during two tasks: 1) a paired associate memory task, and 2) a motion detection task. These tasks were expected to evoke opposite BOLD responses in regions of the DMN and visual attentional network (VAN). Memory search was expected to evoke positive BOLD responses in the DMN, and visual search during the motion detection task was expected to evoke negative BOLD responses in the DMN. In opposition, memory search was expected to evoke negative BOLD responses in the VAN, and visual search during the motion detection task was expected to evoke positive BOLD responses in the VAN (Sestieri et al., 2010). We expected that if hypoxia specifically reduced neural activity in DMN regions, this would lead to diminished performance and smaller BOLD responses in the paired associate memory task. However, if neurovascular coupling is affected by hypoxia, then performance would not necessarily be affected and BOLD response in the DMN would be diminished, or even reversed, during task performance.

4.4 Methods

4.4.1 Participants

Twenty-four healthy adults (17 males) were recruited into the study (mean (SD); age, 23(2) years; height, 177 (9) cm; body mass, 75.4 (13.3) kg; education, 18 (2) years). Participants had not travelled to altitude ($\geq$ 1500 m) in the preceding six months and had no medical contraindications (anaemia, cardiovascular disease, hypertension, uncontrolled metabolic disease, claustrophobia, cardiac pacemakers or any other metal implants). Female participants were studied during the early follicular phase of their menstrual cycle, or the placebo phase of oral contraceptives. All participants provided written informed consent. Ethical approval was granted by the Ethics Committee of the School of Sport, Health, and Exercise Sciences at Bangor University.

4.4.2 Study design

To determine the effect of hypoxia on rCBF at rest and during cognitive tasks, the study followed a double-blind repeated-measures counterbalanced cross-over design. Participants were blinded to experimental condition throughout, and researchers were blinded at point of data processing and analysis. Participants completed an encoding and familiarisation session for the cognitive tasks the day before each experimental trial. Experimental trials consisted of 3 h 30 min exposure to normoxia (fraction of inspired oxygen; $F_IO_2 = 0.209$) or hypoxia ($F_IO_2 = 0.120$) with an arterial spin labelling (ASL) scan to measure resting rCBF and fMRI to measure task-evoked activations during a paired associate memory task (to activate the DMN and deactivate the VAN) and a motion detection task (to deactivate the DMN and activate the VAN). Experimental trials were separated by 10 (7) days.

4.4.3 Experimental protocol

A schematic representation of the experimental trial procedures is depicted in Figure 4.1. Participants completed the first 2 h of each exposure in a temperature and humidity-controlled environmental chamber (Hypoxico Inc; NY), and the final 1 h 30 min of each exposure in a Magnetic Resonance Imaging (MRI) scanner. Throughout transportation to the MRI suite and the MRI scanning procedure, participants wore a leak-free face mask connected to a two-way Hans Rudolph valve with an inspiratory port connected via Falconia tubing to a 1000 L Douglas bag (containing $F_IO_2 = 0.209$ or $F_IO_2 = 0.120$, dependent on trial condition).

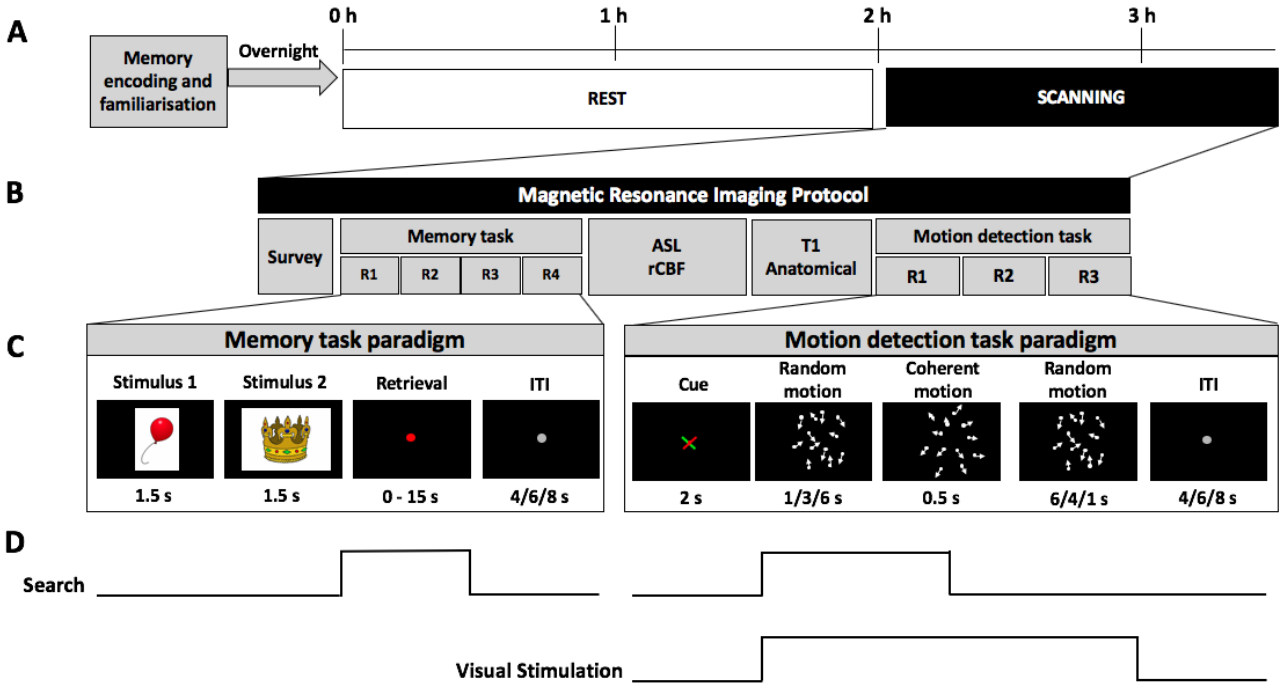


Figure 4.1: Schematic representation of experimental trial procedures. Experimental procedures are presented as (A) an overview of the experimental trial, (B) in reference to magnetic resonance imaging (MRI) scans, (C) as an overview of the cognitive task paradigms, and (D) with representation of the regressors of interest modelled in the general linear model (GLM). Participants were counterbalanced according to environmental condition (normoxia/hypoxia) and cognitive task type (memory/motion detection). In each experimental trial, participants completed 4 runs of 25 memory task trials (R1-R4), and 3 runs of 38 motion detection task trials (R1-R3), separated by an ASL scan to assess resting regional cerebral blood flow, and a T-1 weighted high-resolution anatomical image scan. ASL, arterial spin labelling; ITI, inter-trial intervals; rCBF, regional cerebral blood flow.

4.4.4 Physiological monitoring

Expired carbon dioxide (CO_2) was sampled from the face mask for 5 min, during the resting ASL scan within the MRI scanner, and analysed by a calibrated fast responding gas analyser (GC-0017 (0-20%) SprintIR CO_2 Sensor; GSS, Cumbernauld, UK), and recorded using CO_2 logging software (GasLab; CO2Meter, Inc.; Florida, USA). End-tidal CO_2 ($P_{ET}CO_2$) was calculated from recorded CO_2 trace using peak detection software (Borchers, 2018). Heart rate and oxygen saturation (S_pO_2) were measured at 30 min intervals for the first 2 h. Heart rate was measured using a 3-lead electrocardiogram (ECG) connected to a high-resolution ultrasound machine (Acuson X300, Siemens Healthcare GmbH; Erlangen: Germany), and S_pO_2 was measured using pulse oximetry (9550 OnyxII; Nonin Medical Inc, Minnesota).

4.4.5 Cognitive tasks

All cognitive task paradigms were written in Octave, using the PsychToolbox 3 (Brainard, 1997; Pelli, 1997).

Encoding and familiarisation

Memory task. Memory was assessed by a paired associates memory task. The day before each experimental trial, participants completed an encoding session, where they were presented with two lists of images each containing 50 associate pairs of items taken from the Rossion and Pourtois (2004) pictorial set, and were asked to commit the pairs to memory. Each list consisted of 25 semantically-related pairs, and 25 semantically-unrelated pairs. Images were presented for 1.5 s each, separated by 0.5 s. To ensure task engagement, participants were asked to provide a rating of the relatedness of the items in each pair on a four-item scale, ranging from "extremely unrelated" to "extremely related". Items were repeated across the two lists within each session, but were not repeated

across the two encoding sessions (one for each experimental trial). To enhance learning, participants completed a two-alternative forced choice (2AFC) recall immediately after studying each list.

Motion detection task. During the encoding session, participants were also familiarised to the motion detection task. The number of familiarisation trials was adjusted as necessary to ensure all participants achieved a minimum 80% accuracy by the end of the session.

Experimental trial tasks

The day after encoding, participants were exposed to either normoxia (F_IO_2 = 0.209) or poikilocapnic hypoxia (F_IO_2 = 0.120).

Memory task. The memory task paradigm was used to determine the effect of hypoxia on memory performance and memory search-evoked BOLD responses. For the paired associate memory task, participants were presented with a cue image followed by a second image that was either the associate pair of the cue image (target) or an image that was not the associate pair of the cue image (foil). The participant was asked to identify whether the second image was the correct associate pair for the cue image. Memory search was assumed to take place in the interval between the presentation of the first image and the participant's response. Participants were encouraged to prioritise accuracy over speed when engaging in memory recall and provide a yes/no judgement with an additional confidence rating (high/low confidence). In each experimental trial, participants completed a total of 100 memory task trials, split into 4 runs of 25 trials each, with jittered inter-trial intervals (ITIs) that were either 4, 6 or 8 s.

Motion detection task. The motion detection task paradigm was used to determine the effect of hypoxia on visual search performance, visual search-evoked BOLD responses, and visual stimulation-evoked BOLD responses. In the motion detection task, each trial began with the presentation of a cue indicating two of the four quadrants to direct attention, followed by motion

stimuli consisting of four random dot kinematograms (RDK) lasting 8 s for each trial. In each RDK, seventy-two 4 pt white dots were presented on a black background, randomly positioned within a 220 pt circular aperture. Dynamic noise was produced by randomly replotting the dots every 12 frames. In 27% of trials, one of the circular patches contained a brief (500 ms) interval of coherent eccentric motion, occurring either early (0.77 s after stimulus onset), middle (3.77 s), or late (6.77 s) in the trial. Coherent motion was produced by translating 100% of the dots each frame (speed = 0.012 aperture/frame). Participants maintained fixation on a central cross hair throughout the trial and pressed a response button using their left thumb when they detected coherent motion (target). Visual search was assumed to take place in the interval between stimulus onset and the participant's response. Visual stimulation was assumed to occur for the duration of the stimulus presentation. In each experimental trial, participants completed a total of 114 motion detection task trials, split into 3 runs of 38 trials each, with jittered ITIs that were either 4, 6 or 8 s.

4.4.6 Imaging methods

All MRI sequences were conducted on a 3 Tesla MRI scanner (Achieva; Philips Medical) using a 16-channel head and neck coil. All imaging sequences were acquired with sensitivity encoding for fast magnetic resonance imaging.

Anatomical images. To provide anatomical brain images for registration of CBF and fMRI BOLD scans, T1-weighted anatomical image scans were acquired in the middle of the cognitive test battery in normoxia and hypoxia trials. High resolution T1-weighted images were acquired as 5 echo MP-RAGE sequences (TE = 3.5, 5.1, 6.8, 8.5, 10.2 ms; TR = 12 ms, TI = 1150 ms; 3D acquisition; field-of-view = 240 × 22 × 130 mm; voxel dimensions = 0.7 × 0.7 × 0.7 mm).

Regional cerebral blood flow (rCBF) at rest. To measure resting rCBF, ASL images were acquired in the middle of the cognitive test battery in normoxia and hypoxia trials. The ASL scans were completed while participants were looking at a white fixation cross presented on a black background. Single-phase ASL

images were acquired using the ASL package provided by Philips Medical. This is based on an echo planar acquisition using signal targeting with alternating radiofrequency (EPISTAR) ASL labelling. Labelling of inflowing blood was achieved through a parallel slab applied 20 mm below the acquisition slices (slab thickness 100 mm, delay 1600 ms). Specifically, each scan consisted of 12 slices at 2 × 2 mm in-plane resolution with 256 × 256 mm field-of-view and 6 mm slice thickness aligned to the AC-PC axis. Slices were acquired as 40 tagged and control pairs with TR of 3 s, and TE of 15 ms, giving a scan time of approximately 4 mins.

Task-evoked fMRI activations. To measure the task-evoked fMRI activations and deactivations, BOLD contrast functional images were acquired using a gradient-echo echo-planar sequence (TR = 2000ms; TE = 30 ms; flip angle = 90°; 3 mm axial slices; 3 × 3 mm in-plane resolution). BOLD images were motion-corrected within and between runs, corrected for across-slice timing differences, resampled into 2 mm isotropic voxels, and warped into a standardized atlas space (Montreal Neurological Institute; MNI; Mazziotta et al., 2001).

4.4.7 fMRI statistical analysis

General linear model (GLM)

A GLM that used an assumed hemodynamic response function provided separate voxel-wise maps of the BOLD activity associated with each cognitive process within the two tasks. Cognitive processes included in the memory task GLM were: memory search (hit), memory search (miss), memory search (correct rejection), memory search (false alarm), stimulus 1 presentation, stimulus 2 presentation, and button press. Cognitive processes included in the motion detection task GLM were: visual search (hit), visual search (miss), visual search (correct rejection), visual search (false alarm), visual stimulation, target presentation (hit), target presentation (miss), and cue presentation.

Memory search-evoked activations were determined during the memory task by modelling regressors for activations occurring between stimulus presentation and the response on hit trials. Visual search activations were determined during the motion detection task by modelling regressors for activations from stimulus onset to target detection on hit trials. Visual stimulation-evoked activations were determined during the motion detection task with a regressor associated with those periods when participants were watching the dots move throughout the entire visual stimulus, rather than isolated until the detection response. In this manner, memory search, visual search, and visual stimulation were modelled against the other task processes, which were included as regressors of no interest.

First-level analyses of individual runs were modelled using the GLM using the FEAT function in the FMRIB software library (FSL). We then conducted a second-level analysis using FSL's fixed effects (FE) model with one contrast per participant to analyse the within-subject repeated measure. Finally, we conducted third-level analyses on the individual participant contrast of parameter estimates (COPEs) using FMRIB's Local Analysis of Mixed Effects (FLAME) model to determine group-level activations and deactivations during normoxia and hypoxia conditions separately, and the differences between the conditions.

Region of interest (ROI) analyses

Peak activations from normoxia maps of the memory and motion detection tasks were used to identify seed coordinates for key regions of the DMN and VAN respectively (Table 4.1). The MNI coordinates from these key regions were inputted to the NeuroSynth database (*www.neurosynth.com*) which provides automated meta-analysis of activation maps from 10,000s of previous studies (Buckner et al., 2011; Choi et al., 2012; Yeo et al., 2011). The NeuroSynth database was used to create a functional connectivity map for each seed coordinate where each voxel represented the probability of functional coactivation with the seed coordinate, calculated by meta-analysis of previous

studies. Region of interest (ROI) masks were created by implementing a threshold of 65% on the functional connectivity maps, thereby including only voxels that have a minimum 65% chance of functional coactivation with the seed region.

Region	MNI Coordinates		
	x	y	z
Default Mode Network (DMN)			
Posterior cingulate cortex (PCC)	-8	-42	+36
Left angular gyrus (Left AG)	-40	-70	+37
Right angular gyrus (Right AG)	+51	-61	+33
Medial prefrontal cortex (mPFC)	-3	+61	+8
Visual Attentional Network (VAN)			
Left middle temporal area (Left MT)	-43	-68	+8
Right middle temporal area (Right MT)	+45	-64	+6
Left frontal eye field (Left FEF)	-29	-5	+48
Right frontal eye field (Right FEF)	+33	-6	+52
Left intraparietal sulcus (Left IPS)	-29	-50	+50
Right intraparietal sulcus (Right IPS)	+28	-50	+52

Table 4.1: Region of interest (ROI) seed coordinates. Coordinates provided are in MNI space. Seed coordinates were inputted to the NeuroSynth meta-analysis database to produce functional activation maps. These functional activation maps were used to create thresholded (65%) ROI masks.

4.5 Results

4.5.1 Manipulation check: hypoxia

Participants showed the expected pattern of physiological responses to the F_IO_2 manipulation. Normobaric hypoxia decreased S_pO_2 compared to normoxia by 18% (95% CI: [-20, -16]; $p < 0.001$), increased heart rate by 13 bpm (95% CI: [7, 18]; $p < 0.001$), and decreased $P_{ET}CO_2$ by 4 mmHg (95%CI [-5, -2]; $p < 0.001$).

4.5.2 Regional cerebral blood flow (rCBF) at rest

ASL-based measures of resting rCBF confirmed that after 3 h of normobaric hypoxia rCBF *increased* in most cortical regions. Consistent with our previous observations (Lawley et al., 2017), hypoxia *decreased* rCBF in the precuneus (PCUN), the angular gyrus (AG), and the PCC (Figure 4.2, blue/light blue; cluster-based corrections: $p < 0.01$), suggesting that posterior nodes of the DMN deviated from changes in rCBF observed in the rest of the cortical mantle. Interestingly the medial prefrontal cortex (mPFC) of the DMN, did not show this decrease in rCBF.

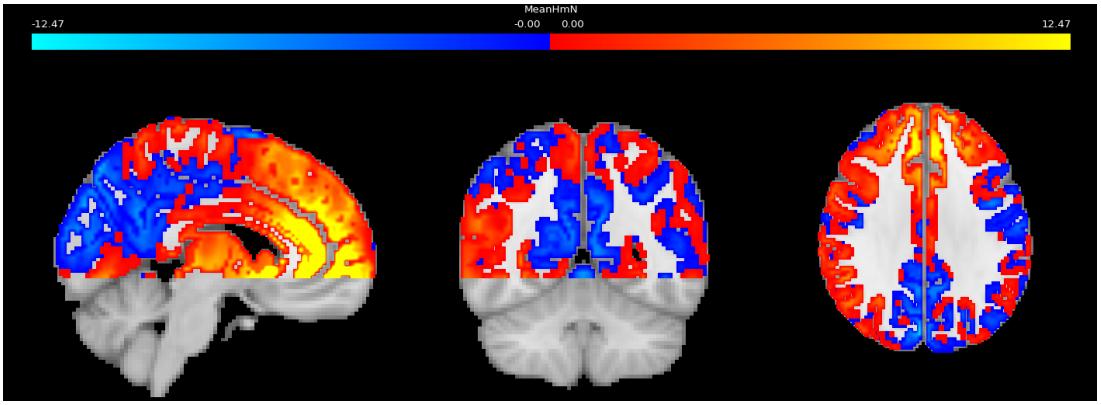


Figure 4.2: Significant clusters of increases (yellow/red) and decreases (blue/light blue) in regional cerebral blood flow (rCBF) at rest in hypoxia, compared to normoxia. rCBF differences between normoxia and hypoxia were determined using the RANDOMISE tool in FSL, setting cluster-based FEW corrections at $p < 0.01$.

To determine whether rCBF reductions could reflect idiosyncratic differences in arterial blood gases, we examined the relationships between hypocapnia and hypoxaemia with local changes in rCBF in the PCC across participants. Individual differences in $P_{ET}CO_2$ did not correlate with the magnitude of rCBF reductions ($r = -0.24$; $p = 0.5$; Figure 4.3A). In contrast, individual differences in S_PO_2 were moderately correlated with with the magnitude of rCBF reductions ($r = 0.48$; $p = 0.07$; Figure 4.3B). Combined, this implicates hypoxaemia as the cause of the observed rCBF reductions, not hyperventilation-induced hypocapnia.

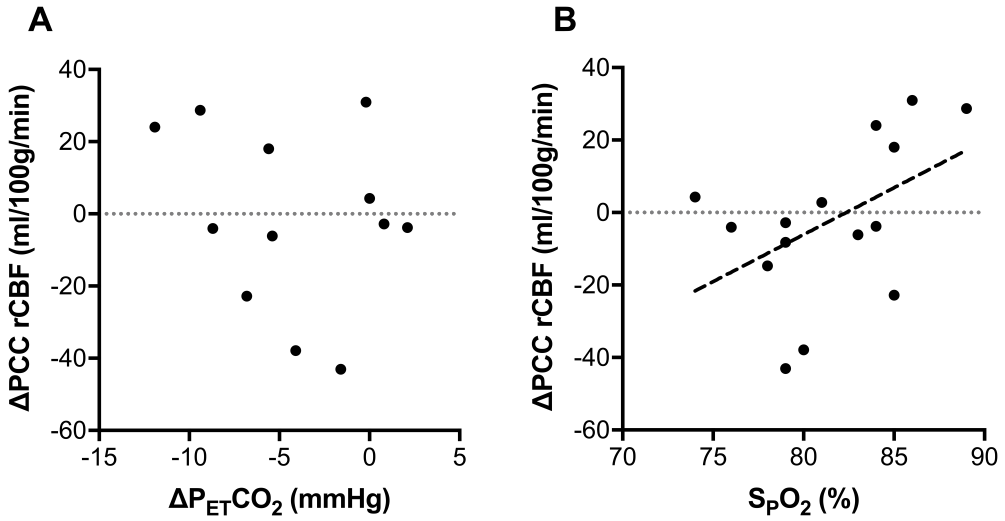


Figure 4.3: Relationships between (A) end-tidal carbon dioxide ($P_{ET}O_2$ and (B) oxygen saturations (S_pO_2) and regional cerebral blood flow (rCBF) decrements. (A) Hypoxia-induced change in $P_{ET}CO_2$ between normoxia was not related to hypoxia-induced change in rCBF to the posterior cingulate cortex (PCC; r = -0.24; p = 0.5), but (B) lower S_pO_2 in hypoxia was moderately related to exacerbated hypoxia-induced decrements in rCBF to the PCC (r = 0.48; p = 0.07).

4.5.3 Cognitive task performance

Our fMRI paradigms included two tasks performed sequentially and counterbalanced across participants. The two tasks consisted of a paired associate memory task to assess memory search performance, and a motion detection task to assess visual search performance. Hypoxia had no appreciable effect on performance in either task (Table 4.2), suggesting participants were able to produce equivalent neural activations in order to sustain task demands in hypoxia compared to normoxia.

4.5.4 Task-evoked fMRI activations

Search-evoked responses in normoxia

The BOLD responses during both memory and visual search match in both location and direction with those reported previously, and confirm that our experimental paradigms activate the expected brain networks in normoxia.

	Normoxia	Hypoxia	95% CI	p
Memory task				
Sensitivity (d')	1.23 (0.57)	1.20 (0.53)	-0.32, 0.09	0.3
Accuracy (%)	73.8 (9.3)	72.4 (9.0)	-4.7, 1.9	0.4
Reaction time (s)	6.4 (1.2)	6.3 (1.2)	-0.24, 0.13	0.6
Motion detection task				
Sensitivity (d')	2.35 (1.01)	2.28 (0.96)	-0.40, 0.27	0.7
Accuracy (%)	82.7 (13.1)	80.1 (14.6)	-6.0, 0.8	0.3
Reaction time (s)	0.747 (0.181)	0.783 (0.249)	-0.031, 0.103	0.1

Table 4.2: Cognitive task performance during normoxia (F_IO_2 = 0.209) and hypoxia (F_IO_2 = 0.120). Values represent group average means (SD). Sensitivity (d') was computed from the proportion of hits and false alarms and represents the extent to which task performance deviates from chance (as a z-score); a higher d' indicates better performance.

BOLD responses during memory search. Figure 4.4A shows the anatomical distribution of memory search BOLD responses in normoxia. Specifically, positive BOLD responses were observed in regions of the posterior DMN, including the PCC and left AG. Activations were also observed in the PCUN, lateral occipital complex (LOC), hippocampus (HIP) and inferior frontal sulcus (IFS). Deactivations were observed in the precentral gyrus (PreCG), post central gyrus (PoCG), supramarginal gyrus (SMG), anterior cingulate cortex (ACC) and Insula. Regions of memory search-evoked activations and deactivations were comparable to those reported in previous studies (Sestieri et al., 2011; Sestieri et al., 2010). In particular, positive BOLD responses were more prominent in the left than the right hemisphere, consistent with known lateralisation for memory search processes. Activations in the LOC have also been previously reported in paired associate memory recall, in keeping with recall-driven reactivation of material-specific sensory cortices (Wheeler and Buckner, 2004; Wheeler et al., 2000).

BOLD responses during visual search. Figure 4.4B shows the anatomical distribution of visual search BOLD responses in normoxia. Specifically, positive BOLD responses were observed in VAN, including the left and right middle temporal areas (MT), right frontal eye field (FEF), and left and right intraparietal sulci (IPS). Responses were also observed in the superior frontal gyrus (SFG), middle frontal gyrus (MFG), PreCG, and lateral occipital cortex (LOC). Visual search related negative BOLD responses were found in the DMN including the

PCC and left and right AG. Deactivations were also observed bilaterally in the SMG, parahippocampal gyrus (PHIP), PCUN, and cuneus (CUN). Visual search-evoked responses were comparable to those previously reported (D'Avossa et al., 2006; Shulman et al., 2003).

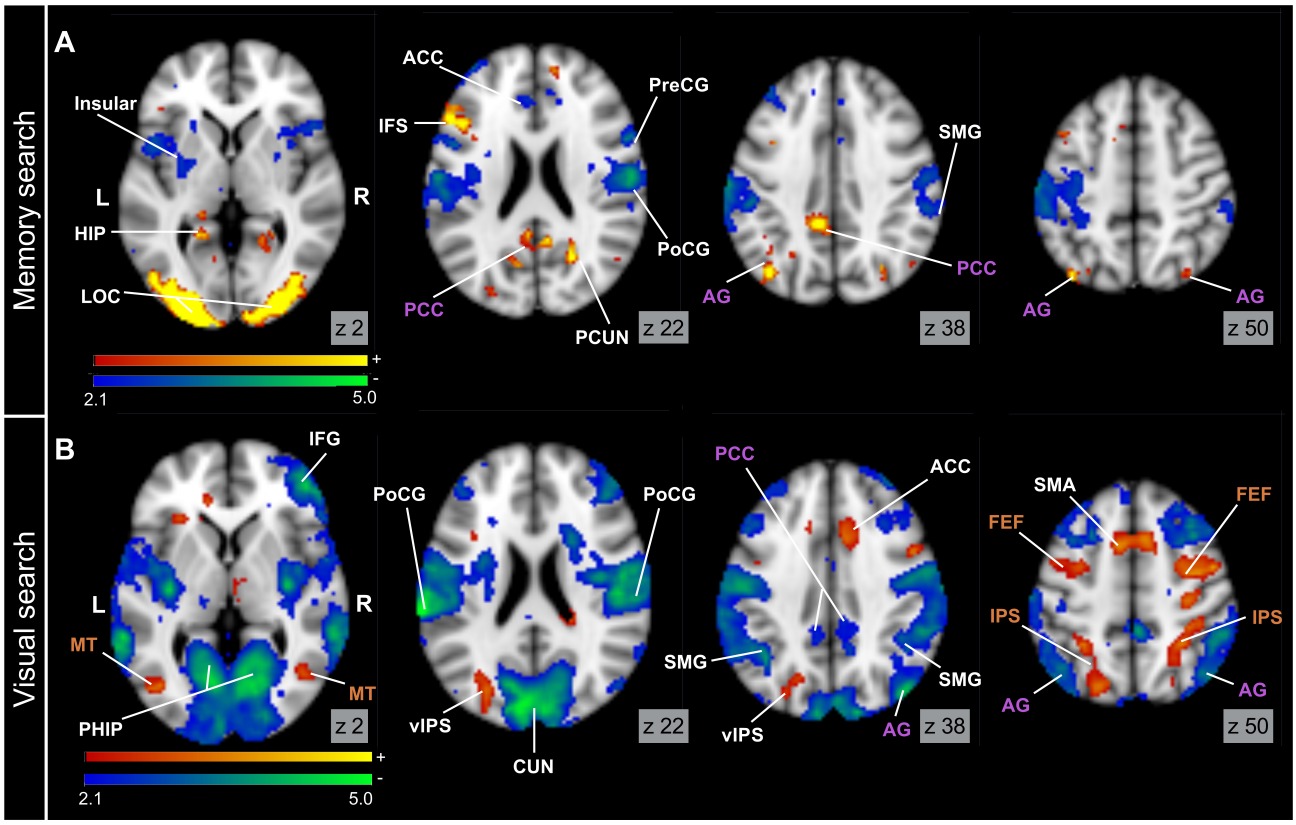


Figure 4.4: Cognitive task manipulation check. (A) Memory search-evoked activations and deactivations and (B) visual search-evoked activations and deactivations. (A) Memory search-evoked activations (warm colours) and memory search-evoked deactivations (cold colours). (B) Visual search-evoked activations (warm colours) and visual search deactivations (cold colours). Purple labels indicate regions of the default mode network (DMN), brown labels indicate regions of the visual attentional network (VAN). All activation maps $p < 0.05$. ACC, anterior cingulate cortex; AG, angular gyrus; CUN, cuneus; FEF, frontal eye field; HIP, hippocampus; LOC, lateral occipital cortex; IFG, inferior frontal gyrus; IFS, inferior frontal sulcus; IPS, intraparietal sulcus; MFG, middle frontal gyrus; MT, middle temporal area; PCC, posterior cingulate cortex; PCUN, precuneus; PHIP, parahippocampal gyrus; PoCG, postcentral gyrus; PreCG, precentral gyrus; LOC, lateral occipital cortex; SMA, supplementary motor area; SMG, supramarginal gyrus; vIPS, ventral intraparietal sulcus.

Effect of hypoxia on task-evoked fMRI activations

Hypoxia significantly altered the task-evoked BOLD response in a number of brain regions.

BOLD responses in the DMN. In contrast to normoxia, during hypoxia we observed negative BOLD responses during memory search in posterior regions of the DMN, and positive BOLD responses during visual search in the same regions. This produced the opposite pattern of BOLD responses to these two tasks compared to the pattern observed in normoxia, as shown in Figure 4.5A and Figure 4.5C. These results indicate that during hypoxia, memory search-evoked BOLD responses were significantly diminished in the PCC and mPFC compared to normoxia. ROI-based analyses (summarised in Figure 4.5B) further demonstrate a negative BOLD response during hypoxia in PCC (normoxia = 0.20 $\pm$ 0.09; hypoxia = -0.13 $\pm$ 0.13; p = 0.02) and right AG (normoxia = 0.11 $\pm$ 0.09; hypoxia = -0.17 $\pm$ 0.13; p = 0.04), with trends for decreased activations in the left AG (normoxia = 0.22 $\pm$ 0.09; hypoxia = 0.03 $\pm$ 0.11; p = 0.09) and mPFC (normoxia = 0.20 $\pm$ 0.20; hypoxia = 0.01 $\pm$ 0.17; p = 0.07) indicating that hypoxia induced a reversal of the usual direction of the BOLD response in these regions.

Figure 4.5C shows maps of the difference between visual search activations in normoxia and hypoxia. Significantly diminished deactivations during hypoxia were found in the PCC, and left and right AG. ROI analyses indicated significantly increased BOLD responses in the left AG (normoxia = -0.14 $\pm$ 0.07; hypoxia = 0.08 $\pm$ 0.07; p = 0.02) and right AG (normoxia = -0.19 $\pm$ 0.09; hypoxia = 0.07 $\pm$ 0.07; p = 0.01), confirming the amplitude of deactivations was decreased in hypoxia. A similar trend was found in the PCC (normoxia = -0.09 $\pm$ 0.07; hypoxia = 0.04 $\pm$ 0.06; p = 0.07). Interestingly, the only DMN region that did not show this trend was the mPFC, also the only region without hypoxia-induced decreases in rCBF at rest (assessed by ASL; Section 4.5.2).

BOLD responses in the visual attentional network (VAN). Hypoxia did not affect the amplitude of negative BOLD responses evoked during memory search in the VAN. However, hypoxia increased the amplitude of positive BOLD responses during visual search in the VAN (as shown in the regional analysis in Figure 4.6C).

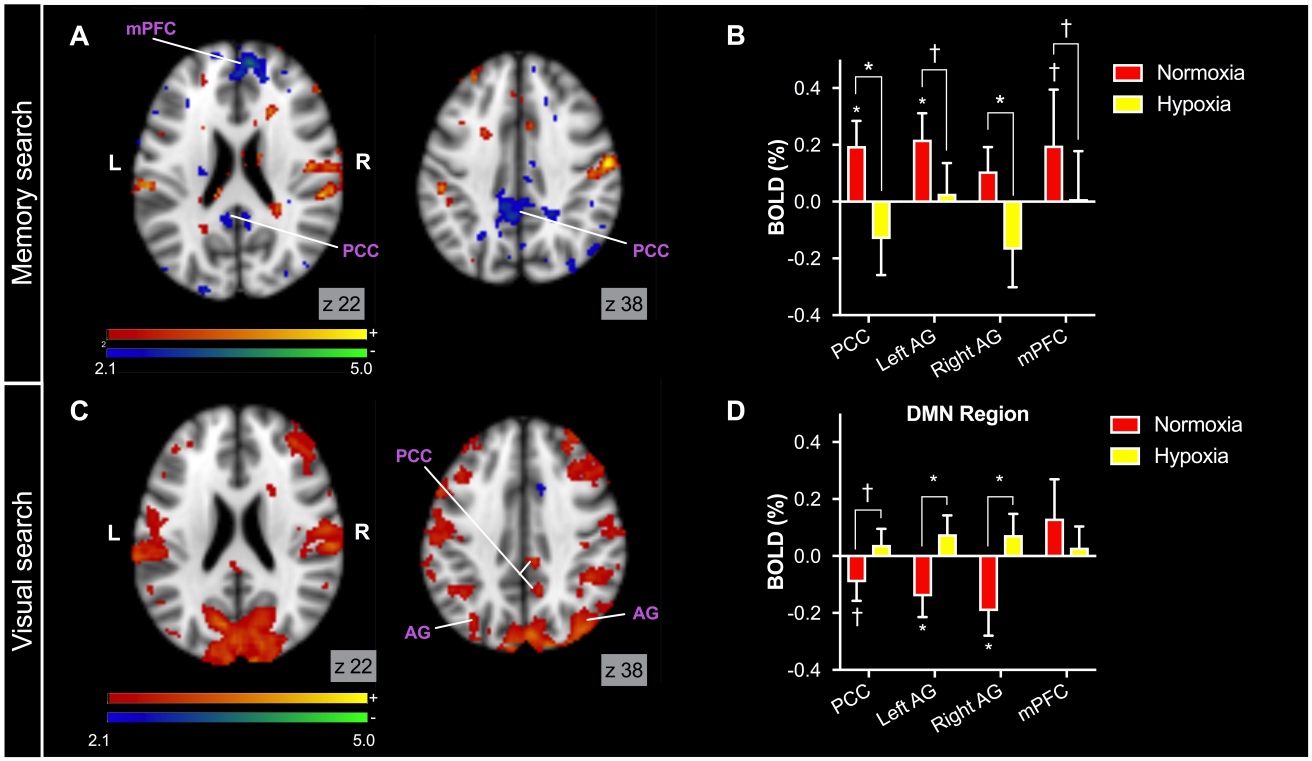


Figure 4.5: Effect of hypoxia on memory and visual search activations in the default mode network (DMN). (A) Contrast maps showing the difference between memory search-evoked activations in normoxia and hypoxia. Warm coloured areas indicate regions with significantly increased activations in hypoxia, cold coloured areas indicate regions with significantly decreased activations in hypoxia. (B) Memory search BOLD response in DMN regions of interest (ROIs). (C) Contrast maps showing the difference between visual search-evoked activations in normoxia and hypoxia. Warm coloured areas indicate regions with significantly increased activations in hypoxia, cold coloured areas indicate regions with significantly decreased activations in hypoxia. (D) Memory search BOLD response in DMN regions of interest (ROIs). AG, angular gyrus; mPFC, medial prefrontal cortex; PCC, posterior cingulate cortex. Error bars indicate SEM; † $p < 0.1$; * $p < 0.05$.

Figure 4.6A shows maps of the difference in memory search activations between normoxia and hypoxia. ROI-based analyses revealed no differences between normoxia and hypoxia during memory search in regions of the VAN (MT, FEF, or IPS; as shown in Figure 4.6B).

Figure 4.6C shows maps of the difference in BOLD response evoked during visual search between normoxia and hypoxia. Hypoxia increased activations in the MT and FEF regions measured by voxel-wise analysis. ROI-based analyses confirmed hypoxia tended to increase visual search activations in the left MT (normoxia = 0.19 ± 0.06; hypoxia = 0.32 ± 0.06; $p = 0.08$), right MT (normoxia = 0.22 ± 0.06; hypoxia = 0.33 ± 0.05; $p = 0.08$), and left IPS (normoxia = 0.13 ± 0.04;

hypoxia = 0.26 ± 0.07; p = 0.06) regions of the VAN. However, hypoxia had no effect on visual search activations in the left FEF (normoxia = 0.15 ± 0.05; hypoxia = 0.15 ± 0.03; p = 0.5), right FEF (normoxia = 0.15 ± 0.05; hypoxia = 0.19 ± 0.05; p = 0.3), or right IPS (normoxia = 0.19 ± 0.05; hypoxia = 0.24 ± 0.04; p = 0.3) when assessed by ROI analysis.

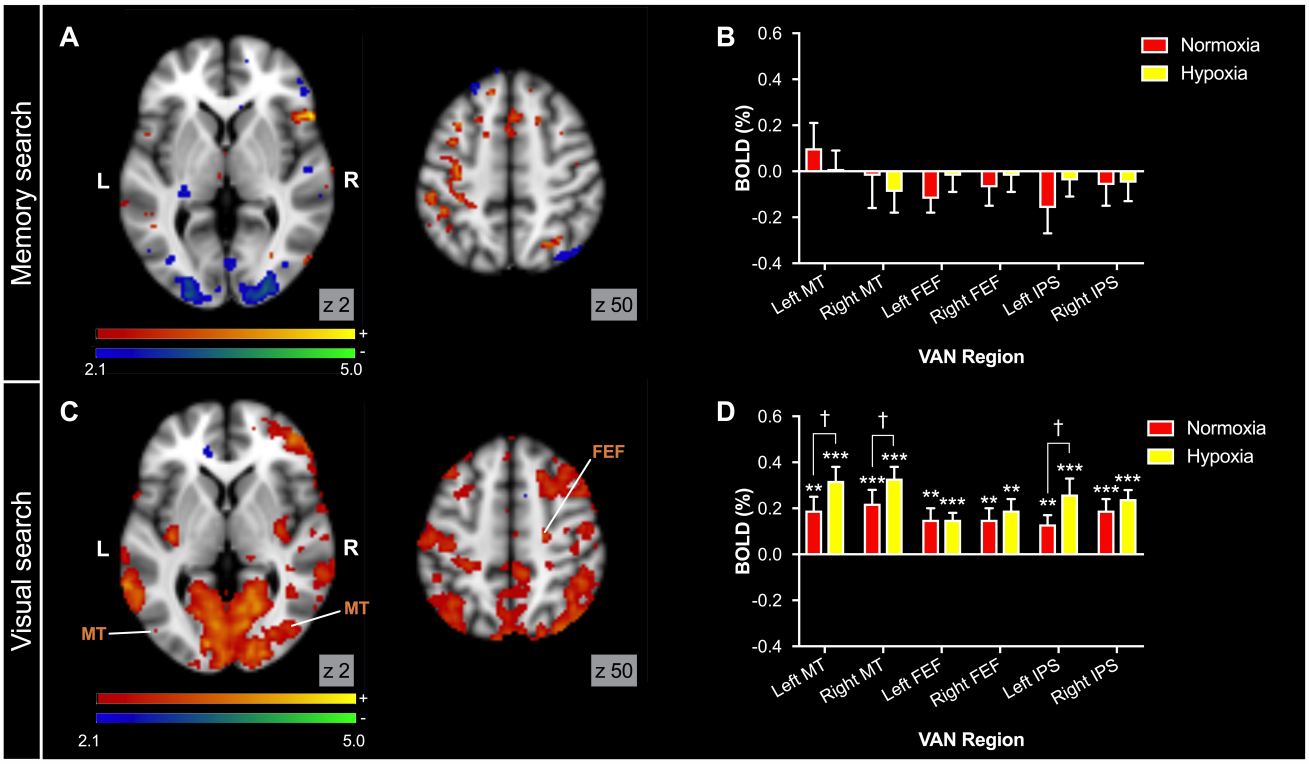


Figure 4.6: Effect of hypoxia on memory and visual search-evoked activations in the VAN. (A) Contrast maps showing the difference between memory search-evoked activations in normoxia and hypoxia. Warm coloured areas indicate regions with significantly increased activations in hypoxia, cold coloured areas indicate regions with significantly decreased activations in hypoxia. (B) Memory search BOLD response in VAN regions of interest (ROIs). (C) Contrast maps showing the difference between visual search-evoked activations in normoxia and hypoxia. Warm coloured areas indicate regions with significantly increased activations in hypoxia, cold coloured areas indicate regions with significantly decreased activations in hypoxia. (D) Memory search BOLD response in VAN regions of interest (ROIs). FEF, frontal eye field; IPS, intraparietal sulcus; MT, middle temporal area. Error bars indicate SEM; † $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

BOLD responses in the visual cortex. To determine the effects of hypoxia on neurovascular responses in the visual cortex, we examined the BOLD response evoked by the visual stimulus in the motion detection task. This component of the BOLD signal reflected activations present throughout the entire visual stimulus, rather than isolated until target detection. Previous findings indicated that visually-evoked CBF and BOLD signal changes are unaffected by hypoxia

(Mintun et al., 2001). Visual stimulation evoked activations in the visual cortex in both normoxia and hypoxia, with substantial overlap between the respective activation maps. Figure 4.7A and 4.7B show the extent of significant BOLD responses in the same axial slice during normoxia and hypoxia respectively. Figure 4.7C displays the overlap between the two maps. ROI-based analyses of the region of overlap confirmed hypoxia had no effect on the magnitude of the activation (normoxia = 0.63 $\pm$ 0.33 %; hypoxia = 0.55 $\pm$ 0.31 %; p = 0.4; Figure 4.7D).

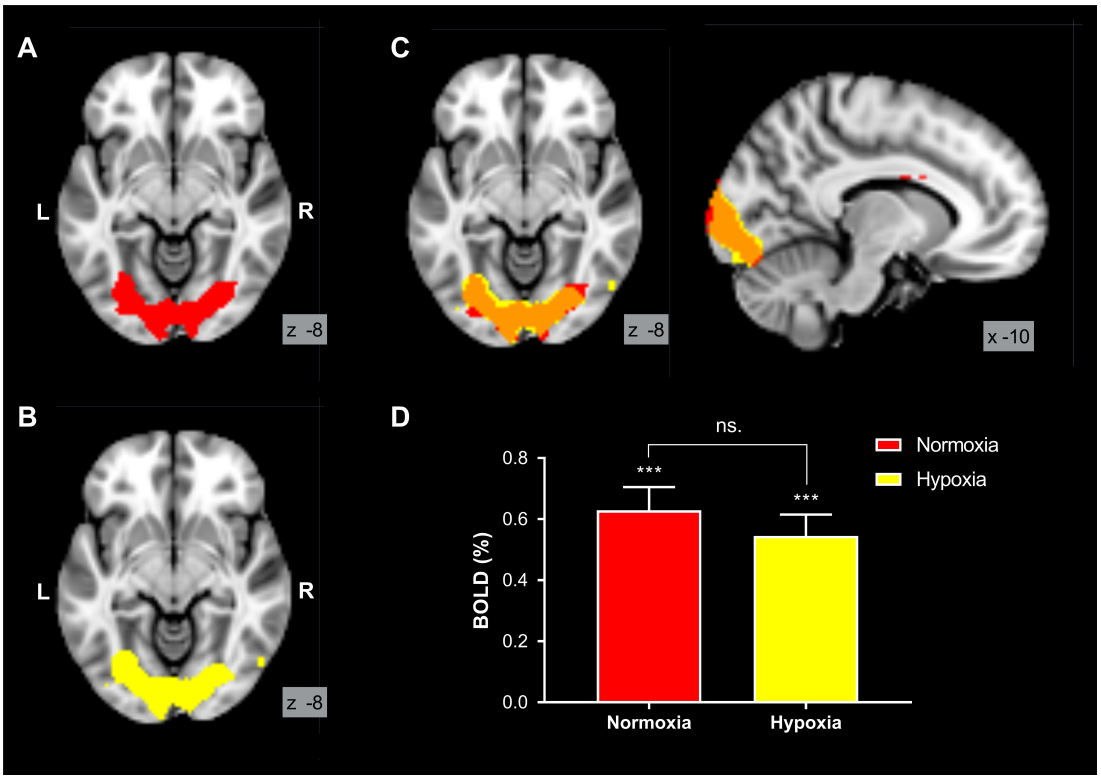


Figure 4.7: Visual stimulation-evoked activations. (A) Visual stimulation-evoked activations in the visual cortex during the motion detection task in normoxia (red), and (B) during the motion detection task in hypoxia (yellow). (C) Normoxia and hypoxia visual stimulation-evoked activation maps displayed substantial overlap (orange) when viewed together. (D) Region of interest (ROI) analyses for this area of the visual cortex revealed significant visual stimulation-evoked activations during visual stimulation in both normoxia and hypoxia (*** $p < 0.001$), with no difference between the conditions (ns.). Error bars indicate SEM; all activation maps $p < 0.05$.

4.6 Discussion

We examined the effects of hypoxia on resting rCBF and task-evoked BOLD responses. Hypoxia-induced changes in both resting rCBF and the task-evoked

BOLD response showed large regional heterogeneity, indicating that hypoxia does not produce the same effects across the cortical mantle. In fact, regions belonging to the DMN, particularly the PCC and AG, showed diminished rCBF at rest during hypoxia, confirming and extending a previous observation from our laboratory, which documented hypoxia-induced decreases in rCBF to PCC (Lawley et al., 2017).

The finding that DMN regions show decreased rCBF during hypoxia, in contrast to widespread increases in rCBF to the rest of the brain, could be interpreted in two different ways. One possibility is that it may reflect a decrease in neural activity in DMN regions during hypoxia, in keeping with the fact that these regions deactivate during processing of environmental stimuli (Raichle, 2015). The second possibility is that it may reflect changes in neurovascular coupling in the face of unchanged neural activity (Hayes and Huxtable, 2012; Nagaoka et al., 2006). In order to gain further insight on the factors that may contribute to the peculiar rCBF changes observed in the DMN at rest, we measured BOLD signals in normoxia and hypoxia during two tasks. The first was a paired associate memory task known to evoke positive BOLD responses in the DMN. The second was a motion detection task known to evoke negative BOLD responses in the DMN. The memory task also evoked negative BOLD responses, and the motion detection task positive BOLD responses, in regions outside the DMN (e.g. the VAN).

Interestingly, we did not observe any appreciable difference in either memory or visual search performance between normoxia and hypoxia, suggesting participants were able to produce equivalent neural activations in order to sustain task demands under these conditions. However, BOLD responses were affected by hypoxia. In particular, regions of the DMN and beyond that showed positive BOLD responses during memory search in normoxia, showed negative BOLD responses in hypoxia. The observation of negative BOLD responses in DMN regions during memory search, despite no impairment in task performance, is puzzling since DMN regions are understood to sustain memory recall (Sestieri et al., 2011; Sestieri et al., 2010), and were shown to be active in normoxia. Although a negative BOLD is commonly understood as reflecting decreases in

neural activity (compared to rest), this is not the only possible interpretation of the negative BOLD responses we observed in hypoxia (Hayes and Huxtable, 2012). Indeed, such an interpretation of the negative BOLD (decreased neural activity) in this case would imply a profound reorganisation of neural activity occurred during hypoxia to sustain memory recall.

Two alternative possible explanations of the observed negative BOLD response in the DMN can be provided. The first is that DMN regions simply undergo uncoupling of vascular responses from neural activity, leading to decreased rCBF and negative BOLD responses in hypoxia that reflect task-evoked increases in oxygen metabolism in the absence of a hyperaemic response. This would be comparable to observations of negative BOLD responses in the visual cortex with experimentally-induced hypotension (Nagaoka et al., 2006). However, aerobic metabolism is thought to contribute very modestly toward the metabolic costs of increased neural activity in the human brain (Fox et al., 1988). An alternative, if speculative, hypothesis stems from an observation that stimulus-evoked increases in neural activity lead to negative BOLD responses during early stages of brain development (Iadecola, 2017). Neonates have been shown to exhibit an inversion of the haemodynamic response (Yamada et al., 2000) caused by post-stimulus vasoconstriction of the pial arteries (Kozberg et al., 2013), which may be related to the fact that the neonatal brain environment is hypoxic relative to the adult brain environment.

What none of these hypotheses accounts for is the heterogeneity of the hypoxia-induced vascular responses in the human cortex. The observed inversion of the BOLD response was specific to the DMN. In fact, hypoxia produced modest increases in activations in the VAN during visual search, and had no effect on activations in the visual cortex during visual stimulation (replicating previous results; Mintun et al., 2001). This supports the notion that the DMN behaves in a different way from other cortical regions (Raichle, 2015). The DMN may be more sensitive to factors that affect CBF responses, such as arterial oxygen and carbon dioxide content, and energetic load elsewhere in the brain. Of particular relevance, these regions are also those most susceptible to neurovascular dysfunction in neurodegenerative diseases (Iadecola, 2004).

Indeed, neurovascular dysfunction of the PCC and PCUN regions precedes cognitive impairment in the progression of dementia (Benzinger et al., 2013; Langbaum et al., 2010).

4.6.1 Limitations

This study is limited by its use of the BOLD response to infer neural activity. The BOLD fMRI signal is dependent on the haemodynamic response, and does not directly measure neural activity (Logothetis, 2002; Logothetis and Wandell, 2004). Therefore, interpretation of the observed responses relies on inference based on assumed relationships. Although definitive interpretation of the precise physiological cause of the differences observed is not possible, this study still provides novel evidence of region-dependent differences in neurovascular responses in hypoxia.

4.7 Conclusion

In conclusion, hypoxia alters rCBF and task-evoked BOLD responses across the human cortex, in a region-dependent manner. Hypoxia-induced changes in resting CBF and task-evoked BOLD responses may reflect shared mechanisms, most likely involving either homeostatic regulation or changes in neurovascular coupling. The reasons behind the anatomical heterogeneity of the effects of hypoxia on cortical rCBF and BOLD responses remain to be elucidated.

4.8 Author contributions

For this research study, G.M.K.Rossetti contributed to conception and design, pilot testing, data collection, data analysis (excluding ASL data), interpretation of results, preparation of figures (excluding Figure 4.2), manuscript drafting,

and manuscript revisions. A full list of authorship contributions is provided in Appendix C.

Chapter 5

General Discussion

5.1 Summary of main findings

This PhD sought to understand physiological responses to the high-altitude environment and identify methods to enhance health and performance at altitude using a combination of rigorous lab-based methods, and applied field methods. This thesis includes three independent research studies, each investigating a different aspect of altitude physiology. Specifically, the first study (Chapter 2) identified that greater sea-level fitness is associated with reduced sense of effort during exercise and better mood on altitude expeditions, but has no association with AMS. A practical application of these findings is that sea-level fitness tests may aid preparation for high-altitude travel. The second study (Chapter 3) established that the popular dietary intervention of nitrate supplementation, far from being an effective ergogenic aid and AMS prophylaxis, increases sense of effort during exercise and exacerbates AMS in hypoxia. The practical application of this study is that dietary nitrate supplementation is not recommended for high-altitude travel. The third study (Chapter 4) utilised MRI methods to identify region-dependent heterogenous neurovascular responses to hypoxia, and provided evidence of hypoxia-induced disruptions to neurovascular coupling that are specific to DMN regions. This study observed reductions in blood flow to DMN regions including the PCC and right AG at rest, and negative BOLD responses in these regions during a DMN-dependent memory task, despite no impairment in task performance. This was in contrast to a compensatory increase in haemodynamic response observed in the VAN during a motion detection task.

5.2 Cardiovascular fitness and high-altitude travel

Chapter 2 identified that a high $\dot{V}O_{2max}$ was associated with a lower sense of effort during exercise in acute hypoxia and at altitude, and reduced fatigue at altitude. Given that fatigue is one of the two most-important early indicators of subsequent death on Mt Everest (Firth et al., 2008), this association with reduced fatigue is important. In addition, this study identified that even simple questionnaire-based estimation of $\dot{V}O_{2max}$ was a better prediction of exercise performance at altitude than sophisticated laboratory testing of hypoxic exercise responses. Since neither test was able to satisfactorily predict AMS, fitness assessments may provide the most useful tool to help individuals prepare for high-altitude travel. This has direct application for medical and outdoor practitioners who may wish to use it to assess a patient or client's readiness for high-altitude travel, or to monitor their preparation. This study also observed relationships between fitness level and physiological responses to hypoxia, which may provide insight into the physiological mechanisms underlying the adaptive response in high-fit individuals.

5.2.1 A blunted HVR may be protective in high-fit individuals

Previous literature has reported regular endurance exercise training (Richalet et al., 2012) and high $\dot{V}O_{2max}$ (Byrne-Quinn et al., 1971) are associated with a blunted HVR. Previous authors proposed this blunted ventilatory response partly explains the observed exacerbated hypoxaemia in highly-trained individuals and contributes to the greater performance decline and elevated altitude illnesses. In contrast, Chapter 2 found a negative association between $\dot{V}O_{2max}$ and HVR, but this was not associated with worse hypoxaemia. Rather, greater exercise ventilation reserve and ventilatory efficiency, were associated with superior exercise tolerance at altitude, and HVR_e tended to explain the relationship between $\dot{V}O_{2max}$ and RPE. This provides scope to interpret this blunted HVR as a beneficial adaptive response, particularly since the oxygen cost of respiratory muscle work (W_B) contributes to the accelerated development of fatigue in

hypoxia (Amann et al., 2007a). At sea level, W_B comprises 5.5% of $\dot{V}O_{2max}$, but W_B accounts for 26% of $\dot{V}O_{2max}$ at 5050 m (Cibella et al., 1999). Further, the requirement for dramatic hyperventilation is one of the primary limitations for exercise at extreme altitude (Bernardi et al., 2006).

Previous studies have shown that the increase in W_B at altitude contributes to the development of both central and peripheral fatigue (Amann, 2012). Amann *et al.* (2007) used proportional assist ventilation to reduce W_B by 35-80% in hypoxia, and demonstrated a substantial (21%) amelioration of hypoxia-induced post-exercise peripheral fatigue. This can be explained since increased W_B induces a vascular shunt towards the respiratory muscles, resulting in reduced blood flow to the locomotor muscles (Harms et al., 1997). The reduction in oxygen delivery to the locomotor muscles was accompanied by a reduction in locomotor muscle $\dot{V}O_2$ for the same workload, suggesting an increased reliance on anaerobic respiration, leading to accumulation of metabolites and peripheral fatigue (Gaskill et al., 2001; Ghosh, 2004).

In addition, excessive hyperventilation may elevate central fatigue by exacerbating hypocapnia, causing hypocapnic cerebral vasoconstriction that outweighs hypoxic vasodilation, limiting CDO_2. Individuals with a blunted HVR are likely to have a lower ratio of PO_2 to PCO_2 (Ainslie and Ogoh, 2010), which is associated with greater CBF at altitude (Lucas et al., 2011). Exacerbated hypocapnia during exercise in hypoxia (following intermittent and chronic hypoxic exposures) is associated with decreased CBF and cerebral oxygenation (Ainslie et al., 2008). This cerebral hypoperfusion may directly contribute to elevated central fatigue (Ogoh et al., 2009). A schematic of this theoretical model relating excessive hyperventilation to peripheral and central fatigue is provided in Figure 5.1.

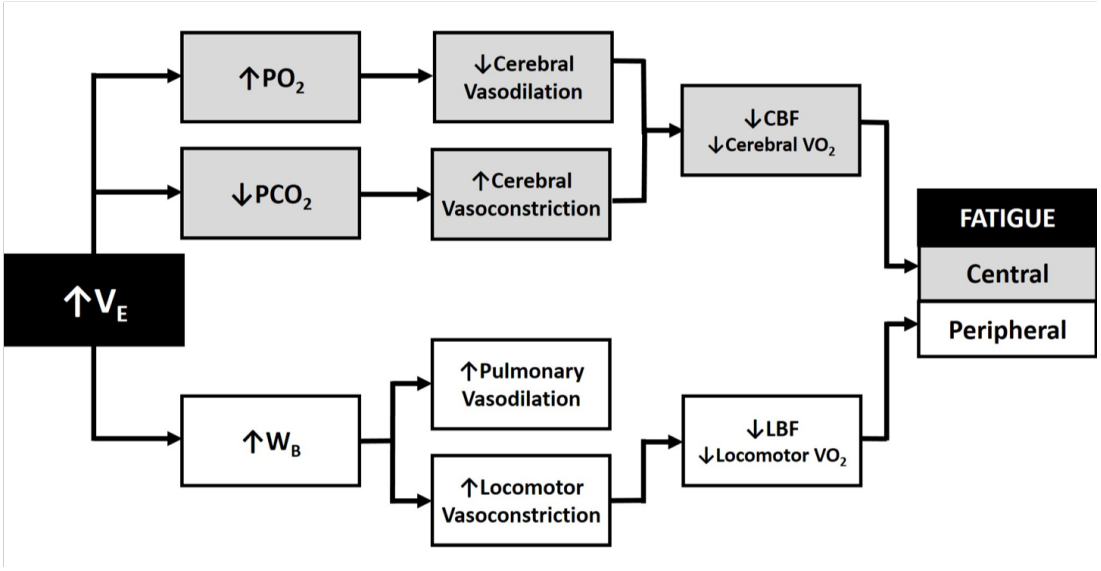


Figure 5.1: Theoretical model of physiological mechanism for relationship between ventilation and fatigue. Excessive hyperventilation (e.g. elevated HVR) could increase central and peripheral fatigue during exercise. CBF, cerebral blood flow; LBF, locomotor muscle blood flow; PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; V_E, ventilation; VO_2, oxygen consumption; W_B, work of breathing.

5.3 Dietary nitrate in hypoxia

Chapter 3 demonstrated dietary nitrate supplementation increases AMS symptom severity, specifically headache, and sense of effort during submaximal exercise, particularly in those who are susceptible to AMS.

5.3.1 Subsequent literature

Since the publication of Chapter 3 in the *Journal of Applied Physiology* (Rossetti et al., 2017b), a field study has been published that investigated the effect of dietary nitrate supplementation on AMS and acclimatisation parameters on a high-altitude expedition. Cumpstey *et al.* (2017) report no difference in AMS incidence or physiological parameters including S_pO_2, ventilation, and blood pressure, between nitrate and placebo groups. However, the data presented in the article show a non-significant trend for increased AMS severity in the nitrate group. The contrasting findings may relate to the smaller sample size in the most recent study, reducing their power to detect an effect. Alternatively,

the different findings may relate to differences in study design. Cumpstey *et al.* used two independent groups, rather than a repeated measures crossover design, and were therefore unable to analyse the effect of nitrate according to AMS susceptibility. This may be important since the effect of increased severity reported in Chapter 3 was driven by an increase in AMS severity in AMS-susceptible individuals (those who experienced AMS in their placebo trial). The effects of dietary nitrate on physiological responses to hypoxia reported in Chapter 3 were also dependent on AMS-susceptibility. Nitrate had opposing effects on physiology in AMS- compared to AMS+, with no uniform effect across the whole sample.

5.3.2 Nitric oxide and oxidative stress

NO is vital to a multitude of physiological processes in the human body, and dietary nitrate supplementation has been shown to have numerous physiological benefits in healthy and clinical populations. Benefits include reduced blood pressure and improved vascular function, leading some authors to refer to it as a "magic bullet" treatment (Jones, 2013). However, Chapter 3 of this thesis demonstrates the potential harmful effects of nitrate supplementation in hypoxia.

To understand why this popular dietary intervention is harmful under these specific conditions, it is useful to consider the other negative physiological responses to hypoxia (e.g. oxidative stress and inflammation). Under normal conditions, endogenous NO helps to maintain endothelial function, largely through the actions of cyclic guanosine monophosphate (cGMP; Hickey and Kubes, 1997). However, under conditions of inflammation or oxidative stress (such as in hypoxia), excessive bioavailability of NO exacerbates production of pro-inflammatory cytokines and increases vascular permeability and edema (Bernareggi et al., 1997; Mcquaid and Keenan, 1997; Mundy and Dorrington, 2000). This includes the upregulation of pro-inflammatory cytokines (such as interleukin-6) that are associated with AMS-susceptible individuals' inability to

mount an anti-inflammatory and anti-permeability response to hypoxia (Julian et al., 2011; Makris et al., 2010).

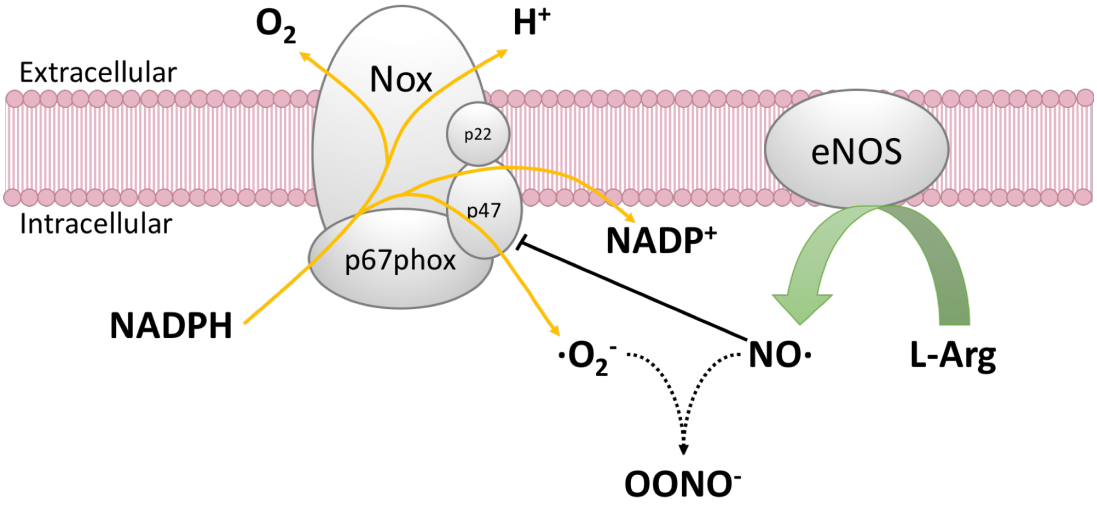


Figure 5.2: Interactions of nitric oxide (NO) with dihydronicotinamide-adenine dinucleotide phosphate (NADPH)-oxidase (Nox) in vascular cells. The vascular Nox complex may contain Nox1 or Nox4, bound to the human neutrophil cytochrome b light chain (p22phox) in the plasma membrane. NADPH-oxidation is activated in hypoxia. NO produced by endothelial nitric oxide synthase (eNOS) reacts with superoxide ($\cdot O_2^-$) to produce the reactive species peroxynitrite ($OONO^-$). L-Arg, L-arginine. Figure adapted from Dusting et al., 2005.

Specifically, hypoxia activates dihydronicotinamide-adenine dinucleotide phosphate (NADPH)-oxidase, resulting in the production of superoxide which combines with NO to form the highly reactive molecule peroxynitrite (Capone et al., 2012; Rathore et al., 2008; Figure 5.2). The production of peroxynitrite results in endothelial dysfunction, failure of endothelium-dependent vasodilation, and propensity for vasoconstriction (Dusting et al., 2005). This is possibly exacerbated by the upregulation of inducible NOS (iNOS) in hypoxia (Kaur et al., 2006), since the NO produced by iNOS is associated with platelet adhesion and inflammation (Ovechkin et al., 2005, 2007), unlike the protective effects of NO from eNOS. This provides a physiological rationale for potential harmful effects of upregulated bioavaibility of NO in hypoxia with implications for severe high-altitude illnesses, since severe high-altitude illnesses are characterised by vascular permeability and oedema. In particular, the pathophysiology of HAPE, where heterogenous pulmonary vasoconstriction has a vital role in development of the condition. Therefore, dietary nitrate

supplementation may not only exacerbate AMS, but also increase the severity and prevalence of severe high-altitude illnesses.

5.4 Regional cerebral physiology in hypoxia

5.4.1 Region-specific alterations in neurovascular coupling

Chapter 1 reported that, according to the existing literature, the maintenance of $CMRO_2$ in hypoxia is achieved through increased CBF without alteration in cOEF. However, the findings of Chapter 4 indicate this mechanism is region- and domain-specific. Hypoxia induced an increase in the BOLD response in the VAN during a VAN-dependent motion detection task, supporting the proposed maintenance of $CMRO_2$ through a compensatory elevation in CBF. This elevation may be necessary to increase the diffusion gradient to enable oxygen extraction form the capillaries. Previous models to explain the BOLD response have proposed the pronounced excess in the CBF response to neural activation is necessary to ensure sufficient oxygen concentration gradient to drive the diffusion of oxygen from the capillaries to the parenchyma (Buxton and Frank, 1997). Since the oxygen content of arterial blood is reduced in hypoxia, the concentration gradient will be decreased at rest, therefore a greater increase in CBF enables the maintenance of oxygen extraction.

In contrast, hypoxia induced negative BOLD responses in the DMN during a DMN-dependent memory task, which could possibly be attributed to a lack of CBF response, but a maintenance of $CMRO_2$ through increases in cOEF. The BOLD response represents the ratio of oxyhaemoglobin to de-oxyhaemoglobin. Based on the model of a CBF overshoot in response to neural activity (the haemodynamic response model), a positive BOLD response indicates neural activation (Logothetis and Wandell, 2004). Therefore, a negative BOLD response may represent deactivation, but could also represent an increase in cOEF to compensate for a failed haemodynamic response to $CMRO_2$ (Hayes and Huxtable, 2012; Nagaoka et al., 2006). This interpretation is controversial since it contrasts

the accepted mechanism in the current literature, and our study lacked a direct measure of neural activity or $CMRO_2$.

Alternatively, the negative BOLD responses may reflect region-specific alterations in metabolism. Specifically, hypoxia-induced reductions in rCBF to DMN regions may result in a shift towards increased contribution of anaerobic metabolism in these regions. Previous studies have investigated the effect of hypoxia on the contribution of anaerobic metabolism in the visual cortex, determined by magnetic resonance spectroscopy (MRS) measurement of lactate, with conflicting results (Tuunanen and Kauppinen, 2006; Vestergaard et al., 2016). However, since these studies are isolated to the visual cortex, they cannot be applied to the DMN-specific alterations observed in Chapter 4, particularly since we demonstrate no change in visual stimulation-evoked BOLD responses in the visual cortex. Further, investigation of lactate alone fails to account for the brain's ability to use supplemental lactate as a glucose sparing fuel, particularly under conditions of hypoxia or hypoperfusion, such as in traumatic brain injury (Welling et al., 2015). Alternative interpretations relating to post-stimulus vasoconstriction of pial vessles, akin to what has been observed in neonates (Kozberg et al., 2013), may also explain this observed phenomenon. However, since the BOLD response is a single measure of a complex, multifaceted physiological process (neurovascular coupling), any mechanistic interpretation is speculative.

5.4.2 Anatomical specificity: implications for neurodegenerative diseases

The region-specific alterations in neurovascular responses in hypoxia are particularly interesting given the regions affected. The regions with the most prominent reductions in resting rCBF were the PCC and PCUN, with negative BOLD responses observed in the PCC during a PCC-dependent memory task. These regions have particular relevance to the development of neurodegenerative diseases such as dementia. An estimated 24 million people

suffer from dementia, providing a major public health concern (Ballard et al., 2011).

The most common form of dementia is Alzheimer's disease (AD; Girouard and Iadecola, 2006), and functional and structural abnormalities in the progression of AD originate from the PCC and PCUN, before extending to other brain regions (Love and Miners, 2016; Miners et al., 2016). Indeed, region-specific hypoperfusion to these brain regions occurs in individuals at risk of AD before disease progression (Langbaum et al., 2010). Further, beta-amyloid deposition and glucose hypometabolism occur in the PCC and PCUN ten years before AD symptom onset (Benzinger et al., 2013). The decline in perfusion exceeds the reduction in metabolic demand, resulting in tissue damage (Love and Miners, 2016). Hypoperfusion and hypometabolism occur first in the PCC and PCUN, despite being distant from the site of primary neural degeneration in AD (the entorhinal cortex; Matsuda, 2001). Hypoperfusion to these regions is not only observed at rest, but attenuation of the haemodynamic response to neural activity is also observed in these regions during AD progression (Girouard and Iadecola, 2006; Iadecola, 2004; Iadecola, 2017). In summary, there is marked similarity in the region-specific physiological characteristics of early AD progression, and the hypoxia-induced effects we observed. Therefore, the hypoxic model may be of use to elucidate reasons for this anatomical specificity governing neurodegenerative diseases.

5.5 Cognition in hypoxia

Chapter 4 assessed the effect of hypoxia on performance on a paired associates memory task and motion detection task. Hypoxia did not affect any measure of performance on either task.

5.5.1 Long-term memory is not affected by hypoxia

Long-term memory is assessed by the ability of an individual to recall information after an extended period of time, usually with a minimum of one overnight sleep (Bell et al., 2014). Chapter 4 illustrates the role of the PCC and AG brain regions in long-term memory retrieval. This observation was expected by design, since a recall task was deliberately chosen to target these brain regions given their reported decreased resting rCBF in hypoxia. However, hypoxia unexpectedly had no effect on memory performance, despite alterations in the neurovascular response to the memory task. This is the first study to investigate the effect of hypoxia on long-term memory recall, providing an unexpected and novel finding.

How can cognitive task performance be maintained despite altered neurovascular function in the responsible brain regions? As discussed in Section 5.4.1, the negative BOLD responses observed in the PCC and AG during the memory task may actually represent the maintenance of task-evoked $CMRO_2$ without hyperaemia. Therefore, performance is perhaps maintained through increases in cOEF to maintain $CMRO_2$ and neural activity. However, the memory task was completed after 2-3 h exposure and lasted a total of only 24 min. Whether this response is sustainable over a longer-duration task, or with a chronic exposure to hypoxia, is unknown. Importantly, as mentioned above, any mechanistic interpretation is speculative until further studies have been able to separate the physiological processes governing neurovascular coupling.

Alternatively, the reason for maintained memory recall performance may relate to memory formation and encoding. Not only responsible for recall (Raichle, 2015), the PCC and AG brain regions are largely responsible for the learning of information at encoding (Liu et al., 2014). The maintenance of neurovascular function in the PCC and AG may be more important for learning, rather than recall. In the study reported in Chapter 4, all encoding trials were completed in normoxia, followed by an overnight rest (also in normoxia). Therefore the preservation of long-term memory performance reported in the study can be

applied to memory recall only. Whether hypoxia influences long-term memory including encoding, remains to be studied.

5.5.2 Visuo-spatial attention is not affected by hypoxia

Visuo-spatial attention enables us to perceive and interact with the world around us. It is essential for the completion of everyday tasks, for example picking up and handling tools or other implements (Farran and Formby, 2011). In Chapter 4, hypoxia had no effect on visuo-spatial attention performance, determined by visual search during a motion detection task. This is in agreement with previous studies that have assessed the effect of *acute* or *short-term* exposure to hypoxia on visuo-spatial attention performance. Caldwell *et al.* (2017) used a series of visuo-spatial puzzles and observed no change in performance from sea level after three or seven days at 3800 m. Similarly, Bonnon *et al.* (1999) reported no difference in visuo-spatial performance between individuals exposed to altitude and sea-level controls.

Although visuo-spatial performance is protected against *acute* hypoxic insults, this is not the same for *chronic* exposures. One study observed the effect of *chronic* hypoxia on a variety of cognitive domains, and identified a significant reduction in visuo-spatial attention performance over a 18-month stay at altitude. Sharma *et al.* (2014) identified significantly impaired visuo-spatial attention performance assessed by the Clock Drawing Test (Shulman, 2000) and Raven Standard Progressive Matrices (Raven et al., 1992). However, this impairment was only present in a subgroup of individuals identified as cognitively impaired at altitude, accounting for only 8% of the study group. In the remaining 92% of participants, chronic hypoxia had no effect on visuo-spatial attention performance.

In conclusion, the finding of no difference in visuo-spatial attention performance reported in Chapter 4 is consistent with previous findings, providing reassurance that the null-effect is not a result of insufficient statistical power. This is further supported by the increase in BOLD response observed in the VAN during the

motion detection task, providing evidence of a physiological compensatory mechanism to maintain performance.

5.6 Finding common ground

5.6.1 Improving oxygen saturations isn't sufficient

Interventions to improve exercise performance and alleviate AMS at altitude are often chosen because they reduce hypoxaemia. However, research presented in this thesis demonstrates that an increase in S_aO_2 is not sufficient to guarantee health or performance benefits. Chapter 2 demonstrated that higher S_pO_2 at rest or during exercise, was not associated with reduced sense of effort during exercise, or an increase in perceptually-regulated exercise. Further, S_pO_2 was not related to peak AMS score, or number of days with AMS. In agreement with these observations, Chapter 3 observed chronic dietary nitrate successfully increased S_pO_2 in AMS-resistant individuals, but this was not associated with any benefit to exercise tolerance. These observations support the hypothesis outlined in Chapter 1, that S_aO_2 does not provide useful insight to differentiate between individuals subjected to the same (or at least similar) hypoxic exposure. This is because it fails to account for differences in other physiological components governing oxygen delivery and extraction.

5.6.2 Methodological consideration: poikilocapnic hypoxia

An important methodological consideration for this thesis relates to the nature of the hypoxic exposures. All studies used poikilocapnic hypoxia for the experimental manipulation, which enabled investigation of the physiological response to the hypoxic environment at altitude (as in Chapter 2), or as a simulation of altitude (as in Chapters 3 and 4).

Since Chapter 2 was an applied field study, the use of isocapnic hypoxia was not possible or appropriate for most elements of the study. However, it is important to note that the study used a modified Richalet test for HVR which assesses ventilatory response to poikilocapnic hypoxia, and is not a true test of oxygen chemosensitivity. Previous studies have reported a blunted oxygen chemosensitivity, but also a blunted hypercapnic chemosensitivity, in athletes compared to controls (Byrne-Quinn et al., 1971). The findings of Chapter 2 confirm that the observed physiological differences in chemosensitivity translate to the real-world ventilatory responses to the high-altitude environment.

In Chapters 3 and 4, $P_{ET}CO_2$ was monitored but not controlled. The purpose of Chapter 3 was to investigate the effect of dietary nitrate supplementation versus placebo, and accordingly all tests were conducted in hypoxia. $P_{ET}CO_2$ values in Chapter 3 were $\sim$33 mmHg, suggesting mild hypocapnia when compared to normocapnic values of 35-40 mmHg (Artru et al., 1989). However, the extent of hypocapnia was no different between nitrate and placebo conditions, and was not affected by AMS susceptibility. The chapter proposed NO-stimulated increase in hypoxia-induced cerebral vasodilation as a potential mechanism for the observed increase in high-altitude headache. It is interesting to note this may have occurred despite hypocapnic cerebral vasoconstrictive drive. Given that this applied study aimed to determine the efficacy of dietary nitrate supplementation for high-altitude travel, it was important to use an experimental manipulation that is closest to the high-altitude environment.

In Chapter 4, 2-3 h poikilocapnic hypoxia induced a 4 mmHg decrease in $P_{ET}CO_2$ (compared to normoxia). We report controversial reductions in blood flow at rest and during cognitive tasks, but it is inappropriate to attribute these reductions to systemic hypocapnia. The affected regions are specific to the DMN, and do not match regions known to be most sensitive to hypocapnia (Ito et al., 2000; Schlünzen et al., 2010). Further, the extent of hypocapnia was not related to the rCBF reductions. In contrast, individual differences in S_pO_2 tended to correlate with the magnitude of rCBF reductions, suggesting hypoxaemia as the cause of the observed rCBF reductions, not hypocapnia.

5.7 Future directions

5.7.1 Understanding physiological mechanisms from Chapter 2: exercise training intervention

Chapter 2 identified possible physiological mechanisms to explain the relationship between fitness and exercise capacity in hypoxia. However, a comprehensive mechanistic interpretation is beyond the scope of this cross-sectional field study, since intervention studies are required to evaluate the direct impact of any proposed preventative measure (Thiese, 2014). A possible future direction could be to investigate the effect of experimentally-improved cardiovascular fitness ($\dot{V}O_{2max}$) on the physiological mechanisms governing exercise performance in hypoxia. An intervention study using combined endurance and high intensity interval training could better determine that $\dot{V}O_{2max}$ *per se*, rather than confounding variables, is responsible for the improvement in exercise performance. In particular, a laboratory-based intervention study would be able to test the hypothesised mechanism depicted in Figure 5.1. Specifically, it could determine whether an improvement in fitness reduces W_B during exercise in hypoxia, and whether this relates to changes in central and peripheral fatigue.

In addition, a recent study identified the limiting factor for $\dot{V}O_{2max}$ was dependent on training status at sea level. In untrained individuals, cardiovascular capacity (determined by locomotor muscle oxygen delivery) exceeds muscle capacity (determined by mitochondrial capacity), while in trained individuals, cardiovascular capacity is the limiting factor for $\dot{V}O_{2max}$ (Gifford et al., 2016). The literature review presented in Chapter 1 stated the mechanism responsible for maintaining oxygen delivery to the muscles is dependent on exercise intensity and modality (Section 1.1.4). This subsequent observation at sea-level highlights the possibility that the primary mechanism may also be dependent on fitness level. Previous studies have identified that high-fit individuals exhibit significantly greater leg oxygen extraction in hypoxia

compared to normoxia for the same absolute intensity (Calbet et al., 2003). In this study, greater oxygen extraction helped overcome reduced leg oxygen delivery in order to ameliorate the decline in leg $\dot{V}O_2$. In addition, the superior tissue extraction may be able to overcome the short capillary transit time associated with an elevated cardiac output (Roca et al., 1992). Future studies should investigate the interaction between fitness level and hypoxia on the physiological limiting factors to exercise performance by comparing oxygen delivery and extraction during exercise in hypoxia, with mitochondrial capacity between fitness groups.

5.7.2 Unanswered questions from Chapter 4: regional heterogeneity in neurovascular coupling

In Chapter 4, hypoxia increased BOLD signal in the VAN during a motion detection task, indicating the maintenance of oxygen delivery despite reduced blood oxygenation. In contrast, hypoxia induced reductions in blood flow to DMN regions including the PCC and right AG that persisted during a DMN-dependent long-term memory task, despite no impairment in memory task performance. Since participants were able to complete the memory task to the same level of performance in hypoxia as in normoxia, I infer neural activity was unchanged in the DMN during memory search in hypoxia. Importantly, performance was not affected on either task, despite evidence of maintained cerebrovascular function during the motion detection task, and evidence of cerebrovascular dysfunction during the long-term memory task. However, previous studies have reported hypoxia-induced impairment on executive function and working memory tasks (Asmaro et al., 2013). To date, no studies have investigated the neurovascular response to these tasks in hypoxia.

The findings of Chapter 4, combined with previous literature, suggest regional heterogeneity in neurovascular responses to hypoxia, with three characteristic responses. These responses consist of; maintained neural activity accompanied by elevated CBF responses to compensate for reduced arterial oxygen supply (as in the VAN), maintained neural activity with an inversion of the haemodynamic

response (as in the DMN), and decreased neural activity resulting in impaired performance (as with working memory tasks, reliant on the SN) (Engström et al., 2013; Li et al., 2012). However, since the BOLD fMRI signal is dependent on the haemodynamic response and does not directly measure neural activity (Logothetis, 2002; Logothetis and Wandell, 2004), these hypothesised responses rely on inference based on assumed relationships.

A previous study identified neurovascular uncoupling in anaesthetised rats, determined by simultaneous electroencephalography (EEG) and fMRI (Sumiyoshi et al., 2012). The authors observed significant reductions in fMRI BOLD signal in response to forepaw stimulation, with unchanged EEG responses. These effects may not directly relate to cognitively-active humans, and were not conducted in relation to the DMN, VAN, or SN. However, the experimental model of investigation is something worth replicating. Simultaneous assessment of EEG and fMRI would confirm whether hypoxia truly induces neurovascular uncoupling, or whether neuroplasticity occurs to meet the cognitive demand through means outside the DMN. This would make it possible to differentiate the location and amplitude of neural activity (measured by EEG) from the CBF response (measured by fMRI). An overview of a potential study design using three cognitive tasks to target each of the hypothesised neurovascular responses to hypoxia is provided in Table 5.1.

Task	Neural network	Hypothesised effect of hypoxia	
Motion detection	Visual attention network (VAN)	Task performance	$\longleftrightarrow$
		Neurovascular coupling	$\longleftrightarrow$
		Neural activity (EEG)	$\longleftrightarrow$
		Haemodynamic response (fMRI)	$\uparrow$
Long-term memory	Default mode network (DMN)	Task performance	$\longleftrightarrow$
		Neurovascular coupling	$\downarrow$
		Neural activity (EEG)	$\longleftrightarrow$
		Haemodynamic response (fMRI)	$\downarrow$
Working memory (DSB)	Salience network (SN)	Task performance	$\downarrow$
		Neurovascular coupling	$\longleftrightarrow$
		Neural activity (EEG)	$\downarrow$
		Haemodynamic response (fMRI)	$\downarrow$

Table 5.1: Overview of potential study design to investigate regional heterogeneity in neurovascular responses to hypoxia. Tasks are presented in reference to the neural networks they stimulate, and the hypothesised responses to hypoxia. DSB, digit-span backward task. $\uparrow$ indicates hypothesised increase; $\downarrow$ indicates hypothesised decrease; $\longleftrightarrow$ indicates hypothesised no effect.

In addition, the use of MRS to measure metabolites, would enable separation of some of the physiological processes governing neurovascular coupling. For example, measurement of the anaerobic metabolite lactate would help determine whether the negative BOLD responses observed in the DMN reflect an increased reliance on anaerobic metabolism (Tuunanen and Kauppinen, 2006; Vestergaard et al., 2016). The use of MRS to measure the concentration of the neurotransmitters gamma-aminobutryric acid (GABA) and glutamate would also provide a more direct measure (compared to BOLD fMRI) of task-evoked increases in metabolism.

5.8 Conclusions

The major conclusions from Chapter 2 are that greater sea-level fitness is related to lower sense of effort during submaximal exercise at altitude and better mood (less fatigue, tension, and confusion) on a high-altitude expedition. Importantly, the lower sense of effort during submaximal exercise in high-fit individuals did not come at the cost of worse AMS or greater arterial oxygen desaturation. This study provides the first empirical evidence to support recent recommendations that people might complete sea-level aerobic fitness training before high-altitude travel (Bärtsch and Swenson, 2013; Burtscher et al., 2015). Low-fit persons may improve their trekking experience by increasing sea-level fitness because it is associated with less effort and better mood during trekking at altitude. The study also indicates that a sea-level fitness assessment could be used to aid preparation for high-altitude travel by enabling better aerobic exercise prescription and identifying those people who might benefit most from the aerobic training. Given that fatigue and confusion are major risk factors for injury and fatality at altitude (Firth et al., 2008), sea-level fitness assessment and exercise training should be considered as part of preparations for high-altitude travel.

The major conclusions from Chapter 3 are that dietary nitrate increases AMS symptom severity, specifically headache, and sense of effort during submaximal

exercise in hypoxia, particularly in those who experience AMS. The consumption of nitrate in those susceptible to AMS raises oxygen cost, ventilation, and blood pressure during walking exercise in hypoxia, and the consumption of nitrate has no beneficial effects in those resistant to AMS. Therefore dietary nitrate is not recommended as an AMS prophylactic or ergogenic aid in nonacclimatised individuals at altitude.

The major conclusions from Chapter 4 are that acute hypoxia induces region-specific heterogenous neurovascular alterations. Acute hypoxia induced reductions in rCBF to DMN regions including the PCC and right AG, which persisted during a DMN-dependent memory task, despite no impairment in memory task performance. The negative BOLD responses observed in these regions may relate to increases in cOEF, post-stimulus vasoconstriction of pial vessels, or alterations in metabolism. In contrast, hypoxia induced increases in visual search-related activations in VAN regions including the MT, FEF, and left IPS. This increase in activation, coupled with no change in visual search performance, indicates the maintenance of oxygen delivery in hypoxia despite reduced blood oxygenation. The DMN appears to be more sensitive to disturbances in neurovascular coupling compared to other brain regions, a finding of particular relevance given the DMN is central to the progression of neurodegenerative diseases.

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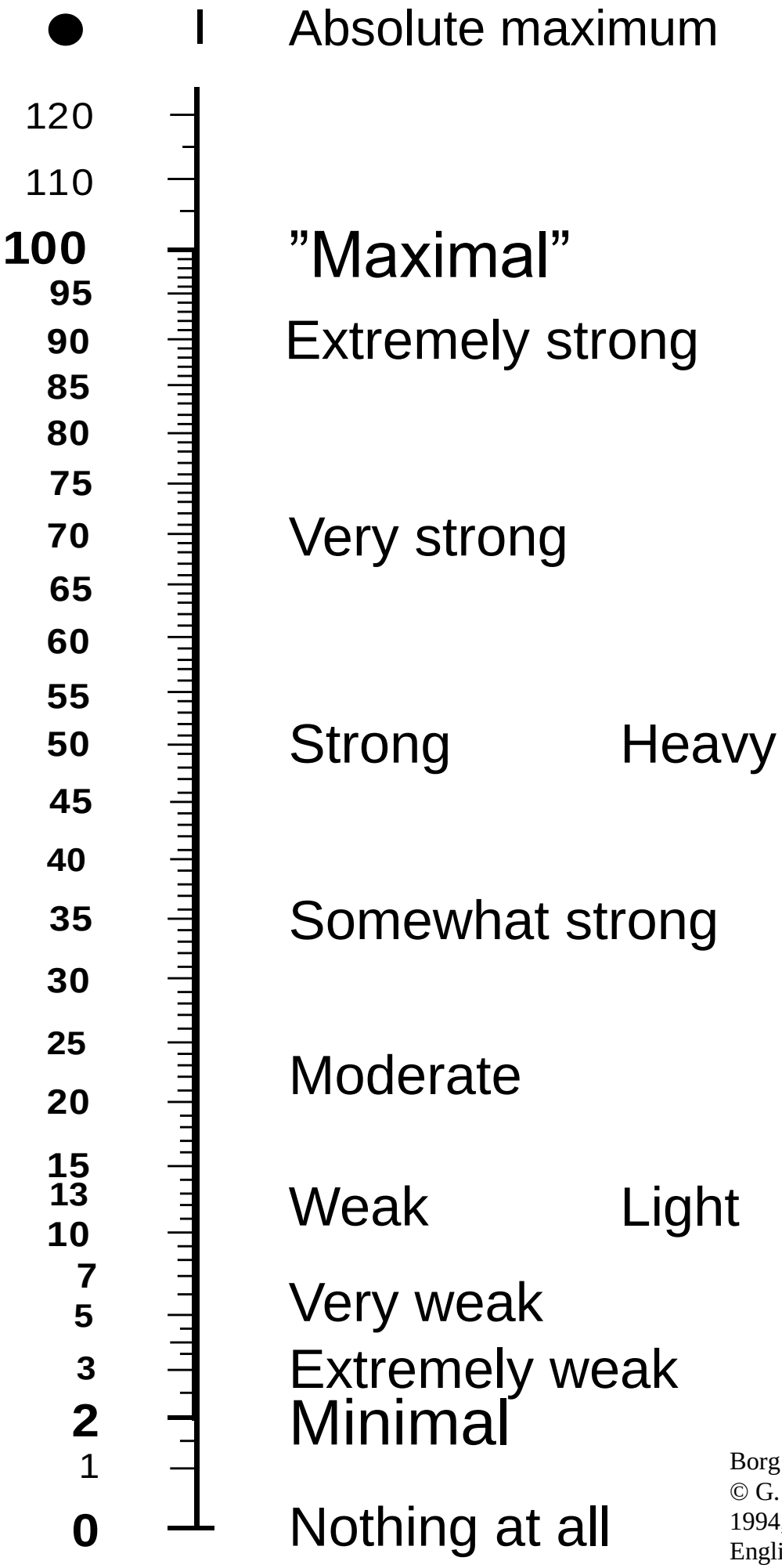
Appendix A

Scales and Questionnaires

Scales and questionnaires included in the thesis are provided overleaf.

1. Sense of effort during exercise
    (a) Rating of Perceived Exertion (RPE): Borg CR100
2. Physical activity
    (a) Self-report physical activity
    (b) Energy expenditure terrain coefficient
3. Psychological mood
    (a) Brunel Mood Scale (BRUMS)
4. High-altitude health
    (a) Acute Mountain Sickness (AMS): Lake Louise Score (LLS)
    (b) Acute Cerebral Mountain Sickness (AMS-C): Environmental Symptoms Questionnaire (ESQ)
    (c) High-altitude headache: Visual analogue scale (VAS)



Borg centiMax (CR100) scale

English

Physical activity

In the table below select the number (0 to 7) that best describes your general activity level for the past month

Do not participate regularly in programmed recreation sport or heavy physical activity		
0	Avoid walking or exertion, for example, always use elevators, drive whenever possible instead of walking	☐
1	Walk for pleasure, routinely use stairs, occasionally exercise sufficiently to cause heavy breathing or perspiration	☐
Participate in recreation or work requiring modest physical activity, such as golf, horseback riding, calisthenics, gymnastics, table tennis, bowling, weighting lifting, yard work.		
2	10 to 60 min per week	☐
3	Over 60 min per week	☐
Participate regularly in heavy physical exercise such as running or jogging, swimming, cycling, rowing, skipping rope, running in place or engaging in vigorous aerobic exercise such as tennis basketball or handball.		
4	Run less than 1 mile (1.6 km) per week or spend less than 30 min per week in comparable physical activity	☐
5	Run 1 to 5 miles (1.6 to 8 km) per week or spend 30 to 60 min per week in comparable physical activity	☐
6	Run 5 to 10 miles (8 to 16 km) per week or spend 1 to 3 h per week in comparable physical activity	☐
7	Run over than 10 miles (16 km) per week or spend over 3 hours per week in comparable physical activity	☐

Trekking logbook – terrain factors guide

Please see below the terrain descriptions and factors. Please record both the descriptor and the numerical factor in your trekking logbook.

Terrain description	Terrain factor
Tarmac	1
Dirt road	1.1
Light brush	1.2
Hard packed snow	1.3
Heavy brush	1.5
Swampy bog	1.8
Loose sand	2.1
Soft snow (15cm)	2.5
Soft snow (25cm)	3.3
Soft snow(35cm)	4.1

To be completed on day 6 (Machhakhola to Jagat) on arrival at camp, after the day's trek

Participant ID:

The Brunel Mood Scale

Below is a list of words that describe feelings. Please read each one carefully. Then cross the box that best describes HOW YOU FEEL RIGHT NOW.
Make sure you answer every question.

		Not at all	A little	Moderately	Quite a bit	Extremely
1.	Panicky	❑	❑	❑	❑	❑
2.	Lively	❑	❑	❑	❑	❑
3.	Confused	❑	❑	❑	❑	❑
4.	Worn out	❑	❑	❑	❑	❑
5.	Depressed	❑	❑	❑	❑	❑
6.	Downhearted	❑	❑	❑	❑	❑
7.	Annoyed	❑	❑	❑	❑	❑
8.	Exhausted	❑	❑	❑	❑	❑
9.	Mixed-up	❑	❑	❑	❑	❑
10.	Sleepy	❑	❑	❑	❑	❑
11.	Bitter	❑	❑	❑	❑	❑
12.	Unhappy	❑	❑	❑	❑	❑
13.	Anxious	❑	❑	❑	❑	❑
14.	Worried	❑	❑	❑	❑	❑
15.	Energetic	❑	❑	❑	❑	❑
16.	Miserable	❑	❑	❑	❑	❑
17.	Muddled	❑	❑	❑	❑	❑
18.	Nervous	❑	❑	❑	❑	❑
19	Angry	❑	❑	❑	❑	❑
20.	Active	❑	❑	❑	❑	❑
21.	Tired	❑	❑	❑	❑	❑
22.	Bad tempered	❑	❑	❑	❑	❑
23.	Alert	❑	❑	❑	❑	❑
24.	Uncertain	❑	❑	❑	❑	❑

For official use only:

Ang: _____ Con: _____ Dep: _____ Fat: _____ Ten: _____ Vig: _____

Lake Louise questionnaire (LLQ)

Please go through each question in turn and rate its corresponding intensity on the 0 - 3 scale.
Where 0 suggests that the symptom is not present and 3 indicates that the symptom is incapacitating

Symptom	Definition	Score
(1) Headache	No headache	0
	Mild headache	1
	Moderate headache	2
	Severe headache, incapacitating	3
(2) Gastrointestinal symptoms	No gastrointestinal symptoms	0
	Poor appetite or nausea	1
	Moderate nausea or vomiting	2
	Severe nausea and vomiting, incapacitating	3
(3) Fatigue and/or weakness	Not tired or weak	0
	Mild fatigue/weakness	1
	Moderate fatigue/weakness	2
	Severe fatigue/weakness, incapacitating	3
(4) Dizziness / lightheadedness	Not dizzy	0
	Mild dizziness	1
	Moderate dizziness	2
	Severe dizziness, incapacitating	3
(5) Difficulty sleeping	Slept as well as usual	0
	Did not sleep as well as I usual	1
	Woke up many times, poor night's sleep	2
	Could not sleep at all	3

Environmental symptoms questionnaire (ESQ)

Please circle the number of each item to correspond to HOW YOU FEEL AT THIS MOMENT. PLEASE ANSWER EVERY ITEM. If you do not have the symptom, circle zero (NOT AL ALL).

	Not al all	Slight	Somewhat	Moderate	Quite a bit	Extreme
1. I feel lightheaded	0	1	2	3	4	5
2. I have a headache	0	1	2	3	4	5
3. I feel dizzy	0	1	2	3	4	5
4. I feel faint	0	1	2	3	4	5
5. My vision is dim	0	1	2	3	4	5
6. My coordination is off	0	1	2	3	4	5
7. I feel weak	0	1	2	3	4	5
8. I feel sick to my stomach (nauseous)	0	1	2	3	4	5
9. I lost my appetite	0	1	2	3	4	5
10. I feel sick	0	1	2	3	4	5
11. I feel hung over	0	1	2	3	4	5

Visual Analogue Scale (VAS)

Please go through each visual analogue scale in turn and mark on the line the intensity/feeling that corresponds to each symptom. Where the beginning of the line indicates no perception/feeling at all and the end of the line indicates maximum perception/feeling.

Headache

None ——— Severe

Gastrointestinal symptoms

None ——— Severe

Fatigue and/or weakness

None ——— Severe

Dizziness / lightheadedness

None ——— Severe

Difficulty sleeping

None ——— Severe

I feel sick

None ——— Severe

Appendix B

MR measures

B.1 Larmor equation

The radio frequency (RF) required to alter energy states, and therefore alignment, of a particle in a magnet is determined by the Larmor equation:

$$RF(\omega) = MGratio(\gamma) \times T(B) \tag{B.1}$$

Where RF, radio frequency; MGratio, magneto-gyric ratio; T, magnetic field in T. For hydrogen (H) at 3T, ω = 127.74 MHz.

B.2 Types of MR scans

B.2.1 T_1 scans

T_1 scans are used for anatomical images, particularly for demonstrating non-pathological anatomy. For T_1 scans, water and cerebral spinal fluid are black, in contrast to fat, which shows up white. T_1 scans measure longitudinal relaxation; how quickly the protons realign with the magnetic field (following the RF pulse). A schematic representation of this realignment is provided in Figure B.1.

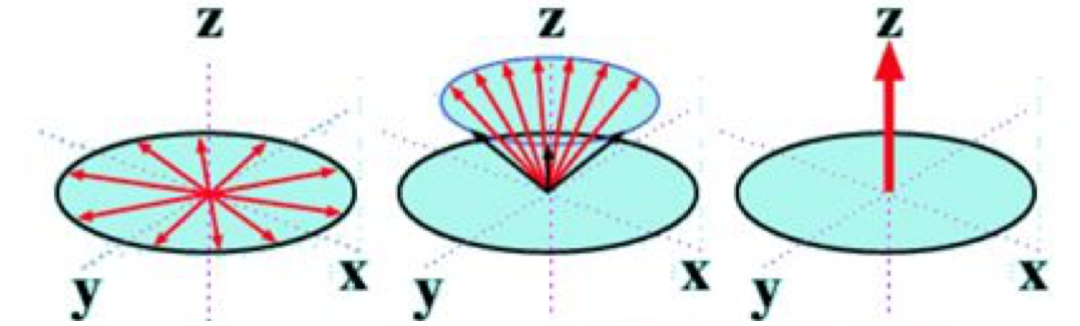


Figure B.1: Schematic representation of longitudinal relaxation. Provides the basis for T_1 MR scans.

B.2.2 T_2 scans

T_2 scans are used for anatomical images, particularly for identifying pathology since most (although not all) lesions are associated with an increase in water content. For For T_2 scans, water and cerebral spinal fluid show up white, in contrast to fat, which is black. T_2 scams measure transverse (or spin-spin) relaxation; how quickly the protons give off energy as they recover their equilibrium. A schematic representation of transverse relaxation is provided in Figure B.2.

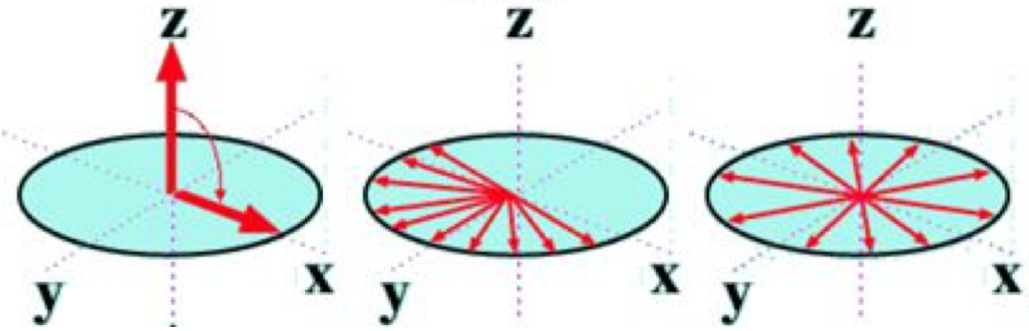


Figure B.2: Schematic representation of transverse relaxation. Provides the basis for T_2 MR scans.

B.2.3 T_2* scans

T_2* scans are used for functional MRI (including measurement of blood oxygen level-dependent [BOLD] responses). T_2* effects arise from local field inhomogeneities that cause faster de-phasing than predicted based on T_2

properties. A schematic representation of T_2* effects is provided in Figure B.3.

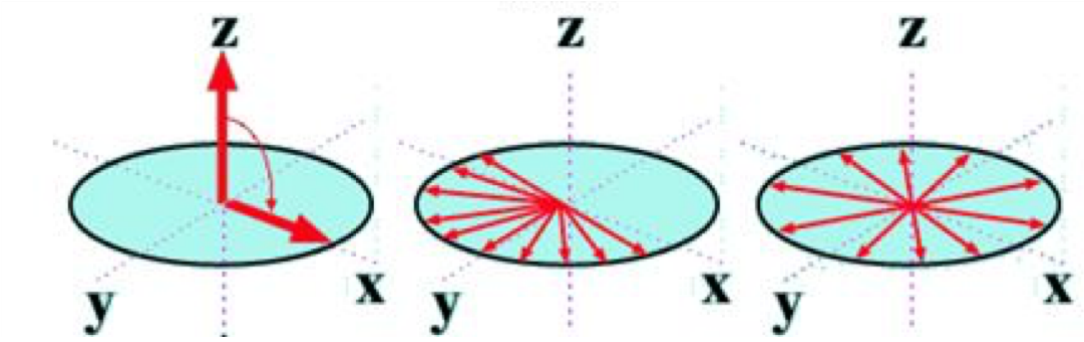


Figure B.3: Schematic representation of T_2* effects. Provides the basis for functional MRI scans.

B.3 MR methodologies included in the thesis

B.3.1 Arterial spin labelling (ASL)

ASL can be used to measure regional cerebral blood flow. Unlike perfusion MRI, ASL is non-invasive since it does not require injection of a contrast agent. ASL uses blood water as the contrast agent to measure perfusion.

Contrast is generated through the use of **radio frequency pulses** to **magnetically label**, or "tag", protons in blood water molecules before the reach the tissue of interest. "Labelling" refers to a change in the magnetic state of the in-flowing spins, either **saturation** or **inversion**. Arterial blood water is often magnetically labelled just below the slice of interest by applying a **180°RF inversion pulse**. This inverts the net magnetisation of the blood water. When the saturated blood water flows into the slice of interest, it exchanges with local tissue water. This exchange reduces the total tissue magnetisation, and therefore the MR signal. The tag image is taken at this point. The experiment is then repeated without labelling the arterial blood to create a control image, and the tag image is subtracted from the control image to produce a perfusion difference image. This perfusion difference image reflects the blood flow because any

tissue that does not contain flow will be similar in the two images. A schematic representation of ASL methodology is provided in Figure B.4.

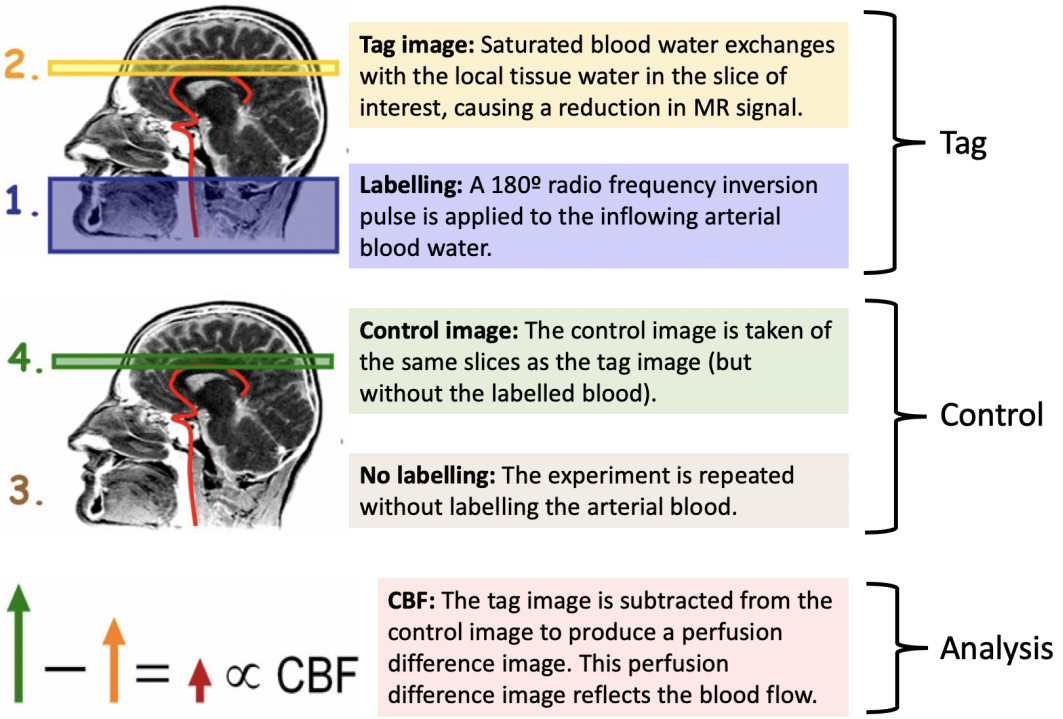


Figure B.4: Schematic representation of arterial spin labelling (ASL) methodology. Provides a non-invasive measure of perfusion.

B.3.2 Blood oxygen level-dependent (BOLD) response

The BOLD response is used in functional MRI (fMRI) to measure the haemodynamic response to metabolic activity.

The BOLD signal is driven by the ratio of oxyhaemoglobin to deoxyhaemoglobin. This is because, for a T_2* sequence, MR signal is greater where blood is oxygenated, and decreased where blood is deoxygenated. The BOLD response relies on an over-compensatory haemodynamic response such that an increase in oxygenated blood (and therefore an increase in signal) represents an increase in metabolic activity. The BOLD response is comprised of five temporal components (provided in text below and in Figure B.5):

Initial dip (0.5-1 s): Transient increase in oxygen consumption before change in blood flow.

Rise: Hyperoxic phase resulting from vasodilation of arterioles.

Overshoot/peak (5-8 s): Over-compensatory response found with blocked designs with extended intervals.

Sustained response (2-3%): Blocked design relies on comparison of sustained activity versus baseline.

Undershoot: Cerebral blood flow (CBF) is more locked to stimuli than cerebral blood volume (CBV). Increased CBV without increased CBF results in a reduction in MR signal. Observed with stimuli >10 s.

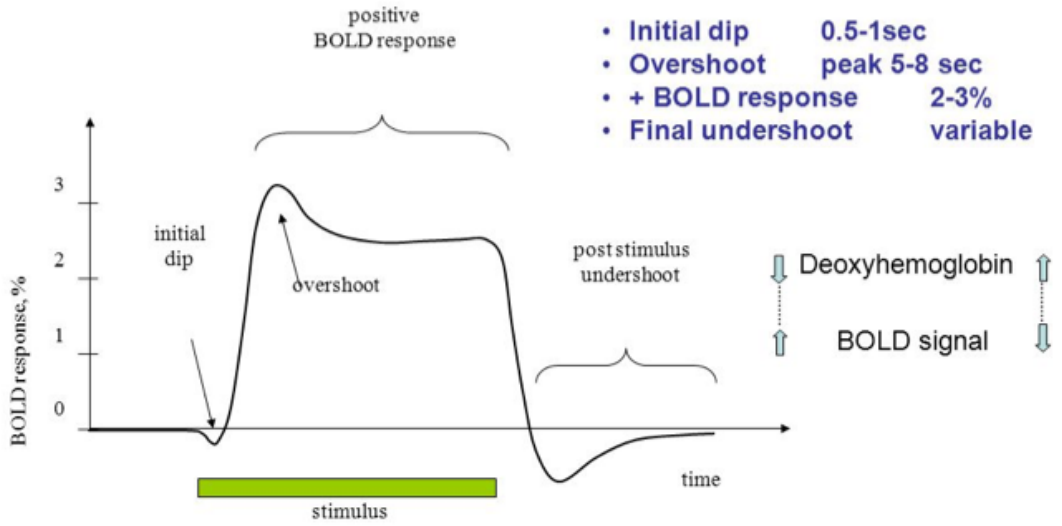


Figure B.5: Schematic representation of blood oxygen level-dependent (BOLD) response. Provides the basis for functional magnetic resonance imaging (fMRI).

Appendix C

Authorship contributions

Authorship contributions are prodivided in detail in the following pages. For Chapter 2, G.M.K.Rossetti contributed to conception and design, pilot testing, data collection, data analysis, interpretation of results, preparation of figures, manuscript drafting, and manuscript revisions. For Chapter 3, G.M.K.Rossetti contributed to conception and design, pilot testing, data collection, data analysis (excluding NO_x chemiluminescence), interpretation of results, preparation of figures, manuscript drafting, and manuscript revisions. Finally, for Chapter 4, G.M.K.Rossetti contributed to conception and design, pilot testing, data collection, data analysis (excluding ASL data), interpretation of results, preparation of figures (excluding Figure 4.2), manuscript drafting, and manuscript revisions.

Author Name	Conception and design	Pilot testing	Data collection Bangor	Data collection Nepal	Data analysis	Interpretation of results	Preparation of figures	Manuscript drafting	Manuscript revisions
Gabriella M.K. Rossetti	X	X	X	X	X	X	X	X	X
Jamie H. Macdonald	X		X			X		X	X
Matthew Smith			X	X	X				
Anna R. Jackson			X	X	X				
Nigel Callender			X	X	X				
Hannah K. Newcombe			X	X					
Heather M. Storey			X	X					
Sebastian Willis			X	X					
Jojanneke van den Beukel				X					
Jonathan Woodward				X					
James Pollard			X	X					
Benjamin Wood		X	X						
Victoria Newton		X	X						
Jana Virian		X	X						
Owen Haswell		X	X						
Samuel J. Oliver	X		X	X		X		X	X

Table C.1: Summary of authorship contributions for Chapter 2. MEDEX2015: Is fitness important for individual's readiness to perform at altitude? Authors are listed according to authorship list in publication (Rossetti et al., 2017a).

Author Name	Conception and design	Pilot testing	Data collection	Data analysis	Interpretation of results	Preparation of figures	Manuscript drafting	Manuscript revisions
Gabriella M.K. Rossetti	X	X	X	X	X	X	X	X
Jamie H. Macdonald	X				X		X	X
Lee J. Wylie				X	X		X	X
Samuel J. Little		X	X		X			
Victoria Newton		X	X					
Benjamin Wood		X	X					
Kieran A. Hawkins			X		X			
Rhys Beddoe			X		X			
Hannah E. Davies		X	X					
Samuel J. Oliver	X		X	X	X		X	X

Table C.2: Summary of authorship contributions for Chapter 3. Dietary nitrate supplementation increases acute mountain sickness severity and sense of effort during hypoxic exercise. Authors are listed according to authorship list in publication (Rossetti et al., 2017b).

Author Name	Conception and design	Pilot testing	Data collection	Data analysis MRI	Data analysis Phys	Interpretation of results	Preparation of figures	Manuscript drafting	Manuscript revisions
Gabriella M.K. Rossetti	X	X	X	X	X	X	X	X	X
Giovanni d'Avossa	X	X		X		X		X	X
Matthew Rogan		X	X	X			X		
Jamie H. Macdonald	X		X			X		X	X
Samuel J. Oliver	X		X			X		X	X
Paul G. Mullins	X	X	X	X		X	X	X	X

Table C.3: Summary of authorship contributions for Chapter 4. Hypoxia modifies neurovascular coupling in the default mode network (DMN). Authors are listed according to authorship list as submitted for publication.