



**THE PHYSIOLOGICAL AND RENAL RESPONSES
TO HYDRATION STATUS IN HYPOXIA**

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ABSTRACT

The purpose of this thesis was to examine the physiological responses to hypoxia in resting and exercising conditions and evaluate how these are influenced by changes in hydration and fluid balance.

Study one showed that lower inspired oxygen fraction induced greater physiological strain, calculated using heart rate and rectal core temperature, and symptoms of altitude illness, when completing rest and exercise bouts of the intermittent walking test using inspired oxygen fractions of 0.21, 0.15 and 0.12.

The second study found that a state of body fluid deficit (hypohydration) induced significant physiological strain and increased altitude illness symptoms, compared to euhydration. While drinking a large bolus of fluid (hyperhydration) induced similar physiological strain to that of hypohydration, with reports of severe headache symptoms.

The third study dehydrated participants to different levels of hypohydration (1%, 2%, 3% of body mass loss) prior to the intermittent walking test. This found significant increases in physiological strain and altitude illness when body mass loss was greater than 2%. While 1% of body mass loss reduced the symptoms of acute mountain sickness (AMS) and headache scores, suggesting minor hypohydration may improve hypoxic tolerance.

Study four showed increases in cellular stress, quantified by heat shock protein 70 (HSP₇₀), and alterations to blood brain barrier (BBB) function when participants rested over 6 hrs in hypoxia. Rehydration in the first 2 hrs of the exposure only delayed the onset of AMS symptoms in individuals who either tolerated or dropped out of all hypoxic trials.

Study five showed how hypoxia and hypohydration induce similar cardiovascular responses and when combined these effects on physiological strain are exacerbated further. Isotonic (77mmol/L NaCl, 150mmol/L glucose) fluid rehydration in the first 2 hrs of exposure was shown to maintain fluid balance, the integrity of the BBB and reduce cellular physiological strain. Water rehydration induced the most severe Lake Louise questionnaire (LLQ) symptoms and notably severe headaches, which is likely to be a result of increased serum 100β values and therefore BBB damage.

Study six monitored hydration indices of participants trekking to Everest base camp over a 14 day period. The study showed individuals drinking approximately $45\text{ml}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ to have lower self reported symptoms of AMS. Individuals suffering with AMS tended to demonstrate a fluid retention when ascending to altitudes of 3500m.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ADH	Antidiuretic hormone
AMS	Acute mountain sickness
ANP	Atrial natriuretic peptide
BBB	Blood brain barrier
BM	Body mass
Ca²⁺	Calcium
FCH₂O	Free water clearance
CHSI	Cumulative heat strain index
Cl	Chloride
CO₂	Carbon dioxide
CpO₂	Peripheral blood oxygen content
ECF	Extracellular fluid
ESQ	Environmental symptoms questionnaire
ESQc	Environmental symptoms questionnaire cerebral score
EU	Euhydration
FEV₁	Forced expiratory volume in 1 second
FI:O₂	Inspired oxygen fraction
FVC	Forced vital capacity
eGFR	Estimated Glomerular filtration rate
HAPE	High altitude pulmonary oedema
HACE	High altitude cerebral oedema
[Hb]	Haemoglobin concentration
Hct	Haematocrit
HEAD	Feeling state headache score
HI	Hypoxic isotonic fluid ingestion trial
HSP70	Heat shock protein 70
HCR	Hypoxic cardiac response
HN	Hypoxic no fluid ingestion trial
HVR	Hypoxic ventilatory response
HR	Heart rate
HW	Hypoxic water ingestion trial

HYPER	Hyperhydration
ICF	Intracellular fluid
K	Potassium
[La]	Blood lactate concentration
LLQ	Lake Louise questionnaire
MVV	Maximal voluntary ventilation
Na	Sodium
NaCl	Sodium chloride
NN	Normoxic no fluid ingestion trial
O₂	Oxygen
PASP	Pulmonary artery systolic pressure
PETCO₂	End tidal carbon dioxide
PETO₂	End tidal oxygen
Posmo	Plasma osmolality
PO₂	Partial pressure of oxygen
PRA	Plasma rennin activity
PSI	Physiological strain index
PV	Plasma volume
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
SaO₂	Peripheral arterial oxygen saturation
TBW	Total body water
THIRST	Perceived thirst
TSS	Thermal sensation scale
Ucol	Urine colour
Uosm	Urine osmolality
USG	Urine specific gravity
Uvol	Urine volume
$\dot{V}E$	Minute ventilation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake

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This thesis is dedicated to my brother, I know you're looking down and helping me along. Your brief life continually drives me to achieve in mine.

DECLARATION

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed: A.J.Richardson

Dated: 18/05/2010

The following published articles have been due to work contained within this thesis:

Chapter IV

Richardson, A., Twomey, R., Watt, P., & Maxwell, N. (2008). Physiological responses to graded acute normobaric hypoxia using an intermittent walking protocol. *Wilderness Environ Med*, 19(4), 252-260.

Chapter V

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Chapter VI

Richardson, A., Watt, P. & Maxwell, N. (2009). The effect of hypohydration severity on the physiological, psychological and renal hormonal responses to hypoxic exercise. *Eur J Appl Physiol*, 106, 123-130

CHAPTER I.

INTRODUCTION

Hypoxia, a state of oxygen deficiency within tissues, may be caused by many environmental and physiological factors. At altitudes above sea level the partial pressure of oxygen (PO_2) reduces and results in less oxygen available for use in respiration, known as hypoxemic hypoxia. When exposed to environmental hypoxia, whether using a nitrogen enriched tent (normobaric hypoxia), pressure chamber (hypobaric normoxia) or altitude (hypobaria), the body immediately responds to the reduced oxygen availability (Lahiri *et al.* 2005). The extent of this physiological response varies between individuals and may be quantified through physiological examination (Burtscher *et al.* 2008b). When at altitude, or during laboratory based hypoxic exposure, individuals are unlikely to be in a constant euhydrated state. Although there is research into the area (Aoki & Robinson 1971; Basnyat *et al.* 1999; Nerín *et al.* 2006; Rennie *et al.* 1993) the effect of hydration state on the human response to hypoxia or altitude is not fully understood, while the control of fluid balance is known to be a key mechanism in altitude and hypoxia tolerance (Basnyat & Murdoch 2003).

This thesis is presented in the following chapters.

Chapter II reviews the literature surrounding the physiological changes that occur with hypoxic exposure at rest and during exercise within the human body. Particular interest is paid to the pathophysiology and prediction of hypoxic illnesses. The review also

investigates the physiological and renal hormonal changes that occur with hydration status and fluid balance and the interaction of these with hypoxia.

Chapter III describes the common methods used throughout all experimental chapters.

Chapter IV presents the first study, which identifies the simple physiological changes that occur at rest and during exercise within hypoxia. This investigation assesses the use of an intermittent walking protocol to allow differentiation between individuals tolerance to acute normobaric hypoxia.

Chapter V uses the intermittent walking test to identify differences in physiological responses and hypoxic tolerance with hydration states above and below that of a euhydrated level.

Chapter VI follows on from Chapter V by investigating the effects of graded hypohydration on physiological responses and tolerance to hypoxia, as Chapter V evidenced the negative consequences of hypohydration within hypoxia.

Chapter VII introduces the role of rehydration within hypoxia and the influence on fluid balance at rest. This chapter also examines the physiological effect of fluid balance states on a longer duration resting hypoxic insult.

Chapter VIII leads on from Chapter VII and examines the effect of fluid balance changes on a hypoxic intermittent walking protocol as detailed in the first three experimental chapters.

Chapter IX is a field based study investigating the changes in hydration while trekking at altitude in a large heterogeneous sample. The chapter examines the physiological and renal responses that occur with acclimatisation to altitude.

Chapter X comparatively discusses all the experimental studies, with particular detail surrounding the influence of hydration on an individual's tolerance to hypoxia and altitude.

CHAPTER II.

REVIEW OF LITERATURE

2. Introduction

This literature review aims to identify the physiological changes that occur with hypoxia, how these relate to individual's tolerance to hypoxic exposure and how this tolerance has been quantified. Physiological markers related to hypoxic tolerance are investigated and evaluated for use within the prediction of hypoxic tolerance through screening tests. Focus will be on the quantification of hypoxic tolerance and pathophysiological mechanisms that relate to acute mountain sickness symptoms through acute hypoxic or altitude exposure.

The review then investigates the maintenance of fluid balance during exercise and hypoxia. Focus of the review will be on the measurement, quantification and physiological consequences of hypohydration, hyperhydration and rehydration. These hydration states will be explored across a range of environmental conditions, with reference to how they may interact with the pathophysiological occurrence of hypoxic tolerance and acute mountain sickness symptoms.

Finally the review will investigate the simulation of altitude exposure through normobaric hypoxia. The common exercise intensities and patterns that are performed within this environment will be evaluated, in order to effectively simulate rest and exercise at altitude.

2.1. Hypoxia

2.1.1 History of Hypoxic Research

Through the invention of the barometer in 1644 by Evangelista Torricelli, the explanation of Boyle's law in 1660, the description of respiratory gases by Lavoisier in 1777 and the connection between low partial pressures of oxygen and high altitude illness by Paul Bert in 1878, science has slowly pieced together the physiological consequences of hypoxia. However, A.M Kellas in 1921 was the first individual to question correctly human ability in hypoxia, in his paper, unpublished until 2001 (Kellas 2001). He debated the possibility of humans summiting what was by then known as the highest mountain in the world, Mount Everest. Kellas, in 1921, identified the main issues associated with climbing Mt Everest, including physical and physiological difficulties, such as acclimatisation protocols, which are still the main logistical and scientific considerations today.

In 1953 Tenzing Norgay Sherpa and Sir Edmond Hillary summited Mt Everest and although non-scientific, the expedition was to show the world how man could push the perceived limits of human physiology. This feat drove L.C.G.E Pugh, the physiologist for the successful 1953 expedition, to further the understanding of high altitude physiology by leading the 1960 Silver Hut expedition. The scientists carried out extensive physiological and neuropsychological measurements on themselves while residing in the silver hut at 5800m for several months. Later, in 1981 the American Medical Research Expedition to Everest (AMREE) recorded physiological data on the summit including electrocardiograms, barometric pressure and alveolar gas.

Operation Everest I (1946) and II (1985), carried out by Charles Houston and colleagues (Houston 1997), were not expeditions but decompression chamber studies measuring a

range of physiological markers, while participants gradually decompressed to the summit of Everest over a period of 4-6 weeks. Although these studies may be ethically controversial now, the invasive measurements generated valuable information regarding the physiological changes that occur with hypobaria. The recent 2007 Caudwell Xtreme Everest expedition, carried out a variety of physiological and neuropsychological tests on two hundred and sixteen individuals walking to Everest Base Camp, while a further fifteen were tested as they climbed to the summit (Grocott *et al.* 2007). The main goal of this research was to relate the acclimatisation process and individual's tolerance to hypoxia, to clinical populations who suffer with hypoxia. The expedition produced the highest arterial blood sample (8500m) (Grocott *et al.* 2009) and highest cardiopulmonary exercise test (8050m) (Grocott *et al.* 2007).

The brief historical outline of hypoxic research demonstrates how a physiological emphasis has developed greater importance within medical research. There is a realisation that by investigating the human physiological responses, adaptations and tolerance to a hypoxic stimulus may allow insight into pathophysiological alterations at altitude and more importantly, the treatment of clinical patients suffering from hypoxia or its consequences.

2.1.2. Acute Mountain Sickness

Acute mountain sickness (AMS) is defined as a syndrome of nonspecific symptoms, thus being subjective (Hackett & Roach 2001), although there is dispute over AMS determination (Bartsch *et al.* 2004). The Lake Louise Consensus Group (Roach *et al.* 1993) suggested AMS is the presence of a headache in an unacclimatised person who has recently travelled to over 2500m and additionally, has one of the following symptoms;

gastrointestinal upset, dizziness, insomnia, lassitude or fatigue. Although symptoms usually occur within 6-10 hours of ascents, cases have been recorded after only 1 hour (Basnyat & Murdoch 2003).

Key factors influencing the development of AMS are the degree of pre-acclimatisation, speed of ascent and altitude (Bartsch *et al.* 1991b; Honigman *et al.* 1993; Houston & Dickinson 1975; Schneider *et al.* 2002). Individual susceptibility also has a major contribution towards the development of altitude illness (Basnyat & Murdoch 2003), yet the physiological predictors of susceptibility are largely unknown (Burtscher *et al.* 2008b). Incidence rates vary from 13% to 40% at altitudes of 3000-4000m (Basnyat *et al.* 1999; Hackett *et al.* 1976; Honigman *et al.* 1993; Maggiorini *et al.* 1990; Vann *et al.* 2005) and increase with severity of altitude exposure (Honigman *et al.* 1993; Vann *et al.* 2005). However, there is evidence to suggest that these incidence rates reduced from 1986-1998, with the increase in AMS awareness causing slower ascent profiles (Gaillard *et al.* 2004).

2.1.2.1. Pathophysiology of Altitude Illness

The mechanisms surrounding AMS development are still widely contested (Bartsch *et al.* 2004; Basnyat & Murdoch 2003; Hackett 1999), causing many papers to criticise the current understanding of the condition and suggest ways that future research could take a valid and reliable mechanistic stance (Bartsch *et al.* 2004; Roach & Hackett 2001).

The literature offers a dearth of research on the hypoxic brain, culminating in many theories and suggestions regarding AMS development (Schoene 2008). Most recent suggestions of the AMS development pathway have been presented by Basnyat &

Murdoch (2003) as shown in Figure 2.1, while cerebral based AMS development is well presented by Schoene (2008). Other authors have presented similar, slightly more detailed flow charts (Hackett & Roach 2001; Roach & Hackett 2001), which all centre around hypoxemia causing either cerebral blood volume, flow or permeability of the blood brain barrier to increase, ultimately causing brain swelling and AMS. Yet, other than the general pathway, this is as detailed as current research can agree at present (Bartsch *et al.* 2004).

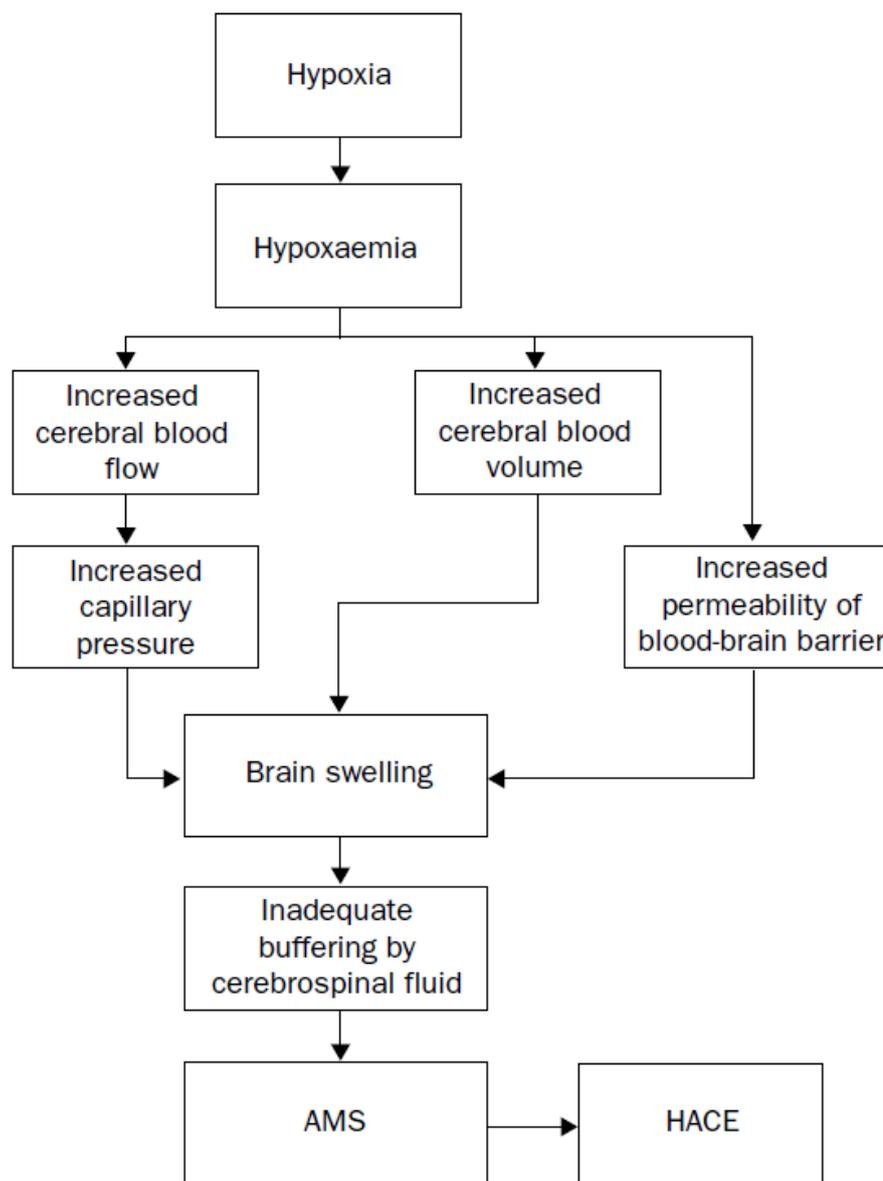


Figure 2.1: Proposed pathophysiology of AMS and HACE by Basnyat & Murdoch (Basnyat & Murdoch 2003).

The cerebral brain swelling theory can be distinguished into two separate sub-theories for an individual's susceptibility, either the ability to cope with the increases in cerebral cell swelling or prevention of swelling (Hackett & Roach 2001) via fluid control, or being able to maintain the integrity of the blood brain barrier (Basnyat & Murdoch 2003; Kaur & Ling 2008). These mechanisms will be discussed individually in sections 2.1.4. Researchers have suggested a 'tight fit' hypothesis in which the cranium to cerebrum ratio may determine whether cerebral swelling can be tolerated (Ross 1985). However, this theory needs far greater empirical evidence before it has any real support, it has been shown fluid control (Loeppky *et al.* 2005a) and swelling (Roach & Hackett 2001) is not the same in all individuals and thus cranium size cannot be the only factor relating to altitude tolerance (Ross 1985).

2.1.2.2. Measuring Acute Mountain Sickness

The diagnosis of AMS has always been problematic due to the nature of reporting subjective symptoms (Bartsch *et al.* 2004). Nevertheless, there have been progressive advancements in the determination of AMS. Singh *et al.* (1969) were the first to acknowledge the presence of headache as the key self - report symptom. This led to the production of scoring systems such as the General High Altitude Questionnaire (Evans, 1966), the Hackett score (Hackett, 1976) and Environmental Symptoms Questionnaire (ESQ) (Sampson *et al.* 1994). Finally in 1991 through a working group consensus, Hackett & Oslz (1991) produced the Lake Louise Scoring system (LLQ), which is the most commonly used AMS scoring scale used presently and referred to as the gold standard method (Roach & Kayser 2007).

Wagner *et al* (2007) used a visual analogue scale (VAS) for patients to self report AMS symptoms. The VAS was trialled on 396 climbers on Mt Whitney, up to 4419m. When compared to the standard LLQ scoring system there was a reasonable correlation ($r=0.65$, $P<0.05$), while it demonstrated a strong test-retest reliability (ICC = 0.996, 95% CI = 0.992 to 0.998). However, the main difficulty with the scale is the population specific issues, such as language and perceptions of words. Dellasanta *et al* (2007) showed in 266 non-Anglophone trekkers that the ESQ and the LLQ identified different people to have AMS (LLQ score >3). While at LLQ scores of >5 showed greater similarity to ESQ, although 20% of cases were still diagnosed differently. It would appear there are still issues with AMS diagnosis when comparing between questionnaires and across population groups.

2.1.2.3. Prediction of Acute Mountain Sickness

No single variable exists that can accurately and reliably predict an individual's tolerance to altitude, hypoxia or hypobaria. Hence, groups have attempted to combine many variables in the hope that a combination of factors can be assessed in order to calculate altitude tolerance.

In a recent review, Burtcher *et al* (2007) compared sixteen prediction tests. Although the review attempts to identify all the factors associated with AMS, variation of protocols, measurement techniques, AMS diagnosis, exercise modes, ascent profiles and poor sample sizes means comparison is difficult. Therefore, Burtcher *et al* (2007) simply identified the test with the highest reported prediction rate, to be the best method; interestingly this test, was the test previously devised by Burtcher *et al* (2004) discussed later in section 2.1.3.3. The authors report that the test with the next highest identification

rate also uses SaO₂ as a means of AMS prediction (Roach *et al.* 1998). Although the study (Roach *et al.* 1998) uses the resting SaO₂ values at 4200m to predict tolerance, which is of little use as many individuals suffer with altitude illness well before 4200m. Burtscher *et al.* (2007) also miss out a key study by O'Connor *et al.* (2004) that implies pulse oximetry has a limited association to AMS, as later described in section 2.1.3.3.

Dissapointingly, Burtscher *et al.* (2007) did not report the recent work by Savourey *et al.* (2007) presumably due to the short time between publications. The prospective study investigated a good range of measures in normobaric hypoxia and hypobaric normoxia before participants climbed at high altitude. Savourey *et al.* (2007) showed that normobaric hypoxic and normoxic hypobaric conditions are physiologically different, and go on to suggest that normobaric hypoxia is practically the most viable. The authors conclude that factors such as end tidal CO₂ (PETCO₂), peripheral O₂ blood content (CpO₂), change in breathing frequency and hypoxic cardiac response (HCR) are necessary in the prediction of AMS. A criticism of the study would be that the subjects were split into two separate expeditions to the Andes and Himalayas and thus, undoubtedly had different ascent profiles, maximal height gained, expedition duration and barometric pressures between groups. Consequently this would mean AMS peak and mean could be different between climbs.

Grant *et al.* (2002) attempted to answer directly whether sea level physiological measures can predict altitude tolerance. The study found poor correlation between the prediction variables and AMS. The study only measured SaO₂, PETCO₂ and hypoxic ventilatory response (HVR) as prediction markers, while other studies have evidence of a greater variety of physiological markers that may indicate AMS susceptibility (Burtscher *et al.*

2008b). Altitude research has no strong evidence of any clear, valid and reliable protocol that may be used to predict later altitude or hypoxia tolerance thus far.

2.1.3. Physiological Effects of Hypoxia

2.1.3.1. Oxygen Sensing in Acute Hypoxia

Acute hypoxia requires an immediate response by the body to regulate oxygen (O_2) supply to the tissues, initiating cardiorespiratory reflexes and enzymatic changes to cope with the reduction in PO_2 . Low PO_2 is first noted by the glomus cells of the carotid body. Research then debates the following process, supporting both the mitochondrial hypothesis and membrane model (Lahiri *et al.* 2005).

The mitochondrial hypothesis explains that calcium (Ca^{2+}) release from mitochondrial endoplasmic reticulum is due to electron transport from the substrate through the respiratory chain becoming degraded at lower O_2 pressures causing mitochondrial membrane depolarization (Lahiri *et al.* 2005) (Figure 2.2).

The membrane model suggests low PO_2 inhibits the plasmalemma potassium (K^+) channels causing depolarization of the glomus cells, increasing the firing frequency (Shirahata & Sham 1999). This depolarization also activates the voltage gated Ca^{2+} channels causing Ca^{2+} influx from the extracellular space, whereby Ca^{2+} is stored at 2×10^4 times greater concentration than in normoxia. This Ca^{2+} release results in a release of neurotransmitters from the carotid body (Lopez-Barneo *et al.* 2004; Shirahata & Sham 1999).

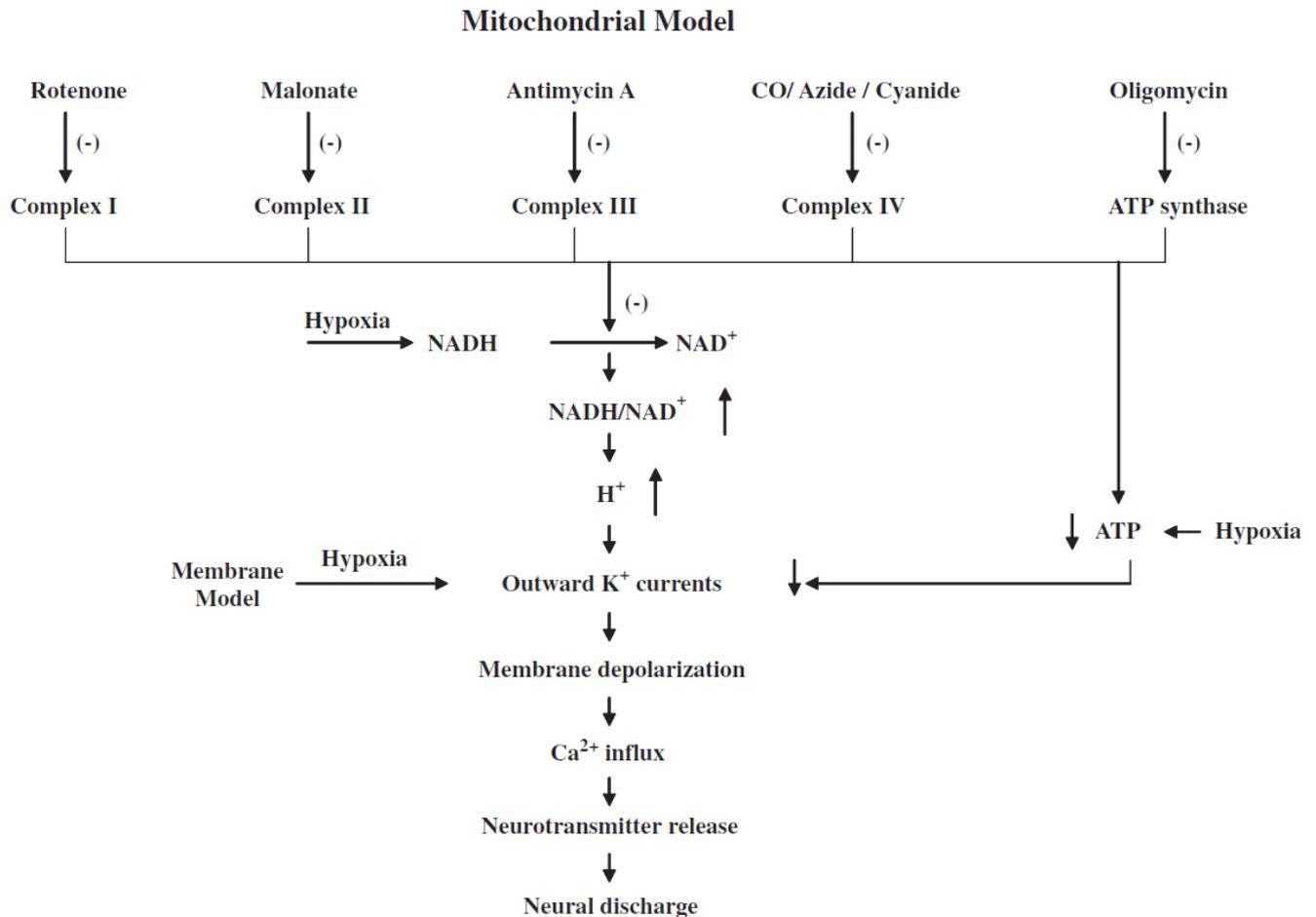


Figure 2.2: The mitochondrial and membrane model of oxygen sensing. Sites of complex I, II, III and IV on the inner mitochondrial membrane. Proposed sites of inhibition of the respiratory chain by specific drugs as indicated. This is followed by H⁺ increase and then the membrane model comes into the picture (Lahiri *et al.* 2006).

The neurotransmitters can be classified into two groups; conventional and unconventional. The conventional neurotransmitters stored in synaptic vesicles activate membrane receptors and local afferent nerves. Unconventional neurotransmitters such as carbon monoxide and nitric oxide directly affect proteins and cytosolic enzymes (Lopez-Barneo *et al.* 2004). Research is unclear about the true mechanisms surrounding neurotransmitter release from the carotid body during hypoxia. Ultimately, they are known to increase

pulmonary vasoconstriction, increase systemic vasodilation and promote excitation of the central chemoreceptors (Peers 2002).

The pulmonary arteries are also capable of noting and responding to reductions in PO_2 . Research (Gurney 2002) suggests that O_2 levels can be sensed in the smooth muscle cells of the artery walls whereby either K^+ channels, Ca^{2+} stores, sarcoplasmic reticulum or Ca^{2+} sensitivity of the contractile proteins act as the primary O_2 sensing mechanism. When hypoxia is sensed the pulmonary arteries tend to respond by vasoconstriction which can be both beneficial, by diverting blood from hypoxic alveoli to ventilated areas, or negative in that vasoconstriction over a long period can cause vascular leakage causing extracellular water retention (Bartsch *et al.* 2005). This is widely suggested to be a major contributing factor in the onset of high altitude pulmonary edema (Loeppky *et al.* 2005a) and possibly, AMS (Bailey *et al.* 2004).

Semenza & Wang (1992) attempted to identify the mechanism initiating the erythropoietic response to hypoxia. This ultimately led to the discovery of hypoxia-inducible factor (HIF), which would later be separated into subunits HIF-1 α and HIF-1 β (Wang *et al.* 1995). Proline residue 402 and 564 in HIF-1 α can be hydroxylated by prolyl hydroxylase (PHD). During normoxia hydroxylation of proline causes the binding of von Hippel-Lindau tumour suppressor protein (VHL). This binding process leads to the degradation of HIF-1 α (Bruick & McKnight 2002). Another factor inhibiting HIF (FIH) hydroxylates asparagine residue in HIF-1 α , reducing its ability to activate transcription by inhibiting binding of the transcriptional coactivator (Lando *et al.* 2002). However, under hypoxic conditions prolyl hydroxylase cannot be activated, allowing HIF-1 α to accumulate and translocate to the nucleus where it may bind to HIF-1 β to form HIF-1. HIF-1 is then

activated when bound to co activators known as CBP/p300 (Smith *et al.* 2008). This process is illustrated in Figure 2.3. HIF-1 was shown to have a central regulatory role at a cellular and systemic level (Semenza 2004). Semenza (2004) documented that HIF regulates several hundred genes that control cell growth, angiogenesis, apoptosis, metabolism and vasomotor activity, to improve the delivery and utilisation of oxygen. Although there has been much research over the seventeen years since HIF's discovery (Semenza 2009a, b), there is still little known regarding the role HIF has in hypoxia tolerance and acclimatisation (Semenza 2006).

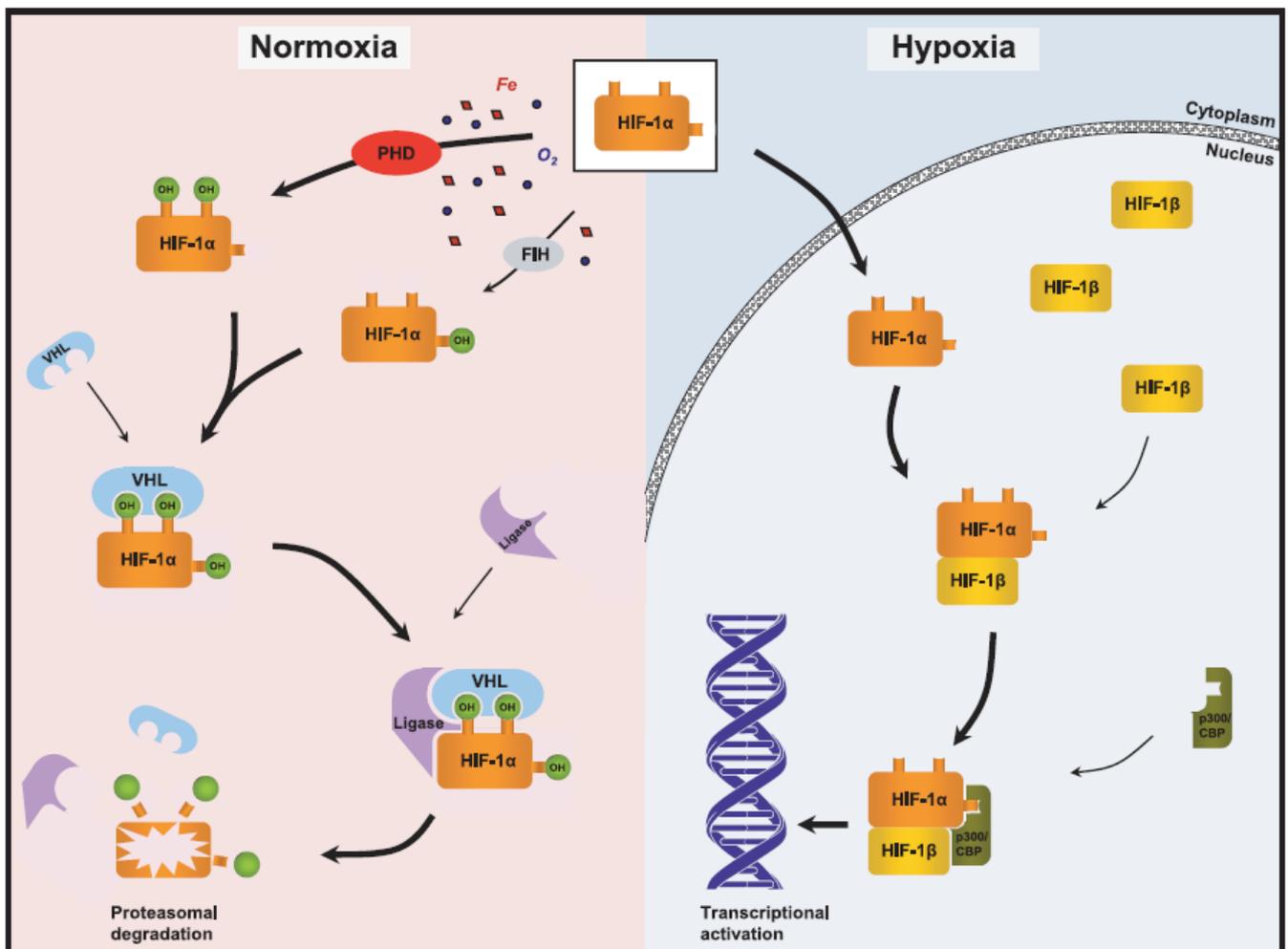


Figure 2.3: Schematic representation of the PHD-VHL-HIF axis. OH denotes hydroxyl group. Adapted from Smith *et al* (2008).

2.1.3.2. Autonomic Responses to Hypoxia

The immediate response to acute hypoxia, investigated in a range of species, is an increase in sympathoadrenal activity (Hainsworth *et al.* 2007).

Rowell & Blackmon (1986) noted a non-significant increase in sympathetic activity when exposing humans to acute bouts of hypoxia (10.4% O₂) over a 20min period and a lower dose (7.6% O₂) for a further 15min in those individuals that were thought to tolerate the higher dose of hypoxia. Limited by ethical considerations, one participant was forced to withdraw due to a bradycardic response. This highlights the individualistic response to hypoxia and the problematic interpretation of data with such low sample size [n=6 and 4 (7.4% O₂)]. However, significant sympathetic nervous activity increases have been reported on a variety of other species (Heistad & Abboud 1980).

Chen *et al* (2008) drove twenty-seven unacclimatised participants from 550m to 3180m over 3 hours. Of the twenty-seven participants, thirteen were diagnosed with at least minor AMS symptom scores, quantified by the Lake Louise Questionnaire (LLQ) score (LLQ >3) after 24hours. The authors (Chen *et al.* 2008) found no significant changes in parasympathetic or sympathetic activity, which they associated with variation in individual responses to altitude tolerance. Although of a longer hypoxia duration, these results are similar to those of Rowell & Blackmon (1986) in regard to individual tolerance and cardiac responses. Clearly, a larger sample size differentiating between hypoxia tolerance through dichotomous groups or a continuous relationship, is needed to assess the acute sympathetic response to hypoxia. Loeppky *et al* (2003a) highlight the relationship that sympathetic activity and AMS may have. Investigating the effect of resting exposure to 423mmHg / 4850m over 8-12hrs in fifty-one participants, they found that AMS

sufferers had a vasodilatory response with a increase in sympathetic activity. The authors concluded that AMS could be associated with augmentation of β -adrenergic tone (Loeppky *et al.* 2003a) also suggested earlier by Rathat *et al* (1992).

Normal physiological responses would predict an acute heart rate increase in response to O₂ decline. Wilkins *et al* (2008) support this suggestion, finding a significant rise in heart rate with hypoxia, while they noted no significant increase with rest or incremental forearm activity in hypoxia, possibly due to a blunted adrenergic response from higher resting adrenaline values, resulting from the downregulation of the adrenoreceptors.

With prolonged exposure over 15 days (Voelkel *et al.* 1981), heart rate at rest and during exercise may reduce in response to the constantly elevated circulating catecholamines (Antezana *et al.* 1994; Favret & Richalet 2007; Savard *et al.* 1995). To support this concept, exposures of 1-15 days were not found to have any significant effect on α_1 (Morel *et al.* 2003) or β -adrenergic receptor density (Kacimi *et al.* 1993). While research by Leon-Velarde *et al* (2001) investigating exposures of greater than 21 days, found a significant decline of 24% β -adrenergic receptor density. Studies (Kacimi *et al.* 1993) of up to 30 days have seen greater declines of 46%. However, receptor sensitivity and post membrane events could also contribute to a redeuction in the effectiveness of adrenaline.

2.1.3.3. Peripheral Arterial Oxygen Saturation

Peripheral arterial oxygen saturation (SaO₂) is known to fall during hypoxia due to low PO₂ reducing haemoglobin's affinity for O₂. Studies have shown greater SaO₂ reductions in AMS susceptible individuals during acute (Bhaumik *et al.*, 2003) and chronic (Roggla *et al.*, 2000) hypoxia. Burtscher *et al* (2004) noted that AMS susceptibles' SaO₂ was 4.9%

lower than non susceptible after a short 20-30 minute exposure at rest (Figure 2.4). This was found to be the closest prediction of AMS to date (Burtscher *et al*, 2007), as described in section 2.4. Roach *et al* (1998) found SaO₂ had a negative correlation with AMS symptoms in one hundred and two climbers at 4200m. At lower altitude of 3100m, Roggla *et al* (2000) found the three AMS sufferers had greater reduction in SaO₂ compared to the thirty-seven non sufferers. Schirlo *et al* (2002) also found significantly lower SaO₂ values in HAPE susceptibles.

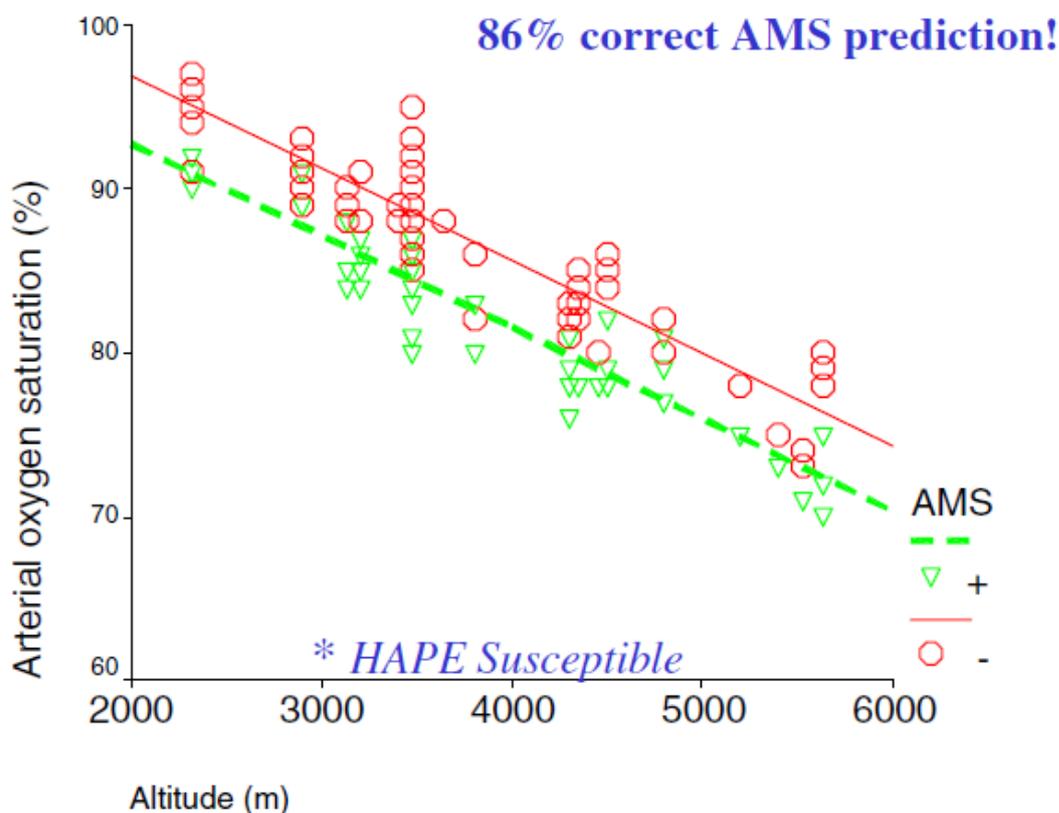


Figure 2.4: Comparison of SaO₂ values at different altitudes between AMS and non-AMS sufferers ($r = 0.86$, $p < 0.05$), adapted from Burtscher *et al* (2004).

However, there are also some studies that have found no significant relationship between SaO₂ and mountain illness (Dubowitz *et al*, 2005; Bartsch *et al*, 2002; Hohenhaus *et al*,

1995; O'Connor *et al*, 2004). Dubowitz *et al* (2005) emphasised that SaO₂ was not related to AMS score in 45 hikers at 3080-4367m. Likewise, Hohenhaus *et al* (1995) explained that SaO₂ was not different between those susceptible to AMS, HAPE or not susceptible when cycling in normobaric hypoxia at 21%, 14% or 12%O₂. Finally, O'Connor *et al* (2004) suggest that SaO₂ via pulse oximetry is not an adequate predictor of AMS, calling for further research into the prediction of AMS using simple techniques.

Interestingly, the studies which found no relationship between AMS and SaO₂ only used participants who had experienced altitude or hypoxia within the last week or less (O'Connor *et al*. 2004; Roach *et al*. 1995; Roeggla *et al*. 1996). An epidemiological study using a large sample of five hundred and fifty trekkers with various ascent profiles, suggested that other than fluid intake, SaO₂ was the only other factor related to AMS incidence. Basynat *et al* (1999) go on to state that a SaO₂ value of <85% at 4243m suggests trekkers are 2.35 times more likely to suffer with AMS (95% confidence interval, 1.55-3.56). Although by ascent to 4243m, development of AMS would have already occurred in the majority of cases, thus have no use as a predictive tool. O'Connor *et al* (2004) suggest the variations in study findings are derived from previous altitude exposure and the ascent profiles undertaken, as ventilatory alterations to altitude follow a separate response time than that of cerebral alterations. Meanwhile, the chronological SaO₂ responses to acute hypoxic exposure have limited evidence (Garcia *et al*. 2000). Furthermore, degree of altitude exposure may also be important with higher altitude studies (Basynat *et al*. 1999; Roach *et al*. 1998) inducing greater ventilatory changes. Further research needs to document alterations in SaO₂ and AMS with ascent and acclimatisation to hypoxic exposure using a large controlled study sample. Measurement techniques, such as pulse oximetry, also require improvements in validity and reliability.

Roach *et al* (2000) found that exercise within hypoxia induces greater hypoxemia and thus exacerbate AMS symptoms (Roach *et al.* 2000) (Figure 2.5). Clearly, further research needs to identify the effect of exercise in hypoxia on physiological and symptomatic alterations, and stipulate the exercise intensities performed.

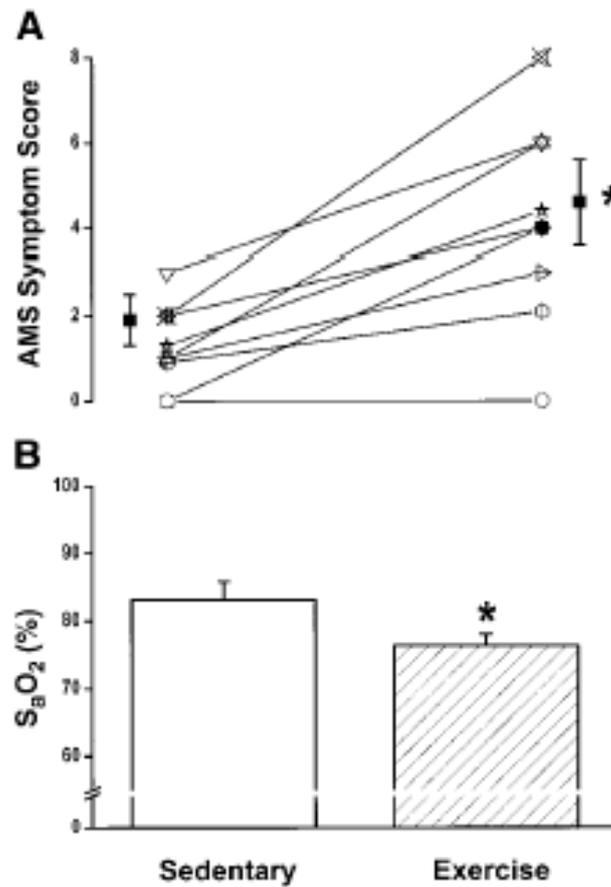


Figure 2.5: Comparison of LLQ and SaO₂ scores after rest and exercise at altitude, adapted from Roach *et al* (2000).

2.1.3.4. Ventilatory Effects of Hypoxia

Barcroft in 1925, was the first to suggest differences in lung development between low altitude and high altitude natives. However, these differences in chest circumference and vital capacity were actually very small when comparing low altitude natives and South American Indians residing above 4500m. Further evidence to reject Barcroft's hypothesis

showed South American Quecha of ~3500m had smaller measurements than the USA as a whole (Stinson & Frisancho 1978). In contrast, Tibetans have been shown to have significantly greater vital capacities than high altitude natives (mean high altitude residency of 8yrs) (Sun *et al.* 1990). This suggests a genetic difference in lung development. Wood *et al.* (2003) showed particularly high peak flows (>139%) in Himalayan residents above 3300m. Similarly, Apte & Rao (2005) found a significantly greater forced expiratory volume in Ladakh natives (~3450m) compared to acclimatised lowlanders when corrected for height.

Although there may be evidence for genetic differences in lung function, which possibly relates to altitude tolerance, there is limited research on lung function and susceptibility to mountain illnesses. Mason *et al.* (2000) investigated the changes in spirometry variables on ascent to 5300m. At sea level spirometry had no predictive value to those later suffering with AMS. Further, there was no correlation between AMS, SaO₂ and the lung function alterations. There are many other studies detailing spirometric changes with ascent to altitude (Loeppky *et al.* 1997; Moore 2000; Pollard *et al.* 1997; Sharma & Brown 2007), evidencing a significant decline in lung function above 3500m, though the link between these changes with ascent profile or from sea level are not, as yet, linked with AMS development.

Ventilation is known to rise during a hypoxic insult (Loeppky *et al.* 1997). In some cases at altitude, ventilation may decrease (Garcia *et al.* 2000), which is known as hypoxic ventilatory depression. This is likely to be a result of hypoxia - induced decline in O₂ sensitivity (Powell *et al.* 1998), though others (Sato *et al.* 1992) suggest this may not be the only mechanism.

This individual ventilatory response may also relate to individual's tolerance to hypoxia. Moore *et al* (1986) was one of the first to suggest that a low hypoxic ventilatory response (HVR) was related to HAPE susceptibility, finding ventilatory blunting for any given SaO₂ in the HAPE symptomatic than non-symptomatic group. Although, the sample sizes (n = 4 + 4) were low when considering such variable responses and accuracy of the pulse oximetry. Bartsch (2002) found that low altitude HVR assessment was not related to AMS in individuals climbing to 4559m over a 3 day period. Although, Bartsch (2002) suggested that failure to increase HVR on immediate exposure to high altitude resulted in severe hypoxemia and AMS, which would support Moore *et al*'s (1986) experiment which only used 4.5hr of simulated altitude. A significant number of studies have since been performed either using altitude or hypoxia to investigate the predictive value of HVR on mountain illness. Studies have found evidence both for (King & Robinson 1972; Matsuzawa *et al.* 1989; Moore *et al.* 1986; Selland *et al.* 1993) and against (Milledge *et al.* 1991; Sutton *et al.* 1976) HVR and the relationship to AMS, or other altitude illnesses. Hohenhaus *et al* (1995) suggested that only HAPE susceptibles have a low HVR, while AMS susceptibles may have a low or high HVR in comparison to the mean non-susceptibles. Bartsch *et al* (2002) suggest that low altitude HVR testing cannot predict AMS, though failure to increase HVR with ascent to high altitude may induce hypoxemia and thus AMS.

Recent research by Bernardi *et al* (2006) on the Italian K2-Everest Expedition (Spring-Summer 2004) found contradictory evidence, suggesting a low HVR allows individuals to ascend higher, as individuals with higher HVR exhaust their maximal voluntary ventilation (MVV) at lower altitude, while individuals with low HVR have greater

ventilatory efficiency and thus ventilatory reserve volume to cope with the highest altitudes (Figure 2.6).

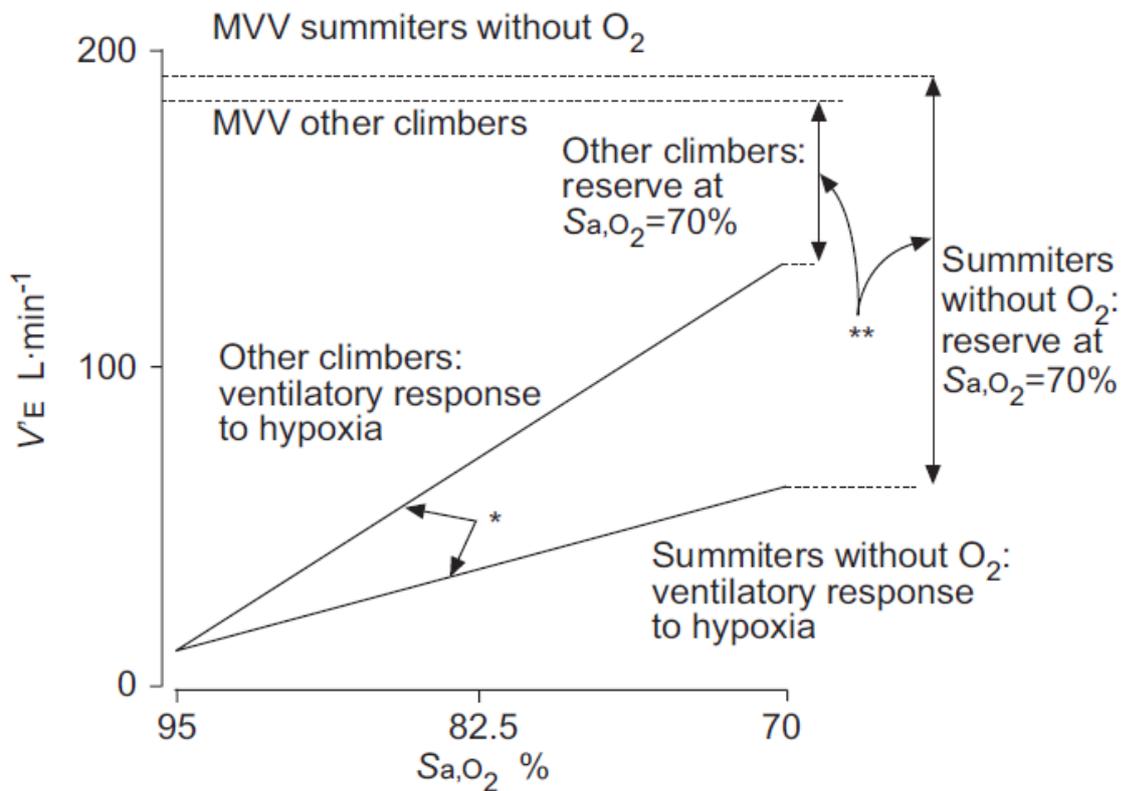


Figure 2.6: Schematic diagram showing the relationship between the ventilatory response to hypoxia and the ventilatory reserve. Data were obtained after fifteen days at altitude (9 days at 5,200 m). The significant differences reported, refer to the difference in HVR mean slopes between the two groups (which were drawn from 95% S_{a,O_2} down to 70%), and to the mean ventilatory reserve. The ventilatory reserve was calculated by subtracting the ventilation reached at S_{a,O_2} of 70% from MVV. *: $p < 0.05$; **: $p < 0.01$. Adapted from (Bernardi *et al.* 2006).

In the most recent HVR study, Bernardi *et al* (2007) attempted to build on their previous work, finding lower HVR in high altitude yoga trainees (Buddhist monks) compared to physically active Sherpas, suggesting HVR is not wholly responsible for performance at extreme altitude as previously described (Bernardi *et al.* 2006). Interestingly, Bernardi *et*

al (2007) showed individuals practicing yoga maintained satisfactory oxygen transport, with decline in haematocrit and haemoglobin, implying ventilatory control and efficiency may be learnt and trained through yoga - based respiratory training to improve hypoxic tolerance.

End tidal CO₂ (PETCO₂) may be an indicator of hypoxic tolerance. Savourey *et al* (1994) found that PETCO₂ was the only physiological variable to correlate with AMS and ESQ score, when comparing a range of markers such as heart rate, SaO₂ and ventilation, at sea level and altitude. Similarly, Reeves *et al* (1993) found sea level PETCO₂ to correlate with altitude PETCO₂ values. However, Muza *et al* (2000) found no relationship between PETCO₂ and AMS, though they found sea level PETCO₂ to correlate positively ($r = 0.57$, $p < 0.05$) with PETCO₂ in hypobaric hypoxia equal to 4300m altitude, when measuring twenty volunteers over a 4hr exposure. Grant *et al* (2002) also showed PETCO₂ had no significant correlation to AMS score in twenty two individuals trekking to 4320m. Although, correlations were stronger at the higher altitudes when AMS scores were greater and there were fewer extraneous factors influencing AMS diagnosis using the scale.

Terblanche *et al* (2004) evaluated the reliability and validity of physiological markers measured in hypoxia. The authors suggest that both cardiac (HCR) and ventilatory (HVR) responses to hypoxia (FIO₂: 0.08) are reliable measures and repeatable between visits (HVR: $r = 0.76-0.92$; HCR: $r = 0.35-0.76$).

2.1.3.5. Measurement of Core Temperature

The four commonly used locations for measurement of core temperature are the rectum, oesophagus, tympanic membrane and intestines. No single location, except arguably hypothalamic temperature, offers a true core temperature due to localised variations in temperature, especially during exercise (Lim *et al.* 2008; Livingstone *et al.* 1983; Sparling *et al.* 1993). Oesophageal thermometry tends to give the most accurate quantification of core temperature changes during exercise due to depth of measurement and close proximity to the heart (Gass & Gass, 1998). In comparison, rectal temperature may be slower to react to exercising changes (Gibson *et al.*, 1981) and may produce values greater than true core temperature due to the proximity to exercising leg musculature and the path of the heated blood from the exercising legs. During severe internal climatic changes, rectal temperature may be the best representation of true core temperature (Livingstone *et al.* 1983). Saltin & Hermansen (1966) showed resting rectal core temperature values were greater than oesophageal by $\sim 0.1^{\circ}\text{C}$, while differences increased to 0.2°C with submaximal exercise of varying intensity. In support, Gibson *et al.* (1981) found rectal temperature was greater by 0.1°C at rest, while Nielsen & Davies (1976) suggest rectal can generate values up to 0.6°C greater than oesophageal after 150 min of cycling.

Other non invasive techniques such as oral, axillary, tympanic and temporal have been compared to rectal core temperature when exercising outdoors in normothermic conditions. Casa *et al.* (2007) found that none of the measures produced values close to rectal temperature, with the limit of difference set at $\pm 0.27^{\circ}\text{C}$. Lefrant *et al.* (2003) compared four measures of core temperature against pulmonary artery temperature and found that rectal ($-0.07 \pm 0.4^{\circ}\text{C}$) and oesophageal ($0.11 \pm 0.3^{\circ}\text{C}$) produced similar temperatures to that of pulmonary artery temperature, in intensive care patients.

2.1.3.6. The Effects of Hypoxia on Core Temperature

Changes in core temperature have been linked to the onset of AMS (Maggiorini *et al.* 1997a; Roggla *et al.* 2000b). However, field based assessment of hypoxic effects on core temperature can be difficult, as environmental conditions tend to induce greater changes in core temperature (Livingstone *et al.* 1983), irrespective of hypoxia extent. At altitude, invasive measurement techniques may not be easily performed, or welcomed by participants. Studies monitoring core temperature changes within hypoxic normothermic conditions within the laboratory have found no such correlation between AMS and body temperature (Loeppky *et al.* 2003a; Roggla *et al.* 2000a). Loeppky *et al.* (2003a) noted that in AMS sufferers' core temperature initially increased by 0.2°C after 1hr, but then decreased by 0.4°C from hours 1-6. In contrast, non AMS sufferers body temperature increased by 0.3°C by 6hrs, opposing the findings of Roggla *et al.* (2000b). The reason for these differences are likely to be the result of exercise and varying environmental conditions within the field based research by Roggla *et al.* (2000a), whilst the controlled laboratory - based test by Loeppky *et al.* (2003) used a far shorter duration. This vasodilatatory response to hypoxia induced through redistribution of blood to the periphery (Weisbrod *et al.* 2001) seems to be an acute response lasting up to 6hrs in AMS sufferers only (Loeppky *et al.* 2003a). This theory is actually supported by Loeppky *et al.* (2003a) whom recorded a rise in both AMS and non AMS groups from 6 hrs onwards.

Maggiorini *et al.* (1997a) examined sixty climbers ascending from sea level to 4559m over 22 hrs. The severe ascent profile was deliberately enforced in an effort to induce AMS, HAPE or HACE. Auxillary temperature was monitored prior to ascent and throughout the three day exposure. The study suggested body temperature was strongly correlated with mountain sickness (Day One $r=0.54$; Day Two $r=0.48$; Day Three $r=0.62$; Day four

$r=0.6$). This finding along with the work of Roggla *et al* (2000b) suggests mountain sickness induces an inflammatory response increasing core body temperature. Further support for this theory comes from recording plasma concentration of interleukin 1 and interleukin 6 in individuals suffering with mountain sickness.

2.1.3.7. Measures of Physiological strain

There are currently a range of physiological strain indices, which have been developed and improved in sensitivity over time. The first, developed in 1945 by Robinson *et al* (1945) called the physiological effect index was developed for workers exposed to hot environments. The index quantified the heat strain as a ratio of heat exposure against the hottest tolerable conditions. Gold (1961) suggested a heat strain index based on heart rate alone, validating the index at rest in hot environments (38-71°C). Hall & Polte (1960) developed an index of physiological strain which was the first to calculate strain based on resting and current heart rate, core temperature and sweat rate. However, the index was validated against heat storage ($r=0.94$) and also used some particularly severe exposures over 1-3hrs (38, 54, 71°C). Moran *et al*'s (1998a) Physiological Strain Index (PSI) uses an equation based on the changes in heart rate and core temperature, generating results of a 1-10 scale. However, it is possible to generate negative PSI values if long periods of rest induce decline in core temperature. Most recently, Frank *et al* (2001) proposed an almost identical Cumulative Heat Strain Index (CHSI). The PSI quantifies strain at the specific time point and therefore acts as a dynamic value, whereas the CHSI quantifies the total strain from resting to the current state, giving a value between 0-1000. There are numerous arguments for the use of either strain index, as there is minimal difference in sensitivity between the CHSI or PSI, while both differentiate between environmental conditions and exercise intensities (Frank *et al*. 2001; Moran 2000). However, there has

been far greater validation of the PSI at various hydration states (Moran *et al.* 1998a), ambient temperatures (Moran *et al.* 1999a; Moran *et al.* 1998b) and between gender groups (Moran *et al.* 1999b), and as a measure of stress (Moran 2000). Further, the use of the PSI has been adopted outside of Moran's research group and used in a variety of studies into heat stress (Bergeron *et al.* 2009; Castle *et al.* 2006) and prolonged endurance activity and rehydration (Sims *et al.* 2007a; Vallier *et al.* 2005) to quantify physiological strain. For the measurement of multiple recordings over a period of time and set time points, it seems as though the PSI is a more valid index of strain induced through a physical or environmental conditions. Yet, there seems to be no hypoxic studies attempting to quantify the physiological strain of hypoxia at rest and during exercise.

2.1.3.8. Heat Shock Proteins

At a cellular level the effects of a physiological stressor may be monitored through adaptive changes in cellular structure and function, or even via alterations in gene expression. However, the measurement of heat shock proteins (HSP) may also allow quantification of a physiological stressor at a cellular level. These proteins occur in all living organisms and are believed to have cytoprotective effects, act as chaperones to cellular proteins and mRNA and may regulate some metabolic pathways. The discovery of these proteins in 1962 suggested that they became active in response to external stressors such as heat. Later it became clear that the HSPs are continually active and numerous external stressors, if sufficient, can upregulate the relevant HSP transcription. A review by Kregel (2002) suggests at least eight factors could promote HSP₇₀ transcription, shown in Figure 2.7.

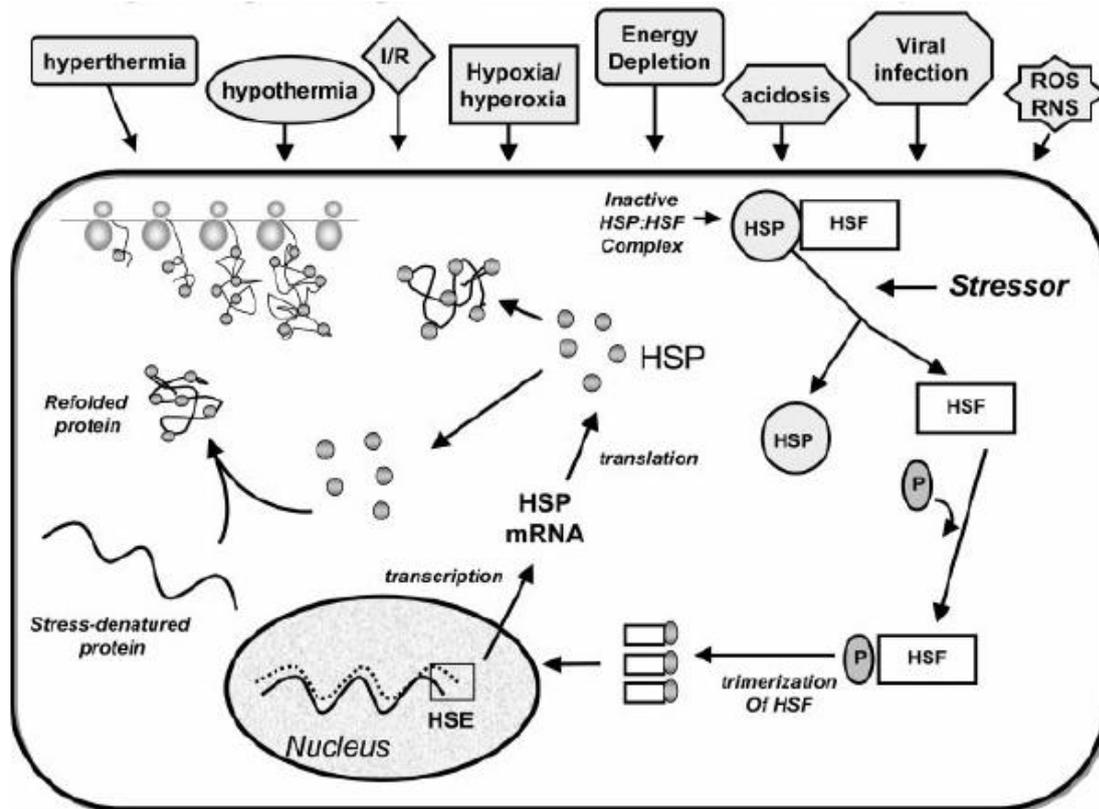


Figure 2.7: A summary of some of the major physiological signals that activate the inducible form of HSP₇₀ synthesis (*top*) and a proposed mechanism for increased HSP₇₀ expression within a cell. Heat shock factors (HSFs), present in the cytosol, are bound by heat shock proteins (HSPs) and are maintained in an inactive state. A broad array of physiological stimuli (“stressors”) are thought to activate HSFs, causing them to separate from HSPs. HSFs are phosphorylated (P) by protein kinases and form trimers in the cytosol. These HSF trimer complexes enter the nucleus and bind to heat shock elements in the promoter region of the HSP₇₀ gene. HSP₇₀ mRNA is then transcribed and leaves the nucleus for the cytosol, where new HSP₇₀ is synthesized (Kregel 2002).

Hypoxia has been shown to promote HSP₂₀ (David *et al.* 2006), HSP₇₀ (Aoe *et al.* 1997; Kawana *et al.* 2000; Mestril *et al.* 1994; Tokyol *et al.* 2005; Turman & Rosenfeld 1999; Zhong *et al.* 2000) and HSP₆₀ (Gupta & Knowlton 2002) expression. Yet these studies

were on rabbits, rats and piglets, human based hypoxia studies into HSP seem limited (Oehler *et al.* 2000), with much of the present literature investigating the HSP expression and thermotolerance (Kregel 2002). Clearly, oxidative stress could be a sufficient stimulus to induce such changes with humans. Oehler *et al.* (2000) showed that expression of HSP₇₀ in human endothelial cells was significantly reduced during hypoxia. However, venous endothelial cells are hypoxically tolerant through constant low oxygen tensions, while in-vitro culture were exposed to supraphysiological oxygen levels, which may have acted as a greater stressor. Tokyol *et al.* (2005) noted increases in HSP₇₀ expression in rabbit's kidneys after exposure to acute hypoxia (2.43 ± 0.2) compared to chronic intermittent hypoxia (1.86 ± 0.14). The study used a moderate exposure of 11% O₂ for 4hrs, in respective to other hypoxic rat studies (Mestril *et al.* 1994; Zhong *et al.* 2000), which tend to use exposures outside the range of human tolerance and ethical standards. In relation to this thesis, it is important to highlight that upregulated HSP₇₀ expression within the hypoxic rat kidney, emphasises systemic hypoxia may cause cellular injury to the human renal system. This finding may be a factor in the alteration of renal function with sufficient hypoxic stress in humans, e.g. as might be expected after a myocardial infarct or cardiac failure.

In comparison to hypoxia, the response of HSPs to exercise is relatively well researched resulting in a range of reviews on the topic (Fehrenbach & Niess 1999; Fehrenbach & Northoff 2001; Kregel 2002; McLemore *et al.* 2005; Noble *et al.* 2008; Powers *et al.* 2001; Salinthon *et al.* 2008; Tupling 2008). The research has shown HSP₇₀ expression is exercise intensity dependent in rats (Milne & Noble 2002) and humans (Liu *et al.* 2000). Further, sufficient aerobic training may mediate a reduction in HSP₇₀ expression when additional stressors arise (Melling *et al.* 2007). Temperature also induces significant

expression of HSP₇₀ when exercise is maintained at an intensity around anaerobic threshold (Kim *et al.* 2004). These changes are determined by an individual's heat storage and thus core body temperature, a resultant of exercise and temperature tolerance combined.

Clearly exercise and hypoxia as single entities, result in HSP₇₀ expression through physiological stress imposed at a cellular level. It remains to be seen if hypoxic exposure in humans is similar to the response in rats (Tokyo *et al.* 2005). While the effect of hypohydration on HSP₇₀ expression remains unresearched and may offer a cellular view of the physiological stress imposed by hypovolemia.

2.1.3.9. Haematological Issues and Changes with Hypoxia

In individuals without nutrient deficiencies, erythropoietin controls the production of red blood cells. Erythropoietin is a glycoprotein, which is produced in the proximal tubular cells of the kidneys and to a lesser extent in the liver (Erslev, 1991). Production of erythropoietin is regulated by HIF-1, as discussed in section 2.1.3.1. Freidmann *et al* (2005) noted a 10-185% increase in erythropoietin production after 4 hrs of hypoxia (FIO₂: 0.15), demonstrating a wide individual variation to the hypoxic insult. While Mackenzie *et al* (2008), found significant increases in erythropoietin after 2hrs (19.3±4.4 to 24.1±5.1mU/mL, $p<0.04$). Rodriguez *et al* (2000) found a similar increase from 8.7 to 13.5 mU / mL over 90mins using a slightly greater hypoxic insult (540hPa / 5000m). The range in resting and hypoxia induced erythropoietin levels are not clear, but may be due to the small (n=5), older (29-50yrs) sample group used by Rodriguez *et al* (2000), in comparison to Mackenzie *et al's* (2008) physically active university student sample group. Prior work by Reinhart *et al* (1991) found that none of the measured parameters of blood

rheology (hematocrit, plasma viscosity, whole blood viscosity) were related to AMS score. Although the authors did note, that at low and high altitude, hematocrit remained lower in non AMS sufferers. Bartsch and colleagues (Bartsch *et al.* 1989a; Bartsch *et al.* 1989b) also noted similar findings. While, Milledge *et al.* (1989) found no correlation between haemoglobin, or packed cell volume and AMS score on ascent to 4300m over two days.

From the research it is clear that haematological adaptations are important for the altitude acclimatisation process (Bartsch *et al.* 2004; Basnyat & Murdoch 2003; Hackett & Roach 2001), however sea level resting hematocrit and haemoglobin values do not have any relationship to an individual's AMS susceptibility or their response to an acute hypoxic stimulus.

2.1.4. The Brain and Acute Hypoxia

Evidence for the effect of hypoxia on the brain was first noted in 1862 by Glaisher (Doherty 2003) when conducting balloon flights to 29,000ft. Through such rapid and severe hypoxia, Glaisher (Doherty 2003) recorded, 'appendicular and later truncal paralysis, blindness, initially preserved cognition, and subsequent loss of consciousness'. Previous hypoxic research such as the 'Operation Everest' studies (Houston *et al.* 1987) also appreciated the role of hypoxia on brain function, yet it is only in the last 20 years that research has had the capabilities to monitor brain impairment by MRI, near infrared spectroscopy (NIRS) or markers of blood brain barrier damage (Wilson *et al.* 2009). These methods now allow a variety of cerebral mechanisms to be investigated. For the purposes of this literature review only blood brain barrier damage and cerebrovascular

blood flow will be covered, as these are currently thought to be most linked to AMS pathophysiology and hypoxia tolerance.

Hornbein's (2001) summary of the brain at altitude emphasises the detrimental effect acute and chronic exposure may have on cognitive function. Yet his summary clearly demonstrates how little is understood with regard to the mechanisms which induce such a response. Thus, much of the research now being carried out within hypoxia and brain research adopts an applied and integrative response to determine mechanistic pathways.

2.1.4.1. The Blood Brain Barrier

Kaur & Ling's (2001) well presented review explains that hypoxia is likely to damage the blood brain barrier, even with acute exposure. Hypoxia initiates the expression of HIF-1 α (section 2.1.3.1), which in turn initiates the transcription of vascular endothelial growth factor (VEGF) (Semenza 2004). VEGF and nitric oxide activate glial cells, which secrete inflammatory cytokines enhancing the permeability of the vasculature resulting in vascular leakage within the brain (Croll *et al.* 2004) and disrupting the BBB tight junctions causing increased permeability of the barrier (Mark & Davis 2002). This increased BBB permeability, allows increased water movement and oedema (Kimelberg 2004), which may be one of the mechanisms for the onset of AMS and/or HACE (Bartsch *et al.* 2004; Roach & Hackett 2001).

Wilson *et al* (2009) present an interesting mechanistic diagram within their recent review on the brain at altitude (Figure 2.8). For the purpose of this thesis the key mechanism the authors illustrate is the accumulation in HIF-1 α and the consequent upregulation of VEGF, which induces membrane damage and angiogenesis, allowing oedema and

ultimately an increase in pressure. Wilson *et al* (2009) also stress the importance of hypoxic vasodilatation which stimulates painfibres of the trigeminal nerve, inducing pain and headache.

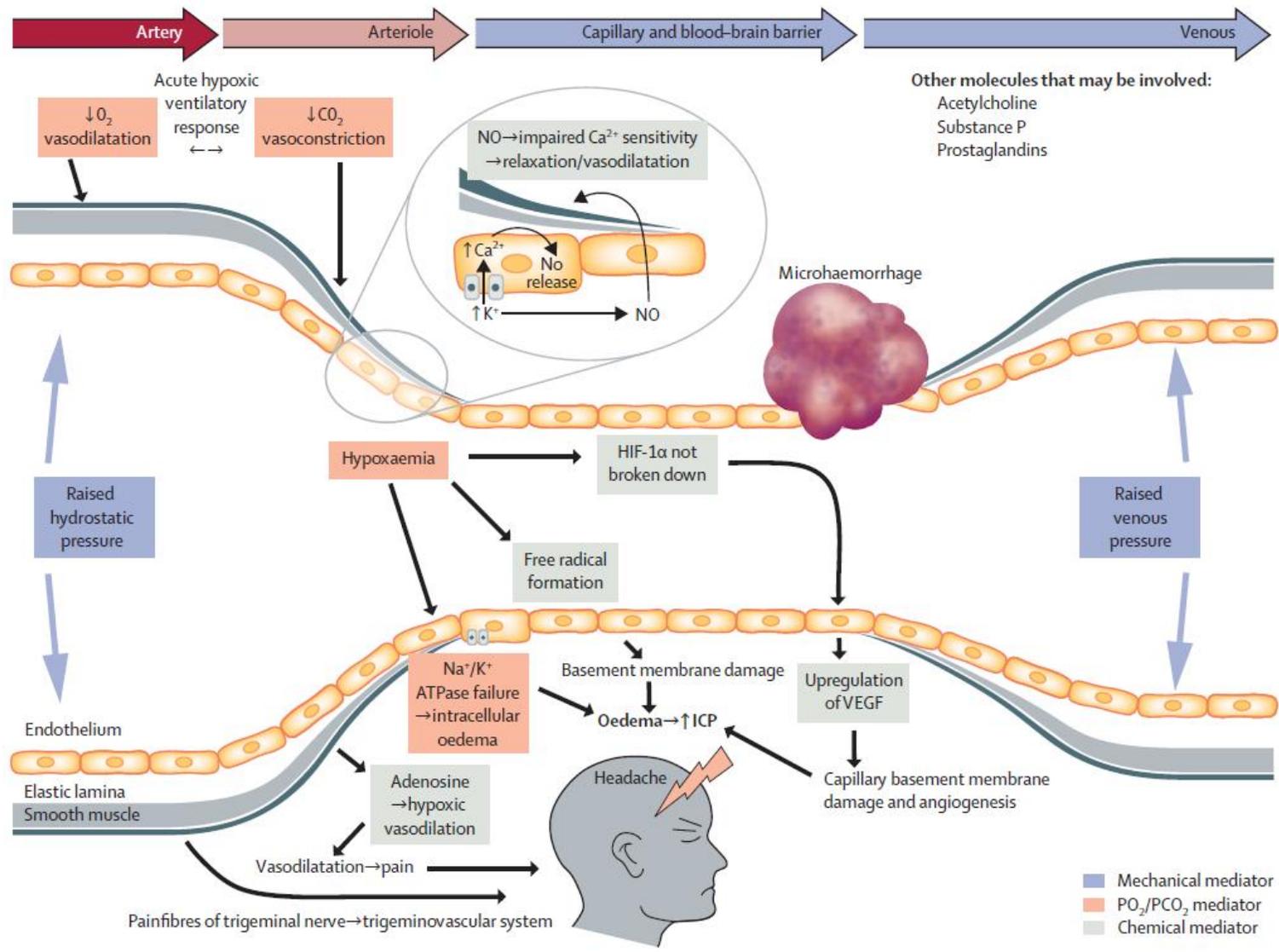


Figure 2.8: Mechanistic understanding of headache development with exposure to hypoxia, adapted from Wilson *et al* (2009).

It is possible to monitor blood brain barrier integrity with a number of techniques, such as contrast enhanced computer tomography or MRI or detecting proteins in the blood that are normally confined to the cerebrospinal fluid resulting from BBB damage and leakage. Tests for the cerebrospinal fluid changes require invasive techniques including lumbar puncture. One marker that has proven useful is serum 100 β , normally stored in brain astrocytes, which is released to the circulation when BBB disruption occurs (Kapural *et al.* 2002). A review by Marchi *et al.* (2004) examined the measurement of peripheral markers for quantification of BBB damage. Serum 100 β was found to be the only marker normally located at higher concentration in the CSF than plasma (Jonsson *et al.* 1999). When BBB integrity is altered, serum 100 β may be the only sensitive marker, which can be measured from blood sampling. Other peripheral markers of BBB integrity such as monomeric-transferrin (Marchi *et al.* 2003), glial fibrillary acidic protein (Stummer *et al.* 1995) and neuron-specific enolase (Reiber 1998) have been investigated, yet their quantifiable representation of BBB damage is not clear (Marchi *et al.* 2004). As the measurement and understanding of serum 100 β , in relation to BBB function is relatively new, normative values are yet to be provided for resting or exercising data (Kapural *et al.* 2002; Marchi *et al.* 2004), to indicate BBB disruption or brain injury. Resting values of 0.01 - 0.003 $\mu\text{g}\cdot\text{L}^{-1}$ are regarded as healthy, while increases of $>0.10\mu\text{g}\cdot\text{L}^{-1}$ indicate some degree of BBB disruption (Kapural *et al.* 2002). Serum 100 β has been found to increase with head trauma (Stalnacke *et al.* 2006; Stalnacke *et al.* 2003), axial vibrations (Otto *et al.* 2000) to the brain and muscle damage (Schulpis *et al.* 2007). However, all of these studies where serum 100 β increased were before and after a sporting performance, such as boxing (Otto *et al.* 2000), football (Mussack *et al.* 2003; Stalnacke *et al.* 2006), ice hockey (Stalnacke *et al.* 2003) or basketball (Schulpis *et al.* 2007; Stalnacke *et al.* 2003). Therefore, the true mechanism causing the serum 100 β rise, whether it be muscle damage, exercise, brain

vibrations, hypoxemia, neurological damage or direct minor head trauma, cannot be ascertained.

Watson *et al* (2005) demonstrated increases up to $0.23\mu\text{g}\cdot\text{L}^{-1}$ in serum 100β when exercising at $60\% \text{VO}_2\text{peak}$ in a warm (35°C) environment, although there was a large participant variation in responses. In a similar warm trial of lower intensity running ($55\% \text{VO}_2\text{peak}$), serum 100β was found to be $0.08 \pm 0.02\mu\text{g}\cdot\text{L}^{-1}$ at rest and increased to $0.20 \pm 0.06\mu\text{g}\cdot\text{L}^{-1}$ when no fluid was given, and $0.13 \pm 0.03\mu\text{g}\cdot\text{L}^{-1}$ with fluid administration equal to sweat loss. However, the changes were not significant due to the range in serum 100β values. This may be due to the lack of accuracy and precision in regards to serum 100β measurement at present, with serum 100β ELISA kit coefficient of variation values at around 7.13% (YK150, Yanaihara Institute INC, Japan).

A significant increase in serum 100β due to a physiological stressor, is currently the strongest evaluative blood marker of BBB integrity available (Kapural *et al.* 2002; Kaur & Ling 2008; Marchi *et al.* 2004; Marchi *et al.* 2003; Mercier & Hatton 2000).

2.1.4.2. Cerebrovascular Blood Flow

Cerebrovascular blood flow (CBF) may also be partly responsible for the development of AMS or HACE, although research has found contrasting evidence. Jensen *et al* (1990) showed no link between AMS onset and the rise in CBF when ascending from sea level to 3475m. Yet Jansen *et al* (1999) showed a significantly greater cerebral hemodynamic response in those suffering with AMS. Likewise, Van Osta *et al* (2005) concluded that AMS sufferers showed greater hemodynamic response to altitude than non sufferers, or Sherpas.

Buck *et al* (1998) showed that only 20 minutes of exposure to simulated hypoxia equal to 4500m increased cerebral blood flow, further blood flow was found to be greater towards the hypothalamus in comparison to other cranial regions. In contrast, exposure to 3000m caused no significant change in cerebral blood flow. The authors suggest that these increases in hypothalamic blood flow may contribute to acute mountain sickness pathophysiology (Buck *et al.* 1998). Brugniaux *et al* (2007) provide evidence for hypoxic exposure induced cerebrovascular vasodilatation, while hypoxia driven hyperventilation decreases the PaCO₂, consequently resulting in cerebral vasoconstriction. The immediate response to acute hypoxia is to increase CBF (Poulin *et al.* 1996) although this response may (Poulin *et al.* 1996) or may not (Steinback & Poulin 2007) be influenced by the regulation of ambient PCO₂. Consequently, these hypoxia induced alterations in CBF may result in either a reduction in blood flow and thus oxygen delivery, or excessive CBF resulting in vascular leakage and/or damage to the BBB. This response, similar to altitude tolerance, is entirely individualistic, dependent upon hypoxic severity and requires further mechanistic investigation, especially with regard to the pathophysiology of AMS and HACE.

2.2. Hydration and Fluid Balance

Euhydration is a state of adequate fluid balance to maintain homeostasis. The terms hypohydration and hyperhydration define states of negative and positive fluid balance, respectively. These states continually change in the body. The term for dynamic fluid loss is dehydration. These states and the physiological alterations they induce will be discussed. However, it is important to stress the difficulty and debate surrounding the measurement and definition of all hydration states.

2.2.1. Assessment of Hydration

The most common method for assessment of hydration state is monitoring of body mass alterations. Body mass, over a short period of time (<4-6hrs) (Armstrong 2005), will not change unless substances enter or leave the body. Based on this, 'baseline' body mass is normally ascertained through a minimum of three body mass measurements on three consecutive days (Armstrong 2005). Armstrong (2005) suggested that body mass may fluctuate by $\pm 0.51 \pm 0.2\text{kg}$, with a group coefficient of variation for repeated days of $0.66 \pm 0.24\%$. To establish accurately the change in body water, water losses through sweat and urine need to be calculated along with fluid intake (Shirreffs 2003). Average daily fluid losses are made up of the gastrointestinal tract (100-200ml), ventilation ($\sim 0.12\text{L}$), urine (2L) and sweat (500ml), totalling 2.5-3L (Sawka 2007). However, these values vary depending upon activity, body size, altitude, temperature and humidity, therefore normative data on fluid losses are difficult to interpret or compare appropriately.

Other markers can be used in the assessment of hydration, yet currently there is no gold standard method to quantify or calculate hydration state (Armstrong 2007). Urine osmolality measures the total solute concentration, per kilogram of solvent, whereas

osmolality is the total solute concentration, per litre of solute. Although urine osmolality is regarded as a reliable measure of solute content and therefore kidney concentration function (Armstrong 2007), it does not give a clear indication of hydration state when analysing a general population, due to variations in diet and fluid intakes. Mantz & Wentz (2003) clearly demonstrated significant differences in mean baseline urine osmolality across European countries. Further, urine excreted from the body has been inside the body for a period of time and therefore may not represent the hydration state at the time of measurement. Likewise, urine osmolality may not be accurate when the body undergoes severe hypohydration and subsequent rehydration (Francesconi *et al.* 1987).

Urine specific gravity (USG) measures the density of the urine sample. A study by Armstrong *et al* (1998) found USG positively correlated with urine osmolality ($r^2 = 0.96$), suggesting that these variables could be used interchangeably. Measurement of urine colour is based on the content of urochrome within the urine. Large urine volume will be more dilute and therefore paler. Armstrong (1998) suggested the use of an eight colour scale, which was found to have a linear relationship with osmolality and urine specific gravity. Although this scale lacks precision, it is cheap, quick to measure and requires no real expertise, ideal for regular checking of hydration state.

Many researchers have attempted to group hydration indices into hydration states, yet no consensus parameters or variables have so far been set. One such attempt by Casa *et al* (2000) (Table 2.1), offers hydration state quantification for athletes. However, groupings and perceptions of minimal dehydration (Casa *et al.* 2000) seem inaccurate and misleading, as dehydrations of this value have been shown to negate endurance performance (Cheuveront *et al.* 2005) and physiological strain (Moran *et al.* 1998a), as

discussed in section 2.2.3. While based on data from other work, the urine colour and USG values seem to be underestimated compared to body mass losses (Cheuveront & Sawka 2005). Casa *et al* (2000) also failed to present osmolality values, which based on other data, would be estimated at <400 mosmol/kg (well hydrated / euhydrated), 700 mosmol/kg (minimal dehydration), 1000 mosmol/kg (significant dehydration) and >1300 mosmol/kg (serious dehydration).

Table 2.1: Categorising hydration state using body weight change, urine colour and specific gravity.

Hydration status	% Body weight change*	Urine colour	USG
Well hydrated	+1 to -1	1 or 2	<1.010
Minimal dehydration	-1 to -3	3 or 4	1.010– 1.020
Significant dehydration	-4 to -5	5 or 6	1.020– 1.030
Serious dehydration	< -5	>6	>1.030

% Body weight change = [(Baseline bodyweight – assessment body weight) / baseline body weight] x 100. USG: Urine specific gravity. Adapted from Casa *et al* (2000).

As consequent of these variations in hydration assessment, ascertaining hydration status requires consideration of all four indices for valid and sensitive assessment when using dehydration and rehydration protocols (Armstrong *et al.* 1998). For research purposes the dehydration protocol undertaken should be the determinant of the set condition, as opposed to setting the condition based on body mass loss as a measure of hydration state. There is clearly a need for large scale research to evaluate urine indices across a range of euhydration, hypohydration and hyperhydration states (Armstrong 2005, 2007; Shirreffs 2003).

2.2.2. Maintenance of Fluid Balance

Fluid balance is essential to ensure homeostasis of a range of regulated processes in the body, water makes up about 60% of body mass. Of that water mass, approximately 66% is intracellular fluid (ICF), sometimes known as cytosol, it is composed mainly of water, dissolved ions, small solute molecules and proteins. Cytosol has a pH of 7.0 with a high concentration of potassium and low concentration of sodium. The other 33% is extracellular fluid (ECF), equating to approximately 15L in an average male, of which blood plasma accounts for 3L and interstitial fluid 12L. This tends to have a comparably lower potassium concentration, higher sodium concentration and a higher pH, approximately 7.4, than cytosol.

Fluid balance is mainly controlled by circulating hormones, which are initiated by the chemoreceptors and baroreceptors. In normal fluid balance, increased intake of sodium chloride (NaCl) or high plasma concentrations of NaCl, due to water loss, increase osmotic movement of water from intracellular fluid to the plasma, as water follows solutes. This rise in blood volume is noted by the atrial walls, through stretch receptors causing release of atrial natriuretic peptide (ANP). ANP is stored and released by cardiac myocytes of the atria in response to atrial distention, raised Na^+ concentration, sympathetic stimulation or angiotensin II. ANP reduces blood volume and therefore, cardiac output and systemic blood pressure, by counteracting the renin-angiotensin system ANP inhibits renin release from the juxtaglomerular apparatus (JGA) and aldosterone secretion from the adrenal cortex. These decrease sodium reabsorption in the distal convoluted tubule and increases pressure in the glomerular capillaries causing greater excretion of sodium and water (Ryan *et al.* 1998). Increased blood volume also decreases renin release by the juxtaglomerular cells thus reducing formation of angiotensin II, in

turn reducing release of aldosterone. This slows reabsorption of Na^+ and Cl^- in the renal ducts while angiotensin II formation also increases glomerular filtration in the kidney tubules. These processes collectively reduce reabsorption of Na^+ and Cl^- and thus free water, to decrease blood volume (Antunes-Rodrigues *et al.* 2004). This complex process is illustrated in Figure 2.9.

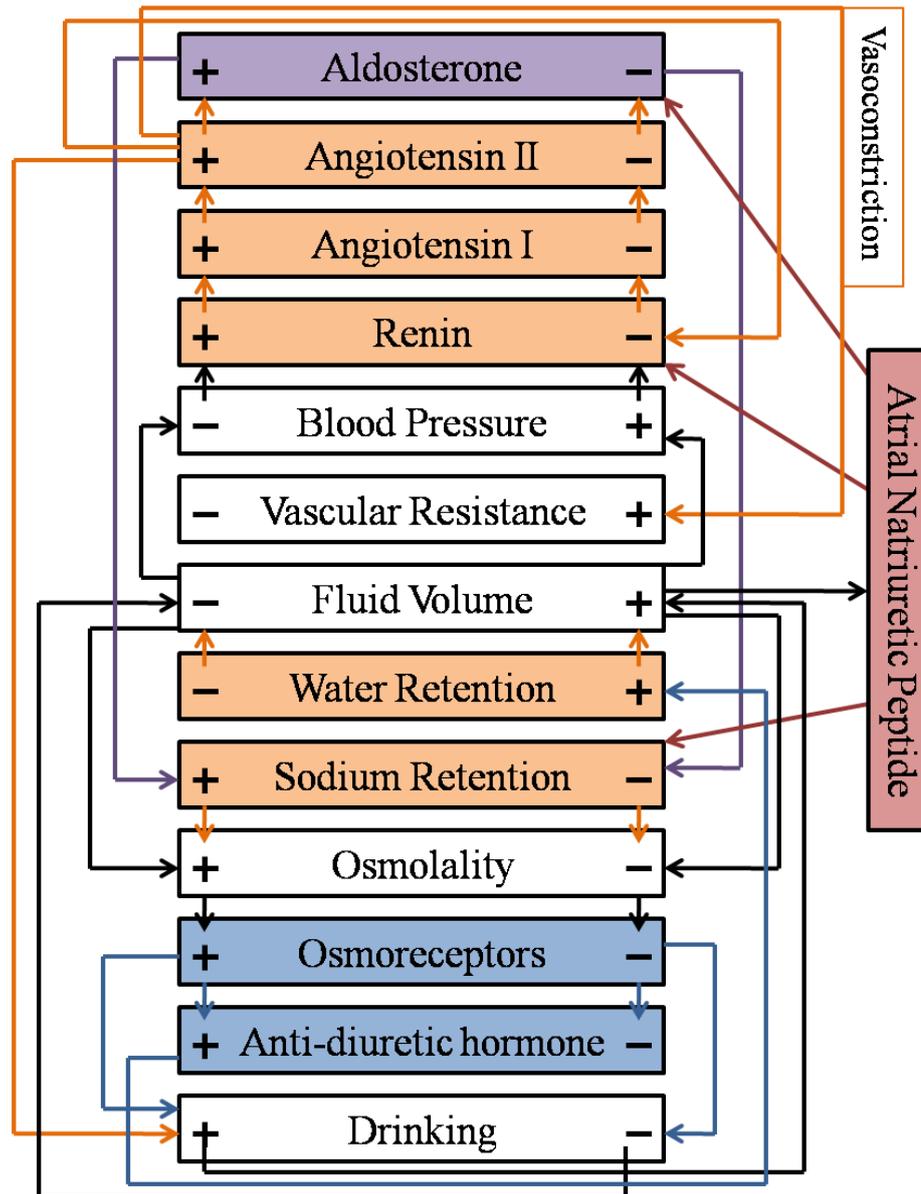


Figure 2.9: Flow diagram of fluid control showing the alterations in fluid control with increases in decreases of the relevant factors. Red denotes cardiac located factors. Orange denotes kidney located factors. Blue denotes hypothalamic located factors.

Dehydration occurs when water loss is greater than water gain causing increased blood osmolality, decreased blood volume, urine production and decreased flow of saliva. These changes stimulate the hypothalamic thirst centre by either stimulation of osmoreceptors in the hypothalamus, or by the decline in blood pressure causing renin release from the juxtaglomerular cells, increasing angiotensin II formation. The thirst centre then responds, increasing fluid intake (Antunes-Rodrigues *et al.* 2004; Ryan *et al.* 1998).

The hormonal responses can also act to reduce dehydration especially if the thirst response is not acted upon. When blood volume is reduced and blood osmolality increases, osmoreceptors stimulate anti-diuretic hormone (ADH), which act to make the collecting ducts of the kidney cells more permeable to water by opening aquaporin II water channel proteins, moving water from the kidney tubules into the kidney cells and back into the blood stream. This results in a small volume of highly concentrated urine in an attempt to lower blood NaCl concentration, the major plasma osmolyte. ADH also targets sudoriferous glands decreasing water loss via perspiration, and arterioles causing elevated blood pressure through vasoconstriction (Ma & Verkman 1999). Aldosterone, a steroid hormone, is synthesized in the adrenal cortex in response to increased angiotensin II, adrenocorticotrophic hormone (ACTH) or increased K^+ levels all proportionately related to the decline in Na^+ levels. Plasma acidosis and detection of atrial pressure reduction by the stretch receptors also promote aldosterone release, which stimulates H^+ secretion in the collecting ducts, normalizing plasma bicarbonate and activates Na^+ retention and K^+ excretion, the activity of the basolateral Na^+/K^+ pump is regulated by these factors by phosphorylation (Antunes-Rodrigues *et al.* 2004).

The hormones regulating body fluid during hypohydration have been widely researched. Francesconi *et al* (1985) investigated subjects hypohydrated by 3, 5 and 7% of body weight, finding that hypohydration greater than 5% correlated with increased plasma renin activity (PRA) and aldosterone concentration. This supports later work by Francesconi *et al* (1989), finding PRA and aldosterone increasing with greater voluntary hypohydration. Roy *et al* (2001), found increases in PRA, angiotensin I, aldosterone and ANP, after a decrease in PV of 14 \pm 3%. However, Maresh *et al* (2004) found fluid controlling hormones were not influenced by pre-exercise hydration state. Maresh *et al* (2004) stress this is an anomaly, as plasma osmolality was significantly greater in the hypohydration trial and this should initiate an ADH response. Yet, significant increases in ADH, aldosterone and PRA were noted with exercise when participants were hypohydrated and no water was consumed over the trial. Montain *et al* (1997) assessed the hormonal response with graded hypohydration of 0%, 3% and 5% body mass loss. Aldosterone and ADH increased with hypohydration severity, which was exacerbated with exercise in aldosterone, but not ADH. Montain *et al* (1997) suggest that ADH alterations are the result of plasma osmolality changes.

2.2.2.1. Monitoring Fluid Balance

The criterion method for measurement of total body water (TBW) is the dilution method, whereby the participant drinks a set amount of deuterium oxide. This then equilibrates with all body water over 1-2hrs (Detailed in section 3.10.1). Body water volume or mass can then be obtained by measuring the tracer levels and the degree of dilution from blood, saliva or urine (Van Kreel *et al.* 1996). Although this is a common and accurate method, the process is very long - winded and requires a great deal of time, often expensive equipment and expertise to analyse the samples (Earthman *et al.* 2007). Biochemical

research has attempted to improve precision, accuracy and streamline the dilution method process, using a variety of methods (Jennings *et al.* 1999). These include: the falling drop method (Schloerb *et al.* 1951); the infrared absorption measurement (Turner *et al.* 1960); freezing point depression (Reaser & Burch 1958); mass spectrometry (Solomon *et al.* 1950) and gas chromatography (Arnett & Duggleby 1963). Much research uses gas chromatography – continuous flow isotope ratio mass spectrometry (GC-CF IRMS) by measuring acetylene gas from the reaction of H₂O with carbide (Van Kreel *et al.* 1996). While ideally the expensive technique of ²H equilibration using a platinum catalyst would be performed (Herd *et al.* 2000).

Extracellular fluid volume (ECF) is also an important measure of fluid balance, which can be ascertained through the measurement of bromide space using either spectrophotometry or high performance liquid chromatography. The protocol for the spectrophotometry technique and the deduction of intracellular fluid volume (ICF) is detailed within section 3.10.2. Numerous studies have used and validated this method (De Lorenzo *et al.* 1997; Earthman *et al.* 2007; Islam *et al.* 1999; Schulz 1993). Similar to the TBW dilution method, the process can be time - consuming, difficult to assess and requires a reasonable level of expertise. Consequently, more simple methods, such as multi-frequency bioelectrical impedance analysis (MFBIA) are being developed.

MFBIA uses electrical impulses of high and low frequencies (range 5-1000kHz) to penetrate the cells, based on resistance and reactance, the Cole-Cole theoretical model (Cole & Cole 1941) can ascertain volume of the separate fluid spaces (De Lorenzo *et al.* 1997). However, this indirect measurement technique has come under much scrutiny over accuracy and reliability due to the reliance on equations (Earthman *et al.* 2007). There

seems to be contrasting evidence for (Hanaki *et al.* 2006; Morel & Jaffrin 2008; Patel *et al.* 1994; Plank *et al.* 1998; Valencia *et al.* 2003) and against (Asselin *et al.* 1998; Chumlea *et al.* 2007; Dioum *et al.* 2005; O'Brien *et al.* 2002) the use of MFBI in predicting TBW or ECF in various populations, while others simply suggest further research is required (Cornish *et al.* 1993). Earthman *et al.* (2007) offer a detailed and expansive review of twenty-two studies comparing measurement of TBW and ECF using deuterium oxide and sodium bromide dilution methods against MFBI. Earthman *et al.*'s (2007) conclusion concurs with others (Chumlea *et al.* 2007; De Lorenzo *et al.* 1997), in that the Cole-Cole method offers a reasonable prediction equation for the majority of adults within a normal body composition range, yet further research into population specific equations is needed. Improvements in general accuracy and precision are required before MFBI can be used as a standard clinical and research practice. Although many studies (Hanaki *et al.* 2006; Morel & Jaffrin 2008; Patel *et al.* 1994; Plank *et al.* 1998; Valencia *et al.* 2003) find a significant correlation ($r = 0.57-0.93$) between MFBI and other techniques it should not automatically suggest MFBI is a good measure of fluid compartment volumes. Greater analysis using Bland Altman plots is required within the literature, while a significance value of $p < 0.01$ may be more appropriate when considering the minimal alterations in fluid volumes and the required accuracy within research and medicine.

2.2.3. The effects of hypohydration in normoxia

There is a wealth of research into hydration during normoxic endurance exercise and the effect on performance. Cheuveront *et al.* (2003) found dehydration of >2% of body weight during normoxic exercise lasting more than 90 minutes in an environment of 20-21°C, impaired endurance performance, although 2% dehydration in shorter duration exercise,

showed no significant effect on sprint performance (Bachle *et al*, 2001). In hotter environments, of 31 – 32°C, any exercise lasting longer than 60 minutes is affected when the body is 2% dehydrated (Below *et al*, 1995). Gonzalez-Alonso & Calbet (2003) suggest this performance decrement is due to dehydration increasing cardiovascular strain through hypovolemia and hyperthermia, reducing muscle metabolism efficiency and negatively affecting neurological function. It is also suggested that dehydration increases muscle glycogen use during continuous exercise, which is believed to come about due to an increased core temperature, reduced oxygen delivery and the increase in catecholamines (Hargreaves *et al*, 1996).

Many different methods have been used to investigate the physiological effects of dehydration. Much of the research attempts to dehydrate participants through exercise or reduced fluid ingestion at least 24 hrs prior to testing. Montain & Coyle (1992) used graded hypohydration over the duration of the test, to investigate the effect on hyperthermia. They concluded that physiological strain via increased heart rate and core temperature, increased linearly with the level of hypohydration. In another study by Montain *et al* (1995) participants were dehydrated to 3 and 5% of body weight 24 hrs prior to testing, when looking at thermoregulatory sweating with exercise intensity. Montain *et al* (1995) suggested that levels of hypohydration created a graded response in thermoregulation, that was independent of the exercise intensity. Moran *et al* (1998a) dehydrated participants to four different levels prior to a 120 min cycling, finding that body water loss above 2.3% caused significant increase in physiological strain (Figure 2.10). This was a similar graded response to that of Montain *et al* (1995). In a more severe dehydration study by Sawka *et al* (1985), eight participants were dehydrated by 3, 5 and 7% body mass loss prior to four bouts of 25 min moderate intensity walking with

10mins recovery in between each bout. As in the previous studies, physiological strain increased linearly with severity of hypohydration. Interestingly, the 7% hypohydration trial noted no further reduction in plasma volume with exercise, instead inducing a significant increase in plasma osmolality. It was highlighted that this rise in plasma osmolality had more significant effect on sweat rate decline than hypovolemia alone. Pichan *et al* (1988) hypohydrated heat acclimatised participants to lower degrees of 1, 2 and 3% body mass loss. Significant increase in physiological strain and VO_2 were only noted with 2% and 3% hypohydration, yet sweat rate only declined with 3% hypohydration. This suggests increases in physiological strain are not dependent upon decline in the sweat response. This is supported by Wyndham & Strydom (1969) who suggest that hypohydration $\geq 3\%$ leads to greater heat storage.

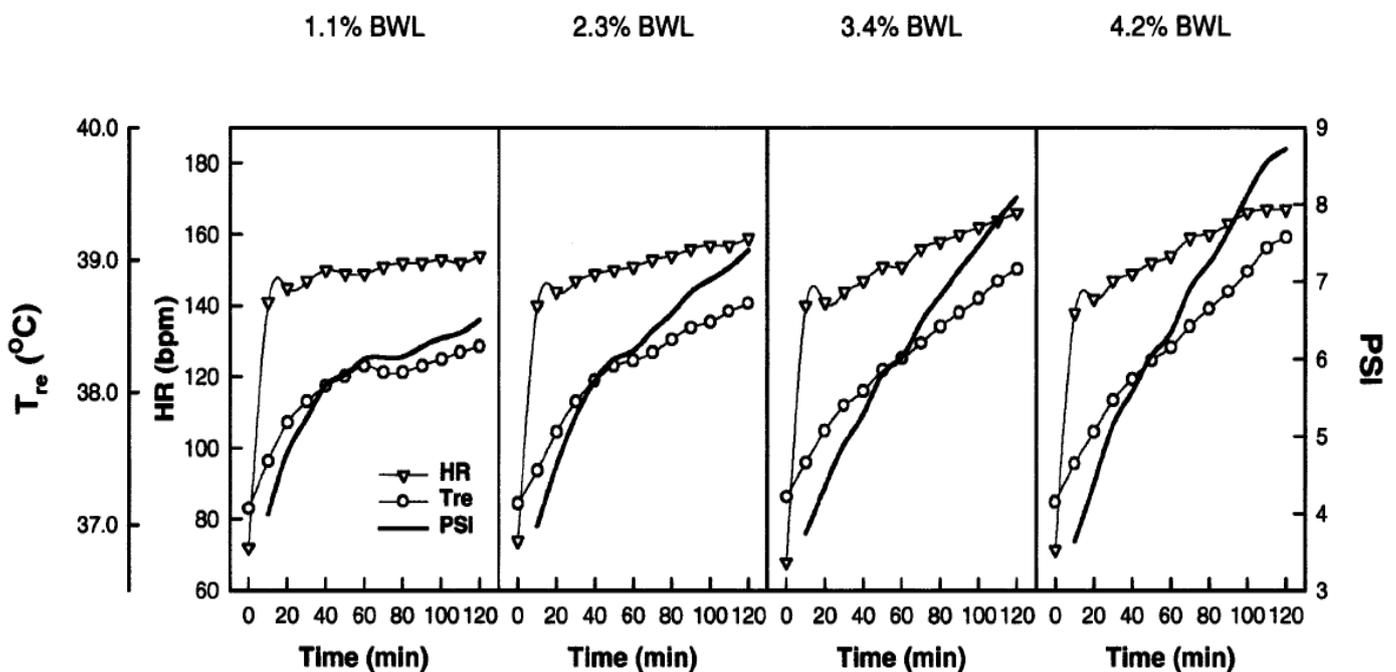


Figure 2.10: Physiological strain index (PSI), calculated from rectal temperature (T_{re}) and heart rate (HR), applied to mean values obtained from eight subjects exposed to heat stress [33°C , 50% relative humidity and 65% maximum O_2 consumption ($\text{VO}_{2\text{max}}$)] at 4

different levels of hypohydration [1.1, 2.3, 3.4, and 4.2% body weight loss (BWL)].

Adapted from Moran *et al* (1998a).

Sawka *et al* (2001) suggest a more linear response to heat storage occurs with graded dehydration. Castelliani *et al* (2001) calculate that for every 1% loss of body mass due the dehydration in the heat, core body temperature increases by 0.2-0.3°C and heart rate by 5-8 beats·min⁻¹, all due to the significant decline in cardiac output via reduced end diastolic volume. Greenleaf & Castle (1971) had previously suggested a rise in core temperature of 0.1°C for every 1% loss in body mass, although these variations may be dependent upon the differences in exercise protocol and climatic conditions. Likewise, Montain *et al* (1998) calculated 0.12°C per 1% loss in body mass, in the heat. While, Buono & Wall (2000) suggest a value of 0.16°C in the heat, but suggest a value of 0.08°C in normoxic normothermic conditions, which no other research seems to offer.

2.2.4. The effects of hyperhydration in normoxia

At sea level, studies investigating performance and hyperhydration have presented contrasting findings, some indicate an improvement in endurance performance in hot or temperatue conditions (Anderson *et al.* 2001; Coutts *et al.* 2002; Easton *et al.* 2007; Montner *et al.* 1996), while others have found no effect (Latzka & Sawka 2000; Magal *et al.* 2003; Marino *et al.* 2003). During exercise, hyperhydration tends to induce significant reduction in physiological strain measures via heart rate and core temperature reductions, in comparison to euhydrated or hypohydrated states (Greenleaf & Castle 1971). However, water hyperhydration studies tend to use exercise heat stress, exacerbating the sweat response that hyperhydration benefits (Latzka *et al.* 1997, 1998).

Studies using hyperhydration tend to use various water loading protocols. Water volumes have been of 20 (Anderson *et al.* 2001), 21 (Marino *et al.* 2003; Melin *et al.* 2002; Riedesel *et al.* 1987), 26 (Montner *et al.* 1996), 29 (Latzka & Sawka 2000; Latzka *et al.* 1997, 1998) and 37ml/kg body mass (Freund *et al.* 1995; O'Brien *et al.* 2005). These volumes tend to be ingested over a period of 30mins (O'Brien *et al.* 2005) to 2.5hrs (Marino *et al.* 2003). Latzka *et al.* (1997) demonstrated that hyperhydration of 29ml/kg body mass increased total body water by approximately 1.5L after 60mins of rest in normoxic normothermia, yet body water returned to pre-ingestion levels after a further 60 mins of exercise in the heat, when no rehydration was allowed.

The majority of hyperhydration studies have used glycerol to promote water retention, preventing the increased urine output which ultimately induces a net fluid loss (Kavouras *et al.* 2006). Nevertheless, glycerol hyperhydration studies show significant increases in urine output independent of fluid regulating hormonal response (Melin *et al.* 2002). Marino *et al.* (2003) demonstrated that glycerol hyperhydration reduces physiological strain to a similar extent as water hyperhydration alone, during 1hr variable intensity cycling. Likewise, Magal *et al.* (2003) found glycerol hyperhydration to improve hydration status, yet this had no performance benefits for sprint, skill and agility tests. In contrast, O'Brien *et al.* (2005) suggest glycerol hyperhydration has greater thermoregulatory benefit during 4hr of cold exposure. This longer exposure may allow time for the water hyperhydration trial to initiate sufficient diuresis to cause negative fluid balance. In comparison, glycerol may retain sufficient fluid over this time to see significant differences.

No studies have investigated the effect of hyperhydration in a hypoxic or high altitude environment. Due to the alterations in renal fluid control within hypoxia, hyperhydration may have an effect through inducing diuresis. Conversely, it may cause additional fluid retention. Westerterp *et al* (1996) suggest a greater fluid load would promote the onset of AMS symptoms through greater extracellular fluid causing rise in cerebral blood volume and intracranial pressure.

2.2.5. Dangers and causes of hyponatremia

Hyponatremia, defined as a significant decline in plasma sodium concentration below 135mmol.L^{-1} , is widely thought to come about due to large sodium chloride losses or water intoxication through retention of large volumes of low sodium fluids (Noakes 1992). Rosner & Kirven (2007) in a recent in-depth review, explain exercise based hyponatremia is commonly brought on through drinking large amounts of water over a long period. This is frequently reported in activities of low to moderate intensity whereby sweat rates are far less than the water volume input (Rosner & Kirven 2007).

To rehydrate or hyperhydrate individuals acutely, as explained in section 3.6, individuals have to drink a large bolus of water, which could cause a hyponatremic state. Still, this seems unlikely as the majority of reported cases are of chronic fluid loading before and during endurance activities; such as a marathon. This low yet prolonged sweat rate, allows individuals to hydrate actively above a euhydrated level. Studies carrying out hyperhydration protocols should be aware of hyponatremic symptoms when over - loading participants with water alone. Nevertheless, the prevalence of hyponatremia is quite low. Studies investigating hyponatremia prevalence of marathon runners in normothermic normoxia suggest prevalence rates of 13% (Almond *et al.* 2005) to 29% (Hew *et al.*

2003). Within an altitude setting hyponatremia is a possibility, as exercise is usually of a low yet prolonged intensity at which individuals can continually drink. Furthermore, trekking groups tend to be actively encouraged to hydrate (Basynat *et al.*, 1999), even though the consequences of this are unknown. As yet, hyponatremia has not been reported in this environment. Although, hyponatremia case studies have been published on low altitude recreational hikers (Backer *et al.* 1993).

2.2.6. Rehydration strategies and fluid balance

In all human beings there is a fundamental need to rehydrate on a daily basis. This only becomes an issue when hypohydration is severe, or when there is limited time for rehydration to take place, which is often the case in sporting or clinical environments. However, at altitude rehydration can be an issue on a daily basis due to lack of clean water, nausea and more importantly the alteration in renal fluid control prior to altitude adaptation. As yet, no research has investigated the effect of rehydration in hypoxic environments, though there is much research into the restoration of fluid balance for exercise under normoxic conditions (Galloway 1999).

Sodium concentration of drink is known to be important in the restoration of fluid balance (Gonzalez-Alonso *et al.* 1992; Merson *et al.* 2008; Nose *et al.* 1988b; Nose *et al.* 1991; Shirreffs & Maughan 2000), water alone causes significant decline in serum osmolality and a consequent diuresis (Costill & Sparks 1973). Yet, research into the optimal rapid electrolyte reloading is still of debate (Merson *et al.* 2008). As recently as 2007, research (Shirreffs *et al.* 2007) has suggested commercially available drinks do not contain sufficient sodium to allow sodium balance and promote adequate fluid retention.

Gonzalez-Alonso *et al* (1992) dehydrated nineteen participants to achieve a 2.5% body mass loss and then rehydrated them in a thermoneutral environment over 2hr using water, diet cola or a carbohydrate-electrolyte solution equal to 100% of their fluid loss. Authors found that water and carbohydrate-electrolyte drinks regained similar percentage body mass, while the diet cola trial gained significantly less in the 2 hours. The fact that participants remained 0.6-0.9kg below their euhydrated body mass, supports Shirreffs's (1996) suggestion that adequate rehydration requires an intake of 150% of body mass loss. The study (Shirreffs *et al.* 1996) demonstrated that only volumes of 150% and 200% of the body mass loss restored fluid balance, sodium ($61\text{mmol}\cdot\text{L}^{-1}$) in the drink ensured adequate retention.

Nose *et al* (1988b) also demonstrated that a 0.45% NaCl drink restored plasma volume significantly greater than water alone and elevated plasma osmolality throughout the sodium drink trials. Merson *et al* (2008) also provided evidence that sodium chloride concentration of 40 or 50mmol/L in drinks lead to greater retention of fluid than water alone. Though this treatment did not significantly improve time to exhaustion at 95%VO₂max 4hr after rehydration. Sims *et al* (2007a) showed isotonic sodium loading (164mmol/L) prior to a run to exhaustion at 70%VO₂max gave lower physiological and perceived strain during exercise, improving exercise capacity in warm conditions. The comparator drink was a very low dose of sodium (10mmol/L), not sufficient to promote fluid retention. Kenefick *et al* (2007) found no difference in aldosterone, ADH, Na⁺, plasma osmolality or fluid retention with tonicity of fluid. This study demonstrates that 0.45% NaCl solution was sufficient to promote fluid retention, while 0.9% NaCl has no additional benefit. Further, there was no difference between oral or intravenous administration for these variables. A very similar study by Castellani *et al* (1998) also

noted no difference in plasma volume change between intravenous hypotonic (0.45% NaCl) and isotonic (0.9% NaCl) solutions.

Greenleaf *et al* (1998) decided that sodium content was more important to plasma volume expansion than the total osmotic content of the fluid ingested. Although sodium concentrations that induce to great an increase a change may be detrimental to exercise performance. The concensus (Schedl *et al.* 1994) suggests sodium ingestion of 60-90mmol/L at a sodium to glucose ratio of 2:3 would allow optimal reabsorption across the gut.

There is little research aimed at understanding the effects of the rate of fluid ingestion on hydration status, most studies using rehydration amounts of 1-3L, depending upon percentage body mass loss, ingested over a period of 1-2 hrs, depending upon the exercise protocol (Gonzalez-Alonso *et al.* 1992; Kavouras *et al.* 2006; Shirreffs *et al.* 2007). Kovacs *et al* (2002) investigated different intake rates of 120% body mass loss. The high rate, giving 60%, 40% and 20% of fluid in hours 1, 2 and 3 respectively, increased plasma volume faster than the lower rate, which ingested at 24%.hr⁻¹ for 5 hrs. However, fluid output was far greater for the high rate dose for 2-4 hrs, consequently there was no difference in plasma volume for either trial by 6hrs. This suggests high rate doses should be given immediately to replenish fluid losses, while the body regulates fluid compartment and plasma volumes irrespective of dosing rates.

Singh *et al* (1990) hypohydrated participants to various degrees (-1, -2, -3, -4%) and then partially rehydrated the -3% and -4% participants to -2% hypohydrated. Singh *et al* (1990) found that the -3% group fully restored plasma volume, while -4% group's plasma volume

was incomplete. This suggests that severe hypohydration, <-3% causes fluid movement from the plasma into the intracellular compartments. This is particularly important in hypoxic exposure when considering the alterations in oxygen carrying capacity and pathophysiology of AMS through the possible rise intracranial pressure inducing headache.

2.2.7. Fluid Balance in Hypoxia

During hypoxia, fluid balance is severely disrupted through changes in hormonal control, due to changes in O₂ concentration and central chemosensitivity, although we do not fully understand the science behind these processes at present. Heyes *et al* (1982) measured the endocrine and renal responses to 1 hr of exposure to an equivalent 5100m hypobaria or hypoxia and found decreased urine flow (-56%), increased urine osmolality (340%), increased ADH (2700%) and decreased blood pressure (-18%) in the hypoxia trial. This data accounts only for the initial response to hypoxia, with circulating hormonal levels plateauing after the initial physical shock of severe acute hypoxia to the body. These changes with hypoxia combine to increase fluid retention, with significantly greater fluid retention occurring in AMS sufferers (Loeppky *et al.* 2005b; Westerterp *et al.* 1996).

Although there are clearly renal function alterations in some, if not all individuals when exposed to hypoxia, there is a great deal of conflicting evidence concerning the mechanisms involved (Pichler *et al.* 2008). Early work suggested glomerular filtration rate (GFR) increased (Berger *et al.* 1949), or stayed the same (Axelrod & Pitts 1952) when breathing hypoxic gas (FIO₂:0.10) for 40-80 mins, while increased diuresis and natriuresis were shown as early as 1961 (Ullman 1961). Later work tended to examine longer duration altitude exposures (Olsen *et al.* 1992; Pichler *et al.* 2008), and these experiments

showed a higher estimated glomerular filtration rate (eGFR) in individuals suffering with AMS (Pichler *et al.* 2008). Non AMS sufferers, high altitude residents and acclimatised individuals have shown a decline in eGFR when at altitude (Rennie *et al.* 2005; Sayarlioglu *et al.* 2005). The effects of altitude on renal function, measured as GFR, remain unclear.

Westerterp *et al.* (1996) found a fluid shift from intracellular to extracellular space of around 1 litre in AMS sufferers when resting at 4350m for four days. This was the first use of deuterium oxide and sodium bromide for the quantification of body water at altitude. Westerterp *et al.* (1996) note that irrespective of hydration status, individuals with AMS showed the greatest shifts in ECF relative to TBW. This finding is supported by Carson *et al.* (1969) who found that those suffering with AMS recorded the greatest fluid shifts, through gain or loss in ECF. Unlike Westerterp *et al.* (1996), Jain *et al.* (1980) noted changes in TBW and ICF on ascent to altitude. The study compared body water fluid compartment changes in eighteen healthy participants between 200m and 3500m. Body compartments were measured at 3 and 12 days, TBW and ICF were reduced at 3 (-3.7% and -3.3% respectively) and 12 days (-4.7% and -4.3% respectively). A significant decrease in ECF (6%) and PV (16%) was noted at day 12. Krzywicki *et al.* (1971) monitored body fluid compartments, using deuterium oxide and sodium thiocyanate, over a six day exposure to 4300m. TBW significantly decreased by 2.25kg over the six days, while there were non-significant increases of 1.27kg in ECF. Consequently, ICF was significantly reduced by 3.52kg, which is considerably more than other research (Westerterp 2001). This may be a consequence of particularly heavy exercise during the exposure.

Reasons for these alterations in fluid balance is believed to centre around the changes in fluid controlling hormones (Loepky *et al.* 2005a). Milledge and Catley (1982) demonstrated that 2 hrs hypoxic exposure (FIO_2 : 12.8) reduced ACE activity due to the alteration in aldosterone and rennin relationship. While longer altitude exposure studies completed by the group supported the alteration in ACE activity, suggesting that elevated angiotensin II may be the cause of this response.

During normoxia ANP is known to inhibit aldosterone secretion, though Westendrop *et al* (1993) explain that this process is disrupted in hypoxia, as hypoxia reduces aldosterone secretion while increasing ANP (Lawrence *et al.*, 1990). Milledge *et al* (1989) suggested that ANP levels at low altitude were negatively correlated with the increase in AMS symptoms when ascending to 4300m over a two day period. Similarly, the initial 24hr urinary excretion was negatively correlated to AMS symptoms (Milledge *et al.* 1989).

Bartsch *et al* (1988) observed ADH increases in AMS sufferers from $1.0 \pm 0.1 \text{ pmol L}^{-1}$ to $2.9 \pm 1.2 \text{ pmol L}^{-1}$ over a 42hr period at 4559m. ADH was also found to increase more with exercise at high altitude in AMS susceptibles compared with non susceptibles ($23.8 \pm 14.4 \text{ pmol L}^{-1}$ vs $3.4 \pm 1.8 \text{ pmol L}^{-1}$) (Bartsch *et al.* 1991a). The reasons for this difference in ADH release between AMS sufferers and non sufferers are unknown. The degree of hypoxic stress over 24 hours has been found to correlate positively with release of ADH (Claybaugh *et al.*, 1982), probably due to the increase in adrenocorticotrophic hormone (ACTH) release caused by hypoxic stress, ACTH is known to initiate the release of ADH and aldosterone (Bartsch *et al.* 1991a).

Bartsch *et al* (1988) found aldosterone significantly increased in AMS sufferers ($191 \pm 7.0 \text{ pmolL}^{-1}$ to $283 \pm 55 \text{ pmolL}^{-1}$), whereas non AMS sufferers noted a significant decrease ($189 \pm 19 \text{ pmolL}^{-1}$ to $111 \pm 17 \text{ pmolL}^{-1}$), while at rest. Bartsch *et al* (1991a) noted greater increase in aldosterone production during exercise in hypoxia in AMS susceptible volunteers compared with those non susceptible ($617 \pm 116 \text{ pmolL}^{-1}$ vs $233 \pm 42 \text{ pmolL}^{-1}$). Bartsch *et al* (1988) found that ANP significantly increased in AMS sufferers from 31 ± 4 to $87 \pm 26 \text{ pmolL}^{-1}$ ($p < 0.001$), while there was no significant change in non sufferers. Bartsch *et al* (1988) concluded that greater changes in ADH and aldosterone were more likely to cause the changes to fluid homeostasis in hypoxia. In contrast, Bartsch's later work (Bartsch *et al.* 1991b) looked at fluid homeostasis over a slow ascent to 4559m and found greater fluid retention and ANP increases in those who had previously suffered with AMS or HAPE, therefore concluding that ANP and atrial widening may be in part, responsible for AMS. Strangely, the study only measured ANP, PRA and aldosterone and thus cannot rule the likely contribution of ADH.

Jack Loeppky, from New Mexico, recently produced some meticulous research into fluid control and altitude. His first related paper (Loeppky *et al.* 1990) investigated the effect of head-down tilt during hypoxic ($\text{PIO}_2 = 81 \text{ mmHg}/4800\text{m}$) exposure, finding tissue oxygenation and cardiopulmonary function to be the only markers significantly affected by head-down tilt in hypoxia. Following on from this, Loeppky *et al* (1993) then examined the effect of eight days of head-down bed rest on fluid control at altitude (3300m). Interestingly, a diuresis and natriuresis were only present for the first four days and curtailed thereafter, mimicking ANP levels. ADH and aldosterone decreased with head-down bed rest, although there was little difference between control and bed rest trials. The authors concluded that plasma catecholamines (adrenaline and noradrenaline)

and ANP were responsible for the fluid shifts resulting from head-down bed rest in hypoxia.

Loeppky *et al* (2005) then investigated the difference in fluid balance between hypobaric, hypoxic and altitude conditions relatively equal to 3500m, using a 10 hrs resting exposure in nine healthy males. After 3 hrs AMS scores were significantly greater in the altitude exposure in comparison with hypobaria or hypoxia, as previously described by Roach *et al* (1996). During altitude exposure ADH was notably lower than when the volunteers were in hypoxia or hypobaria, in contrast aldosterone was significantly greater in hypobaria. Loeppky *et al* (2005) suggest greater fluid retention at altitude, caused by elevated ADH and unchanged aldosterone is due to a synergistic relationship between the two hormones and lower barometric pressure.

Finally, Loeppky's (2005a) recent study examined fifty-one participants, resting in a hypobaric (4880m) environment over a 12 hr period, monitoring fluid controlling hormones, electrolytes and fluid shifts. Body fluid volume increased at 3 hrs, while plasma Na^+ decreased after 6 hrs. Na^+ and K^+ were found to reduce further through the 12 hr experimental period, while, as expected, ANP increased and aldosterone decreased. Retrospectively, participants were placed into AMS groups based upon peak LLQ scores (susceptibles: mean LLQ = 7.4, range 5-11), non susceptible: mean LLQ = 1, range = 0-2.5) leaving sixteen participants within each group. Data analysis showed AMS susceptibles to have greater $\text{Na}^+:\text{K}^+$ at 6 hrs, while their ADH significantly increased over time. Furthermore, ADH reduced in non susceptibles, showing significantly lower ADH levels in non susceptibles from 90 mins onwards. The authors concluded that AMS susceptibles retain a greater fluid volume due to rise in ADH preventing adequate diuresis

in the early stages of exposure. This fluid retention response is illustrated in Figure 2.11, showing the fluid input, output and net fluid balance over the 12 hr exposure. However, the grouping of AMS susceptibles is a debatable subject, with many individuals suggesting that AMS, as a continuous variable, should be analysed as such (Roggla *et al.* 2000a; Royston *et al.* 2006). Loeppky *et al.* (2005a) offered little explanation regarding the mechanisms that surround the increase in ADH in susceptible subjects, except the suggestion that sensation of nausea was greater in those individuals, though nausea, as a single entity was not measured. Although nausea is known to increase ADH levels (Otto *et al.* 2006), it may not be the key factor in increasing ADH and fluid retention, but rather a product of it.

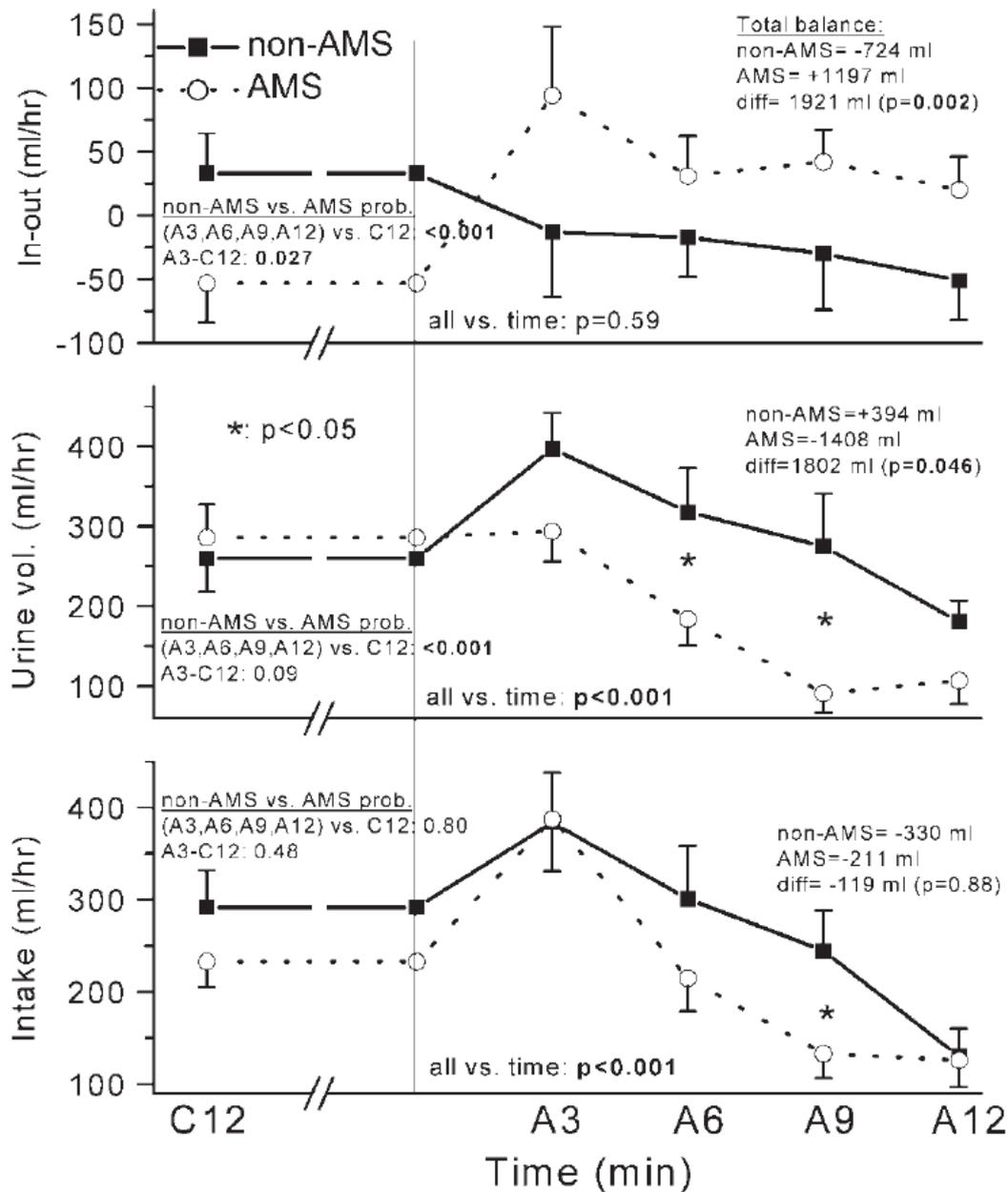


Figure 2.11: Water balance from fluid intake and urine production during baseline, control and over 12 hrs at altitude by both groups. Error bars indicate standard error of the mean. C12 is the value over a 3- to 4-h period on the late afternoon-early evening of the control day, and A3, A6, A9, and A12 indicate the end of the 3-h intervals at altitude, ending at about the same time of day as the C12 measurements, adapted from Loeppky *et al* (2005a). Significance is shown for differences (diff) between acute mountain sickness (AMS) and non-AMS for changes in measurements from baseline. Vol = Volume; In-out = net balance.

2.2.7.1. Fluid Balance during hypoxic exercise

A study by Bocqueraz *et al* (2004) used 60 minutes cycling at a constant relative workload (55% and 75% VO_2max) during hypoxic and normoxic exposures. Trials at 55% VO_2max showed no change in most fluid controlling hormones, with the exception of ANP, which showed a minor increase. While both 75% VO_2max exposures induced significant rise in ADH, PRA and aldosterone, although significantly greater increases were noted in the normoxic trial. In contrast, ANP was greater in the hypoxic exposure, suggesting ANP may have a different time course for release or breakdown than other fluid controlling hormones. The authors concluded that fluid control is influenced by exercise once a threshold is reached, irrespective of oxygen availability. The results also suggest the hypoxic exposure is causing a fluid retention in comparison to normoxia, which has been noted in AMS sufferers. Bocqueraz *et al* (2004) failed to compare AMS symptom scores, SaO_2 or differences in fluid control between subjects, which may have identified subject variations and being such a short test fluid retention would have been minimal. Still, the test emphasises that exercise intensity alters fluid control and that duration of hypoxia greater than 60mins may highlight changes in ANP as well as other fluid controlling hormones.

Bouissou *et al* (1987) found that during trials of 40, 60, 80 and 100% VO_2max under hypoxic and normoxic conditions, plasma renin activity increased with workload while aldosterone did not change. Catecholamine concentrations were no different between conditions. However, Bouissou *et al* (1987) did record greater plasma solute content and osmolality under hypoxia, concluding that hypoxia stimulates greater fluid electrolyte loss from the vascular space while greater K^+ loss from the active muscle must also be initiated. In a later, yet slightly simpler study by Bouissou *et al* (1988), six participants

cycled at 65% VO_2max for 1hr under normoxic or hypoxic conditions. There was no difference in plasma ACE, ACTH, aldosterone or catecholamine release between conditions, though PRA was significantly greater during the hypoxic trial. This indicates that hypoxia alters the PRA mediated release of aldosterone. Maher *et al* (1975) came to the same conclusion when comparing similar dependent variables using a protocol of 20min of light (40% VO_2max) and moderate (75% VO_2max) cycling under hypoxic or normoxic conditions. A 6hr exercise protocol by Meehan (1986) caused no change in urine or serum osmolality, Na^+ , K^+ or ADH, while aldosterone and PRA fell with exercise. Yet, the study only used an exercise protocol of 1mph on a 0% gradient and allowed drinking throughout the protocol. The study found no difference between normoxic and hypoxic (12.5% O_2) protocols. In contrast, Koulmann *et al* (1999) assessed fluid balance in six participants over 1hr of normoxic or hypoxic cycling using deuterium oxide turnover. Expected performance decrements were noted with hypoxia. No differences in elimination of ingested water were seen, when assessed using the same relative power. However, the 20ml D_2O dosage was given as part of a high concentration glucose (12.5%) drink, which would have induced similar retention effects for both conditions, possibly disguising the 'normal' effect of exercise in hypoxia.

To demonstrate the effect of altitude and exercise on renal and endocrine function, Olsen *et al* (1992) used a short duration (160 min) intermittent exercise test of increasing intensity at sea level and then after a 3 day ascent to 4350m. Olsen *et al* (1992) demonstrated that the antidiuretic effects of exercise were the same at altitude and sea level, concluding that GFR was maintained irrespective of hormonal changes, unless the exercise is particularly severe (>56% of maximum work rate). However, the short duration between exercise bouts may not have allowed sufficient time to differentiate a

reponse from separate intensities. Also, Olsen *et al* (1992) reported only very minor AMS symptoms even though the ascent rate was particularly severe, indicating a highly tolerant homogenous group.

The exercise response alone tends to induce greater renal alterations than hypoxia. However, longer duration hypoxic exposures including exercise protocols are still required as much of the limited research available focuses on exposures ~1hr, insufficient to cause significant renal alterations.

2.2.7.2. Hypohydration at altitude

Research on hydration during exercise in hypoxic conditions is less common than that aimed at hydration status and the development of AMS (Cumbo *et al.* 2002). Few studies have looked at hypohydration prior to exercise in hypoxia, even though mountaineers on an expedition will often progressively dehydrate over a period of days through inadequate fluid intake (Piccoli *et al.* 1996).

Hypohydration research at sea level suggests there may be links between hypohydration severity and fluid control, relating to AMS pathophysiology. Carter *et al* (2006) investigated the effect of hypohydration and heat stress on cerebral blood volume while standing. The study showed that hypohydration reduced cerebral blood flow and therefore, may induce orthostatic intolerance. In a hypoxic environment cerebral flow volume is thought to rise due to fluid retention and exacerbate AMS headache symptoms (Hackett 1999). This suggests that cerebral blood flows above and below a normal level, induced through poor fluid control, may exacerbate symptoms such as fatigue, lightheadedness,

dizziness and nausea, associated with orthostatic intolerance and AMS. Hence, some studies have used orthostatic control to predict AMS susceptibility.

Aoki & Robinson (1971) were the first to investigate the influence of hydration on AMS. The study found that dehydration, induced through furosemide administration, had no effect on AMS symptoms when exposed to two days of simulated altitude at ~4000m. This was in comparison to a placebo trial and administration of vasopressin in oil to maintain hydration. Though inducing states of hypohydration or euhydration through exogenous supplementation may influence fluid controlling hormones, which are now thought to be of significant influence on hypoxic tolerance (Loeppky *et al.* 2005a). Aoki & Robinson (1971) highlighted the notion that dehydration may in fact have a beneficial effect on AMS, yet no study has gone on to investigate such a hypothesis. Aoki & Robinson (1971) also suggested the importance of solute and internal water balance and redistribution of blood flow in AMS pathogenesis, in relation to the increase in cerebral blood volume and brain water tissue. Nearly 40 yrs onwards and research still tentatively centres around this theory.

In 1993, Journal of Wilderness Medicine published Viewpoints for water intake at high altitude (Rennie *et al.* 1993). This short article offered interesting opinions from the altitude medicine community. Although there was no consensus, the viewpoints clearly demonstrated the difficulty in differentiating between dehydration through lack of fluid intake and alterations in fluid balance through a diuretic or anti-diuretic response. This, in combination with a lack of controlled research at altitude or within the laboratory at the time of publication, meant that some of the views centred around anecdotal explanations. Others offered some explanations and suggested further research was necessary.

After a transported ascent to 4,350m Westerterp *et al* (1996) monitored ten participants over a four day period at altitude. The study found reduced water intake of around 80% of sea level fluid consumption in those that suffered with AMS. While those that remained asymptomatic drank 100-105% of the fluid consumed at sea level. To support this finding, Basnyat *et al* (1999), using a cross-sectional prospective study on 550 trekkers in the Khumbu valley, reported that raising fluid intake above 3L per day decreased the risk of AMS (odds ratio 1.57; 95% confidence interval, 1.02-2.40). In order to measure many participants, the study could not control for ascent profiles, cardiovascular fitness, food intake or previous altitude exposures. As a consequence, Basnyat *et al* (1999) highlights the need for further research into hydration maintenance at altitude using controlled trials. Basnyat *et al* (2001) also suggests that hydration plays a significant role in AMS pathophysiology and encourage further research into this area within a letter to the Editor of the New England Medical Journal. A view which other highly esteemed research leaders, including Peter Hackett and Robert Roach, support (Basnyat *et al*. 2001).

Bartsch *et al* (1993) combined data from three previous studies to determine the need for fluid intake at altitude. They found that there was no difference in fluid input between those with and without AMS, however, there was a significantly greater diuresis and natriuresis in those without AMS symptoms ($p < 0.05$). Similar to the study of Basnyat *et al* (1999) there was limited control over the ascent profiles, while the authors (Bartsch *et al*. 1993) noted wide variation in fluid intakes between groups (2-4L/24hr).

Olsen *et al* (1992) suggested a protocol to investigate renal responses to acute hypoxia using rest and exercise. Authors used a short 40 min rest bout followed by three bouts of

20 min exercise of increasing intensities (18 ± 2 , 37 ± 4 and $56\pm 6\%$ W_{\max}), separated by 20 min of rest (Figure 2.12). Olsen *et al* (1992) measured biochemical markers of renal function to determine whether exercise intensity in hypoxia influenced renal function via fluid regulating hormones. Although this protocol enables a short yet feasible duration to ascertain changes in hypoxia, the exercise bouts are incomparable and do not ascertain whether physiological changes are the result of an exercise intensity greater than steady state or the immediate hypoxic cardiac or ventilatory response. Comparing different exercise intensities may be of use over longer duration exposures once acclimatisation occurs or over multiple exposures, yet over a short duration while acclimatisation continues, would be inappropriate.

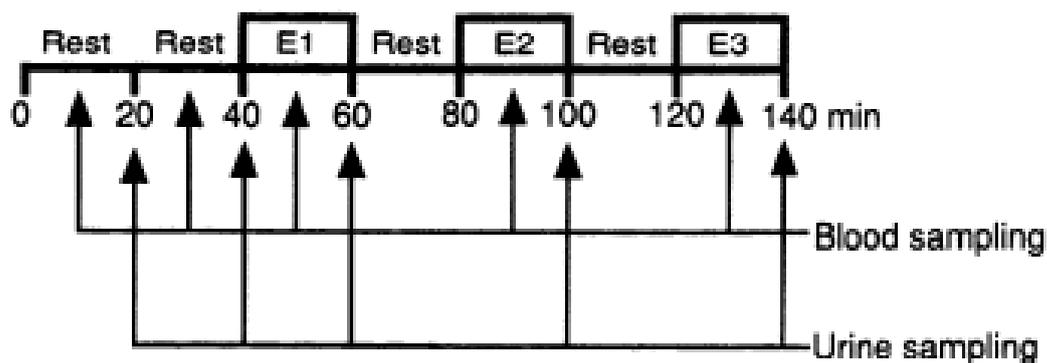


Figure 2.12: Experimental protocol employed by Olsen *et al* (1992). Exercise periods E1, E2 and E3 at sea level corresponded to 18 ± 2 , 37 ± 4 and $56\pm 6\%$ of maximal exercise (W_{\max}) respectively and at high altitude to 24 ± 4 , 41 ± 6 and $57\pm 4\%$ of high altitude W_{\max} respectively.

The most recent study on hydration at altitude by Nerin *et al* (2006), showed that over a seven day walk to 7000m, individuals experiencing AMS demonstrated lower daily urine outputs (AMS = 1336ml; Non AMS = 1655ml). There were no significant difference in

fluid retention between AMS and non AMS sufferers (AMS = 41%; Non AMS = 43%), which contrasts much of the previous AMS and fluid balance work. However, the study was poorly controlled and analysed, with each of the nine participants results taken and compared on separate days, no alterations made for participants attaining significantly different altitudes and only very simple fluid input and output monitored. The study did not report hydration markers, fluid shifts or other possible markers of altitude intolerance other than total LLQ score.

2.3. Exercise at Altitude

Individuals visiting altitude tend to be either trekking or skiing, thus to test hypoxic tolerance a test should effectively mimic normal activity over a set protocol by using set rest : exercise bouts, activity mode, exposure duration and level of hypoxic exposure.

To induce similar responses to altitude, an acute hypoxia study needs to use a moderate intensity constant workload long enough to cause adequate strain and induce minor AMS symptoms. Richardson *et al* (2008b) looked at the physiological responses to an acute 10min bout of 12.8%O₂, showing a 34% decline in SaO₂, 28% rise in heart rate and no change in respiratory rate, all measures returned to baseline within 3mins post hypoxia. Burtcher *et al* (2004) found that 20-30 minutes was the minimum duration required to see any change and plateau in SaO₂ at rest in poikilocapnic hypoxia and went on to show that low SaO₂ after 20-30mins predicted AMS susceptibility in 86% of participants. Gunig *et al* (2000) suggest the optimal duration may be slightly longer, around 90 minutes when investigating HAPE susceptibility and pulmonary artery systolic pressure during hypoxic supine cycling, although the study only looked at three time points (45, 90, 240 minutes). Selland *et al* (1993) found 4 hours exposure enough to note significant decline in HAPE sufferer's lung function variables. Therefore it seems possible to ascertain individuals' immediate hypoxic response from 90mins, irrespective of AMS definitions. Ideally, a test for AMS would be over a longer period as symptoms normally take at least 6-12 hrs to develop once at a greater altitude (Basnyat & Murdoch 2003). However, a laboratory based screening test of this duration is simply not feasible for any physician or physiologist to carry out, and thus a shorter intensive test inducing hypoxic and physiological stress would allow differentiation between participants able to cope with hypoxia and those unable.

Ainslie's field based work (Ainslie *et al.* 2002a; Ainslie *et al.* 2003; Ainslie *et al.* 2002b) on low altitude hill walking has provided much of the physiological evaluation on this activity since Pugh's work in the 1960's (Pugh 1964b, 1967). However, the studies tend to quantify the physiological cost of walking within wet, cold or windy environments as opposed to long duration controlled lab based experiments. Both Pugh *et al.* (1967) and Ainslie *et al.*'s (2002) work suggest that hill walking requires around 50% VO_2max , though this value ranges between 30-60% VO_2max . Weller *et al.* (1997a, b) also produced similar studies with far greater sample sizes, supporting Pugh's (Menier & Pugh 1968; Pugh 1964a; Pugh 1967) low powered work. Yet at altitude it is thought that walking speed is reduced due to the increased physiological strain from hypoxia. Thus a speed equal to 40% VO_2max in normoxia would be more feasible within an altitude setting, equating to around 50% VO_2max based on the reduction of VO_2max at altitude and change in walking speed (Wehrlin & Hallen 2006).

Further support for this hypothesis comes from Cymerman *et al.* (1981) whom exercised four young men at 4,300m with various loads and at various gradients. Authors concluded that the optimum intensity of exercise was 48% of altitude VO_2max . Further analysis of data from the study, demonstrates the physiological demands of walking on a gradient, inducing relatively greater physiological strain than external load carriage (Table 2.2). Irrespectively, Cymerman *et al.* (1981) highlight that inducing a physiological strain around 50% of altitude specific VO_2max is more important for exercise application or research, than specifying gradients or loads.

Table 2.2: The physiological responses to walking on different gradients, with different loads, at 4,300m altitude.

External Load, kg	Grade, %	Exercise Intensity, W	HR, beats·min ⁻¹	$\dot{V}O_2$ (STPD), ml·kg ⁻¹ ·min ⁻¹	$\dot{V}E$ (BTPS), l·min ⁻¹	Oxygen Pulse, ml·beat ⁻¹
0	0		120 ±5	10.5 ±0.3	29.4 ±1.0	7.2 ±0.3
0	8	72 ±4	143 ±5	17.6 ±0.5	52.8 ±3.0	10.0 ±0.7
0	16	144 ±8	165 ±6	27.5 ±0.6	94.6 ±7.6	14.1 ±1.3
15	0		129 ±5	11.5 ±0.3	33.3 ±0.9	7.2 ±0.2
15	8	85 ±4	153 ±6	18.7 ±0.5	60.7 ±4.0	10.4 ±0.9
15	16	170 ±4	172 ±7	27.0 ±1.0	113.7 ±8.1	12.8 ±1.2
30	0		129 ±6	12.8 ±0.4	39.7 ±1.6	8.0 ±0.7
30	8	98 ±4	152 ±6	22.7 ±0.7	78.8 ±6.2	11.8 ±1.0
30	16	196 ±4	170 ±5	29.6 ±1.5	128.9 ±7.7	14.7 ±1.2

Values are subject means \pm SE across days measurements taken in subjects walking at 1.12 m·s⁻¹ at an altitude of 4,300 m with different external loads and treadmill grades. HR, heart rate; $\dot{V}O_2$, O₂ uptake; $\dot{V}E$, minute ventilation.

During hiking, gradients change considerably, though to make the test comparable a constant gradient would be easier to maintain and allow investigation of physiological changes during steady state exercise. While a constant gradient would cause adequate strain on the body (Minetti *et al*, 2002; Roach *et al*, 2002) and exacerbate AMS symptoms (Roach *et al*, 2002). Minetti *et al* (2002) suggest a gradient of 20% as the optimal gradient for a mountain pathway, causing the lowest metabolic cost per vertical metre. Though Minetti explains in this (Minetti *et al*. 2002) and a previous article (Minetti 1995) that at altitude, where there is a reduction in metabolic power, optimal gradient is significantly reduced. Therefore a gradient of 10%, which Minetti *et al* (1993) defined as the metabolic

optimum gradient in 1993, would be more applicable as it also represents a feasible gradient that can be reproduced on most universal treadmills.

2.4. Literature Review Summary

Exposure to hypoxia or altitude induces a physiological response, which may manifest as deleterious symptoms of headache, lethargy, nausea and dizziness, dependent upon individual's tolerance to hypoxia. Irrespective of an individual's hypoxic responses, a state of hypohydration increases physiological strain through a combination of factors. The role of fluid balance within hypoxia remains to be explained, although there is clearly evidence that the control of fluid movement and balance is important within AMS pathophysiology (Hackett *et al*, 1982). The effects of hydration state and fluid balance during rest and exercise within a hypoxic environment need further investigation.

CHAPTER III.

GENERAL METHODS

This chapter describes the materials and methods used in all experimental chapters. In experimental chapters using additional or modified measures, these will be described within the specific methods section.

3.1. Health and Safety

All experiments in this thesis were approved by the University of Brighton Research Ethics Committee before testing was carried out. The first five experimental chapters were carried out within the University of Brighton, Welkin Laboratories. Specific health and safety issues in the sixth experimental chapter are stated (section 9.3). There were at least two experimenters present throughout each test, at least one of which was qualified at first aid and had completed an intermediate life support course. Use of the hypoxic chamber also required at least two experimenters; one within the hypoxic chamber attending to the participant and one experimenter outside the chamber ensuring both individuals within the hypoxic chamber were safe.

Control of Substances Hazardous to Health (COSHH) sheets were completed for every powder or solution used within the study. Risk assessments were also completed for use of all labs, hypoxic facilities, exercise and invasive techniques such as cannulation, venepuncture and rectal thermometry.

All apparatus used was cleaned before and after use. Falconia tubing, mouthpieces and nose clips were soaked in Virkon disinfectant (1%, Antec Int. Suffolk, UK) for at least 10

minutes. These were then thoroughly rinsed prior to use. Electrical equipment contacting the body such as heart rate monitors or saturation probes were cleaned using alcohol wipes, after each use. Rectal thermistors and all other non-reuseable waste were disposed of by incineration. Sharps, such as needles and cannulae from the measurement of blood were disposed of in a designated sharps bin.

Experimental trials were started after the participants provided written informed consent as shown in Appendix 1-3. For each individual study, participants were provided with a participant information sheet detailing the study design and the participant requirements. Participants were also invited to ask questions regarding the study, before they consented to undertaking the research. Participants were informed that they could withdraw from the study at any time without providing justification or explanation.

Experiments were stopped if any of the following criteria were met:

- the participant asked to stop the test. Participants were not required to give any reason for this;
- the experimenter felt it appropriate to stop the test whether it be for equipment problems or the participant displaying signs of illness such as chest pain, dyspnea, nausea, vomiting, generic pain/discomfort, faintness or dizziness;
- the rectal core temperature increased by more than 2°C from the starting value;
- the rectal core temperature decreased by more than 1.5°C from the starting value; or
- the rectal core temperature increased to >39.7°C

These rectal core temperature safety limits are set by the University of Brighton Research Ethics Committee.

3.2. Participants and recruitment

Physically active individuals were recruited for each study and participated after giving written informed consent (Appendix 1), as approved by the University of Brighton Research Ethics Committee. Participants had not performed exhaustive exercise in the 2 days prior to each trial and had not consumed alcohol or caffeine during a period of at least 24 hours immediately preceding the study. Participants were non-smokers and had not spent any time above 2000m in the preceding 2 months. Participants were explained verbally the protocol, dangers and discomforts possible, while also given a participant information pack to read. Participants could then decide if they still wanted to take part.

3.3. Familiarisation trials

3.3.1. Body Fat Assessment

Assessment of body fat was measured using Harpenden callipers (Harpenden, Idass, England) and calculated via the Jackson and Pollock (1978) seven site skinfold body fat assessment.

3.3.2. Determination of Walking Speed for the intermittent walking protocol

Walking speed, on a 10% gradient at 50% VO_2max , was predicted using a 10 min steady state walking test with intensity based on the heart rate: oxygen uptake relationship. Participants walked at 4, 4.5 and $5\text{km}\cdot\text{hr}^{-1}$ to quantify heart rate, values were then extrapolated and compared to the heart rate and VO_2 values attained during the VO_2max test.

3.3.3. Determination of Lactate threshold and Maximal Oxygen Uptake

Participants completed a lactate threshold and maximal oxygen uptake test, using an incremental running protocol, as validated by Weltman et al (1990). Initial increments of 1kph every 3 min were used until lactate threshold (lactate increase >1mM) was reached. The test then continued at increments of 1kph every minute until volitional exhaustion, heart rate plateaued within 8bts/min of their aged predicted maximum heart rate or participants reported a rating of perceived exertion value of 19-20. Gas analysis (section 3.7.3) was carried out on all the expired gas samples throughout the test. The maximum oxygen uptake value was regarded as the $\dot{V}O_{2max}$.

3.4: The Intermittent Walking Test

Based on previous research (Olsen *et al*, 1992) the intermittent walking test involved testing a range of physiological markers over the 125 min test duration. The test included a 35 min rest period followed by three 20 min exercise phases separated with 5 min rest intervals and a final 20 min recovery phase, as shown in Figure 3.1. Exercise involved participants walking on a treadmill (PP55Sport, Woodway, Germany) at a speed equal to sea level 50% VO_{2max} , at a gradient of 10%, which was calculated in the steady state walking test (section 3.3.2).

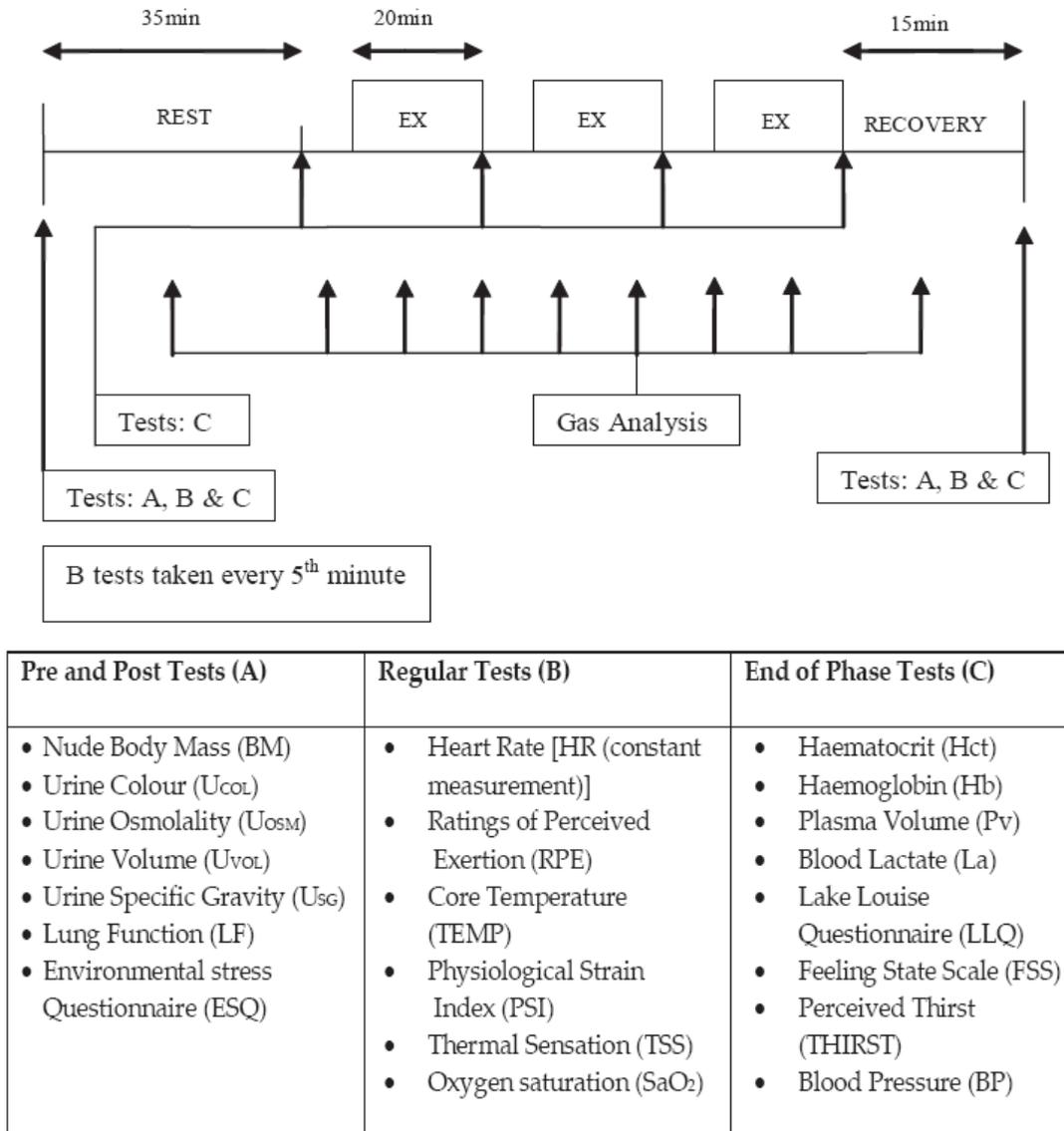


Figure. 3.1. A schematic diagram showing the rest, exercise periods and measurement time points during the Intermittent Walking Test.

3.5 Controlling the Hypoxic Environment

For experimental chapters IV to VI a hypoxic tent (Colorado Altitude Training, Colorado, USA) was filled with nitrogen enriched air by nitrogen generators (Colorado Altitude Training, Colorado, USA) (Figure 3.2). The four generators each produced up to 10L/min of nitrogen enriched gas. This volume could be controlled to limit the inspired oxygen fraction (FIO_2) within the tent. FIO_2 was constantly monitored throughout each trial by connecting sample tubing from the tent to a gas analyser (Servomex 1400, Servomex Group Ltd, Crowborough, England). For the longer duration trials in Chapter VII a British Army gas mask (S10, British Army, UK) was worn and connected to the nitrogen generators, as shown in Figure 3.3. The gas mask was connected to the gas analyser sample line via the built-in drinks tube to constantly monitor inspired O_2 and CO_2 . Chapter VIII used a purpose built hypoxic chamber (The Altitude Centre, UK), which also used nitrogen enriched air to generate and control hypoxia.

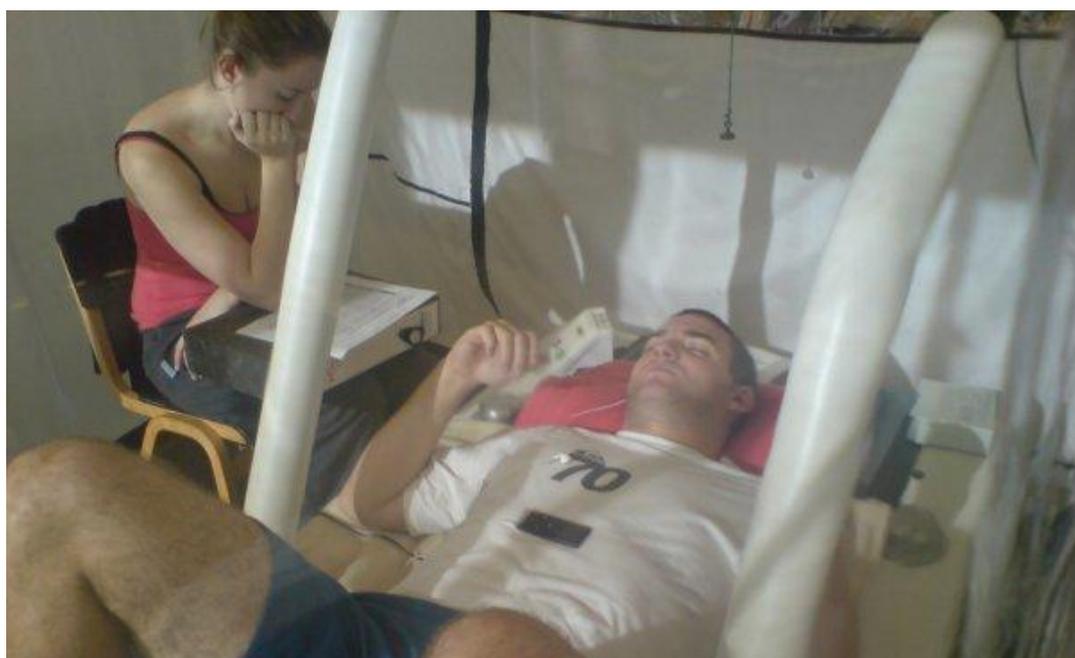


Figure 3.2: Participant resting during a hypoxic intermittent walking protocol within the tent.



Figure 3.3: Participant wearing a British Army gas mask to generate hypoxic conditions over a long duration protocol.

3.6 Hydration protocols

Euhydration: Participants completed 1 hr of high intensity running ($\sim 75\% \text{VO}_2\text{max}$) 15 hrs prior to the intermittent walking test, while wearing warm clothing (hat, gloves, jumper, trousers). Nude body mass was measured pre and post exercise. Participants were then encouraged to rehydrate over the 15 hours preceding the intermittent walking test, by consuming 150% of sweat loss during the hour prior to exercise (Shirreffs *et al.* 2007).

The following protocols were used to induce hypohydration throughout the experimental chapters and then referred to as hypohydration protocol 1, 2 or 3 within each chapter.

Hypohydration1: Participants completed the exercise as in the euhydration trial and were required to consume fluid equal to half the amount lost as sweat, over the 15 hrs preceding the intermittent walking test.

Hypohydration2: Participants completed the exercise as in the euhydration trial and were fluid restricted during exercise and for 15 hrs preceding the intermittent walking test.

Hypohydration3: Participants completed 1.5 hrs exercise and were fluid restricted during exercise and for 24 hrs preceding the intermittent walking test.

Hyperhydration: Participants completed the exercise and consumed fluid as in the euhydration condition, on arrival to the laboratory participants were asked to consume a bolus of water equal to $28\text{ml}\cdot\text{kg}^{-1}$ body mass over a period of 40 mins (Latzka *et al.* 1998), prior to pre test measures.

3.7. Generic variables

3.7.1 Body Mass

Nude body mass was recorded using electronic scales (WB110PMA, Tanita, Netherlands), sensitive to 50g. The scales were calibrated prior to each day of testing using a known weight. For the studies using hydration trials, establishing ‘normal’ or ‘baseline’ body mass required measurement of nude post morning void body mass on seven consecutive mornings. Cheuveront *et al* (2004) suggested a minimum of three measurements is necessary to ascertain a valid baseline body mass as daily variation in adults was quantified as 0.51 ± 0.2 kg.

3.7.2.1 Urine Markers

Participants were asked to void their bladder contents into a measuring jug so urine volume could be recorded. Urine was then poured into a clear sterile 30ml sample container. The urine container was then held up to natural light and urine colour was assessed against the Urine Colour Chart (Armstrong 2000), illustrated in Figure 3.4. This was performed by at least two individuals to prevent experimenter visual error or bias.



Figure 3.4: A urine colour chart (Armstrong, 2000)

Table 3.1: Validity and reliability testing for urine osmolality, urine specific gravity and urine colour, when comparing between twenty measures of the same urine sample on the same day and a further twenty measures of one urine sample on the following day from the same participant. SD = Standard deviation, CV = Coefficient of variation.

	<i>Osmolality</i> (<i>mosm.kg⁻¹</i>)		<i>Specific Gravity</i>		<i>Colour</i>	
Day	1	2	1	2	1	2
Mean	355	367	1.008	1.009	2	2
SD	5.23	5.9	0.0007	0.0006	0	0
CV (%)	1.4	1.6	0.07	0.05	0	0
Diff in the Mean (%)	3.48		0.0614		0	
Between Day CV (%)	1.03		0.072		0	
Correlation (<i>r</i>)	0.562*		0.443*		1*	

* Denotes significant Pearson's correlation comparing measures from separate days.

3.7.2.2 Urine Specific Gravity

Urine specific gravity was assessed using a refractometer (Specific Gravity Refractometer Model 32, Atago, USA). A small volume (~2ml) of urine was placed onto the glass window and the window flap closed. The refractometer was then held up to the light, while the experimenter looked through the eye piece and recorded the values from the scale within. The refractometer was calibrated daily using distilled water (USG = 1.000).

3.7.2.3. Urine Osmolality

Urine osmolality was measured using a micro osmometer (Micro-osmometer 3300, Advanced Instruments Inc, USA). A small volume (50µL) of urine was collected using the osmometer pipette. The pipette was then placed inside the micro-osmometer whereby

the sample osmolality was measured using water freezing point depression. The micro osmometer was calibrated daily using distilled water and was also calibrated prior to each study using solutions of known osmolality.

3.7.3. Gas Analysis

Participants wore mouth pieces connected to Falconia tubing and Douglas bags (Type 343, Georg Fischer, Switzerland), while the gas samples were collected over approximately 40s for exercising samples and 60s for resting samples. The Douglas bags were then analysed using a infra-red capnograph gas analyser (Servomex 1400, Servomex Group Ltd, Crowborough, England) and values for oxygen uptake (VO_2), carbon dioxide (VCO_2), minute ventilation (V_E) and respiratory exchange ratio (RER) were calculated using the Haldane equation and recorded. Breathing frequency was measured and tidal volume calculated from V_E . Technical error of measurement (TEM) was calculated for the primary investigator. TEM for VO_2 , VCO_2 and V_E were 0.029L, 0.021L and 0.211L respectively, when measuring five separate gas samples on a resting participant on two consecutive days.

Equation 3.1: Calculation of tidal volume

$$\text{Tidal Volume} = V_E / \text{Breathing Frequency}$$

3.7.3.1 Peripheral Arterial Oxygen Saturation

Peripheral arterial oxygen saturation was estimated using a fingertip pulse oximeter (Nonin 2500, Nonin Medical Inc, Minesotta, USA). Values were recorded after a period of 10s wearing the pulse oximeter to allow values to settle.

3.7.3.2 Lung Function

Lung function was measured standing upright, using a Vitalograph (Gold Standard Vitalograph, Vitalograph Ltd, Buckinghamshire, England). Participants completed the lung function trials in a resting state in a normoxic environment. Each lung function test was performed a minimum of three times to ensure participants gave their best effort. Forced expiratory volume in 1 sec (FEV1), forced vital capacity (FVC), maximal voluntary ventilation (MVV) and vital capacity (VC) were recorded from the test that achieved the largest FVC.

3.7.3.3 Hypoxic Ventilatory Response

Hypoxic ventilatory response (HVR) was calculated using the slope of the regression line from the change in minute ventilation against the change in SaO₂, multiplied by -1 to generate a positive value.

Equation 3.2: Calculation of hypoxic ventilatory response

$$\text{HVR} = \Delta V_E / \Delta \text{SaO}_2$$

3.7.3.4 Cardiovascular and Thermoregulatory Measures

Rectal temperature was measured using a rectal thermistor probe inserted 10 cm past the anal sphincter (Henleys Medical Supplies Ltd, Welwyn Garden City, England). Heart rate R-R intervals measured constantly throughout every study using a polar heart rate monitor (S810, Polar, Kempele, Finland).

Equation 3.3: Calculation of physiological strain index

$$PSI = 5(T_{ret} - T_{re0}) \cdot (39.5 - T_{re0})^{-1} + 5(HR_t - HR_0) \cdot (180 - HR_0)^{-1}$$

Whereby T_{ret} and HR_t were simultaneous measurements taken at any time during the exposure and T_{re0} and HR_0 were the initial measurements (Moran *et al.* 1998a).

3.7.3.5 Hypoxic Cardiac Response

Hypoxic cardiac response (HCR) was calculated using the slope of the regression line from the change in heart rate (HR) against the change in SaO_2 , multiplied by -1 to generate a positive value.

Equation 3.4: Calculation of hypoxic cardiac response

$$HCR = \Delta HR / \Delta SaO_2$$

3.7.4. Perceptual Scales

3.7.4.1. Environmental Symptoms Questionnaire

A modified sixty-five question Environmental Symptoms Questionnaire (ESQ) (Wright *et al.* 1985), with two sleep based questions extracted, was used (Appendix 5). This questionnaire was produced by the United States Army as a method of quantifying a range of environmental stressors. Development of this questionnaire allowed specific scores to be calculated from the whole questionnaire. ESQ cerebral score (ESQc) is thought to represent closely symptoms of AMS. This is calculated by taking the weighted sum of fifteen cerebral-based questions (Sampson *et al.* 1983).

3.7.4.2. Lake Louise Questionnaire Score

The Lake Louise Questionnaire (LLQ) (Roach *et al.* 1993) was used with the sleep question extracted (Appendix 4). AMS symptoms were calculated using the sum of four questions scored 0-3, including; headache, gastrointestinal upset, fatigue or weakness, dizziness or lightheadedness. Scores of >5 are thought to represent AMS, the value of which is used in this thesis. Although this has come under much scrutiny (Bartsch *et al.* 2004) and is also inclusive of the sleep based questions, which are often irrelevant during acute exposures.

3.7.4.3. Thermal Sensation Scale

Thermal sensation measures as devised (Gagge *et al.* 1967) and validated by Gagge *et al.* (Gagge *et al.* 1969), allows the quantification of perceived thermal sensation (Appendix G), using a 0-10 scale. This scale has been widely used and validated against more sophisticated and complex measures of thermal sensation (Zhang *et al.* 2004).

3.7.4.4. Perceived Thirst

The Perceived Thirst Scale (Engell *et al.* 1987) allows the quantification of perceived thirst, using a 1 to 9 scale. This was validated against graded hypohydration of 0%, 3%, 5% and 7% loss in body mass and against various fluid intake levels by ad libitum drinking. Further studies have used (Kenefick *et al.* 2008; Maxwell *et al.* 2009) and validated this scale (Maresh *et al.* 2004).

3.7.4.5. Rating of Perceived Exertion

Rating of perceived exertion (RPE) (Borg 1998) quantifies perceived exertion during exercise. Validation studies of this have shown the scale to correlate positively with

exercise intensity (Kolkhorst *et al.* 1996; Pandolf *et al.* 1984) throughout the many versions of the Borg scales (1973, 1982, 1998) (Chen *et al.* 2002). The most recent meta-analytical review (Chen *et al.* 2002) of all these studies investigating the Borg RPE scale validity, suggests the validity and reliability is entirely dependent upon the type of exercise and environmental stressor. However, when examining the validity over a wide range of exercise types, intensities and environments, the RPE scale is still significantly positively correlated with exercise intensity ($r = 0.8-0.9$) (Chen *et al.* 2002).

3.8. Haematological Measures

3.8.1. Finger Tip Blood Sampling

Finger tip blood samples were collected from the third finger of the participant's non dominant hand. The finger was cleaned using alcohol wipes and pricked using a finger prick pen (Accucheck Softclix Pro, Roche, Lewes, England). The first appearance of blood was wiped away for blood to then be collected with the relevant implement depending on sample type as detailed in sections 3.8-3.9. Reliability sampling and analysis was carried out prior to experimental studies. Blood was collected for lactate, glucose, haemoglobin and haematocrit analysis and assessed same day and between days on one participant, as detailed in Table 3.2. Blood analysis is detailed in section 3.8.2 to 3.8.4. Analysis showed all capillary blood sampling to have less than 5% typical error.

3.8.2 Blood Lactate

Fingertip capillary whole blood was collected in lithium heparin coated microvette tubes (Microvette CB300, Sarsedt, Akteingesellschaft & Co, Numbrecht, Germany) and analysed immediately using a lactate and glucose monitor (YSI Model 2300 Stat Plus, Yellow Springs, Ohio, USA).

3.8.3 Haemoglobin

Fingertip capillary blood was collected using Hemocue slides (B-Hemoglobin Microvettes, Hemocue, Angelhom, Sweden) and then placed into a portable Hemocue haemoglobin device (B-Hemoglobin Photometer, Hemocue, Angelhom, Sweden). This device quantified haemoglobin to $1\text{g}\cdot\text{L}^{-1}$. The device was calibrated prior to each sample using a Hemocue calibration slide (B-Hemoglobin Calibration Slide, Hemocue, Angelhom, Sweden) of a known value ($131\text{g}\cdot\text{L}^{-1}$).

3.8.4 Haematocrit

Blood for measurement of haematocrit was collected in heparinised capillary tubes (Hawksley & Sons LTD, Lancing, England) from either finger prick blood sampling or directly from the syringe, for experiments in Chapter VII and Chapter VIII. Blood was collected in triplicate to allow for breakages of the delicate capillary tubes and to obtain an averaged sample value. Capillary tubes were spun in a centrifuge (Hematospin 1300, Hawksley & Sons LTD, Lancing, England) at 2000rpm for 1 min. Haematocrit was then measured using a haematocrit capillary tube slide measure (Hawksley Reader 01502, Hawksley & Sons LTD, Lancing, England) by measuring the red blood cells from the separated blood plasma.

Table 3.2: Validity and reliability testing for blood lactate, glucose, haematocrit and haemoglobin, when comparing between ten samples on the same day and a further ten samples on the following day from the same participant. Blood lactate (Exercising) was measured while the participant performed 15 minutes of walking (5kph) on a 10% gradient. SD = Standard deviation, CV = Coefficient of variation.

	<i>Lactate (mmol.L⁻¹)</i>		<i>Lactate (mmol.L⁻¹)</i>		<i>Glucose (mmol.L⁻¹)</i>		<i>Haematocrit (%)</i>		<i>Haemoglobin (g.dL⁻¹)</i>	
	<i>(Exercising)</i>									
Day	1	2	1	2	1	2	1	2	1	2
Mean	1.01	1.03	1.49	1.44	4.08	4.02	45.1	45.2	13.4	13.3
SD	0.05	0.06	0.15	0.11	0.08	0.11	0.41	0.32	0.09	0.13
CV (%)	6.0	5.8	7.3	7.7	1.8	1.3	0.9	0.7	0.7	0.9
Diff in the Mean (%)	1.7		2.8		2.5		0.001		0.7	
Between Day CV (%)	4.533		9.117		1.181		0.524		0.66	
Correlation (<i>r</i>)	0.445*		0.004		0.523*		0.621*		0.402*	

* Denotes significant Pearson's correlation, comparing day 1 to day 2.

3.8.5 Venepuncture Blood Sampling

All venepuncture samples were taken from the antecubital fossa using a sterile needle (21G needle, Sarsedt, Akteingesellschaft & Co, Numbrecht, Germany) and syringe (B&D Plastipak 30ml Syringe, Hamburg, Germany). Prior to venepuncture the participant was placed into a sitting or lying position, an appropriate arm was chosen and the area cleaned using alcohol wipes. A tourniquet was used to select a vein and allow insertion of the needle. The necessary volume of blood (typically 10ml) was drawn into the syringe.

Blood was immediately transferred into 5ml EDTA (32.332, Sarsedt, Akteingesellschaft & Co, Numbrecht, Germany) or 5ml clear centrifuge tubes (32.320, Sarsedt, Akteingesellschaft & Co, Numbrecht, Germany) so the blood could be spun in a centrifuge

(5804R Centrifuge, Eppendorf, Hamburg, Germany) for 10 min at 5000rpm at a temperature of 5°C. The plasma or serum was then immediately separated from the blood and placed in 2ml microtubes. Three aliquots per sample point were stored in the freezer (-86°C VIP Series, Sanyo Biomedical, Loughbrough, UK) at -86°C for no longer than 3 months before biochemical analysis.

3.8.6 Cannulation

Participants were placed into a lying position and an appropriate hand was chosen and cleaned with alcohol wipes. A tourniquet was used to select a vein and a cannula (18G I.V. Catheter, Biovalve, Vygon, UK) was then inserted into the dorsal aspect of the hand. The cannula was then flushed with 20ml of saline solution (0.9% Sodium Chloride I.V. Infusion, Baxter Healthcare LTD, Norfolk, UK). A syringe (B&D Plastipak 30ml Syringe, Hamburg, Germany) was then used to draw blood via the cannula. A further 20ml of saline was used to flush the cannula after each sample was taken, which was equal to the amount of blood extracted.

3.8.7 Plasma Volume Change

Change in plasma volume (PV) was calculated from haematocrit (Hct) and haemoglobin ([Hb]), between each time point using the following equation of (Dill & Costill 1974), where 'A' was the first blood sample and 'B' was the second;

Equation 3.5: Calculation of plasma volume change

$$\Delta PV\% = 100 \left[\frac{[HbA] (1 - HctB \times 10^{-2})}{[HbB] (1 - HctA \times 10^{-2})} \right] - 100.$$

3.8.8 Peripheral Oxygen Blood Content

Peripheral arterial oxygen saturation (SaO₂) was measured using a finger tip pulse oximeter (Nonin 9550 Onyx II, Nonin Medical Inc, Minnesota, USA). Peripheral O₂ blood content (CpO₂) was calculated from [Hb] and SaO₂ using the following equation;

Equation 3.6: Calculation of peripheral blood oxygen content

$$CpO_2 = [Hb] \times SaO_2 \times 1.34.$$

3.8.9. Plasma Osmolality

Plasma osmolality was measured using a micro osmometer (Micro-osmometer 3300, Advanced Instruments Inc, USA). A small volume (50µL) of plasma was collected using the osmometer pipette. The pipette was then placed inside the micro-osmometer whereby the sample was measured using freeze point depression. The micro osmometer was calibrated daily using distilled water and was also calibrated prior to each study using solutions of 200mosm·kg⁻¹, 250mosm·kg⁻¹ and 300mosm·kg⁻¹.

3.9. Biochemical Analysis using Enzyme-Linked Immunoassay (ELISA) kits

3.9.1. Standard ELISA Analysis

Aldosterone, ADH, ANP, HSP₇₀ and serum 100β were all measured from plasma using an Enzyme Immunoassay (ELISA) kits, sensitivity and CV% are described in Table 3.3. The serum 100β ELISA had a measurement range of 98-6300pg·ml⁻¹ and offered limited precision based on the estimated size of change (Kaur & Ling 2008). Values for all ELISA kits were read on a microplate reader (ELX800 Universal Microplate Reader, Biotek Instruments INC, Vermont, USA) at a wavelength of 450nm.

Table 3.3: Description of ELISA kits used with the sensitivity and Coefficient of Variation (CV%) as detailed by the respective manufacturers.

<i>Measure</i>	<i>Details</i>	<i>Sensitivity</i>	<i>CV%</i>
ANP	1-28 Alpha, Phoenix Europe GMBH, Germany	0.14 ng·ml ⁻¹	<5
ADH	Arg8, Phoenix Europe GMBH, Germany	0.04 ng·ml ⁻¹	<5
Aldosterone	DB52001, IBL, Germany	10 pg·ml ⁻¹	<5
HSP ₇₀	EKS-715, Stressgen, Assay Design, USA	90 pg·ml ⁻¹	5.9
Serum 100β	YK150, Yanaihara Institute INC, Japan	98 pg·ml ⁻¹	7.13

3.9.2. Urine Creatinine Analysis

Urine creatinine was measured using a colorimetric microplate assay (Oxford Biomedical Research, USA) based on the Jaffe reaction (Bonsnes & Taussky 1945). The urine creatinine was diluted, mixed and reacted with picric acid under alkaline conditions, causing a quantifiable orange colouration. The absorption spectroscopy (PU8670 VIS/NIR, Philips, USA) was set at a wavelength of 500nm.

3.10. Fluid Compartment Measures

Multi-frequency bioelectrical impedance analysis (MFBI) (Xitron 4000, San Diego, CA) was used to monitor TBW and ECF, using the Cole-Cole theoretical model (Cole & Cole 1941), calculating ICF by subtracting ECF from TBW. Measurements of MFBI were only performed once the participant had lay still for at least 45min, the same lying position was performed for every measurement. A study (Armstrong *et al.* 1997) using the same Xitron monitor, equation and similar white male participants found TBW values to correlate ($r = 0.93$) with deuterium analysis derived TBW values. The MFBI technique was found to underestimate by 0.97L while the error (SD of the bias) was 1.18L. In

contrast ECF was shown to be over-estimate MFBIA by 1.07L when compared to the bromide method, while the techniques correlated ($r = 0.93$). This represents an error of approximately 5% for both TBW and ECF using MFBIA. Subsequently, this technique has come under much scrutiny over accuracy and reliability (Earthman *et al.* 2007), thus deuterium oxide was also used to assess TBW and sodium bromide to quantify ECF; the gold standard measurement technique of fluid compartments (Armstrong 2007).

Two hours prior to testing participants were given 10ml of 99.9% deuterium oxide (Sigma Aldrich, UK) and 1ml/kg of body mass 6g% sodium bromide (Sigma Aldrich, UK), 25ml of water was then used to wash the container and given to the participant to consume. Blood samples to assess plasma deuterium and bromide were taken immediately prior to dosage and then at the same time points as the other blood measures. When used in such small doses these substances are regarded as harmless (Janssen *et al.* 1997; Nielsen *et al.* 1971; Van Kreef *et al.* 1996), although COSHH sheets were completed.

3.10.1. Deuterium Oxide Analysis

Deuterium oxide was measured from blood plasma using isotope ratio mass spectrometry by HNR Cambridge. Studies using the same technique have found coefficient of variation to be between 0.0% and 2.4% for duplicate samples (Dioum *et al.* 2005).

The following equation was used to calculate TBW, where δ_{pre} and δ_{post} are the pre and post test deuterium values [$\delta = \text{delta Vienna} - \text{standardised mean ocean water (V-SMOW)}$], δ_{dose} and δ_{tap} are the content of the tap water and deuterium dose, W is the amount of water diluting the dose, A is the amount of deuterium oxide administered to the participant, a is the amount of dose diluted for analysis, 18.02 is to convert TBW is moles

to kilograms, 1.04 is a correction factor to account for the exchange of deuterium with the labile hydrogens in the body other than TBW.

Equation 3.8: Calculation of total body water using deuterated water

$$\text{TBW (kg)} = (((W \times A) / 18.02a) \times ((\delta_{\text{dose}} - \delta_{\text{tap}}) / (\delta_{\text{post}} - \delta_{\text{pre}}))) \times 18.02 / 1.04$$

3.10.2. Bromide Analysis

Bromide was measured from serum using infrared spectrophotometry (PU8670 VIS/NIR, Philips, USA). Each blood sample was deproteinised with 1.0ml of ice cold 0.6M perchloric acid and centrifuged at 5000rpm for 10min at 4°C. The supernatant was then separated and 500µl added to a test tube containing 500µL of 0.6% sodium chloride and 500µL of 0.0375% gold chloride, a wavelength of 450nm was then used to measure the samples exactly 3 min after the addition of the supernatant. Concentrations were derived from a standard curve (Islam *et al.* 1999). Intra-assay and inter-assay variations have been previously calculated at 3% and 5-10% respectively (Zdolsek *et al.* 2005).

3.11. Filtration

3.11.1. Glomerular Filtration Rate

Estimated glomerular filtration rate (eGFR) was calculated using creatinine clearance, as described by (Levey *et al.* 2003).

Equation 3.9: Calculation of glomerular filtration rate from serum creatinine

$$\text{eGFR} = 186 \times \text{serum creatinine}^{-1.154} \times \text{Age}^{-0.203} \times [1.21 \text{ if black}] \times [0.742 \text{ if female}].$$

3.11.2. Free Water Clearance

Free water clearance (CH_2O) was calculated using the following equation, whereby V was the urine flow rate and U_{osm} and P_{osm} are urine and plasma osmolality.

Equation 3.10: Calculation of free water clearance

$$CH_2O = V - ((U_{osm} / P_{osm}) \times V).$$

3.12. Statistical analysis

Data was checked for normality and sphericity and was adjusted using the Huynh-Feldt method. All data was analysed using a standard statistical package (SPSS version 14 for Windows, 2005). Data was reported as mean \pm SD, with the significance level set at $p < 0.05$. Any specific statistical analyses that were necessary to each study are detailed in the relevant experimental chapter. For chapters IV-VIII, two way ANOVA with repeated measures was used to identify significant effect of the condition and or time point. Additionally, one way ANOVA with repeated measures was used to compare between conditions, bonferroni pairwise comparisons compared separate conditions.

3.13. Pilot Work

Prior to the experimental studies, much time was spent ensuring the appropriate hypoxic conditions were controllable and maintainable throughout exposure protocols, particularly while multiple individuals were within the tent and exercising. Through continual alterations in generator flow rates and tent ventilation, based on continuous hypoxic tent gas sampling (detailed in section 3.7.3) the O_2 and CO_2 content were well controlled.

Other pilot work surrounded the manipulation of hydration state through prior dehydration and fluid restriction. Based on the work by Montain & Coyle (1992) and Sawka *et al.* (1984, 1985) and through monitoring dehydration over periods of exercise and fluid restriction the set protocols were established (section 3.6). Similarly, previous protocols by Latzka & Sawka (2000) and Latzka *et al.* (1997, 1998) were assessed prior to the hyperhydration condition used in Chapter IV.

Longer duration resting hypoxic trials were also performed prior to experimental studies (Chapter VII and VIII) on six individuals, to test whether there was a particular response times for SaO₂, heart rate, LLQ and ventilation to resting hypoxia, as suggested by Swenson *et al* (1995). The responses found were clearly individualistic, yet most severe symptoms were seen from ~4hours onwards, similar to the findings of Loeppky and colleagues (Loeppky *et al.* 2005a; Loeppky *et al.* 2003b; Loeppky *et al.* 2005b).

3.14. Powers Analysis

Testing in hypoxia can be time consuming and of some discomfort for participants, while the cost and consequent feasibility is also important to consider.

To carry out post hoc powers analysis, the effect size of the data must be quantified using the following equation, where μ^1 is mean of sample one, μ^2 is the mean of sample two, Σd^2 is the sum of the differences and 'n' is the sample size.

Equation 3.11: Calculation of Effect Size

$$\text{Effect Size} = (\mu^1 - \mu^2) / ((\Sigma d^2) / n)$$

Using Cohen's d or f for a t-test or ANOVA respectively, the relationship between effect size and statistical power for that test can be established. Effect sizes are often grouped into low, medium and high. Subsequently, post hoc data from Chapter IV was used to complete powers analysis (95% confidence interval), to ascertain the optimum number of participants for the later studies. The study of twelve participants found effect sizes of ~ 0.8 for the main physiological markers, suggesting a sample size of between 8 and 12 participants [LLQ (n=8), SaO₂ (n=10), heart rate (n=12), rectal core temperature (n=9)] to find significant difference between hypoxic conditions. Data from Chapter V found similar powers analysis sample size values when comparing between different hydration states using the same physiological markers [LLQ (n=7), SaO₂ (n=15), heart rate (n=9), rectal core temperature (n=7)]. From the powers analysis data, a sample size of eight participants was used from Chapters V to VIII.

CHAPTER IV.

PHYSIOLOGICAL RESPONSES TO GRADED ACUTE NORMOBARIC HYPOXIA USING AN INTERMITTENT WALKING PROTOCOL

4.1 Abstract

The study aimed to measure the physiological responses to acute normobaric hypoxia during an intermittent walking protocol. Twelve active healthy male participants completed a 125 min test that involved rest and walking (50% $\dot{V}O_{2max}$) during normoxic [20.93%O₂ (NORM)] and two hypoxic conditions [14%O₂ (HYP1) and 12%O₂ (HYP2)]. A range of physiological markers were measured throughout the test. LLQ scores and ESQc scores were used as a measurement of AMS symptoms. Oxygen saturation (SaO₂) ($r=-0.72$), thermal sensation scale ($r=0.61$), heart rate ($r=0.63$), perceived thirst ($r=0.49$), core temperature ($r=0.44$), rating of perceived exertion ($r=0.79$), feeling state ($r=0.88$) and Δ body mass ($r=0.68$) all positively correlated with the highest LLQ ($p<0.05$). Heart rate (NORM 124±9; HYP1 132±12; HYP2 142±13) and SaO₂ (NORM 97±1; HYP1 84±4; HYP2 77±6) were significantly different between the three conditions during the exercise phases (data presented at 20mins of exercise). A range of physiological markers are associated with symptoms of AMS following brief periods of hypoxic exposure. These physiological markers are significantly altered with severity of hypoxic exposure.

Key Words: Hypoxia, Acute Mountain Sickness, Intermittent Exercise

4.2 Introduction

The extent of the physiological response to hypoxia is known to vary when travelling above 2500m (Bartsch *et al.* 2004). In some individuals the decline in the PO₂ results in the development of AMS, although this can depend on the ascent style, altitude, prior history of AMS and an individual's altitude tolerance (Bartsch *et al.* 2004). The Lake Louise Consensus Group (Roach *et al.* 1993) defined AMS as the presence of headache in an unacclimatized person who has recently arrived at an altitude above 2500m plus the presence of one or more of the following: gastrointestinal symptoms (anorexia, nausea, or vomiting), insomnia, dizziness, and lassitude or fatigue.

Physiological variables associated with the onset of, and susceptibility to, AMS include: increased body temperature (Roggla *et al.* 2000a), decreased SaO₂ (Burtscher *et al.* 2004), increased end tidal CO₂ (PETCO₂) (Reeves *et al.* 1993), decreased peripheral blood oxygen content (CpO₂) (Savoirey *et al.* 2007), increased hypoxic cardiac response (HCR) (Savoirey *et al.* 2007) and increased change in lung function (Ge *et al.* 1997). A review by Burthscher *et al.* (2008b) suggests SaO₂ at 30 minutes exposure is currently the most reliable predictor of AMS. However, at present there is no specific test which quantifies hypoxic tolerance over an acute exposure, even though there have been many attempts at predicting hypoxic tolerance and many suggested physiological variables and protocols. Research needs to identify and validate one appropriate test protocol to allow comparison between studies and responses to interventions.

Pathophysiological mechanisms of AMS are widely debated, current thinking centres on the oxidative stress of hypoxia causing hypoventilation, reduced gaseous exchange (Bartsch *et al.* 2002), changes in fluid controlling hormones (Loeppky *et al.* 2005a) and

possible increased permeability in the central nervous system (Bailey *et al.* 2004). These mechanisms tend to induce AMS over a period of hours, although (Hackett & Roach 2001) suggest that AMS symptoms may be seen within 1 hour, while others have shown AMS to occur after 1-2 hours of arrival to altitudes of approximately 3500m via plane (Purkayastha *et al.* 1995) or cable car (Erba *et al.* 2004). Research tends to overlook the immediate responses to hypoxic exposure and concentrates on longer term adaptation, or indeed maladaptation with resulting mountain maladies.

This study aimed to investigate some physiological responses to acute normobaric hypoxia during rest and exercise. Relating physiological changes to subjective AMS symptoms will help develop our understanding of the physiological processes that determine tolerance to acute hypoxia. It was hypothesised that acute physiological changes during an intermittent walking test would be sensitive to normoxic and hypoxic conditions. It was also hypothesised that acute physiological responses to a short duration, intermittent walking test at simulated altitude may be used to indicate tolerance to acute hypoxia.

4.3 Methods

Twelve physically active male participants of 27 ± 7 yrs, height 182 ± 7 cm, mass 85 ± 6 kg, body fat 11.0 ± 1.6 %, VO_2 at lactate threshold 27 ± 5.1 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and VO_2max of 54.5 ± 11.3 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ attended the laboratory in a euhydrated state on four separate occasions. The first visit was a familiarisation session involving anthropometric measures, cardiovascular fitness assessment and assessment of walking speed at 50% VO_2max (Section 3.3.1 & 3.3.2). The next three visits involved an intermittent walking test (Figure 4.1) in different conditions [$20.93\% \text{O}_2$ /sea level (NORM),

14% O₂/3200m (HYP1) and 12% O₂/4300m (HYP2)]. The physiological measures and the time points at which they were recorded are shown in Figure 4.1 and explained in detail within Section 3.

The order of the tests was randomised, determined by a Latin squares design. Each test was separated by a seven day “wash out” period. Two-way ANOVA with repeated measures was used to identify effect of the condition and the time point on the physiological measures. One-way ANOVA with repeated measures was used to compare between conditions, bonferroni pairwise comparisons compared between the conditions. Pearson’s product moment correlation was used to compare the relationship of LLQ scores and physiological measures.

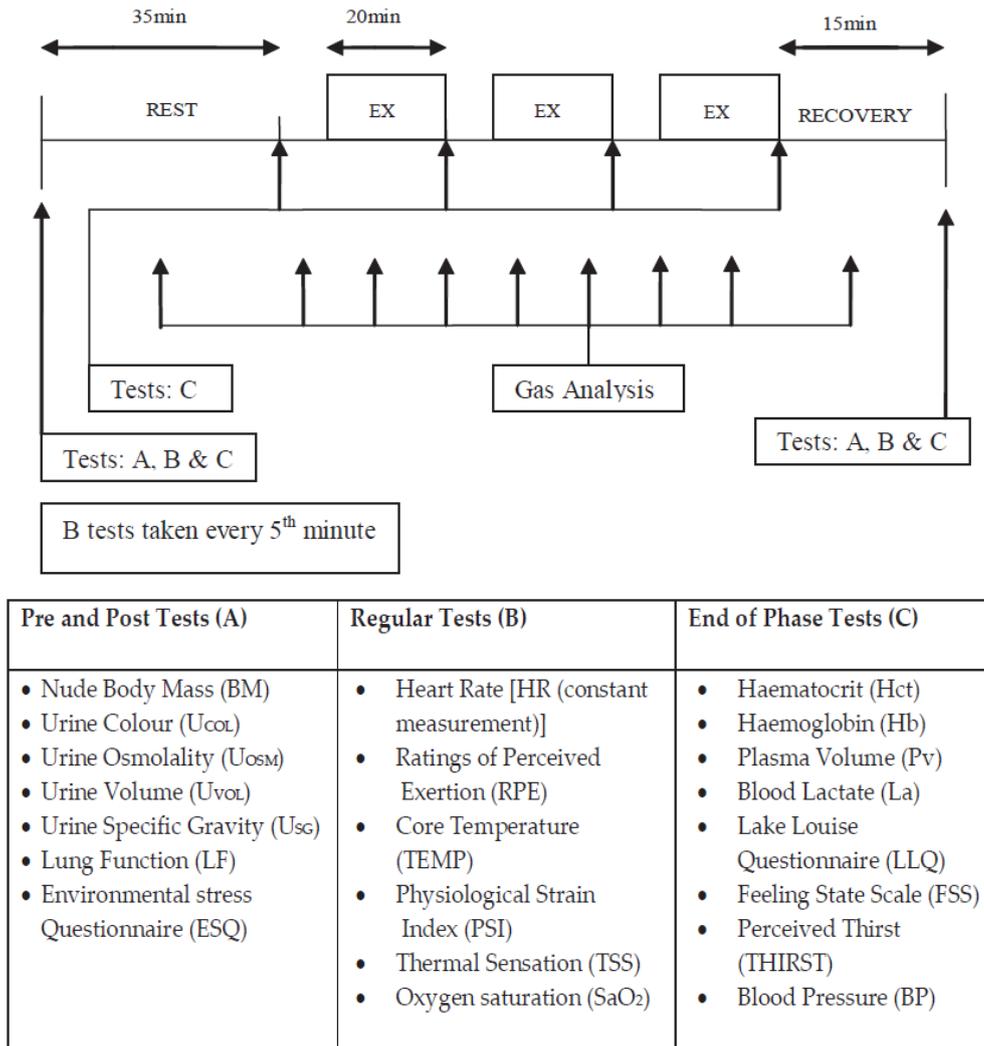


Figure 4.1: Schematic of the experimental design, showing the measurements and timings. Arrows denote measurement points.

4.4 Results

All participants (n=12) completed NORM and HYP1 trials, although in HYP2 one participant was extracted from the tent at 40 minutes after briefly losing consciousness. This participant made a full recovery but their data was not used for the analysis. Walking speed, set at 50% $\dot{V}O_2$ max ranged between 5.4 and 6.2km·hr⁻¹.

Table 4.1. Comparison of AMS markers between the three conditions, (Mean \pm SD). FEEL (feeling state), HEAD (headache), BREATHE (ease of breathing), STOMACH (stomach discomfort) represent the visual analogue scores for the respective questions. * denotes significance ($p < 0.05$), f and p values assessed using one way repeated measures ANOVA. 20.93% O₂ (NORM), 14% O₂ (HYP1), 12% O₂ (HYP2)

Variable	NORM	HYP1	HYP2	$f(p)$ Values
LLQ	0.58 \pm 0.6	1.58 \pm 1.3	3.9 \pm 2.3	16.42 (0.000)*
ESQc	0.03 \pm 0.07	0.26 \pm 0.2	0.6 \pm 0.3	13.25 (0.000)*
Δ FEEL (cm)	0.62 \pm 1.3	0.83 \pm 1.9	1.8 \pm 2.6	1.81 (0.189)
Δ HEAD (cm)	0.04 \pm 0.4	0.62 \pm 1.5	1.74 \pm 2.2	5.69 (0.011)*
Δ BREATHE (cm)	0.34 \pm 0.9	1.1 \pm 2.0	2 \pm 2.3	2.78 (0.085)
Δ STOMACH (cm)	0.30 \pm 0.7	0.39 \pm 1.0	1.1 \pm 1.7	1.92 (0.17)

AMS Markers

AMS markers, including the highest LLQ score over the test (LLQ) (Fig 4.2), difference in ESQc (Fig 4.3) and difference in headache visual analogue scale pre and post testing increased with greater hypoxia, while the highest values recorded during HYP2 (Table 4.1). LLQ and Δ ESQc ($r=0.839$, $p < 0.001$), LLQ and Δ HEAD ($r=0.727$, $p < 0.001$) and Δ ESQc and Δ HEAD ($r=0.903$, $p < 0.001$) were all found to correlate positively.

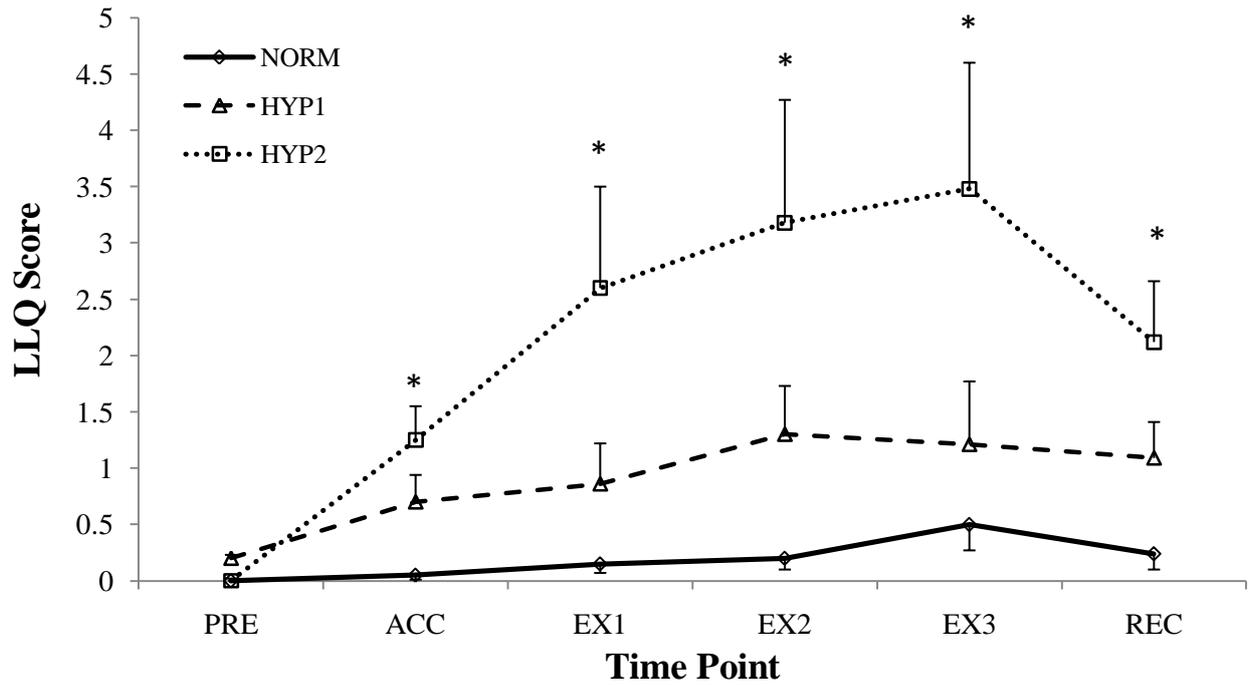


Figure 4.2: LLQ score during the experimental protocol across conditions. * denotes significant difference between conditions ($p < 0.05$). 20.93% O₂ (NORM), 14% O₂ (HYP1), 12% O₂ (HYP2)

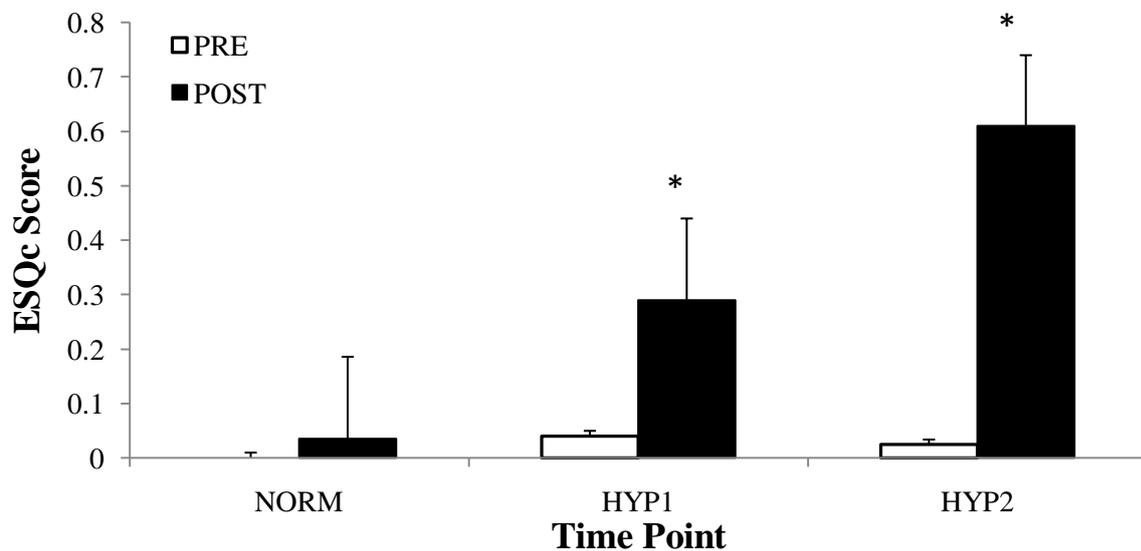


Figure 4.3: Change in ESQc score between test conditions. Y error bars are shown. * denotes significant difference between PRE and POST values for each condition ($p < 0.05$).

Gas Exchange Measures

VO_2 and VCO_2 were not found to be different between conditions, or correlate with AMS markers. Respiratory exchange ratio and minute ventilation were found to increase with severity of hypoxic conditions during rest ($p<0.05$) and positively correlated with both LLQ and ΔESQ_c after exercise and recovery ($p<0.05$). SaO_2 was found to decrease with hypoxic conditions (Figure 4.4) and negatively correlated with both LLQ and ΔESQ_c for all time points during the test ($p<0.05$).

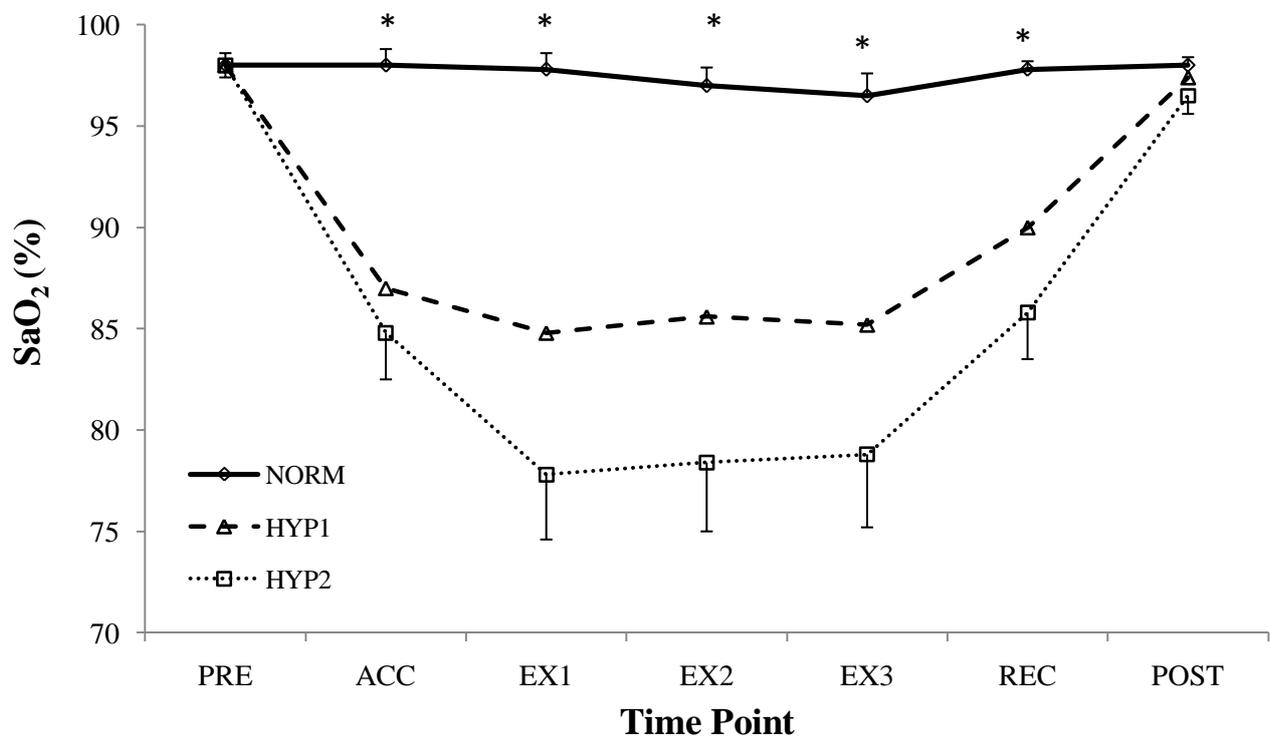


Figure 4.4: Change in mean SaO_2 over the test duration. Y error bars are shown * denotes significant difference between conditions ($p<0.05$). 20.93% O_2 (NORM), 14% O_2 (HYP1), 12% O_2 (HYP2)

Physiological Strain Index

Heart Rate and core temperature were found to be significantly greater with the more severe hypoxic conditions ($p<0.05$) (Fig 4.5). Heart rate and core temperature positively

correlated with both ESQc and LLQ during exercise and recovery ($p<0.05$). Consequently, physiological strain during rest, exercise and recovery was found to be greater with further hypoxia and correlated with both LLQ and ESQc ($p<0.05$). Core temperature, heart rate and physiological strain, were found to correlate positively with the corresponding LLQ score during exercise and recovery ($p<0.05$).

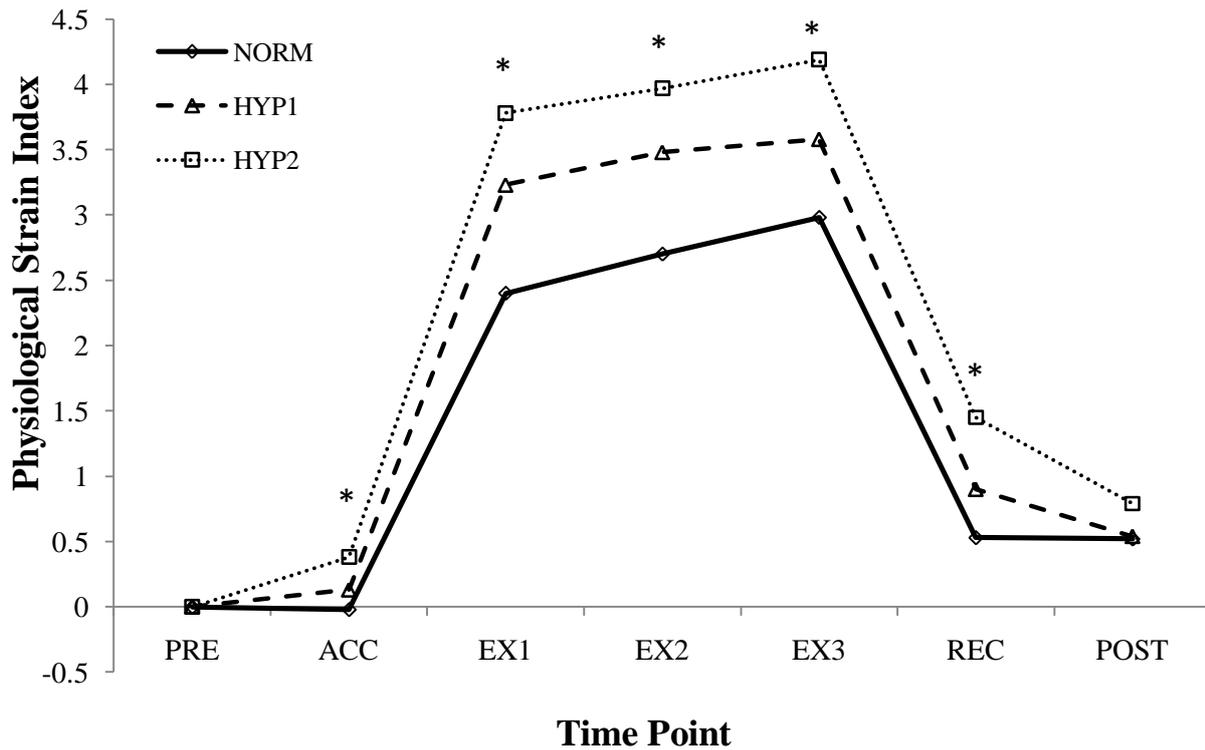


Figure 4.5: Change in mean PSI over the test duration. * denotes significant difference between conditions ($p<0.05$). 20.93% O₂ (NORM), 14% O₂ (HYP1), 12% O₂ (HYP2)

Blood Markers

Resting (ACC: NORM $1.12 \pm 0.23\text{mmol.L}^{-1}$; HYP1 $1.34 \pm 0.24\text{mmol.L}^{-1}$; HYP2 $1.44 \pm 0.34\text{mmol.L}^{-1}$) and exercise blood lactate (POST: NORM $1.82 \pm 0.43\text{mmol.L}^{-1}$; HYP1 $2.74 \pm 0.65\text{mmol.L}^{-1}$; HYP2 $3.07 \pm 0.98\text{mmol.L}^{-1}$) values were found to rise with hypoxic conditions ($p<0.05$). There was no significant difference in any of the other blood

markers between conditions. Exercising blood lactate values positively correlated with LLQ and Δ ESQc ($p < 0.01$)

Urine Measures

Post test urine volume (NORM 166 ± 84 ml; HYP1 264 ± 83 ml; HYP2 349 ± 94 ml) was found to increase with hypoxic conditions ($p < 0.05$), but did not correlate with AMS markers. No other urine measures were found to be different between conditions or correlate with the AMS markers.

Perception Scales

Perceived thirst and thermal sensation were found to increase significantly with hypoxia during exercise, recovery and post test ($p < 0.05$). RPE was significantly increased with hypoxic conditions for all exercise time points ($p < 0.05$). LLQ and Δ ESQc were found to correlate positively with perceived thirst during the third exercise bout and in recovery, thermal sensation during the final two exercise bouts in recovery and post test, and all RPE time points ($p < 0.05$).

Anthropometrics

Initial anthropometric or cardiovascular fitness markers, including body fat percentage, body mass, body mass index, height, lactate threshold and VO_2 max, did not correlate with AMS scores. Change in body mass, as a result of sweating, negatively correlated with both LLQ ($r = 0.681$, $p < 0.001$) and ESQc ($r = 0.667$, $p < 0.001$) (Fig 4.6) and was found to increase with more severe hypoxia ($p < 0.05$). Lung function values were not found to be different between conditions, nor to correlate with any AMS markers.

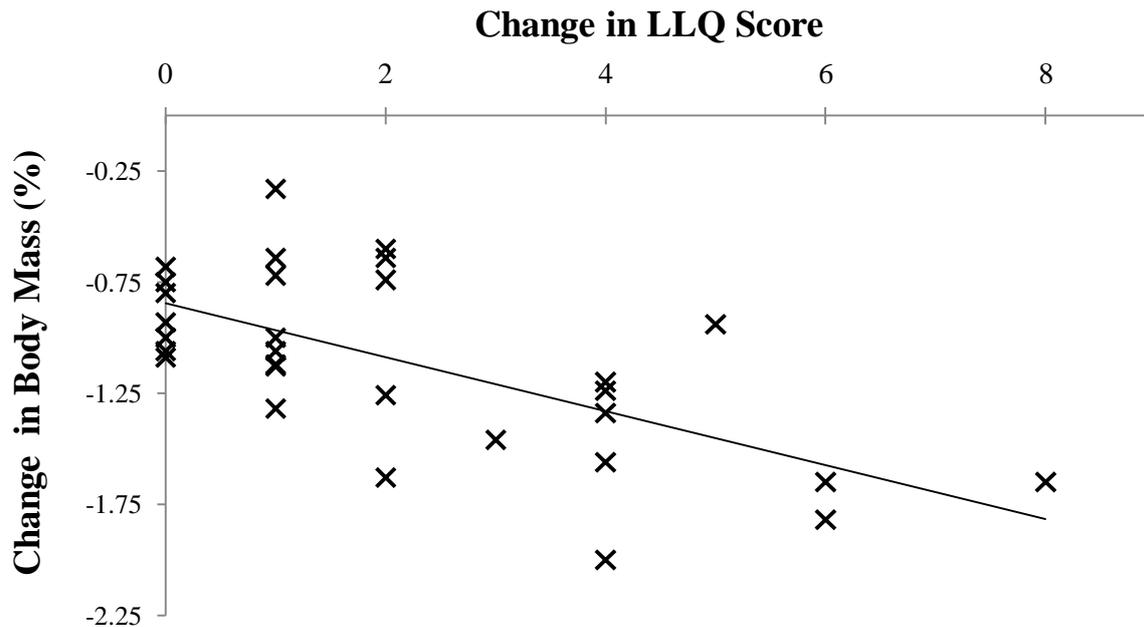


Figure 4.6: LLQ against ΔBM ($r=0.681$, $p<0.000$). $\Delta\text{BM} = 0.1258\text{LLQ} - 0.8764$

4.5 Discussion

This study aimed to investigate the physiological responses of the human body to acute hypoxia and determine whether they are associated with the presence of AMS. Physiological strain, thermal sensation scale, heart rate, perceived thirst, core temperature, rating of perceived exertion, feeling state and loss of body mass increased and SaO_2 decreased with severity of hypoxia. These measures were also found to be related to the presence of AMS symptoms.

Cerebral AMS symptoms, necessary in the diagnosis of AMS, are suggested to occur after 6-12 hours of hypoxic exposure (Houston & Dickinson 1975; Singh *et al.* 1969), with symptoms worsening over a period of days (Ward *et al.* 2000). In contrast, four subjects experienced LLQ scores of >4 (including headache symptoms), at some point in the HYP2 test, while five subjects had an $\text{LLQ}_{\text{MEAN}} >3$. This may be due to the moderate to high intensity exercise performed, which is known to exacerbate AMS symptoms (Roach

et al. 2000). However, three of the subjects had LLQ scores of 3 after only 35 min of rest. This supports work by Purkayasther *et al* (1995) and Erba *et al* (2004) who reported AMS in individuals within 1-2 hours, when exposed to acute altitude. Fig 4.2 and Fig 4.3 also highlight the effect greater hypoxic stress has on AMS symptoms, suggesting a hypoxic threshold for AMS symptom development.

SaO₂ was found to be the closest correlate of AMS markers over the test duration, supporting previous research (Bircher *et al.* 1993; Burtcher *et al.* 2004). While, research using slow ascent rates over a few days also shows correlation between SaO₂ and the onset of AMS (Roach *et al.* 1998). However, many acute hypoxic studies contradict these findings (O'Connor *et al.* 2004; Roach *et al.* 1995; Roeggla *et al.* 1996). In the current study, individuals most severely affected by hypoxia saw the lowest SaO₂ values; the participant withdrawn from HYP2 recorded SaO₂ values of 64% during rest, while at HYP1 the subject's SaO₂ (81-87%) and AMS (LLQ = 2; ESQc = 0.6) values were similar to the other subjects.

Heart rate, core temperature and physiological strain index correlated well with Δ ESQc, highest LLQ score and LLQ scores at their respective time points, suggesting physiological strain index may be a useful measure in future research. However, the difficulty of measuring physiological strain may prevent measurement of core temperature as regularly as lab based research.

Rise in core temperature has been shown to be proportional to mountain illness severity, finding a temperature increase of $0.5 \pm 0.6^\circ\text{C}$ in individuals with LLQ score ≤ 3 , $1.2 \pm 0.6^\circ\text{C}$ in those with >3 and $1.7 \pm 0.6^\circ\text{C}$ in those with cerebral edema (Maggiorini *et al.* 1997b).

Similar studies have also reported AMS sufferers experiencing body temperature increases of $\sim 1^{\circ}\text{C}$ (Roggla *et al.* 2000a). The current study recorded significant increases in temperature with severity of hypoxia, which correlated with AMS symptoms. Difference in mean core temperature of $\sim 0.3^{\circ}\text{C}$ between HYP1 and HYP2 for all time points during the test suggests the additional hypoxic stress caused greater physiological stress, in terms of heat strain, resulting in significantly higher AMS symptom scores for HYP2. The four individuals recording the highest LLQ_{MEAN} score also recorded the highest average temperature for all time points, suggesting core body temperature rise may be related to the onset of AMS symptoms. In contrast, Loeppky *et al.* (2003a) found core temperature to decline from 1 to 6hrs with resting hypoxic exposure, attributing the decline to catecholamine induced rise in vasodilation. However, during the current study, the response to exercise may have altered this catecholamine and vasodilatory response. While the study by Loeppky *et al.* (2003a) allowed participants to control room temperature, with AMS sufferers allowing the lowest room temperature.

The heart rate response to hypoxia is widely accepted as an immediate rise with hypoxic exposure (Marshall 1994), with reduction in resting heart rate over a period of days as oxygen-carrying capacity of the blood improves and pH is reduced toward pre-altitude exposure values (Mazzeo *et al.* 1995). The current study would support this finding since we observed an immediate rise in resting and exercising heart rate, with heart rate elevated in proportion to the severity of hypoxia, as was found in a similar study (O'Connor *et al.* 2004). Studies by Loeppky *et al.* (2003a) and O'Connor *et al.* (2004) have also measured a positive correlation between AMS markers and heart rate ($p < 0.05$). The pathophysiological link between heart rate and AMS symptoms is not clear, though early rise in sympathetic tone due to orthostatic intolerance has been suggested (Loeppky *et al.*

2003a). Similarly, in prolonged hypoxic exposure, Krasney (1994) suggested brain distortion, due to a rise in intracranial pressure, causes chemoreflexes to increase sympathetic activity. Unlike Loeppky *et al* (2003a) that demonstrated greater blood pressure in AMS sufferers, the current study found no significant change in blood pressure that would indicate orthostatic intolerance.

Change in body mass was found to be the closest correlate to both ESQ_c and LLQ. The causal relationship is unclear, though it is thought that greater physiological stress, exacerbated by a poorer response to hypoxia, could cause a greater sweat rate. In contrast, research suggests hypoxia directly suppresses sweat gland function, through depressed cholinergic stimulation (Dipasquale *et al.* 2002) and a decrease in acetylcholine release during hypoxic exposure (Elizondo 1973).

Post test urine volume or change in urine volume did not correlate with Δ ESQ_C or LLQ, similar to other research (Miledge *et al.* 1989). Therefore, the study cannot support the hypothesis that hypoxia may influence hormonal orthostatic control. The lack of significant findings may be due to the low subject number and some variation in pre test hydration status. Furthermore, the test protocol did not allow participants to void their bladder until post test, preventing voluntary diuresis and therefore, not recording an individual's urinary response to hypoxia. It is also possible that the short test duration may not have allowed enough time for fluid control to be influenced.

Further research could identify the influence of fluid or orthostatic control on acute physiological responses and development of AMS symptoms, while further validation of the intermittent walking test using greater sample size, is also necessary.

4.6 Conclusion

Many physiological parameters are influenced by severity of hypoxia, these increases in physiological stress may contribute to the development of AMS symptoms. Measurement of these physiological variables using an intermittent walking or during acute exposure to normobaric hypoxia, may allow determination of hypoxic tolerance. Measures of physiological strain and SaO₂ are the closest correlates of LLQ and therefore warrant further investigation.

CHAPTER V

HYDRATION AND THE PHYSIOLOGICAL RESPONSES TO ACUTE NORMOBARIC HYPOXIA

5.1 Abstract

The effect hydration status has on exposure to hypoxia is unclear. The purpose of the study was to identify how hydration status, above and below euhydrated levels affects the physiological responses and onset of acute mountain sickness (AMS) symptoms, during acute normobaric hypoxia. Eight males completed intermittent walking tests under normobaric hypoxic conditions ($FIO_2 = 0.13$) after controlled hyperhydration (HYPER), hypohydration (HYPO) and euhydration (EU) protocols. A range of physiological, psychological and altitude illness markers were monitored throughout the 125min exposure. Heart rate, core temperature, peripheral arterial oxygen saturation, urine osmolality and mean self reported Lake Louise Questionnaire AMS scores (LLQ) were significantly different between EU, HYPO and HYPER, respectively and closely correlated with environmental symptoms questionnaire (ESQ), LLQ and headache scores ($p < 0.05$). Other measures of ventilation and lung function were also significantly different between hydration conditions ($p < 0.05$). Hydration state above and below euhydration has detrimental consequences on physiological strain and onset of AMS symptoms when exposed to acute normobaric hypoxia.

Key Words: Hypohydration, Hyperhydration, Physiological Strain Index, Acute Mountain Sickness

5.2 Introduction

Acute hypoxia can cause a decline in oxygen delivery, a rise in sympathetic activity (Hainsworth *et al.* 2007), peripheral vasoconstriction (Fukuda-Matsuda *et al.* 2007) and a consequent decline in aerobic capacity (Favret & Richalet 2007) and rise in heat storage (Roggl *et al.* 2000a), as demonstrated in Chapter IV. Similarly, hypohydration causes hypovolemia, peripheral vasoconstriction, compensatory rise in sympathetic activity and a consequential decline in oxygen delivery and heat dissipation (Cheuveront *et al.* 2005). Hence, this increase in physiological strain with hypoxic insult, noted in Chapter IV, is likely to be exacerbated with alterations in hydration state. Also in Chapter IV sweat rate through changes in body mass, was shown to positively correlate to LLQ score (Figure 4.6). As well as the physiological strain increasing sweat rate, this indicates that dehydration may be important in the development of AMS symptoms.

Further, risk of dehydration is greater at altitude due to increased water vapour loss, energy expenditure and ventilation (Westerterp 2001). High altitude backpackers have been shown to be hypohydrated at the start of a day's hike and progressively dehydrate with each day (Rozier 1998), while mountaineers can dehydrate by 5.5% over 3 weeks (Piccoli *et al.* 1996). At sea level >2% hypohydration reduces endurance performance and further, hypohydration can have a significant effect on health and cognitive function (EJCN 2003). However, little research has investigated the effect of hydration status during hypoxia (Nerín *et al.* 2006) in adult (Basnyat *et al.* 2001; Rennie *et al.* 1993) and paediatric (Basnyat *et al.* 1998) populations, causing much debate within the scientific community. The only study to control hydration at simulated altitude, found no evidence to support hydration maintenance as a countermeasure to the observed effects (Aoki & Robinson 1971). In a retrospective study, Basnyat *et al.* (1999) found that drinking less

than 3000ml per day increased the risk of AMS by 60%, and suggested drinking up to 5L·day⁻¹ would reduce AMS incidence.

In contrast, fluid retention, through antidiuresis has also been linked to the onset of AMS symptoms (Loeppky *et al.* 2005a). Over-hydrating may increase extracellular fluid volume and induce greater intracranial pressure and headache (Roach & Hackett 2001). Conversely, hyperhydration may reduce the physiological stress of hypoxia through increased stroke volume, vasodilation and heat dissipation. Sea level studies have contrasting findings with some that show hyperhydration to improve cardiovascular and thermoregulatory markers when euhydration cannot be maintained in other conditions (Latzka & Sawka 2000), while others demonstrate no effect (Latzka *et al.* 1997, 1998; Marino *et al.* 2003).

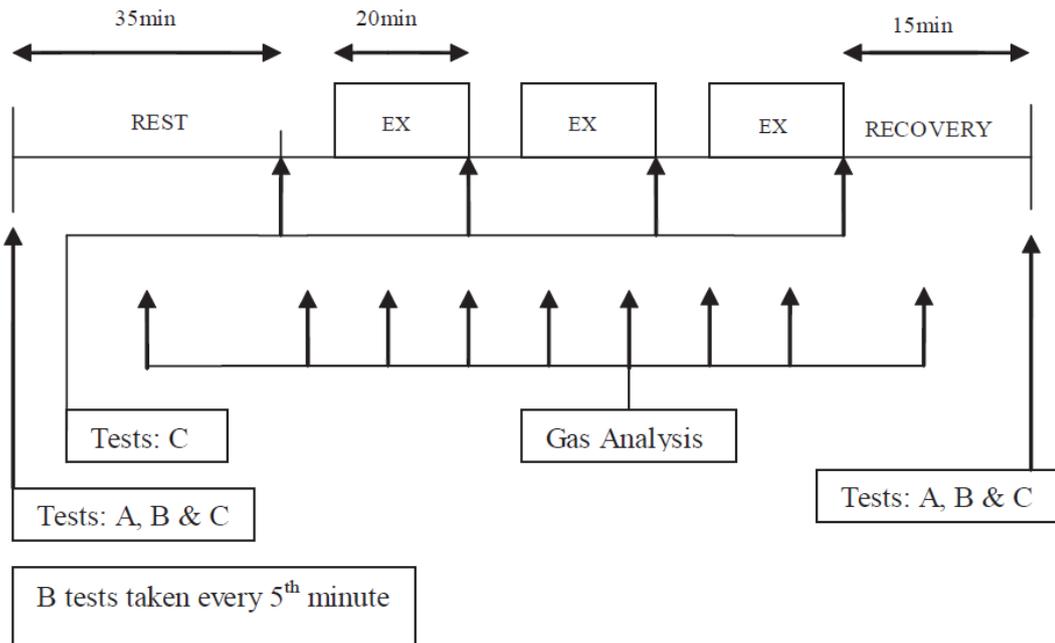
It is hypothesised that hydration, above and below a euhydrated state, increases the physiological strain and onset of AMS symptoms, during a 125 minute acute normobaric hypoxic intermittent walking test.

5.3 Methods

The study required eight physically active males 20 ± 1 yrs, of height 182 ± 4 cm, mass 89 ± 13 kg, body fat 13 ± 3 % and $\dot{V}O_2\text{max}$ of 43 ± 7 ml·kg⁻¹·min⁻¹ to attend the laboratory on four separate occasions. The first visit was a familiarisation session involving anthropometric measures, cardiovascular fitness assessment and assessment of walking speed at 50% $\dot{V}O_2\text{max}$ (Section 3.3.1 & 3.3.2). The next three visits involved an intermittent walking test (Figure 5.1) in a hypoxic environment ($FIO_2 = 0.13$) under three different hydration protocols [euhydrated (EU), hypohydrated (HYPO), hyperhydrated

(HYPER)]. In the EU condition participants completed one hour of moderate intensity running 15 hours prior to the intermittent walking test, while wearing warm clothing (hat, gloves, jumper, trousers). Participants were then encouraged to rehydrate over the 15 hours by consuming 150% of sweat loss during the hour exercise. For the HYPO conditions participants completed the exercise as described for the EU trial, but were fluid restricted during exercise and for 15 hours preceding the intermittent walking test. For the HYPER condition participants completed the exercise and consumed fluid as described for the EU condition, but on arrival to the laboratory participants were asked to consume a bolus of water equal to $28\text{ml}\cdot\text{kg}^{-1}$ body mass over a period of 40 minutes (Latzka, 1998), prior to pre test measures.

Various physiological and perceptual markers were measured over the intermittent walking test, as described in Section 3 and illustrated within Figure 5.1. The order of the tests was randomised, determined by a Latin squares design. Each test was separated by a seven day wash out period. Two way ANOVA with repeated measures was used to identify significant effect of the condition and or time point. One way ANOVA with repeated measures was used to compare between conditions, bonferroni pairwise comparisons compared separate conditions. Paired T-tests were used to compare pre and post values of urine and physiological measures.



A Tests	B Tests	C Tests
<ul style="list-style-type: none"> • Nude Body Mass (BM) • Urine Colour (Uc) • Urine Osmolality (Uo) • Urine Volume (Uv) • Urine Specific Gravity (Usc) • Lung Function (LF) • Environmental stress Questionnaire (ESQ) • Haematocrit (Hct) • Heamoglobin (Hmg) • Plasma Volume (PV) • Blood Lactate (Lac) • Plasma Omolality (Po) 	<ul style="list-style-type: none"> • Heart Rate (HR) (constant measurement) • Ratings of Perceived Exertion (RPE) • Rectal Temperature (Tr) • Physiological Strain Index (PSI) • Thermal Sensation Scale (TSS) • Oxygen saturation (Sp O₂) • Hypoxic Cardiac Response (HCR) • Peripheral O₂ Blood Content (CpO₂) • Hypoxic Ventilatory Response (HVR) 	<ul style="list-style-type: none"> • Heamoglobin (Hb) • Lake Louise Questionnaire (LLQ) • Feeling State Scale (FS) • Perceived Thirst (T) • Blood Pressure (BP)

Figure 5.1: Schematic of the experimental design, showing the measurements and timings. Arrows denote measurement points.

5.4 Results

Hydration protocols induced different pre-test body mass and hydration markers, causing hydrations of approximately 0%, -2% and +2% of normal body mass for the EU, HYPO and HYPER conditions respectively, as shown in the Table 5.1.

Heart rate, core temperature and PSI were found to be significantly different between hydration conditions ($p<0.05$) and over time ($p<0.05$) (Figure 5.2). During exercise heart rate and PSI were higher in the HYPO and HYPER conditions, but not different from each other. Core temperature was significantly lower throughout the HYPER trials, compared to either the EU or HYPO conditions. None of the time points correlated with AMS symptom markers.

Perceptual markers of strain including, rating of perceived exertion (EU 12.9 ± 1 ; HYPO 14.2 ± 2 ; HYPER 14.1 ± 1) and thermal sensation scale [at rest (EU 4.5 ± 0.5 ; HYPO 4.3 ± 0.6 ; HYPER 4.2 ± 0.4), during exercise (EU 5.5 ± 0.7 ; HYPO 6.1 ± 0.2 ; HYPER 6 ± 0.8)] were found to be greater in HYPO and HYPER than EU conditions ($p<0.05$). As expected, perceived thirst [pre test (EU 2.5 ± 0.9 ; HYPO 6.8 ± 2 ; HYPER 0.8 ± 0.3), post test (EU 4.8 ± 1 ; HYPO 8.7 ± 3 ; HYPER 3.6 ± 1)] was increased with an increased water deficit ($p<0.05$).

Table 5.1: Mean \pm SD pre and post hydration markers for euhydrated (EU), hypohydrated (HYPO) and hyperhydrated (HYPER) conditions. * Denotes significant difference between EU and HYPO conditions, # Denotes significant difference between EU and HYPER conditions, \diamond Denotes significant difference between HYPO and HYPER conditions, \dagger denotes significant difference between pre and post values ($p < 0.05$).

	<i>Eu</i>		<i>Hypo</i>		<i>Hyper</i>	
	Pre	Post	Pre	Post	Pre	Post
Body Mass (kg)	89.7 \pm 13.8 *#	88.56 \pm 13.5 *# \dagger	87.6 \pm 13.8 \diamond	86.9 \pm 13.7 \diamond \dagger	91.7 \pm 13.9	88.7 \pm 13.7 \dagger
Body Mass Loss from normal (%)	0.1 \pm 0.02 *#	-	-2.1 \pm 0.3 \diamond	-	1.92 \pm 0.6	-
Urine Specific Gravity	1.013 \pm 0.006 *	1.015 \pm 0.001 *# \dagger	1.027 \pm 0.002 \diamond	1.026 \pm 0.003 \diamond	1.009 \pm 0.006	1.001 \pm 0.001 \dagger
Urine Osmolality (mosm \cdot kg $^{-1}$)	290 \pm 111 *	340 \pm 110 *# \dagger	1064 \pm 60 \diamond	1029 \pm 116 \diamond \dagger	204 \pm 66	101 \pm 36 \dagger
Urine Colour	2 \pm 0.46 *	2 \pm 0.9 *#	5 \pm 0.06 \diamond	5 \pm 1.06 \diamond	1.8 \pm 0.99	1 \pm 0
Plasma Osmolality (mosm \cdot kg $^{-1}$)	309 \pm 4	309 \pm 13	313 \pm 11	318 \pm 7 \diamond	311 \pm 9	308 \pm 7
Urine Flow (L \cdot hr $^{-1}$)	-	0.283 \pm 0.103 *#	-	0.082 \pm 0.075 \diamond	-	0.696 \pm 0.0405
Free Water Clearance (L \cdot hr $^{-1}$)	-	-0.0091 \pm 0.001 *#	-	-0.189 \pm 0.183 \diamond	-	-0.464 \pm 0.092
Sweat Rate (L \cdot hr $^{-1}$)	-	0.34 \pm 0.22 *	-	0.22 \pm 0.15	-	0.28 \pm 0.26

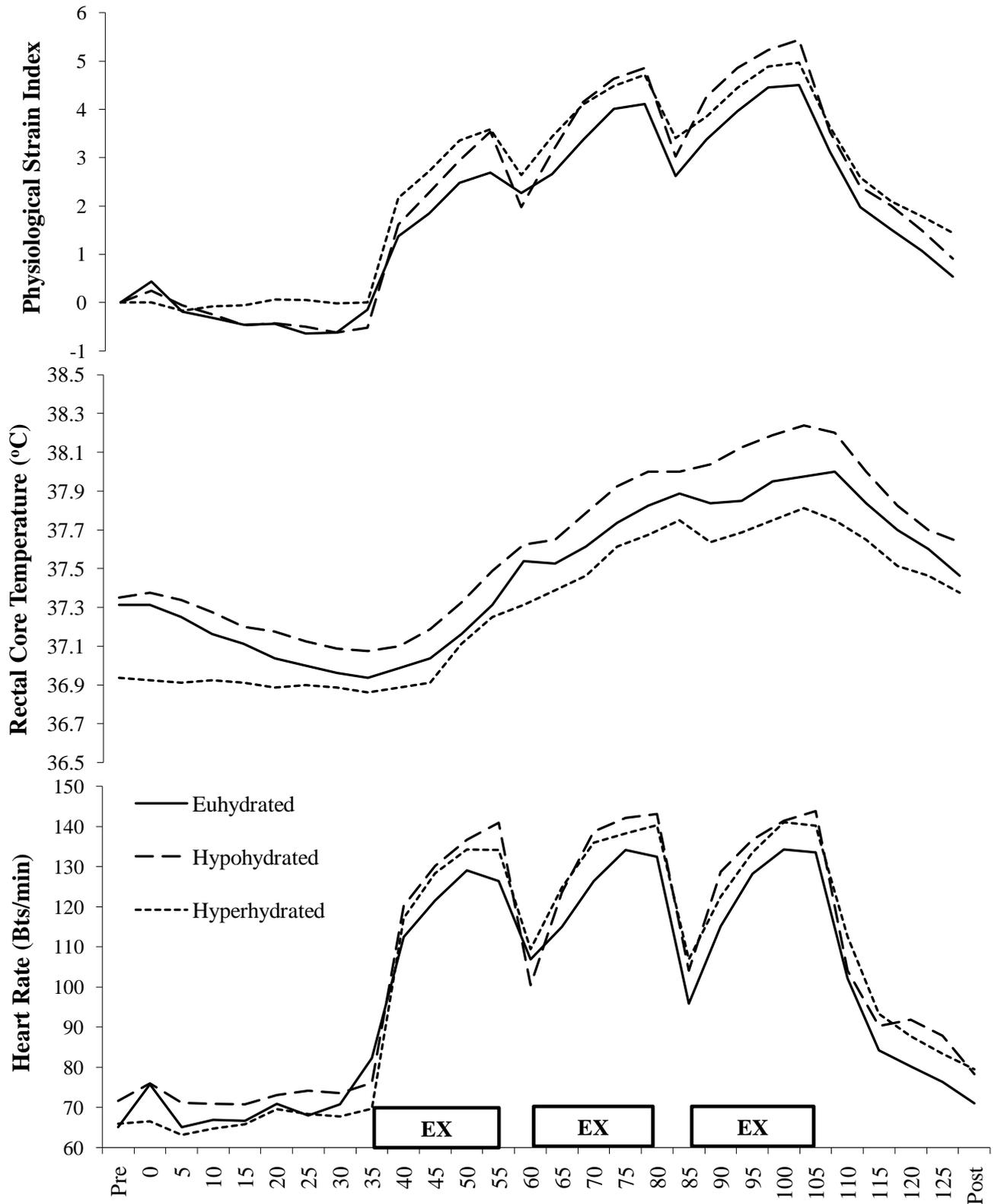


Figure 5.2: Heart rate, rectal core temperature and physiological strain index time course for the three hydration conditions. The three 20 min exercise phases are indicated above the X axis.

SaO₂ was different between conditions ($p<0.05$), with HYPER ($75\pm 1\%$) significantly less than EU ($79\pm 3\%$) and HYPO ($80\pm 3\%$) during all exercise time points ($p<0.001$). Derivatives of SaO₂ such as hypoxic cardiac response and hypoxic ventilator response were not found to be different between conditions. However, hypoxic ventilatory response during the 2nd and 3rd bout of exercise positively correlated with mean LLQ scores ($r=0.510$, $p<0.05$; $r=0.498$, $p<0.05$, respectively). Minute ventilation during exercise (EU 38.2 ± 4.1 ; HYPO 40.5 ± 6.2 ; HYPER 44.4 ± 6.2 L·min⁻¹) was significantly different ($p<0.05$), while other ventilatory markers, such as breathing frequency and tidal volume, showed no difference with hydration.

Urine measures showed pre test differences between conditions (Table 5.1). Post test values for urine specific gravity, osmolality and colour all rose in EU, declined in HYPER and were maintained in HYPO. There was no difference in plasma osmolality between conditions, though total urine volume and free water clearance increased with greater hydration (Table 5.1). Plasma osmolality values were found to be higher than normal, even though values were remeasured in duplicate after recalibration of the micro-osmometer.

Blood measures of hematocrit, haemoglobin and change in plasma volume showed no difference between conditions and did not change over time. Peripheral blood oxygen content was no different between conditions although values after the 40 minutes rest negatively correlated with all AMS measures [LLQmean ($r=-0.642$, $p<0.05$), LLQ peak ($r=-0.634$, $p<0.05$), ESQc ($r=-0.689$, $p<0.05$).

Forced vital capacity (FVC) was not different between conditions. Forced expiratory volume in 1 second (FEV1) was different between conditions post test (EU 4.84 ± 0.31 ; HYPO 5 ± 0.34 ; HYPER 5.4 ± 0.64 L) ($p < 0.012$), hence pre and post test change (EU -0.045 ± 0.002 ; HYPO 0.18 ± 0.001 ; HYPER 0.316 ± 0.004 L) in FEV1 was significantly different between conditions ($p < 0.05$). However, FEV1/FVC values showed no difference between conditions or over time, though FEV1/FVC pre and post difference correlated with LLQ mean ($r = 0.564$, $p < 0.05$), LLQ peak ($r = 0.639$, $p < 0.05$) and ESQc ($r = 0.646$, $p < 0.05$) scores.

ESQc increased with hypoxia ($p < 0.05$) and the greatest increases from pre to post intermittent walking test were seen in HYPO (EU 0.21 ± 0.28 ; HYPO 0.51 ± 0.23 ; HYPER 0.38 ± 0.3). LLQ increased over time and rose significantly with exercise in all conditions, returning near to pre exercise with 20 minutes of rest (Figure 5.3). Some HYPO and HYPER trials presented with pre test LLQ and ESQc values greater than EU values, therefore change in markers was calculated. LLQ mean, peak and ESQc score (EU 0.21 ± 0.2 ; HYPO 0.44 ± 0.2 ; HYPER 0.55 ± 0.2) were all significantly greater in HYPO and HYPER than EU ($p < 0.05$).

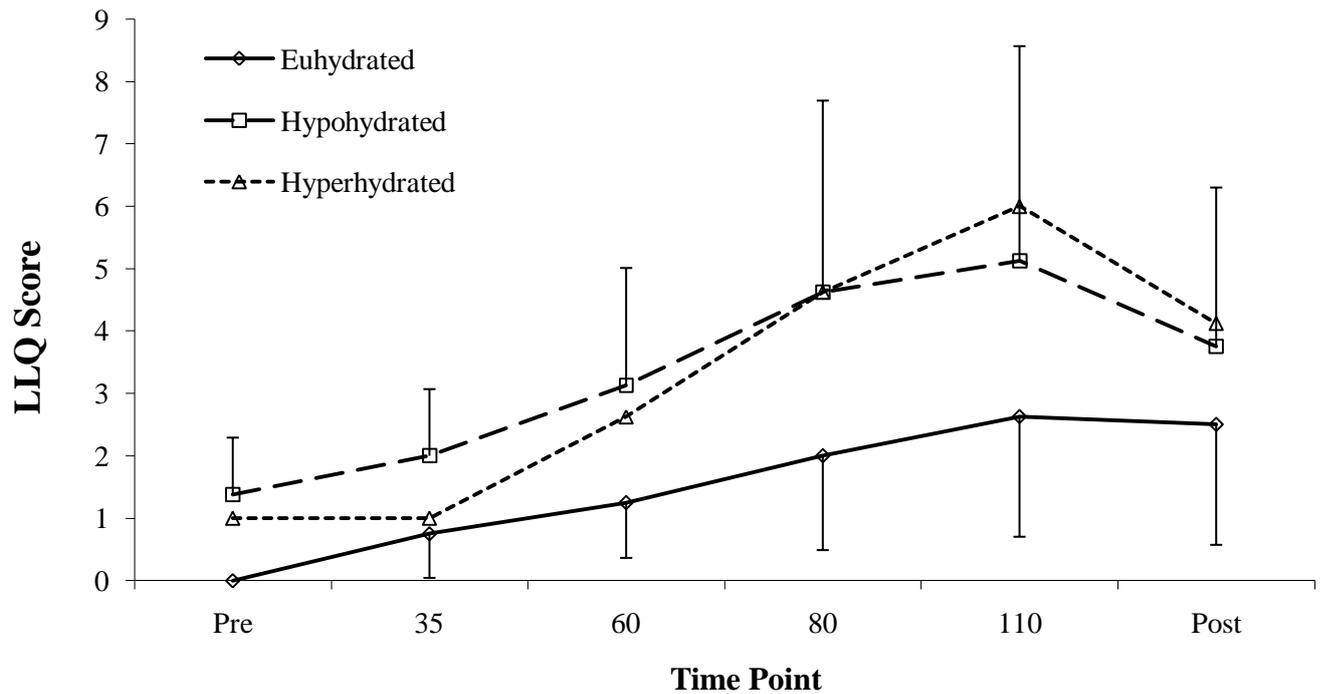


Figure 5.3: Lake Louise questionnaire score over the intermittent walking test between each hydration condition. Y error bars are shown.

5.5 Discussion

This study aimed to identify the effects of hydration status on the physiological responses to intermittent exercise under hypoxic conditions. The results clearly show greater physiological strain, hypoxemia, AMS symptoms and change in feeling states, in hydrations above and below euhydration.

At rest HYPO and HYPER showed higher and lower heart rates, respectively, than EU conditions, as has been previously noted (Nadel *et al.* 1980). Elevated HR during HYPO, as compensation for a decline in end diastolic ventricular volume and thus maintenance of cardiac output (Sawka *et al.* 1984), was only notably different between conditions at rest. This may be due to significant decline in HYPER SaO₂ during hypoxic exercise causing significant strain and sympathetic adrenergic response. Consequently heart rate would rise

to meet oxygen delivery demands, negating the reductions in cardiovascular strain, which was previously noted with hyperhydration during exercise in hot environments (Latzka *et al.* 1998). In HYPO, rise in sympathetic activity induced metabolic heat production while low plasma volume caused peripheral vasoconstriction cumulatively increasing heat storage, resulting in HYPO producing the greatest rest and exercising core temperatures. This may also be responsible for the heart rate drift noted in HYPO, while other conditions maintained steady state after 10 mins of exercise. Core temperatures for HYPER were maintained constantly lower than EU or HYPO throughout the test, in response to elevated plasma volume promoting vasodilation and enhanced heat dissipation. The general trend for reduced and increased core temperatures has been previously reported with hyperhydration (Dini *et al.* 2007) and hypohydration (Montain *et al.* 1998) respectively in all conditions, except hypoxia. From this study, hypoxic exercise seems to have similar detrimental effects as exercise in the heat (Sawka *et al.* 2000). Continual rise in core temperature for all conditions, shows an inability to dissipate heat at such inspired oxygen fraction, irrespective of hydration state.

Perceptual scales highlight these physiological changes, with rating of perceived exertion raised above EU values for both HYPO and HYPER. In contrast, TSS was not different between conditions, even with constant differences in core temperature of $\sim 0.4^{\circ}\text{C}$ throughout the trials. Visual analogue scales and AMS symptom scores showed both HYPO and HYPER to elicit similar adverse effects on feeling state and particularly headache, in comparison to EU. While initially HYPO induced worse symptoms which continued to develop over the test, HYPER from 60 minutes onwards caused the most severe headache and LLQ scores (Figure 5.3). Mechanisms behind headache development are not proven here, yet it is conceivable that this headache may be due to the resultant

increase in intracellular fluid and thus, cell swelling within the brain, notably increasing 60 to 80 mins post fluid ingestion. Increases in cerebral arteriole constriction with hypoxic exercise would aggravate cerebral hypoxia, yet no differences in ventilation were noted between hydration conditions. It is unclear whether headache severity was exacerbated due to hypoxia, as previous sea level hyperhydration studies have presented similar findings (Latzka *et al.* 1998), though headaches have not been reported in hyponatremia (Rosner & Kirven 2007). Clearly mechanisms causing this acute headache are not related to the chronic fluid retention based AMS pathophysiology that Roach & Hackett (Roach & Hackett 2001) describe, yet it is clear that over drinking whilst at altitude may induce headache and exacerbate AMS symptoms. Hence, for diagnosis of AMS, patients should be in an established state of euhydration, as previously suggested (Basnyat & Murdoch 2003). SaO₂ is thought to be the main predictor of wellness or performance during hypoxia (Burtscher *et al.* 2008b), though hydration differences suggest SaO₂ may only be reliable when individuals are in a state of euhydration.

Suggested fluid intake values of up to 5L a day (Basnyat *et al.* 1999) seem sensible based on EU exercising sweat rates of 0.6L·hr⁻¹ found in this study. While others suggest a more feasible 3L when at altitude (Basnyat *et al.* 1999). The intake of the 5L clearly needs to be spread throughout the day, in order to maintain a state of euhydration and optimal fluid balance. This may or may not have a detrimental effect on physiological strain and onset of AMS symptoms, when exposed to hypoxia for longer duration (Loeppky *et al.* 2005a). Further, the ingestion of water alone may have detrimental consequences inducing diuresis and fluid compartment alterations. The hypohydration level used in this study, may only replicate the deficits induced over a day. It remains unclear as to whether physiological responses to hypoxia are altered with severity of hypohydration or whether

a critical hypohydration level exists whereby tolerance and performance within hypoxia are significantly reduced from euhydrated values.

This study used a small sample of young physically active adult males, unrepresentative of the general population. Also, the study did not use a normoxic control to compare the effects of hydration states on normoxic exercise. However, much work (Latzka *et al.* 1997; Montain & Coyle 1992; Sawka *et al.* 1984; Sawka *et al.* 2001) has been published on exercise within normoxic and hyperthermic environments and the sole purpose of the study was to identify the effect of hydration states above and below euhydration during hypoxic intermittent exercise.

5.6 Conclusion

Although AMS tends to occur over a period of hours and may be the result of multifactorial processes, induction of greater physiological strain is only likely to exacerbate AMS symptoms (Roach *et al.* 2000). Over-hydration may induce symptoms of headache and gastrointestinal discomfort. This study clearly shows that hydration state above and below euhydration has detrimental consequences on physiological responses, psychological feeling states and self reported AMS symptoms. Correlation of urine measures highlights the ease at which hydration status can be evaluated. Based on the findings from this study, maintaining urine colour at ~2, specific gravity <1.015 or osmolality <400 mosm·kg⁻¹ with small, but regular fluid intakes should reduce the physiological strain during acute hypoxic exposure. Further, well controlled research using greater sample size is required to ascertain whether hydration state is influential over a chronic exposure, while the mechanistic association between hypohydration and hypoxia also needs further exploration.

CHAPTER VI.

THE EFFECT OF HYPOHYDRATION SEVERITY ON THE PHYSIOLOGICAL, PSYCHOLOGICAL AND RENAL HORMONAL RESPONSES TO HYPOXIC EXERCISE

6.1 Abstract

Evidence of the effect of dehydration on physiological responses to hypoxia is limited. The purpose of this study was to determine the effect of hypohydration severity on physiological, renal hormonal and psychological responses to acute hypoxia. Eight males completed intermittent walking tests (IWT) under normobaric hypoxic conditions ($FI O_2 = 0.13$) after completing four separate hypohydration protocols, causing reduction in body mass of approximately 0% (EU), 1% (H1), 2% (H2) and 3% (H3). Physiological and psychological markers were monitored throughout the 125min test. Fluid controlling hormones were measured pre and post exposure. Heart rate (HR), core temperature, peripheral arterial oxygen saturation (SpO_2), minute ventilation and urine osmolality were found to be significantly different between hydration conditions and correlated with Lake Louise Questionnaire score (LLQ) ($p < 0.05$). LLQ score increased with hypohydration severity above H2 (EU 1.3 ± 1 ; H1 1.2 ± 1 ; H2 2.7 ± 2 ; H3 3.9 ± 2) ($p < 0.001$). Antidiuretic hormone and aldosterone increased over the test, but were not different between hydration conditions ($p < 0.05$). Atrial natriuretic peptide showed no change over time, nor with conditions. Therefore, renal hormones are not influenced by hypohydration severity during moderate intensity hypoxic exercise. Hypohydration of $>2\%$ body mass loss induces greater physiological strain during hypoxic exercise and may cause rise in symptoms such as fatigue, headache, nausea and lightheadedness.

Key Words: Dehydration, acute mountain sickness, physiological strain

6.2 Introduction

Anecdotally, tolerance to hypoxia is thought to be reduced by dehydration, although there is little evidence to support (Basnyat *et al.* 2001) or reject (Aoki & Robinson 1971; Nerín *et al.* 2006) this hypothesis (Basnyat *et al.* 2001; Basnyat *et al.* 1998; Rennie *et al.* 1993). Chapter V demonstrated that hydration above and below a state of euhydration increased physiological strain and perceptions of AMS symptoms.

Under normoxic conditions, hypohydration causes elevated thermoregulatory and cardiovascular strain and decreased endurance capacity (Sawka *et al.* 2000). However, it is unclear whether these mechanisms are linearly influenced by severity of dehydration (Sawka *et al.* 1985). Increases in heat storage and autonomic function may occur linearly with hydration status (Montain & Coyle 1992; Sawka *et al.* 1985), or at a threshold hypohydration (Wyndham & Strydom 1969). The physiological toll of hypoxia and hypovolemia is likely to reduce oxygen delivery further, resulting in a significant rise in cardiac output and metabolic heat production. A concomitant reduction in heat dissipation should further increase heat storage, inducing greater physiological strain. It is unclear whether the magnitude of physiological strain will follow the severity of dehydration.

Hormonal control of fluid balance in hypoxia may involve increases in antidiuretic hormone (ADH) (Bartsch *et al.* 1991a) and atrial natriuretic peptide (ANP) (Bartsch *et al.* 1988) in AMS susceptible individuals, ADH release has been positively correlated with the degree of hypoxic stress (Claybaugh *et al.* 1982). Loeppky *et al.* (2005a) showed ADH changes to occur from 90 minutes during hypoxic rest. Paradoxical alterations in the renin-angiotensin system's control of aldosterone have also been noted in hypoxia (Lawrence *et al.* 1990). Consequently, fluid retention may tend to occur in those unable to

adapt as effectively when exposed to hypoxia or altitude (Loeppky *et al.* 2005a). In normoxic conditions ADH, aldosterone and ANP undergo an exercise intensity dependent rise (Freund *et al.* 1991). However, hypoxia seems to suppress the response of aldosterone and plasma renin activity to exercise (Zaccaria *et al.* 1998), while others have found hypoxia to have no influence on either hormone during mild exercise (Meehan, 1985).

Responses to dehydration have not been well documented during hypoxia, even though hormonal regulation of fluid is thought to relate to an individual's tolerance to hypoxia. Chapter V demonstrated that hypohydration can induce a rise in physiological strain and symptoms of AMS, yet it is important to quantify the response to graded hypohydration severities. It is hypothesised that hypohydration severity alters the physiological, hormonal and psychological responses to a 125min intermittent walking test under normobaric hypoxic conditions.

6.3 Methods

Eight physically active males 20 ± 1 yrs, of height 184 ± 3 cm, mass 82 ± 7 kg, body fat $13 \pm 2\%$, and $\dot{V}O_{2\max}$ of 45.7 ± 6 ml·kg⁻¹·min⁻¹ attended the laboratory on five separate occasions. The first visit was a familiarisation session involving anthropometric measures, cardiovascular fitness assessment and assessment of walking speed at 50% $VO_{2\max}$ (section 3.3.1 & 3.3.2). The next four visits involved an intermittent walking test (Figure 3.4.1) in a hypoxic ($FIO_2=0.13$) environment under four different hydration protocols [euhydrated (EU), hypohydrated to ~1% body mass loss (H1), hypohydrated to ~2% body mass loss (H2), hypohydrated to ~3% body mass loss (H3)]. For the EU condition participants completed 1 hr of high intensity running (~75% $VO_{2\max}$) 15 hrs prior to the intermittent walking test, while wearing warm clothing (hat, gloves, jumper, trousers).

Nude body mass was measured pre and post exercise. Participants were then encouraged to rehydrate over the 15 hours preceding the intermittent walking test, by consuming 150% of sweat loss during the hour prior to exercise (Shirreffs *et al.* 2007). In H1 participants completed the exercise as in the EU trial and were required to consume fluid equal to half the amount lost as sweat, over the 15 hrs preceding the intermittent walking test. In H2 participant completed the same exercise but consumed no fluid over the exercise bout or for the 15 hrs prior to testing. For the H3 condition participants completed 1.5 hrs exercise and were fluid restricted during exercise and for 24 hrs preceding the intermittent walking test.

Physiological and perceptual markers were monitored throughout the intermittent walking test, these are described within Section 3 and illustrated in Figure 6.1. In addition to the previous chapters, the study monitored fluid controlling hormones such as ADH, ANP and aldosterone pre and post hypoxic exposure. Blood, pre and post test, was taken from the antecubital fossa using a 10ml syringe and 21 gauge hypodermic needle. Methods for blood taking, storage and analysis are detailed in section 3.6.4.

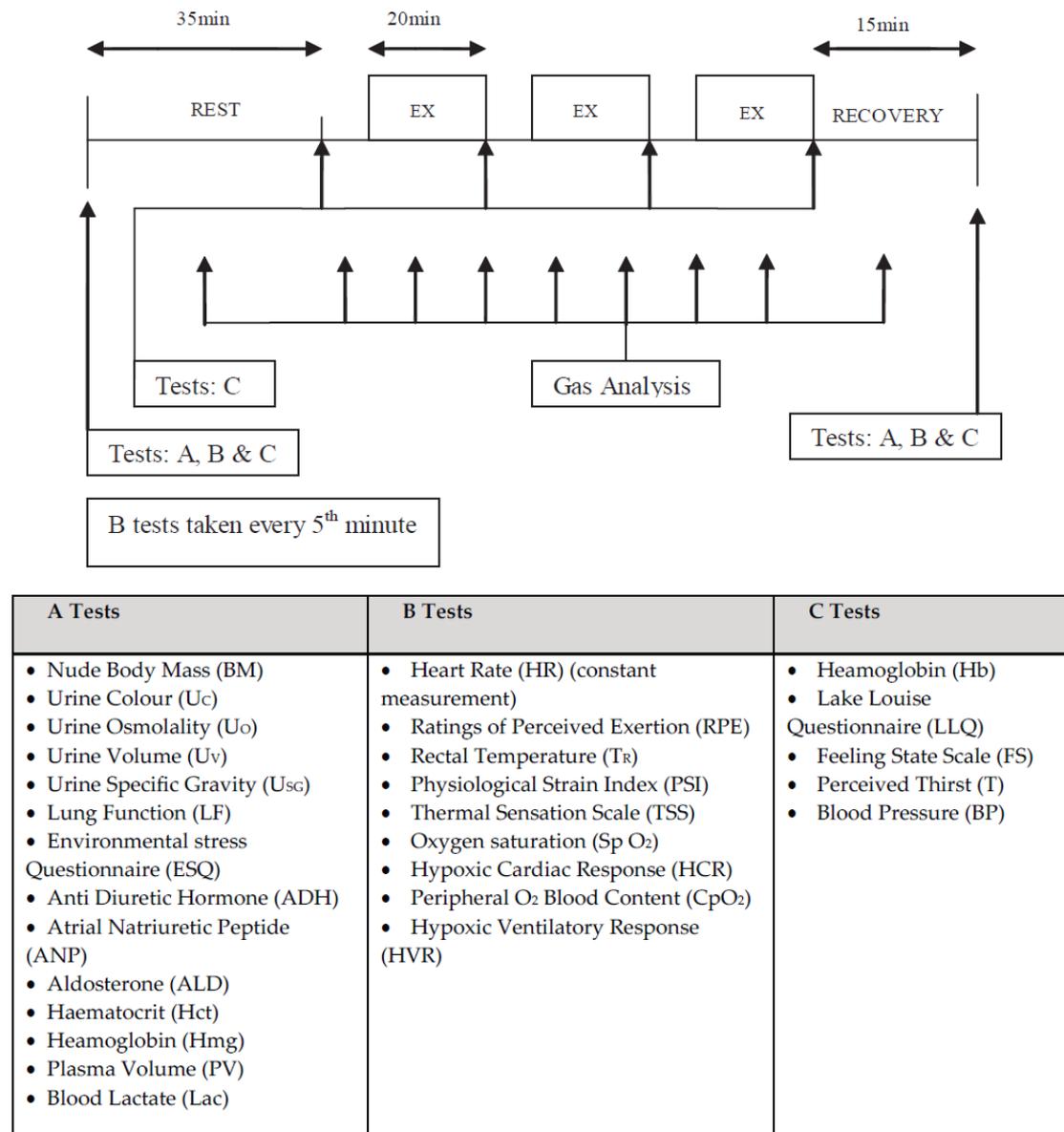


Figure 6.1: Schematic of the experimental design, showing the measurements and timings. Arrows denote measurement points.

Statistics

The order of the tests was randomised, determined by a Latin squares design. Each test was separated by a seven day 'wash out' period. Two way ANOVA with repeated measures was used to identify significant effect of the condition and or time point. One way ANOVA with repeated measures was used to compare between hydration conditions, bonferroni pairwise comparisons compared between separate conditions. Paired T-tests

were used to compare pre and post values of urine, blood and physiological measures.

6.4 Results

Dehydration protocols reduced body weight by approximately 0%, -1%, -2% and -3% in EU, H1, H2 and H3 trials, respectively. Urine markers were also used to indicate hydration state as shown in Table 6.1. All post IWT urine values correlated with LLQ mean and LLQ peak. Renal hormones showed no differences between conditions, although ADH and aldosterone values significantly increased over the test. Hct and [Hb] also showed no difference between conditions, though [Hb] did increase over the test duration ($p<0.001$).

At rest heart rate was approximately $3\text{bts}\cdot\text{min}^{-1}$ greater per 1% loss in body mass, when hypohydrated by $\geq 2\%$. During exercise there was greater variation in heart rate over time and between conditions. Significant difference was found during the last two stages of each exercise bout between EU and H1 and the H2 and H3 conditions ($p<0.05$). Heart rate at exercise time points correlated with LLQ mean, LLQ peak and ESQc score. Rectal core temperature and physiological strain index were not different between conditions, but did change over time ($p<0.005$, Figure 6.2) and correlated with AMS measures during exercise ($p<0.05$). Both rectal core temperature and physiological strain were significantly greater in H2 and H3 compared to EU and H1 conditions from the second bout of exercise onwards ($p<0.05$).

Table 6.1: Mean \pm SD pre and post hydration markers for EU, H1, H2 and H3 conditions. Symbols to denote significant difference to H1 = #, H2 = \diamond , H3 = Ω . † denotes significant difference between pre and post values ($p < 0.05$). Antidiuretic hormone (ADH), atrial natriuretic peptide (ANP), aldosterone (ALD).

	<u>Eu</u>		<u>H1</u>		<u>H2</u>		<u>H3</u>								
	Pre	Post	Pre	Post	Pre	Post	Pre	Post							
Body Mass (kg)	82.4 \pm 7.3	$\diamond\Omega$ †	81.4 \pm 7.2	$\diamond\Omega$	81.4 \pm 6.9	Ω †	80.7 \pm 7.1	Ω	80.4 \pm 6.6	†	79.8 \pm 6.6	79.3 \pm 6.5	†	78.7 \pm 6.5	
Body Mass Loss from normal (%)	0.08 \pm 0.01	# $\diamond\Omega$	-		-0.968 \pm 0.46	$\diamond\Omega$	-		-1.981 \pm 0.68	-		-3.098 \pm 0.87	-		
ADH (pg ml ⁻¹)	1.7 \pm 0.47	†	3.75 \pm 0.87		1.82 \pm 0.44	†	5.21 \pm 1.01		2.19 \pm 0.53	†	5.66 \pm 0.91	2.25 \pm 0.51	†	5.72 \pm 0.97	
ANP (pg ml ⁻¹)	27.6 \pm 5.5		30.4 \pm 4.03		26.6 \pm 3.57		27.4 \pm 2.88		24 \pm 3.74		26.8 \pm 1.3	21 \pm 5.14		25.2 \pm 3.11	
ALD (pg ml ⁻¹)	97.6 \pm 24.7	†	188.6 \pm 44.6		110.8 \pm 26.9	†	197.4 \pm 47.2		125.6 \pm 33.8	†	207 \pm 51.9	135.8 \pm 37.8	†	230.4 \pm 64.2	
Urine Specific Gravity	1.008 \pm 0.005	# $\diamond\Omega$ †	1.010 \pm 0.003	# $\diamond\Omega$	1.022 \pm 0.004	$\diamond\Omega$ †	1.020 \pm 0.002	$\diamond\Omega$	1.027 \pm 0.001	†	1.025 \pm 0.003	1.029 \pm 0.003	†	1.028 \pm 0.005	
Urine Osmolality (mosmo kg ⁻¹)	236 \pm 43	# $\diamond\Omega$ †	452 \pm 185	# $\diamond\Omega$	838 \pm 202	$\diamond\Omega$ †	824 \pm 150	$\diamond\Omega$	1059 \pm 59	†	1029 \pm 112	1069 \pm 103	†	1059 \pm 136	
Urine Colour	2 \pm 1.2	$\diamond\Omega$ †	2 \pm 0.64	# $\diamond\Omega$	3 \pm 0.83	Ω †	3 \pm 0.64	Ω	4 \pm 0.53	†	4 \pm 0.46	Ω	5 \pm 0.83	†	5 \pm 1.06
Plasma Osmolality (mosm kg ⁻¹)	285 \pm 3.0		286 \pm 9.3		286 \pm 3.3		286 \pm 5.3		288 \pm 5.7		288 \pm 8.1	288 \pm 6.7		289 \pm 8.4	
Urine Flow (ml min ⁻¹)	-		3.42 \pm 1.6	# $\diamond\Omega$	-		1.25 \pm 0.63		-		1 \pm 0.25	-		0.814 \pm 0.285	
Free Water Clearance (L hr ⁻¹)	-		2.95 \pm 1.4	# $\diamond\Omega$	-		0.96 \pm 0.53	Ω	-		0.709 \pm 0.21	-		0.561 \pm 0.212	
Sweat Rate (L hr ⁻¹)	-		0.65 \pm 0.11	# $\diamond\Omega$	-		0.38 \pm 0.04		-		0.34 \pm 0.01	-		0.33 \pm 0.02	

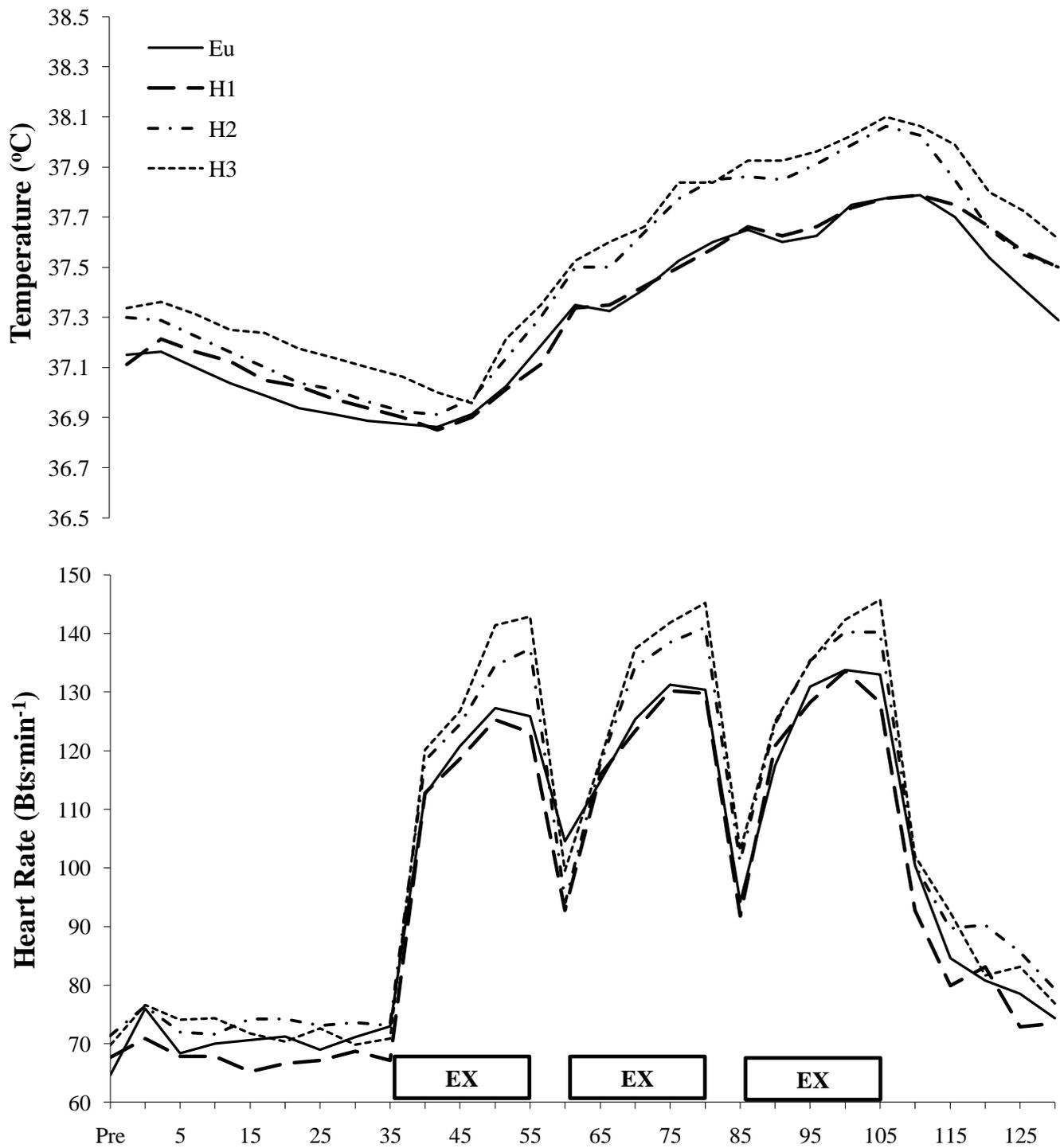


Figure 6.2: Mean rectal core temperature and heart rate measured every 5th minute of the intermittent walking test, comparing between the four hydration conditions. Exercise bouts (EX) are highlighted on the X axis.

Ratings of perceived exertion (EU 11 ± 1 ; H1 11 ± 1 ; H2 13 ± 2 ; H3 14 ± 2) were not different between conditions, but did increase over time ($p < 0.0001$), while all time points correlated with all AMS symptom markers ($p < 0.01$). Thermal sensation [at rest (EU 4.3 ± 0.5 ; H1 4.1 ± 0.4 ; H2 4.3 ± 0.5 ; H3 4.4 ± 0.5), during exercise (EU 5.6 ± 0.5 ; H1 5.8 ± 0.3 ; H2 5.8 ± 0.4 ; H3 5.9 ± 0.9)] also showed no difference between conditions, but did increase over time ($p < 0.05$). Perceived thirst increased with severity of hypohydration ($p < 0.001$), increased with time ($p < 0.001$) and correlated with LLQ_{mean} ($r = 0.54$), LLQ_{peak} ($r = 0.59$) and ESQ_c score ($r = 0.65$) ($p < 0.01$). Visual analogue headache, breathing and feeling state scales were all different between conditions ($p < 0.01$) and increased with time ($p < 0.0001$), stomach feeling states showed no differences. AMS symptoms measures including LLQ mean ($p < 0.01$), LLQ peak ($p < 0.01$) and post test ESQ_c score (EU 0.2 ± 0.2 ; H1 0.2 ± 0.2 ; H2 0.5 ± 0.1 ; H3 0.7 ± 0.1) ($p < 0.01$) all increased with hypohydration severity and correlated together ($p < 0.01$) (Figure 6.3). Individual LLQ question scores showed all symptoms except gastrointestinal stress, to increase over the intermittent walking test. Headache and fatigue/lethargy scores were significantly different between hydration conditions for the majority of time points ($p < 0.05$) (Table 6.2).

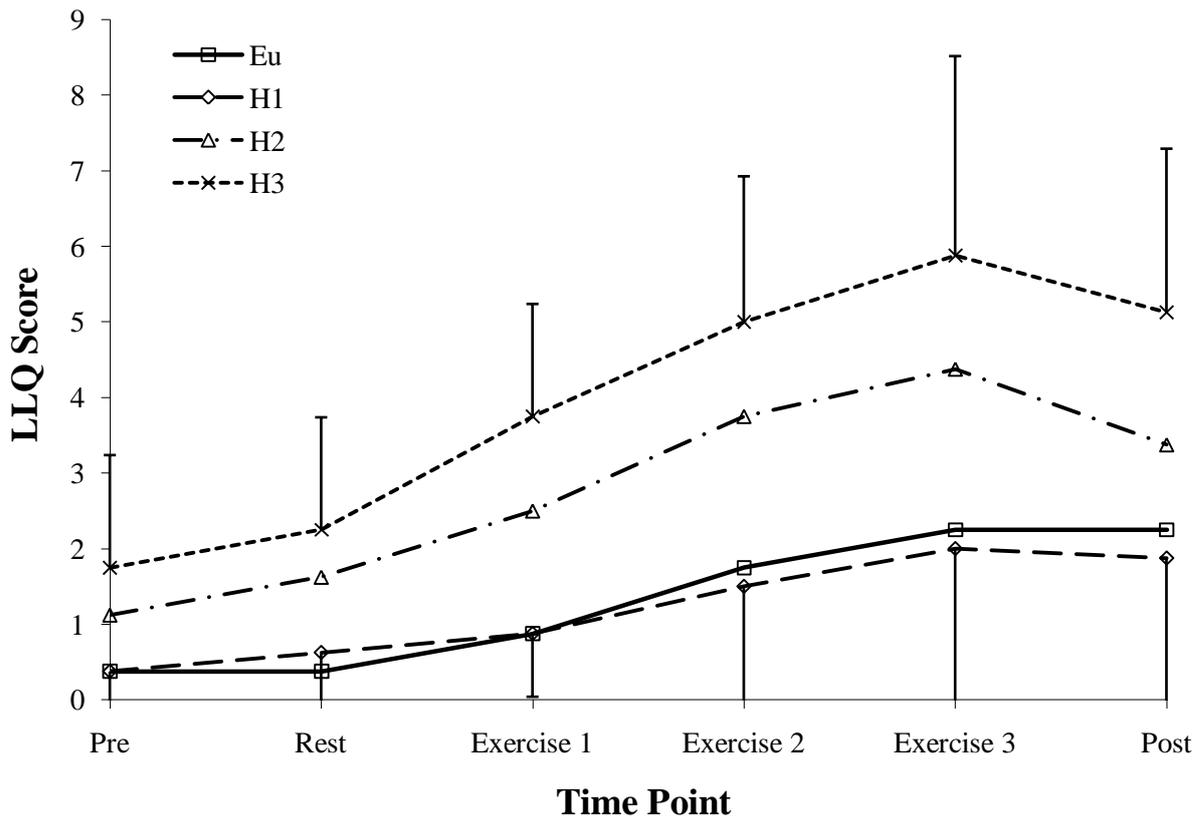


Figure 6.3: Lake Louise Questionnaire score over the intermittent walking test between each hydration condition. Y error bars are shown.

Ventilation markers such as SaO_2 , tidal volume and breathing frequency were not different between conditions or over time. However, SaO_2 tended to be lower with hypohydration severity except in H1 [rest (EU 85 ± 4 ; H1 86 ± 2 ; H2 82 ± 4 ; H3 $82 \pm 4\%$), exercise (EU 80 ± 4 ; H1 79 ± 3 ; H2 79 ± 4 ; H3 $77 \pm 4\%$)]. Similarly, minute ventilation was greater with hypohydration severity, but was also reduced in the H1 condition [rest (EU 17.4 ± 2 ; H1 14.2 ± 1 ; H2 17.4 ± 4 ; H3 $15.8 \pm 3 \text{ L} \cdot \text{min}^{-1}$), exercise (EU 36.2 ± 6 ; H1 30.4 ± 3 ; H2 37.8 ± 11 ; H3 $38.5 \pm 4 \text{ L} \cdot \text{min}^{-1}$)]. SaO_2 was the only ventilation marker to correlate with any AMS measures ($p < 0.05$). Derivatives such as HCR (Figure 6.4), HVR (Figure 6.5) and CpO_2 showed no difference between conditions. No lung function values showed significant change between conditions or over time.

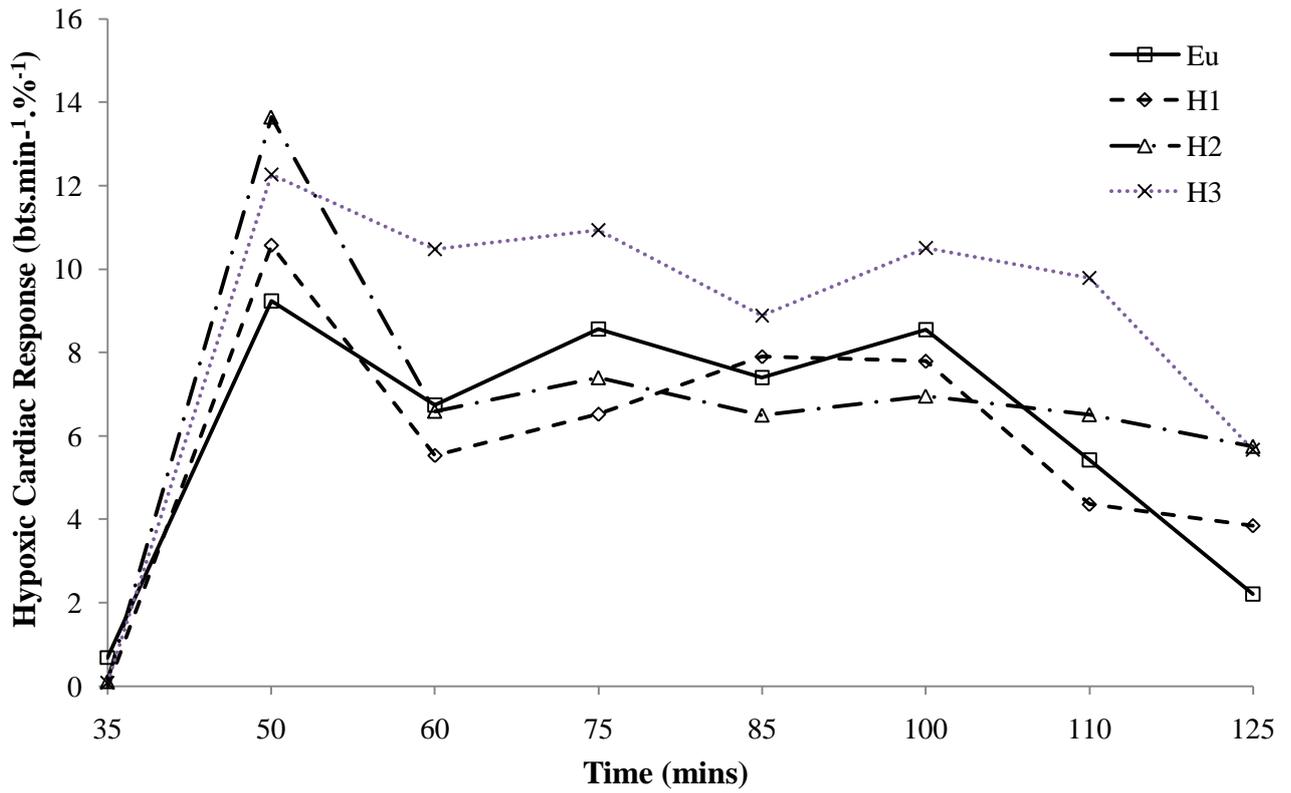


Figure 6.4: Hypoxic cardiac response from the resting phase onwards.

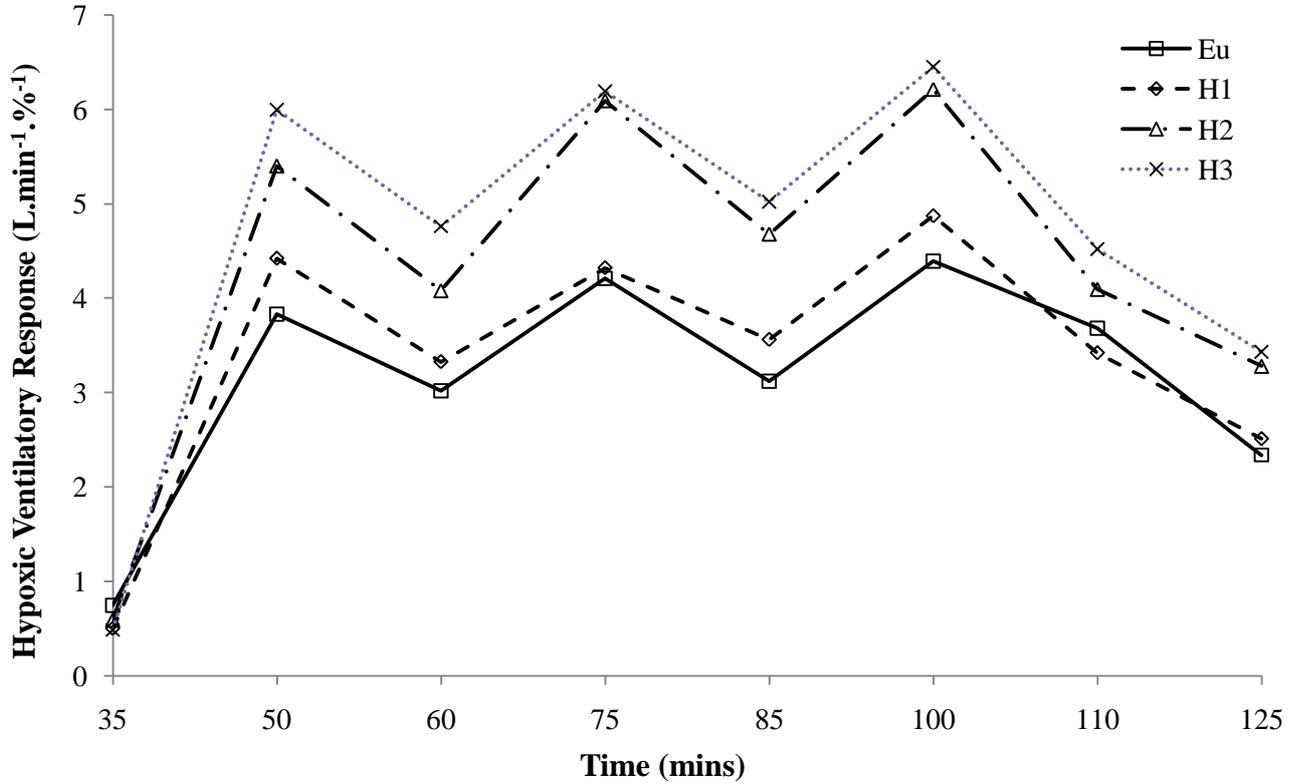


Figure 6.5: Hypoxic ventilatory response from the resting phase onwards.

Table 6.2: Comparing Individual Lake Louise Questionnaire symptom scores (Mean \pm SD) between EU, H1, H2 and H3 hydration conditions, for all time points throughout the intermittent walking test. Symbols to denote significant difference to H1 = #, H2 = \diamond , H3 = Ω . † denotes significant increase from pre intermittent walking test values ($p < 0.05$).

<i>LLQ Symptom Condition</i>	<i>Pre</i>		<i>Rest</i>		<i>Exercise 1</i>		<i>Exercise 2</i>		<i>Exercise 3</i>		<i>Post</i>	
<u>Headache</u>												
EU	0.2 \pm 0.04	$\diamond\Omega$	0.2 \pm 0.05	$\diamond\Omega$	0.4 \pm 0.2	$\diamond\Omega$	0.5 \pm 0.2	$\diamond\Omega$ †	0.5 \pm 0	$\diamond\Omega$ †	0.5 \pm 0.2	Ω †
H1	0.2 \pm 0.04	$\diamond\Omega$	0.4 \pm 0.2	$\diamond\Omega$	0.3 \pm 0.2	$\diamond\Omega$	0.4 \pm 0.1	$\diamond\Omega$	0.3 \pm 0.1	$\diamond\Omega$ †	0.3 \pm 0.1	Ω †
H2	0.5 \pm 0.2	Ω	0.9 \pm 0.3	Ω †	1 \pm 0.3	Ω †	1.3 \pm 0.6	†	1.3 \pm 0.6	†	0.9 \pm 0.4	Ω †
H3	1 \pm 0.3		1.4 \pm 0.3	†	1.6 \pm 0.6	†	1.9 \pm 0.8	†	2 \pm 0.8	†	1.9 \pm 0.9	†
<u>Fatigue/lethargy</u>												
EU	0.1 \pm 0.02	$\diamond\Omega$	0.1 \pm 0.02	$\diamond\Omega$	0.2 \pm 0.1	$\diamond\Omega$	0.8 \pm 0.4	$\diamond\Omega$ †	1 \pm 0.4	$\diamond\Omega$ †	1 \pm 0.5	Ω †
H1	0.1 \pm 0.02	$\diamond\Omega$	0.1 \pm 0.06	$\diamond\Omega$	0.3 \pm 0.1	$\diamond\Omega$	0.8 \pm 0.3	$\diamond\Omega$ †	1 \pm 0.6	$\diamond\Omega$ †	0.9 \pm 0.5	Ω †
H2	0.4 \pm 0.1		0.3 \pm 0.1		0.7 \pm 0.3	Ω †	1.8 \pm 0.8	Ω †	1.9 \pm 0.7	Ω †	1.5 \pm 0.9	Ω †
H3	0.5 \pm 0.2		0.4 \pm 0.2		1.2 \pm 0.6	†	2.1 \pm 1	†	2.4 \pm 1	†	2.1 \pm 0.9	†
<u>Lightheadedness/dizziness</u>												
EU	0.1 \pm 0.04		0.1 \pm 0.03	Ω	0.2 \pm 0.1	Ω	0.5 \pm 0.3	†	0.7 \pm 0.4	Ω †	0.7 \pm 0.3	†
H1	0.1 \pm 0.04		0.1 \pm 0.05	Ω	0.2 \pm 0.1	Ω	0.4 \pm 0.2		0.7 \pm 0.3	†	0.7 \pm 0.4	†
H2	0.2 \pm 0.1		0.3 \pm 0.1		0.4 \pm 0.2		0.6 \pm 0.3	†	0.8 \pm 0.4	†	0.8 \pm 0.4	†
H3	0.3 \pm 0.1		0.4 \pm 0.1		0.8 \pm 0.3	†	0.8 \pm 0.4	†	1.2 \pm 0.8	†*	1 \pm 0.7	†
<u>Gastrointestinal Stress</u>												
EU	0		0.01 \pm 0.01		0.03 \pm 0.02		0.03 \pm 0.02		0.04 \pm 0.03		0.03 \pm 0.03	
H1	0		0.02 \pm 0.04		0.03 \pm 0.03		0.02 \pm 0.01		0.02 \pm 0.02		0.02 \pm 0.02	
H2	0.04 \pm 0.02		0.1 \pm 0.02		0.2 \pm 0.1		0.2 \pm 0.1		0.3 \pm 0.2		0.1 \pm 0.04	
H3	0.1 \pm 0.05		0.1 \pm 0.03		0.3 \pm 0.1		0.3 \pm 0.2		0.3 \pm 0.3		0.2 \pm 0.1	

6.5 Discussion

This study aimed to investigate the effect of hypohydration severity on physiological, hormonal and psychological responses to an intermittent walking test in normobaric hypoxic conditions. Although many of the markers did significantly change with hypohydration severity, the response does not seem to be as linear as similar normoxic study reports (Montain & Coyle 1992).

The current study did not find a linear response between core temperature and dehydration. Although studies of similar exercise intensity in the heat found increases of around 0.10°C (Greenleaf & Castle 1971) to 0.15°C (Sawka *et al.* 1985) for 1% increase in dehydration. However, Wyndham & Strydom (1969), claim that a loss in body weight of 3% induces significant increase in heat storage. In the current study hypohydration of 2% seems to induce a significant rise in core temperature. This continual increase in heat storage via diminished heat dissipation may be responsible for the heart rate drift in H2 and H3 during exercise. However, for the first exercise bout, increases in temperature were not different between conditions, suggesting other factors such as reduced venous return and cardiac filling are in part responsible for heart rate drift. Wingo *et al.* (2005) explained that heart rate drift may be a consequent of increased relative metabolic intensity, known to increase with hypohydration severity (Nybo *et al.* 2001) and hypoxia.

Hormones regulating renal function showed no difference between hydration conditions, this may be due to low sample size and range in participant resting values, although there were clearly differences between hydration conditions (Table 6.2). This lack of change is likely to result from plasma osmolality not altering between conditions. Senay

(1979) suggests plasma osmolality controls the response to hypohydration, elevating plasma ADH. It is proposed that the hypohydrations induced were sufficient for the body to maintain plasma osmolality and volume through reperfusion from the intracellular space, as similar research has used significantly greater hypohydration levels (Nadel *et al.* 1980; Sawka *et al.* 1984; Senay 1979).

An exercise induced rise in ADH and aldosterone was noted for all conditions. This response to exercise is consistent with other normoxic work, whereby exercise of $>60\%VO_2\text{max}$ is thought to induce renal hormone release through adrenocorticotrophic elevation (Virtu 1992). However, hypoxia is thought to suppress ADH (Claybaugh *et al.* 1978) and aldosterone (Bouissou *et al.* 1987) release irrespective of exercise (Zaccaria *et al.* 1998). Plasma osmolality did not significantly alter with exercise, even though it is widely thought plasma osmolality stimulates the release of renal hormones (Takamata *et al.* 2000). This suggests plasma osmolality is not the solitary initiator of renal hormone release and that sympathoadrenal activity alone may allow sufficient stimulus. However, it is also conceivable that renal hormone release over the test duration combined with the blood measurement recorded 40 min after exercise completion, may have been sufficient to reduce plasma osmolality to near resting levels.

Sweat gland function is also thought to be suppressed through depressed stimulation of the eccrine gland (Dipasquale *et al.* 2002). In the current study sweat rates were greater in EU, but were not different between H1, H2 and H3, suggesting renal fluid retention with all body water deficits. This is not consistent with the lack of change in plasma osmolality, which is thought to mediate sweat rates (Nadel *et al.* 1980; Sawka *et al.* 1984).

Lack of change in ratings of perceived exertion and thermal sensation would indicate that participants felt no additional physical difficulty with the severity of hypohydration during exercise. Yet participant's perception of headache, lightheadedness and lethargy increasing with severity of hypohydration, generating greater LLQ and ESQ values, indicates the occurrence of cerebral and/or orthostatic changes. Furthermore, participants reported no symptoms of gastrointestinal upset with such acute hypoxic insult (Table 6.2), while visual analogue values for stomach pain also did not alter in any condition. The use of the LLQ allows quantification of these symptoms, yet by definition (Bartsch et al. 2004) AMS takes several hours of rest at a greater altitude to diagnose. Therefore, these values cannot be interpreted as AMS without assessing individual's responses to a longer duration resting hypoxic exposure.

Minor hypohydration (H1) showed core temperature and heart rates similar to euhydrated values, while headache scores were constantly lower than all other conditions. This suggests such minimal hypohydration has little effect on physiological strain. The increase in symptoms of headache, dizziness, fatigue and nausea are seemingly caused from the greater physiological strain induced through exercising while more severely hypohydrated. Further research needs to investigate the cerebral mechanisms acting around this minor hypohydration. While longer duration resting exposure is needed to effectively monitor renal responses to hypohydration during hypoxic exposure.

6.6 Conclusion

Hypohydration severity greater than 2% of body mass, increases physiological strain during exercise. Hormones regulating fluid balance increase with hypoxic exercise but

do not alter with hypohydration severity. Individuals exercising within hypoxic environments should ensure they are adequately hydrated, as in a normoxic environment, so to prevent rise in physiological strain and reduce the likelihood of symptoms such as headache, fatigue, nausea or lightheadedness.

CHAPTER VII

INFLUENCE OF REHYDRATION ON PHYSIOLOGICAL AND RENAL RESPONSES TO REST IN HYPOXIA

7.1. Abstract

Fluid balance is thought to be a factor in the pathophysiology of altitude illness, yet fluid balance during acute exposure to hypoxia has not been previously explored. Eight healthy participants were hypohydrated 24hrs prior to testing, using exercise and fluid restriction, causing a ~2.5% loss in body mass. Participants then rested in either normoxia (NH) or hypoxia (HH) (FIO₂: 0.125) for up to 6 hrs, with no fluid replacement. Participants also completed two other hypoxic trials, rehydrating with either water (HW) or isotonic fluid (HI) in the first 2 hrs of exposure. Each trial was separated by seven days. Measures of body water, fluid compartments, serum 100β, HSP₇₀, anti diuretic hormone (ADH) and urine hydration markers were taken at 2 hr intervals. HR, SaO₂, core temperature and perceptual scales were monitored throughout the trials. Rehydration increased body water and extracellular fluid compartment volumes, maintained with isotonic fluid rehydration. Serum 100β and HSP₇₀ values increased with hypoxic exposure [Serum 100β (HH=0.13 ± 0.05μg/L, HW=0.11 ± 0.05μg/L, HI=0.11 ± 0.06μg/L); HSP₇₀ (HH=0.51 ± 0.08pg/L, HW=0.24 ± 0.07pg/L, HI=0.21 ± 0.08pg/L)], no differences between rehydration trials were found. LLQ score correlated positively with serum 100β ($r = 0.5524$, $p < 0.05$). Although rehydration has an effect on the physiological responses to hypoxic exposure, it is the individualistic hypoxic tolerance that has the greatest influence on physiological changes to the blood brain barrier function, cellular physiological strain and fluid regulating hormones.

Key Words: Altitude, fluid, serum 100b, HSP70, Body water

7.2. Introduction

Hypohydration is common at altitude (Basnyat *et al.* 1999), previous chapters have shown physiological strain and symptoms of AMS to increase with severity of hypohydration below 2% of body mass loss, with acute hypoxia (Chapter V & VI). Although, evidence from research contests the effect of dehydration at altitude (Basnyat *et al.* 1999; Basnyat *et al.* 1998; Rennie *et al.* 1993; Van Kreel *et al.* 1996). While in a clinical setting patients are often hypoxemic (van Beest *et al.* 2008) and hypovolemic through traumatic blood/fluid losses (Grocott *et al.* 2005), making direct comparisons difficult.

The renal response to hypoxia, mediated by the peripheral chemoreceptors (Swenson *et al.* 1995), has the most notable consequences. Individuals successfully adapting to hypoxia exhibit increased free water clearance, while those that suffer with AMS tend to show anti-diuresis and anti natriuresis, increasing total body water by ~1.2L over 12 hours (Loeppky *et al.* 2005b). This is a consequent of hypoxia-induced ADH release, although conflicting research supports (Loeppky *et al.* 2005a) and rejects (Swenson *et al.* 1995) the role of these hormones in AMS pathophysiology.

Body fluid shifts may occur with hypoxia, although measurement technique makes field-based monitoring difficult. Thus, few studies have investigated fluid control in simulated hypoxia (Loeppky *et al.* 2005a). Multi frequency bioelectrical impedance analysis allows more simple measurement of the fluid compartments, allowing the time course of hypoxic fluid balance to be established (Earthman *et al.* 2007). However, accuracy and sensitivity via prediction equations have been questioned (Armstrong 2005). Thus combined use of the deuterium oxide and bromide methods (Islam *et al.*

1999) to measure total body water and extracellular fluid volume respectively, would be needed to monitor fluid shifts accurately.

Fluid retention may cause a rise in cerebrovascular blood flow in combination with increasing permeability of the BBB through the hypoxia induced release of bradykinin, histamine, nitric oxide, arachidonic acid and vascular endothelial growth factor (VEGF) (Basnyat & Murdoch 2003; Kaur & Ling 2008). This may result in cerebral cell swelling, rise in intracranial pressure and ultimately AMS symptoms (Hackett & Roach 2001). Serum 100β , an indicator of increased BBB permeability (Marchi *et al.* 2004), may ascertain the immediate changes in BBB with hypoxic insult. Yet, the only study (Bailey *et al.* 2009) to measure this observed no changes in circulating serum 100β with 6 hrs of hypoxic exposure.

Physiological strain has been shown to increase with acute hypoxia (Chapter IV) and hypohydration severity (Chapter VI), however quantification of the physiological strain at cellular level is limited (Liu *et al.* 2000; Melling *et al.* 2007). HSP_{70} has been studied in normothermic and hot environments (Kregel 2002), yet data from hypoxic environments tends to be limited to animals (Aoe *et al.* 1997; Kawana *et al.* 2000; Mestral *et al.* 1994; Tokyol *et al.* 2005; Turman & Rosenfeld 1999; Zhong *et al.* 2000). Tokyol *et al.* (2005) showed HSP_{70} to increase significantly within the kidney when exposed to hypoxia, suggesting the response may be related to the alterations in renal function within hypoxia. This biochemical marker of physiological strain may give a mechanistic indication of the cellular strain hydration and hypoxia may induce as single and combined entities.

Due to renal responses and hypohydration influencing physiological strain and AMS symptoms, rehydration techniques in hypoxia may be important. Intake of large hyposmolar fluid volumes may be as detrimental as hypohydration (Chapter V & VI) altering fluid compartments. High sodium solutions replenish extracellular fluid compartments greater than water alone (Nose *et al.* 1988a), while urine output is reduced via sodium induced increase in ADH (Mitchell *et al.* 2000). Fluid replenishment using different isotonic solutions has shown varying improvements in cardiovascular function (Kenefick *et al.* 2007; Sims *et al.* 2007a), which is essential during hypoxic exposure to reduce physiological strain. However, retention of too much fluid may also cause detrimental symptoms (Basnyat & Murdoch 2003). By looking at the fluid movement mechanisms during acute hypoxia in a state of hypohydration, fluid balance and high intracellular fluid volume, it may be possible to identify the detrimental fluid controlling mechanisms that induce physiological strain and the onset of AMS symptoms during hypoxic exposure.

This study aims to quantify the effect of rehydration solution on fluid compartments when exposed to hypoxia at rest and to identify whether rehydration can reduce physiological strain and AMS symptoms and whether these changes are related to alterations in cellular strain and blood brain barrier permeability. The first hypothesis states that the type of rehydration solution will alter the time course of fluid balance in hypoxia. The second hypothesis states that regulation of fluid balance is related to physiological strain and onset of AMS at rest.

7.3. Methods

Eight physically active participants of 21 ± 1 yr, height 171 ± 6 cm, mass 79.7 ± 10.4 kg, $VO_{2\max}$ 49.3 ± 13 ml \cdot kg $^{-1}\cdot$ min $^{-1}$ completed four trials under hypoxic [Hypoxic Hypohydration (HH), Hypoxic Water Rehydration (HW), Hypoxic Isotonic Fluid Rehydration (HI)] and normoxic [Normoxic Hypohydration (NH)] environmental conditions. Participants lay in either a normoxic ($FIO_2=0.2093$) or normobaric hypoxic ($FIO_2=0.12$) environment for 6 hrs after completing a dehydration and fluid restriction trial. To induce hypohydration participants completed 1 hr of high intensity running ($\sim 75\%$ $VO_{2\max}$) 24 hrs prior to testing, while wearing warm clothing (hat, gloves, jumper, trousers). Nude body mass was measured pre and post exercise. Participants were then fluid restricted for the 24hrs up to testing, but were allowed to maintain normal solid food intake.

Over the first 2 hours of exposure, HW and HI trials required participants to consume 25% of body mass loss every 20 minutes of either pure water (HW) or a 77mmol/L sodium chloride (NaCl) and 150mmol/L glucose isotonic solution (HI), drinking a total of 150% of body mass loss. HH and NH trials required complete fluid restriction for the 24 hours and throughout the 6 hour exposure. Hypoxic conditions were created by participants wearing gas masks (S10 Gas Mask, British Army, UK) connected to nitrogen generators (Colorado Altitude Training, Colorado, USA). Throughout the exposure physiological measures, perceptual scales and acute mountain sickness scores were taken (Figure 7.1). The order of the tests was randomised, determined by a Latin squares design. Each test was separated by a seven day 'wash out' period.

Blood Markers

Blood (20ml) was taken from the antecubital fossa, pre deuterium and sodium bromide loading, pre exposure and every 2 hrs from the start of exposure. Blood was centrifuged in EDTA tubes to obtain plasma for assessment of plasma osmolality, HSP₇₀, ADH and deuterium oxide. Blood was also collected, stored and spun in clear tubes to obtain serum for measurement of creatinine, sodium, serum 100β and bromide. Full details of the blood analysis are in section 3.8.

Fluid Compartment Measures

Multi-frequency bioelectrical impedance analysis (MFBI) (Xitron 4000, San Diego, CA) was used to monitor TBW and ECF, using the Cole-Cole theoretical model (Cole & Cole 1941), calculating ICF by subtracting ECF from TBW. Measurements of MFBI were only performed once the participant had lay still for at least 45min, the same lying position was performed for every measurement. Measurement time points are illustrated in Figure 7.1 and further details of the MFBI technique are in Section 3.10.

Two hours prior to hypoxic exposure participants were given 20ml of 99.9% deuterium oxide and 6mg·kg⁻¹ sodium bromide, 25ml of water was then used to wash the container and given to the participant to consume. Blood samples to assess deuterium and bromide were taken immediately prior to dosage and then at the measurement time points in Figure 7.1. Measurement of deuterium oxide from blood plasma required gas chromatography- isotope ratio mass spectrometry. TBW was then derived from equation 3.8 (page 95). Bromide was measured from serum, using infrared spectrophotometry. Each blood sample (5ml) was deproteinised in 1.0ml of ice cold 0.6M perchloric acid and centrifuged at 5000rpm for 10min at 4C. The supernatant was

then separated and 500 μ l was added to a test tube containing 500 μ L of 0.6% sodium chloride and 500 μ L of 0.0375% gold chloride, wavelength was measured exactly 3 minutes after the addition of the supernatant. Concentrations were derived from a standard curve (Islam et al. 1999).

Statistics

Two way ANOVA with repeated measures was used to identify significant effect of the rehydration condition and or time point. One way ANOVA with repeated measures was used to compare between rehydration conditions, bonferroni pairwise comparisons compared between separate rehydration conditions. Once the participants had or were withdrawn from the test, the data from that point onwards was not used in the analysis. Participant withdrawals for each condition (HH n=4; HI n=5; HW n=5; NH n=8) are shown in Table 7.1. After withdrawl the last recorded LLQ score (9) was used for the remainder of the test, so AMS symptom score could remain comparable be individuals of various tolerance. LLQ score of 9 was the highest value allowed, due to ethical limits set by the University of Brighton Research Ethics Committee.

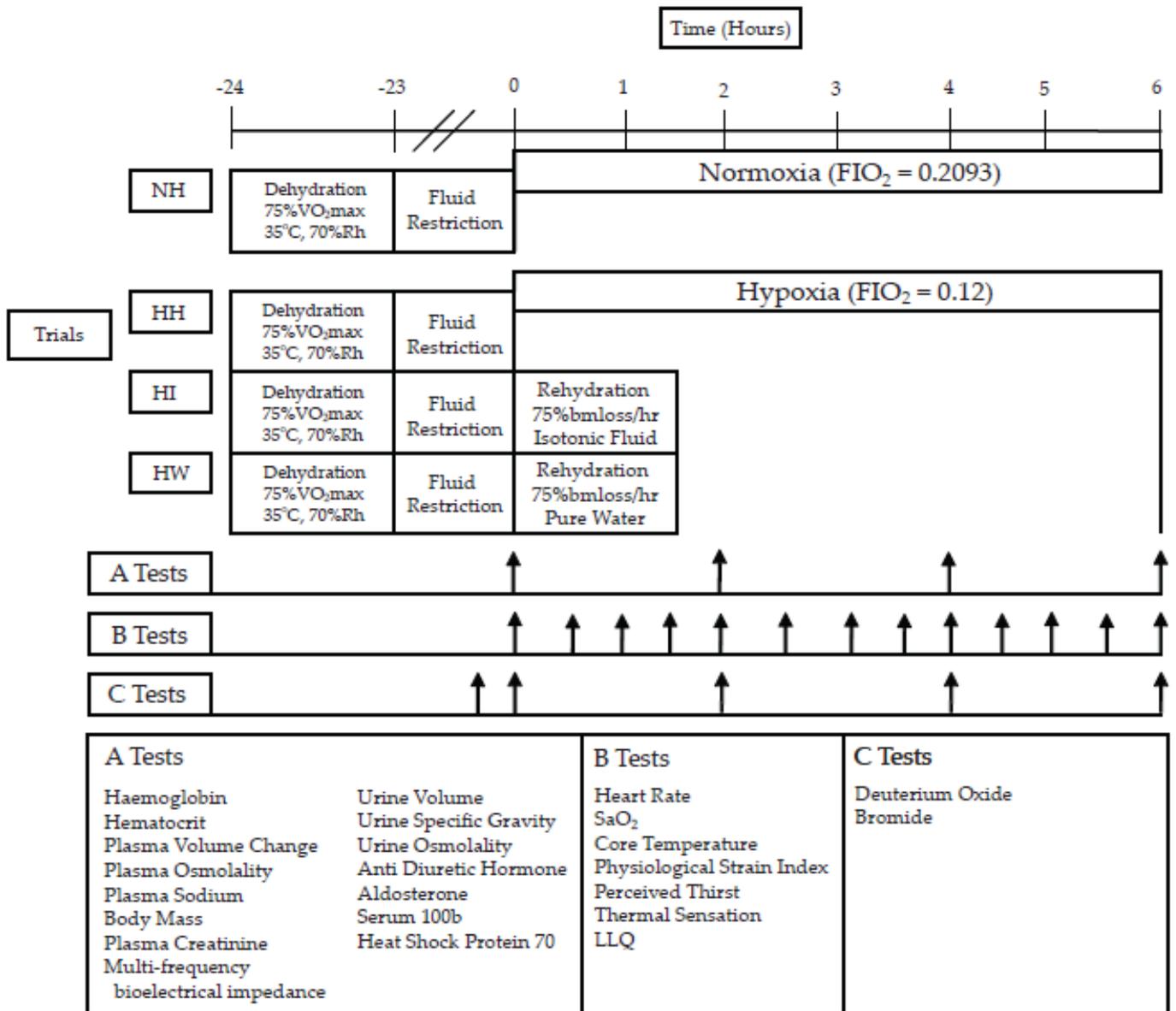


Figure 7.1: Schematic of the experimental design, showing trial protocols, measurements and timings. Arrows denote measurement points. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

7.4. Results

Physiological Responses

Tolerance to the hypoxic insult, through quantification of physiological responses or test duration, was individualistic. All participants took part in all conditions. However, the time at which participants withdrew or had to be withdrawn from the testing varied between conditions and participants (Table 7.1).

Table 7.1: Time to dropout (hours), for each participant, in each condition. A value of 6 hours indicates that the participants did not drop out.

<i>Participant Number</i>	<i>HI</i>	<i>HW</i>	<i>HH</i>	<i>NH</i>
1	6	6	6	6
2	6	6	6	6
3	4.45	4	4	6
4	6	6	5.30	6
5	6	6	6	6
6	5	4.45	2.45	6
7	3.30	3.30	3.15	6
8	6	6	6	6
Mean \pm SD	5.20 \pm 1	5.10 \pm 1	4.50 \pm 1	6 \pm 0

Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Heart rate showed no significant difference between conditions or over time, although hypoxic conditions were significantly greater than the NH trial ($p < 0.001$) from 2hrs onwards (Figure 7.2).

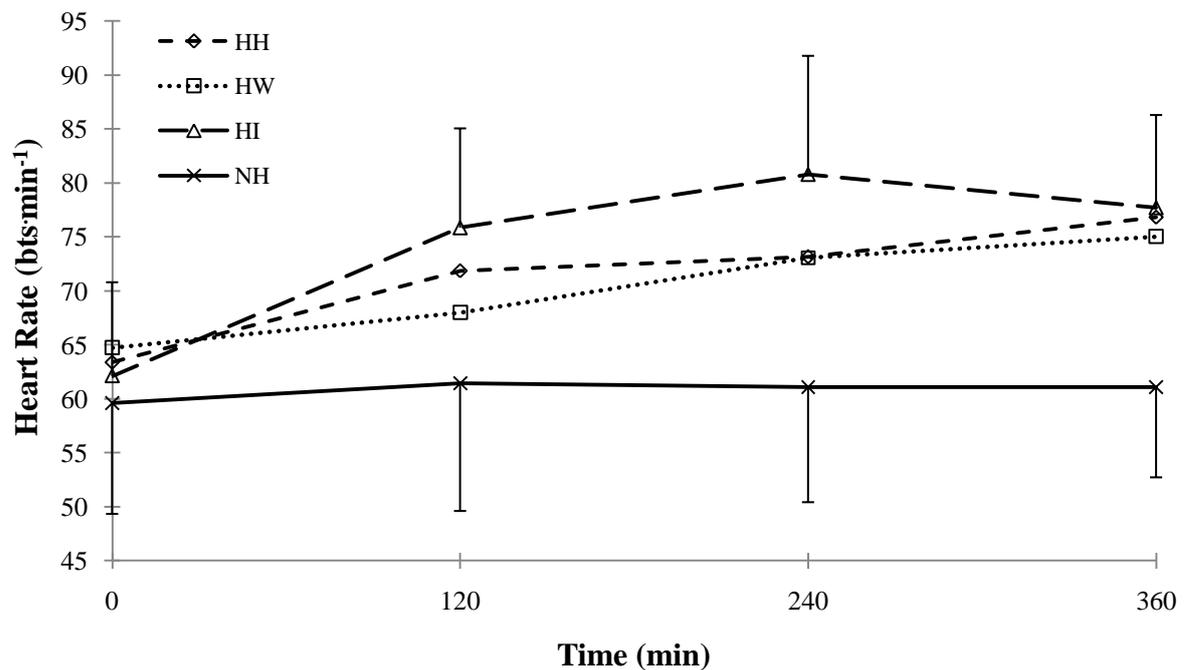


Figure 7.2: Heart rate over time for all conditions. Y error bars are shown. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Core temperature showed no significant changes over time ($f=2.327$, $p=0.107$) or between conditions ($f=1.75$, $p=0.188$) and ranged between 37.05°C and 37.35°C , with a mean \pm SD of $37.18 \pm 0.39^{\circ}\text{C}$ for all time points and all conditions. As a consequent, PSI was also not significantly different over time or between conditions, although hypoxic conditions induced significantly greater physiological strain than the NH trial ($p<0.001$).

SaO₂ was significantly lower in hypoxic trials compared to NH, but there was no significant difference between the hypoxic trials. SaO₂ values for all hypoxic trials did not change over time from 2hrs onwards ((HI $87\pm 1\%$; HW $86\pm 2\%$; HH $85\pm 2\%$; NH

98±0.5%). Subsequently, HCR was also not different between hypoxic trials or over time.

Haematological Markers

Plasma osmolality was significantly less in HW and HI conditions from 2hrs onwards (HI 287±6mosm·kg⁻¹; HW 288±7mosm·kg⁻¹; HH 295±6mosm·kg⁻¹; NH 292±5mosm·kg⁻¹), this did not significantly increase over time. There were significant changes in plasma volume in HI and HW conditions, which were maintained in the HI trial (Figure 7.3). Other blood markers such as hematocrit and haemoglobin showed no significant change over time and were not different between conditions.

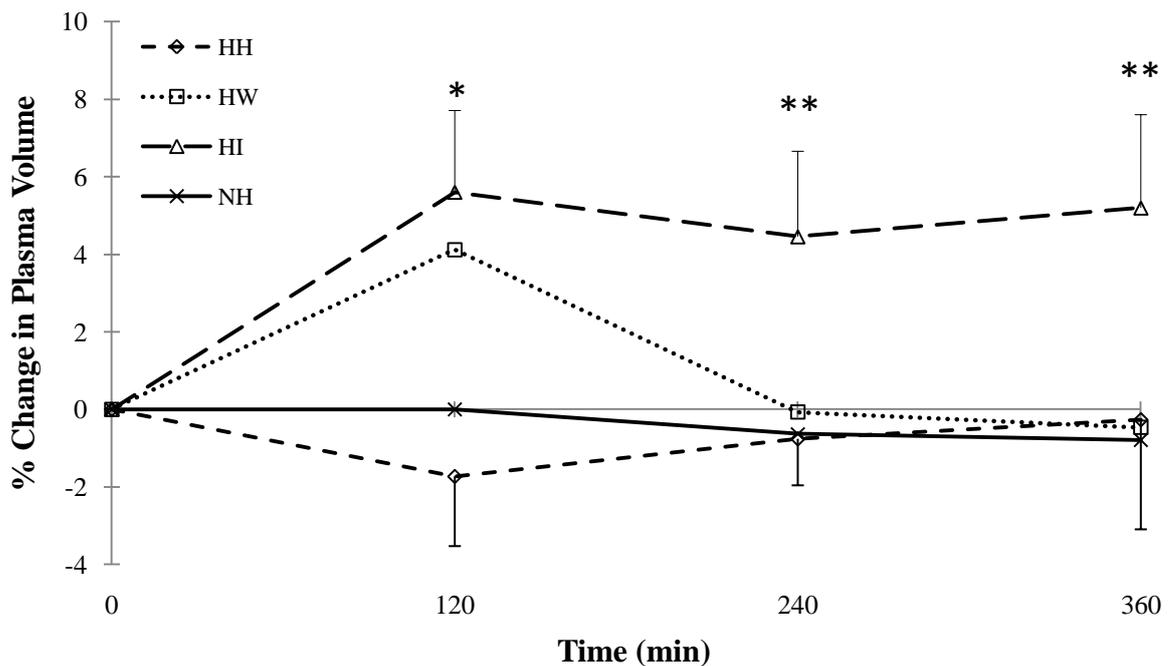


Figure 7.3: Change in plasma volume over time and between conditions. Y error bars are shown. * denotes significant difference between HI and the HH and NH trials. ** denotes significant difference between HI and all other trials. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Serum 100β increased in all hypoxic conditions, although there was no difference found between rehydration trials. HSP_{70} also increased in hypoxic trials, while there was no significance between rehydration trials (Table 7.2).

Table 7.2: Serum 100β and HSP_{70} values pre and post test for each of the trial.

*Denotes significant difference between pre and post test values.

	<u>HH</u>		<u>HW</u>		<u>HI</u>		<u>NH</u>	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Serum 100β ($\mu\text{g/L}$)	.05 \pm .03	.17 \pm .09*	.05 \pm .02	.13 \pm .07*	.05 \pm .01	.11 \pm .07*	.06 \pm .04	.05 \pm .02
HSP_{70} (pg/L)	.39 \pm 0.1	0.9 \pm .1*	.45 \pm .08	0.69 \pm .08*	.42 \pm .1	0.63 \pm .08*	.43 \pm .1	0.44 \pm .1

Heat Shock Protein 70 (HSP_{70}), Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Fluid Balance

Inducing hypohydration prior to investigation in all conditions caused loss of body mass (HI 2.6 \pm 0.3kg; HW 2.5 \pm 0.3kg; HH 2.5 \pm 0.3kg; NH 2.5 \pm 0.4kg) and change in markers of hydration state [Urine osmolality (HI 1039 \pm 92mosmo \cdot kg⁻¹; HW 1024 \pm 51mosmo \cdot kg⁻¹; HH 1020 \pm 80mosmo \cdot kg⁻¹; NH 1015 \pm 100mosmo \cdot kg⁻¹); Urine colour (HI 4.75 \pm 0.7; HW 4.25 \pm 0.7; HH 4.25 \pm 0.7; NH 4.5 \pm 0.5); Urine specific gravity (HI 1.024 \pm 0.002; HW 1.024 \pm 0.002; HH 1.024 \pm 0.001; NH 1.024 \pm 0.001)] to that of ‘baseline’ values. No difference was observed between conditions for these variables.

As expected, changes in body mass were significantly different over time and between conditions, representative of the fluid intake and glomerular filtration rate (Figure 7.4). In contrast, TBW using MFBIA was not different between conditions or over time,

although there were increases from pre to 120 min in HI and HW, in comparison to hypohydration trials ($p < 0.05$). HI was also greater than all other trials post test ($p < 0.05$). Pre dosage deuterium samples were found to be contaminated when analysed and consequently were not used for determination of TBW. Analysis of Δ SMOW alone (HH = 287.949; HI = 93.4093; HW = 140.905; NH = 128.43) demonstrated no significant differences between conditions due to large variation between participants.

Extracellular fluid volume using MFBIA showed no difference between all conditions as a whole ($f = 0.678$, $p = 0.597$) or over time ($f = 0.307$, $p = 0.820$). This was also found using the bromide technique. Yet, there were significant differences between individual conditions, as illustrated in Figure 7.4.

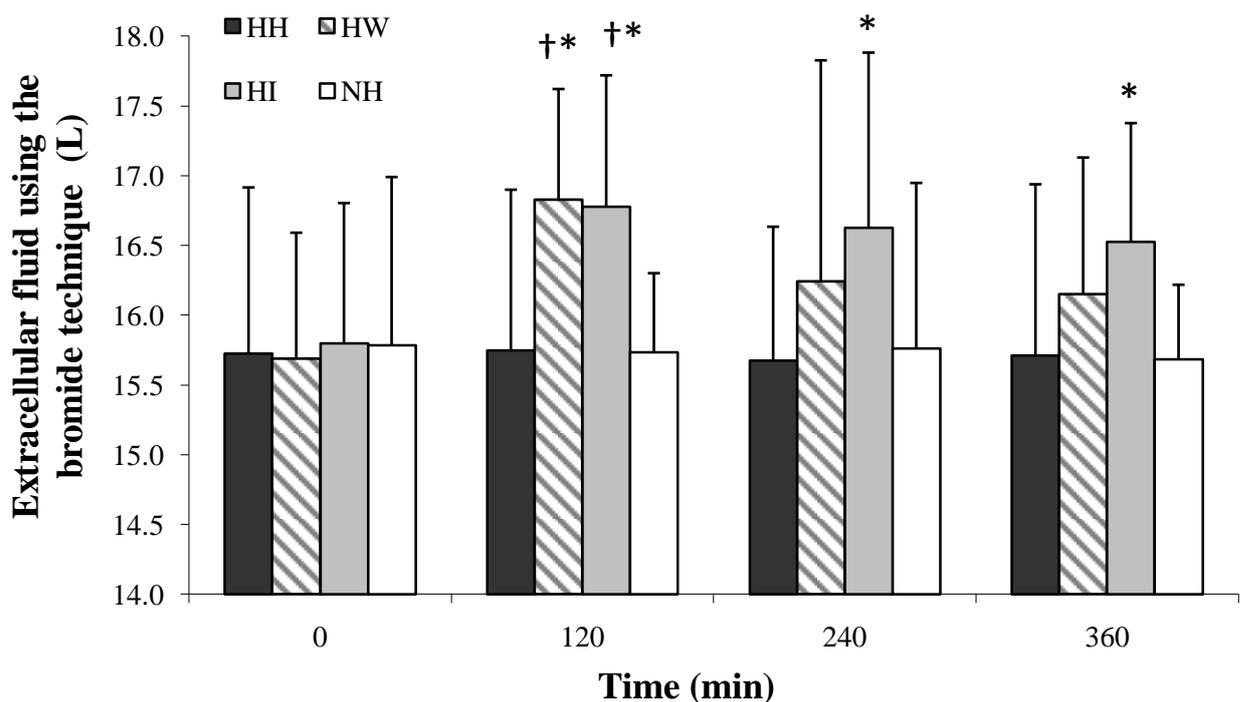


Figure 7.4: Extracellular fluid volume between conditions and over the duration of the test. Y error bars are shown. * denotes significant difference in comparison to HH and NH conditions. † denotes significant increase from previous time point. Hypoxia

Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

There was a positive correlation between extracellular fluid volumes measured using MFBIA and the bromide method ($r=0.96$, $p<0.001$). Although when compared using the Bland Altman plot (Figure 7.5), it is apparent that MFBIA offers values of ~3% body mass greater than the bromide method. As a product of TBW and ECW, ICF was also notably greater at 120 min in HI and HH compared to hypohydration trials, while HI was greater than all other trials post test, yet when statistically analysed over the whole data set and over time, there were no significant differences.

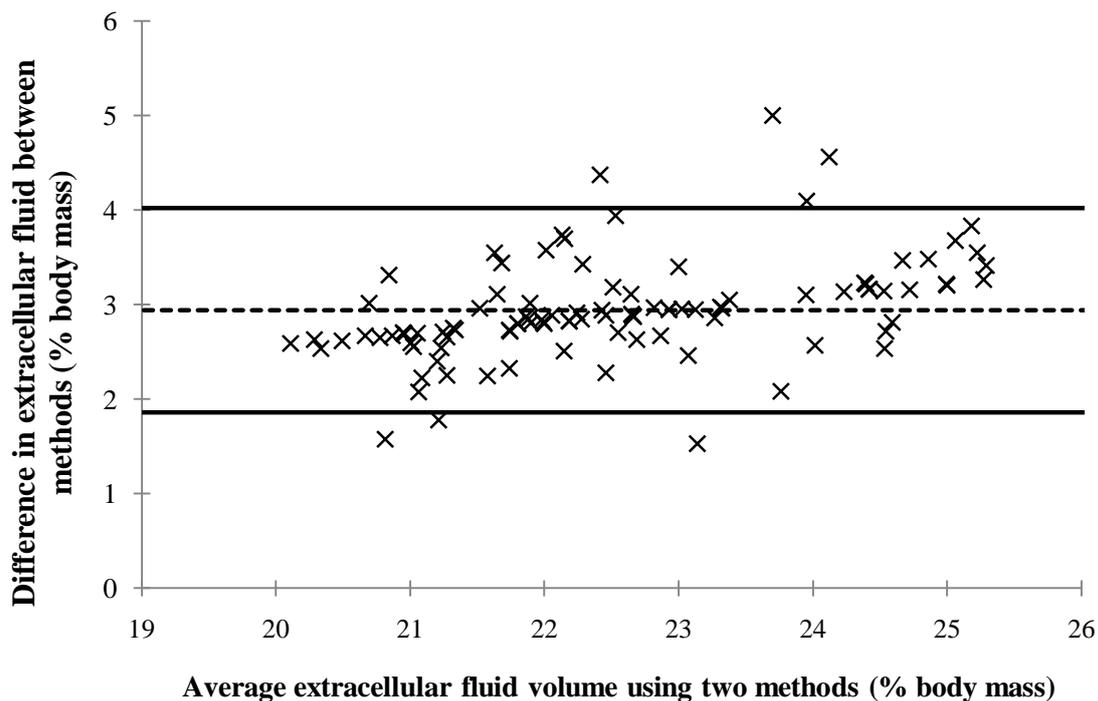


Figure 7.5: A Bland Altman plot comparing the use of multi-frequency bioelectrical impedance analysis against the standard bromide method, for determination of extracellular fluid volume expressed as a percentage of baseline body mass. Solid lines

indicate ± 2 standard deviations of the methods difference. Dotted line indicates the mean of the differences.

ADH declined with rehydration and increased with exposure to hypoxia when hypohydrated. HW and HI significantly decreased over time. HW was significantly less than HH and NH from 120 mins onwards. HI was less than both NH and HH from 240 min onwards (Figure 7.6).

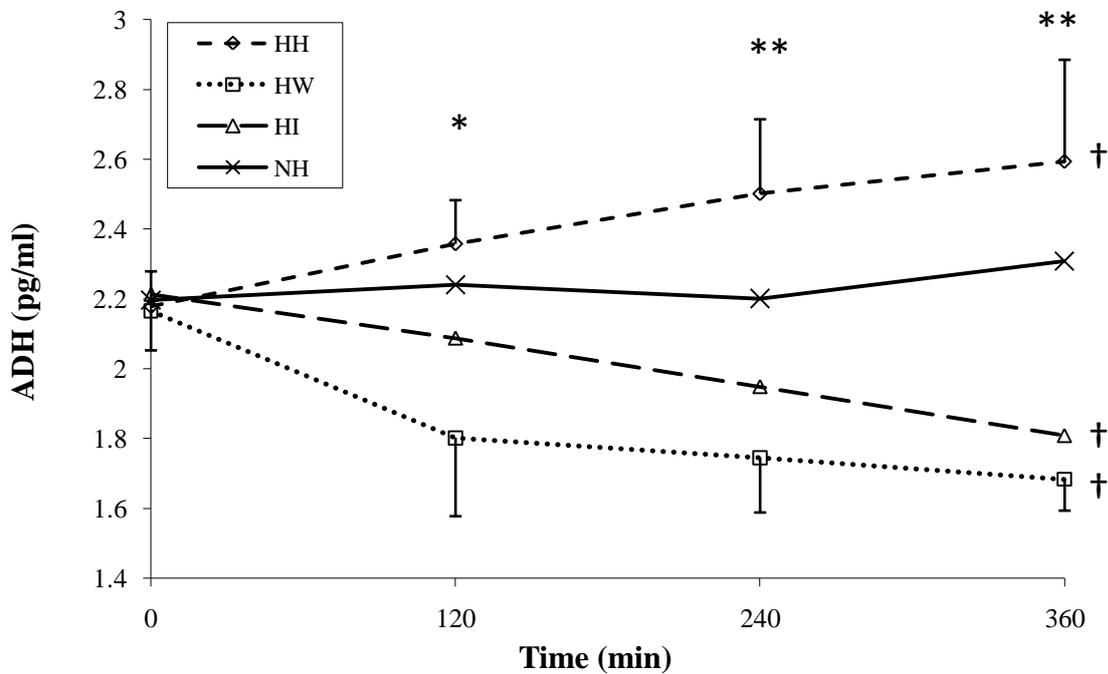


Figure 7.6: Change in antidiuretic hormone (ADH) over time and between conditions. Y error bars are shown. * denotes significant difference between HW and NH. ** denotes significant difference between NH and the HI and HW trials. † denotes significant difference over time. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Glomerular filtration rate was significantly different between conditions from 2hrs onwards and over time ($f=16.273$, $p<0.001$), while free water clearance was only significantly greater at the 2hr time point ($p<0.001$). Thus urine output data demonstrated significant difference between conditions from 2hrs onwards in all urine markers except urine volume at 6hrs. There was also significant difference between conditions when analysed separately, as explained in Figure 7.7.

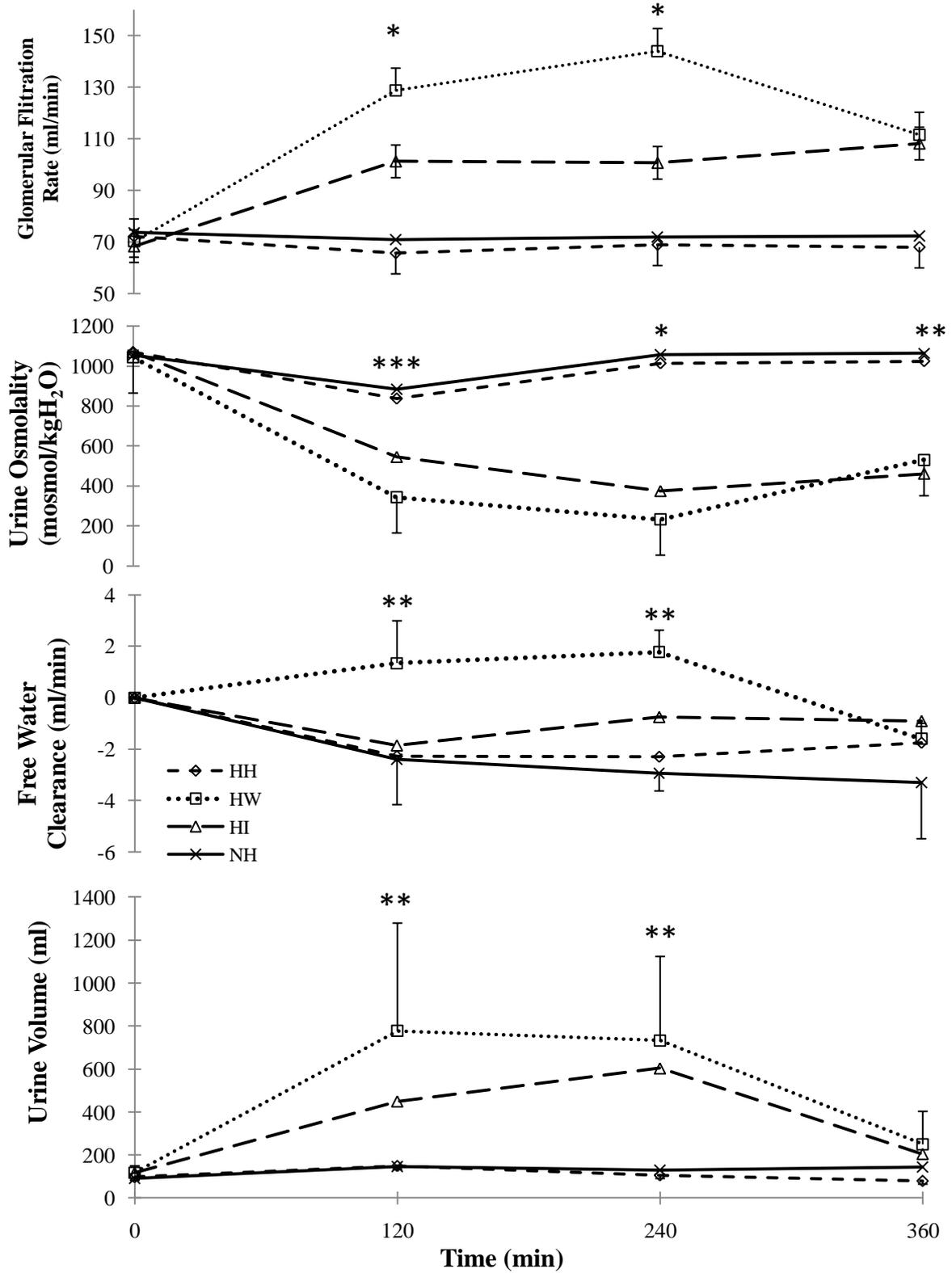


Figure 7.7: Urine volume, urine osmolality, glomerular filtration rate and free water clearance over the test duration between all four conditions. Y error bars are shown. * denotes significant difference for the HW condition in comparison to all other

conditions. ** denotes significant difference for the HW and HI conditions in comparison to HH and NH conditions. *** denotes significant difference for the HW condition in comparison to the HH and NH conditions ($p < 0.05$). Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Perceptual Responses

As all participants that dropped out reached LLQ scores of between 8 and 9 prior to dropping out, the last data point values were left in for statistical analysis, making the LLQ profile over time comparable (Figure 7.8). Including LLQ data from the last point prior to drop out generates significantly greater mean values than those of the remaining sample continuing to 6hrs (HI 2.3 ± 2.9 ; HW 2.6 ± 2.3 ; HH 2.7 ± 3). The HH condition generated significantly greater LLQ values than the other conditions from 135-150mins, while HH was significantly greater than the NH conditions from 2hrs. From 210mins onwards, LLQ scores were greater in all hypoxic than normoxic conditions ($p < 0.05$). In these hypoxic conditions LLQ significantly increased over time ($f = 12.242$, $p < 0.001$) (Figure 7.8). Figure 7.8 illustrates the linear increase in all hypoxic trials, with the linear rise in HW and HI starting approximately 2hrs after the HH trial. The plateauing of the LLQ values was a result of maximal LLQ scores being reached and participants dropping out, indicating a ceiling effect from the questionnaire measures. There was also a noticeably large variation in LLQ response between participants.

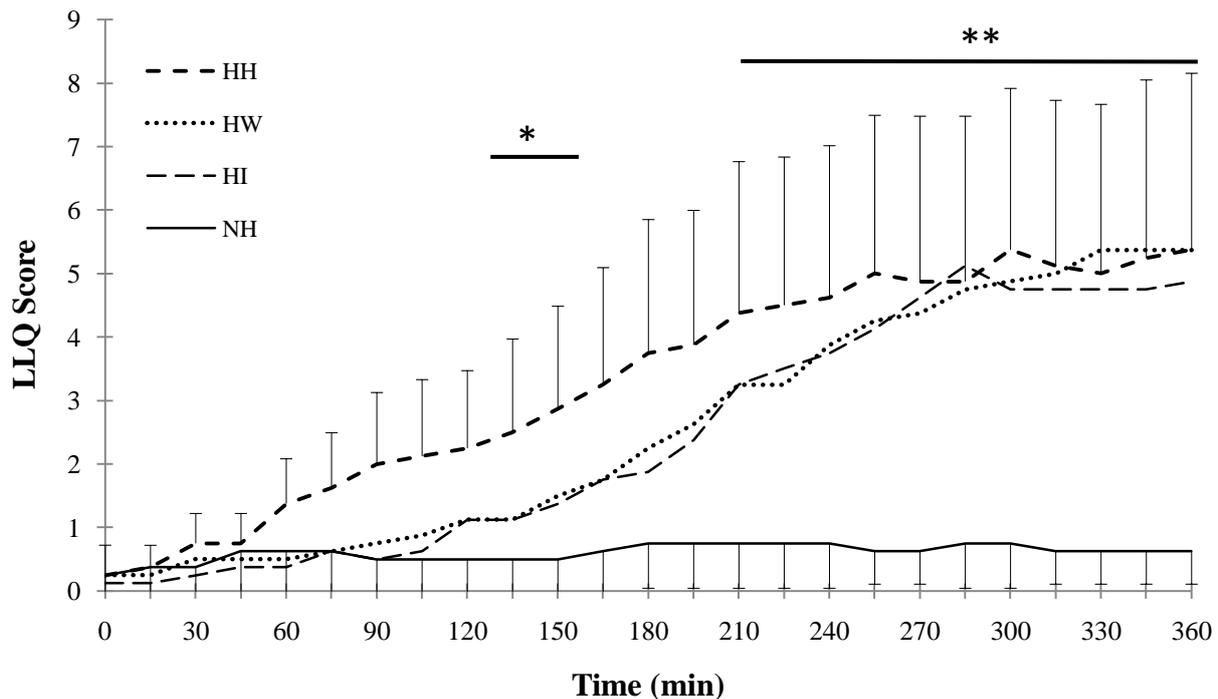
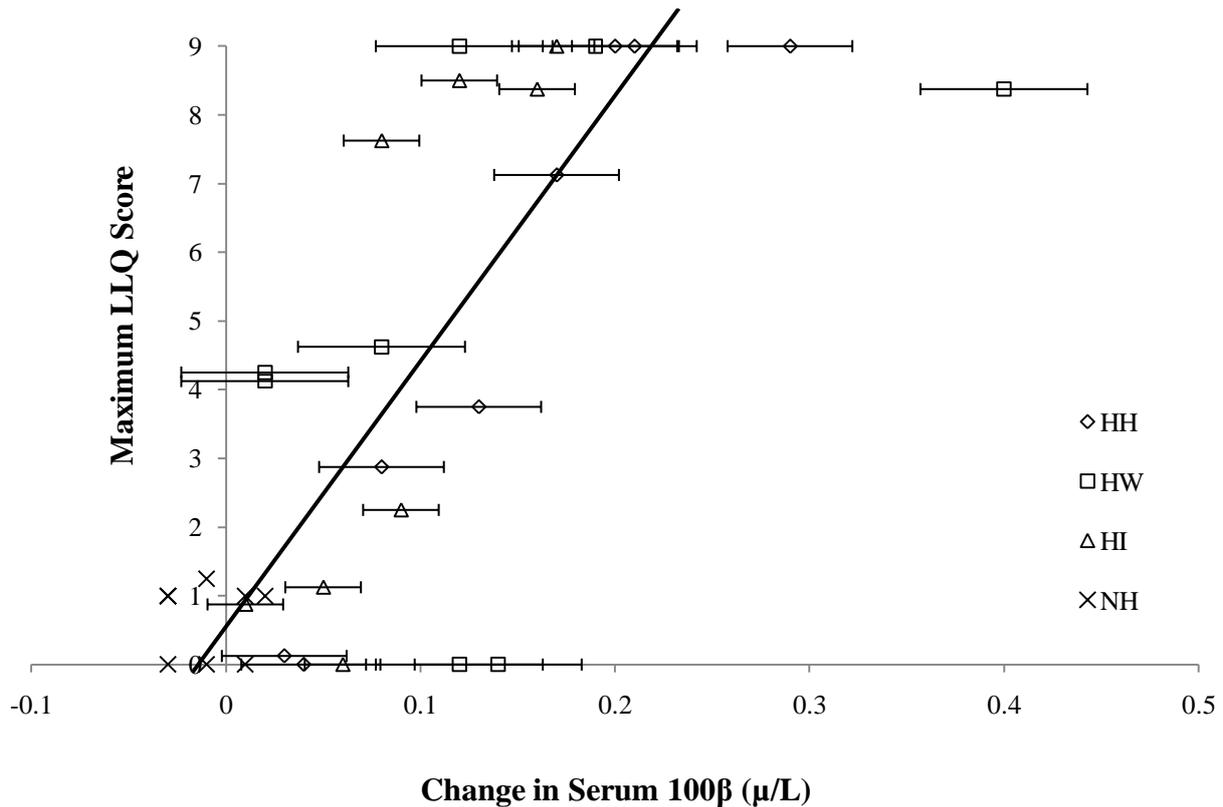


Figure 7.8: Change in Lake Louise Questionnaire Score (LLQ) over the test duration between all four conditions. Y error bars are shown. * denotes significant difference for the HH condition LLQ scores over the selected time period, in comparison to all other conditions. ** denotes significant difference for the NH condition LLQ scores over the selected time period, in comparison to all other conditions ($p < 0.05$). Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

LLQ score was found to be positively correlated to serum 100β values irrespective of conditions ($r^2 = 0.682$, $p < 0.05$). However, there was no significant correlation when conditions were analysed separately, suggesting an individualistic hypoxic tolerance has the greater influence on physiological alterations of the BBB and increases in LLQ scores (Figure 7.9).



7.5. Discussion

This experiment aimed to quantify the time course of fluid balance with rehydration in hypoxia while at rest and investigate the regulation of fluid balance and its mechanistic relationship to physiological strain and onset of AMS symptoms.

Due to variation in participant drop out times, data from these participants could not be statistically analysed and may have had a bearing on variables particularly at the final 6 hour time point, which only a few participants reached (Hypoxic Hypohydrated n=4; Hypoxic Isotonic n=5; Hypoxic Water n=5; Normoxic Hypohydrated n=8) (Table 7.4.1). LLQ scores would have been affected by these dropouts. Figure 7.9 demonstrates comparable linear slopes for all hypoxic trials, with HH increases starting at time 0, whereas HW and HI trials induced LLQ increases later, from around 2hrs. However, the noticeable plateau in values that starts around 300min indicates a ceiling effect whereby participants dropping out scored between 8 and 9 at their last time point and those continuing did not see any further change in severity of headache, nausea, lethargy or light-headedness. This suggests tolerance to hypoxia is actually quantifiable earlier than 6 hrs, which is the standard time stated for altitude illness onset (Basnyat & Murdoch 2003). While this supports Burtscher's (Burtscher 2008; Burtscher *et al.* 2004; Burtscher *et al.* 2008b) suggestions that a short term screening test could predict altitude tolerance, although as also explained by Burtscher (Burtscher *et al.* 2008b) 20-30 mins seems too short. Further, the participants dropping out tended to drop out in all hypoxic trials suggesting an almost dichotomous grouping of 'tolerants' and 'non tolerants'. However, this dichotomous simplification seems to be influenced by fluid balance as there were differences in LLQ and drop out times between rehydration conditions.

As expected, estimated glomerular filtration rate (eGFR) and free water clearance (FCH₂O) increased with the intake of water, while eGFR and FCH₂O values did not significantly increase above HH or NH trials at 2 and 4hrs in the HI condition (Figure 7.8). This slower rate of gastric emptying in the HI trial, maintained TBW and ECF volumes higher than pre test values. ECF declined from 4 to 6hrs in HW as a result of excessive low concentration urine outputs. Irrespective of rehydration condition, urinary measures were not different when exposed to hypoxia alone (Figure 7.4.6). Similarly, maintenance of ECF or reduction in TBW has little benefit on tolerance to resting hypoxic exposure, as previously suggested by others (Loeppky *et al.* 2005a; Loeppky *et al.* 2005b). While the study cannot support the work by Picher *et al.* (2008) who demonstrated eGFR to be greater in those suffering with AMS. Likewise, no urinary measures correlated with LLQ values. Significant difference in TBW and ECF maintenance may be of greater physiological and perceptual significance when exercising in hypoxia.

There were notably greater ADH values in the HH compared to the NH trials, as a result of a continuous linear increase in ADH over the 6 hr HH trial. Meanwhile, NH remained at a similar level throughout. HI and HW ADH values decreased linearly over time, but not to the extent previous normoxic studies have suggested (Kenefick *et al.* 2007), given the rehydration protocol. Differences in ADH response to hypoxia have been noted (Bartsch *et al.* 1988; Heyes *et al.* 1982; Loeppky *et al.* 2005a; Meehan 1986), while further studies have gone onto suggest that ADH may decrease in individuals tolerant to hypoxia (Loeppky *et al.* 2005b). Loeppky *et al.* (2005b) suggested this rise in ADH resulted from the onset of nausea. However, Loeppky *et al.* (2005b) did not measure perception of nausea, while the current study found no link to the rise in nausea and

changes in ADH values. Heyes *et al* (1982) recorded a 2700% increase in ADH values with a 1hr hypoxic (FIO₂:0.105) exposure. These values are far greater than any noted in this study (65% difference in NH vs HH at 6hrs), even with hypohydration and hypoxic exposure combined. This is possibly due to Heyes *et al* (1982) using well-hydrated participants starting with much lower resting ADH levels and a far more severe hypoxic exposure. Heyes *et al* (1982) explain this substantial increase is due to hypotension and nausea initiating the release of ACTH and subsequent release of ADH. Researchers have suggested that it is the early perceptions of the environment that may govern the ADH and, therefore, diuretic response to hypoxic exposure (Loeppky *et al.* 2005a; Loeppky *et al.* 2003a). They suggest that perceptions of apprehension, discomfort or stress can initiate ADH release and ultimately induce a fluid retention response, causing further feelings of discomfort and nausea.

Heart rate was raised with all hypoxic exposures and was greatest in the HI trial. Core temperature and thermal sensation were no different throughout, indicating that the resting hypoxic exposure was of insufficient strain to induce heat storage, when compared to previous exercising studies (Chapter V; Chapter VI). This also suggests that there was no reduction in physiological strain with rehydration of any sort, although perceptually there appears to be a benefit with thirst and subjective LLQ assessment. This may be due to the low sensitivity of the physiological strain index at rest in a normothermic environment. Heart rate and core temperature as single entities have low CV (<1%), yet the use of physiological strain as a calculated variable considering alterations from pre test values, designed for quantification of physiological strain in hot environments with continuous or intermittent exercise (Gotshall *et al.* 2001; Moran *et al.*

1998b), lacks sensitivity when core temperature changes are small and possibly negative (Gotshall *et al.* 2001).

Plasma HSP₇₀ was measured to demonstrate that the hypoxic insult induced a physiological strain at a cellular level. Hypohydration alone caused no significant increase in HSP₇₀, yet when hypohydrated during hypoxia, HSP₇₀ release was significantly increased above the values of the rehydration conditions. HSP₇₀ expression has been widely demonstrated to increase with exercise (Noble *et al.* 2008; Salinthonne *et al.* 2008) or with exposure to heat (Kim *et al.* 2004). Yet this study is the first to demonstrate the change in HSP₇₀ release at rest due to hypoxia and hydration as single and combined entities. This HSP over-expression has been suggested to be a protective mechanism against the cellular hypoxia. Marber *et al.* (1995) found that HSP₇₀ over-expression increased the resistance to myocardial ischemia in transgenic mice. The combined effects of hypohydration and hypoxia, even when resting, may have induced greater sympathetic activity to reduce hypoxemia, although SaO₂ values were not significantly different between hydration conditions. Likewise, there was no significant correlation between HSP₇₀ release and physiological strain or heart rate, which may have been due to the minimal variations in physiological indices at rest and large range in HSP₇₀ release.

Serum 100β demonstrated significant increases with exposure to hypoxia, yet lack of precision in the measurement technique (CV = 7.14%) and such small changes in serum 100β values prevented any noticeable trends occurring with regard to rehydration condition. This is in contrast to the only other study measuring serum 100β and rehydration (Watson *et al.* 2006), which noted ~0.07μgL⁻¹ lower serum 100β values in

participants maintaining a euhydrated state, during long duration endurance activity, while their study had similar CV (7.2%). However, hypoxia shows some effect on BBB integrity. Figure 7.9 tentatively demonstrates a relationship between change in serum 100β and peak LLQ score (limited to 9). All pre exposure values fell below the measurement range ($98\text{pg}\cdot\text{ml}^{-1}$), so accurate calculation of change is problematic. Yet these resting values in healthy participants would be expected (Kapural *et al.* 2002). Bjursten *et al.* (2010) recently demonstrated an increase in serum 100β of up to 122% over a 7 day ascent to 4559m. Similar to the current study Bjursten *et al.* (2010) also showed serum 100β values to correlate positively ($r = 0.45$, $p < 0.05$) with LLQ scores suggesting that BBB permeability has a role within the pathophysiology of AMS. All hypoxic exposure tests induced similar serum 100β values to those caused by boxing (Otto *et al.* 2000), football (Stalnacke *et al.* 2006), ice hockey (Stalnacke *et al.* 2003) and endurance activity in a warm environment (Watson *et al.* 2005). However, these increases are in contrast to the study by Bailey *et al.* (2009), which observed no changes in serum 100β after 6hrs of resting hypoxic exposure. While MRI (Kallenberg *et al.* 2007; Kallenberg *et al.* 2008; Schoonman *et al.* 2008) and doppler (Van Osta *et al.* 2005) studies have not evidenced disruption to the BBB with hypoxia.

The interpretation of the data from this study indicates that resting acute normobaric hypoxic exposure evoked a physiological stress response, although response characteristics and intensities were individualistic. Reasons for the individualistic response, irrespective of fluid balance alterations, are difficult to ascertain from the data. There is no clear physiological differentiation between those tolerating the full hypoxic exposure and those who could not. While in a resting state the sensitivity of the physiological and perceptual measures is of even greater importance, and may have also

contributed to the lack of significant findings. Clearly the rehydration protocols had some bearing upon tolerance time or physiological stress response. It is evident that the maintenance of a euhydrated state, which is sustained for longer with isotonic fluid ingestion, allows greater sweat loss, heat dissipation, reduced sympathetic response and ultimately may reduce physiological strain and the chance of suffering with symptoms of nausea, lightheadedness, fatigue, lethargy and headache. Yet, ultimately tolerance to a resting hypoxic insult is individualistic in nature and consequently deserves further investigation.

7.6. Conclusion

Rehydration using either water or isotonic fluid has some immediate benefit on physiological and perceptual strain when resting in hypoxia. However, the individualistic response to hypoxia makes differentiation between rehydration effects on BBB changes, cellular physiological strain or fluid regulating hormones difficult. Rehydration may show greater influence when humans undergo sufficient physiological strain through hypoxic exercise, reducing the almost dichotomous groupings noted within this study.

CHAPTER VIII.

INFLUENCE OF REHYDRATION ON PHYSIOLOGICAL AND RENAL RESPONSES TO AN INTERMITTENT WALKING TEST IN HYPOXIA

8.1. Abstract

It is thought that fluid balance has a role in the pathophysiology of altitude illness. While fluid balance is known to alter with hypoxia and exercise, their combined effects have not been investigated using a controlled acute exposure. Seven healthy participants were hypohydrated 24hrs prior to testing, using exercise and fluid restriction, causing a ~2.5% loss in body mass. Participants then rested in either normoxia with no fluid replacement (NH) or hypoxia (FIO₂: 0.12) with no fluid (HH), water (HW) or isotonic fluid (HI) replacement over the first 2 hrs of exposure. After 200 mins of rest, participants completed an intermittent walking protocol at 50% VO₂max. Each trial was separated by seven days. Measures of body water, fluid compartments, serum 100β, Heat Shock Protein (HSP₇₀), Anti diuretic hormone (ADH) and urine hydration markers were taken pre and post exposure. Heart rate, peripheral arterial oxygen saturation (SaO₂), core temperature and perceptual scales were monitored throughout the trials. Fluid compartment volumes increased with rehydration and remained at similar values in the isotonic rehydration trial. HSP₇₀ values increased in all trials (HH=1.48, HW=1.00, HI=0.95, NH=0.46 pg/L), though increases were significantly greater in hypoxic trials. Serum 100β also increased in all hypoxic trials (HH=0.28, HW=0.25, HI=0.09, NH=-0.005 μg/L). Physiological strain was significantly greater in the last 20min walking phase of the HH condition. Core temperature remained ~0.2 - 0.4°C higher in the hypohydrated conditions. Hypoxia and hypohydration induced a similar

physiological strain, which was exacerbated when the two were combined. Rehydration reduced physiological strain. The hypoxic disruption of the blood brain barrier increased with excessive water intake, suggesting isotonic rehydration is necessary to reduce physiological strain and prevent symptoms of headache and nausea.

Key Words: Altitude, walking, serum 100b, HSP₇₀, physiological strain

8.2. Introduction

Chapters V and VI demonstrated that rehydration to a euhydrated state is essential in order to prevent the exacerbation of physiological strain or perceptions of AMS symptoms. Chapter VII evidenced the immediate benefit of rehydration, irrespective of tonicity. However, the resting exposure may have been of too little physiological and perceptual consequence for some participants, while it was clearly too much for others.

Many studies have found solutions of greater tonicity to improve rehydration (Gonzalez-Alonso *et al.* 1992; Merson *et al.* 2008; Nose *et al.* 1988b; Sims *et al.* 2007a), while others dispute the physiological improvement with ingested solution tonicity (Kenefick *et al.* 2007; Mitchell *et al.* 2000). Chapter VII demonstrated that rehydration solutions of greater tonicity allow greater fluid retention and thus a more sustained maintenance of hydration state, which would be of greater importance with exercise-induced increases in sweat response, sympathetic activity, peripheral vasoconstriction and hormonal alterations, as described in chapters IV to VI. Performance improvements due to rehydration solutions have been noted by some (Sims *et al.*, 2007), but not others (Merson *et al.* 2008). Sims *et al.* (2007) found isotonic sodium loading of 164mmol/L prior to a run to exhaustion at 70%VO₂max, to reduce heart rate, core temperature and perceived strain during exercise through maintaining elevated plasma volume,

consequently improving time to exhaustion in warm conditions. However, there is no known research detailing the benefit of rehydration on hypoxic exercise, although there is clearly evidence to suggest its importance (Basnyat *et al.* 1999; Basnyat *et al.* 2001; Nerín *et al.* 2006; Richardson *et al.* 2009a; Richardson *et al.* 2009b).

Chapter VII illustrated the change in BBB permeability in some individuals unable to tolerate hypoxia, which could be related to physiological strain at a cellular level. For others, hypoxia had little physiological consequence, therefore the addition of exercise may induce sufficient stimulus to increase physiological strain causing greater alterations in BBB permeability. Such strain may also demonstrate more notable differences in rehydration strategies.

This study aimed to assess the effect of isotonic versus water rehydration strategies on fluid compartment alterations, physiological responses and onset of AMS symptoms during an intermittent walking test in acute normobaric hypoxia.

8.3. Methods

Seven physically active participants of 21 ± 3 yr, 173 ± 6 cm, 69.9 ± 9 kg and $VO_{2\max}$ of 45.4 ± 6 ml·kg⁻¹·min⁻¹ attended the laboratory on five separate occasions. The first visit was a familiarisation session involving anthropometric measures, cardiovascular fitness assessment and assessment of walking speed at 50% $VO_{2\max}$ (Section 3.3.1 & 3.3.2). In the next four visits participants completed an extended intermittent walking protocol using different rehydration types under hypoxic [Hypoxic Hypohydration (HH), Hypoxic Water Rehydration (HW), Hypoxic Isotonic Fluid Rehydration (HI)] and normoxic [Normoxic Hypohydration (NH)] environmental conditions. Prior to these

trials participants were dehydrated and fluid restricted, as detailed in Section 3.6 as Hypohydration³, although the current study used only 1hr of dehydration exercise.

The exposure protocol involved ingestion of a deuterium oxide and sodium bromide bolus (section 3.10.2) 2hrs prior to exposure. The participants then rested in a half lying position for 2hrs40min and then completed three 20min exercise bouts of walking at 50%VO₂max on a 10% gradient. Over the first 2hrs of exposure, HW and HI trials required participants to consume 25% of body mass lost every 20 min of either pure water (HW) or a 77mmol/L sodium chloride (NaCl) and 150mmol/L glucose isotonic solution (HI), drinking a total of 150% of body mass loss. HH and NH trials required complete fluid restriction for the 24 hrs and throughout the 4hr 5min exposure. The hypoxic (FIO₂=0.12) and normoxic (FIO₂=0.2093) conditions were induced and controlled using a hypoxic chamber (The Altitude Centre, London, UK). Throughout the exposure physiological measures, perceptual scales and acute mountain sickness scores were taken (Figure 8.1). The order of the tests was randomised, determined by a Latin squares design. Each test was separated by a seven day 'wash out' period.

Blood Markers

Blood (20ml) was taken from the antecubital fossa using, pre deuterium and sodium bromide loading, pre exposure, 2hrs into exposure and post exposure (shown in Figure 8.1) and collected in EDTA tubes. Blood plasma was analysed for plasma osmolality, HSP₇₀, ADH, deuterium oxide, plasma osmolality. Blood was also collected and spun in clear tubes to provide serum for measurement of creatinine, sodium, serum 100β and bromide. Plasma and serum samples were stored in microtubes at -86°C until analysis. Details of the blood analysis are given in Section 3.8.

Fluid Compartment Measures

Fluid compartments were measured as detailed in section 3.10, using multi-frequency bioelectrical impedance analysis, deuterium oxide and sodium bromide methods. Time points for these measures are highlighted in the schematic (Figure 8.1).

Statistics

Data was checked for normality and sphericity and was adjusted using the Huynh-Feldt method. Two way ANOVA with repeated measures was used to identify significant effect of the rehydration condition and or time point. One way ANOVA with repeated measures was used to compare between rehydration conditions, bonferroni pairwise comparisons compared between separate rehydration conditions. Once the participants had or were withdrawn from the test, the data from that point onwards was not used in the analysis. Participant withdrawals for each condition (HH n=7; HI n=6; HW n=5; NH n=7) are shown in Table 8.1. After withdrawl the last recorded LLQ score (9) was used for the remainder of the test, so AMS symptom score could remain comparable be individuals of various tolerance. LLQ score of 9 was the highest value allowed, due to ethical limits set by the University of Brighton Research Ethics Committee. All data was analysed using a standard statistical package (SPSS version 14 for Windows, 2005). Data was reported as mean \pm SD, with the significance level set at $p < 0.05$.

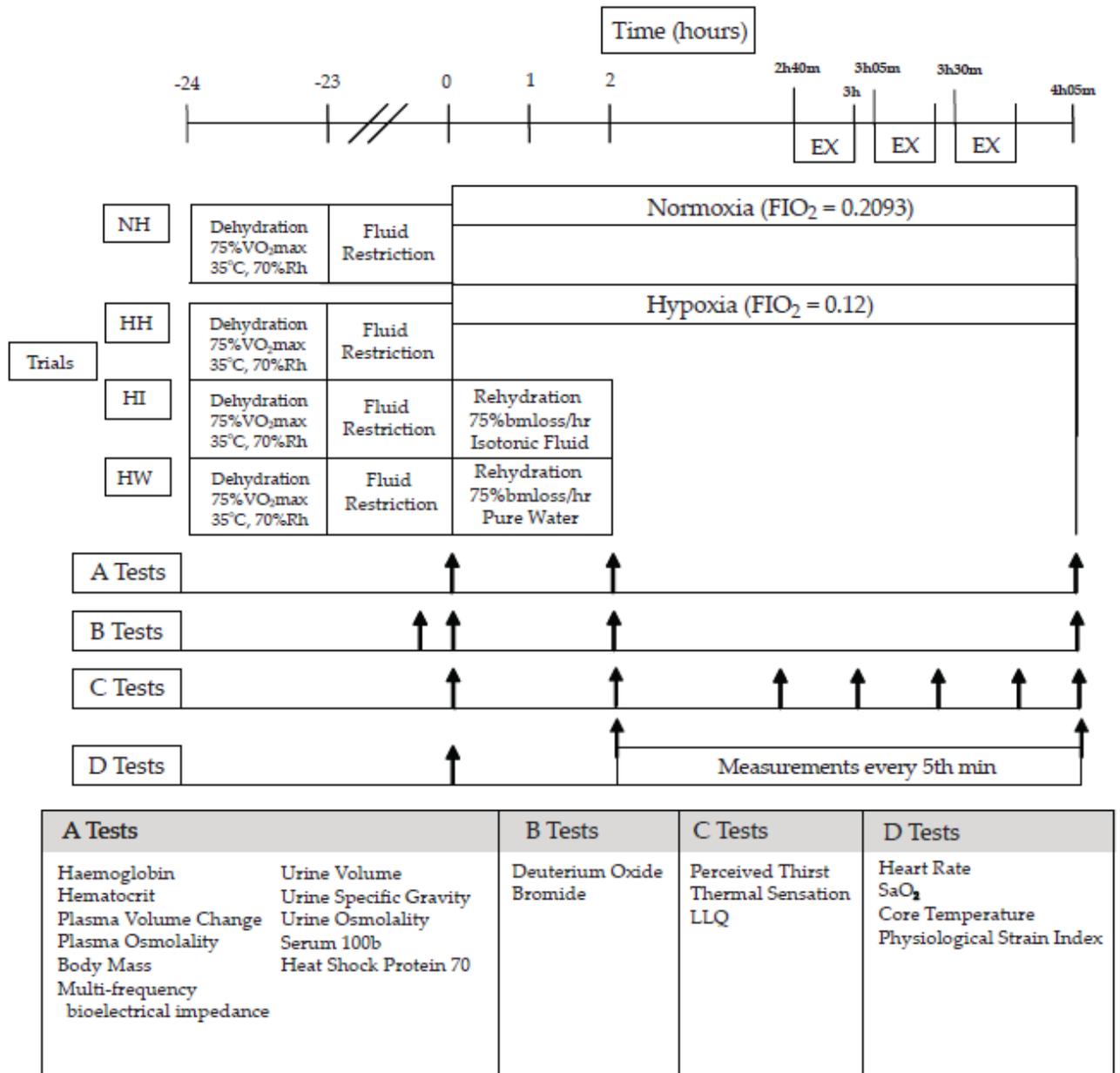


Figure 8.1: Schematic of the experimental design, showing trial protocols, measurements and timings. Arrows denote measurement points. The time axis highlights the 20 min bouts of exercise (EX) interspersed with 5 min rest. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

8.4. Results

All seven participants took part in the study, with an average walking speed at 50% VO_2max of $5 \pm 0.6\text{km/hr}$ (range 4.3–5.8km/hr) at 10% gradient. The exercise and fluid restriction trials 24 hours prior, induced body mass decline of $2.5 \pm 0.6\%$ other urine markers of hydration state are shown in Table 8.1. Five participants completed all trials, two participants dropped out of the HW at time points 120min and EX1. One of these participants also dropped out of the HI trial at the same point (EX1), displaying severe nausea and dizziness within 5 mins of starting exercise.

Table 8.1: Time to dropout (hours), for each participant, in each condition.

<i>Participant Number</i>	<i>HI</i>	<i>HW</i>	<i>HH</i>	<i>NH</i>
1	6	6	6	6
2	6	6	6	6
3	2.85	2.85	6	6
4	6	6	6	6
5	6	2	6	6
6	6	6	6	6
7	6	6	6	6

Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Severe headaches were reported during exercise in HW trials. Figure 8.4.1 demonstrates the onset of the severe LLQ symptoms within certain individuals when undertaking hypoxic exercise. All hypoxic conditions reported significantly greater LLQ scores than normoxic trials ($p < 0.05$), even with large variation between participants.

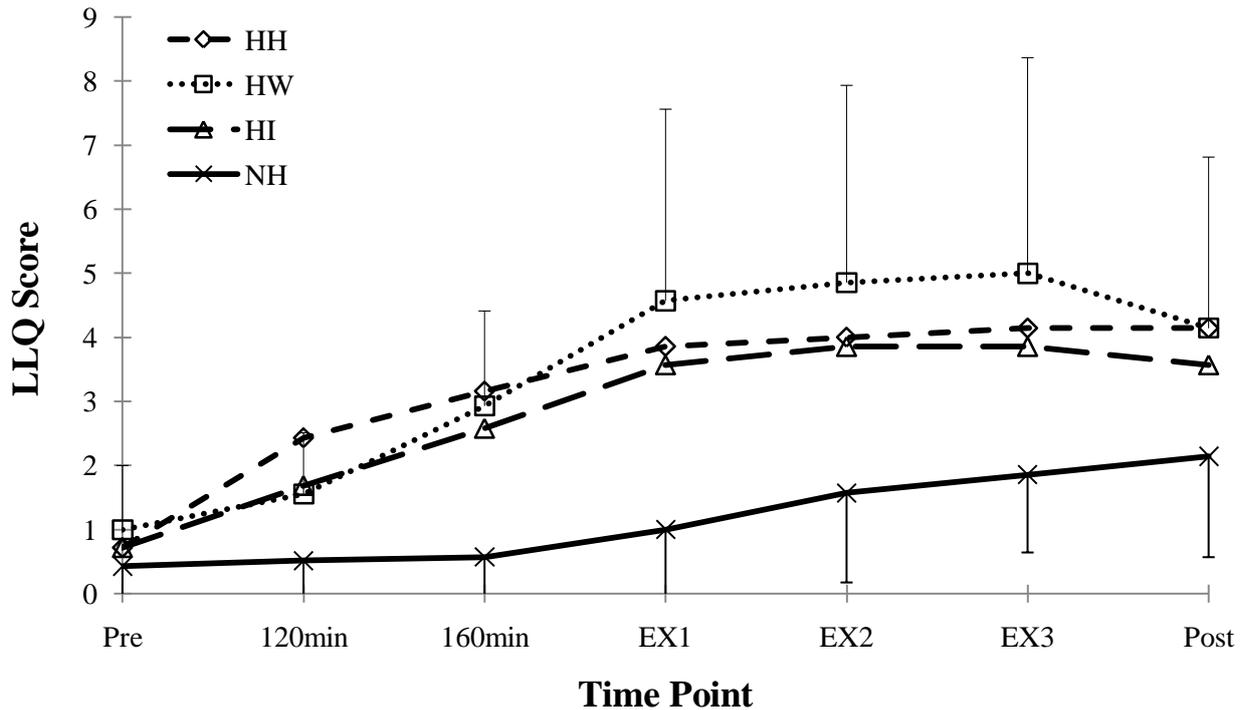


Figure 8.2: LLQ score over the set time points and between conditions. Y error bars are shown. HH = Hypoxia Hypohydrated, HI = Hypoxia Isotonic, HW = Hypoxia Water, NH = Normoxia Hypohydrated.

Fluid Balance

ADH did not significantly alter in any conditions by 120 min. Though significant ADH increases were noted in the HH and NH conditions post exercise ($p < 0.05$), while HW and HI remained similar to pre test values (Table 8.1). As a consequence, urine flow was significantly greater in the HW condition at 120 min ($f = 12.45$, $p < 0.05$). Post test there was no difference between HW and HI, though they both remained significantly greater than HH and NH. Similarly sweat rate was greater in HW and HI than the hypohydrated conditions (Table 8.2).

Table 8.2: Mean \pm SD pre, 120min and post hydration markers for NH, HH, HI and HW conditions. † denotes significant difference from pre test values values ($p < 0.05$).

	<i>HH</i>			<i>HW</i>			<i>HI</i>			<i>NH</i>		
	Pre	120 min	Post	Pre	120 min	Post	Pre	120 min	Post	Pre	120 min	Post
Body Mass (kg)	71.2 \pm 9.1	70.9 \pm 9.1†	70.1 \pm 9.1†	71.4 \pm 9.1	72.9 \pm 9.2†	72.0 \pm 8.6†	70.6 \pm 8.8	72.1 \pm 9.2†	71 \pm 8.8†	71.2 \pm 9.3	71 \pm 9.2†	69.9 \pm 9.3†
ADH (pg·ml ⁻¹)	2.22 \pm .2	2.49 \pm .21	5.64 \pm .33†	2.15 \pm .13	1.79 \pm .22	2.50 \pm .16	2.17 \pm .11	1.98 \pm .08	2.90 \pm .16	2.19 \pm .14	2.23 \pm .14	4.54 \pm .38†
Urine Osmolality (mosmo·kg ⁻¹)	1032 \pm 94	1054 \pm 59	1078 \pm 279	1004 \pm 135	270 \pm 290†	60 \pm 27†	941 \pm 65	290 \pm 259†	291 \pm 174†	921 \pm 118	931 \pm 115	1045 \pm 131
Plasma Osmolality (mosm·kg ⁻¹)	299 \pm 2	301 \pm 2	305 \pm 4	299 \pm 5	288 \pm 4	292 \pm 2	301 \pm 3	293 \pm 5	292 \pm 2	300 \pm 1	301 \pm 2	304 \pm 3
Urine Flow (ml·min ⁻¹)	-	1.8 \pm 1	1.5 \pm 1	-	9.1 \pm 3.8	5.4 \pm 5.8	-	6.9 \pm 3.2	7.6 \pm 6.3	-	1.4 \pm .4	1.8 \pm .8
Free Water Clearance (ml·hr ⁻¹)	-331 \pm 527	-527 \pm 343	-270 \pm 214	-319 \pm 658	319 \pm 658	357 \pm 375	-228 \pm 222	198 \pm 419	211 \pm 406	-244 \pm 136	-364 \pm 164	-377 \pm 167
Glomerular Filtration Rate (ml·min ⁻¹)	64 \pm 12	59 \pm 13	50 \pm 10	63 \pm 14	136 \pm 27	87 \pm 24	66 \pm 11	107 \pm 24	94 \pm 19	61 \pm 15	62 \pm 17	54 \pm 14
Sweat Rate (ml·min ⁻¹)	-	-	7.8 \pm 4.6	-	-	16.1 \pm 8.9	-	-	14.8 \pm 7.6	-	-	6.7 \pm 4.9
Plasma Volume (% Δ)	-	-0.9 \pm .8	-1.8 \pm 1.9	-	0.5 \pm 2	1.4 \pm 1	-	0.6 \pm 2	2.5 \pm 1	-	-0.2 \pm .7	-1.3 \pm .7

Antidiuretic hormone (ADH), Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Extracellular fluid volume was not different over time irrespective of measurement technique. ECF using MFBIA demonstrated no significant alterations over time, or between conditions. However, bromide technique values demonstrated significant change in HW trials by 120 min ($f=10.23$, $p < 0.05$), although only HI maintained the increase in ECF volume (Figure 8.3). Pre dosage deuterium analysis found the samples to be contaminated preventing determination of TBW. Analysis of Δ SMOW values (HH = -41.3; HI = 162.3; HW = 123.2; NH = -91.1) showed no significant difference between conditions, though data trends demonstrate fluid retention with the hypohydration trials and a large fluid turnover in the rehydration trials. However, MFBIA demonstrated changes in TBW pre and post testing (Figure 8.4), though these were not found to be significant. ECF volumes using MFBIA were only related to bromide analysis pre testing ($r=0.702$). Post test ECF ($r=0.183$) and TBW ($r=0.104$) had no correlation to MFBIA values, as a result of participant variation, fluid loss and movement.

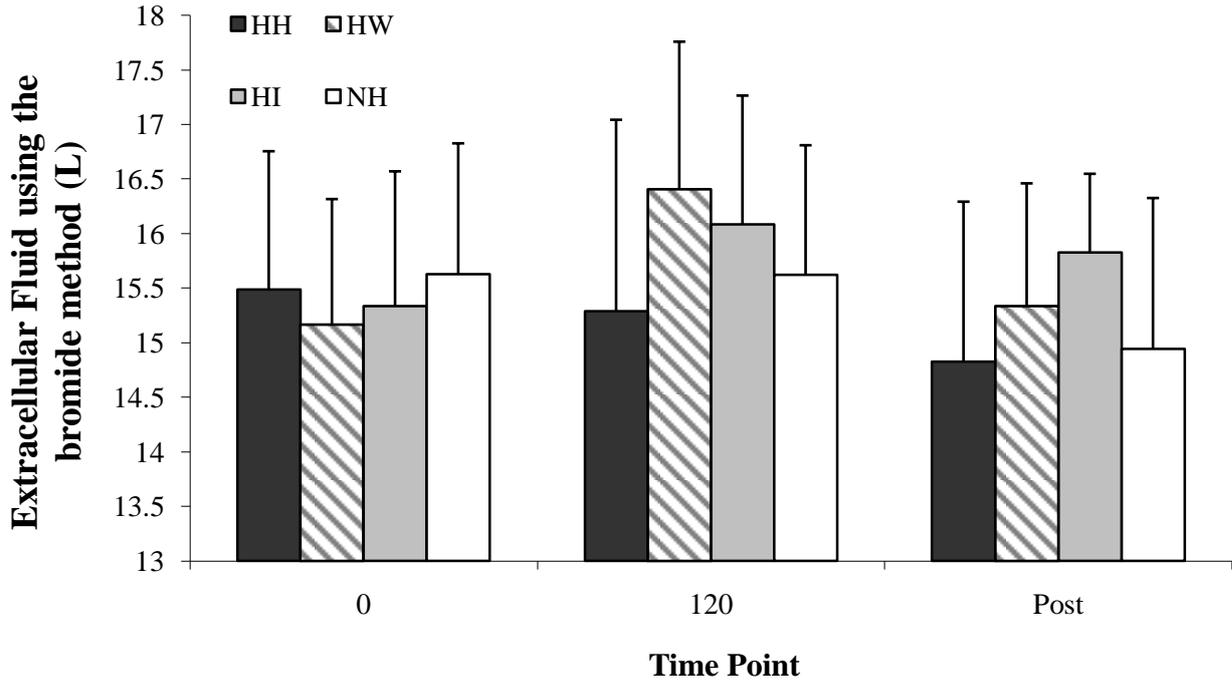


Figure 8.3: Extracellular fluid volume between conditions and over the duration of the test. Y error bars are shown. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

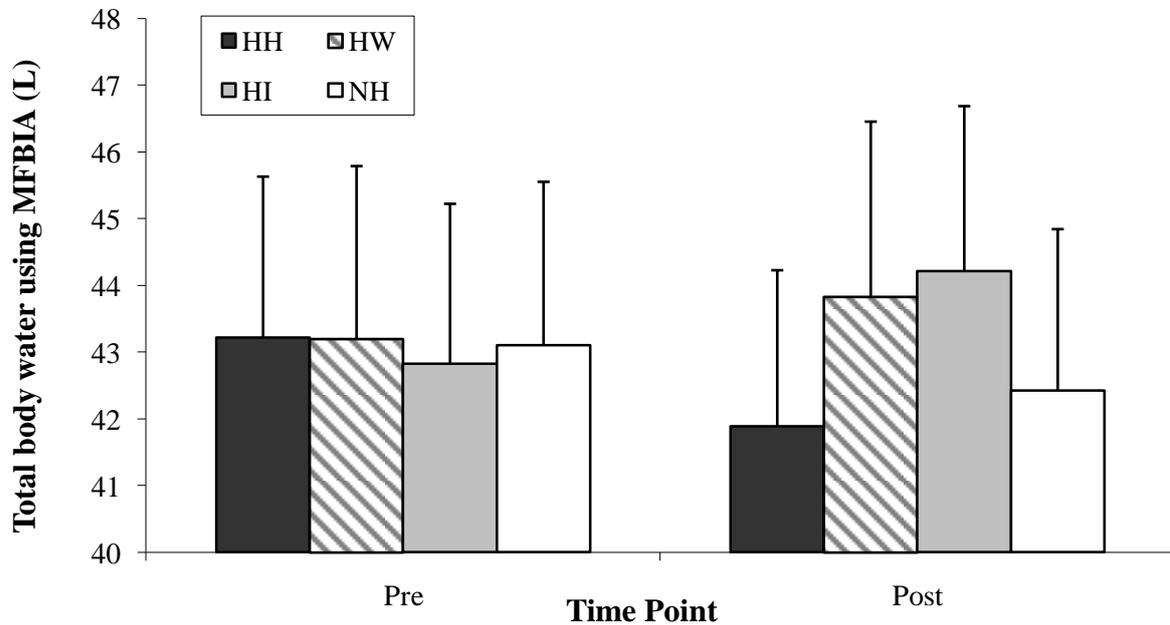


Figure 8.4: Total body water volume pre and post test and between conditions. Y error bars are shown. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Physiological Responses

SaO₂ was not significantly different between any of the hypoxic conditions, although HH values on entry to hypoxia and HW values at EX2 and EX3 were notably lower, shown in Figure 8.5.

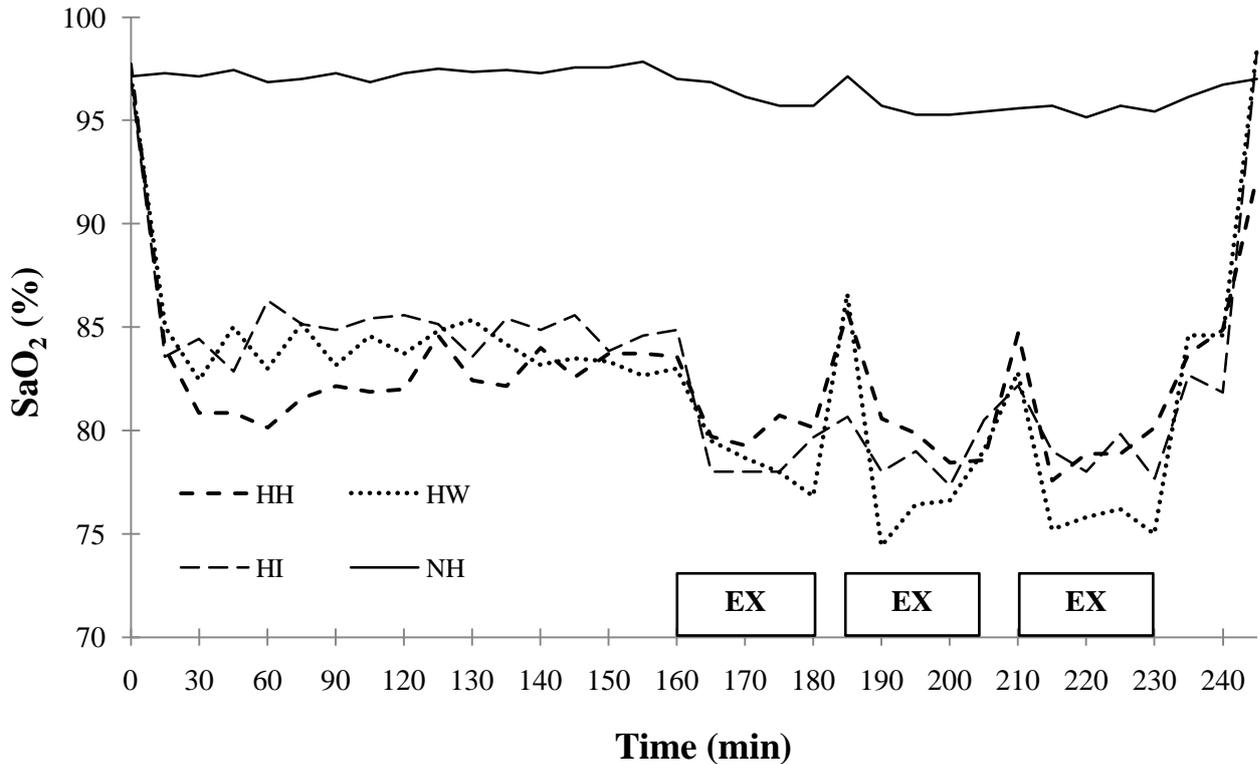


Figure 8.5: Peripheral arterial oxygen saturation (SaO₂) over time and between conditions. Exercise periods are shown (EX), Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Rectal core temperature was significantly greater at rest in the hypohydrated conditions by approximately 0.2°C, this difference rose to 0.4°C with the onset of exercise. Heart rate was significantly greater in hypoxic than normoxic conditions at rest by approximately 15bts·min⁻¹, yet differences were not evident during exercise. There was no difference in heart rate between hypoxic conditions. There was no significant

difference in PSI between any conditions until EX3 whereby HH was significantly greater than all other conditions. These changes are illustrated in Figure 8.6.

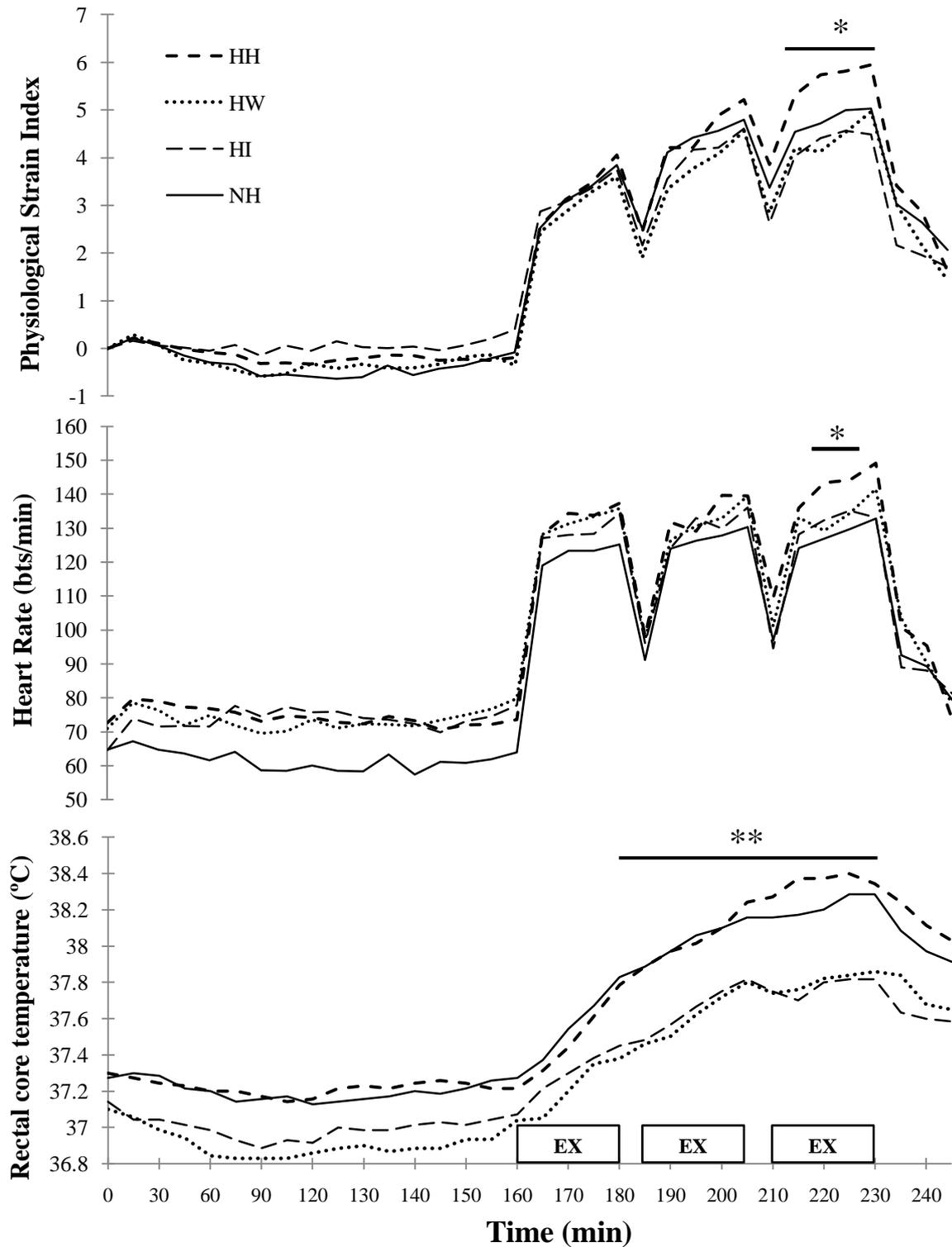


Figure 8.6: Indices of physiological strain over the test duration and between conditions. Exercise periods are shown (EX). * denotes significant difference over that

time period between HH and all other trials. ** denotes significant difference between NH and HH and the HI and HW conditions over that time period. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

HSP₇₀ increased significantly in all hypoxic trials, with HH demonstrating greatest increases, yet this was not significantly greater than other hypoxic trials. In HW and HH conditions, S100 β significantly increased above NH condition values (Table 8.3).

Table 8.3: Changes in serum 100 β (S100 β) and Heat Shock Protein 70 (HSP₇₀) between conditions. † denotes significant difference from pre test values values ($p<0.05$).

*Values below measurement technique range.

	<i>HH</i>		<i>HW</i>		<i>HI</i>		<i>NH</i>	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
HSP ₇₀ (pgL ⁻¹)	0.46±.11	1.94±.38†	0.45±.12	1.45±.13†	0.43±.14	1.38±.12†	0.41±.13	0.87±.13
S100 β (μ gL ⁻¹)	0.044±.03*	0.32±.08†	0.055±.02*	0.30±.09†	0.041±.02*	0.14±.09	0.057±.009*	0.052±.02*

Serum 100 β positively correlated with HSP₇₀ when analysed using all conditions together ($r=0.59$, $p<0.05$). When analysed as single conditions the HI condition reported positive correlation between HSP₇₀ and serum 100 β ($r=0.775$, $p<0.01$), yet no such correlation was found with HH ($r= 0.06$), HW ($r=0.119$) or NH ($r=0.149$) (Figure 8.7).

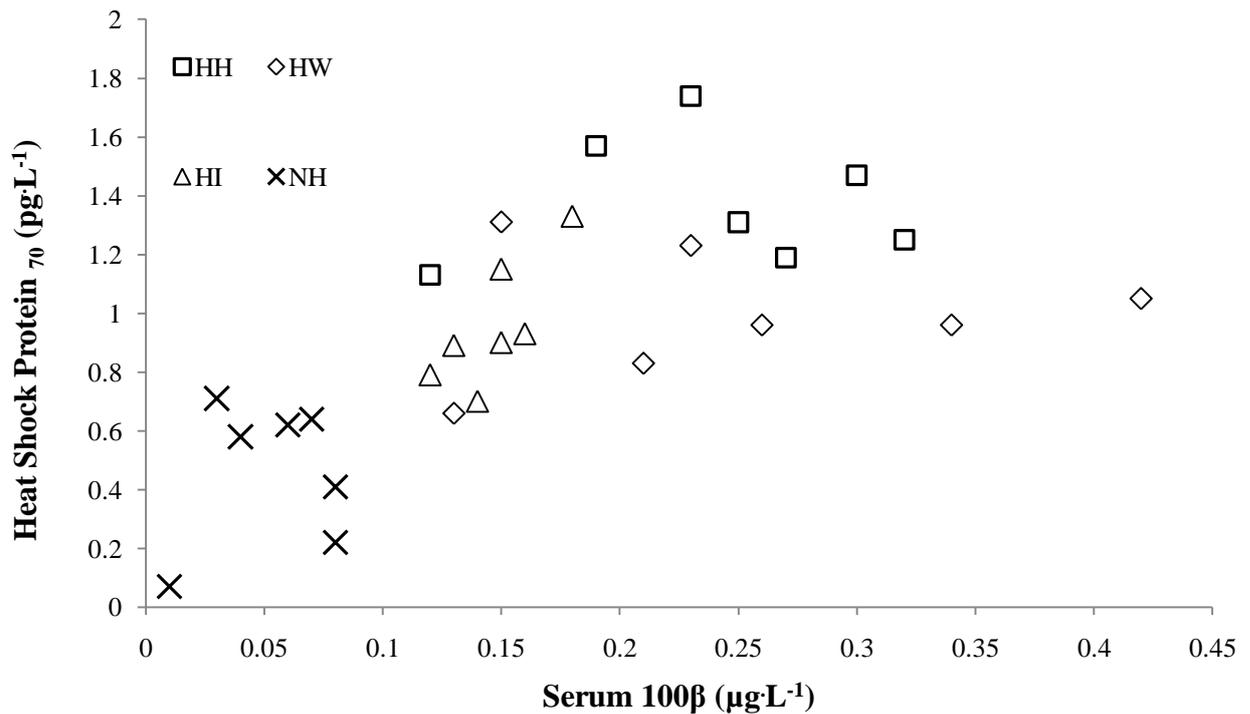


Figure 8.7: Change in plasma heat shock protein 70 against the change in plasma serum 100β over the test duration, between the four conditions. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

8.5. Discussion

This experiment aimed to investigate the effect of fluid balance on the physiological responses to hypoxic exercise and relate these findings to the development of AMS symptoms. Hypoxic tolerance of the participants within this study was notably greater than that in Chapter VII. Only two participants within this sample dropped out, both in the HW condition and one also within the HH condition. Mean LLQ values were also notably lower, though, as with all previous experimental chapters, there was clearly a wide variation in regard to hypoxia tolerance, irrespective of rehydration. The HW trial induced the greatest increase in LLQ scores, with many reporting severe headaches and nausea (Figure 8.2), which increased with the onset of exercise. This immediate nausea with exercise is possibly a result of excessive fluid within the gut, as a consequence of

slower gastric emptying rates in the two smallest participants (based on body mass, and height). This response has not been noted in previous experimental chapters, suggesting that the response is a result of excessive rehydration as opposed to hypoxic exercise. Though other studies (Noakes & Speedy 2006; Rosner & Kirven 2007) using excessive fluid intakes have documented such symptoms.

Core temperature was consistently 0.2°C greater in the hypohydration trials than the rehydration trials, throughout the rest phase. This difference increased to 0.4°C with exercise and the resulting relative increase in core temperature, similar to other published values (Greenleaf & Castle 1971; Sawka *et al.* 1985). The HH trial induced significantly greater values than NH only within the third stage of exercise. This is possibly as a result of greater fluid losses through ventilation, sweat rate and relative metabolic intensity due to hypoxia (Nybo *et al.* 2001). As a consequent, heart rate was also significantly greater for the third stage of exercise. At rest, hypoxia induced an increase in heart rate of approximately 18bts·min⁻¹, irrespective of hydration state. This demonstrates a hypoxically-induced sympathetic drive, as opposed to a thermoregulatory mechanism, in order to maintain sufficient O₂ delivery. This difference in normoxic and hypoxic heart rate response is reduced with exercise and in contrast the rehydration conditions initially induce the greatest sympathetic response. It is summarised that this is in response to hemodilution and the consequent need to increase cardiac output via heart rate, as a result of the blood's inefficient oxygen carrying capacity. By the third phase, greater sweat losses, fluid compartment equilibration and cardiovascular strain induced through heat storage accumulate to cause a large rise in heart rate for the hypohydrated conditions. This is evidenced by core temperature and

heart rate changes combining to significantly elevate physiological strain for the HH condition in the third exercise phase, above all other conditions (Figure 8.6).

Pulse oximetry data was similar to that presented by Imray *et al* (2005), for the given exercise intensity and hypoxic exposure. The greatest desaturation occurred in the HW trial. Differences between conditions were only noted in the second and third exercise phases, possibly due to the reduced efficiency in alveolar O₂ diffusion as a result of increased heart rate and hemodilution. Nevertheless, this reduced O₂ delivery was not significant enough to induce a sympathetic response to that of hypohydration alone. Further research into this response could compare exercising muscle and cerebral oxygenation, as performed by Nielsen *et al* (1999) in sea level studies.

Watson *et al* (2005) demonstrated increases in serum 100β of 0.20μg·L⁻¹ with endurance cycling in a warm environment. While a later study (Watson *et al.* 2006) demonstrated greater serum 100β rise with dehydration when performing similar activity. The results of these two studies support the findings of the current study in that hypohydration during exercise increases the release of serum 100β. Though from the values there is clearly a greater serum 100β release as a result of hypoxia, in comparison to hydration state or exercise performed, as supported by the previous resting exposure study (Chapter VII). While the majority of research monitoring serum 100β (Bailey *et al.* 2009), diffusion weighted MRI (Kallenberg *et al.* 2007; Kallenberg *et al.* 2008; Schoonman *et al.* 2008) or doppler (Van Osta *et al.* 2005) have observed no BBB disruption with hypoxia. The one study to support the increase in serum 100β with altitude exposure demonstrated an increase of 122%, less than that of the current study (~400% in HI condition), though this may be a result of the severe acute exposure

within the current study compared to the chronic slow ascent in Bjursten *et al's* (2010) study. While the precision and accuracy in measurement of normoxic serum 100β at rest, makes presentation of absolute concentrations ($\mu\text{g}\cdot\text{L}^{-1}$) more applicable. The results also demonstrate that participants undergoing hypoxic exercise when hypohydrated or rehydrated using water, had far greater circulating serum 100β levels than individuals suffering axial vibrations (Otto *et al.* 2000) or direct head trauma as a result of sporting activity (Stalnacke *et al.* 2006; Stalnacke *et al.* 2003), suggesting a severe disruption of the BBB with hypoxic exercise.

HSP_{70} and serum 100β correlated when conditions were not considered ($r = 0.63$, $p < 0.05$). Yet, when rehydration conditions were analysed separately only the hypoxia condition using isotonic rehydration found serum 100β and HSP_{70} to correlate. This may suggest that HSP_{70} and serum 100β are more closely related when in a euhydrated state and fluid balanced. Therefore, when individuals are euhydrated, physiological strain at a cellular level may influence the development of AMS. Hypoxic water replacement trials induced a greater rise in serum 100β but no significant increases in HSP_{70} , which may have resulted from a reduced cellular stress due to rehydration. While serum 100β increased as a result of more fluid available to exacerbate BBB disruption and allowing the lower concentration fluid to pass into the brain, increasing intracranial pressure and inducing the reported severe headaches. Conversely, HH noted higher serum 100β values through inducing greatest physiological strain at a cellular level as demonstrated by the greatest HSP_{70} expression. These relationships are illustrated within Figure 8.7, though each condition requires further research to support or explain this mechanistic response.

Plasma osmolality was maintained close to pre test values over the testing in all conditions, although rehydration conditions were lower post test than hypohydration conditions. These small alterations in plasma osmolality may be due to participant variations, while fluid reperfusion from the intracellular space also acts to limit alterations in plasma osmolality. Previous chapters have demonstrated similar findings (Chapter VI), while studies finding significant alterations in plasma osmolality have tended to use greater hypohydration severities (Nadel *et al.* 1980; Sawka *et al.* 1984). Meanwhile, normoxic rehydration studies have found significant declines in plasma osmolality (Kenefick *et al.* 2006; Nose *et al.* 1988b). Nose *et al.* (1988b) further demonstrated that FCH_2O followed plasma osmolality. Even though plasma osmolality changes were not deemed significant, free water clearance followed similar alterations to plasma osmolality, supporting Nose *et al.*'s (1988b) work. As expected, plasma volume increased with rehydration and decreased with hypohydration trials. Plasma volume declined most in HH, while HI increased and then maintained plasma volume for the test duration. The alterations in plasma volume closely follow the alterations in ADH and consequently urinary free water clearance. ADH was no different between HI and HW conditions. All conditions increased with exercise, which was exacerbated in the hypohydrated trials. The significantly greater ADH in HH compared to NH demonstrates that hypoxia alone induces an increase in ADH as found with other studies (Heyes *et al.* 1982; Loeppky *et al.* 2005a; Loeppky *et al.* 2005b; Meehan 1986).

TBW and ECF volumes were shown to decline in the HH condition, while NH remained the same demonstrating that hypoxia alone has an influence on fluid compartment volumes (Figure 8.3 & 8.4). Isotonic rehydration maintained TBW and ECF volumes from 120 mins onwards. While water increased the volumes in the short term, post

testing compartment volumes were not significantly greater than the pre test hypohydrated state. This illustrates the importance of fluid tonicity to maintain a state of euhydration and the insufficiency of water rehydration when consuming a large bolus prior to exercise, as demonstrated in previous research (Shirreffs *et al.* 2007; Shirreffs & Maughan 1997; Sims *et al.* 2007a; Sims *et al.* 2007b).

One limitation of the current study is the small sample size, especially with regard to the variations in participants' tolerance to altitude. However, the controlled nature of the study and invasive techniques make studying a greater sample difficult. Irrespectively, the data presented here demonstrates there is a mechanistic relationship between fluid balance and hypoxic tolerance. Future work, using greater sample sizes or MRI brain scanning to identify BBB functional alterations, may be able to relate these physiological responses to individual's hypoxic tolerance and demonstrate the mechanistic changes with alterations in fluid balance.

8.6. Conclusion

Hypoxia ($FIO_2:0.12$) and hypohydration (2.5% body mass loss) induce a similar physiological strain, which is exacerbated when the two are combined. Rehydration reduces this physiological strain at a cardiovascular and cellular level. Within hypoxia, it is important that isotonic fluid ($NaCl\ 77mmolL^{-1}$ and $glucose\ 150mmolL^{-1}$) is ingested in order to prevent excessive disruption to the BBB, which increases with physiological strain and/or excess fluid, causing symptoms associated with AMS, such as severe headache and nausea, to be exacerbated.

CHAPTER IX.

FLUID RESPONSES AND TOLERANCE TO ALTITUDE EXPOSURE

9.1. Abstract

Fluid control at altitude has been linked to altitude tolerance. This study aimed to quantify the time course of hydration indices, physiological markers and acute mountain sickness (AMS) development over the ascent to Everest Base Camp at 5300m from sea level. Twenty-three participants completed the ascent and all tests without medication, out of the 42 participants who started the ascent. Fluid output, intake, urine specific gravity, urine colour, body mass, oxygen saturation (SaO₂) and Lake Louise questionnaire (LLQ) score were measured daily over the 14 day ascent. Participant data was split into those who suffered (AMS; LLQ \geq 6, $n = 7$) or did not suffer with AMS (No AMS; LLQ \leq 3, $n = 11$) over the ascent. Body mass significantly declined in all participants (AMS = 4.75 ± 0.8 ; No AMS = $3.69 \pm 0.5\%$ Body mass loss). Individuals suffering with AMS had a notable increase in body mass with ascent to 3400m. Urine output averaged $1993 \pm 230\text{ml}\cdot\text{d}^{-1}$ over 6 urinations. Hydration indices were not different in AMS sufferers, though there was greater fluid turnover in non AMS sufferers. Fluid intake negatively correlated with LLQ score ($r=0.659$, $p<0.05$), which suggests drinking $\sim 45\text{ml}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ may prevent AMS onset. Data demonstrates a need for adequate drinking at altitude, which may induce diuresis and prevent fluid retention, which seems to be related to AMS development.

Key Words: Fluid intake, urine, body mass, trekking, drinking.

9.2. Introduction

Previous research has shown hydration state and fluid balance to influence physiological and perceptual responses to acute hypoxia (Chapters V & VI) and altitude tolerance (Basnyat *et al.* 1999; Basnyat & Murdoch 2003). Hydration requirements seem particularly important in AMS symptom prevention when exercise is performed (Chapters VII & VIII). Yet, the effect that longer duration altitude exposure has on hydration state, or fluid balance lacks empirical evidence due to poor control of studies, low sample size, variable ascent profiles, illness and homogenous groups (Bärtsch *et al.* 1998; Basnyat *et al.* 1999; Basnyat *et al.* 2001; Nerín *et al.* 2006). Previous work has attempted to quantify appropriate hydration rates at altitude to maintain fluid balance, however by their own admission the study offered only speculative conclusions (Basnyat *et al.* 1999). Nerin *et al.* (2006) demonstrated no difference between AMS sufferers and non-sufferers urine output, fluid input and fluid retention. Although the sample size was low, ascent rate was not controlled and hydration markers were not recorded. Basnyat *et al.* (1999), using a cross-sectional prospective study on 550 trekkers in the Khumbu valley, reported that raising fluid intake above 3L per day decreased the risk of AMS (odds ratio 1.57; 95% confidence interval, 1.02-2.40). While other publications have debated the role of hydration in AMS pathophysiology, suggesting further research is needed (Basnyat *et al.* 2001).

Body mass changes have been discussed in regard to field (Westerterp & Kayser 2006; Westerterp *et al.* 1994), or laboratory-based (Rose *et al.* 1988; Westendrop *et al.* 1993; Westerterp-Plantenga *et al.* 1999; Westerterp *et al.* 2000a) studies, suggesting that hypoxia induces a significant decline in body mass from all cellular components. However, the contradictory evidence (Macdonald *et al.* 2009; Tanner & Stager 1998) on

body composition at altitude is a result of small sample sizes, observational designs, pre and post testing methods and the variation in altitude tolerance.

The thesis so far has demonstrated the need for euhydration over an acute hypoxic exposure. However, the ecological validity in the previous chapters is low. If the findings of this work are to be related to altitude exposure, then field based evidence of the hydration requirements and the importance of fluid balance needs to be demonstrated at altitude over an appropriate sojourn. By monitoring urine output and hydration status in a larger heterogeneous sample using identical ascent profiles, it may be possible to establish the effect of altitude exposure on fluid balance, hydration status and body mass. This may then be related to individuals' tolerance to altitude, through comparison of physiological and perceptual variables.

9.3. Methods

Forty two (21 males, 21 females) participants attended a familiarisation day whereby they met other individuals on the Everest Base Camp treks, were explained the testing protocols and completed sea level testing. On the familiarisation day participants gave written informed consent prior to sea level testing. The study was accepted by both the University of Brighton and University College London Research Ethics Committees. All participants were examined by a physician prior to sea level testing, to ensure they were of satisfactory health. Participants were told not to take any medication throughout the trek, unless they indicated the medication within the diary. Any participants taking acetylzolamide, diamox, dexamethasone or any other known medication that may improve altitude tolerance were extracted from the data set. Ascent profile is illustrated in Figure 9.1, all participants completed the same ascent and descent profile. No

participants were allowed to venture more than 200m above or below the set ascent profile on rest days. Participants were encouraged to perform minimal physical activity on 'rest' days.

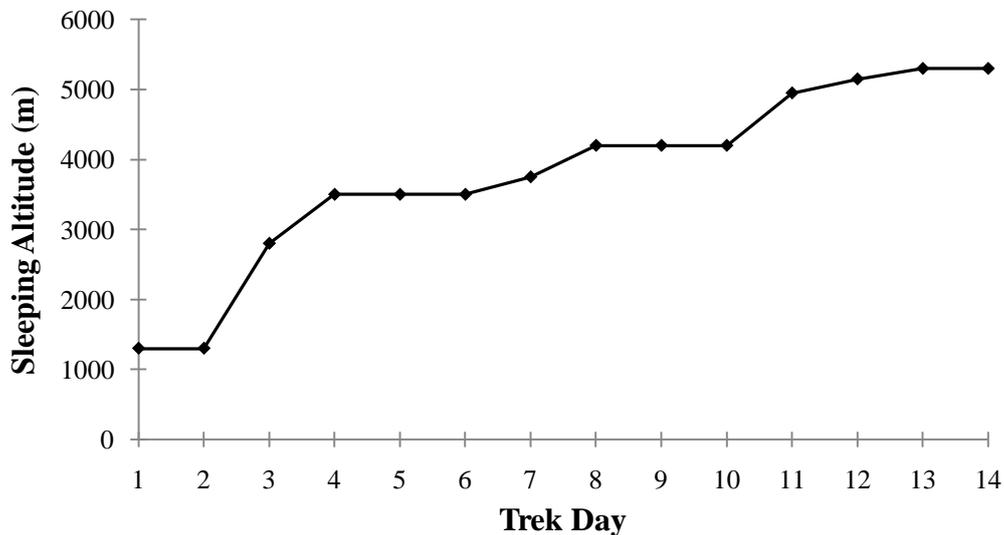


Figure 9.1: Ascent profile for all trekking and rest days.

Resting blood pressure (Omron 705IT, Omron Healthcare, UK), heart rate and SaO₂ (Nonin 9500 Onyx Pulse Oximeter, Minnesota, USA) was recorded every morning in their diaries. Participants recorded LLQ scores in their diaries, every morning (section 3.7.4.2.). All diary entries were completed prior to breakfast and any daily caffeine intake.

Urine volume was collected in a measuring jug and recorded, with urine colour, every urination throughout the trek. USG was measured every first daily void and every evening void prior to dinner (~5pm). USG and colour were measured as explained within section 3.7.2. Body mass was recorded every morning using mechanical scales

(Salter 145, Tonbridge, UK), participants wore similar clothing and no shoes for every body mass measurement.

Statistics

Data was checked for normality and sphericity and was adjusted using the Huynh-Feldt method. Participants were split into AMS sufferers (AMS; $LLQ \geq 6$, $n = 7$) or AMS non-sufferers (No AMS; $LLQ \leq 3$, $n = 11$) using the participants peak LLQ score reported over the ascent. The remaining five participants recording moderate peak LLQ scores ($LLQ = 4-5$) were extracted from the data set with regard to AMS group comparisons. Independent T-tests were used to compare between AMS groups. Paired T-tests were used to compare pre and post values and USG samples. Pearson's correlation was used to assess relationship between physiological measures and LLQ scores. All data was analysed using a standard statistical package (SPSS version 14 for Windows, 2005). Data was reported as mean \pm SD, with the significance level set at $p < 0.05$.

9.4. Results

Of the 42 participants on the Everest base camp treks, 23 (12 males) participants completed a full set of urine output, fluid input data and did not take any medication throughout the trek. Only these twenty three data sets were therefore analysed. Participants were aged 43 ± 15 y, of height 168 ± 8 cm and body mass 70.2 ± 13 kg. LLQ score increased with altitude in both groups, yet there was significant difference in peak (AMS= 6.7 ± 1 ; No AMS= 2.2 ± 1) and mean (AMS= 2.8 ± 0.7 ; No AMS= 1.1 ± 0.7) LLQ scores between AMS groups ($p < 0.05$) (Figure 9.2).

Body mass was not different between the groups (AMS = 72 ± 12 kg; No AMS = 66 ± 9 kg) and throughout the treks. Body mass decreased in all participants. Average body mass loss by the end of the trek, expressed as a percentage of sea level body mass was $4.28 \pm 0.7\%$. The AMS group recorded greater body mass loss (AMS = $4.75 \pm 0.8\%$; No AMS = $3.69 \pm 0.5\%$), though this was not significant (Figure 9.3).

Urine output was not different between groups, with a whole group average daily output of 1993 ± 230 ml over 6 ± 0.7 urinations per day. The number of urinations per day was not different between groups. Daily urinations significantly increased above baseline values on days 8, 9 and 10 for both groups ($p < 0.05$). This trend was also evident from the urine output data, increasing above baseline values on day 9 in both groups (Figure 9.3). Net fluid balance fluctuated daily due to the variations in exercise and, consequently, sweat rate. However, Figure 9.3 demonstrates that net fluid balance increased by day 7 in the AMS group.

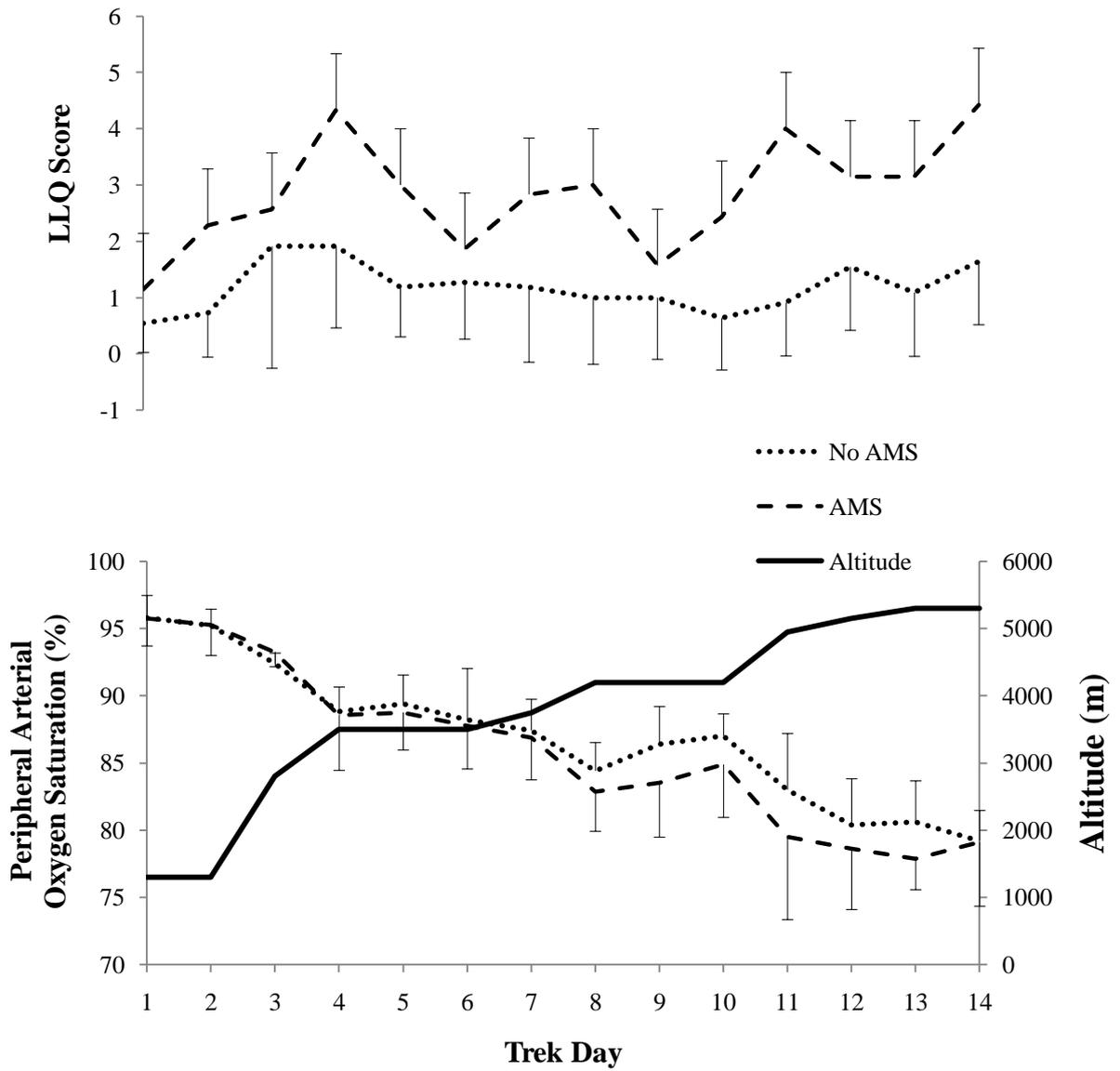


Figure 9.2: SaO₂ and LLQ scores between AMS groups over the test duration. Ascent profile is shown on the secondary y axis. Y error bars are shown.

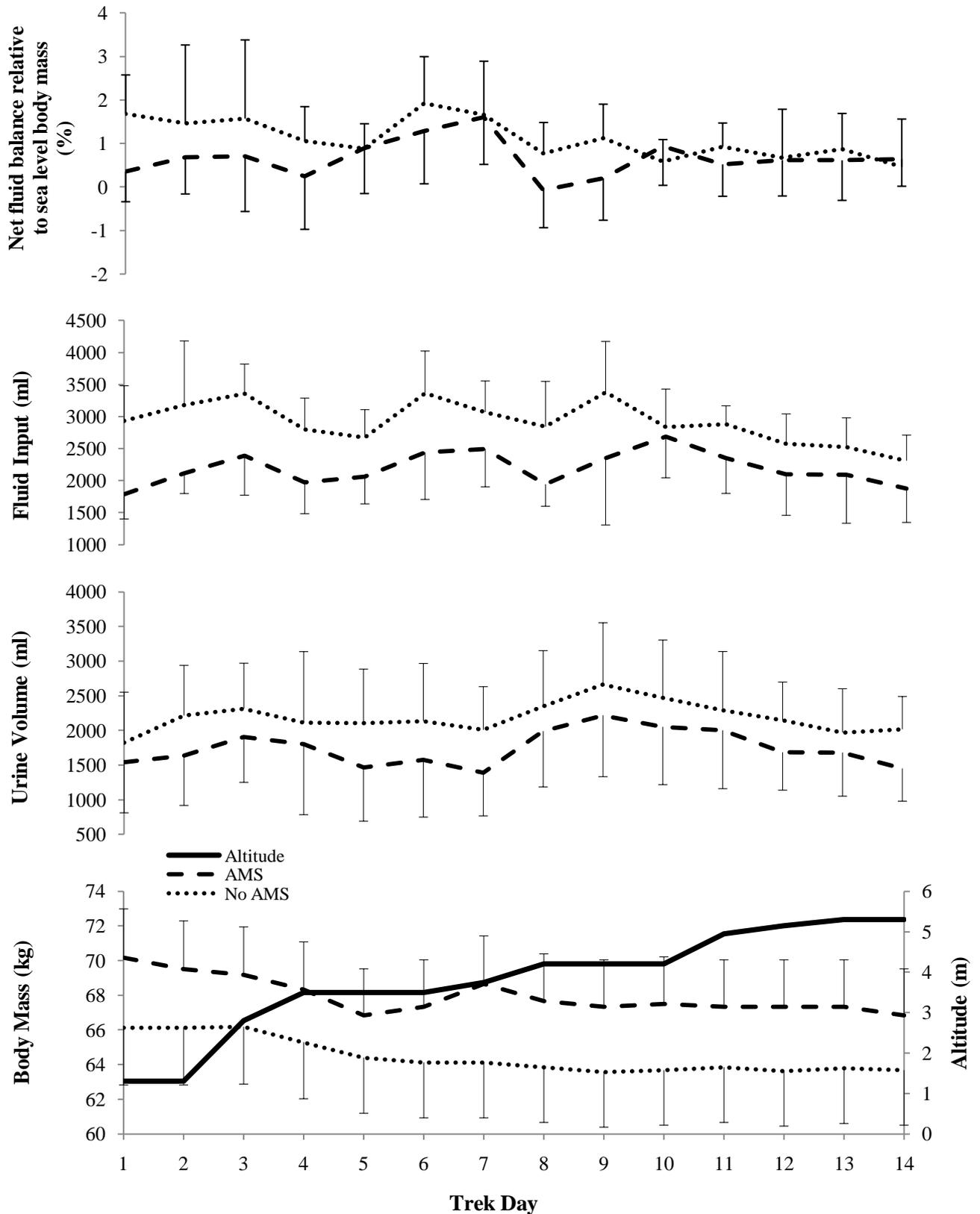


Figure 9.3: Changes in body mass, daily urine volume and net fluid balance over the duration of the ascent, between AMS groups. Ascent profile is shown on the secondary y axis. Y error bars are shown.

The AMS group had lower average fluid input over the test duration ($2276 \pm 311\text{ml}$) than the non AMS group ($2908 \pm 331\text{ml}$), though this was not significant. When expressed relative to body mass fluid intakes between AMS groups were significantly different (AMS $32 \pm 3\text{ml}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$; No AMS $45 \pm 5\text{ml}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$), while fluid input was significantly inversely correlated with peak LLQ for all the participants (Figure 9.4).

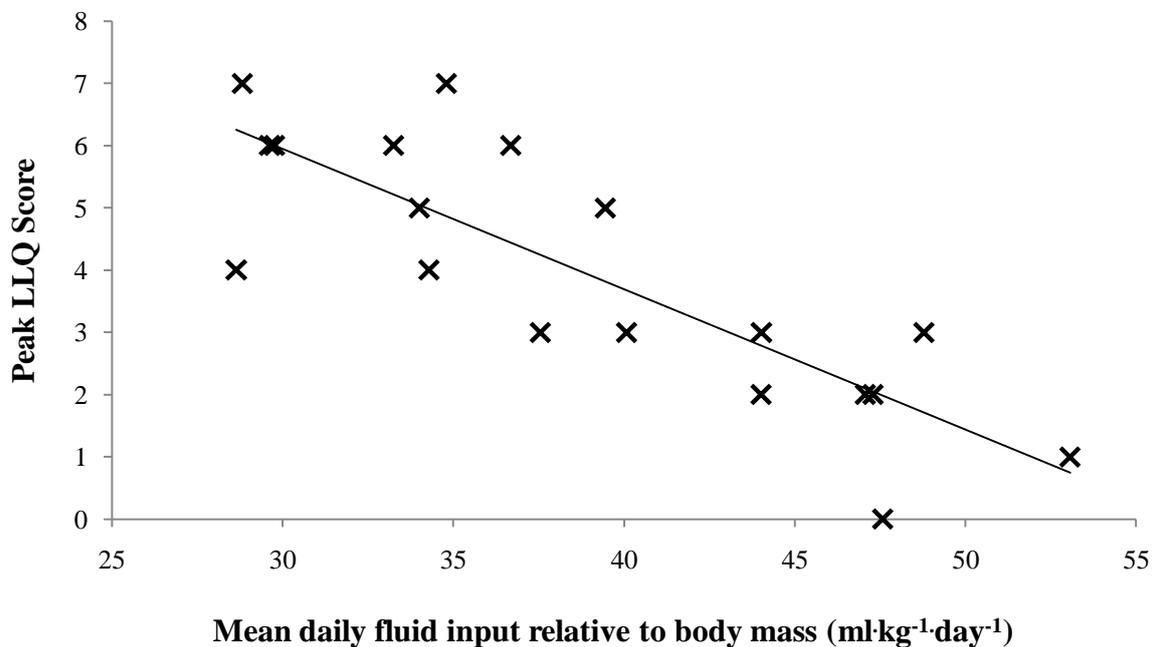


Figure 9.4: Peak LLQ score against mean fluid input relative to body mass throughout the trek and for each participant ($r^2 = 0.6591$, $y = -3.0569x + 50.6$).

Urine specific gravity was not different between groups in either the morning (AMS = 1.013 ± 0.003 ; Non AMS = 1.014 ± 0.001) or evening (AMS = 1.011 ± 0.004 ; Non AMS = 1.009 ± 0.002) samples (Figure 9.5). Average daily urine colour presented similar trends to the USG values (AMS 3.1 ± 0.4 ; Non AMS 3.2 ± 0.3).

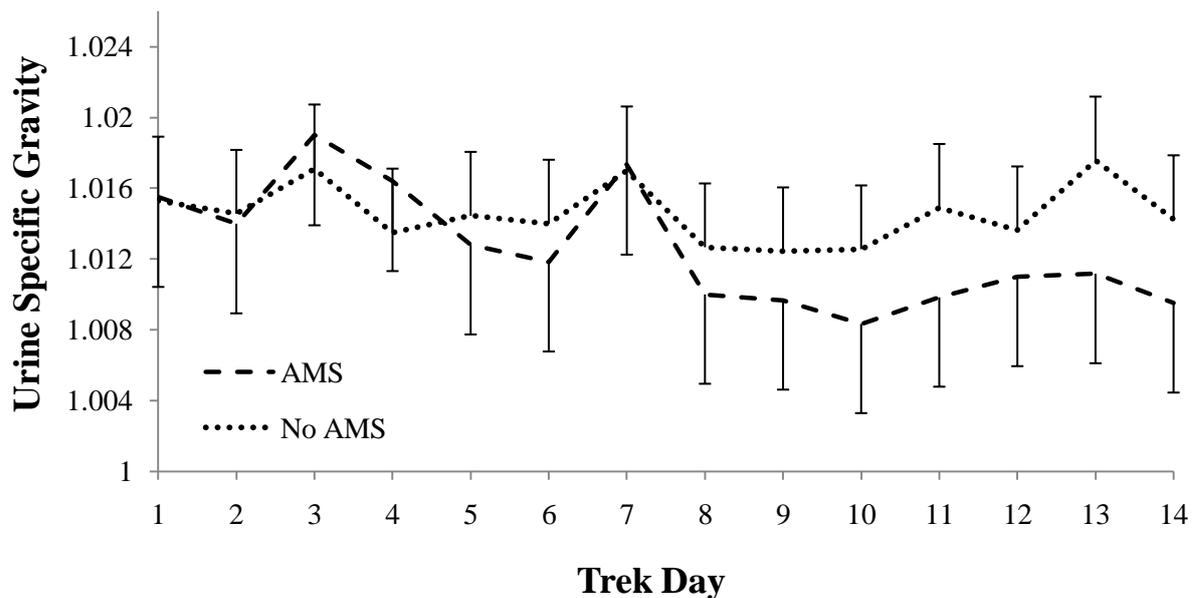


Figure 9.5: Urine specific gravity for the first void, over the ascent duration between AMS and non AMS groups. Y error bars are shown.

9.5. Discussion

The field study attempted to quantify the fluid intake and output of individuals that did and did not suffer with AMS on ascent to 5300m over 14 days.

Fluid intake is thought to be of importance in protecting against AMS. This study did not find a significantly lower fluid intake in those suffering with AMS. Similar average fluid intakes ($2800 \pm 979\text{ml}$), were recorded by Nerin *et al* (2006) in soldiers climbing to various altitudes, but found no significant difference in those with or without AMS. Basynat *et al* (1999) suggested that drinking $>3000\text{ml}$ would reduce the chance of suffering with AMS, when surveying a large sample climbing to various altitudes over various time frames. This suggestion is tentatively supported by Figure 9.4, which demonstrates that individuals ingesting higher relative average fluid intake, subjectively expressed lower peak LLQ scores over the trek duration. These individuals ingested

fluid at rates of approximately $45\text{-}50\text{ml}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ or $3150\text{-}3600\text{ml}\cdot\text{day}^{-1}$ in a 70kg individual. However, fluid intake values may lack accuracy and precision as they were self reported by the participants, although the importance of accuracy was stressed.

Despite similar intake values, the current study found greater urine output values ($1993 \pm 230\text{ml}$) than that of Nerin *et al's* (2006) study ($1557 \pm 758\text{ml}$). This is likely to be a consequent of the lower altitude, slower ascent rate and greater amount of rest days in the current study. In contrast to Cumbo *et al* (2002) who demonstrated significantly greater USG values in AMS sufferers, the present study demonstrated that the trekkers maintained USG and therefore, a hydrated state throughout the trek irrespective of AMS grouping. Thus body mass loss must be the result of energy balance alterations and/or minor losses in total body water through increased ventilation as altitude and subsequent physiological strain increase (Hannon *et al.* 1969; Richardson *et al.* 2008a; Surks *et al.* 1966; Westerterp 2001; Westerterp *et al.* 1996). Krzywicki *et al* (1971) noted that body mass loss occurred over a six day exposure to 4300m, irrespective of calorific intake, indicating that body water loss was an adaptive mechanism to hypoxic exposure. Previous theories by both Surks *et al* (1966) and Hannon *et al* (1969) suggested a decline in plasma volume was only the resultant of body fluid shifts. Subsequently research has demonstrated body fluid losses with altitude exposure in humans (Macdonald *et al.* 2009). Yet, linking this adaptive hypohydration response to AMS prevalence, irrespective of fluid intake, remains to be shown many years on. Macdonald *et al* (2009) recently demonstrated a 2.4kg loss in body mass over a 21 day expedition, of which 35% was decline in TBW. However, measurements were taken one day pre and one day post expedition, preventing the assessment of fluid balance and body mass time course. The current study demonstrates a body fluid loss in all

participants, irrespective of AMS, while calorific intake was not controlled. Bartsch *et al* (1998) conclude that AMS can develop without water retention and thus is not a pathophysiological factor of AMS, though they go on to suggest that AMS susceptibles may develop a fluid retention due to rise in physiological strain imposed by hypoxemia and exercise. The current study shows no difference in fluid retention over the trek period between AMS groups, yet on days five, six and seven, whereby participants were exercising above 3000m for the first time, there was an increase in fluid retention in AMS susceptibles. The non AMS group also noted considerable fluctuations in urine volume over this period, though body mass steadily declined irrespectively, demonstrating control of fluid balance, regardless of ventilatory, sympathetic or hormonal alterations induced. From the data, it is difficult to distinguish the effect altitude or exercise as single factors in this retention. However, the trend occurs from day five (a rest day at 3400m) onwards, suggesting the severity of altitude is important, while a period for fluid to build is also necessary.

Although the study had a reasonable heterogenous sample size, controlled ascent profiles and prophylaxis, the large variation in individual responses to altitude, caused a lack of significant findings. Further, the grouping of AMS and non AMS sufferers is controversial (Altman & Royston 2006), causing debate as to how these should be dichotomised, if at all. Also, the prevalence of AMS within the study was lower (39% of participants recording a peak LLQ ≥ 5) than other studies have reported (Hackett & Rennie 1979; Hackett *et al.* 1976). This is likely to be a consequent of the conservative ascent profile and classification of AMS (Roach & Kayser 2007).

A limitation of the study was the control of fluid input. Participants were instructed to measure out all drinks, yet the accuracy of participant measurements could not be fully controlled. However, the measurement of body mass would still give indication of less subtle changes in fluid retention or excretion. Further, variation in ingested fluid may also influence retention/excretion rate. Participants drank a range of fluids, the volumes of which would be impossible to analyse independently, while subtraction of caffeine from individuals' diets may have induced headaches irrespectively.

Field based testing generates a high ecological validity, inducing certain physiological and psychological changes that are not possible to cause within the lab, though researchers have tried (Houston 1997). However, measurements at altitude are difficult and costly, with a high participant withdrawal rate due to many uncontrollable issues. Further, findings from the data set are difficult to attribute to a single entity due to the number of confounding variables that compile over a period of days at altitude. Tests within the laboratory can accurately and reliably quantify the responses to a hypoxic stimulus, yet it is difficult to establish whether these measured responses are representative of an altitude environment. Laboratories at high altitude continue to be used (Grocott *et al.* 2007), yet still there are issues with sample sizes, withdrawals and the number of confounding variables that amount over the days getting to and staying at the laboratory. These issues will improve with the development of more accurate portable measuring devices and the use of large scale altitude research expeditions. Further research may also consider relating the fluid responses to individuals ACE II genotype, which has been related to individual's tolerance to altitude and is known to influence blood pressure and body fluid control (Thompson *et al.* 2007).

9.6. Conclusion

Adequate fluid intake of $\sim 45 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ is important in AMS prevention, yet ultimately AMS is dependent upon individual susceptibility. The role of fluid retention in AMS pathophysiology and the control of fluid homeostasis is important within AMS research, yet these still remain unclear after many years of research.

CHAPTER X.

GENERAL DISCUSSION

This general discussion will briefly present the principle findings of each study, the pertinent physiological issues arising from the studies, future research paths and finally, the practical applications of this PhD to individuals exposed to hypoxia and the importance of maintaining fluid balance.

10.1. Principle findings

Study 1 The purpose of this study was to develop an intermittent walking protocol, which could be used to determine an individual's physiological responses to hypoxia. This study showed that a lower inspired oxygen fraction induced greater physiological strain and symptoms of altitude illness, when completing rest and exercise bouts of the intermittent walking test. Change in body mass over the test duration negatively correlated with LLQ scores. The intermittent walking test demonstrated a good degree of construct validity, as it was sensitive to the different levels of hypoxia.

Study 2 In the second study participants were hyperhydrated or hypohydrated prior to completing the intermittent walking test as performed in study one. The results showed hypohydration, a state of body fluid deficit, to induce significant physiological strain and increase altitude illness symptoms, compared to euhydration. Likewise, hyperhydration induced physiological strain increases and reports of severe headaches similar to hypohydration. This study demonstrated the need for euhydration in hypoxia

supporting previous literature (Basnyat *et al.* 1999; Basnyat *et al.* 1998; Rennie *et al.* 1993).

Study 3 The third study dehydrated participants to different levels of hypohydration (1%, 2%, 3% of body mass loss), prior to completing intermittent walking tests in hypoxia. The results showed significant increases in physiological strain and altitude illness when body mass loss was greater than 2%. Previous work has suggested significant performance decrements (Cheuveront *et al.* 2005) and increases in physiological strain (Wyndham & Strydom 1969) when dehydrated to this degree in normoxia. However, -1% of body mass loss reduced the symptoms of AMS and headache scores, suggesting minor hypohydration may improve tolerance to acute hypoxia, while causing no significant increase in physiological strain.

Study 4 The fourth study used dehydrated participants (2.5% body mass loss) 24 hrs prior to completing a 6 hr, hypoxic or normoxic exposure at rest. Participants either remained hypohydrated or were rehydrated with water or isotonic fluid. This study demonstrated that long term hypoxic exposure while at rest caused significant increases in cellular stress and alterations to blood brain barrier function. There was little difference between the hypoxic conditions in regard to rehydration strategies. However, hypohydrated hypoxic conditions showed greater and earlier drop-out than other conditions. Although, individuals that dropped out withdrew in all hypoxic exposures, demonstrating an almost dichotomous response of those that did or did not tolerate the hypoxic exposures.

Study 5 The fifth study used a longer term hypoxic exposure as in the fourth study, with the addition of the intermittent walking test in the last 125mins of exposure. This demonstrated the effect of exercise on physiological strain at a cellular level and specifically, how hypoxia and hypohydration induce similar cardiovascular responses, which when combined, further exacerbate physiological strain. Isotonic fluid rehydration was shown to maintain fluid balance, the integrity of the BBB and cause a lower cellular physiological strain. Water rehydration induced the most severe LLQ symptoms and notably severe headaches. This may be related to the increased serum 100β values with water rehydration in hypoxia. Yet this response had no relationship to increased physiological strain at a cardiovascular or cellular level. This suggests fluid balance has some responsibility for AMS symptom development, rather than increases in physiological strain.

Study 6 The sixth study was part of a research expedition (Caudwell Xtreme Everest 2009) which investigated the hydration of participants trekking to Everest base camp (5300m) over a 14 day period, by monitoring fluid intake, output and indices of hydration. The study tentatively showed individuals drinking more fluid, in the region of $45\text{ml}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, to have lower self reported symptoms of AMS, supporting previous research (Basnyat *et al.* 1999). Furthermore, individuals suffering with AMS tended to demonstrate fluid retention when ascending to altitudes above 3500m, as previously described by others (Bartsch *et al.* 1991b; Bärtsch *et al.* 1998; Loeppky *et al.* 2005a; Westerterp *et al.* 1996).

10.2 Issues arising from the studies

The thesis attempts to describe and explain the physiological and perceptual responses to hypoxia and altitude at rest and during exercise, while also considering the role of fluid balance and hydration throughout these exposures. Prior to the production of this thesis there had been many attempts to create an AMS or altitude tolerance test using various physiological variables, protocols and parameters. Burtscher *et al* (2008b) compared the use of these tests in predicting AMS in later altitude or hypoxic exposures. Burtscher *et al* (2008b) state that SaO₂ after 30 mins of hypoxia is currently the strongest predictor of AMS (86%), but explain that far more research is needed to generate a reliable prediction test. Altitude research has tended to use various exposure protocols or exercise bouts making comparison between studies difficult. Even if AMS prediction is not possible, the development of a standard hypoxic test to quantify tolerance or intervention strategies would be of use to allow comparisons. In the first study the intermittent walking test was developed and used and may offer an applicable and sensitive test, which can be completed within a lab over a short duration.

Aside from the measurements of such physiological variables or prediction tests is the overall pathophysiology of AMS. By understanding this process and the underlying alterations occurring with hypoxia, accurately predicting altitude tolerance may be a far greater possibility. Numerous models explaining the pathophysiological process have been presented, some focusing on the mechanistic changes, while others have opted for an integrative approach. The most recent, presented by Burtscher *et al* (2008a) (Figure 10.1) illustrates the importance of sympathetic changes with hypoxic exposure that influence the renal response, anti-diuresis and development of AMS.

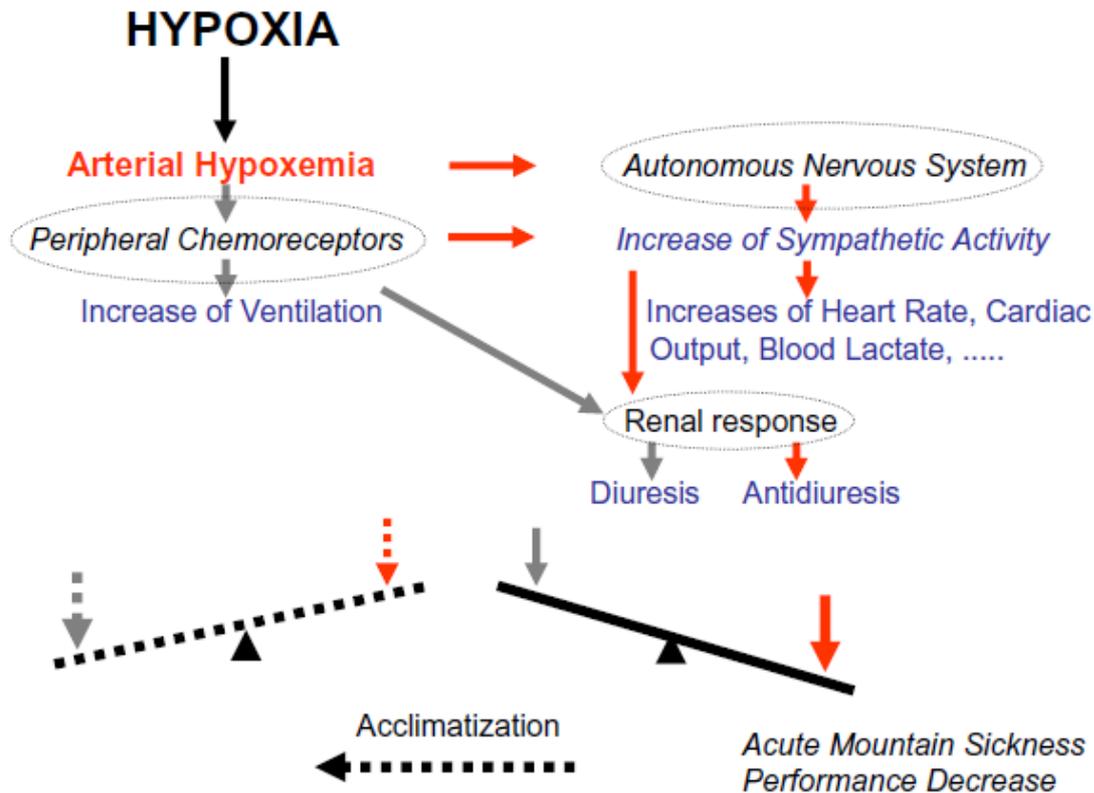


Figure 10.1: Schematic overview of selected responses to acute hypoxia (altitude), which may adapt during preacclimatization (Burtscher *et al.* 2008a).

However, this particular model offers little explanation to the processes and mechanisms causing such acclimatisation and essentially why some individuals tolerate altitude and some do not. In this thesis a variety of physiological and perceptual variables were compared to the symptoms associated with AMS. Participants were exposed to various hypoxic severities and states of hydration and fluid balance, allowing the role of these sympathetic, ventilatory and renal responses to be investigated in regard to their influence in an individual's tolerance to altitude.

Similar to the mechanisms of AMS development, the role of hydration at altitude also requires much further scientific attention. The earliest published debate on the issue

(Rennie *et al.* 1993) explains that individuals that have a diuretic response at altitude feel well, while forcing down fluids will not encourage a diuresis, it is not possible if the individual is in a hypohydrated state. A more recent debate (Basnyat *et al.* 2001) suggests adequate hydration, causing a clear urine colour, is needed to reduce the chance of altitude illness. Yet, researchers highlight the lack of scientific evidence supporting this notion. Articles have attempted to quantify the effect of hypohydration on hypoxic exposure through controlled trials (Aoki & Robinson 1971). While others have performed field based longitudinal (Nerín *et al.* 2006; Westerterp *et al.* 1996) or cross-sectional (Basnyat *et al.* 1999) measures of fluid input and output, and compared these to reported AMS symptoms. However, none of these have answered whether hypohydration influences AMS development and quantified the optimal amount an individual should drink while exposed to hypoxia or at altitude.

The following sections will explain how this thesis has attempted to address these issues, add empirical evidence to the existing literature, and based on the findings, produce a model integrating the physiological and perceptual responses of the relevant systems.

Physiological Consequence

The cardiovascular system is the first to respond to a hypoxic stimulus (Chen *et al.* 2008), while hypohydration is also known to induce rise in sympathetic activity (Montain & Coyle, 1992). As expected, heart rate, core temperature and therefore physiological strain index were greater with severity of hypoxia (chapter IV) and hypohydration (chapters V & VI). However, within chapter VIII the rehydration conditions were not different to the hypohydration conditions for heart rate or

physiological strain until the third exercise phase. This suggests that hemodilution caused heart rate to increase, similar to the tachycardic effect of hypohydration. By the third phase of exercise, differences in heart rate between rehydration and hypohydration conditions were similar to that of euhydration and 3% hypohydration in chapter VI. This is likely to be the result of hemoconcentration nearing optimal levels via exercise induced sweat and urine losses. Roach *et al* (2000) explain that increasing physiological consequence, whether through exercise or hypohydration, will worsen the degree of hypoxemia and therefore increase the chance of AMS development, which is illustrated in Figure 10.1 (Burtscher *et al.* 2008b). The earlier chapters (IV, V & VII) of this thesis demonstrate this notion, although the later chapters cannot support this link as a result of the significant variability over long duration studies.

During exercise in hypoxia, hypohydration tended to increase core temperature by 0.1°C per 1% of body mass lost. This is similar to increases of 0.1°C (Sawka *et al.* 1985) and 0.15°C (Greenleaf & Castle, 1971) seen with hypohydrated participants exercising at a similar intensity in the heat. Core temperature rises have been related to rises in AMS, Maggiorini *et al* (1997b) reported increases in core temperature of 0.5°C in mild and 1.2°C in severe AMS cases. While Roggla *et al* (2000b) suggests increases of 1°C in AMS sufferers. These field-based values are in excess of the average difference between normoxia and hypoxia (FIO₂:0.12) in core temperature during rest (0.2°C) and exercise (0.5°C) found within this thesis, suggesting that core body temperature rises chronically. This may be as a result of intracellular fluid shifts reducing sweat efficiency and therefore, increasing heat storage over time. In contrast, Loeppky *et al's* (2003) acute hypoxic study found core temperature to be significantly

less in AMS sufferers. Although, participants could control the ambient temperature and core temperature was measured orally.

As demonstrated in previous research on rabbits (Tokyo *et al.* 2005), rats (Zhong *et al.* 2000) and humans (Oehler *et al.* 2000), HSP₇₀ expression is upregulated with acute hypoxic exposure. These increases are independent of exercise or core temperature alterations (chapter VII vs VIII), as shown previously (Kim *et al.* 2004; Melling *et al.* 2007). The influence of HSP₇₀ upregulation on AMS development has not previously been described. This thesis reports a relationship between peak LLQ score and HSP₇₀ changes over the exposure, indicating that cellular physiological consequence is of importance to AMS symptom development (chapters VII and VIII). Loeppky *et al.* (2003) have reported hypoxia induced catecholamine release to be greater in AMS sufferers, which would increase HSP₇₀ upregulation. This is linked to the increases seen in heart rate and core temperature with severity of hypoxia and hypohydration. When comparing chapters VII and VIII, HSP₇₀ was significantly greater with exercise for all conditions (Figure 10.2), while hypohydration (2.5% body mass loss) only induced significantly greater increases than the rehydration trials when at rest. The opposite might have been expected, as hypohydration should exacerbate exercise-induced increases in physiological cost. This suggests that carriage of the additional fluid load increases the physiological consequence, increasing HR to hypohydration condition levels in chapter VIII. Another reason may be the cellular damage such that hypohydration and rapid rehydration induces, while hemodilution and the consequent rise in the sympathetic response could also explain this.

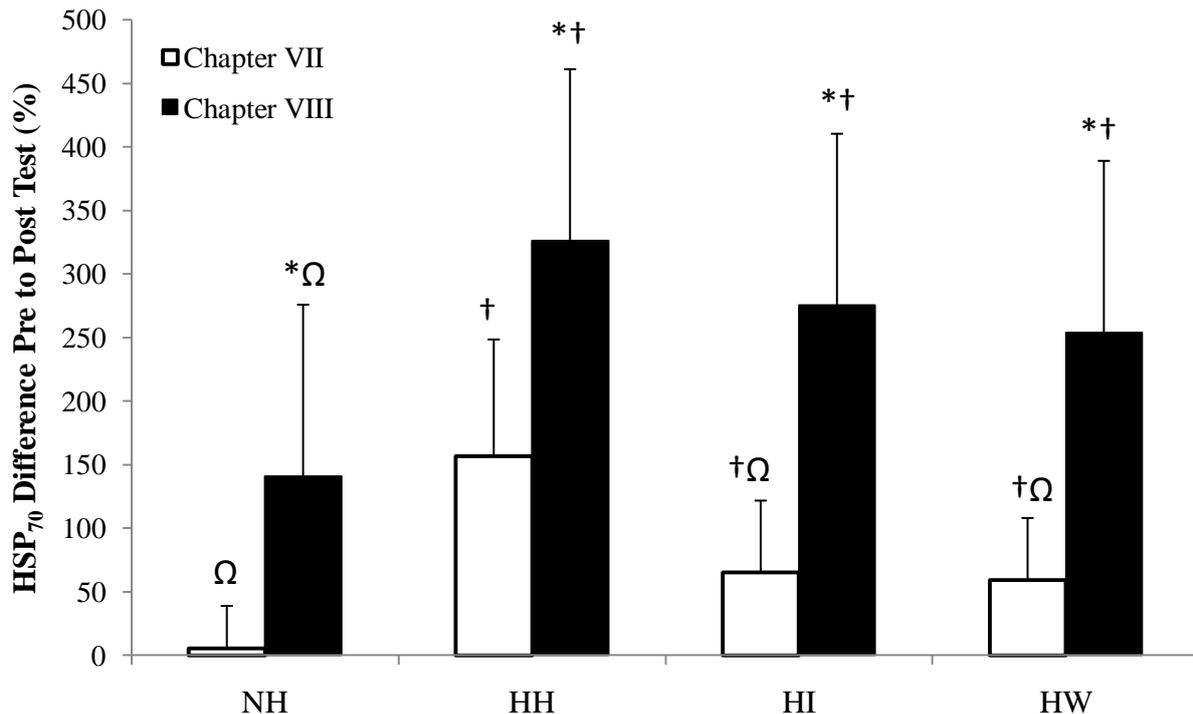


Figure 10.2: Comparison of HSP₇₀ concentration change between resting (Chapter VII) and exercising (Chapter VIII) exposures to hypohydration in normoxia (NH), hypoxia (HH) and with rehydration using isotonic fluid (HI) and water (HW). * denotes significant difference between Chapter VII and Chapter VIII. † denotes significant difference to NH. Ω denotes significant difference to HH. Hypoxia Hypohydrated (HH), Hypoxia Isotonic (HI), Hypoxia Water (HW), Normoxia Hypohydrated (NH).

Renal Responses

It has been suggested that an anti-diuretic response to hypoxia increases the susceptibility to AMS symptoms (Burtscher *et al.* 2008a). Loeppky *et al.* (2005a, b) found increases in fluid retention were related to AMS symptoms. Authors attributed this relationship to a 50% greater ADH increase in AMS sufferers than non sufferers, causing a decline in FCH₂O, corroborating with other studies (Heyes *et al.*, 1982; Bartsch *et al.*, 1991a). In contrast one study reported a higher eGFR when suffering with AMS (Pichler *et al.*, 2008). Though the study used the cystatin-C method, which is

unaffected by exercise, as opposed to creatinine in the current studies (chapter VII & VIII), and did not control fluid or sodium intake. Similarly, Bartsch *et al* (1998) explain that sodium and water retention, due to ADH increases, is related to AMS onset. Though, by inducing AMS with fast (helicopter) ascent to 4559m and then showing no difference in urinary responses between AMS sufferers and non sufferers, Bartsch *et al* (1998) demonstrated that renal responses are not the sole pathphysiologic factor in AMS. This renal response cannot be supported by the acute tests completed within the first five experimental chapters. No difference was found in FCH₂O or eGFR with hypoxia. This is possibly due to the short duration exposure and the comparison of hypohydrated states, which caused initial eGFR and FCH₂O to be low, while there was a large variation in participants' responses. Further, eGFR and FCH₂O were not correlated with increases in AMS. Yet, longer term analysis of fluid balance within chapter IX evidenced this response after five days of altitude exposure whereby AMS sufferers reported alterations in fluid input and output, and exhibited minor fluid retention while non sufferers body mass continued to steadily decline. Although this observation was in a controlled, field-based study using a reasonable sample size, the applicability of this should be questioned, as so many other factors such as diet and exercise are influential, when exposed to altitude. These factors have far greater influence on renal function than the effect of the hypoxic stimulus alone, which as evidenced by chapters VII & VIII, causes no significant short term renal response at rest or during exercise. Twelve years on from Bartsch *et al*'s (1998) paper, there is still no unequivocal answer as to whether these renal alterations are a cause or consequence of AMS, due to the difficulty in controlling these factors in a large sample group in the field.

As demonstrated in chapters V to IX, euhydration is essential in order to reduce physiological strain and symptoms of LLQ, irrespective of the underlying mechanisms. Intake values shown within chapter IX indicate that individuals should drink $45\text{ml}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$. However maintenance of euhydration, as shown in chapters VII & VIII, requires drinking of small quantities regularly or ingestion of isotonic fluid to maintain reasonable hemoconcentration and fluid balance. Meanwhile drinking the volume suggested in chapter nine, as water or a large proportion of it within a short period may induce severe headache symptoms reported in chapter eight's water rehydration condition and chapter five's hyperhydration condition.

Cerebrovascular Alterations

The mechanism inducing these headaches seems to be related to the increase in serum 100β , as shown in chapter VIII (Figure 8.4.6). Serum 100β release ranged between participants, as also found by Watson *et al* (2005). This indicates an individualistic response, yet it is unclear as to why certain individuals may have greater BBB disruption than others. Bjursten *et al* (2010) showed serum 100β to correlate positively with LLQ score using seven participants measured on five separate occasions on ascent to 4559m. This relationship is similar to that shown in chapter VII and supports the suggestion that BBB disruption has a role in AMS pathophysiology, whether over a chronic or acute exposure. While recent work measuring serum 100β (Bailey *et al.* 2009) and MRI (Kallenberg *et al.* 2007; Kallenberg *et al.* 2008; Schoonman *et al.* 2008) suggests that hypoxia does not disrupt the BBB. In the current study, increases in ECF via water intake seem to exacerbate BBB disruption. Bailey *et al* (2009) postulate that hypoxia does not cause disruption to BBB integrity, but in fact changes in cerebral autoregulation due to alterations in redox homeostasis, is responsible for AMS

development. Basynat & Murdoch (2003) illustrated the importance of blood volume and permeability of the BBB within their review of altitude illnesses (chapter II, Figure 2.1). Wilson *et al* (2009) present within their review, a mechanistic diagram of headache development through hypoxic exposure (chapter II, Figure 2.8). Wilson *et al.* (2009) proposed that among other factors, hypoxia induces vasodilatation, activating the trigeminal nerve, causing a headache. Further, the authors (Wilson *et al.* 2009) explain that HIF-1 α accumulates, upregulating VEGF and causing membrane damage, which would be exacerbated by exercise (Imray *et al.* 2005) as supported by chapter VII & VIII. Oedema can then develop, and increase intracranial pressure, which would be exacerbated with excessive fluid (Kimelberg 2004).

When comparing chapters seven and eight, it appears that exercise at 50%VO₂max has no significant effect on serum 100 β release. Similarly, there seems to be no relationship between serum 100 β release and physiological strain at either a cellular or cardiovascular level. This evidence collected within chapters VII and VIII contrasts the findings of chapter IV to VI in which heart rate and physiological strain index were closely related to the increases in self-reported symptoms of AMS. These comparisons demonstrate that in order to get a true reflection of hypoxic tolerance that would be expected in a longer duration exposure to altitude, only resting exposure actually causes symptoms of AMS that are related to the onset of BBB disruption. Hypoxic exercise may simply induce cardiovascular and ventilatory stress, causing rise in symptoms of fatigue, nausea and dizziness that would be expected at such intensity with low oxygen availability, shown in chapters IV, V & VI. Though, it is thought that hypoxic exercise would increase the accumulation of HIF-1 α , VEGF upregulation and ultimately membrane damage (Croll *et al.* 2004; Imray *et al.* 2005; Kaur & Ling 2008; Wilson *et*

al. 2009). The investigations within this thesis may be of too short a duration to demonstrate this developmental response.

Integrative Model

The integration of the systems presented and the findings obtained in this thesis support the presence of certain processes in AMS development. Figure 10.3 brings together these suggestions into one model, to illustrate the role of these different systems and the influence hydration state may have. From the figure it is evident that the severity of hypoxia can alter, and as chapter IV demonstrates, the physiological and perceptual responses are exacerbated by the severity of hypoxia. Likewise, hydration state can also fluctuate and subsequently induces a rise in physiological consequence, through heart rate and core temperature, as body water is lost. As a product of increasing the physiological consequence respiratory systems respond and subsequently require renal compensation to excrete alkylotic byproducts. The renal system is also driven by the renal hormones, as discussed in this thesis, physiological consequence and hydration state can directly alter the ADH response, which is essential to the control of excretion or retention. By reducing the physiological consequence, ADH rise is attenuated. Likewise, rehydration would reduce physiological consequence but also decrease ADH directly, stimulating a diuretic response. However, hypohydration can induce the opposing response, increasing ADH and physiological consequence, initiating a retentive response. Over-hydrating or rehydrating with water may well reduce ADH but the excessive fluid intake may have a similar acute effect to the retention response, as noticed in chapters V and VII.

From this thesis and other research (Bartsch *et al.* 1998) it is clear that the renal response to hypoxia is not the only factor in AMS development. The thesis demonstrates the direct effect of hypoxia on BBB permeability, allowing a greater amount of fluid is able to cross into the brain, inducing intracranial pressure, which would be exacerbated with fluid retention or excess fluid intake described. Similar models developed (Roach & Hackett 2001) have proposed that cranial cerebrospinal fluid increases as a result of lower cerebrospinal compliance, causing a rise in intracranial pressure. Authors (Roach & Hackett 2001) suggest that this process is the differentiating factor in AMS susceptibility. Findings in this thesis cannot support nor rule out this process. Roach & Hackett (2001) also suggest BBB permeability is increased as a result of greater cerebral blood flow and volume. These suggestions are similar to Basynat & Murdoch (2003) who illustrate (Figure 2.1) that the increase in brain swelling leads to inadequate buffering of the cranial cerebrospinal fluid, though a 'no AMS' response is not proposed. Bailey *et al's* (2009) recent suggestion of changes in cerebral autoregulation, requires far greater research, yet would still imply fluid balance alterations influence AMS development. Evidence from this thesis implies that BBB permeability is related to AMS symptom development, irrespective of individuals' cerebrospinal compliance, and therefore it is the movement of fluid which is of primary importance.

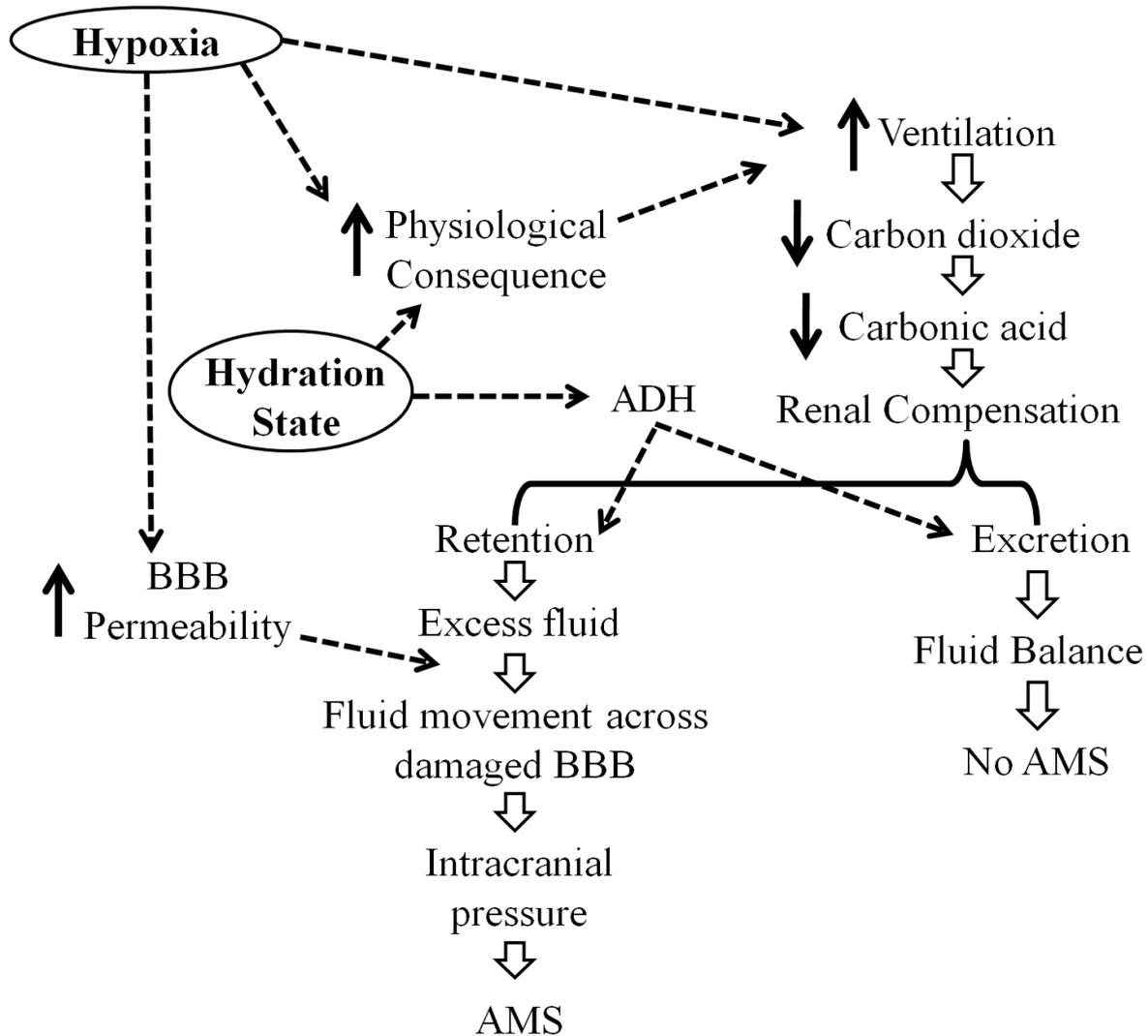


Figure 10.3: Integrative model of AMS development in relation to physiological consequence, renal responses and cerebral alterations assessed within this thesis. Acute Mountain Sickness (AMS), Blood Brain Barrier Permeability (BBB). $\text{---}\rightarrow$ indicates suggested relationships based on the findings of this thesis. \downarrow indicates the process pathway based on previous literature discussed throughout the thesis. \uparrow indicates an increase in the relevant physiological variable.

Acute Mountain Sickness or Altitude Tolerance?

Throughout the thesis there has been an attempt to relate the physiological, renal and hormonal responses to hypoxia with AMS symptoms. By definition, AMS requires exposure to altitude above 2500m for at least 6 hrs, though other authors state that it is possible to induce AMS within 1 hr. Subsequently, the studies presented are only presenting the symptoms induced, whether these symptoms are, or are not AMS is irrelevant as AMS itself is a series of self reported non specific symptoms. In reality diagnosis is often a case of whether the individual reports feeling unwell with introduction to altitude and if there is no other plausible explanation, AMS is diagnosed. From the literature and work in all chapters of this thesis, it appears that the individualistic response to altitude is not as simple as whether someone does or does not tolerate altitude. Individual participants tend to respond to altitude in a similar way each time, yet these responses are by no means dichotomous. Much of the research presented within the literature analyses and interprets altitude tolerance as either those that are suffering with AMS or those that are not, as presented within chapter nine. Irrespective of the definition of AMS, the questionnaire used or the appropriate LLQ score to diagnose AMS, there is clearly a continuous response to hypoxia or altitude and therefore should be analysed as such. Dichotomous groupings with the moderate LLQ scores removed from the data set eradicates much of the data and masks the fact that individuals can have a moderate response to altitude. Correlation or stepwise multiple regression of LLQ score against physiological markers or mechanisms, seems a more appropriate analytical method. Demonstrated within chapter IX, dichotomous analysis may mask important findings, such as the effect of fluid intake between AMS groups whereas the analysis of the whole data set using correlation allowed the effect of daily fluid intake to be demonstrated (Chapter IX, Figure 9.4).

10.3. Future Research

This thesis demonstrates that individuals have various responses over a short-term test, surely making it possible to predict tolerance using the physiological responses of a large sample (>150 participants) to a short term screening test and comparing these with their response to a long duration controlled trial. The current research attempting this uses inappropriately small sample sizes, while the field based comparisons are poorly controlled using various ascent profiles or past altitude illness history (Burtscher *et al.* 2004; Burtscher *et al.* 2008b; Grant *et al.* 2002; Savourey *et al.* 2007).

Fluid balance during altitude exposure requires further investigation. Previous studies have investigated the role of fluid regulating hormones or fluid compartments pre and post exposure, even though the effects of altitude tend to be over days and with introduction to >3500m, as illustrated in chapter IX (Figure 9.4). Those that have monitored fluid balance over multiple time points at altitude, have used small samples, which when considering variation in individual's tolerance, is unlikely to demonstrate anything conclusive. Assessing the role of fluid loading for hypohydrated and hypoxic healthy individuals would be best within a multi-day hypoxic exposure, as the number of extraneous variables can be controlled more thoroughly than with field-based exposures, similar to that of the Operation Everest trials (Joanny *et al.* 2001; Sutton *et al.* 1983; Westerterp *et al.* 2000b). Analysis of the eGFR, FCH₂O and hormones regulating fluid balance (PRA, ADH, aldosterone, ANP) responses in a large sample with a regulated calorie, sodium and fluid intake, would allow greater mechanistic interpretation of the renal influence in AMS development.

As shown in chapter VI, minor dehydration of 1% body mass loss may have no effect on reported symptoms of AMS or physiological consequence. Though this suggestion requires a great deal of further study in regard to fluid movements and the mechanisms by which a minor dehydration would improve tolerance. Further, this response may be due to carrying less and the resulting decline in energetic cost. From the data presented within this thesis it seems evident that an excessive fluid load is in part responsible for the development of symptoms associated with AMS, via BBB disruptions and fluid movement.

Furthermore, this thesis did not attempt to quantify fluid movement across the BBB, even though there has been much speculation regarding the mechanisms surrounding headache development. Alterations to the BBB and fluid shifts can be quantified using MRI (Kallenberg *et al.* 2007), which is expensive and brings about many logistical issues with regard to hypoxia. Other work (Ainslie *et al.* 2008; Imray *et al.* 2005; Wilson *et al.* 2009) presents a far more detailed mechanistic approach to the investigation of intracranial pressure and AMS development, from which the data generated by this more applied thesis supports.

10.4. Practical application of findings

The development of the intermittent walking test and its use throughout the thesis suggests it may be a useful tool for altitude tolerance screening, as previously discussed (chapter IV; Burtscher *et al.* 2008b). However, it may also be of use when comparing hypoxic training interventions such as intermittent hypoxic exposure to improve hypoxic tolerance. Investigating the physiological responses to the hypoxic intermittent walking test pre and post hypoxic training could identify whether the individual has improved their acute hypoxic response, as described in chapter IV. This may be of use to mountaineers or even individuals performing at moderate altitude.

From all chapters and particularly chapter V and VI, it is evident that pulse oximetry has a reasonable variability ($\pm 2\%$) when the individual is euhydrated. When the individual is in a state of hypohydration, hyperhydration or rehydrating, their SaO₂ may be significantly different from normal levels. This is important in regards to research into pulse oximetry, while from a clinical perspective it further demonstrates the importance of euhydration when monitoring SaO₂.

Ascent rate is one of the few issues altitude physiology supports as a key determinant of altitude tolerance (Basynat & Murdoch, 2003). Slower ascent rates allow a greater acclimatisation phase, reducing the daily physiological consequence and chance of suffering with AMS, irrespective of the individual's susceptibility. The findings of this thesis would support any reduction in physiological consequence whether through reducing daily activity through walking speed, height gain or load carriage, or the severity of exposure to hypoxia.

Using the LLQ scoring system to identify individuals with AMS symptoms was useful to this thesis, although the symptoms of AMS can be due to many things other than AMS itself. The thesis simply supports previous work (Basnyat & Murdoch 2003), whereby AMS can only be diagnosed correctly when an individual is fully rested and in a euhydrated state. Exercise and hypohydration will only exacerbate symptoms used within the LLQ score.

Hydration monitoring via USG or urine colour seems to have no benefit when trekking at altitude, as there was no difference in hydration markers between AMS groups. Accurate recording of body mass may be useful in identifying when individuals are retaining fluid, which would then suggest the pathophysiological development of AMS. This should be particularly important with introduction to ~3500m whereby fluid retention tends to develop.

The key practical application of this work is the need for adequate fluid intake when in hypoxia or at altitude. This should be a quantity of approximately $45\text{ml}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ or $3\text{L}\cdot\text{d}^{-1}$ and consumed in small but regular boluses. Intake of excessively large fluid boluses is likely to have negative consequences. An increased fluid load would increase energetic cost and consequently physiological strain. Excessive low concentration fluid loading may also increase the fluid available to move across the BBB and subsequently increase intracranial pressure, as previously discussed (Figure 10.3). The fluid consumed should be isotonic, or at least have reasonable electrolyte (Sodium $>60\text{mmol/L}$) content allowing compartment equilibration, retention and minimal effect on the hemocentration at any given time. This will ultimately allow the individual to maintain a state of euhydration and fluid balance. However, drinking fluid in this manner will not

prevent AMS developing, yet it will help reduce physiological strain and consequently the likelihood of membrane damage, fluid movement and headache development.

10.3. Conclusions

It is proposed that this thesis and its dissemination via publication and conference presentations, will help to explain the need for hydration when resting or exercising within hypoxia or at altitude. Previous articles have conveyed the need for research into hydration status, fluid balance and their role in altitude tolerance, subsequently causing discussion within the area. Clearly, this thesis evidences the need for hydration and the control of fluid balance within a hypoxic environment and consequently opens the door for other research within this area of study.

CHAPTER XI.

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APPENDIX 1

Informed Consent (Generic Form)

UNIVERSITY OF BRIGHTON

Participation Consent Form

An Investigation into the

- I agree to take part in this research which is to assess the
- The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.
- I have had the principles and procedures explained to me and I have also read the information sheet. I fully understand the principles and procedures.
- I am aware I will be required to:
 - Rest in a hypoxic chamber while breathing a different composition of air
 - Insert a flexible rectal thermometer 10 cm into my anus
 - Give urine samples
 - Have blood samples taken at intervals
 - Answer a Lake Louise and Environmental Stress questionnaire.
- I understand that any confidential information will be seen only by the researchers and will not be revealed to anyone else.
- I understand I am free to withdraw from the investigation at any time, without needing to provide a reason.

Name (please print).....

Signed.....

Date.....

Witnessed by experimenter (please print).....

Signed.....

Date.....

APPENDIX 3

CHELSEA SCHOOL
UNIVERSITY OF BRIGHTON

NAME:

DATE:

EXPERIMENTER:

YES/NO

- | | |
|---|-------|
| 1. HAVE YOU HAD ANY KIND OF ILLNESS OR INFECTION IN THE LAST 2 WEEKS? | ----- |
| 2. ARE YOU TAKING ANY FORM OF MEDICATION? | ----- |
| 3. DO YOU HAVE ANY FORM OF INJURY? | ----- |
| 4. HAVE YOU EATEN IN THE LAST HOUR? | ----- |
| 5. HAVE YOU CONSUMED ANY ALCOHOL IN THE LAST 24 HOURS? | ----- |
| 6. HAVE YOU PERFORMED EXHAUSTIVE EXERCISE IN THE LAST 48 HOURS? | ----- |

IF THE ANSWER TO ANY OF THE ABOVE QUESTIONS IS YES, THEN YOU MUST CONSULT A MEMBER OF STAFF BEFORE UNDERGOING ANY FORM OF EXERCISE TEST

SIGNATURE OF SUBJECT:

APPENDIX 4**LAKE LOUISE QUESTIONNAIRE****Self Report Questionnaire:**

- | | | |
|---------------------------------|---|--|
| 1. Headache | 0 | No headache |
| | 1 | Mild headache |
| | 2 | Moderate headache |
| | 3 | Severe headache, incapacitating |
| 2. Gastrointestinal symptoms | 0 | No gastrointestinal symptoms |
| | 1 | Poor appetite or nausea |
| | 2 | Moderate nausea or vomiting |
| | 3 | Severe nausea & vomiting, incapacitating |
| 3. Fatigue and/or weakness | 0 | Not tired or weak |
| | 1 | Mild fatigue/ weakness |
| | 2 | Moderate fatigue/weakness |
| | 3 | Severe fatigue/ weakness, incapacitating |
| 4. Dizziness / light headedness | 0 | Not dizzy |
| | 1 | Mild dizziness |
| | 2 | Moderate dizziness |
| | 3 | Severe dizziness, incapacitating |

APPENDIX 5

Environmental Symptoms Questionnaire (ESQ)

Circle the number of each item to correspond to HOW YOU HAVE BEEN FEELING.

0 = No symptom, 5 = Extreme symptoms

PLEASE ANSWER EVERY ITEM.

1.	I feel light headed.	0	1	2	3	4	5
2.	I have a headache.	0	1	2	3	4	5
3.	I feel sinus pressure.	0	1	2	3	4	5
4.	I feel dizzy.	0	1	2	3	4	5
5.	I feel faint.	0	1	2	3	4	5
6.	My vision is dim.	0	1	2	3	4	5
7.	My coordination is off.	0	1	2	3	4	5
8.	I am short of breath.	0	1	2	3	4	5
9.	It is hard to breathe.	0	1	2	3	4	5
10.	It hurts to breathe.	0	1	2	3	4	5
11.	My heart is beating fast.	0	1	2	3	4	5
12.	My heart is pounding.	0	1	2	3	4	5
13.	I have chest pain.	0	1	2	3	4	5
14.	I have chest pressure.	0	1	2	3	4	5
15.	My hands are shaking or trembling.	0	1	2	3	4	5
16.	I have a muscle cramp.	0	1	2	3	4	5
17.	I have stomach cramps.	0	1	2	3	4	5
18.	My muscles feel tight or stiff.	0	1	2	3	4	5
19.	I feel weak.	0	1	2	3	4	5
20.	My legs or feet ache.	0	1	2	3	4	5
21.	My hands, arms, or shoulders ache.	0	1	2	3	4	5
22.	My back aches.	0	1	2	3	4	5
23.	I have a stomach ache.	0	1	2	3	4	5
24.	I feel sick to my stomach (nauseous).	0	1	2	3	4	5
25.	I have gas pressure.	0	1	2	3	4	5
26.	I feel constipated.	0	1	2	3	4	5
27.	I feel warm.	0	1	2	3	4	5
28.	I feel feverish.	0	1	2	3	4	5
29.	My feet are sweaty	0	1	2	3	4	5
30.	I am sweating all over.	0	1	2	3	4	5
31.	My hands are cold.	0	1	2	3	4	5
32.	My feet are cold.	0	1	2	3	4	5
33.	I feel chilly.	0	1	2	3	4	5
34.	I am shivering.	0	1	2	3	4	5
35.	Parts of my body feel numb.	0	1	2	3	4	5
36.	My skin is burning or itchy.	0	1	2	3	4	5
37.	My eyes feel irritated.	0	1	2	3	4	5
38.	My vision is blurry.	0	1	2	3	4	5
39.	My ears feel blocked up.	0	1	2	3	4	5
40.	My ears ache	0	1	2	3	4	5
41.	I can't hear well.	0	1	2	3	4	5
42.	My ears are ringing.	0	1	2	3	4	5
43.	My nose feels stuffed up.	0	1	2	3	4	5
44.	I have a runny nose.	0	1	2	3	4	5
45.	I have a nose bleed.	0	1	2	3	4	5
46.	My mouth is dry.	0	1	2	3	4	5
47.	My throat is sore.	0	1	2	3	4	5

48.	I am coughing.	0	1	2	3	4	5
49.	I have lost my appetite.	0	1	2	3	4	5
50.	I feel sick.	0	1	2	3	4	5
51.	I feel hungover.	0	1	2	3	4	5
52.	I am thirsty.	0	1	2	3	4	5
53.	I feel tired.	0	1	2	3	4	5
54.	I feel sleepy.	0	1	2	3	4	5
55.	I feel wide awake.	0	1	2	3	4	5
56.	My concentration is off.	0	1	2	3	4	5
57.	I feel more forgetful than usual.	0	1	2	3	4	5
58.	I feel worried or nervous.	0	1	2	3	4	5
59.	I feel irritable.	0	1	2	3	4	5
60.	I feel restless.	0	1	2	3	4	5
61.	I am bored.	0	1	2	3	4	5
62.	I feel depressed.	0	1	2	3	4	5
63.	I feel alert.	0	1	2	3	4	5
64.	I feel good.	0	1	2	3	4	5