Hormonal Adaptation to Acute and Chronic Hypoxia

The role of brain natriuretic peptide and stress hormones in the diagnosis and etiology of altitude illness

Adrian John Mellor

Doctorate of Medicine

Institute of Cellular Medicine, Newcastle University

December 2014

Preface

Many people travel to mountainous regions for exploration and recreation. This imposes a physiological challenge and brings about changes in every organ system within the body. This physiological challenge is unique within healthy individuals and may provide an insight into mechanisms relevant to ill health. This work aimed to investigate mechanisms behind the hormonal and physiological adaptation to high altitude and why, in some individuals, this extreme physiological challenge leads to serious and potentially life threatening illness.

For the military rapid deployment of elite forces to high altitude can result in altitude illness, the need for aeromedical evacuation and a degradation of the ability to perform an operational role. For those travelling to mountainous areas for recreational reasons the lack of a diagnostic test for altitude illness may lead to unnecessary, dangerous and expensive evacuation to lower altitudes.

Brain natriuretic peptide (BNP) is released from the heart in response to ventricular wall stress and has a well-established role in diagnosing heart failure. BNP increases with raised pulmonary artery systolic pressure (PASP), raised PASP is a key feature of high altitude pulmonary oedema (HAPE) and as such BNP may be raised with HAPE. Furthermore BNP is expressed in hypoxic cells and may therefore be raised as a marker of altitude illness.

During the work for this thesis investigating BNP in 48 subjects on an ascent to 5129m, it has been demonstrated that there is a significant rise in BNP with those with acute mountain sickness vs. those without. RoC analysis for this change in BNP demonstrated an area under the curve (AUC) of 0.601 for detecting AMS and an AUC of 0.675 for detecting severe AMS. Similarly the AUC was 0.645 for BNP detecting a PASP greater than 40 mmHg. It is important to note that these findings were across all subjects, i.e. the pretest probability of a positive result was low. In asymptomatic populations the AUC for BNP in detecting mild left ventricular systolic dysfunction is 0.6 for women and 0.72 for men.

These findings raise the possibility that BNP may be a useful diagnostic adjunct to current scoring systems in identifying significant pathology at high altitude. Specifically a low or normal BNP could be used to “rule out” altitude illness making an alternative diagnosis more likely and reducing the risk of the patient remaining at altitude. During the course of the study one subject who developed HAPE was monitored with serial BNP and cardiac echo studies which was reported in the International Journal of Cardiology and shows the utility of this approach.

Amongst the other findings of the studies that make up this thesis the importance of exercise in adaptation to high altitude was highlighted. Subjects reporting high perceived exertion scores (Borg RPE $\geq$15 which is around the aerobic threshold) had significantly different values of stress hormones (cortisol and copeptin), as well as higher scores for acute mountain sickness, lower oxygen saturations and higher heart rates. These physiological effects were present post exercise but also persisted the following day at rest.

This finding highlights that future studies relating to physiological adaptation to high altitude need to use relevant exercise intensities and may explain some of the inconsistencies in other published studies.

Structured Abstract

Data was collected in two main phases;

1. A field study undertaken in the Cordillera Real Region of Bolivia.
2. Laboratory investigation of terrestrial, hypobaric and normobaric hypoxia.

Phase 1

The aim of phase one of the studies was to investigate the endocrine changes (specifically brain natriuretic peptide (BNP), arginine vasopressin (AVP), cortisol, catecholamine and copeptin) with ascent to high altitude. These changes were investigated during a "real world" trekking expedition to Bolivia. Other potential markers for AMS namely high-sensitivity C reactive protein (hs-CRP), high-sensitivity cardiac troponin T (hs-cTnT) and neutrophil gelatinase associated lipocalin (NGAL) were also studied.

The overarching hypothesis was that Acute Mountain Sickness (AMS) would be associated with fluid retention and elevated BNP and that changes in other hormones involved in fluid balance such as AVP, copeptin and cortisol may also reflect AMS.

The hypotheses investigated included;

- That BNP would rise with high altitude (HA) illness and be associated with both AMS and a high pulmonary artery systolic pressure (PASP).
- That copeptin would accurately reflect changes in AVP at altitude and therefore have utility in future field studies as a surrogate for AVP.
- That AMS would be associated with either elevated copeptin/AVP or with failure to suppress the copeptin/AVP response to exercise.
- Inflammation and/or rises in PASP occur with the development of high altitude illness and would lead to a detectable rise in hs-cTnT, NGAL or hs-CRP.
- The dynamic changes in stress hormones associated with exercise are key in the pathogenesis of altitude illness.
- The physiological stress of HA would lead to a rise in cortisol. This would contribute to fluid retention and be linked to the development of AMS.
- There would be no difference between total body water (TBW) measured by single or multifrequency bioimpedance analysis.

Methods:

This was an observational study with data collection at sea-level and then subsequently over a 10 day ascent from 3800m to 5129m. Daily physiological data was collected with more detailed investigation performed at 3833m, 4450m and 5129m. At these altitudes, data was collected post exercise (after the ascent) and at rest (the following day). Venous blood samples were collected for hormonal assays. BNP and NGAL were analysed in situ using point of care testing technology. Assessment of TBW was performed using bioimpedance devices and pulmonary artery systolic pressure (PASP) was estimated using transthoracic echocardiography.

Results and implications:

50 subjects were recruited to the study, 48 of whom were studied at 3833m and 4450m with 47 subjects studied at 5129m. Results presented as mean (range, SD) unless otherwise stated.

Significant findings were:

Oxygen saturations fell with ascent to altitude to a nadir of 79% (SD 4.4., range 68-88) at 5129m.

Fluid balance showed no significant change with altitude or relation to AMS.

Plasma osmolality did not change with ascent.

AVP and copeptin did not increase until extreme altitude (5129m) and both increased by a greater degree in those recording higher levels of perceived exertion. This was despite no change in osmolality which suggests a non-osmotic stimulus to their secretion.

Cortisol secretion increased at 5129m and may support the possibility that the rise in copeptin and AVP was related to physiological stress at extreme altitude.

Plasma normetanephrine increased with increasing altitude reaching a maximum of 1423.72 (786.0, 355–4159 pmol/L) at 5129m.

Thirst proved difficult to quantify and the visual analogue scale used showed no correlation with total body water, osmolality or AVP.

BNP (and NT-proBNP) increased with increasing severity of AMS as assessed by Lake Louise Score (LLS).

BNP (and NT-proBNP) were higher in those with a high PASP.
Hs-cTnT showed an increase with PASP and but no association with AMS.
Hs-CRP showed an increase with altitude but no association with AMS.
NGAL showed no association with change in altitude.
A consistent finding was an evident separation between subjects recording a low Borg score and those recording a Borg rating consistent with “hard work”. Subjects recording a Borg score $\geq$ 15 (“hard work”) had higher cortisol, AVP, copeptin and an increased LLS. This led to investigation of an additional hypothesis that a higher rating of perceived exertion will lead to an increase in reported AMS symptoms. This was found to be the case with significantly lower SpO_2 at rest, higher heart rates and higher LLS in those reporting a Borg RPE $\geq$ 15.

Phase 2

The difficulties of conducting a field study of this nature, especially where controlling exercise is so difficult, prompted investigation as to the comparison between laboratory conditions (normobaric or hypobaric hypoxia) with the real world.

Hypothesis:

1. There will be no difference in the response to hypoxia in normobaric, hypobaric or “real world” environments.

Methods:

Sixteen subjects were recruited and underwent cardiopulmonary exercise testing at baseline, at the Torino hut in the Italian Alps (3380m), in a normobaric hypoxic chamber and hypobaric chamber at equivalent simulated altitudes. Subjects performed 2 hours’ work on a cycle ergometer at 60% altitude adjusted maximal oxygen uptake. Data were collected on heart rate, oxygen saturations, oxygen uptake, RPE (Borg), LLS and lactate.

Results:

Sixteen subjects were recruited. Fifteen completed testing in the Alps, 14 in normobaric hypoxia and 6 in hypobaric hypoxia.

There were no differences in heart rate, RPE, oxygen uptake or lactate across the three hypoxic environments.

Summary:

During the course of this research there was a progression from trying to understand the importance of individual hormonal changes in the pathophysiology of AMS, to identifying the role of exercise as a very important factor and then looking at laboratory models to replicate the physiology of high altitude.

Acknowledgements

Work of this nature can only be undertaken, at a relatively late stage of a career, with the support and understanding of many people. I would like to acknowledge the support of the Defence Medical Services and friends and colleagues who have supported my absence on trips collecting data in Bolivia and the Alps, picking up additional clinical work while I have been away. The support of the DMS in

funding this line of investigation and recognizing its' importance to Defence has been of great support during the completion of these studies.

Without the academic supervision from Lt Col Dave Woods and Lt Col (now retired) Chris Boos I could not have achieved what I have. It has been an interesting and at times frustrating journey but one which I am pleased to have completed and I recognize the huge amount of effort they have contributed throughout this project. The statistical advice, especially with regard to measures of fluid balance and Bland Altmann plots from Chris was critical in completing chapter 4 of this thesis.

Within the University and Royal Victoria Infirmary I am in debt to Dr Steve Ball, Margaret Brodie, Anne Burnett and several others who have instructed me in assay techniques and kept track of thousands of samples from field studies. The annual appraisals by Dr Simon Baudouin and Dr Petros Perros have been both good fun and hugely informative, lending encouragement, direction and critical appraisal in equal measure. At James Cook, I am grateful for the assistance of Ashlie Pearson, for her help in checking and preparing the manuscript.

I acknowledge the collaboration with John O'Hara and others at Leeds Beckett University who shared the burden of the 3 way comparison study and provided the sports science expertise that made this part of the thesis novel and so very interesting.

This work is dedicated to my wife Nicola without whos' understanding of my obsession with mountains, mountaineering and physiology life would not be the joy that it is.

Authors' statement

The strategy used in developing this thesis was to submit papers relating to the work completed for individual chapters to peer reviewed journals. As a result of this a significant amount of the work has been published during the course of the MD work.

Key works used for the thesis are highlighted below;

Significant parts of chapter 4, notably the discussion on multi versus single frequency bioimpedence methods are published in Boos, C. J., D. A. Holdsworth, D. P. Hall, A. Mellor, J. O'Hara and D. R. Woods (2014). "Comparison of two methods of assessing total body water at sea level and increasing high altitude." Clin Physiol Funct Imaging 34(6): 478-484. This manuscript used data from the field study. I had a significant role in design, data collection and manuscript preparation. I deferred first and senior authourship to Drs' Woods and Boos as the statistical work and interpretation required considerable input from them. I acknowledge this elsewhere in the thesis.

The case report in chapter 5.5 has been previosuly published; Boos, C. J., D. A. Holdsworth, D. R. Woods, K. Green, J. Naylor and A. Mellor (2013). "Cardiac biomarkers and high altitude pulmonary edema." Int J Cardiol 167(3): e65-66. This was a case occurring during the field study and I was responsible for patient management, data collection and manuscript preparation. Dr Boos appears as first author as his echo skills and cardiology background are essential to interpreting the findings.

Data relating to the use of BNP and troponin as biomarkers in high altitude illness (chapters 5 and 7) are previously published as Mellor, A., C. Boos, D. Holdsworth, J. Begley, D. Hall, A. Lumley, A. Burnett, A. Hawkins, J. O'Hara, S. Ball and D. Woods (2014). "Cardiac Biomarkers at High Altitude." High Alt Med Biol, 2014, 15(4):452-458

During the course of planning the field study I was invited to submit a review fo the methodology used and difficulties to the Journal of the Royal Army Medical Corps. This work was subsequently published as Mellor, A. and D. Woods (2014). "Physiology studies at high altitude; why and how." J R Army Med Corps 160(2): 131-134. Parts of the manuscript are used for chapters 2 and 3.

The investigation of the relationship of AVP to copeptin (chapter 6) has been published as Mellor, A. J., C. J. Boos, S. Ball, A. Burnett, S. Pattman, M. Redpath and D. R. Woods (2014). "Copeptin and arginine vasopressin at high altitude: relationship to plasma osmolality and perceived exertion." Eur J Appl Physiol. 2015 Jan;115(1):91-8. doi: 10.1007/s00421-014-2994-7.

Data collected during the field study formed significant parts of chpater 9 (parts 1 and 2) and has been published as Mellor, A. J., D. R. Woods, J. O'Hara, M. Howley, J. Watchorn and C. Boos (2014). "Rating of Perceived Exertion and Acute Mountain Sickness During a High-Altitude Trek." Aviat Space Environ Med 85(12): 1214-1216.

Finally, the description of respiratory physiology in chapter 1.2 is taken from a review article in the JRAMC published by C. Sandberg and J. Naylor (JRAMC 2011; 157. 29-32). I was originally senior author on this paper and wrote the first draft manuscript before electing not to appear as an author. I am grateful to Drs Sandberg and Naylor for acknowledging my contribution and giving their consent to me using the text.

Abbreviations used

SpO_2	Oxygen Saturation As Measured With Pulse Oximetry
PiO_2	Partial Pressure Of Oxygen,
FiO_2	Fraction Of Oxygen In The Air
PB	Barometric Pressure
PH_2O	Saturated Vapour Pressure Of Water
SaO_2	Arterial Oxygen Content
VO_2 max	Maximum Oxygen Consumption
DMS	Defence Medical Services
SBP	Systolic Blood Pressure
HPV	Hypoxic Pulmonary Vasoconstriction
HAPE	High Altitude Pulmonary Oedema.
PAO_2	Alveolar Oxygen Tension,
PiO_2	Inspired Oxygen Tension,
$PACO_2$	Alveolar Carbon Dioxide Tension
RQ	Respiratory Quotient.
CSF	Cerebrospinal Fluid
HCVR	Hypercapnic Ventilatory Response
HA	High Altitude
AVP	Arginine Vasopressin
HVR	Hypoxic Ventilator Response
HCVR	Hypocapnic Ventilator Response
AMS	Acute Mountain Sickness
ESQ	Environmental Symptom Questionnaire
AMS-C	Acute Mountain Sickness – Cerebral score
HACE	High Altitude Cerebral Oedema
CXE	Caudwell Extreme Everest
NP	Natriuretic Peptides - Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP)
NT-pro BNP	N Terminal Prohormone of BNP
MI	Myocardial Infarction
APE	Acute Pulmonary Embolus

CABG	Coronary Artery Bypass Grafting
RVSP	Right Ventricular Systolic Pressure
PASP	Pulmonary Arterial Systolic Pressure
AVP	Arginine Vasopressin
SL	Sea Level
WDT	Water Deprivation Test
ACTH	Adrenocorticotrophic Hormone
RPE	Rating Of Perceived Exertion
HR	Heart Rate
NGAL	Neutrophil Gelatinase Associated Lipocalin
PRA	Plasma Renin Activity
AUC	Area Under The Curve
ROC	Receiver Operator Characteristics
Hs-cTnT	High-sensitivity Cardiac Troponin T
Hs-CRP	Highly Selective C Reactive Protein
AKI	Acute Kidney Injury
ARF	Acute Renal Failure
ECLIA	Electro-Chemiluminescence Immunoassay
SST	Serum Separator Tube
RIA	Radioimmunoassay
BIS	Bioimpedance Spectroscopy
TBW	Total Body Water
ECW	Extra Cellular Water
ICW	Intracellular Water

Contents

List Of Figures And Tables

Figures:

Tables:

Chapter 1. Introduction

Summary

The understanding of hormonal changes pertinent to high altitude illness relies upon an understanding of normal physiology at sea level and at high altitude. As exercise is an almost inevitable part of ascending to altitude the changes that occur with exercise are also of crucial importance.

This chapter provides an introduction to those changes in order to set the context of subsequent chapters.

1.1 Definitions of High Altitude

- Intermediate Altitude (1500m – 2500m)
  Physiological changes occur, SpO_2>90%, altitude illness possible but rare
- High Altitude (2500m – 3500m)
  Altitude illness common with rapid ascent
- Very High Altitude (3500m – 5800m)
  Altitude illness common, SpO_2<90%, marked hypoxia with exercise
- Extreme Altitude (>5800m)
  Marked hypoxia at rest, progressive deterioration, permanent survival not possible

1.2 Normal Physiology Of High Altitude

Unacclimatised lowlanders arriving at high altitude experience profound hypoxia. Oxygen saturations on arrival at 3800m average 90% and, after a trek to 5200m over several days, average 81% (Mellor, Boos et al. 2014). This physiological challenge brings about multisystem changes to adapt to hypoxia and maintain oxygen delivery. Many of these adaptations are beneficial but if unchecked can lead to the development of altitude illness.

Atmospheric pressure changes at high altitude with consequent reduction in the available fraction of oxygen. This effect is further exaggerated by the fact that inspired gases are warmed and moistened as they pass through the nose, mouth and trachea. The saturated vapour pressure of water is constant despite the change in pressure and this reduces the partial pressure of inspired oxygen still further as described in the equation below.

$PiO_2 = FiO_2 (PB – PH_2O)$

Where PiO_2 is the partial pressure of oxygen, FiO_2 is the fraction of oxygen in the air, PB is barometric pressure and PH_2O is the saturated vapour pressure of water.

At, for example, 5800m PB is 380mmHg and therefore the PiO_2 is reduced to 0.21(380-47) = 70mmHg. This contrasts with a PiO_2 of 0.21(760-47) = 150mmHg at sea level.

Adaptive mechanisms develop over time on exposure to hypoxia to mitigate against these effects and include cardiovascular, respiratory and endocrine responses.

1.2.1 *Cardiovascular Responses To Altitude*

Cardiac Output - On acute exposure to a hypoxic environment it is generally accepted that cardiac output increases. This makes good physiological sense; as the partial pressure of oxygen falls in the atmosphere then so does the arterial oxygen content (SaO_2) and in order to maintain oxygen delivery to the tissues cardiac output must increase.

Once acclimatised cardiac output returns towards sea level values both at rest and for any given work rate, except at a maximal level where cardiac output and maximum work rate is reduced (Reeves, Groves et al. 1987, Richalet 2010). This seems paradoxical but is probably due in part to the fact that maximum oxygen uptake (VO_2 max) is reduced at extreme altitude because oxygen transfer from the lung and to the tissue is limited by diffusion, rather than perfusion. This means that the uptake of oxygen at the alveolar/capillary membrane and offload at the capillary/cell membrane is limited by the properties of the membrane rather than the flow of blood to those areas (alternative explanations discussed below).

Heart Rate - Acute hypoxia causes an increase in both resting and exercising heart rate, due to increased sympathetic drive. The higher the altitude then the greater

the increase in heart rate. As a subject acclimatises the resting heart rate generally returns to that of sea level values up to an altitude of approximately 4500m. On exercise however, even in acclimatised subjects, heart rate for a given work load is greater than at sea level except at maximal exercise where maximal heart rate is reduced compared to sea level values.
There are a number of different theories as to why maximal heart rate should be lower at altitude compared to sea level. Pugh et al noted in 1964 that oxygen consumption at maximal heart rate at altitude was reduced compared to sea level values, suggesting for the given work rate much was achieved using anaerobic respiration (Pugh, Gill et al. 1964). Later Richalet and colleagues suggested the reduction is a physiological adaptation that reduces cardiac work in environments with limited oxygen availability - it has been shown that hypoxia down regulates β-adrenergic receptors in animal models (Richalet, Merlet et al. 1990). This adaptation of limited maximum heart rate seems likely to be beneficial given the diffusion limitation of oxygen uptake in the lungs.

Interestingly breathing oxygen at high altitude may reduce the heart rate for a given work load to below sea level values (Pugh, Gill et al. 1964). This may be due to the higher haemoglobin levels (see later) and therefore increased oxygen content in comparison to sea level.

Blood Pressure - Blood pressure shows a variable and highly individual response to acute exposure to altitude; overall there is usually an increase for the first few weeks when lowlanders travel to altitude. This is probably due to an increase in the sympathetic drive and vascular tone. Conversely lowlanders resident at altitude for some years show a decrease in both systolic and diastolic pressures and subjects with known systemic hypertension often show an improvement in blood pressure (Marticorena E 1969). On the Defence Medical Services (DMS) expedition to Aconcagua in 2007 resting systolic blood pressure (SBP) increased from 116 +/- 15 mmHg at sea level to 136 +/-13 mmHg at 4250m (Hooper, Levett et al. 2010). The Birmingham Medical Research Expeditionary Society similarly

report changes in SBP of 131+/-23 mmHg at sea level increasing to 145 +/- 23 mmHg at 3450m (Rhodes, Chesterman et al. 2011).

Pulmonary Circulation - Increases in pulmonary vascular resistance secondary to hypoxic pulmonary vasoconstriction (HPV) lead to pulmonary hypertension at altitude. This is seen in subjects exposed to acute hypoxia, acclimatised lowlanders and high altitude natives alike.
In certain disease states such as asthma and chronic obstructive pulmonary disease, HPV is of value in that it limits ventilation/perfusion mismatch i.e. in areas of the lung that are not being ventilated HPV occurs diverting blood away from these regions. However at altitude, with global hypoxia, HPV serves no purpose and is thought to be the major cause of high altitude pulmonary oedema (HAPE).

During Operation Everest II (Sutton, Maher et al. 1983) pulmonary vascular pressures were measured using pulmonary artery flotation catheters. The pulmonary vascular pressure gradient (mean pulmonary arterial – pulmonary wedge) was measured at different cardiac outputs at various altitudes. At rest the gradient increased with increasing altitude but most striking was the increase in slope of pressure gradient against cardiac output at the higher altitudes (Groves, Reeves et al. 1987). This indicates the marked increase in resistance at high altitude.

The pulmonary hypertension associated with altitude leads to right ventricular and atrial hypertrophy. Changes associated with this hypertrophy are often seen on the ECG with right axis deviation of the QRS axis and increase in amplitude of the P wave in lead II.

Exercising at Altitude - Cardiac output for a given workload returns towards sea level values in acclimatised subjects (except at maximal work rates), the heart rate remains increased and stroke volume is reduced.

Uniquely at extreme altitude both oxygen loading at the alveolus and offloading at the muscles becomes diffusion limited (West 1982, Calbet and Lundby 2009). The diffusion limitation at the muscle level is a relatively new concept, previously it was thought that the work able to be done by muscles at altitude was determined by the oxygen delivery to that muscle. It seems likely that diffusion is the limiting factor at extreme altitude even though the average distance over which oxygen has to diffuse from the capillary to the mitochondria is reduced due to muscle fibres reducing in size (Lundby, Pilegaard et al. 2004) and increasing capillary density (Mizuno, Savard et al. 2008).

Maximal Oxygen Consumption - Maximal oxygen consumption (VO_2 max) in acclimatised subjects at altitude is reduced. The reason is unclear but may be caused by a reduction in muscle mass or the increased blood viscosity at altitude leading to interference in capillary blood flow and oxygen exchange at the tissue level. Other reasons suggested include the diffusion limitation in the lung or exercising muscle (discussed above). An alternative explanation is that blood flow to the muscles of locomotion is reduced as a consequence of the increased demand of the respiratory muscles. Cibella et al looked at the increased oxygen needs of the respiratory muscles at altitude due to the increased workload. They found that at altitude breathing accounted for 26% of VO2 max compared to only 5.5% at sea level (Cibella, Cuttitta et al. 1999)

1.2.2 *Respiratory Changes*

Ventilation is regulated by three stimuli; carbon dioxide (CO_2), oxygen (O_2) and pH. These stimuli are sensed and transduced by peripheral and central chemoreceptors. The efferent response is co-ordinated by the medullary respiratory centres.

The carotid bodies are highly vascularised areas of tissue and respond to decreasing levels of oxygen dissolved in the blood. At normal arterial oxygen tensions they have little output but as the partial pressure falls below 8kPa output increases rapidly. The effector response to the detected hypoxia is increased

ventilation via the cardiorespiratory centre in the medulla. The resulting increased minute volume serves to reduce alveolar partial pressure of carbon dioxide. This increases the available alveolar oxygen as dictated by the alveolar gas equation (below).

$PAO_2 = PiO_2 – PACO_2/R$

Where PAO_2 = alveolar oxygen tension, PiO_2 is the inspired oxygen tension, $PACO_2$ = alveolar carbon dioxide tension and R = respiratory quotient.

This effect of increased minute ventilation due to hypoxia is known as the hypoxic ventilatory response (HVR).

The response to pH, and by extension carbon dioxide, is largely regulated by the central chemoreceptors. These chemoreceptors are on the ventrolateral surface of the medulla and respond to pH changes in the cerebrospinal fluid (CSF).

As plasma carbon dioxide levels rise the gas diffuses freely into the CSF and increases the concentration of H+ ions. The blood brain barrier is much less permeable to H+ ions and bicarbonate than it is to carbon dioxide. Thus changes in the partial pressure of carbon dioxide in arterial blood are reflected quickly in the CSF. The effector response of the central chemoreceptors is to increase ventilation via stimulation of the medullary respiratory centre. This is the hypercapnic ventilatory response (HCVR).

Prolonged exposure to the hypoxia of altitude causes a respiratory alkalosis. The result of this would ordinarily be to reduce respiratory drive via the peripheral chemoreceptors. Renal compensation occurs and HCO_3- is excreted in the urine. Subsequent loss of HCO_3- from the plasma creates a concentration gradient of HCO_3- from CSF to plasma and HCO_3- moves out of the CSF. This reduction in CSF HCO_3- causes an increase in free H+ ions in the CSF which then stimulate the central chemoreceptors which effects an increase in ventilation to reduce CO_2

levels and hence H+ levels within the CSF. Physiologically this occurs to ensure that CSF pH remains constant. With adaptation the level of CO_2 at which increased ventilation will occur is reset to lower levels and the HCVR response curve has been shifted to the left, producing a much more rapid response to smaller changes in CO_2 (Kellog 1963).

1.3 Altitude Illness

It is not surprising that, given the degree of physiological changes at high altitude (HA), specific health effects can occur. Altitude illness is typically classified as acute (onset within hours to a few days) or chronic (onset after years of habitation at HA). Chronic mountain sickness is characterised by polycythaemia and is not considered further.

Acute mountain sickness (AMS) is a collection of symptoms including headache, anorexia, nausea and vomiting, poor co-ordination with fatigue and difficulty sleeping. It is most frequently diagnosed according to the Lake Louise Score (Hackett and Oelz 1992). Pathognomic to the condition is the presence of a headache and the scoring system requires the presence of a headache and other symptoms in order to generate a score from 0 – 18 (table 3.1). A score of $\geq$3 is taken to indicate mild AMS and $\geq$6 severe AMS. This is purely a self-reported score and is the principal method used to diagnose the condition. This is described in more detail in chapter 2.

Diagnosis of AMS is based on a recent gain in altitude, a headache plus at least one of the other symptoms.
A total score of 3-5 is diagnostic of AMS, a score of $\geq$ 6 is diagnostic of severe AMS.

Symptom	Severity	Score
Headache	No headache	0
	Mild headache	1
	Moderate headache	2
	Severe headache	3
Gastrointestinal Symptoms	None	0
	Poor appetite or nausea	1
	Moderate nausea +/- vomiting	2
	Severe nausea +/- vomiting	3
Fatigue and/or weakness	Not tired or weak	0
	Mild fatigue or weakness	1
	Moderate fatigue or weakness	2
	Severe fatigue or weakness	3
Dizziness/lightheadedness	Not dizzy	0
	Mild dizziness	1
	Moderate dizziness	2
	Severe, incapacitating dizziness	3
Difficulty sleeping	Slept as well as usual	0
	Did not sleep as well as usual	1
	Woke many times, poor sleep	2
	Could not sleep at all	3

Table 1.1 Lake Louise Score for the Diagnosis of Acute Mountain Sickness

An alternative self-reported system is the AMS-C score of the Environmental Symptom Questionnaire (ESQ) (Sampson, Cymerman et al. 1983). The AMS-C score of the ESQ is assessed according to the method outlined by Sampson et al (Sampson, Cymerman et al. 1983). In essence the AMS-C score is composed of 11 questions graded from 0-5 covering lightheadedness, nausea, dizziness, headache etc. Each symptom is given a factorial weighting, the resultant scores

totalled and then multiplied by 0.1927 to give a score in the range 0-5. AMS is defined as a score ≥ 0.70.

AMS is usually a self-limiting disease and recovers after a rest at the new altitude or more rapidly with descent. Occasionally the condition leads to the more serious High Altitude Cerebral Oedema (HACE), which is usually preceded by AMS and probably part of a spectrum of disease (Hackett and Roach 2004). High altitude pulmonary oedema is a separate entity which results from pulmonary capillary damage and oedema secondary to raised pulmonary artery pressures (Hall, Duncan et al. 2011). The pathophysiological mechanisms underlying the development of AMS and indeed HACE are not completely understood. It does seem, however, relatively consistent that fluid retention and increased plasma aldosterone (which is responsible for sodium and fluid retention) occur in AMS (Hackett, Rennie et al. 1982, Bartsch, Maggiorini et al. 1991, Bartsch, Pfluger et al. 1991).

The incidence of AMS is largely related to the speed of ascent and ranges from 84% with tourists flying direct to the Everest View hotel in Namche Bazaar at 3860m to 30% at 6190m on Denali in North America (Barry and Pollard 2003). Predicting AMS is problematic. Factors such as age, obesity, pre-existing disease and fitness level are all poor indicators of HA performance. There is no simple relationship to hypoxia as measured by pulse oximetry, although post exercise oxygen saturations may be more useful (Karinen, Peltonen et al. 2008).

1.4 Natriuretic Peptide Physiology

1.4.1 *Normal Physiology*

Atrial Natriuretic Peptide (ANP), Brain Natriuretic Peptide (BNP) and C Type Natriuretic peptide (CNP) are structurally related peptides that, along with urodilatin comprise the natriuretic system. ANP, BNP and CNP share a 17-amino acid internal ring structure and have a role in fluid homeostasis, opposing the renin-angiotensin-aldosterone system and are secreted from cardiomyocytes (Liang, Kapoun et al. 2007). Atrial natriuretic peptide (ANP) was the first cardiac related

natriuretic peptide to be discovered. It is stored in granules in the cardiac atria with a high concentration in the atrial appendage and secreted in response to an increase in atrial distension. The main stimulus seems to be increased atrial transmural pressure. ANP is known to rise acutely under hypoxic conditions in both animal and human models.

BNP was first described in 1988 by Sudoh in porcine brain (Sudoh, Kangawa et al. 1988). It is very species specific (unlike ANP). BNP is stored in cardiomyocytes as proBNP, once release is stimulated proBNP is cleaved into the active hormone BNP and the inactive peptide NT-proBNP. 60-80% of BNP comes from ventricular tissue (Espiner 1994). Resting levels of BNP, in young people at sea level, are an average of 7.1 pg/ml (Woods, Hooper et al. 2011). Levels may rise within two minutes of a stimulus but are transistory as there is limited storage capacity. Plasma half-life is 22 minutes (Davidson and Struthers 1994).

Brain natriuretic peptide (BNP) is secreted as a prohormone and cleaved into the biologically active BNP and the more stable N Terminal prohormone (NT-pro) BNP. Comparative studies demonstrate that BNP and NT-proBNP give equivalent results in a large population of patients with cardiac disease (Park, Baek et al. 2010) and in healthy subjects at high altitude (Woods, Begley et al. 2012).

BNP levels rise with age. Mean BNP levels in males are (Felker, Petersen et al. 2006):

26.2 pg/ml in those aged 55-64 years.

31.0 pg/ml in those aged 65-74 years.

63.7 pg/ml in those aged 75 years and older.

Corresponding levels for NT-pro BNP means (male subjects) are (Galasko, Lahiri et al. 2005)

28 pg/ml in those 45-59

53 pg/ml in those over 60

Women without CHF tend to have higher BNP and NT-pro BNP levels than males of the same age

BNP levels show positive correlation with increases in left ventricular end diastolic pressure, pulmonary artery occlusion pressure and pulmonary artery pressure (Davidson and Struthers 1994). The most important stimulus to BNP release is cardiac stretch (Hall 2005) but BNP also increases with an increased sodium diet, increased blood pressure, chronic renal failure and with increases in catecholamines (Espiner 1994, Hall 2005). Recently BNP levels have been linked to markers of inflammation (see below) and it may have use as a measure of the acute phase response rather than be a specific cardiac marker in some settings. The actions of BNP include the following;

- Renal – increase urinary excretion of sodium
- Cardiovascular system – decrease intravascular volume, decrease SVR, increase cardiac output, decrease right atrial pressure, pulmonary artery occlusion pressure (PAOP) and possibly coronary vasodilation
- Endocrine – inhibit aldosterone secretion, decrease renin activity
- Central Nervous System – possibly acts as a neurotransmitter in CVS control centres

A BNP rise of 20-30 pmol/l results in a two-fold increase in sodium excretion and suppressed renin and aldosterone activity. When infused into human subjects doses resulting in plasma levels 20 fold above baseline, but within the range seen in heart failure and other diseases, decrease mean arterial pressure, pulmonary artery occlusion pressures and right atrial pressures (Espiner 1994).

1.4.2 BNP As A Disease Marker

Due to the fact that BNP is released from the ventricles in response to ventricular stress most interest has focused on its use as a marker of heart failure. In this setting a BNP value of 100pg/ml is highly suggestive of cardiac failure and it can be used to discriminate between pulmonary or cardiac causes of shortness of breath (Felker, Petersen et al. 2006). Whether BNP or NT-pro BNP is measured

seems to have no relationship to its utility as a marker and both are used throughout the literature. The majority of the published literature focuses on the use of BNP and NT-proBNP in heart failure, both for risk stratification (Baggish, van Kimmenade et al. 2008) and response to treatment (Karlstrom, Alehagen et al. 2011).

Not surprisingly natriuretic peptides have also been investigated as markers of outcome after cardiac surgery and after myocardial infarction (MI) (Eliasdottir, Klemenzson et al. 2008, Fazlinezhad, Rezaeian et al. 2011). The levels of BNP recorded after acute MI are very high indeed 4436.63 (+/- 6188.2 pg/ml) whereas more subtle changes are reported with RV dysfunction after acute pulmonary embolus (APE) (527 – 1300 pg/ml being associated with RV dysfunction)(Pieralli, Olivotto et al. 2006). A pre-operative NT-proBNP of >1028 pg/ml in patients undergoing coronary artery bypass grafting (CABG) was associated with increased hospital mortality, severe circulatory failure and decreased long term survival (Holm, Vidlund et al. 2013). These findings are probably reflective of the fact that cardiac failure is an indicator of poor outcome after MI or a risk factor for cardiac surgery and is associated with a high BNP. However, the same remains true for non-cardiac surgery and other conditions (see below).

In patients with community acquired pneumonia, BNP has been demonstrated to be a powerful independent predictor of death and treatment failure with BNP levels of (median) 439.2pg/ml in the non survivors v's 114.3 in the survivors ($p<0.001$) (Christ-Crain, Breidthardt et al. 2008). Levels of BNP have also been shown to be high in rheumatoid arthritis and systemic lupus erythematosus despite no obvious cardiac disease (Karadag, Calguneri et al. 2007, Provan, Angel et al. 2008).

NT-proBNP has been used as a biomarker to assess risk of death in intensive care units (ICU). De Geer and colleagues found an odds ratio for 30 day mortality of 2.6 (95% CI 1.5 to 4.4) for those with NT-proBNP above 1380 ng/ml vs. those below that threshold (De Geer, Fredrikson et al. 2012). In patients with Systemic Inflammatory Response Syndrome following CABG, Kerbaul found very high levels

of NT-proBNP with peak values of 8887 pg/ml (range 2940 – 29372)(Kerbaul, Giorgi et al. 2004). BNP seems to specifically increase in septic shock rather than shock per se (Wolff, Haase et al. 2007).

BNP has a role in modulating the inflammatory mediators in macrophages (Chiurchiu, Izzi et al. 2008) and with inflammation, BNP is stimulated at the transcriptional and translational levels (Ogawa and de Bold 2012). This may explain the high levels in septic shock and other conditions where there is no obvious cardiac pathology. BNP (and NT-proBNP) correlates with other inflammatory markers such as interleukin 6, C reactive protein and tumour necrosis factor (Mehra, Uber et al. 2006, Haugen, Chen et al. 2007, Vila, Resl et al. 2008, Guo, Barany et al. 2009, Jensen, Ma et al. 2010, Vaz Perez, Doehner et al. 2010, Moraes, Ribeiro et al. 2013).

In summary BNP seems to act as a modulator of acute inflammation as well as being released in response to ventricular stretch.

1.4.3 BNP At High Altitude

BNP has a role in inducing a natriuresis and diuresis (albeit mild) and reducing renin and aldosterone secretion (Hall 2005) which are effects that should be beneficial at HA. BNP also has pulmonary vasorelaxant activity, attenuating acute hypoxic pulmonary vasoconstriction and reducing pulmonary hypertension, (Cargill and Lipworth 1995). This effect could protect against the development of HAPE which is associated with an increase in capillary pressure and pulmonary vasoconstriction (Maggiorini, Melot et al. 2001). These actions make it a likely candidate hormone to be involved in acclimatisation.

In animal models, isolated rat hearts increase ANP and BNP with an acute (30 minutes) hypoxic exposure (Toth, Vuorinen et al. 1994). Cultured adult rat cardiomyocytes have demonstrated an increase in BNP mRNA expression after the induction of systemic hypoxia (Weidemann, Klanke et al. 2008), as have rats exposed to hypobaric hypoxia equivalent to an altitude of 5500 metres (Nakanishi,

Tajima et al. 2001). Ex vivo studies of human ventricular myocytes cultured under hypoxic conditions also demonstrated an increase in the synthesis and secretion of BNP (Casals, Ros et al. 2009). Results of in vivo human studies are mixed; in one report involving 10 human subjects there was no apparent rise in BNP despite oxygen saturations of 75-80% being induced (Cargill, McFarlane et al. 1996) whilst a more recent study with humans exposed to acute hypoxia, inducing oxygen saturations (SpO_2) of around 82% over 60 minutes, a 9% rise in NT-proBNP was reported (Due-Andersen, Pedersen-Bjergaard et al. 2008). Both these studies involved breathing hypoxic gas mixtures in normobaricity.

Considering these findings, and that there are BNP receptors on the adrenal gland (Nawata, Ohashi et al. 1991) and that BNP causes a reduction in aldosterone secretion from the adrenals (McGregor, Richards et al. 1990, Hall 2005) (which as discussed above has beneficial effects at HA) it is not surprising that there has been recent investigations regarding BNP at HA. BNP rises with prolonged exercise under normoxic conditions (Hew-Butler, Noakes et al. 2008) and ANP, a related peptide, rises in acute hypoxia and altitude illness (Bartsch, Shaw et al. 1988, Cargill, McFarlane et al. 1996). It therefore would seem reasonable to expect that HA would lead to a rise in BNP.

However, two field studies have found no increase in BNP or NT-proBNP measured during rest at high altitude. Feddersen and colleagues (Feddersen, Ausserer et al. 2009) found no rise in BNP at rest measured the day after arrival at a new altitude with ascent up to 5050 m and SpO_2 of 84.5+/-1.3%. Similarly, another study (Toshner, Thompson et al. 2008) found no rise in NT-proBNP in 10 healthy subjects at 5200 m. These subjects had ascended to 5200 m by vehicle following a 5 day acclimatization period at 3650 m. Samples were again taken at rest with a mean SpO_2 on the day of sampling of 77.6% i.e. lower than that associated with a rise in NT-proBNP with acute hypoxia (Due-Andersen, Pedersen-Bjergaard et al. 2008). Moderate exercise at SL is generally not associated with a rise in BNP or NT-proBNP (Nicholson, Richards et al. 1993, Marumoto, Hamada et al. 1995) and neither is high intensity short duration exercise (Hew-Butler, Noakes

et al. 2008). However, prolonged endurance exercise at SL such as running an ultramarathon (Scharhag, Herrmann et al. 2005, Hew-Butler, Noakes et al. 2008) or prolonged cycling (Scharhag, Meyer et al. 2008) may cause a rise in BNP or NT-proBNP. It is notable that Scharhag found that NT-proBNP levels measured the day after intense cycling have returned to baseline, pre-exercise levels, a fact that could explain the negative findings of Feddersen and colleagues at HA who only measured BNP at rest the day after arrival at a new altitude. Similarly, the acclimatisation at 3650 m followed by vehicular ascent to 5200 m with samples taken at rest the next day could explain the negative findings of Toshner and colleagues. Studies by the DMS hypoxia study group have investigated the BNP response with exercise in the field setting. In 2009 we undertook a field study at altitude in Nepal during the course of a trek to Everest Base Camp, using an ascent profile of a typical recreational trek. BNP was measured using point of care monitors (Alere Triage Biosite Pro, Cheshire, UK) which reduced any reliance on electrical power or requirement to freeze samples. Following this we reported that BNP (mean+/-SEM) rises between sea-level (7.1+/-1 pg/ml) and 5150 metres (17.7+/-5.1 pg/ml, range 6.70-119 pg/ml, p=0.001) in a cohort of 23 subjects (Woods, Hooper et al. 2011). We also found that mean Lake Louise scores (mean+/-SEM) for those with a BNP response (defined as a rise in BNP above 5pg/ml) versus no BNP response at 5150 m were significantly different: 3.3+/-0.5 vs. 0.75+/-0.5 (p=0.034) on day 10 and 3.3+/-0.4 vs. 0+/-0 (p=0.003) on day 11. The major difference in our study was that the research took place during a trek, with BNP samples taken on completion of that exercise stimulus, rather than a study where mechanized transport had been used to gain altitude.

In 2011 we ran a second field study with 20 subjects repeating the same trek to Everest base camp. This time blood samples were taken, centrifuged and frozen in addition to using a point of care monitor. BNP and NT-proBNP (pg/ml, mean+/-SEM) rose significantly from Katmandu (approximately 1300m) (9.2+/-2 and 36.9+/-6.6 respectively) to arrival at 4270 m after exercise (16.6+/-4 and 152+/-56.1, p=0.008 and p<0.001 respectively) and remained elevated the next morning at rest (28.9+/-9 and 207.4+/-65.1, p=0.004 and p<0.001 respectively). At 5150,

immediately following ascent to and descent from 5643 m BNP and NT-proBNP were 32.3+/-8.8 and 301.1+/-96.3 ($p=0.003$ and $p<0.001$ versus Kathmandu values respectively) and at rest the following morning were 33.3+/-9.7 and 258.9+/-89.5 ($p=0.008$ and $p=0.001$ versus Kathmandu values respectively). NT-proBNP and BNP correlated strongly at 5150 m (rho 0.905, $p<0.001$ and rho 0.914, $p<0.001$ for resting and post-exercise samples respectively (Woods, Begley et al. 2012). At 5150 m BNP levels were significantly higher among the four subjects with severe (LL score>6) AMS (58.4+/-18.7) compared to those without (BNP 22.7+/-8.6, $p=0.048$). There were significant correlations between change in body water from baseline to 5150 m with both BNP and NT-proBNP (rho 0.77, $p=0.001$, rho 0.745, $p=0.002$ respectively). This data suggests that exercise is important in provoking a BNP response and that the BNP response may in part be associated with fluid retention. As BNP is a natriuretic peptide and would ordinarily stimulate a diuresis rather than causing fluid retention it seems likely that an additional mechanism, such as myocardial stretch, may have a role.

1.5 Arginine Vasopressin Physiology

1.5.1 *At Sea Level*

Arginine vasopressin (AVP) is a hormone concerned with volume and osmolality maintenance. It is also called antidiuretic hormone as it was originally thought that its actions were the result of two separate substances one vasopressor and one antidiuretic.

AVP is synthesised in cells which have their cell bodies in the hypothalamus and axons projecting into the posterior pituitary, the magnocellular neurons. The cell bodies in the hypothalamus are divided into supraoptic nuclei and paraventricular nuclei. 80-90% of the supraoptic nuclei secrete AVP. The paraventricular nuclei have a more complex neuroendocrine function and a subgroup of these (parvocellular neurons) release AVP to the posterior pituitary via the pituitary portal system which acts on the adenohypophysis leading to an increase in adrenocorticotrophic hormone (ACTH) and subsequently cortisol. This is an important part of the “stress response”. Vasopressin is a nonapeptide with a 6

amino acid ring and a 3 amino acid tail. It is synthesised as a precursor molecule consisting of the nonapeptide, hormone specific neurophysin and an additional glycopeptide (copeptin).

There are two main stimuli to AVP secretion; changes in plasma osmolality and changes in blood pressure or plasma volume. These stimuli act on the magnocellular body to generate an action potential which travels down the long axon to the posterior pituitary. An increase in Ca^{2+} causes secretory granules to then fuse with the cell membrane and be extruded. At physiological pH there is no binding of the AVP to its' neurophysin so all AVP is free in the circulation. Synthesis and transport of AVP to the posterior pituitary are linked which increases transport in response to increased synthesis. There is, however, asynchronicity in these events such that it takes a couple of days for a peak level of mRNA transcription which means that stores of AVP become depleted and may take a while to be replaced after a prolonged stimulus. The main action of interest to fluid balance at HA is the action to facilitate the reabsorption of water by increasing the permeability of the distal collecting tubule mediated via V2 receptors. When AVP binds to V2 receptors in the collecting duct cyclic AMP is activated which brings about the opening of water channels "aquaporins" which allow water to pass into the hypertonic renal medulla. Dissociation of AVP then decreases cyclic AMP levels which causes internalisation of the aquaporins. There is both an acute (minute by minute) response and a chronic increase in the number of channels in response to prolonged stimulation.

Receptor	Action
V1	vasoconstriction
V2	increase in permeability of collecting ducts and an increase in Von Willebrand Factor and Factor VIII
V3 (or V1b)	increase in ACTH secretion

Table 1.2 Actions of AVP

AVP also has a role (along with angiotensin II) in regulating thirst. Thirst is stimulated by changes in osmolality and plasma volume.

There is a rapid, linear change in AVP in response to changes in osmolality whereas a 10% change in blood volume or blood pressure is required to make a difference. Changes in volume amplify the response of AVP to a given change in osmolality. Once blood volume has been lost and BP cannot be maintained there is a sudden and exponential increase in AVP.

The primary function of AVP is in water homeostasis and maintaining osmolality. Sensors in the "osmostat", an area of the organum vasculosum of the lamina terminalis (OVLT) and in the wall of the 3rd ventricle are sensitive to changes in osmolality. These areas are outside the blood brain barrier. Clinically, damage to OVLT e.g. in head injury leads to an inability to maintain osmolality. Normal plasma osmolality is 280 – 295 mOsm/Kg H_2O and is predominantly due to Na+. Basal levels of AVP are maintained at 0.5-2.0 pg/ml. AVP release is very sensitive to changes in plasma osmolality with a 1% change in plasma osmolality leading to a change in AVP release, water reabsorption and increasing urine osmolality. If there is no AVP urine volume is 18-20 l per day, with 0.5-1 pg/ml urine volume is reduced to 4 l per day.

Of relevance to high altitude medicine are the facts that glucocorticoids inhibit AVP and dexamethasone is an important treatment for AMS and HACE (there is a rise in cortisol at extreme altitude) (Woods, Davison et al. 2012) and secondly, that nausea and vomiting are a potent stimulus for AVP secretion. The latter are a feature of AMS and may amplify fluid retention and create a vicious spiral of worsening symptoms.

1.5.2 *Copeptin*

Copeptin is a 39 amino acid long glycopeptide first described by Holwerda in 1972 (Holwerda 1972). AVP is secreted as a 164 amino acid prohormone consisting of

a signal peptide, AVP, neurophysin II and copeptin. Copeptin forms the C terminal part of pro AVP and can be referred to as CT-proAVP.

As it is a prohormone it is much more stable in plasma than AVP and also stable at room temperature and far easier to assay than AVP. Whereas AVP needs to be measured by radio-immunoassay over at least 3 days, copeptin has an automated assay taking 3 hours. More importantly for field studies Morgenthaler showed only a 20% loss in recoverable copeptin after 7 days storage at room temperature (Morgenthaler, Struck et al. 2006).

Copeptin levels in healthy volunteers are 1-12 pmol/l (median <5pmol/l) with men having consistently slightly higher values than women (by 1 pmol/l). There is no correlation with age. In the setting of exercise AVP has been shown to correlate with copeptin during endurance running events and in exercise tests on patients with cardiovascular disease (Maeder, Staub et al. 2010, Hew-Butler, Hoffman et al. 2011). Copeptin has not been measured at high altitude nor compared with AVP release at HA.

In shock states the non-osmotic release of AVP is mirrored by increases in copeptin. In a baboon model of haemorrhage copeptin increases from 7.5 pmol/l to peak at 269 pmol/l and closely follows AVP (Morgenthaler, Muller et al. 2007). Clinically copeptin rises quickly after a myocardial infarction and is higher in those who die. Copeptin has also been shown to predict outcome in respiratory tract infections, strokes and head injury (Muller, Morgenthaler et al. 2007, Kleindienst, Brabant et al. 2010, Morgenthaler 2010). AVP also increases after acute MI and it is unclear whether this is due to the stress response or as a result of cardiac dysfunction or relative under-filling.

1.5.3 *At High Altitude*

AVP is the key hormone involved in reducing free water excretion at the kidney and is crucial in determining fluid balance at SL. Normally at SL a rise in osmolality or a marked decrease in plasma volume would lead to a rise in AVP secretion from

the posterior pituitary and a reduction in free water loss at the kidney thereby maintaining osmolality. It seems intuitive that changes in this system will be important at high altitude where an initial diuresis is crucial in acclimatization. Plasma osmolality rises at HA, secondary to the diuresis (Blume, Boyer et al. 1984, Bestle, Olsen et al. 2002, Maresh, Kraemer et al. 2004). However in spite of this most of the literature reports no accompanying rise in AVP in the normal process of acclimatisation. In the presence of a documented rise in osmolality from 291 mOsmol/kgH_20 at SL to 299 mOsmol/kgH_20 at 4300m (after two days) and 302 (after 20 days) plasma AVP did not change (Maresh, Kraemer et al. 2004). Another report demonstrated a rise in osmolality from 290 mOsmol/kgH_20 to 295 mOsmol/kgH_20 at 5400m and 302 at 6300m (with an average 26.5 days above 5400m) with no accompanying rise in AVP (Blume, Boyer et al. 1984). Such evidence suggests a reset osmotic threshold for AVP release that again facilitates the diuretic process at HA that allows a reduction in plasma volume and hence increase in oxygen carrying capacity of a given volume of blood. Other studies support the notion that at HA a given vasopressin concentration requires a higher osmolality compared with SL. Bestle and colleagues found that over eight days at 4559m AVP was suppressed compared to SL despite an increase in plasma osmolality from 291 mOsmol/kgH_20 to 296 mOsmol/kgH_20. This was associated with a reduced urine volume in the first two days. These subjects were transported to this altitude and all had AMS on arrival. Some studies have even found a reduction in AVP on acute exposure to moderate altitude (2000m) (Porchet, Contat et al. 1984). Interestingly, high-altitude natives (2,600 m) have a greater resting AVP than sea-level natives (Ramirez, Pineda et al. 1998), again suggesting a dynamic hormonal response dependent on length of HA exposure. One study has gone further and examined the AVP response to water deprivation (which would normally stimulate a rise in AVP) at HA. The response to water deprivation appears to remain intact at HA. A 24 hour water deprivation test (WDT) with acute altitude exposure (day 2 at 4300m) and prolonged altitude exposure (day 20 at 4300m) still caused a rise in AVP in response to a rise in osmolality (Maresh, Noble et al. 1985). Interestingly though the rise in AVP with acute altitude exposure peaked at 16 hours and then fell to baseline during WDT but persistently

rose throughout WDT after prolonged altitude exposure. Further the increase in AVP to higher osmolalities (>300 mOsmol/kgH_20) was greater after more prolonged exposure, demonstrating a change in the relationship between AVP and osmolality dependent on acclimatisation. This may reflect the greater osmolality achieved in the latter WDT but could also reflect a dynamic change in AVP response to plasma osmolality according to acclimatisation. In other words, despite the WDT inducing an acute rise in AVP, the AVP response is "capped" with acute altitude exposure to restrict water retention. However, with further acclimatisation this effect is lost, presumably because the desperate need to reduce plasma volume to maintain oxygen delivery per given volume of blood has been overridden by other factors, such as a primary increase in red cell mass. Although it is suggested that plasma volume re-expands with chronic exposure to HA the osmolality in this study was 291 mOsmol/kgH_20, 299 mOsmol/kgH_20 and 302 mOsmol/kgH_20 at SL, day 2 and day 20 respectively. Rather than a re-expansion of plasma volume with prolonged exposure there may simply be a levelling off once initial acclimatisation has occurred.

AVP has been implicated in AMS (Hackett, Forsling et al. 1978, Blume, Boyer et al. 1984, Bartsch, Maggiorini et al. 1991, Loeppky, Icenogle et al. 2005). With acute (8-12 hour) exposure to a simulated 4880m altitude, subjects with high AMS scores (n=16) versus those with low AMS scores (n=16) demonstrated a rise in AVP at rest within 90 minutes of exposure. This was followed by a reduced urine output within 3 hours and subsequent fluid retention. In those subjects with AMS a positive fluid balance of 1.2 l was recorded versus a negative balance of 0.7 l in those without AMS. The low AMS score subjects with the negative fluid balance appear likely to be due to the fall in AVP that was recorded in those subjects (Loeppky, Icenogle et al. 2005). These findings are very supportive of the central role an early diuresis has in acclimatisation, albeit in a very acute setting, and the influence the AVP response has is critical to this. Earlier evidence is supportive of this concept: a more pronounced AVP response in subjects with AMS symptoms after 3–4 hours of simulated HA has previously been recorded (Claybaugh, Wade

et al. 1982); and a tendency for higher AVP in HAPE sufferers versus controls has also been suggested (Cosby, Sophocles et al. 1988).

1.6 Physiological Effects of Exercise

In order to assess changes related to HA it is important to be able to review the results in terms of normal "sea level" responses to exercise. In this way investigations can separate the HA specific changes to those related to exercise, exercise being an intrinsic part of many (if not all) recreational trips to HA. Exercise produces changes in cardiorespiratory parameters that are beyond the scope of this introduction. The changes in specific hormones and factors that may be of interest in altitude illness will be considered at sea level and at HA.

1.6.1 *Effects of Exercise at Sea Level*

During exercise blood flow to the muscle is increased. This is largely achieved by local factors and vasodilation but is augmented by neurohumoral mechanisms that drive an increase in cardiac output and alterations in blood flow (e.g. favouring muscle over gut).

Key mechanisms that will be considered as relevant to altitude illness are:

- Cortisol/ACTH "stress response"
- Catecholamine response
- AVP response

Cortisol response

The effects of cortisol are to increase gluconeogensis, free fatty acid mobilisation and glycogen storage. The hormone also maintains circulatory function and is a key hormone involved in the adaptation to stress. Cortisol can, therefore, be expected to rise with exercise. One of the key features of the stress response is to retain salt and water, the converse adaptation required for successful acclimatisation to altitude.

The increase in cortisol with exercise depends upon both intensity and duration of exercise stimulus. Bonen found that 10 to 30 minutes of gentle exercise at 3mph produced no change in cortisol (measured as urinary cortisol excretion) whereas the same duration of exercise at 7.5mph produced a significant elevation in cortisol excretion with 30 minutes (Bonen 1976). Van Bruggen and colleagues found that cortisol responses increased significantly for both serum (40.4%; P = .001) and saliva (170.6%; P = .007) only in response to high-intensity exercise (VanBruggen, Hackney et al. 2011). In a progressive exercise test to exhaustion cortisol significantly ($p \leq 0.05$) increased from 284 +/- 38 nmol/L at rest to 311 +/- 39 nmol/L at exhaustion but was not significantly elevated at a workload equivalent to 60% of VO_2 max (Buono and Yeager 1991). Salivary cortisol does not rise with low and moderate (at a workload equivalent to 44.5 and 62% VO_2 max) effort, but does rise significantly with more strenuous (equivalent to 76% VO_2 max) exercise (Jacks, Sowash et al. 2002).

Cortisol exhibits diurnal variation and this has an influence on changes in relation to exercise. Exercise related changes are blunted (i.e. not additive) to other diurnal variation. Exercise after a midday meal, when cortisol levels rise produces a much reduced increase in cortisol when compared to morning exercise (Brandenberger and Follenius 1975). The importance of taking into account diurnal variation is important. Hill and colleagues found a modest increase (5.7%) in cortisol at 40% VO2 max effort but when account was taken of diurnal variation they concluded that cortisol levels actually fell in response to exercise at that intensity (Hill, Zack et al. 2008).

Catecholamine response to exercise

Catecholamines increase heart rate, myocardial contractility, respiratory rate, mobilise energy sources and have a myriad of other effects beneficial when exercising. It is therefore not surprising that catecholamine levels increase with exercise.

Even with very brief (but severe) exercise, catecholamine levels are raised. Brooks found that a 30 second stimulus at a high power output of 424.8 +/- 41.9 W (mean +/- SD) the plasma catecholamine concentrations increased from 2.2 +/- 0.6 to 13.4 +/- 6.4 nmol.l-1 ($p<0.001$) and 0.2 +/- 0.2 to 1.4 +/- 0.6 nmol.l-1 ($p<0.001$) for norepinephrine and epinephrine respectively (Brooks, Burrin et al. 1988). Catecholamines start to increase at very low intensity exercise (with norepinephrine increasing before epinephrine) and this increase continues in line with exercise intensity to exhaustion. Concentrations of norepinephrine can reach 40x resting vbalues after a 400m race (Zouhal, Jacob et al. 2008).

The timing of catecholamine measurement is also important as levels continue to increase after exercise with values increasing tenfold during the recovery period (Dimsdale, Hartley et al. 1984). In a review of the literature Zouhal concluded that there is an enhanced catecholamine response in well trained individuals, a phenomena referred to as the "sports adrenal medulla". The same review also concluded that a variety of factors have a significant effect on catecholamine levels including posture, which muscle groups were involved in the exercise test, emotional state and familiarity with the test environment. Gender has no significant effect on catecholamine levels in untrained subjects, at rest or with exercise (Zouhal, Jacob et al. 2008).

AVP and sea level exercise

AVP is the main hormone involved in maintainance of osmolality during exercise. Factors such as fluid restriction or avaliability of fluid during exercise will have a bearing on osmolality changes but it also appears that there are non-osmotic stimuli that affect AVP levels. Hew-Butler in two studies showed a 3-4 fold rise in AVP during ultramarathon running despite no change in plasma sodium (Hew-Butler, Jordaan et al. 2008, Hew-Butler, Hoffman et al. 2011). A similar magnitude of change and evidence of non-osmotic stimuls to secretion has been found by other authors, Melin reported a 4.8 fold increase in AVP with exercise at a level equivalnet to 80% VO_2 max (Melin, Eclache et al. 1980) whilst Inder et al found a higher rise in AVP during exercise to exhaustion despite no change in osmolality

as exhaustion was reached (Inder, Hellemans et al. 1998).

1.6.2 Effects of Exercise at High Altitude

Few studies have examined the endocrine response to exercise in hypoxia. Bouissou found that hypoxia provoked a two fold increase in adrenocorticotrophic hormone (ACTH) levels during exercise (breathing a gas mixture equivalent to 3000m altitude) (Bouissou, Fiet et al. 1988). Interestingly there was a lack of response, in terms of cortisol, which did not change. This suggests a blunting of the cortisol response to ACTH at altitude. In previous work, in a large number of trekkers at up to 5000m, we have shown a reduction in cortisol at 4270m before an increase at higher altitudes (5000m). This is interesting in that a reduced cortisol response to ACTH would be beneficial and prevent a steroid induced retention of salt and water. In this study there was also a weak inverse correlation between SpO_2 and cortisol (rho=-0.451, p=0.004) at 5000m. Although a lower SpO_2 may be a stressor that increases cortisol, the correlation, albeit statistically significant, was not a particularly strong one and more subjects would be needed to confirm this finding (Woods, Davison et al. 2012).

Catecholamines increase with exercise in hypoxia in both human (Clancy, Critchley et al. 1975, Strobel, Neureither et al. 1996) and animal experiments (Warner and Mitchell 1991). Norepinephrine levels may contribute to the enhanced respiratory drive that is seen in hypoxia and this is supported by the fact that yohimbine (a pre synaptic alpha 2 blocker which enhances norepinephrine secretion) increases ventilatory response in humans (Clark, Galloway et al. 1997). Acid base changes can affect catecholamine secretion with acidosis potentiating catecholamine release. At altitude the usual acid base position is that of a compensated respiratory alkalosis so no net effect can be anticipated (Goldsmith, Iber et al. 1990).

Interestingly pre-existing anxiety traits may have an impact on catecholamine levels at higher exercise intensities (Peronnet, Blier et al. 1986). This may be particularly relevant at HA, where anxiety is a common feature.

The AVP response to exercise may also be important. AVP increases with exercise at altitude (Appenzeller and Wood 1992, Olsen, Kanstrup et al. 1992) and may contribute to water retention. This effect may have pathological consequences: 30 minutes of exercise on arrival at HA (4559 m) induces a greater rise in AVP in those with AMS than those without (Appenzeller and Wood 1992, Olsen, Kanstrup et al. 1992). This could lead to a “viscious cycle” of illness increasing stress and secretion of antidiuretic hormones, increasing salt and water retention and thus potentiating AMS.

One interesting findng of relevance is that AVP levels are suppressed by dexamthasone, which is a highly effective treatment for AMS and HACE (Coiro, Volpi et al. 2011).

In summary ANP and possibly BNP rise in hypoxia. BNP also rises as a result of inflammatory processes. Previous studies of BNP at high altitude have not produced a clear conclusion and exercise seems to be an important element in the BNP response. NP changes may also be related to fluid retention with consequent atrial and ventricular distension. AVP is a key hormone in the regulation of fluid balance and this appears to be suppressed (leading to a diuresis) at HA. Measuring AVP is difficult whereas the C terminal part of the proAV hormone (copeptin) can be measured by a simple assay and corrleates well with AVP in a variety of settings. This has not been investigated at HA. The effects of exercise complicate the endocrine response to hypoxia by creating an additive physiological stress. AVP and cortisol both have dual roles in fluid balance and stress response, whilst catecholamine and BNP release are linked.

Chapter 2. Diagnosis of High Altitude Illness; Why a Biomarker is Important

2.1 Introduction

2.2 Biomarkers for AMS

2.1 Introduction

Incidence of AMS, HACE and HAPE.

As can be seen in figure 2.1 the incidence of AMS varies substantially in different mountainous areas of the world. This is related largely due to the altitude gained, ease of access and rapid transport to high altitude. In Alaska where the ascent of Denali (6194m) requires a long slow ascent over a glacier from around 2500m the incidence is relatively low despite the high altitude eventually gained. By contrast in Hawaii where rapid road transport can be used to access an observatory at 4205m the incidence is 80% (Barry and Pollard 2003).

On Kilimanjaro in Tanzania, Karinen studied 130 trekkers attempting to climb to the 5895m summit. Overall the incidence of AMS was 75% and the incidence of HACE was 18% (Karinen, Peltonen et al. 2008). As 52,000 attempt to climb Killimanjaro annually the numbers at risk of potentially fatal HACE are very significant. However, despite this very high risk of HACE the number of deaths on the mountain is reportedly around 10 climbers per year (various internet sources) suggesting Karinen over diagnosed the problem or that local factors such as heightened awareness or ease of descent mitigates the risk.

In a previous study, conducted in Nepal by the DMS, out of 20 subjects, five had AMS at 3400m, and at 4300 m seven subjects had AMS, one of which was severe. At 5150m seven subjects had AMS, with four subjects developing severe AMS and two further subjects with mild AMS after a brief trek to Kalapathar (5600m)(Woods, Begley et al. 2012).

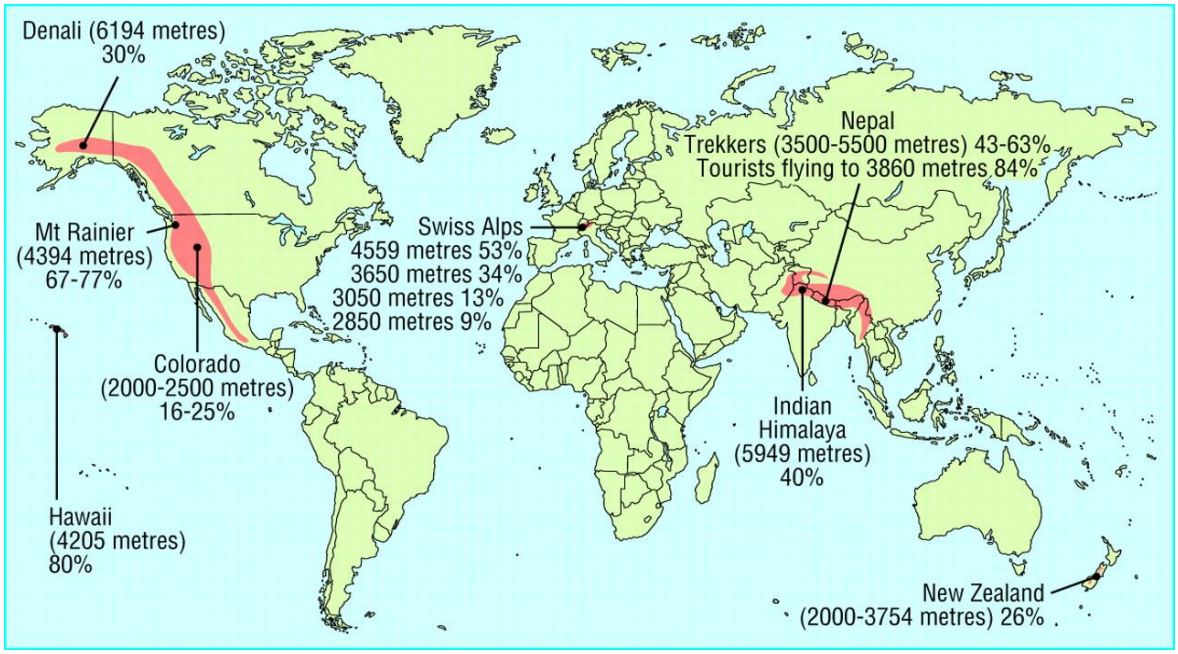


Figure 2.1 Incidence of AMS globally (Barry and Pollard 2003)

AMS is not only a problem at extreme altitude with 25% of conference attendees at a medical conference in the Rocky Mountains at elevations of around 2000m (Montgomery, Mills et al. 1989) reporting symptoms attributable to AMS, half of these took medication. With more than 120 million people visiting the European Alps each year at similar altitudes (source - http://wiki.answers.com/Q/How_many_people_go_to_the_alps_each_year?#slide=2 accessed Jan 2014) this is a significant public health problem.

Potential cost implications

The British Mountaineering Council has recently investigated helicopter rescue in Nepal and found (in terms of diagnosis of significant illness) :

“The picture becomes even more confused when the evacuated trekker reaches Kathmandu. A well-regarded doctor working at Kathmandu clinic that sees many evacuated trekkers splits patients arriving by helicopter into three categories: 20 per cent definitely required evacuation (usually because of HAPE or HACE); 60 per cent fell into a range of uncertainty (diagnosing altitude conditions is very difficult

once the patient is back in oxygen-rich air); and 20 per cent didn't require evacuation at all.

The numbers are striking. The doctor, who requested anonymity, reports seeing up to 15 patients evacuated by helicopter every day in the peak season. That means at least three have been flown unnecessarily at a cost of around $5,000 each. And that's just one clinic." (https://www.thebmc.co.uk/helicopter-rescue-nepal accessed Jan 2014).

A test that excluded high altitude related illness would be as useful as a test to diagnose the condition in reducing this cost burden as well as reducing the risk to rescuers.

Military Importance

Since the end of the cold war there has been a shift in conflict from large organised Armies to smaller "asymmetric" threats from ideologically motivated groups rather than nation States. It stands to reason that these groups will seek shelter in inhospitable regions, away from easy surveillance and communication and mountains provide just such a haven. During the initial war in Afghanistan US forces fought at HA to displace the Taliban. An important battle during this phase was Op Anaconda, fought in the Shahi-Kot valley, where 2700 US and Afghan soldiers faced 1000 Taliban fighters at altitudes of 2500 – 3500m. During this phase of the conflict 8% of casualties evacuated by the US were as a result of altitude illness (Peoples, Gerlinger et al. 2005).

The problem of identifying when troops are ready to deploy at HA is exacerbated by the fact that the only diagnostic criteria for AMS is the self-reported Lake Louise Score (Hackett and Oelz 1992). A self-reported score may not be useful with a cadre of highly motivated and committed soldiers and the score also shares many features with anxiety questionnaires. A biochemical test or predictor for altitude illness, or acclimatisation would be useful. The NATO Review of high altitude medicine in the Military (NATO RTM 146, Review of Military Mountain Medicine Technology and Research Barriers, Sep 2011, accessed on line 20 Dec 2014

http://natorto.cbw.pl/uploads/2011/9/$$TR-HFM-146-ALL.pdf) identifies the need for a capability for the "*early detection and diagnosis of altitude illness prior to onset of severe symptoms that is independent of the victim reporting their symptoms"* and this " *will reduce altitude illness impact on mission and medical support and evacuation requirements*".

Diagnosis of Acute Mountain Sickness

In order to conduct research into AMS the problem must be able to be defined in terms of specific symptoms, signs and clinical tests.

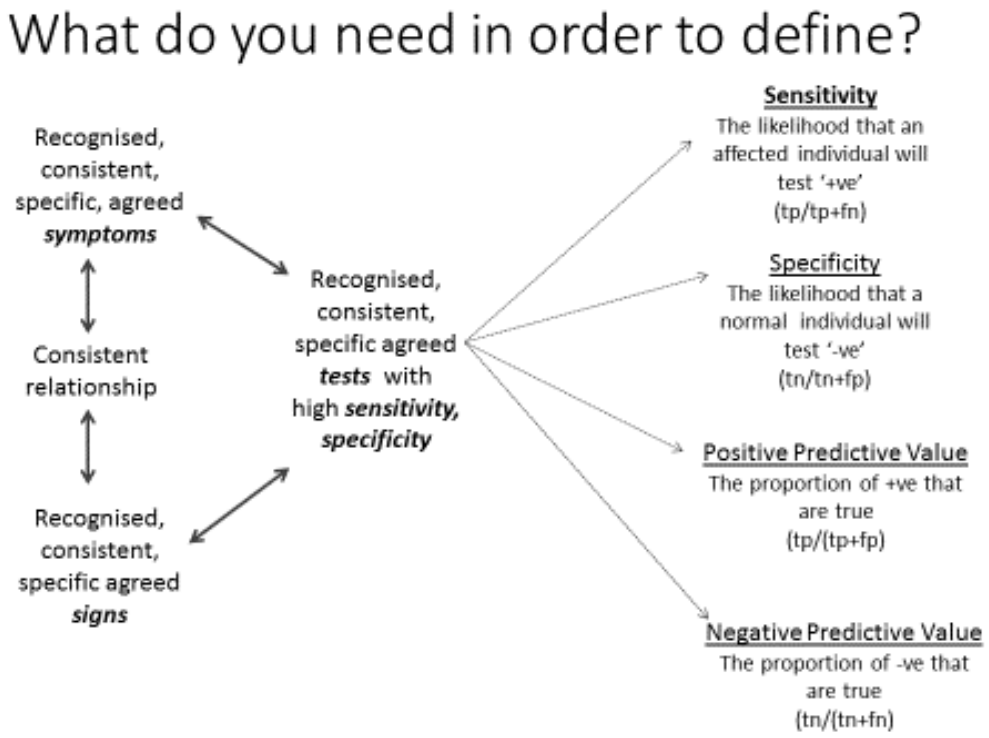


Figure 2.2 Defining the problem (with thanks to Prof Hugh Montgomery)

With AMS the major difficulty is the lack of a defining "gold standard" for diagnosis, with largely subjective symptoms, no hard signs and variable data from easily performed tests (eg SpO_2).

Lake Louise Score

The most frequently used standard for the diagnosis of AMS at the present time is the Lake Louise Acute Mountain Sickness Scoring System (Roach, Bartsch et al. 1993). This is widely used for both clinical diagnosis and research. The score was devised by expert opinion of a committee during the Eighth International Hypoxia Symposium in Lake Louise in 1993 (LLS) (Roach, Bartsch et al. 1993), and it was based in part on a previous discussion of criteria for AMS during the Seventh International Hypoxia Symposium in 1991 (Hackett and Oelz 1992).

The consensus statement states the following;
A diagnosis of AMS is based on a recent gain in altitude, at least several hours at the new altitude, and the presence of headache and at least one of the following symptoms: gastrointestinal upset (anorexia, nausea, or vomiting), fatigue or weakness, dizziness or light-headedness and difficulty sleeping (table 1.1). A score of three points or greater on the AMS self-report questionnaire alone, or in combination with the Clinical Assessment score, constitutes AMS. The Clinical Assessment score is made by an examiner who examines for changes in mental status, ataxia, and peripheral edema. Finally, there is a Functional Score, which can be self-reported or elicited by the examiner. The question asked is this: "Overall, if you had any symptoms, how did they affect your activity?" This is graded 0 through 3.

Most research papers only report the self-reported score values.

One weakness of using the LLS is that it is self-reported and therefore likely to be altered by a subjects' expectation or aspiration. Bartsch has noted that only 61% of the mountaineers reporting a LLS>5 actually said they felt ill (Bartsch, Bailey et al. 2004).

The mechanism behind high altitude headache and sleep disturbance may be distinct and the scoring system may be flawed in considering these two variables together. Macinnis has recently reported LLS in a large group of Nepalese pilgrims

(n=491). He found the sleep quality item of the LLS was weakly related to the other items of the LLS and that removing the sleep component of the score improved the internal consistency of the test (Macinnis, Lanting et al. 2013). Recent work using clustering methodology to identify symptom patterns in AMS has found that there is poor association between sleep pattern and other symptoms (Hall, Maccormick et al. 2014).

Headache is a predominant feature of AMS (Roach, Kayser et al. 2011) and using the LLS a diagnosis of AMS cannot be diagnosed without it. Other scores do not require the presence of a headache and altitude related illness can occur in the absence of a headache (West 2011). Perhaps the best justification for including headache is that it reduces the background noise from self-reporting, for example without headache a trekker who was sleeping badly and anorexic as a result of a gastrointestinal upset could very easily be diagnosed as having severe AMS.

Another scoring system, which was introduced earlier, is the Environmental Symptoms Questionnaire (ESQ) (Sampson, Cymerman et al. 1983). This has 67 items and is therefore more cumbersome to complete. For this reason a shortened form has been developed which uses 11 items of the ESQIII questionnaire for the calculation of the acute mountain sickness – cerebral (AMS-C) score. Each item is graded 0-5 (not present to extremely severe). Each item then has a factorial weight given in parentheses:

light-headed (0.489)
headache (0.465)
dizziness (0.446)
feeling faint (0.346)
dim vision(0.501)
off-coordination (0.519)
feeling weak (0.387)
sick to stomach (0.347)
loss of appetite (0.413)
feeling sick (0.692)

feeling hung over (0.584)

To obtain the AMS-C score, the sum of all item scores multiplied by the respective factorial weight is multiplied by 5 and divided by 25.95. An AMS-C score of 0.7 and above is used to identify subjects suffering from AMS.

Dellasanta found that diagnosis of AMS by the AMS-C score was similar to that diagnosed as severe AMS by the LLS (Dellasanta, Gaillard et al. 2007). However there was some difference in a significant number of cases, 14% of cases diagnosed by LLS were not diagnosed by AMS-C and 6% diagnosed by AMS-C were not considered AMS by the LLS. One probable confounder in this study is that he used the entire ESQIII with 67 questions and some trekkers were non-English speakers.

Maggiorini found that at 4559 m the prevalence of AMS was 40% and 39%, when measured by the AMS-C and LLS respectively. (Maggiorini, Muller et al. 1998)

2.2 Biomarkers for AMS

An ideal biomarker would have certain characteristics;

- Detection of the key features of the disease
- Differentiation between diseases presenting in a similar way
- The ability to stratify severity of the disease
- Reliable, reproducible, simple and inexpensive methodology
- The biological samples used should be easily accessible

There is some evidence that AMS is an inflammatory process (Richalet, Hornych et al. 1991, Kleger, Bartsch et al. 1996, Grissom, Zimmerman et al. 1997, Klausen, Olsen et al. 1997, Hartmann, Tschop et al. 2000, Roggla, Moser et al. 2000, Harris, Wenzel et al. 2003, Bailey, Kleger et al. 2004, Tissot van Patot, Leadbetter et al. 2005, Behn, Araneda et al. 2007, Gertsch, Lipman et al. 2010, Julian, Subudhi et al. 2011, Eide and Asplund 2012, Gertsch, Corbett et al. 2012, Lipman, Kanaan et al. 2012, Nussbaumer-Ochsner, Schuepfer et al. 2012, Zafren 2012, Gertsch,

Holck et al. 2013, Lipman and Kanaan 2013) and as such inflammatory markers may be useful as biomarkers.

CRP, Troponin, NGAL, NT Pro-BNP, BNP (and potentially, in the near future, copeptin) can all be measured by point of care testing and so are attractive possible biomarkers for altitude illness. This will be developed in the subsequent chapters.

Chapter 3. Observational field study of hormonal adaptation to high altitude

Summary

This study was undertaken in a remote and high altitude environment in the Cordillera Real region of Bolivia and provided the bulk of the data for this thesis. The study involved 50 subjects over a period of 5 weeks and the collection of over 2000 blood samples and 300 cardiac echocardiogram studies.

This chapter develops the rationale for performing studies in such an environment, the difficulties and the factors (such as subject selection) pertinent to the data used in subsequent chapters.

3.1 Introduction

The specific details of assays used and data collection will be covered in detail in the relevant chapter. The purpose of this chapter is to act as an overview, highlighting the difficulties of research of this nature and the scope of the investigations undertaken.

History of the association of science and mountaineering.

Anecdotal accounts of medical problems at high altitude go back to the ancient Chinese around 2000 years ago and mountain exploration and scientific enquiry have always gone hand in hand. First ascensionists of Mont Blanc, the physician Michel Gabriel Paccard (1757–1827) and local crystal hunter Jacques Balmat, (1762–1834) were motivated by the ambition to make scientific observations on the summit, including barometric pressure, when they climbed the mountain in 1786. The first scientific work on altitude related physiology was published by Paul Bert in Paris in 1878, “La Pression Barometrique” (Bert 1878). Bert’s work was primarily done in hypobaric chambers and he demonstrated that hypoxia was the prime danger of hypobaric conditions. High altitude observatories and laboratories were built on the two highest mountains in the Alps (Mont Blanc and Mont Rosa) during the late 1800’s (Richalet 2001). Significant events occurred at these laboratories including work on the ventilatory response to hypoxia and what is probably the first recorded case of HAPE. Expeditions with a significant medical aim (rather than science as a secondary aim of the expedition) began with a Pike’s Peak Expedition in 1911. This expedition was led by J.S. Haldane (the famous Scottish

physiologist) and was the first to measure the fall in alveolar carbon dioxide during two weeks at altitude (West JB 2007). The landmark field study was the “silver hut” study in 1960-1. A laboratory was built at 5800m in the Everest area and inhabited for 6 months and data collected up to 7440m on Makalu (Milledge 2010). This project led on to the American Medical Research Everest Expedition in 1981 (led by Professor John B West), where barometric pressure was measured on the summit of Everest for the first time and alveolar gas samples taken (West 2010). In the recent past the Caudwell Xtreme Everest expedition took 208 trekkers to Everest base camp, 15 climbers onto Everest and 8 to the summit in a $2^1/_2$ month long expedition (Grocott, Martin et al. 2010). Data was collected on energetics, exercise tests and microcirculatory function. Arterial blood gases were taken at 8400m showing profound hypoxia and hypocarbia (mean (range) KPa PaO2 3.28 (2.55 – 3.93) and PaCO2 1.77 (1.37-2.1) (Grocott, Martin et al. 2009).

Association with military mountaineering

The Joint Services Expeditions Trust was set up in September 1969 to encourage Joint Service adventurous expeditions which have a significant scientific element. The Trust give grants to support scientific research of all kinds (not exclusively medical) during adventurous training expeditions. The purpose of the grants are “to further the efficiency of Her Majesty’s Armed Forces through the development of personal and leadership qualities by supporting adventurous and arduous expeditions for the purpose of carrying out research of a scientific nature (including social sciences), and disseminating the knowledge gained as a result thereof” (DIN 2007-06-09 The Joint Services Expedition Trust published July 2007). Military expeditions have had a history of including medical research including performing some valuable field studies, notably the first measurement of ANP at altitude and early work on the hypoxic ventilatory response (Milledge, Broome et al. 1988, Milledge, Thomas et al. 1988, Milledge, Beeley et al. 1989). Indeed Griffith Pugh’s knowledge of exercise in hypoxia and in extreme environments, gained during military service in World War 2 and beyond, has been credited as the difference that made the ascent of Everest in 1953 possible (Tuckey 2013).

Mountains are natural borders between countries and therefore potentially areas of conflict. During the early 1960's the Chinese and Indian armies faced each other across the Himalayas (China invaded in 1964). The Indians realised that their troops were at a great disadvantage when compared to the Chinese who were resident at high altitude on the Tibetan plateau. During the conflict the Indian troops suffered greatly with high altitude illness and much work was published during that period by the Indians (Singh, Kapila et al. 1965, Singh, Khanna et al. 1969). Other potential high altitude flash points exist, India and Pakistan have high altitude military posts on the Siachen Glacier in the Karakorum Himalayas and a significant proportion of Afghanistan and northern Pakistan is over 3000 m. Future conflicts are likely to be fuelled by a lack of resources (e.g. oil and gas) and climatic changes (MoD Strategic Trends Programme. 4th Edition. Global Trends out to 2040. Published 2010). This is likely to result in conflict in high altitude regions as populations migrate and exploit currently untapped resources.

Shock and hypoxia is a part of all critical illness. Even with good pre-hospital airway care and ventilation trauma patients frequently present to the Emergency Department in the hospital in Camp Bastion with a profound base deficit indicating tissue hypoxia (22 major ballistic injuries, median base deficit 7, median SpO_2 99.5%) (Mellor and Woods 2012). Inducing physiological shock in healthy subjects is ethically difficult, however enabling individuals to take part in an expedition during which they will inevitably become profoundly hypoxaemic may provide an acceptable approach for both investigators and subject. As an alternative chamber studies have been used to replicate the altitude profiles of Everest ascents. These studies are immensely costly and ethically challenging. To run a hypobaric chamber requires a team of two operators and medical cover around the clock, a typical Everest ascent takes 8 weeks. The ethics of subjecting individuals to 8 weeks of enforced captivity during which they are very likely to become unwell is questionable. Chamber studies can only replicate the hypobaric elements of mountaineering and not the relationship of hypoxia to exercise, dehydration or dietary changes. Contrast this with expeditions where those taking part do so of their own free will and would often undertake the trek with or without the science

element. There are far fewer ethical conflicts. Furthermore through combining a few days of research (as planned days throughout the expedition) and still being able to achieve other aims, in terms of trekking or climbing, individuals taking part in expeditions can achieve research aims. In many ways the military is ideally placed to lead scientific expeditions and as stated above has a long history of doing so. The Joint Services Adventurous Training scheme supports adventurous training financially (typically split equally with contributions from public funds, non-public funds and personal contributions) and further grants are available to expeditions fulfilling suitable scientific aims through the Joint Services Expeditions Trust.

Challenges of high altitude field research.

Mountainous regions of the world are generally poorly developed in terms of road, rail, telephone and power supplies. This lack of resource produces significant challenges to the planning and execution of field research.

Temperature falls in a predictable way with altitude; this is referred to as the lapse rate. Typically in clear skies the rate is 1°C per 100m, with very cold temperatures at high altitude. However due to the thinner atmosphere the radiated solar energy is very high leading to a huge difference in temperature between that in direct sunlight and shade, with almost inevitable sub-zero temperatures after dark. The consequence of this is that maintaining samples or equipment within a reasonable temperature range is difficult. This large temperature range has implications for power supplies. Cold batteries rapidly loose charge or do not generate sufficient power for operating equipment, batteries need to be kept warm (usually in a pocket by day or sleeping bag overnight) if they are to have the predicted output. Even in use equipment can cool rapidly and thought given to maintaining temperature for example, placing equipment on a hot water bottle or inside a down jacket.

Despite the small size and relative portability of modern medical equipment power remains a real issue. Even in well-travelled areas such as the Khumbu valley on the Everest Base Camp trail, there is relatively little reliable electricity. Most tea

houses allow trekkers to recharge from a solar panel or their domestic system (usually solar charged battery rather than generator) for a fee. This supply may not be adequate to charge medical equipment. Solar panel technology has improved dramatically over the last few years but the supply may still be of inadequate output to power a lap-top computer, additionally solar panels are most effective when left stationary but on many expeditions most of the day-light hours are spent trekking. Compact generators can be used but are heavy (usually at least 25Kg, a full porter load where they have to be carried on foot) and sufficient clean fuel may be difficult to obtain.

Disposal of waste (e.g. sharps, contaminated clinical waste) will be difficult in remote areas and at least require carrying in and out.

Carrying out research is often not seen by all the expedition members as the most important aspect on an expedition. During the DMS expedition to Vellicitos and Aconcagua in 2007, few subjects felt able to perform a step test at 0400 in the morning, when it was -5 ºC and oxygen saturations were in the low 80's, however all the team managed to find motivation to climb to the summit of Vellicitos later that morning! Careful planning of the expedition itinerary is required in order to ensure that individual expectation is matched to the scientific aims. Scheduling days for investigation allows work to be carried out in sun light, with functioning batteries or solar power, rather than trying to perform measurements at the end of a long days trekking, in the dark when it's well below freezing. Caudwell Extreme Everest (CXE) overcame this problem by having 4 fixed "laboratories" carrying out investigation along the trek to base camp. This provides an ideal solution but can only be used by large scale expeditions (CXE had 208 trekkers and was the largest project of its' kind ever undertaken)(Grocott, Richardson et al. 2007).

Whilst the leap from high altitude research in healthy subjects to critically ill octogenarians on an intensive care unit is not immediately apparent, high altitude research can help to investigate areas relevant to critical illness. This informs at both the basic science a clinical level. Richard Feynman eloquently observed that

“nature uses only the longest threads to weave her patterns, so that each small piece of her fabric reveals the organization of the entire tapestry” (Fennymann 1967). High altitude research adds strands to the tapestry, and therefore the whole picture is more likely to emerge

3.2 Study Overview

This field study took place in the Cordillera Real region of Bolivia. This was chosen to give a provocative but ethically acceptable ascent profile in terms of the risk of developing AMS, relatively easy access and transport, attractive climbing objectives and most importantly, a range of altitude for study that were comparable with altitudes used on previous studies.

The expedition was planned to use the trek into the mountains as the exercise component. Whilst this was not a standardised exercise challenge it provided the “real world” stimulus that is of interest.

3.2.1 *Ethics*

Ethical approval for the field study, including all aspects of data collection, was obtained from MoD Research Ethics Committee in April 2012 (68/Gen/09 3rd amendment dated 05/04/2012).

3.2.2 *Subjects*

Subjects were all Service personnel and the expedition was organised as military Adventurous Training allowing subjects to be partially funded and have time for the expedition. Subjects were recruited by advertising within the military using Defence Instructions, word of mouth, newsletters within the Defence Medical Services etc.

Fifty subjects were recruited, one subject was unable to take part in the study at very short notice and one subject was withdrawn at the first study altitude due to difficulty taking blood.

Previously published data from field studies in Nepal suggested an observed rate of severe AMS at similar altitudes of around 20% (4 subjects of 20) (Woods, Begley et al. 2012). It was therefore anticipated that an n=50 would lead to a high enough incidence of AMS to be able to identify any differences between a cohort with severe AMS and those without.

All subjects were medically fit and in date for their annual military fitness test (which comprises a 1.5 mile run). Demographic data are presented in Table 3.1 below.

3.2.3 *Baseline data collection*

Baseline physiological data (height, weight, total body water (TBW), 1.5 mile run time, SpO_2, blood pressure, respiratory rate) were collected on all subjects prior to leaving the UK.

A sub-group of 20 subjects took part in a trek in Snowdonia (6 hours with 600m ascent) during which baseline data was collected including phlebotomy and cardiac echocardiograms using the field study protocol. The demographics for this sub group are presented below;

	Sea level Control group	High Altitude group	P value
Age (SD)	35.1 ± 11.1	35.0 ± 9.0	0.98
Range	21-54	22-54	
Height (cm)	172.2 ± 8.1	175.6 ± 9.50	0.14
Weight (Kg)	77.14 ± 15.2	77.8± 14.0	0.87
1.5mile run time (minutes)	10.19 ± 1.5	9.85 ± 1.2	0.42
Smoking history			

- current	1	3	
- non smokers	19	45	0.52
Sex			
- males	13	34	
- females	7	14	0.71

Data is expressed as mean (standard deviation) P values is expressed for comparison of sea level versus high altitude group

Table 3.1 Subject demographics

3.2.4 *Field Study*

La Paz is the highest capital city in the world, the airport is at around 3800m. This was one of the main factors in choosing this region in which to conduct the study. Research was conducted following a trek and then again at rest the following day in accordance with the schedule below.

Day 1	Arrive La Paz airport 4061m, hotel **3681m**
Day 2	Lake Titicaca[1] **3833m** following 6 hour trek with 600m height gain
Day 3	Lake Titicaca rest **3833m**
Day 4	Prep La Paz **3681m**
Day 5	Road head[2] **4450m** following 4 hours trek and 200m height gain
Day 6	Road head **4450m** - research (rest)
Day 7	BC **4710m**
Day 8	BC **4710m**
Day 9	Research camp[3] **5129m** following 4 hour trek with 400m height gain

Day 10 Rest at research camp **5129m** (rest)

[1]. Lake Titicaca (Hotel Esmeralda)

Altitude 3833m

Location 16 deg 09 mins 56.92 s 69 deg 05 mins 23.88 s

[2]. Road Head Camp

Altitude 4,450m (research hut), 4,430 (camp spot)

Location: 16° 4'8.03"S 68°27'9.45"W

[3]. High Camp

Altitude 5,129m

Location: 16° 0'35.17"S 68°23'53.36"W

Summarised graphically the acclimatisation profile is shown in figure 2.1;

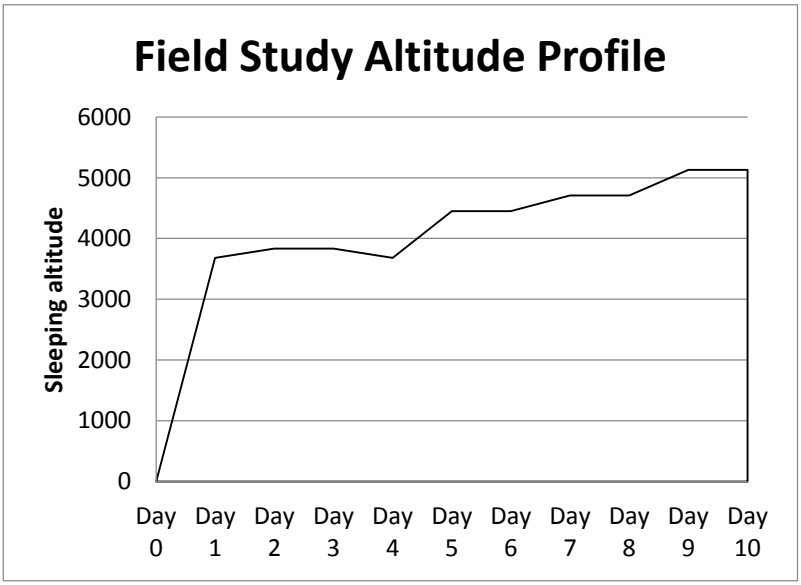


Figure 3.1 Field study altitude profile

3.3 Field Study Protocols

The investigations carried out are outlined in the table below and will be discussed in more detail in the relevant chapters of this thesis.

Basic physiological data and scoring systems for altitude illness (LLS and AMS-C) where carried out each morning and evening by the subjects. The LLS and AMS-C are discussed elsewhere in more detail (Chapter 2).

The subjects rating of perceived exertion (RPE) was recorded according to the score developed by Borg (Borg 1970). This was recorded retrospectively at the end of each exercise study day (day 2,5,9). Subjects were asked to record how much they felt they had exerted themselves according to the scale below;

Score	Effort	Perception
6	20%	
7	30%	Very,very light (rest)
8	40%	
9	50%	Very light (walking)
10	55%	
11	60%	Fairly light
12	65%	
13	70%	Somewhat hard – steady pace
14	75%	
15	80%	Hard
16	85%	

17	90%	Very hard
18	95%	
19	100%	Very very hard
20	Exhaustion	

Table 3.2 15 point Borg RPE scale (Borg 1970)

The scale starts at 6 as the scale is related to heart rate (approximately HR = RPE x 10). This scale has not been validated at high altitude however at sea level it has been found that there is a correlation between RPE and heart rate, lactate levels, $\%VO_2$ max and breathing rate (Noble, Borg et al. 1983). Correlation with heart rate was reported to be good in over 2000 cardiopulmonary exercise tests (rho 0.72) (Scherr, Wolfarth et al. 2013). A Borg score of 15 represents “hard effort” and is above the recommended level for exercise in non-trained athletes (Graham, Atar et al. 2007). For this reason data was divided into two cohorts low Borg (<15) and high Borg ($\geq$15).

	Day 0	1	2	3	4	5	6	7	8	9	10
Altitude	Baseline	3681	3833	3833	3681	4450	4450	4710	4710	5129	5129
Comment			Post exercise	Rest		Post exercise	Rest			Post exercise	Rest
Venous Blood – Serum and plasma separated Point of care BNP & NGAL											
Vital signs (HR, RR, BP, SpO2)(post trek & following morning)											
LLS (post trek and following morning)											
Total body water (after rehydration)											
Body weight											
Haematocrit											
24 hour urine volume											
	= test performed										

Table 3.3 Subject testing schedule for Bolivia field study

At each post-exercise and rest study altitude (days 2,3,5,6,9,10) approximately 25 mls of venous blood was taken. This was allowed to clot where appropriate, immediately centrifuged and frozen for unstable hormones (such as AVP and catecholamines) and frozen within 3 hours in the case of serum for osmolality, $[Na^+]$ e.t.c. BNP and NGAL were measured on a point of care monitor (Biosite Triage Pro, Alere, Cheshire). Overall at each study point 9 cryovials were collected as follows:

Lithium heparin blood tube – plasma decanted into 2 cryovials for AVP assay.
EDTA blood tube – plasma decanted into 4 cryovials for catecholamines (x2), copeptin and plasma rennin

Serum Separator tube – 3 cryovials for cortisol, osmolality and urea and electrolytes.

Echocardiography was performed by a trained technician on a Vivid I machine (GE HealthcareTM Vivid I) post-trek and at rest at the main study altitudes. PASP was estimated from the maximum velocity of the trans-tricuspid gradient using continuous wave Doppler imaging added to a fixed value of 5 mmHg (Yock and Popp 1984), in the UK, by a Consultant Cardiologist (Dr Chris Boos, Poole and Bournemouth NHS Trust) blinded to other data.

Total body water (TBW) was measured using both single frequency (SF) and multiple frequency (MF) devices. SF-BIA was undertaken using The Non Invasive Cardiac System (NICaS®, Israel) which calculates TBW and non-invasive cardiac output (CO). MF-BIA was performed using a tetrapolar technique with a QuadScan® 4000 (Bodystat, Douglas, United Kingdom) (Castillo Martinez, Colin Ramirez et al. 2007). This is considered in more detail in chapter 4.

The cold chain was maintained by the use of two Engel 35l freezers. These ran from a generator by day and on a 12v battery overnight at a monitored temperature

of -20ºc throughout the study. In transit the freezer was carried, connected to a battery by a team of two porters.

3.4 Conclusion

This was a logistically challenging study but one which investigated hormonal changes during the “real world” physiological challenge of a high altitude trek. This study formed the main piece of work for this thesis. Forty-eight subjects were studied on six research days collecting a total of 2600 cryovials of plasma, almost 300 cardiac echos and around 6000 physiological observations.

Chapter 4 - Fluid Balance At High Altitude

Summary

Fluid retention is a key feature of altitude illness and a diuresis is an important part of the process of acclimatisation. Measuring total body water at high altitude is difficult and many techniques cannot be used for practical reasons. Bioimpedance (BIA) techniques, either multifrequency or single frequency, are attractive to use on field studies as they are non-invasive, straightforward and require lightweight portable equipment. Recording body mass and fluid balance are also simple techniques. In this study single and multifrequency BIA was compared and data collected on fluid balance and body mass. A simple visual analogue score for thirst was included to investigate the utility of this as a surrogate for other measures of hydration.

Hypotheses

- Altitude illness would be associated with an increase in TBW and a rise in AVP (and copeptin)
- There would be no difference between TBW measured by single or multifrequency BIA.

4.1 Introduction

The most commonly appreciated response to HA exposure is the increase in concentration of haemoglobin mediated by a hypoxia-driven increase in erythropoietin. However, whilst the rise in erythropoietin takes place within a few hours, changes in red cell mass take several weeks and lag behind the crucial early changes in fluid homeostasis. In the acute stages of HA exposure, changes in fluid balance with a marked natriuresis and diuresis are key to initial acclimatization (Zaccaria, Rocco et al. 1998, Hildebrandt, Ottenbacher et al. 2000). This process reduces total body water by up to 3 litres and leads to a reduction in plasma volume which increases the concentration of haemoglobin per volume of blood. The effect is to increase the oxygen carrying capacity of a given volume of blood compared to sea-level and partially compensates for the reduced partial pressure of oxygen that occurs at HA. A frequently reported feature of altitude illness reported in the literature is a failure of this diuresis to occur (Singh, Khanna

et al. 1969, Hackett, Rennie et al. 1982, Hamilton, Cymmerman et al. 1986, Ramirez, Bittle et al. 1988, Milledge, Beeley et al. 1989) highlighting the central importance of fluid balance and the endocrine response to HA in both acclimatisation and the pathogenesis of altitude illness. The maintenance of salt and water homeostasis is regulated by a number of hormones with a complex relationship between those hormones, catecholamines and other stimuli such as exercise. Arginine vasopressin (AVP) has a predominant action to retain free water through altering the permeability of the distal collecting tubule. As already discussed a diuresis is an essential component of altitude adaptation and it seems likely that AVP is an important hormone in acclimatisation. Normally at SL a rise in osmolality or a marked decrease in plasma volume would lead to a rise in AVP secretion from the posterior pituitary and a reduction in free water loss at the kidney thereby maintaining osmolality.

Our groups previous work in Nepal (using single frequency bioimpedance spectroscopy (BIS)) showed a significant ($p = 0.001$) reduction in percentage total body water (TBW) between 1,300 and 5,150 m (57.6 ± 1.3; 56.24 ± 0.97; 55.88 ± 0.86 and 54.8 ± 0.97 at 1,300, 3,400, 4,300 and 5,150 m, respectively). In those with AMS as judged by the LL score at 5,150 m, TBW was 56.1% in AMS cases and 52.9% in those without AMS but this was non-significant ($p = 0.089$) (Woods, Hooper et al. 2011). For those with severe AMS, although numbers were small (4 subjects), the difference in TBW was significant ($p=0.047$) (figure 4.1).

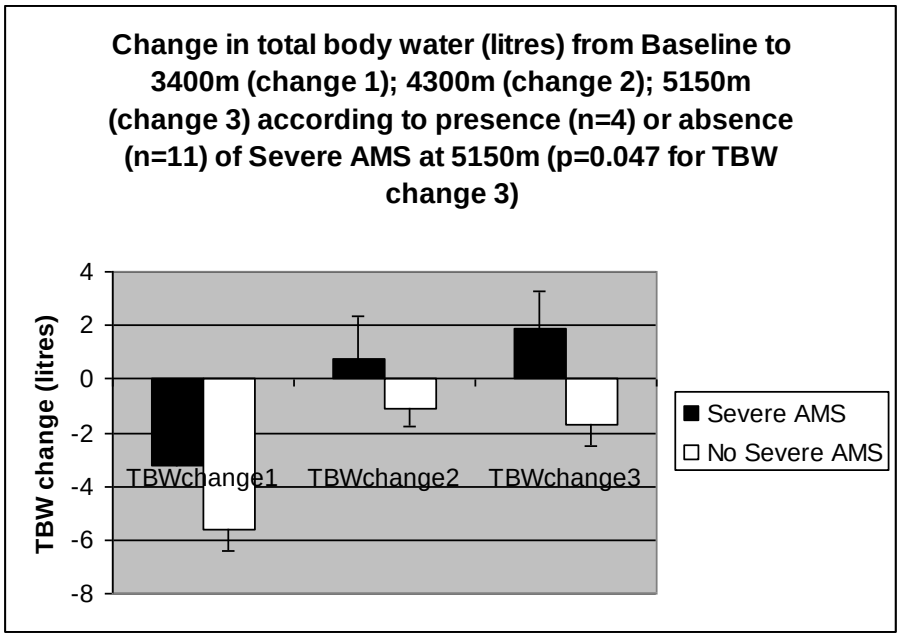


Figure 4.1 Change in TBW at altitudes up to 5150m

Changes in catecholamines, cortisol, AVP and exercise intensity can all have effects on TBW. It is therefore important to understand fluid shifts to understand the mechanisms behind changes in these hormones. In a practical, clinical sense, a simple reliable measure of TBW may have clinical utility in making a diagnosis of AMS as fluid retention is key in this process. Our previous data in 2009 and 2011 showed significant changes as a group but the intra-individual variation was too great to use single frequency BIS for individual assessment.

4.2 Measuring Fluid Balance At High Altitude

In a comprehensive review of techniques for measuring TBW Armstrong (Armstrong 2007) makes the point that any assessment technique provides a singular measure of what is a complex and dynamic process. He states that "total body water approximates "euhydration" when morning body weight is near the normal baseline, fluid intake is adequate, urine colour is pale and urine volume is normal". For the reasons we have seen above this state my never be reached during a trek at HA. Table 4.1, taken from this review, outlines 13 methods of measuring hydration status.

Hydration assessment technique	Body fluids involved	Cost	Time	Technical expertise required	Portability	Likelihood of adverse event
Stable isotope dilution	All (ECF and ICF)	3	3	3	3	2 or 3 [b]
Neutron activation analysis	All	3	3	3	3	2
Bioelectrical Impedance spectroscopy	Uncertain	2	3	2	2	1
Body mass change [c]	All	1	1	1	1	1
Plasma osmolality [d]	ECF	3	2	3	3	2
% plasma volume change	blood	2	2	3	3	2
Urine osmolality	Excreted urine	3	2	3	3	1
Urine specific gravity	Excreted urine	1	2	2	1	1

Urine conductivity	Excreted urine	2	2	2	3[e]	1
Urine colour	Excreted urine	1	1	1	1	1
24 hr urine volume	Excreted urine	1	1	1	1	1
Salivary flow rate, osmolality, protein	Whole, mixed saliva	2-3	2	3	2-3	1
Rating of thirst	Hypothalamus	1	1	1	1	1
Key to rating		1=little 2=moderate 3=great	1=little 2=moderate 3=great	1=little 2=moderate 3= great	1=portable 2=moderate 3=not portable	1=low 2=moderate 3=high

b depending on type of isotope (i.e. radioactive, stable, non-radioactive)

c using a floor scale

d freezing point depression method

e portable hand held meters are available

Table 4.1 13 methods of measuring hydration status (Armstrong 2007)

Of these methods above body mass will change on a prolonged trekking sojourn due to anorexia and/or a relative lack of calorie intake. Osmolality may change as a result of the hormonal changes at HA, independent of hydration status which would also affect percentage plasma change and urine measurements. Neutron activation analysis involves the use of highly specialized equipment and is not appropriate for a field setting. Collection of 24 hour urine would, at the very least, prove logistically challenging to carry the extra weight and volume for a large number of subjects!

Of the methods available, practical solutions to the measurement of TBW at HA are BIS, use of stable isotope dilution (such as deuterated water) or the rating of thirst.

Isotope dilution is generally considered to be the gold standard for measurement of TBW (Armstrong 2007) and has been used previously to assess TBW in subjects exposed to simulated high altitude (Loeppky, Icenogle et al. 2005). However, equilibration of the radioisotope takes several hours and this technique assumes that volume of body fluid compartments remains stable during equilibration. It can also only be performed once over a two week period. These conditions are impossible to generate in remote field environments at HA. Isotope dilution techniques are also expensive (£250 per test) and laborious with a relative long learning curve and the time taken for isotopes to equilibrate prevents frequent, serial measurements of TBW. BIS overcomes several of these shortcomings and has the distinct advantage of being a non-invasive, portable and very quick and easy to perform. This creates several opportunities for HA research where changes in TBW and its associated compartments may be linked to the pathogenesis of AMS and possibly HAPE (Woods, Begley et al. 2012). Estimation of TBW using both single and multi-frequency (SF and MF) BIS has been shown a very high correlation with isotope dilution methods though differences in absolute values of TBW may be observed (Lukaski, Johnson et al. 1985, O'Brien, Baker-Fulco et al. 1999, Carlsson, Bosaeus et al. 2004, Powers, Choi et al. 2009, Haas, Schutz et al. 2012). Furthermore TBW using SF-BIA has strongly correlated with that from MF-BIS (Donadio, Consani et al. 2005). It was on this basis that

we chose to directly compare these two BIS devices that had potential for increased use at HA.

The Non Invasive Cardiac System (NICaS) (NICaS®, Israel) system has adapted the principles of SF-BIA to also measure key cardiac performance (eg cardiac index) and haemodynamic measures (eg systemic vascular resistance index) with ECG monitoring creating further options for cardiac (eg in heart failure) and HA research not available from the Quadscan device (Paredes, Shite et al. 2006, Tanino, Shite et al. 2009). However, the device only measures TBW and does not provide information on compartmental extra cellular water (ECW) or intracellular water (ICW) which is of value in assessment of hydration which is also important to HA research. The QuadScan® 4000 (Bodystat, Douglas, United Kingdom) (QuadScan) utilises the concept of MF-BIS. The principle of BIS is dependent on the current frequency applied. At low frequencies, the current cannot bridge the cellular membrane and will pass predominantly through the extracellular space. At higher frequencies penetration of the cell membrane occurs and the current is conducted by both the ECW and ICW (Kyle, Bosaeus et al. 2004). Using MF-BIS technology to measure the varying impedance at higher and lower frequencies and by applying predictive equations, it is possible to estimate TBW, ECW and ICW (Donadio, Consani et al. 2005).

BIS is based on the fundamental principle that resistance or impedance (Z) to the flow of an electrical current through the body is dependent on the length of the conductive path, the volume of the conductive material, and the resistivity of the conductive material (Kyle, Bosaeus et al. 2004). The actual parameter measured with BIS is the voltage (V) that is produced between two electrodes located most often at sites near to, but different from, the sites where a small clinically undetectable electrical current (I) is introduced. Impedance is calculated from the ratio V/I. In humans, only body water, with its dissolved electrolytes, will conduct a current. Hence, using the assumption that the resistivity of the conductive material is constant, and using height (H) to estimate the path length water TBW can be estimated by measuring impedance to the flow of a small current. Specific prediction equations have been developed to evaluate body TBW from height and height and impedance and

variable regression equations have evolved to include weight and sex amongst other factors (Kyle, Bosaeus et al. 2004).

4.3 Experimental method

Participants were studied in the supine position on a non-conductive surface, with arms and legs abducted at a 30°-45° angle from the trunk to avoid medial body contact by upper and lower extremities. Electrode positions were carefully cleaned prior to use (Powers, Choi et al. 2009).

SF-BIS was undertaken using NICaS which calculates TBW and non-invasive CO. For TBW calculation an electrical current of 1.4 mA with a 32 kHz frequency wave is passed through the patient via two pairs of tetrapolar electrodes with one pair placed at left wrist above the radial artery, and the other pair placed on the contralateral side above the posterior tibialis artery (Paredes, Shite et al. 2006, Tanino, Shite et al. 2009). TBW was calculated by adopting the Kushner formula to the NICaS as follows (Kushner, Schoeller et al. 1992:

TBW in litres= 0.42 x Height^2 / Bessel Resistance + 4.96 for women
TBW = 0.42 x Height^2 / Bessel Resistance + 8.30 for men).

MF-BIS was performed using a tetrapolar technique with a QuadScan® 4000 (Bodystat, Douglas, United Kingdom) (Castillo Martinez, Colin Ramirez et al. 2007). Two electrodes were placed on the left wrist with one just proximal to the third metacarpophalangeal joint (positive) and one on the wrist next to the ulnar head (negative) on the left hand side. Two electrodes were placed on the ipsilateral ankle with one just proximal to the third metatarsophalangeal joint (positive) and one between the medial and lateral malleoli (negative). Total body electrical impedance to an alternate current (0.2mA) was assessed at four different frequencies (5, 50, 100, and 200 kHz) (Donadio, Consani et al. 2005). At the 50 kHz frequency TBW (and Fat-Free Mass) can be predicted using the manufacturer's software after entering the subjects age, sex, height and weight.

Statistical Analysis (BIS)

The Kolmogorov-Smirnov test was undertaken to assess normality of all continuous data. The results were presented as mean ± standard deviation (SD). As TBW was normally distributed paired comparisons of TBW measures were performed with a paired T test and correlations were quantified using the Pearson correlation coefficient ± 95% confidence interval (CI). Comparisons of continuous data from >3 groups were performed using ordinary or repeated measures ANOVA as appropriate with a Tukey post-test. A p value of <0.05 was considered significant for all comparisons.

Agreements between NICaS (SF-BIA) and QuadScan 4000 (MF-BIA) for TBW measurement were assessed using Bland-Altman plots (Bland and Altman 1986) in which the difference between the two values were compared with the average values from the comparative two readings. The bias was defined as the mean ± SD of the difference between the readings. Reasonable agreement was defined as <5% of readings being within 1.96SD (95% CI) from the mean.

Fluid Balance Recordings

During the 24 hours post exercise subjects recorded their fluid balance in terms of input and output. Subjects recorded the volumes (mls) of all liquids consumed and measured urine output in a measuring jug. This enabled a measure of overall fluid balance during resting conditions and free access to food and water. The intention was to collect this data to examine the correlation between fluid balance and the development of AMS and changes in AVP and copeptin. TBW was only measured on the rest day as the predominant changes following a trek would be related to exercise and dehydration rather than to adaptive mechanisms relating to acclimatization. For this reason we could not directly relate TBW directly to fluid balance. This is also the reason why the numbers and incidence of AMS reported differ to those reported in other chapters.

Thirst Visual Analogue Score

Thirst perception can be measured on a visual analogue score (VAS) or a categorical rating. Rolls has published the use of a 10 cm scale (Rolls, Wood et al. 1980). Subjects responded to the statement “how thirsty are you now” with a

mark on the horizontal line. “Not at all” was a mark at the 0cm end of the scale whilst “very thirsty” anchored the 10 cm end. This scale was found to track changes in plasma osmolality. For the field study this VAS was slightly modified for the rating from “no thirst” to “worse thirst ever”, this was to reflect the fact that subjects were asked the question “how severe was your thirst at the thirstiest part of the day”. On initial investigation it was clear that there was a great deal of variability in the subjects’ response to the question, with some subjects consistently recording high levels of thirst whilst others were seldom reporting any thirst. For this reason the change in thirst was modified by subtracting the day 4 score from other scores recorded. Day 4 was a rest day in La Paz with free access to water and no physical exertion.

4.4 Results

4.4.1 *Comparison of methods of measuring total body water with BIA*

The average age was 34.5 ± 9.3 years with an age range of 21-54 years (70.2% male). Body weight was 77.0 ± 14.6 kg at sea level, 77.6 ± 13.9 kg at 3833m post exercise, 77.5 ± 14.0 kg at 3833 at rest, 76.9 ± 13.80 at 4450m and 76.1 ± 13.4 kg at 5129m (figure 4.2). On repeated measures ANOVA this difference was significant between all altitudes and 5129m ($p<0.0001$). The comparative sequential changes in TBW for the NICaS and QuadScan at sea level and increasing HA are shown in figure 4.3. At sea level there was no paired difference between TBW measurement with NICaS (SF-BIA) compared with the QuadScan 4000 (MF-BIA) device respectively (40.5 vs 40.7l; $p=0.76$) (fig 4.4). At 3833m at both exercise (40.2 vs 41.8l; $p<0.0001$) and rest (39.3 vs 41.3; $p<0.0001$) NICaS readings were lower than that for the QuadScan device respectively (figure 4.4). The resting TBW readings at 4450m (38.7 vs 40.9; $p<0.0001$) and 5129m (39.7 vs 41.2l; $p=0.004$) were again lower with NICaS than with the QuadScan respectively (fig 4.4). There was an overall significant difference in TBW measurements between NICaS (39.8 ± 8.5) and QuadScan (41.5 ± 7.7; mean difference -1.7l: $p<0.0001$).

There were strong correlations between the QuadScan and the NICaS systems for TBW assessment at sea level ($r=0.90$; 95% CI 0.78 – 0.95: $p<0.0001$) and at HA ($r=0.92$; 0.89 - 0.94: $p<0.0001$). There was also a strong overall correlation between the two methods ($r=0.91$; 0.88 - 0.93: $p<0.0001$) (fig 4.5). There was a

stronger correlation between TBW and total body weight with the QuadScan (r=0.91, 95% CI: 0.89 - 0.93:p<0.001) than with the NICaS system (r=0.83; 95% CI: 0.79 - 0.87: p<0.0001) (figures 4.6 and 4.7).

On Bland-Altman comparisons of the two methods of TBW assessment the values for TBW at sea level were higher with QuadScan than with NICaS measurements (mean difference [bias] 0.2l; 95% limit of agreement -6.6 to 6.7l) (figure 4.8). This difference was greater at HA (mean difference 1.91; 95% limit of agreement -3.7 to 7.5l) (fig 4.8).

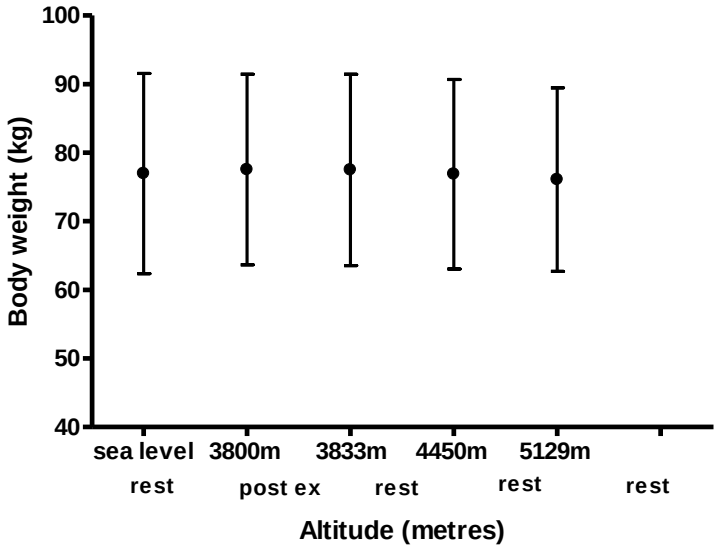


Figure 4.2 Changes in body weight at sea level and at exercise (Ex) and rest at increasing altitudes

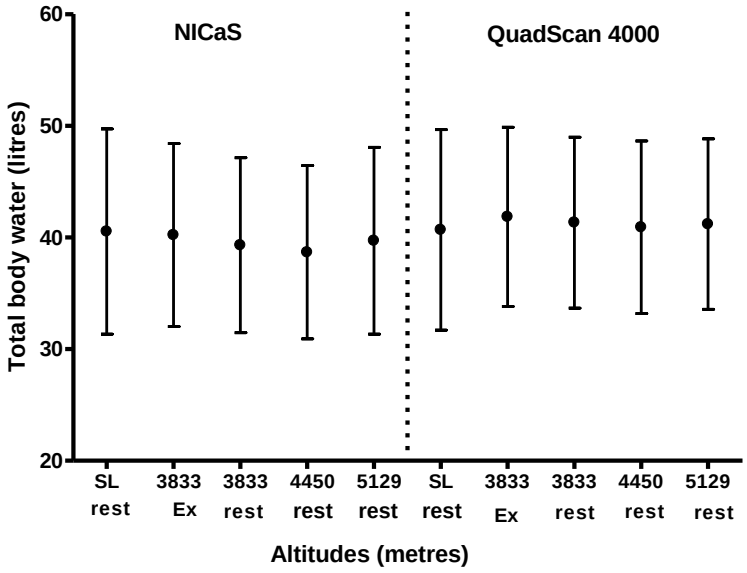


Figure 4.3 Comparative changes in total body water for the NICaS versus QuadScan at sea level and at exercise (Ex) and rest at increasing altitudes

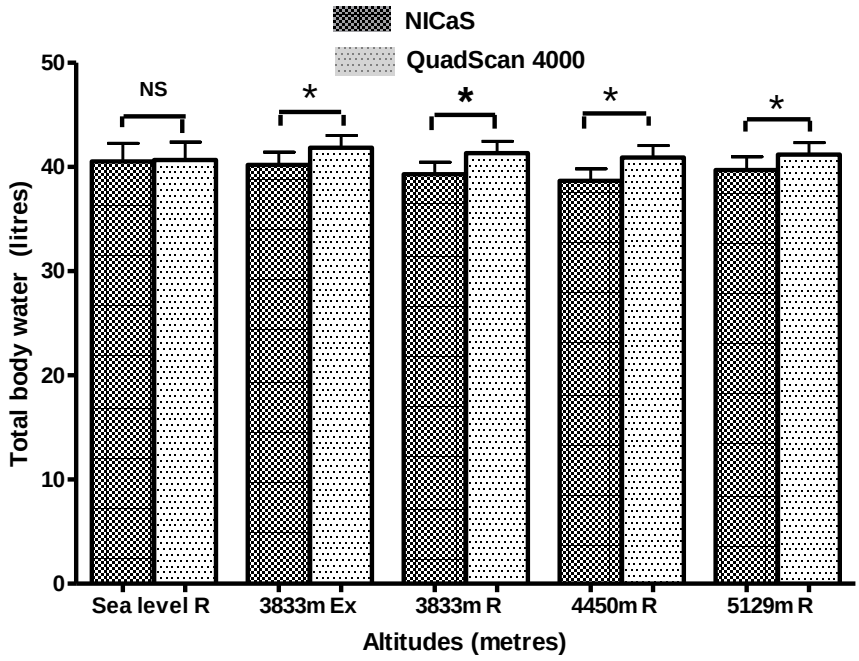


Figure 4.4: Paired changes in data for quantification of total body water at sea level and successive altitudes (* significant on paired testing)

Figure 4.5 Correlation between total body water measurements using NiCAS and BodyStat at sea level and at exercise (Ex) and rest at increasing altitudes

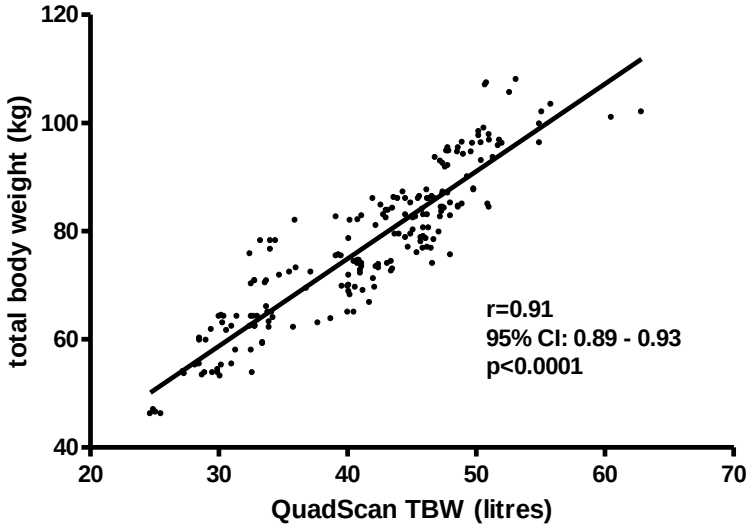


Figure 4.6: Correlations between total body water using the QuadScan and total body weight across all study points

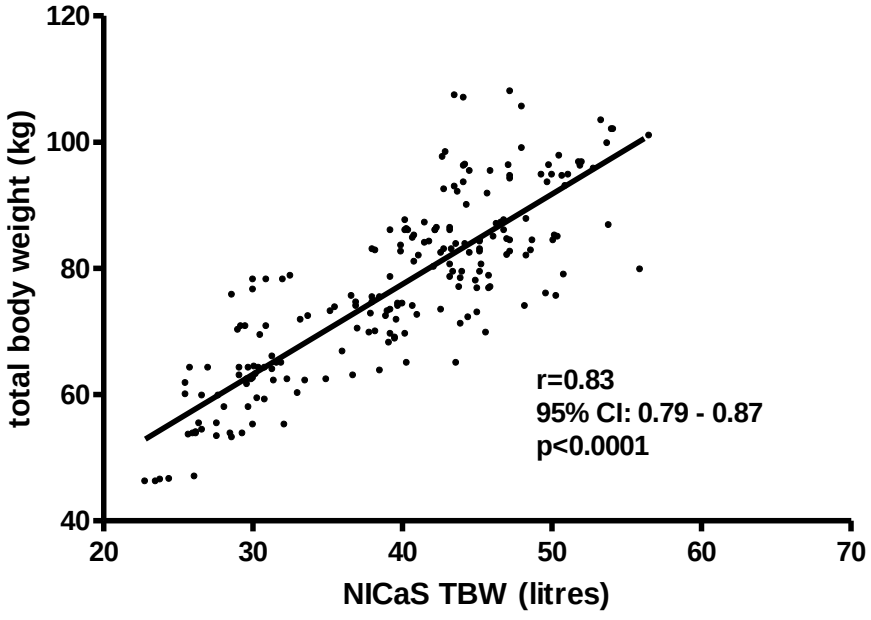


Figure 4.7 Correlations between total body water using the NICaS system and total body weight across all study points

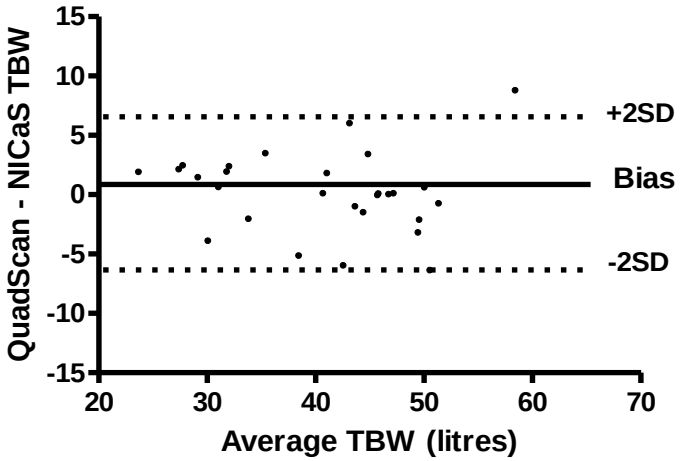


Figure 4.8 Bland-Altman plots comparing total body water measurements with the NICaS versus the QuadScan system at sea level

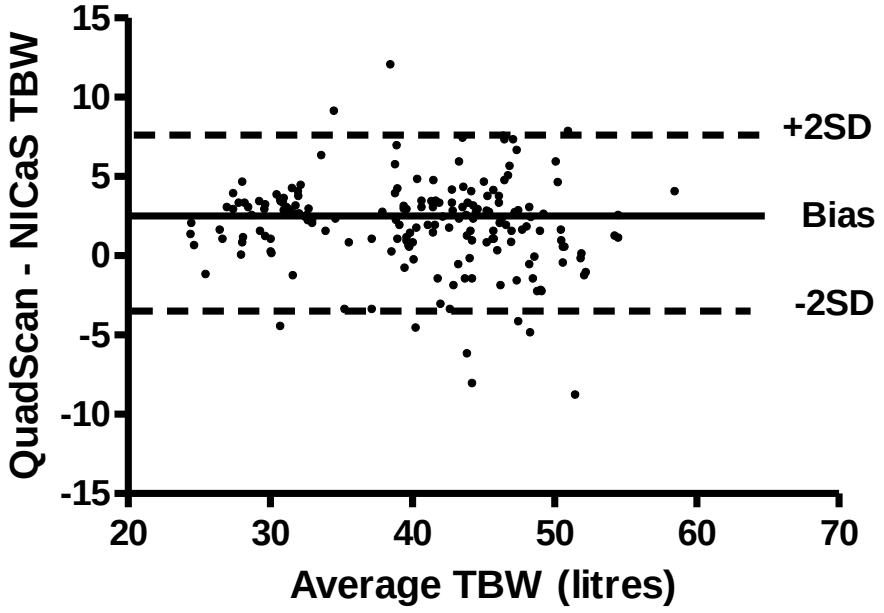


Figure 4.9 Bland-Altman plots comparing total body water measurements with the NICaS versus the QuadScan system high altitude

4.4.2 *Fluid balance measurements*

Altitude	3833m	4450m	5129m
N	48	48	45
Balance	675 (785)	767 (731)	817 (748)
	-2150 - +1800	-1025 - +2730	-800 - +2700

Table 4.2 Fluid balance recorded at each study altitude. Data presented as mean(SD) range, mls.

Although there was a trend towards a more positive fluid balance with increasing altitude there was no statistical significance (38 vs. 44 p= 0.555, 38 vs. 51 p=0.343, 44 vs. 51 p=0.88)

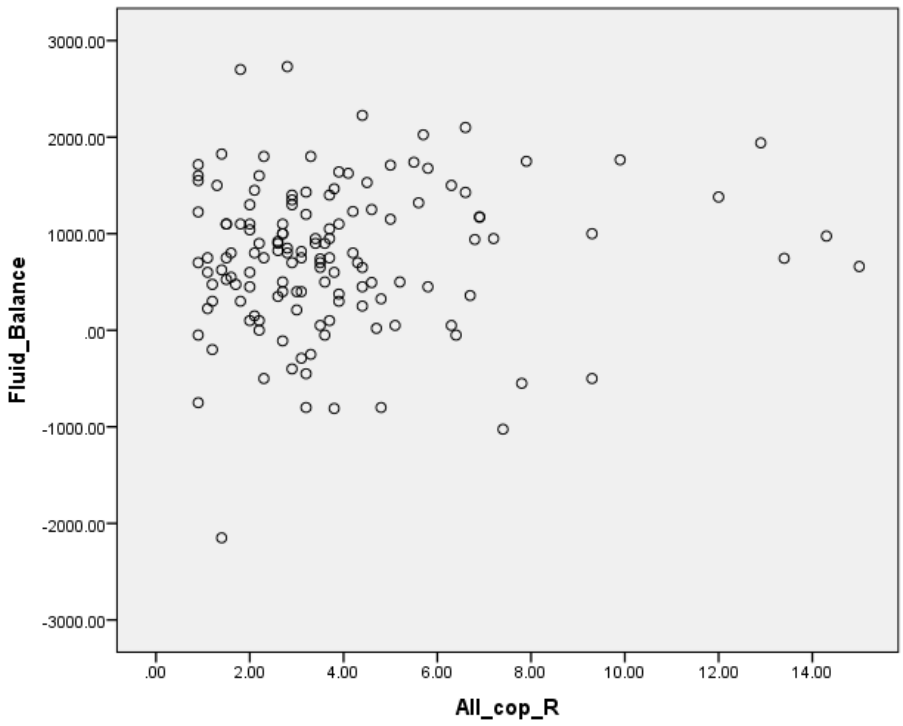


Figure 4.10 Relationship of copeptin to fluid balance

There appeared to be no correlation between fluid balance and copeptin (rho 0.090 p=0.296)

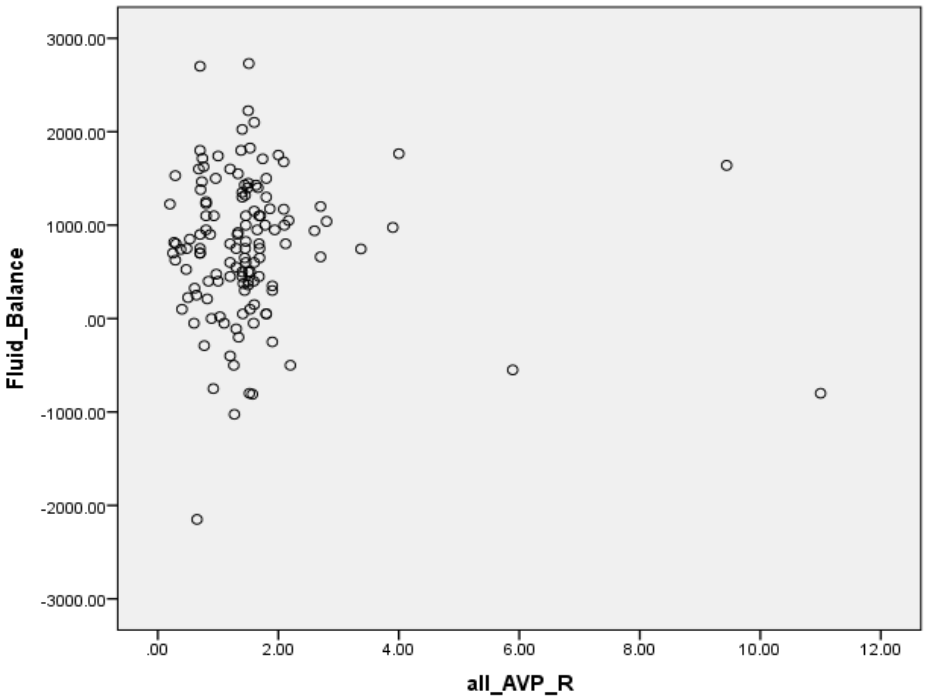
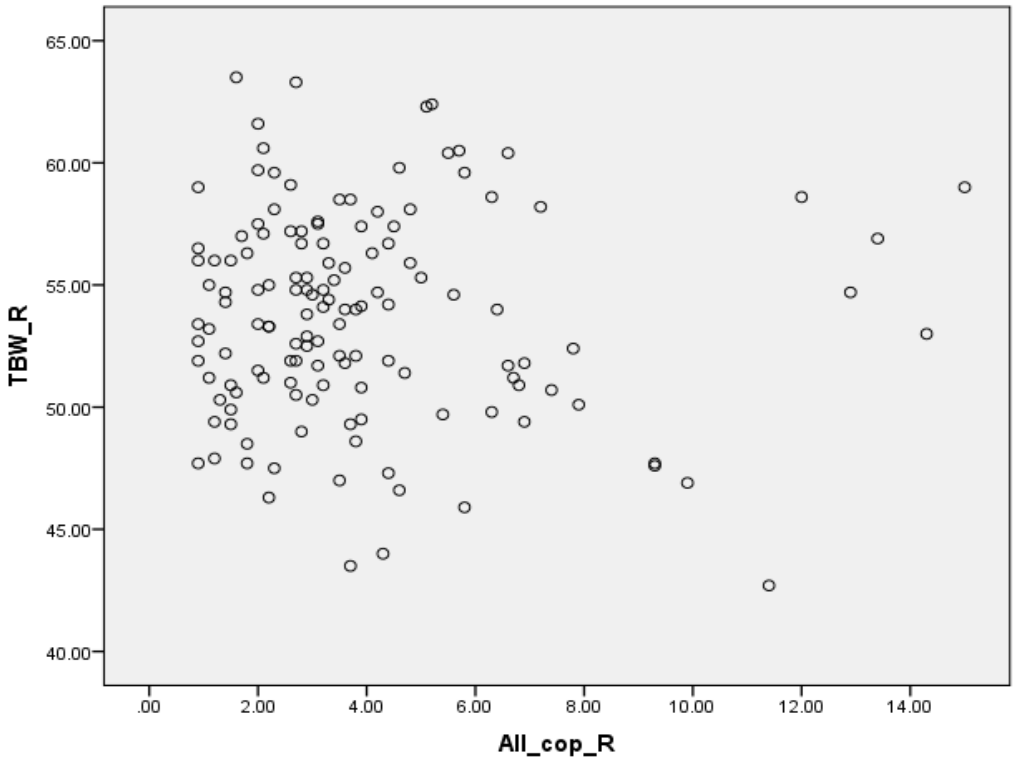


Figure 4.11 Relationship of AVP to fluid balance

Likewise there was no relationship between AVP and fluid balance recorded (rho -0.061 p=0.8)



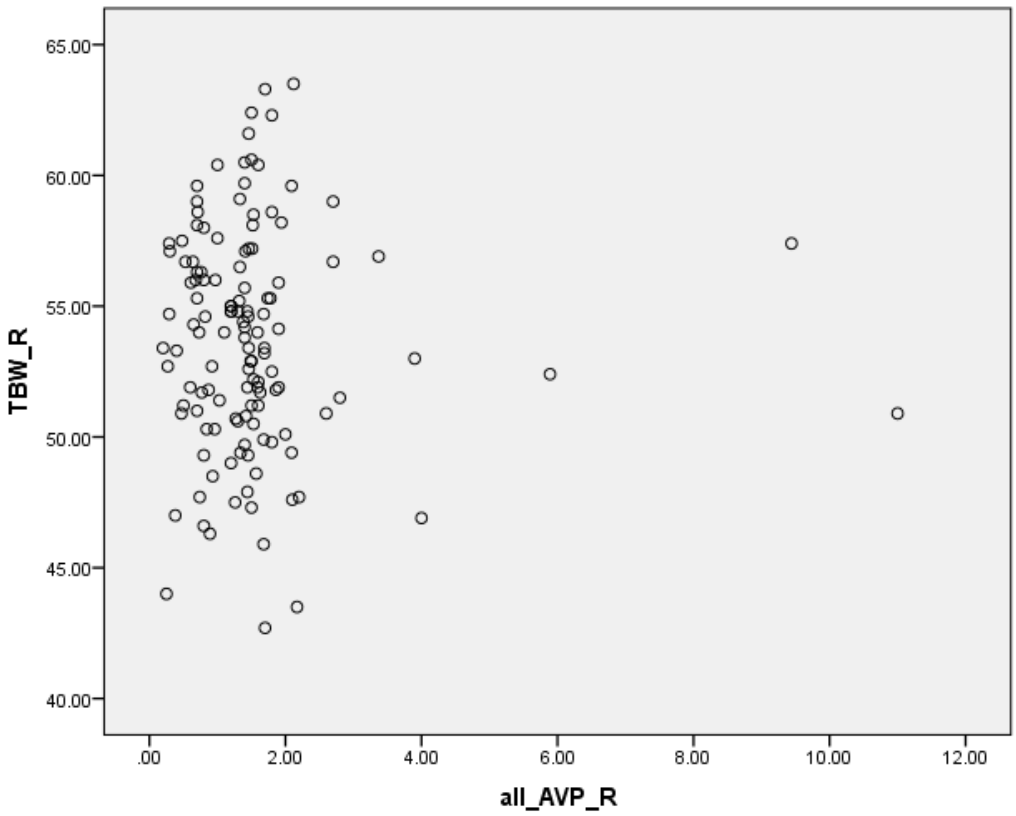


Figure 4.12 Relationship of TBW to copeptin and AVP when recorded on Quadscan device

Total body water as recorded on the Quadscan device showed no relationship to AVP or copeptin (rho -0.022, p=0.8 and rho -0.039, p=0.66 respectively).

Serum sodium measured at rest at each altitude showed no correlation with AVP or copeptin (rho -0.029 p=0.75 and rho 0.037, p=0.67 respectively).

There was no difference in mean fluid balance between those with AMS (n=37) and those without (n=100) (mean +748 mls v's 761 p=0.925).

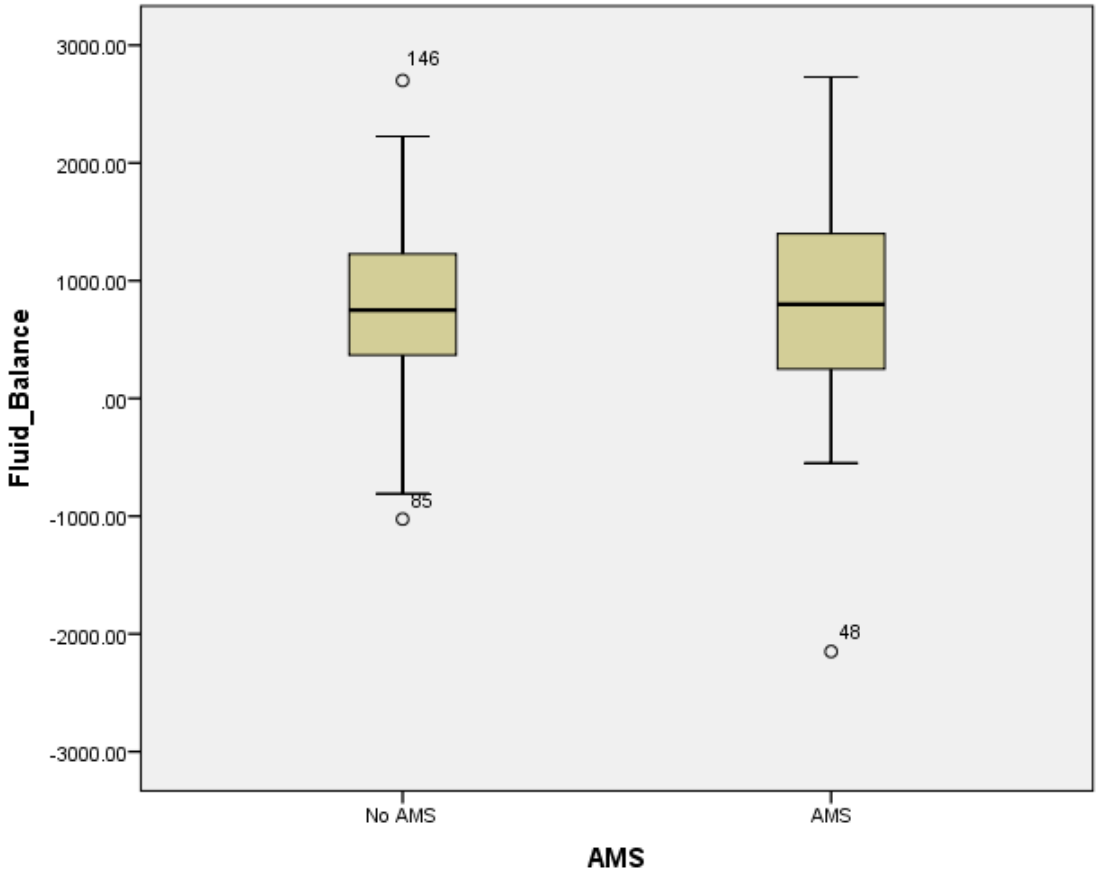


Figure 4.13 Fluid balance in groups with AMS (LLS≥3) or no AMS

There was no difference in fluid balance for those with severe AMS (n=11) v's those without (n=126) (mean +771 v's +608 mls p= 0.494).

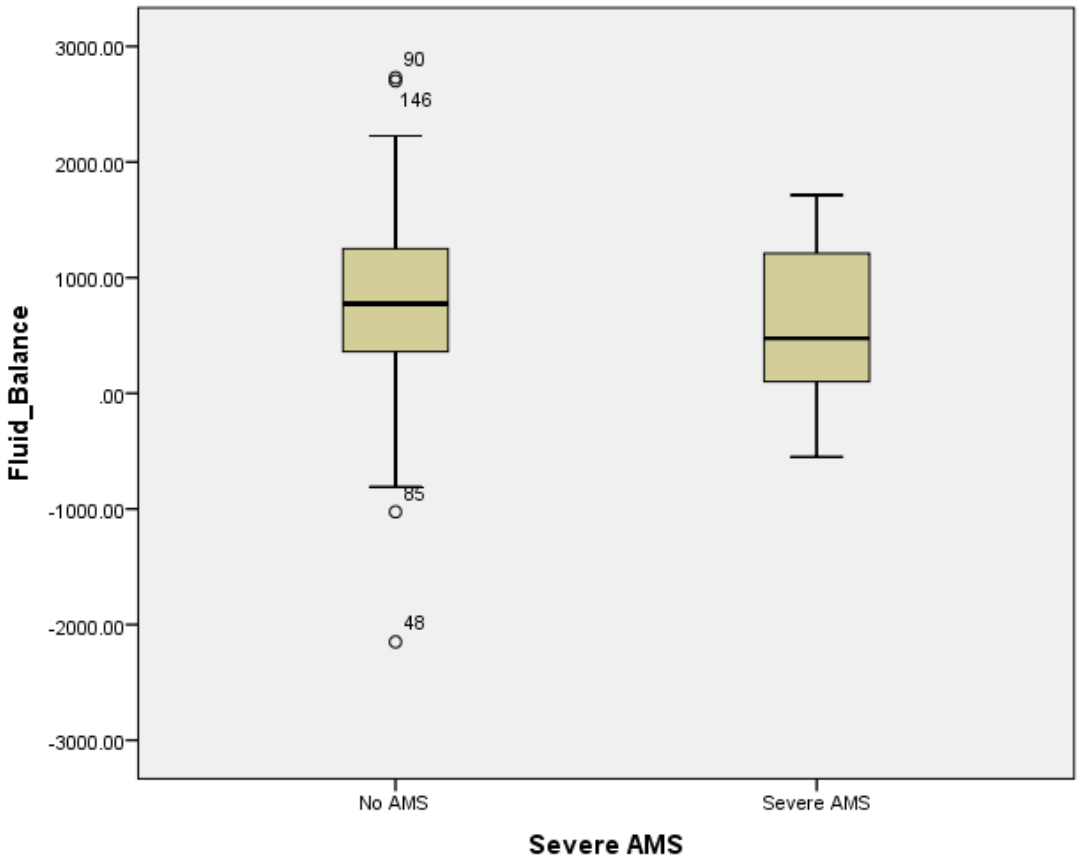


Figure 4.14 Fluid balance in groups with severe AMS (LLS≥6) or no AMS

4.4.3 *Thirst VAS*

The VAS for thirst was completed on 279 out of 288 study points.

These showed great variability between individuals. In an effort to see if the VAS score was meaningful within individuals the score was modified by subtracting the value recorded on day 4. Day 4 of the field study was a day of preparing equipment in La Paz, involved no physical exertion and was in close proximity to free fluids, therefore subjects should have been fully hydrated and not unduly thirsty on this day.

The unmodified mean thirst recorded was 35.52mm SD 18.5 and a range of 80mm. When modified by subtracting day 4 result the mean VAS recorded was 5.4 with an SD of 16.5 a range from -70 to +53 mm.

Thirst did not correlate with osmolality either with or without the modification to the score (Pearson's rho 0.106 p=0.08 and rho 0.076 p=0.21)

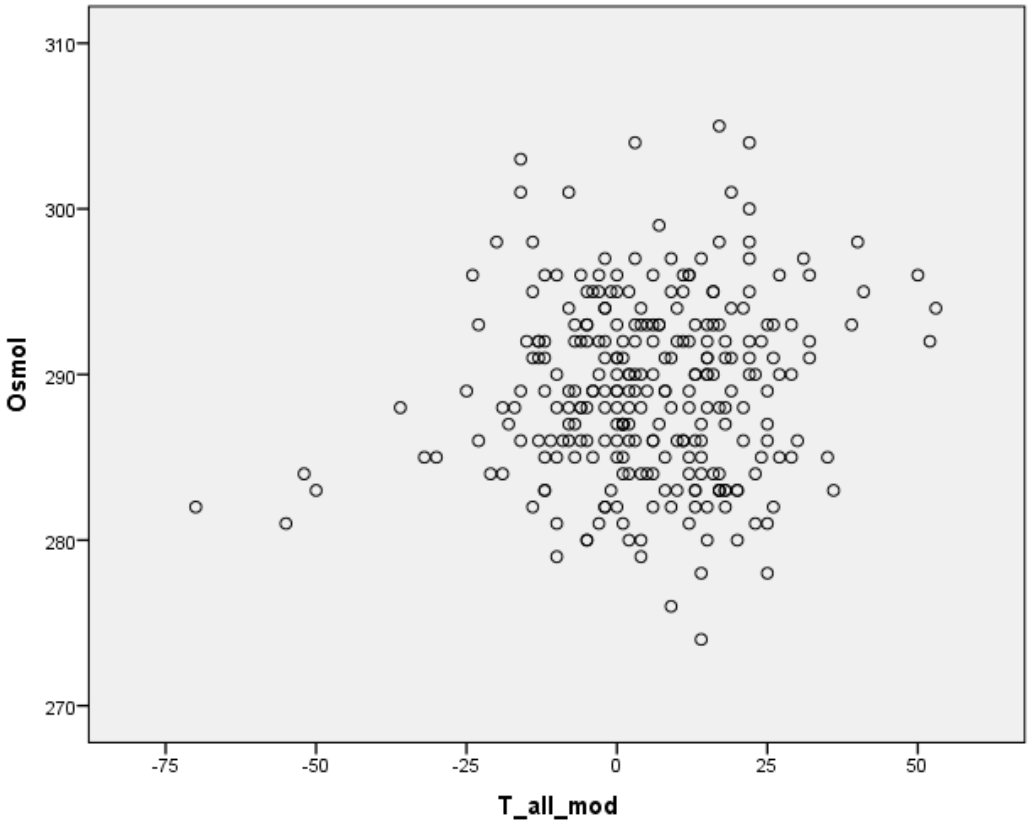


Figure 4.15 Distribution of thirst score when modified with osmolality (Pearson's rho =0.106).

There was no correlation with AVP or copeptin with either modified thirst rating (rho -0.068, p=0.279 and -0.073, p = 0.23 respectively) or the unmodified thirst score (rho 0.125, p = 0.045 and 0.1009 p = 0.072)

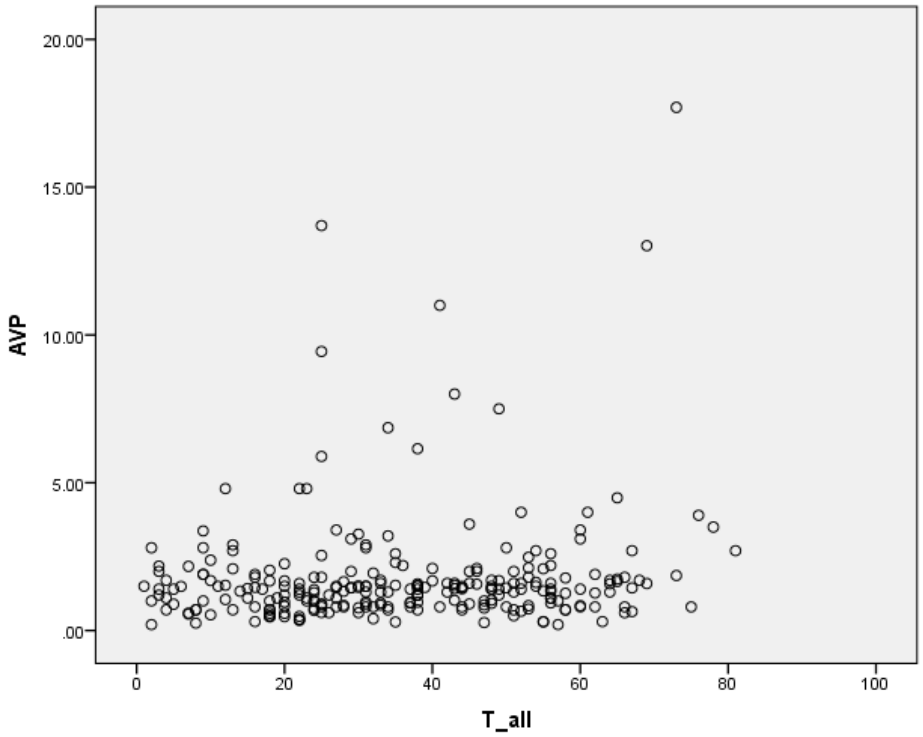


Figure 4.16 AVP correlation with unmodified thirst (Pearson's rho 0.125)

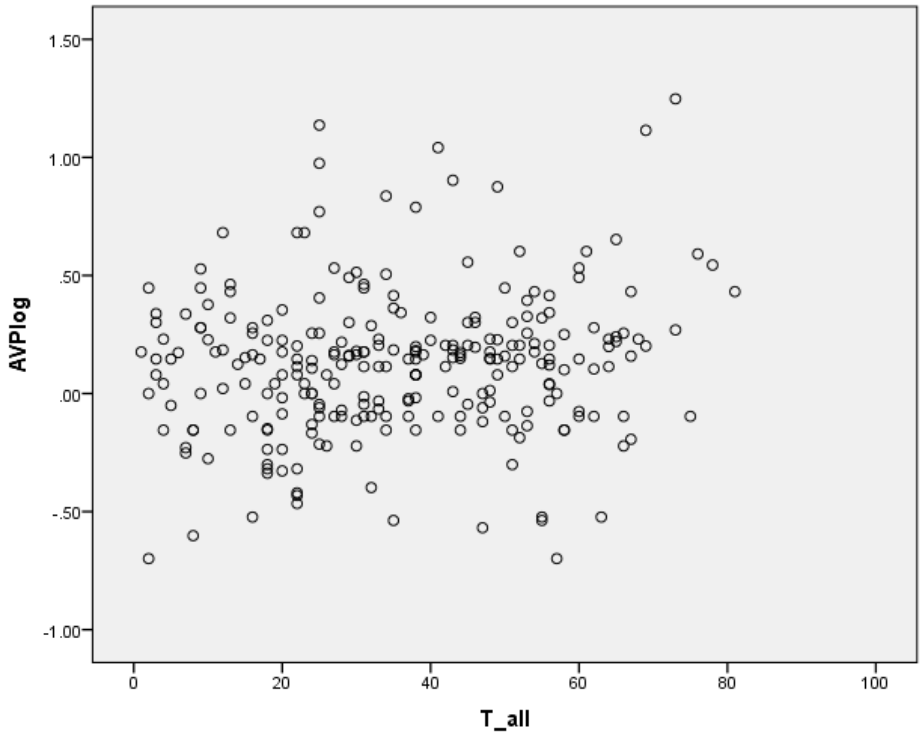


Figure 4.17 Log AVP plotted against unmodified thirst.

4.5 Discussion

There have been very few published studies comparing TBW assessment using SF-BIS and MF-BIS (Donadio, Consani et al. 2005) and this study focussed on the individual BIS methodologies relative to isotope dilution methods and anthropometric estimations rather than direct comparisons of the two BIS techniques. In a previous study of 52 healthy subjects (26 men and 26 women) without known disease SF-BIS agreed well with deuterated water though TBW values were 0.7l higher with deuterium dilution (Haas, Schutz et al. 2012). Interestingly, as in our study, there were relatively wide limits of agreement (mean ± SD) which was greater in men than women (-5.9 to 4.2 vs -3.5 to 2.3). Nevertheless, the methods were highly correlated in both men and women respectively (r^2=0.90 and 0.76).

The small mean difference of 0.2l between the NICaS and the Quadscan 4000 at sea level, observed in our current study, is in keeping with the work of Haas and Donadio cited above. For example, Donadio et al (Donadio, Consani et al. 2005) noted (n=19) higher post-dialysis TBW readings (+0.45l: 95% CI -0.71 to 1.61l) with the BodyStat QuadScan 4000 than that with another SF-BIS analyser (STA-BIA, Akern, Florence) among a group of haemodialysis patients, with a very high linear correlation between the methods (r=0.967). In our study, there was a further increase (overall difference 1.9 l, <5%) in TBW at HA but the strong correlation between the two methods remained the same. There are a number of factors that may explain the observed differences in TBW and it is not possible to know necessarily whether the QuadScan over-estimated TBW or the NICaS underestimated it as comparisons were not made to a reference isotope dilution measure of TBW for the reasons explained earlier. However, we did reference TBW to body mass which is a validated surrogate index for the changes in TBW (Baker, Lang et al. 2009). The QuadScan 4000 appeared to more strongly correlate with body weight than NICAS and more closely reflected the changes in TBW throughout the study. The increased difference in TBW measures between the two devices at HA may relate to the differences in their prediction equations and frequencies used and the impact of changing environmental and physiological conditions on the TBW values obtained (Kyle, Bosaeus et al. 2004). The small differences in electrode placements between the two devices could have influenced the findings but we used the electrode

placements as recommended by the manufacturers and according to previously published work (Paredes, Shite et al. 2006, Tanino, Shite et al. 2009). The NICaS SF-BIA utilises an adapted Kushner equation for TBW quantification (Kushner, Schoeller et al. 1992). The Kushner equation has shown a very strong agreement with that quantified by the gold standard of deuterium dilution (r = 0.996, SEE = 1.47 kg) across a number of differing subject groups. However, it was observed in the validation cohort that the differences increased at higher body weights and underestimated TBW by 7% and no information was provided in this paper on the examination altitude. During the Bolivian field study there was reduction in weight at the highest altitude of 5129m which could have influenced the findings but this would not explain the observed differences in at the two lower altitudes of 3833m and 4450m. Of importance there was no further difference in TBW measures on the post exercise sample at 3833m.

This study had a large sample size performed in a very difficult HA environment and at multiple altitudes (sea level, 3833m, 4450m and 5129m) and the assessment of exercise (at 3833m). There are also a number of limitations of this study that should be acknowledged. In this study SF-BIS was compared with MF-BIA and not to gold standard measures of TBW. As stated earlier, measurement of deuterated water would not have been possible given the environmental conditions of this study. It has been observed that BIS measurement can be influenced by diet and environmental conditions such as ambient and skin temperature and humidity which could not be controlled for in this field study (Lukaski, Johnson et al. 1985, Ellis, Bell et al. 1999). However, these factors would have been equally applicable to both methods which were performed at similar time points. It would have been attractive to have assessed the comparative values of other biometric assessments such as ICW and ECW however these are not available on the NICAs system. The two methods of TBW assessment do use differing frequencies and TBW prediction equations which would apply equally across all the study altitudes.

The fluid balance data did not support a failure of diuresis in those developing AMS. Again this may have been for a number of reasons, fluid intake may not have been accurately recorded, some subjects may have taken more oral rehydration during the trek or have been less inclined to drink during the night

and there may have been a variation in fluid taken in food. This demonstrates the difficulty of performing this sort of study in field conditions rather than in the controlled conditions of a lab.

The fact that copeptin and AVP showed no correlation with fluid balance or TBW is supportive of the discussion later in this thesis that the main stimulus to secretion is through non-osmotic stimuli. Further evidence for this is provided by the lack of correlation between plasma sodium and AVP or copeptin.

The use of the VAS score as a surrogate for hydration status was not successful. This may be for a number of reasons. Firstly our modification of the scale and asking for a rating at the end of the day differs from previous methodology used. Whilst in Rolls study this VAS did track osmolality the time course was much more acute with 11 recording of the VAS score over 1 hour (Rolls, Wood et al. 1980). A 37 point categorical Likert (in the form of responding to questions as to how strongly one agrees or disagrees) rating has been used for thirst sensations but this would be impractical to use in the field (Engell, Maller et al. 1987). A more useful modified Likert rating has been used as a research tool for pre-gastroscopy fasting and may prove useful in future field studies (no thirst/mild thirst/moderate to severe thirst) (Greenfield, Webster et al. 1996) to at least group subjects.

As a conclusion to this study, we found there was good overall agreement between TBW assessments using the SF-BIA NICaS system compared with the MF-BIA BodyStat Quadscan 4000, although the 9% confidence intervals around these agreements were quite wide. Whilst the agreements between the two methods remained unchanged with HA the absolute difference in TBW measurements was greater at both rest and exercise at HA. As the MF-BIA Bodystat Quadscan 400 tracked changes in body mass with more accuracy than the SF device this device seems to be the device of choice for future investigations. The VAS scale proved to be useless in this setting and further work should evaluate the modified Likert score used by Greenfield

Chapter 5. BNP as a Biomarker for Altitude Illness

Summary

BNP has many effects that may make it a beneficial adaptive hormone involved in acclimatisation. It also has been shown to rise in a variety of pathological conditions associated with increased right heart pressures or inflammation and may therefore rise as a marker of high altitude illness. During the field study in Bolivia the BNP and NTPro-BNP levels of trekkers ascending to altitude were measured and compared to PASP and AMS scores. Data was analysed to produce RoC curves for the utility of BNP or NTPro-BNP in predicting AMS or high PASP. RoC analysis for the NPs in severe AMS revealed an AUC for BNP of 0.675 (95% CI 0.553-0.797, p=0.013) and for NT-proBNP an AUC of 0.686 (95%CI 0.56-0.813, p=0.008). An NT-proBNP of 80.5pg/ml had a sensitivity of 61% and a specificity of 69% for detecting severe AMS. For detecting a PASP >40mmHg showed an AUC of 0.645 (95% CI 0.557-0.732, p=0.001) for BNP with an AUC of 0.648 (95% CI 0.566-0.730, p=0.001) for NT-proBNP. These figures are similar to the utility of BNP for detecting heart failure in healthy populations and must be seen in the context of a relatively low pre-test probability of any subject having AMS. Both BNP and NTPro-BNP show potential to aide in the diagnosis of high altitude illness.

Hypothesis

That BNP would rise with HA illness and could be used as a diagnostic test.

5.1 Introduction

As stated earlier in this thesis (chapter 1) there are a number of reasons to suppose that brain natriuretic peptide (BNP) may change with altitude and be a marker for high altitude diseases. To recap briefly these are;

- Effects that induce a natriuresis, diuresis and reduce secretion of renin and aldosterone – all of which would help acclimatisation to high altitude (Hall 2005).
- Has pulmonary vasodilator effects which would reduce any pathological rise in PAP at HA and therefore prevent the formation of HAPE. BNP

levels are related to PAP in a number of diseases (Nagaya, Nishikimi et al. 2000, Elstein, Nir et al. 2005, Corte, Wort et al. 2010).

- The related peptide, atrial natriuretic peptide, is known to rise at HA (Rock, Kraemer et al. 1993, De Angelis, Ferri et al. 1996).
- Prolonged endurance exercise induces a rise in BNP (Elstein, Nir et al. 2005, Scharhag, Herrmann et al. 2005, Hew-Butler, Noakes et al. 2008, Scharhag, Meyer et al. 2008).
- BNP increases in a variety of inflammatory states, correlates with other inflammatory markers (Chiurchiu, Izzi et al. 2008, Inoue, Kawai et al. 2010, Dimitroulas, Giannakoulas et al. 2012) and is a powerful predictor of death and morbidity following surgery and other illness (Christ-Crain, Breidthardt et al. 2008, Eliasdottir, Klemenzson et al. 2008).
- BNP is also elevated after acute brain injury (Sviri, Soustiel et al. 2006).

Previous studies in humans at high altitude have not shown a consistent picture (table 5.1).

Reference-	N	Altitude	BNP/NtPro	Exercise	PASP	Change in BNP	conclusion
(Due-Andersen, Pedersen-Bjergaard et al. 2008)	20	Not stated PaO_2 5.8+/-0.5 KPa	NTPro-BNP	No	No	↑	Hypoxaemia stimulates BNP rise
(Cargill, McFarlane et al. 1996)	10	Not stated SpO_2 75-80%	BNP	No	No	No change	
(Gao, Wang et al. 2013)	46	>3000m	NTpro	Not stated	No	↑ HA or HAPE, ↓ with rx	NTPro may be a biomarker
(Toshner, Thompson et al. 2008)	10	5200m	NTPro	No	Yes	None despite inc PASP	NTpro BNP does not rise
(Feddersen, Ausserer et al. 2009)	14	5050m	BNP	Yes (trek)	No	↑	Correlated with severity of AMS but not diuresis

(Woods, Hooper et al. 2011)	32	5150m	BNP	Yes (trek)	No	↑	A rise in BNP was associated with AMS
(Woods, Begley et al. 2012)	20	5150m	BNP & NT Pro	Yes (trek)	No	↑	BNP associated with AMS
(Woods, Mellor et al. 2013)	20	5150m	BNP & NT Pro	Yes(trek)	Yes	↑	High NT pro BNP identified those with PASP>40
(Boos, Holdsworth et al. 2013)	1 (case report)	3833m	BNP	Yes	Yes	↑↑↑	↓with treatment, useful clinical marker

Table 5.1 Previous human studies measuring BNP in hypoxia

Six of the eight studies have found a rising BNP with altitude and studies looking at specific pathologies (such as HAPE) have generally found a rise in NTPro-BNP with rises in PASP (Woods, Hooper et al. 2011, Woods, Begley et al. 2012, Boos, Holdsworth et al. 2013, Gao, Wang et al. 2013, Woods, Mellor et al. 2013). The one exception is the study by Toshner (Toshner, Thompson et al. 2008) and the Apex 2 study group at 5200m in Bolivia. In this study a cohort of 10 individuals were studied at sea level and then 5200m, whilst their mean NTPro-BNP dropped with ascent (despite increasing mean PASP) only means were compared and their data shows several outliers with either high NTPro-BNP or high PASP. Only 2 subjects reported a score consistent with severe AMS, 2 subjects showed a dramatic rise in NTPro-BNP and 3 subjects developed a PASP above 40mmHg. It is impossible to know whether these are the same subjects and demonstrates the importance of investigating outliers in these studies rather than comparing means.

A recent study in Chinese moving from lowland areas to the Tibetan plateau has found a rise in NTPro-BNP in subjects with HAPE (Gao, Wang et al. 2013) and significantly, this is reduced with treatment.

Overall this suggests a mechanism behind the disease (i.e. raised PASP in HAPE), a marker that rises with the mechanism identified (BNP) and one which can readily be measured.

5.2 Methods

The study methods are described elsewhere (Chapter 3).

BNP was analysed in the field on a Biosite Triage point of care monitor (Alere Ltd, Stockport, UK) using a Triage cardiorenal test kit. The Triage® cardiorenal test is a point-of-care, fluorescence-based immunoassay used which gives a rapid (15 minutes) quantitative measurement of BNP in the range 5-5000 pg/ml and NGAL in a range from 60 to 1,300 ng/ml. This technology was used as it is very suitable for a point of care system to be used in areas with limited power, access to cold storage and calibration equipment. Similar technology does exist for the measurement of NTPro-BNP but we chose to measure this using a lab based test on a serum sample that had been frozen at -20°C. The NTPro-BNP

assay was performed using the Roche NT-proBNP assay (Roche Diagnostics, Mannheim, Germany) with a range from 5 to 35000 pg/mL and a coefficient of variation at a mean NT-proBNP of 474 pg/mL of 5.8%.

As the maximal PASP response in healthy subjects to hypoxia is thought to be 40 mm Hg and as non-HAPE susceptible individuals have a mean rise of PASP to 37+/-4 mmHg during exercise in hypoxia (Grunig, Mereles et al. 2000) data were also analysed according to a PASP cut-off of 40mmHg. A ROC (receiver operating characteristic) analysis was done to investigate the utility of the NPs in identifying AMS/severe AMS and a PASP >40 mmHg.

For statistical calculations the software package SPSS 21.0 was used. Subjects were excluded who took drugs to aid acclimatization (n=3). Parametric or non-parametric statistical tests were applied after performing the Shapiro-Wilks statistic. Changes in dependent variables between altitudes were initially tested by Student's paired t test or Wilcoxon signed ranks test. For independent variables an independent-samples t test or Mann Whitney test was used. Either a within subjects ANOVA or a Friedman test was performed to investigate any serial changes in measures (eg PASP or BNP) with ascent. A correlation analysis between BNP and NT-proBNP was performed using Spearman's rank correlation.

5.3 Results

All data presented as mean, SD (+/- range where appropriate) pg/mL. The subjects were 35.2±9.1 years old, 175.5±9.5 cm in height and 77.5±13.9 kg in weight. 45 subjects reached the highest study altitude (5129 m).

LL scores

Repeated measure (RM) ANOVA revealed a significant change in LL score with ascent ($p<0.001$). Over the six study time points at HA 65 LL scores (24.4%) were consistent with AMS (LL score≥3) and 18 (6.8%) consistent with severe AMS (LL score≥6).

	Altitude (m)	Sea Level	3833	4450	5129
SpO_2	**Rest**	98 (1.34, 94-99)	88 (4.2, 75–93)	86 (4.96, 62-93)	81 (4.4, 66-90)
	Exercise	97 (1.53, 94-99)	84 (4.95, 71-94)	81 (6.28, 53-90)	79 (4.4, 68-88)
PASP (mmHg)	**Rest**	20.2 (7.0, 10-34.4)	27.2 (10.8, 12-54)	32.5 (8.5, 17-56)	33.2 (9.1, 17-54)
	Exercise	23.1 (6.4, 13.1-38)	29.8 (11.6, 12-58)	31.5 (9.8, 16.7-63)	33.9 (11.6, 17-63)
NT-ProBNP (pg/mL)	**Rest**	43 (29, 10-116)	137 (175, 16-1195)	50 (47, 7-270)	85 (92, 8-479)
	Exercise	62 (43, 14-174)	102 (64, 13-289)	36 (29, 5-134)	75 (121, 6-730)
BNP (pg/mL)	**Rest**	8.9 (7, 5-34.7)	13.4 (16.6, 5-111)	6.5, (3.8, 5-24.1)	11.6 (9.7, 5-40.9)
	Exercise	8.9 (5.9, 5.2-27.7)	12.3 (11.1, 5-8.3)	6.3 (4.2, 5-29.6)	8.0 (5.9, 5-33.8)

Data presented as mean (SD, range)

Table 5.2 BNP, NT-proBNP and PASP at each altitude.

5.3.1 BNP and NT-proBNP

BNP and NT-proBNP values for each study point are detailed in Table 5.2. NT-proBNP and BNP significantly correlated (rho 0.763, p<0.001)

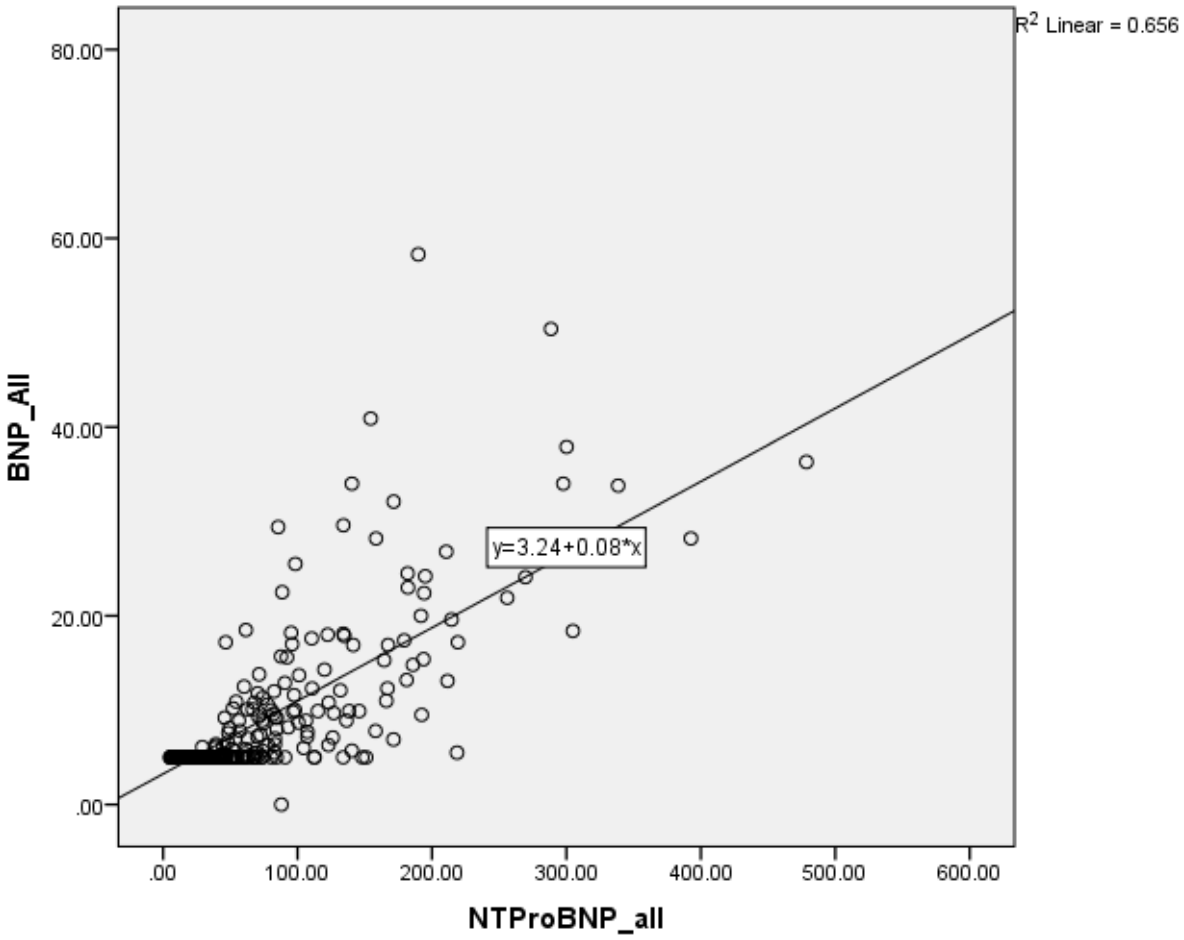


Figure 5.1 Correlation between BNP and NT-ProBNP across all time points

NT-proBNP (p<0.001), but not BNP, rose modestly following exercise at near-SL in the UK. Repeated measures analysis demonstrated a significant change in BNP and NT-proBNP with ascent (P<0.001 for both). Peak BNP and NT-proBNP levels were seen at 3833 m. There was no significant difference in BNP at rest or post-exercise between SL and 5129 m. NT-proBNP was significantly higher at rest at 5129 m vs SL (p=0.008) but not following exercise.

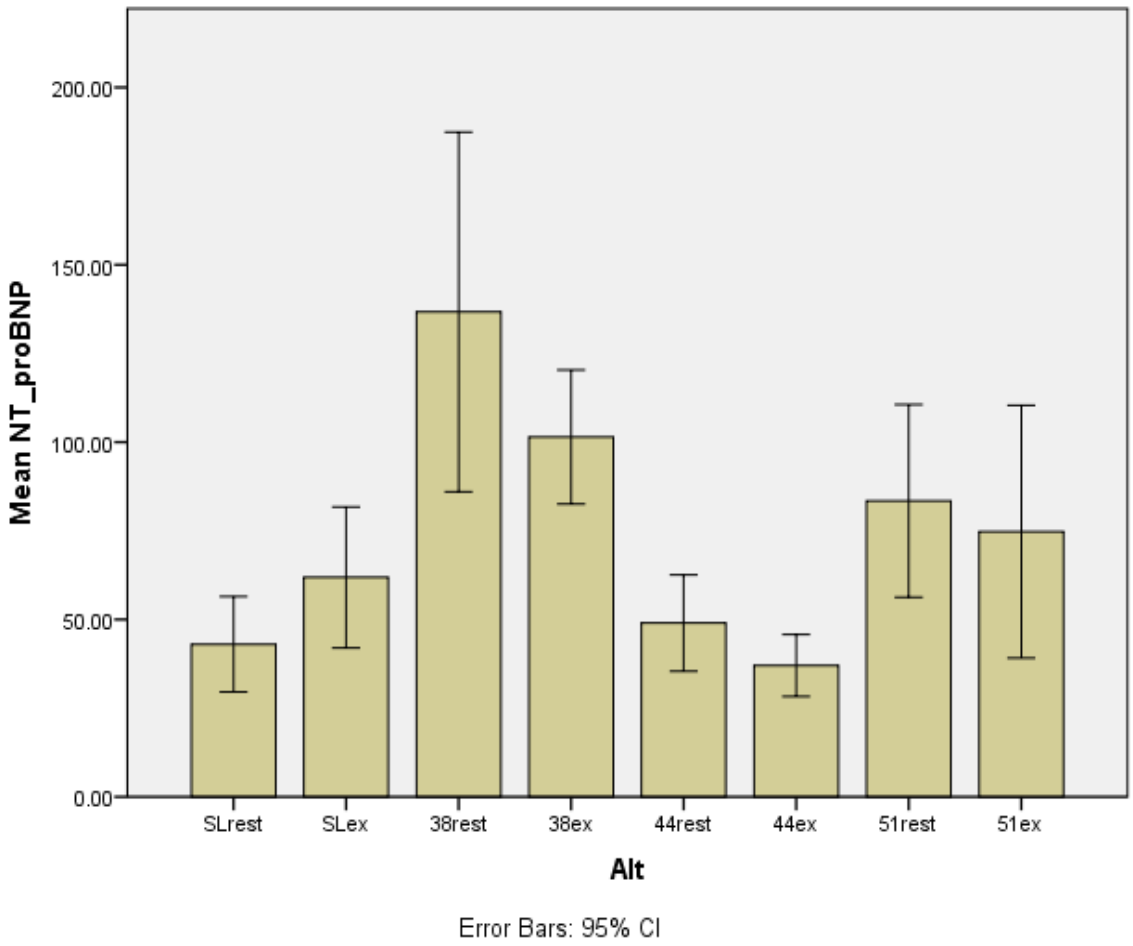


Figure 5.2 NTPro-BNP changes at each altitude

5.3.2 *AMS scores and BNP/NT-proBNP*

At altitude there were 266 simultaneous recordings of a LL score at HA with a simultaneous BNP and NT-proBNP. NT-proBNP and BNP were significantly higher on those occasions when LL score was consistent with AMS (n=65, 24.4%) vs. those without (NT-proBNP: 113±164 vs 72±78, p=0.034; BNP 13.1±16 vs 8.7±7, p=0.008). On those occasions when LL scores were consistent with severe AMS (n=18, 6.8%) NT-proBNP and BNP were also significantly higher than those without (NT-proBNP: 161.2±264 vs 76.4±82.5, p=0.008; BNP 17.1±25 vs 9.2±8, p=0.007).

ROC analysis (to investigate the utility of BNP and NT-proBNP in detecting AMS and severe AMS) revealed an AUC of 0.601 (95% CI 0.519-0.682, p=0.015) for BNP with an AUC of 0.588 (95% CI 0.506-0.67, p=0.034) for NT-proBNP in AMS.

ROC analysis for the NPs in severe AMS revealed an AUC for BNP of 0.675 (95% CI 0.553-0.797, p=0.013) and for NT-proBNP an AUC of 0.686 (95%CI 0.56-0.813, p=0.008). An NT-proBNP of 80.5 had a sensitivity of 61% and a specificity of 69% for detecting severe AMS.

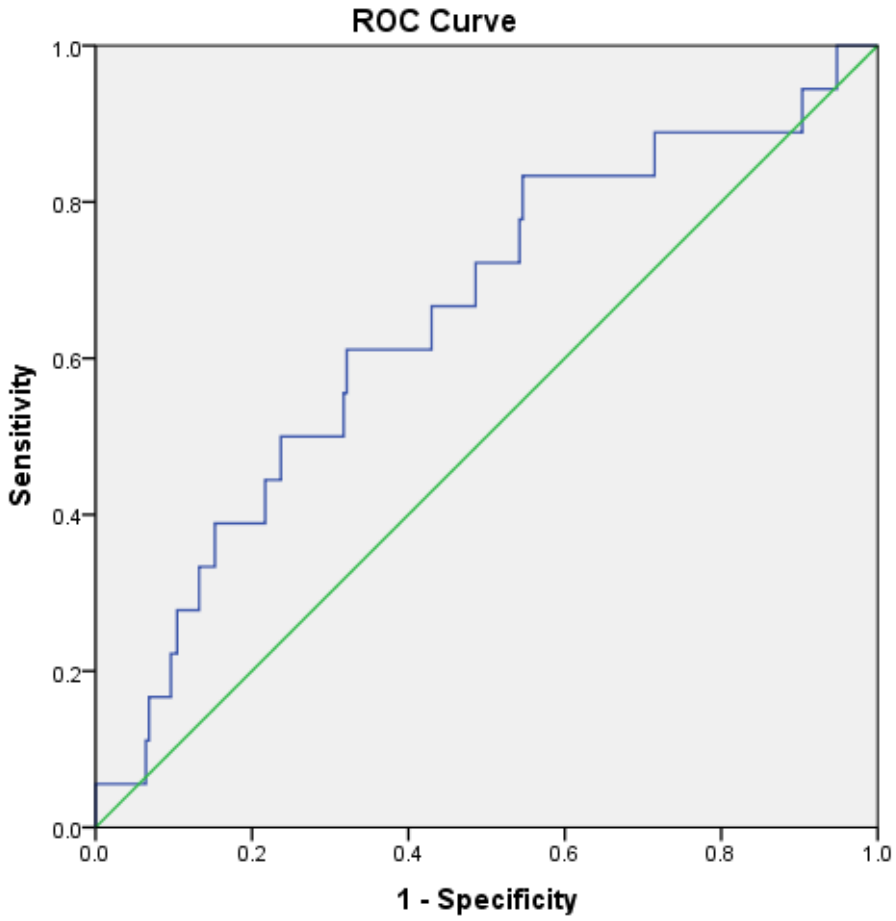


Fig 5.3 Roc Curve for NT-proBNP as a marker in severe AMS (AUC 0.686)

5.3.3 *PASP and BNP/NT-proBNP*

At altitude there were 245 echocardiographic assessments of PASP with simultaneous BNP or NT-proBNP values. Of these 52 (21%) had a PASP≥40 mm Hg. Both NT-proBNP and BNP were significantly higher in those with a high PASP vs those without (NT-proBNP: 137±195 vs 71.8±68, p=0.001; BNP 15.3±18.1 vs 8.7±6.6, p=0.001).

ROC analysis (to investigate the utility of BNP and NT-proBNP in detecting a PASP≥40 mm Hg) revealed an AUC of 0.645 (95% CI 0.557-0.732, p=0.001) for BNP with an AUC of 0.648 (95% CI 0.566-0.730, p=0.001) for NT-proBNP.

Peak BNP and NT-proBNP occurred at 3833 m at rest. At that point there were 6 subjects with a PASP≥40 mm Hg. Their NT-proBNP and BNP were higher than those with a lower PASP (NT-proBNP: 370+/-413 vs 101+/-72, p=0.003; BNP: 37.5+/-38 vs 10+/-6.7, p=0.003).

5.4 Discussion

There was good correlation between NT-proBNP and BNP suggesting that (as in other conditions) these markers can be used with equal confidence.

There was a peak rise in NT-ProBNP and BNP at 3833m, this was associated with peak LLS at 3833m post exercise where mean LLS recorded was approximately twice that at 4450m or 5129m after exercise (2.9 vs. 1.4 p= 0.001 vs 4450m and 0.002 vs. 5129m). While it could be argued that the diagnostic ability of the NPs in detecting AMS or a high PASP is not at present clinically robust it must be noted that the test was applied in all subjects, irrespective of symptoms, at HA. As such the pre-test probability of detecting either condition will naturally be low. The clinical utility of the NPs in detecting asymptomatic left ventricular systolic dysfunction in apparently healthy populations is also highly variable with BNP values between 45-50 pg/ml giving 95% specificity but very low sensitivity (13-27%)(Vasan, Benjamin et al. 2002). The AUC for BNP in detecting mild systolic dysfunction is around 0.60 in women and 0.72 in men (Goetze, Mogelvang et al. 2006). For an NT Pro-BNP this study found an AUC of 0.686, with a value of 80.5 pg/ml providing a sensitivity of 61% and specificity of 69% for severe AMS. One reason our data was not stronger is that the pre-test probability of an individual having the disease was low. For the diagnosis of cardiac failure NPs have an AUC of between 0.804 and 0.883 (Boldanova, Noveanu et al. 2010) if breathless patients presenting to the emergency room are tested. One of the major problems with this line of investigation is the lack of a gold standard against which to assess the test. The Lake Louise Consensus score is quoted as having a sensitivity of 78% and a specificity of 93% for a LLS $\geq$4 being consistent with a diagnosis of acute altitude illness when compared to

AMS-C and AMS clinical score (Maggiorini, Muller et al. 1998). Of note it is interesting that Maggiorini uses a LLS $\geq$4 rather than the usual score of $\geq$3 to diagnose AMS.

Figure 5.2 shows that NT-ProBNP rose with exercise at sea level yet rises at rest at HA. This is explained by the time course of the measurements. For the control group rest samples were taken, there was an ascent followed by descent over several hours. There was therefore a delay from peak exercise stress to sampling. The DMS hypoxia study group has previously shown that brief, profound hypoxia does not produce a BNP result if tested at 3 hours post hypoxia (Woods, Hooper et al. 2011). More importantly the rest samples in Bolivia were taken the following day so a period of exercise took place followed by overnight rest and prolonged exposure to the hypoxic environment. This has implications for optimum timing of any sample for either clinical or research purposes.

Diagnosing HAPE in the field can be a significant clinical challenge and although a high PASP is a key feature of HAPE (Maggiorini, Melot et al. 2001, Bartsch, Mairbaurl et al. 2005) echocardiography is not typically available. A surrogate biochemical marker could therefore be extremely useful and facilitate both early detection and diagnosis.

NT-proBNP has also recently been found to fall with treatment of HAPE in Chinese subjects (Gao, Wang et al. 2013) suggesting the possibility that the NPs may have a role in monitoring the response to treatment similar to that in CCF.

Prolonged endurance exercise leads to increases in NT-proBNP (Scharhag, Herrmann et al. 2005, Hew-Butler, Noakes et al. 2008, Scharhag, Meyer et al. 2008). It has previously been reported (Toshner, Thompson et al. 2008) that following arrival by vehicle at 5200 m (after 5 days acclimatization at 3600 m) no rise in NT-proBNP occurred despite a rise in PASP. This emphasizes the importance of prolonged exercise at HA in the release of NPs. It would seem likely that exercise at HA will increase the myocardial oxygen gradient, potentiate myocardial hypoxaemia, and be a greater stimulus to NP release. However this hypothesis does not explain the lag to BNP rise - brief hypoxia to a nadir of an SPO2 of 62% does not increase BNP within 3 hours (Woods, Hooper et al. 2011).

NT Pro-BNP would appear to be the best biomarker for further investigation. NT-proBNP is more stable in plasma and has a longer half-life therefore values measured are higher and a change more likely to be recognized. The challenge for future studies evaluating biomarkers at HA is to further delineate the pathological from the physiological by investigating those who are symptomatic and where the differential diagnosis may lie between respiratory infection or HAPE. A biomarker that could detect an abnormally high PASP or HAPE at HA could significantly improve diagnosis, management and outcome, similar to that seen with the use of biomarkers in CCF and acute coronary syndromes.

5.5 Clinical relevance - Case Report Subject 42(Boos, Holdsworth et al. 2013)

The potential clinical utility of measuring natriuretic peptides at HA is illustrated by the case of subject 42, a 39 year-old-male participant on the research study. He presented with headache, fatigue and nausea, following a six hour trek from 3,200m to 3,833m. He had flown into La Paz, Bolivia at 3,650m from the UK 48 hours earlier. He subsequently developed orthopnea and significant nocturnal breathlessness leading to insomnia and the onset of central chest tightness by the morning of day three. He was an experienced mountaineer. In 2003 he had suffered a right central retinal vein occlusion at 7,000m on Everest, descending immediately, with spontaneous resolution after four weeks at sea-level. He had subsequently climbed to $\geq$4,000m on multiple occasions over the ensuing nine years. He was an athlete and had completed the Marathon des Sables and several mountain marathon events. He has no other past medical history and was on no medicine to aid acclimatisation or other medication.

On examination, he was obviously breathless and tachypnoeic with a SpO_2 of 76% and had bilateral inspiratory crepitations with severe AMS (LLS 9). He underwent a portable transthoracic echocardiogram (Vivid I, GE Healthcare™, Amersham, Bucks, UK). This revealed significant pulmonary hypertension and right ventricular dilatation with a peak PASP of 54.0 mmHg (Table 7.3). Brain natriuretic peptide (BNP) levels, measured on point-of-care testing (Alere Triage® BNP, Alere Ltd, Cheshire, UK) were significantly raised at 111 pg/ml (normal 5-30pg/ml). Subsequently laboratory investigation showed his NT-proBNP to be 1195 pg/mL at the same time. He was diagnosed with HAPE.

Descent would have been extremely difficult requiring an initial ascent of 300m, furthermore the location he was in required a ferry crossing to return to the mainland – an option that was not available in the evening when the diagnosis was made. Hence, he was kept at altitude and treated with stat oral nifedipine 10mg bid and monitored regularly. Within four hours he had started to improve and slept better that night with resolution of his chest pain by the following morning.

On day four he improved further and continued by vehicle to 4,450m. His PASP, pulmonary vascular resistance, and right ventricular diameter fell (Table 5.3), with normalization of his BNP measurements by day five. He went on to climb to 6,000m just ten days later. Two months later, following his return to sea-level, his stored serum, taken at presentation, was analysed for high-sensitivity cTnT (hs-cTnT) levels (Roche Diagnostics, Burgess Hill, UK; (Table 5.3). His levels were significantly elevated at 43.7 ng/l (normal <14.0ng/l). He also had a repeat echocardiography and a cardiopulmonary exercise test including full 12-lead ECG analysis (Medical Graphics®, Ultima O2, MN, USA). This showed normal PASP, right ventricular size and supra-normal functional exercise capacity (peak VO_2 130% predicted) with no evidence of dynamic ECG changes. At that time he also had a hs-cTnT within the normal range.
This case reports the first case of HAPE where simultaneous echocardiography and testing for BNP and cTnT were obtained at HA during the actual clinical event in a known healthy individual. The patient fulfilled the diagnostic criteria for HAPE which requires the presence of at least two typical symptoms (dyspnoea, cough, decreased exercise and chest tightness) and two recognised clinical features (chest crepitations or audible wheeze, central cyanosis) (Roach, Bartsch et al. 1993).

Our patient presented with HAPE and an associated pathological rise in both point-of-care BNP and latterly available NT-proBNP and hs-cTnT levels. It has been shown that HAPE-susceptible subjects generate higher PASP compared with control subjects, at equivalent HA owing to enhanced hypoxia-driven pulmonary vasoreactivity, which might explain their rapid response to oral Nifedipine (Sartori, Vollenweider et al. 1999, Schoene 2008).

The significant increases in PASP, BNP and hs-cTnT, in this case occurred, despite normal cardiac output and normal estimated left ventricular filling pressures. The rise in BNP and hs-cTnT were not dissimilar to that observed in genuine clinical heart failure and myocardial infarction respectively. The cause for this rise is uncertain and may relate to the degree of PASP increase, right ventricular overload or severity of AMS (Nagaya, Nishikimi et al. 1998, Schoene 2008, Woods, Begley et al. 2012). The availability of echocardiography and simple point-of-care testing for BNP in this case supported the diagnosis and allowed the patient to be safely managed without the need for descent.
His BNP has returned to normal levels in 24 hours yet his PASP remained elevated. At the same time his NTpro-BNP was still elevated (in the highest 5% of the cohort). Whilst his PASP remained high his RV dimensions and calculated pulmonary vascular resistance had both started to fall. This may reflect the complexity of measuring resistance in the pulmonary vasculature (with PASP being a crude measure) and the fact that BNP has a shorter half-life than NT-proBNP, hence NT-proBNP values would have reflected the events of the last 24 hours.

	Altitude			
	Sea Level	3800m		Sea level
Variables	Baseline	At presentation	24 hours later	Two months later
Respiratory rate (minute^{-1})	14	28	18	14
Heart rate (minute^{-1})	44	88	78	43
Blood pressure (mmHg)	135/65	111/73	119/73	122/70
SpO_2 (%)	98	76	89	100
Lake Louise AMS score	0	9	1	0
Right ventricular end diastolic diameter (cm)	4.1	5.2	4.7	4.4
Pulmonary artery systolic pressure (mmHg)	16.1	54.0	50.4	21.6
Pulmonary artery resistance (dyn.s/cm^{-5})	37	226	142	96
Cardiac output (L minute^{-1})	4.4	6.1	7.1	5.4
Estimated left ventricular filling pressure	6.9	4.7	5.1	7.2
BNP level pg/ml	-	111	<5	<5
NT-ProBNP pg/ml		1195	192.3	
Hs-cTnT level, ng/l	-	43.7	36.7	<14

Table 5.3. Time-dependent changes in clinical, echocardiographic and hormonal values with presentation

Chapter 6 - Copeptin and AVP

Summary

As previously described fluid balance is a key part of adaptation to high altitude. Arginine vasopressin (AVP, also known as anti-diuretic hormone) may play a key role in this. The assay of AVP is a complex and time consuming radioimmuno assay and therefore has practical difficulties. Copeptin is C terminal proAVP and can be assayed in an inexpensive, automated way. Confirmation that copeptin values over the duration of a high altitude field study relate to AVP is therefore an important question.

Hypothesis;

Copeptin and AVP would increase together with rising osmolality at HA and be associated with AMS.

6.1 Introduction

As discussed in the introduction an early diuresis is a key part of acclimatisation to altitude.

Anti-diuretic hormone was the name given to the hormone involved in salt and water regulation at the kidney. More recently this hormone has been discovered to have a wider range of properties and has been renamed arginine vasopressin (AVP). Its' release is stimulated by osmoreceptors in the "osmostat", an area of the organum vasculosum of the lamina terminalis and in the wall of the 3rd ventricle that are sensitive to changes in osmolality. Normal plasma osmolality is 280 – 295 mOsm/Kg H2O, the greatest contribution to this is plasma sodium, along with glucose, urea and potassium. Basal levels of AVP are 1.15 – 2.4 pmol/l when osmolality is below 285mOsmol/Kg (Knoepfelmacher, Pradal et al. 1997). A 1% change in osmolality leads to a change in AVP release. AVP increases with increasing plasma osmolality and acts to facilitate the reabsorption of water by increasing the permeability of the distal collecting tubule. Non-osmotically stimulated secretion also occurs as part of the stress response, with high levels in critical illness and multiple trauma patients (Westermann, Dunser et al. 2007). There is evidence that there is an alteration of the osmotic stimulus to AVP release (with relatively suppressed AVP at HA) and also that elevated AVP is associated with HA illness (more fully described in other chapters). Furthermore newly developed

competitive AVP antagonists (such as Tolvaptan) offer a potential therapeutic option for treatment or prophylaxis of altitude illness, if a pivotal role for AVP can be proven. These reasons make AVP an extremely interesting hormone in terms of HA physiology.

The major problem with studying AVP is that the assay is complex, costly and time consuming. Additionally AVP is an unstable nonapeptide with a short (5-15 minutes) half-life in plasma. It is also highly bound to platelets and present in small amounts in the circulation. It can only be assayed by radioimmunoassay, a process which takes 3 days and is available in very few centres. This makes AVP a difficult peptide to study, especially in samples collected in the field.

Over the past decade an assay has been developed for copeptin, the C terminal part of pro AVP.

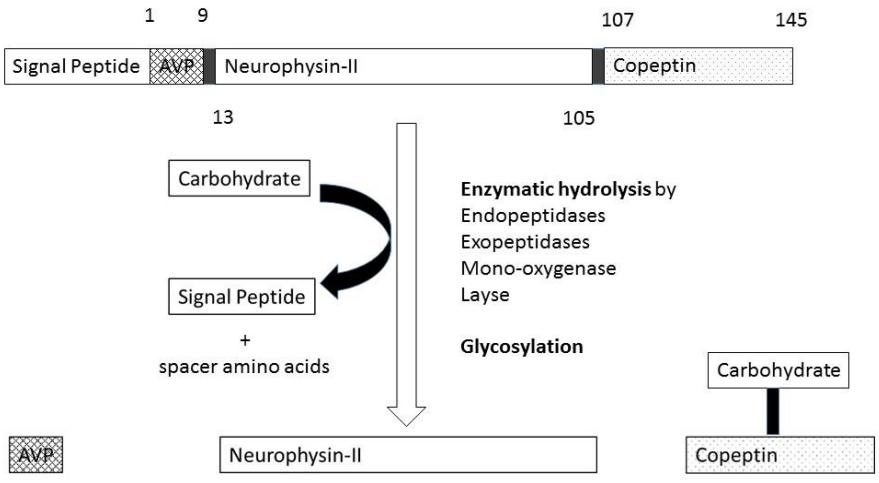


Figure 6.1 The processing of preproAVP (adapted from Elshafi. Annual Review and Research in Biology 2013)

Copeptin has the advantage of being more stable both in vivo and ex vivo. It can be measured by an automated process (see below) which takes less than one hour. Copeptin can be stored in a variety of media (EDTA, heparin, citrate or serum separator tubes). Analysis of 5 patients samples in serum, citrate,

heparin – plasma and EDTA showed mean values between 90 and 110% of the original after 7 days storage at room temperature (Morgenthaler, Struck et al. 2006), with a similar range of 93 – 110 % after two weeks at 4 ºC. The same authors found that samples could be subjected to 4 cycles of freezing and thawing with no change in the copeptin level measured. This is obviously a major advantage over AVP assays for use in a field setting.

Copeptin has been looked at in a number of studies and shown to correlate with AVP (table 6.1).

Author	Correlation (rho)	P value	N	Sample group
Morgenthaler (Morgenthaler, Struck et al. 2006)	0.74	<0.0001	39	Patients with sepsis
	0.31	0.008	71	Healthy controls
	0.78	<0.0001	110	Both groups combined-
Balenescu (Balanescu, Kopp et al. 2011)	0.8	<0.0001	20	Healthy subjects during water and salt loading study
Hew-Butler (Hew-Butler, Hoffman et al. 2011)	0.55	<0.0001	74	Ultra marathon runners
Jochberger (Jochberger, Morgenthaler et al. 2006)	0.282	0.017	70	Healthy controls
	0.73	<0.001	25	Critically ill, septic patients
	0.518	<0.001	36	SIRS patients
	0.725	<0.001	96	Post cardiac surgical patients
Jochberger (Jochberger, Dorler et al. 2009)	0.614	<0.001	60	ICU patients

Table 6.1. Previously published relationship between AVP and copeptin

The field study carried out in Bolivia was the first time that copeptin measurement at HA had been reported and was in a relatively large cohort (potentially 340 data points). The aim of this study was to support the fact that samples could be handled in an appropriate way for the copeptin assay and to provide evidence for AVP being a surrogate of AVP thus enabling the simpler and easier assay to be used in future studies.

There is a suggestion that copeptin (being more stable in the plasma) may reflect more long term changes in plasma volume whereas acute dehydration or stress, nausea etc. (non-osmotic stimuli to AVP) may cause a greater change in AVP secretion (Hew-Butler, Hoffman et al. 2011). This would be reflected in an increase in AVP relative to copeptin and an increase in the AVP:Copeptin ratio.

Importantly Jochberger found a significant but weak correlation (rho=0.33) in healthy controls with a stronger correlation in the septic patients where copeptin and AVP values were higher (rho=0.8) in septic patients (Jochberger, Morgenthaler et al. 2006). This suggests that the correlation is stronger in the stimulated state – healthy controls had a copeptin of 6+/-3 pmol/l whereas in critically ill patients the copeptin was 87 +/- 77 pmol/l. Following endurance exercise there is a good correlation between copeptin and AVP (rho=0.55) (Hew-Butler, Hoffman et al. 2011). One problem with studying these hormones at HA is that levels of AVP are thought to be suppressed (see discussion below) from already low resting levels in the un-stimulated state.

This was the first study to investigate the relationship between copeptin and AVP at high altitude in a large cohort of participants and to assess the utility of the copeptin assay as a surrogate for AVP at HA.

6.2 Methods

The study design and subject demographics are outlined in Chapter 3. To briefly review 20 subjects studied on a near sea level trek formed the control group. Fifty subjects were studied at 6 time points, at 3833 m, 4450m and 5129m (following a trek and at rest).

Data was collected for plasma osmolality, AVP, copeptin at each study point (and at near sea level in 20 subjects).

Plasma osmolality – samples were collected by venesection form an antecubital fossa vein in a serum separator tube (SST). This tube was allowed to clot and then centrifuged within 3 hours. Serum was pipette into a cryovial and immediately frozen to -20ºC.

AVP – samples were collected as above into a Lithium Heparin collection tube. These samples were immediately centrifuged, pipetted and frozen at -20ºC. 1ml of plasma was required for each assay.

Copeptin – samples were collected as above into an EDTA tube. These samples were centrifuged, pipetted and frozen within 30 minutes. Fifty µl was required for each assay.

Subjects recorded daily physiological variables and perceived exertion during the treks as part of the study. The Borg perception of exertion was recorded (Borg 1970) at the end of each day to record the hardest perceived exertion experienced during the day.

6.2.1 *AVP Assay*

Arginine Vasopressin Assay method

Assay of plasma AVP is difficult due to its' low concentrations (this is specifically a problem at HA where AVP secretion is suppressed) and short half-life in plasma. Newcastle University/RVI Hospital has developed a solid phase radioimmunoassay (RIA) for AVP based on reaction with antibodies raised in a rabbit model (Burd, Weightman et al. 1984). As part of this MD I personally analysed the majority of the AVP samples from the field study in the laboratory at the RVI. The plasma samples require extraction by reversed phase solid phase extraction method before incubating with AVP antibody and then a further incubation with ^{125}I-AVP complex.

Principles of solid phase extraction

Sample preparation - The sample is prepared from a lithium heparin blood sample centrifuged and the plasma frozen within 30 mins. This is a crucial step due to the short half-life of AVP. One ml of plasma is required and is initially acidified to reduce any protein binding of the AVP. The solid phase extraction uses a Sep-Pak® C18 non polar sorbent with the samples drawn through via a vacuum eluting system.

Cartridge Conditioning – For reversed phase sorbents the cartridge must be preconditioned and 5ml of methanol is used for the AVP assay. This is preceded and followed by 10 mls de-ionised water. This step is necessary to allow the solvent to penetrate the hydrophobic surface and wet the sorbent and increase the surface area of sorbent available.

Cartridge Loading – The acidified plasma sample is then applied to the extraction column. It is important at this stage to monitor the rate of flow through the column as too high a rate leads to sample breakthrough and poor reproducibility.

Wash step – The cartridge is then washed with 4% acetic acid. This removes unwanted material on the matrix whilst leaving the AVP bound to the sorbent.

Elution step – The AVP bound to the cartridge is eluted with methanol into a receiving tube. This produces a sample of approximately 4 mls which must be taken down to complete dryness. Once dry, the samples can be stored in a freezer until sufficient numbers have been accumulated to run an assay.

Principles of RIA

Radioimmunoassay is a technique first described by Yalow and Bersen to assay plasma insulin (Yalow and Berson 1959). In short this technique involves introducing a known concentration of radiolabelled antigen and antibody to a sample. The labeled antigen then competes with the antigen in the sample to form complexes. When the excess radiolabelled antigen is removed the remaining radioactivity is related to the concentration of antigen in the sample. By creating known standards a binding curve can be generated which allows the value of antigen in the patient sample to be calculated.

AVP RIA Technique

The dried samples must first be resuspended in buffer 800µl of assay buffer. The buffer is made by dissolving 3.028g TRIS(hydroxymethyl)methylamine and 0.1g sodium azide in 400mls distilled water in a beaker. To this 1.75g Bovine serum Albumin fractition V (Sigma A9647) is dissolved. This is then adjusted to be pH 7.4 by adding 5M HCl and made up to a volume of 500mls in a volumetric flask. The tubes are then centrifuged to remove sediments at 1500RPM at 4ºC for 15 mins. 200µl of this is then taken off into another tube (and duplicates created) and incubated with the "first antibody" (AVP-Ab) at 4 ºC for 24 hours. This antibody has been generated in rabbits by a method described by Burd in 1984 (Burd, Weightman et al. 1984). During this incubation period the rabbit antibody forms complexes with the AVP (antigen) in resuspended sample. After 24 hours 100 µl ^{125}I-AVP is added to the mixture. Radiolabelled iodine is chosen as this readily binds with the tyrosine residues in proteins. This ^{125}I-AVP will then form complexes with any rabbit AVP-Ab in a competitive fashion with the sample AVP. The labeled AVP is supplied as (3-[125I}iodotyrosyl)Vassopressin[Arg 8] (Perkin Elmer cat no NEX128010UC). This is allowed to incubate at 4ºC for 18 hours. The final step is to add 100µl anti rabbit Sac Cel (anti rabbit anti-IgG serum) to the tubes. This precipitates the Ab-Ag complex. The tubes then have 1ml of de-ionised water added to dilute the supernatant and are centrifuged for 15 minutes (3000 rpm 4ºC). The final complexes then appear as a white precipitate in the tubes with a clear supernatant. These tubes are then inverted to pour off the supernatant and left briefly to dry. These samples are then counted on a gamma counter (Cobra gamma counter programme 13). This programme initially generates a calibration curve from 10 standards (see below) and calculates AVP from a position on that curve. Background count is also measured and taken into account. Results are reported in pmol/l.

QC samples

QC samples are prepared from commercially available sources (Bio Rad Hypertension marker 1 and 2) and prepared at 3 dilutions.

Preparation of standards

The standards are prepared from 0.5mg AVP standard (Ferring AB, Malmo, Sweden). This is made into Stock AVP standard (1280pg/ml) and is stored at -20 ºC in 0.5ml aliquots. The Stock standard is initially made up to 2 mls to give a concentration of 320pg/ml. A series of 10 tubes are prepared labeled S1 to 10 to which 800µl of assay buffer is added. Tube S10 then has a further 400µl of buffer and 400µl diluted stock standard to make up 1600µl volume. Serial dilution is now performed adding 800µl from each tube in tern i.e. 800µl from S10 to S9, 800µl from S9 to S8.

Potential errors

This is an old assay still using antibody generated in a rabbit over 10 years ago. It involves multiple complex steps during extraction and the RIA process and additionally AVP has a very short half-life in plasma. The method has an intra assay co-efficient of variation of 4% and a functional sensitivity of 0.3 pmol/l (laboratory data, RVI, Newcastle,UK).

6.2.2 *Copeptin assay*

The measurement is automated and carried out on a B·R·A·H·M·S KRYPTOR compact PLUS. The technology used is based on TRACE™ Technology (Time-Resolved Amplified Cryptate Emission), which measures the signal that is emitted from an immunocomplex with time delay. As this is a trademarked process the description in the following two paragraphs is taken from the product data sheet supplied by the company. The basis of the TRACE™ Technology is non-radiative energy transfer from a donor (a cage-like structure with a terbium ion in the centre) to an acceptor, which is part of a chemical protein. The proximity of donor (cryptate) and acceptor when they are part of an immunocomplex and the spectral overlap between donor emission and acceptor absorption spectra intensify the fluorescent signal of the cryptate and extend the life span of the acceptor signal, permitting the measurement of temporally delayed fluorescence.

When the sample is excited with a nitrogen laser at 337 nm, the donor (cryptate) emits a long-life fluorescent signal in the millisecond range at 620 nm,

while the acceptor generates a short-life signal in the nanosecond-range at 707 nm. When the two components are bound in an immunocomplex, both the signal amplification and the prolongation of the life span of the acceptor signal occur at 707 nm, so that it can be measured over µ-seconds. This long-life signal is proportional to the concentration of the analyte to be measured. Non-specific signals, e.g. the signals of the short-life and unbound acceptor and the medium-specific interference signals conditional upon the natural fluorescence of the sample, are eliminated by temporal delay of the fluorescence measurement. The signal generated by the cryptate at 620 nm serves as an internal reference and is measured simultaneously with the long-life acceptor signal at 707 nm which is the specific signal. Interfering influences, e.g. from turbid sera, are automatically corrected by means of the internally calculated ratio of the intensities at these wavelengths.

Technical data:

Sample volume..... 50 µl
Incubation time..... 14 min
Results are given in..... pmol/l
Direct measuring range..... 0.9…500 pmol/l
Measuring range with automatic dilution..... 0.9…2 000 pmol/l
Sample type..... serum, plasma (EDTA, heparin)
Kit stability on board..... 15 days
Assay principle..... sandwich

6.3 Results

Samples were available for 308 samples for AVP and 321 for Copeptin. This is from a potential maximum of 328 samples. The missing samples were a result of difficulties with venesection in some cases and the fact that a higher sample rate was achieved for copeptin is reflective of the fact that whilst 2 ml of blood is required for AVP assay (to give 1ml of plasma) only around 100 µl is required for copeptin.

When this initial data was analysed there were some significant outliers (being more than 3 SD from the mean). For this reason values greater than 22.36 for AVP and 68.72 for copeptin were excluded. Values excluded are listed below (table 1) and were checked for the AVP/Copeptin ratio (mean 0.77 across all samples) and any other features that might identify that case as a clinical outlier. Only one case had a high AVP, which was lower than a (high) copeptin, however this subject showed no other signs of being a clinical outlier so the data was excluded.

Sample no.	AVP (pmol/l)	Copeptin (pmol/l)	Action
SL Ex 07	45.4	5.2	AVP excluded
5200 R 10	35.4	2	AVP excluded
5200 Ex 33	63	2.3	AVP excluded
5200 Ex 31	27.8	13.9	AVP excluded
4200 R 46	35.9	1.7	AVP excluded
3500 Ex 33	31.3	87.7	AVP and copeptin excluded
3500 Ex 23	1.7	353.1	Copeptin excluded

Table 6.2: AVP and copeptin outliers

The revised data for AVP and copeptin was distributed as shown in the table below (values pmol/l)

	n	Mean	Median	SD	Range	Kolmogorov-Smirnov Statistic	P value
AVP	301	1.87	1.40	2.29	0.1 – 21.50	0.260	<0.001
Copeptin	319	5.46	3.80	5.42	0.9 – 53.3	0.212	<0.001

Table 6.3: Revised AVP and copeptin distribution data

Both AVP and copeptin had values clustered around low levels with significant outliers as illustrated below. The data for both was not normally distributed .

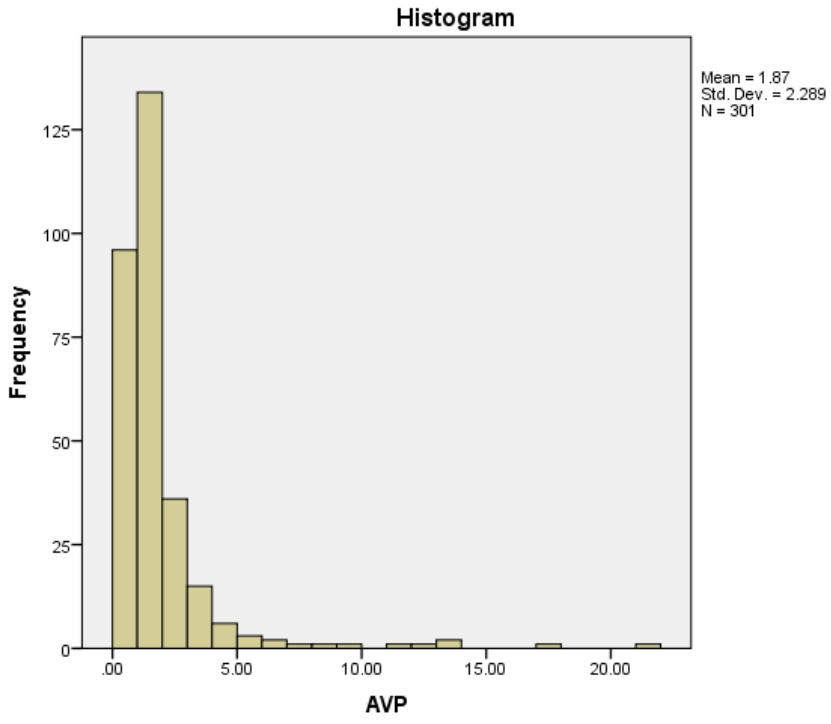


Figure 6.2. AVP value distribution (AVP in pmol/l)

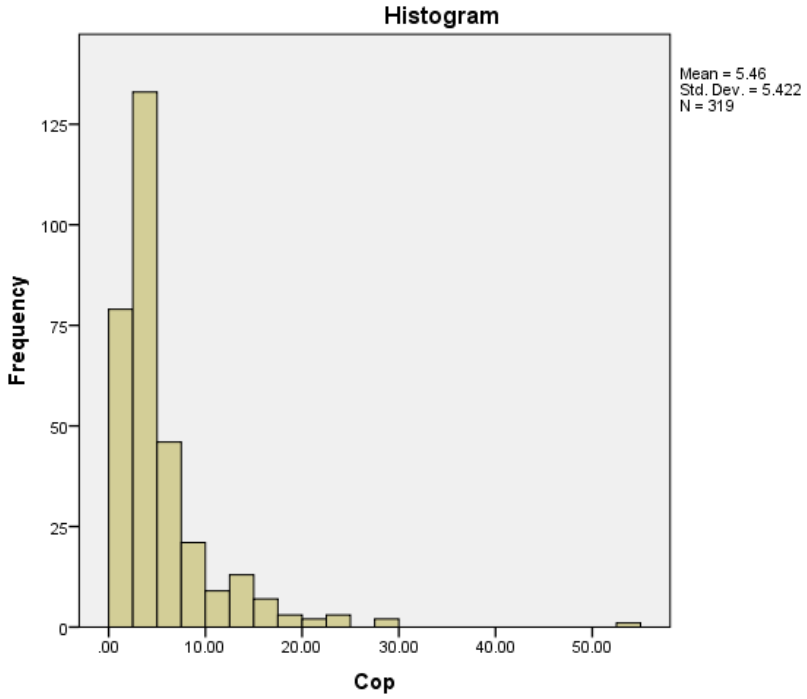


Figure 6.3. Copeptin (cop) value distribution (Copeptin in pmol/l)

There were no significant differences between AVP and copeptin between males and females.

	Male	Female	P Value
AVP			
-N	210	91	
-value	1.4 (1.8, 0.1 – 2.6)	1.8 (2.4, 0.2 – 17.7)	0.155
Copeptin			
-N	222	97	
-value	3.9 (4.68, 0.9 – 28.7)	3.1 (0.9 – 53.3)	0.155

Independent samples Mann Whitney U test

Table 6.4: Distribution of AVP and copeptin by gender

Across all time points AVP correlated with copeptin with a Spearman's rho =0.621 (p<0.0001).

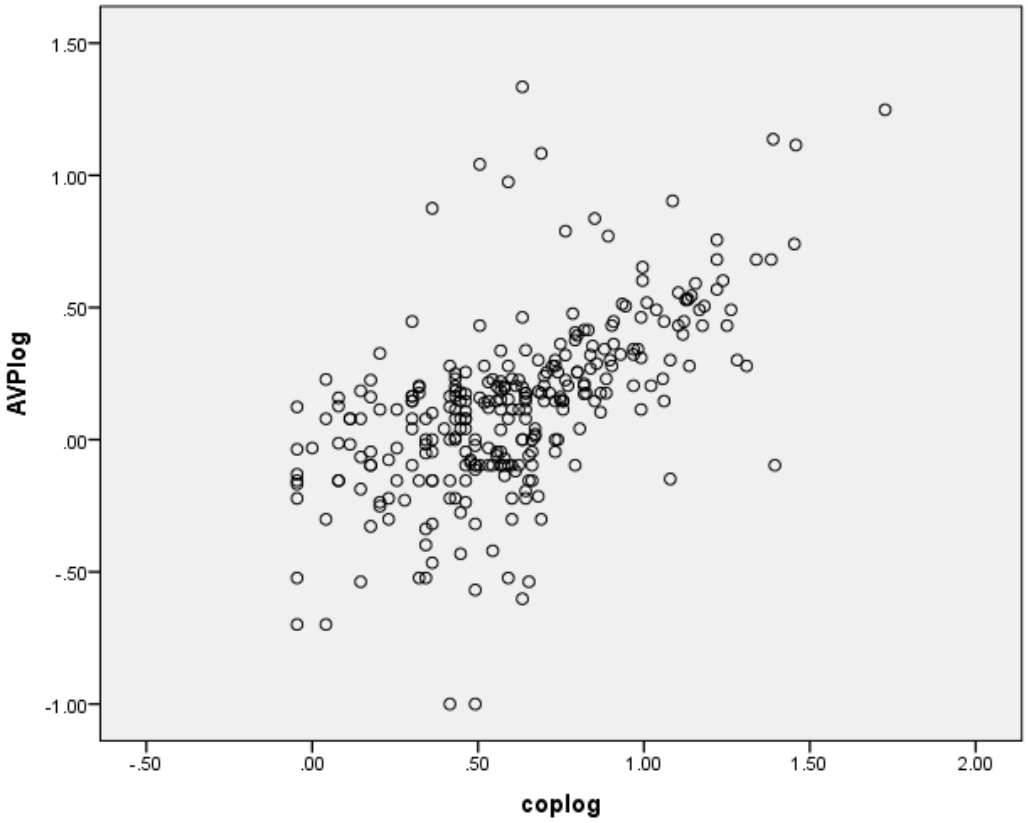


Figure 6.4 Distribution of AVP and copeptin plotted as log values for clarity.

This correlation held true across the altitudes with the exception of at rest at 4500m (3833m exercise Spearman's rho 0.459 p=0.002, rest rho0.414 p=0.004, 4500m exercise rho 0.812, p<0.0001, rest rho 0.295 p=0.064, 5129m exercise rho 0.834 p<0.0001, rest rho 0.663, p<0.0001).

The relationship with exercise of copeptin was maintained at all altitudes with an increase from resting levels. AVP showed a significant increase with exercise only at SL and 5129m. Whilst copeptin levels increased with exercise overall values were significantly reduced compared to sea level values at 4500m at rest and exercise and at 3833m following exercise. Copeptin showed a significant increase with exercise at 5129m.

	Sea Level	3833m	4450m	5129m	P value
Copeptin (pmol/l)					
-rest	3.5 (0.9-13.7)	3.3 (0.9-13.4)	3.2 (0.9-12.9)	3.4 (0.9-15.0)	0.48
-exercise	5.5 (2.6-28.4)	4.2 (1.2-53.3)	3.7 (1.0-8.7)**	6.6 (1.0-24.5)	0.007
AVP (pmol/l)					
-rest	0.9 (0.1-3.3)	0.9 (0.3-5.9)	1.5 (0.8-9.4) **	1.5 (0.2-11.0)	<0.001
-exercise	1.9 (0.1-21.6)	1.1 (0.3-17.7)*	1.1 (0.3-13.0)	2.0 (0.2-13.7) ##	0.002
Plasma Osmolality (mOsmol/Kg H_2O)					
-rest	286 (281-293)	291(279–299)	286 (276 – 298)#	286 (281 – 298)##	0.002
-exercise	296 (284 - 304)	293(281 – 300)*	292 (283 – 305)**	286 (274 – 303)***	<0.001

Data presented as median and range. P value for Kruskal-Wallis (non-parametric ANOVA) shown with post-test for significance: * 3833m vs SL, ** 4450m vs SL, ***5129m vs SL; # 3833m vs. 4450m, ## 3833m vs. 5129m

Table 6.5 Copeptin, AVP and Osmolality changes with rest and exercise at the study altitudes

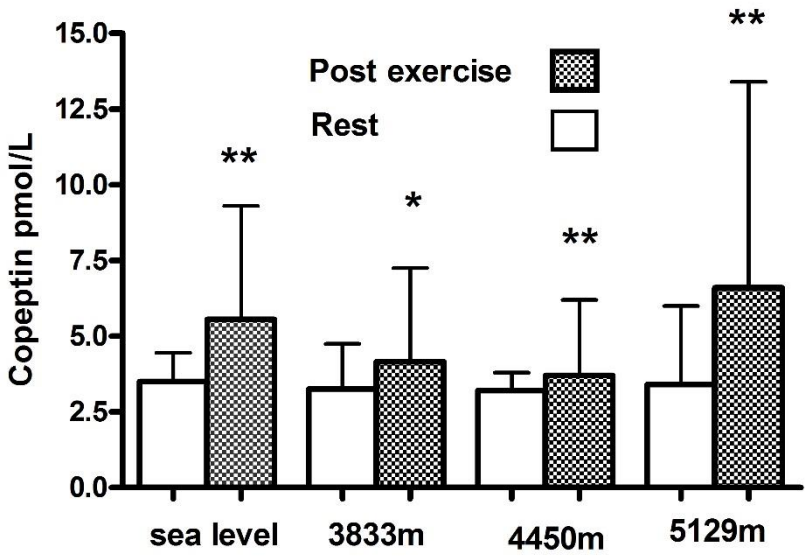


Figure 6.5 Changes in Copetin levels (median, interquartile range) during exercise and rest across increase high altitude. * refers to significant difference ($p<0.05$) on paired sampling,** ($P<0.001$)

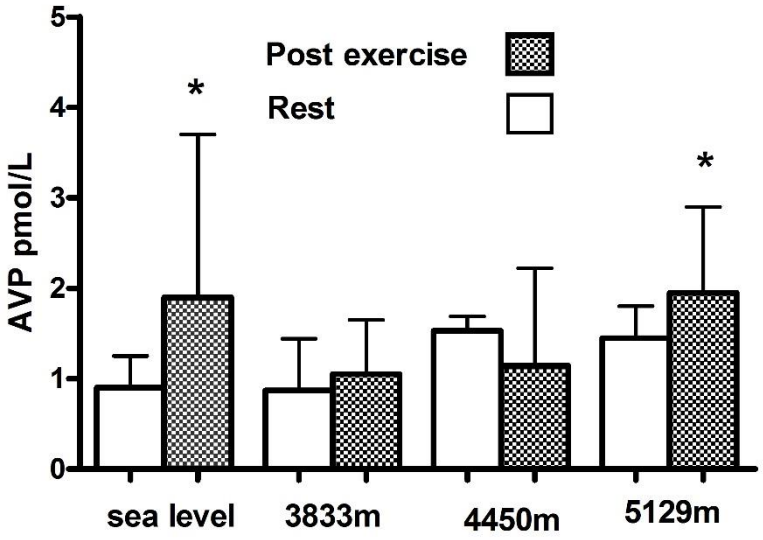


Figure 6.6 Changes in AVP levels (median, interquartile range) during exercise and rest across increase high altitude. * refers to significant ($p<0.05$) difference on paired sampling ** ($P<0.001$)

Altitude (m)	Sea Level		3833		4450		5129	
	N		N		N		N	
Rest	18	3.7 (2.25-31.0)	46	3.78 (0.75-17.2)	40	1.9 (0.41 – 5.83)	44	2.89 (0.29 - 6.7)
Exercise	18	4.14 (0.4-26)	44	4.16 (0.31 – 31)	42	2.98 (0.94 – 7.6)	42	3.45 (1.08 – 10.74)

Table 6.6 Copeptin/AVP ratios

There is no difference in copeptin or AVP values in the subjects developing AMS or severe AMS group (table 6.7).

	No AMS	AMS	Severe AMS	P Value
AVP				
-N	192	46	17	
-value	1.4 (0.2 – 13.7)	1.4 (0.2 – 11.0)	1.4 (0.6 – 17.7)	0.55
Copeptin				
-N	206	48	17	
-value	3.7 (0.9 – 24.5)	3.1 (0.9 – 24.8)	5.0 (0.9-53.3)	0.44

Table 6.7 AVP and copeptin levels (pmol/L) in subjects with acute mountain sickness (AMS)

If the headache component of the LLS is examined in more detail there is no difference in copeptin between those with or without headache, either at all time points (p=0.31) or at 5129m where the copeptin is highest (p=0.556).

Altitude (m)	**3833**			**4450**			**5129**		
	None	Mod	Sev	None	Mod	Sev	None	Mod	Sev
Rest	23	25	0	36	11	1	19	15	9
Exercise	18	28	2	29	17	0	31	15	1

Table 6.8 Frequency of headache (number of observations, none = 0, moderate = 1, severe $\geq$ 2)

Only 3 subjects reported nausea at 5129m (copeptin values 1.3, 11.50, 13.6 v's median 6.6. These numbers are too small to interpret meaningfully.

Oxygen saturations fell with ascent to altitude and were lower after exercise than at rest. Mean oxygen saturations at 3833m were 84% post exercise, 88% at rest (p value v's rest value p<0.001), 4450m 82 % post exercise, 86% at rest (p<0.001) and at 5129m 79% after exercise and 81% at rest (p=0.014). There was no correlation between SpO_2 and copeptin (rho -0.073), or AVP (rho -0.114).

Body weight was 77.0 ± 14.6 kg at sea level, 77.6 ± 13.9 kg at 3833m post exercise, 77.5 ± 14.0 kg at 3833 at rest, 76.9 ± 13.80 at 4450m and 76.1 ± 13.4 kg at 5129m (figure 1). On repeated measures ANOVA this difference was significant between all altitudes and 5129m (p<0.0001). This represents weight lost during the expedition as opposed to fluid lost during the day as osmolality did not change at 5129m.

Cortisol values are reported in chapter 8 of this thesis. Cortisol correlated with copeptin at 5129m (rho 0.358 p=0.014).

The mean Borg score for the trek to 3833m was 13.15 (SD 1.92), for the trek to 4500m it was 12 (SD 1.8) and for the trek to 5129m it was 14.98 (SD 1.98). The trek to 5129m was perceived as significantly harder than either of the lower treks ($p<0.0001$ v's both). The trek to 3833 m was perceived as harder than the trek at 4500m ($p<0.0001$).

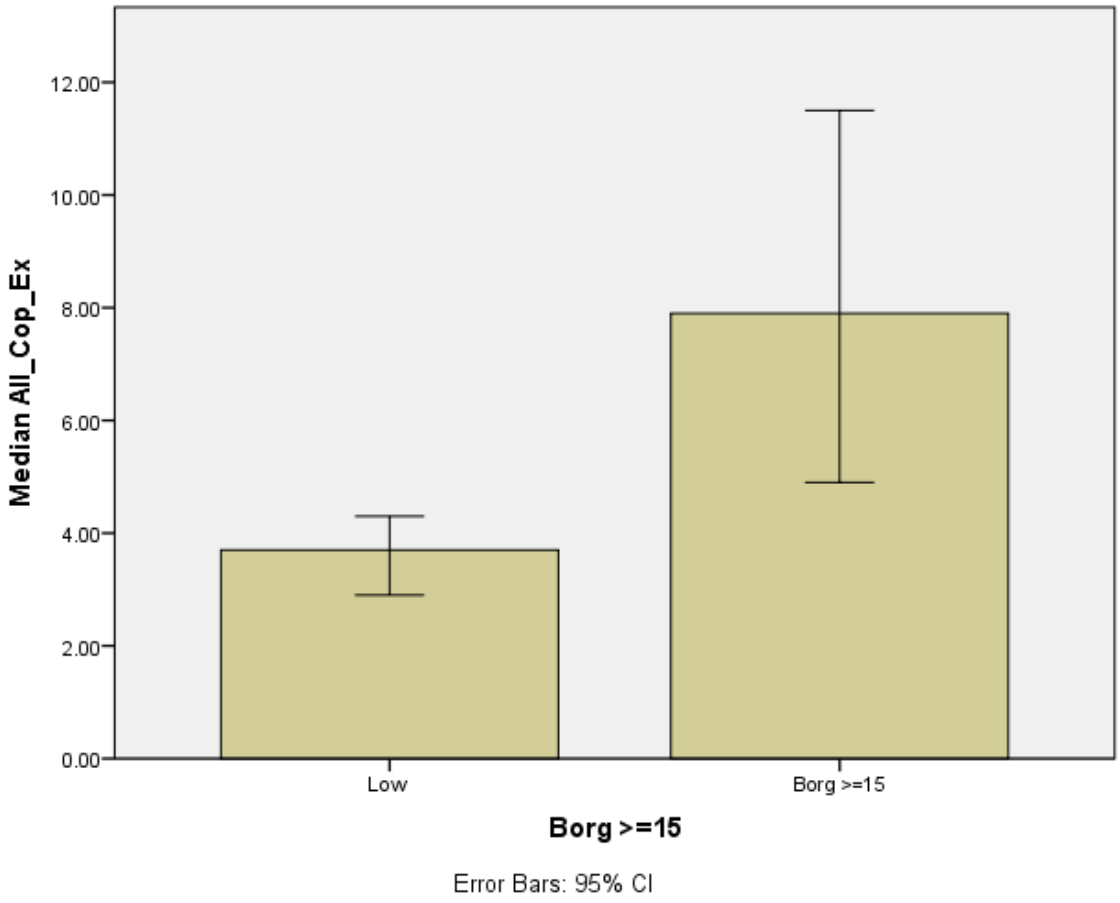


Fig 6.7 Copeptin in the groups with Borg < 15 vs. ≥15

If a cut off of 15 on the Borg score, a rating of "hard effort 80%" is taken then the median copeptin in the high group is 7.9 (n = 38 range 1.1 – 24.8) v's 3.7 (n=94 range 1 -24.2) $p<0.0001$ independent samples Mann Whitney U test) There is a weak but statistically significant correlation between the Borg RPE score at each time point and the copeptin (rho 0.245 p=0.005).

If post exercise osmolality is divided according to Borg RPE category then those reporting a Borg ≥ 15 have a mean osmolality of 288 vs. 291 if Borg < 15 P=0.011 (fig 6.8).

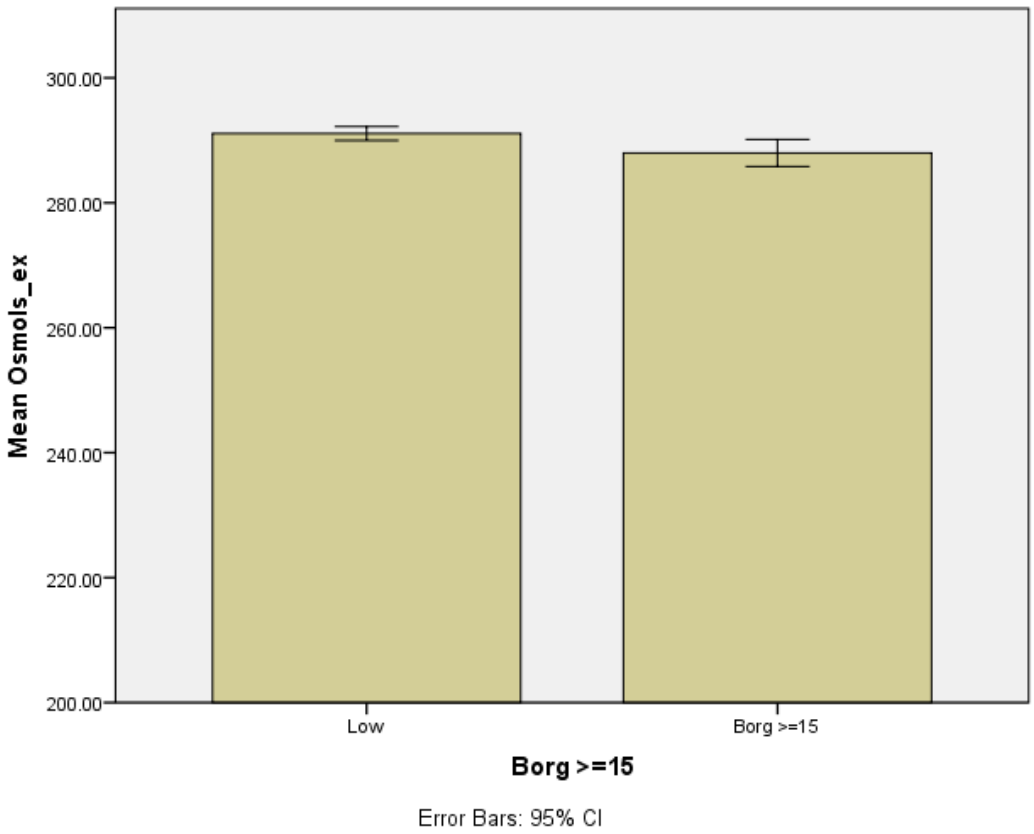


Fig 6.8 Plasma osmolality (mOsmols/L) in the groups with Borg < 15 vs. ≥15

7.4 Discussion

The main findings of this study were that: AVP and copeptin correlate at HA; the AVP and copeptin response to exercise is subdued at altitude until extreme altitude (>5000 m) is reached; AVP and copeptin are not associated with AMS as assessed by LLS; AVP and copeptin secretion at HA are stimulated by non-osmotic rather than osmotic stimuli. The stability of copeptin would favour its use as a surrogate for AVP secretion in future HA field studies.

The median quoted normal value for copeptin in healthy individuals is 4.2 pmol/l (median range 1-13.8 pmol/l). Jochberger found values of 5.9 pmol/l (+/-2.9) in a population of healthy controls (although much higher values in septic patients) (Jochberger, Morgenthaler et al. 2006). This compares well with the data presented ranging from 3.2 to 6.6 pmol/l, with a sea level median value of 3.5 pmol/l. This data is unique in that it demonstrates equal copeptin values in males and females. Other data shows a difference in values between males and females (Morgenthaler, Struck et al. 2006, Bhandari, Loke et al. 2009).

Bhandari found that males had a higher value than females [median (range): 4.3 (0.4-44.3) compared with 3.2 (1.0-14.8) pmol/l; P<0.001]. His study population were from a heart failure screening project although cardiac disease was excluded. Morgenthaler published figures of 5.2 pmol/l for males and 3.7 pmol/l for females, the age of his subjects is not clear. The population in this study is young and fit and that may explain the difference.

Across all time points AVP and copeptin correlated strongly. This is in keeping with the correlations quoted in other papers (Jochberger, Morgenthaler et al. 2006, Morgenthaler, Struck et al. 2006, Jochberger, Velik-Salchner et al. 2009, Balanescu, Kopp et al. 2011, Hew-Butler, Hoffman et al. 2011) and shows that copeptin is a useful surrogate for AVP in the challenging environment of a HA field study. In other studies copeptin and AVP have correlated more highly in sepsis rather than at rest (Jochberger, Morgenthaler et al. 2006) i.e. when there was more stimulus to AVP release. The strongest correlation we found was at 5129m after exercise, when copeptin was at its' highest value and the weakest when copeptin was at its' lowest value (4450m rest).

A significant finding is that copeptin did not rise outside the normal range despite very significant physiological stress. Copeptin levels in patients with systemic inflammatory response syndrome are reported at a mean (sd) of 88 (+/- 89) pmol/l (Jochberger, Morgenthaler et al. 2006). In fact the median copeptin at rest was lower than that at sea level (statistically significant at 4450m) and lower than sea level following exercise at 3833m and 4450m. This damping down of the copeptin/AVP response most probably represents an adaptive neurohumoral response to HA given the detrimental effects of an antidiuresis during acclimatisation to altitude. At higher altitudes (5129m) copeptin rises to above the equivalent value at sea level. Our hypothesis is that this is due to the extreme physiological stress of a perceived hard trek over-riding this damping down of the copeptin response resulting in a normal post exercise rise in copeptin, similar to that seen at sea level. This is similar to the change that is seen in cortisol with suppression of release at rest and post exercise at 4270m but a significant rise at 5150m (Woods, Davison et al. 2012) and the suppression in aldosterone secretion at similar altitudes (Bouissou, Fiet et al. 1988). We did see a large rise in post exercise cortisol at 5129m at a

time of day when we would have expected a dip due to diurnal variation. It is significant that this occurred in tandem with a rise in copeptin (link copep and cortisol or AVP and cortisol) and was more marked with those subjects who had exerted themselves hard. This shows a pronounced stress response to hard exertion at extreme altitude.

The fact that we did not observe a rise in osmolality over time at altitude conflicts with other published data. In a series of 7 subjects osmolality increased to 299.6 +/- 2.2 mOsmol/l over 2 days exposure to 4300m. Despite this AVP did not change, suggesting suppression of the AVP response (Maresh, Kraemer et al. 2004). Bestle found that 8 days at 4559m increased plasma osmolality (+5.3 +/- 1.4 mOsmol/kgH2O) and decreased AVP from 1.14 +/- 0.16 to 0.38 +/- 0.06 pg/ml (Bestle, Olsen et al. 2002). This study was a small number of subjects (n=8) and transport to the Magherita hut where the research was conducted took place by helicopter. As long ago as 1984 Porchet showed a marked decrease in AVP in 15 male workers ascending 2000m by cable car. AVP went from 3.8 (+/_0.4) pg/ml to 0.9 (+/-0.1) pg/ml within 2 hours of reaching altitude (Porchet, Contat et al. 1984). Despite this osmolality did not change. This ascent required no physical exertion and the workers were presumably well acclimatised to the altitude which may explain the pronounced fall in AVP which was not offset by any stress response. Our study is the largest study of its' kind involving the real world stimulus of trekking exercise and allowing subjects access to fluids ad libitum. The data shows that exercise stimulates a rise in osmolality and copeptin at 3833m and at 4500m. The trek to 5129m was perceived to be much more strenuous although the horizontal distance covered was only 1.59km. Why osmolality didn't rise on the ascent to 5129m compared with that to 4500m is interesting. One possible explanation is that the trek to 3833m and 4500m was preceded by a 3 hour coach journey and took place during the heat of the day at lower altitude whereas the trek to 5129m took place in cooler conditions from a basecamp with all subjects well hydrated. In fact the finding was that osmolality was reduced in those reporting a high Borg RPE whereas copeptin increased. This seems counterintuitive but may be because those exercising at a higher intensity may have taken on-board more fluids during the trek. Although body mass fell at 5129m this was not due to dehydration as osmolality was not increased. This was most likely due to

progressive weight loss caused by anorexia at high altitude. The significant rise in AVP and copeptin at 5129 m following exercise was the greatest rise seen at any altitude yet was clearly due to non-osmotic stimulation. This was perceived by the subjects as the hardest trek during the study period. Possible non osmotic causes behind the copeptin and AVP rise may include exertional headache or nausea, anxiety or the stress of a steep climb to a new altitude. Whilst there was no relationship between copeptin or AVP and AMS, nausea or headache as recorded at the end of the day, the effect of transient exercise effects (in terms of nausea etc) cannot be entirely discounted.

The change in the neuroendocrine response above 5000m is of interest. It is widely acknowledged that whilst most people can acclimatise to altitudes up to 5000m not everyone can acclimatise beyond that. It is unlikely to be pure coincidence that 5000 m is also the altitude of the highest permanent human habitation. Anand reports a clinical picture of congestive cardiac failure occurring in otherwise fit Indian Army soldiers deployed at extreme altitudes for long periods (Anand, Chandrashekhar et al. 1993). These soldiers demonstrated fluid retention and increases in cortisol levels. His conclusion was that the neuroendocrine response to the stress of cold, hypoxia and exertion was important in the development of this “subacute mountain sickness”. Our findings support this hypothesis with the rise in copeptin not occurring as a result of changes in osmolality and presumably reflecting neuroendocrine stress. Furthermore the rise in cortisol (and associated AVP rise) would create an anti-diuresis which may be the mechanism behind Anand’s observations. Many studies are conducted at altitudes below 5000m with or without exercise which may explain inconsistencies in the published data. In chamber studies involving a 2 hour ascent to and 2 hour stay at 6000m equivalent altitude (without an exercise stimulus) Okazaki found a fourfold increase in AVP associated with a rise in cortisol and osmolality (Okazaki, Tamura et al. 1984). In contrast Blume (Blume, Boyer et al. 1984) found no change in AVP during a prolonged period at 6300m on a mountaineering expedition to the Everest region. However, these samples were taken in the morning, after a nights rest and in well acclimatised individuals. In fact Blume’s data suggest a suppression of AVP response (as osmolality increased to 302 mOsmol/KgH2O) even at extreme altitude.

In this dataset oxygen saturation is only very weakly correlated with LLS (n=273 rho = -0.135 p=0.026) and not at all with copeptin or cortisol. This fits the published evidence that oxygen saturations are not predictive of AMS as discussed in Chapter 8.1.

It appears that the main stressor is that of exercise and the exercise intensity. There was a highly significant difference between the median values of copeptin dependant on the Borg RPE rating. This data suggests that with increased exercise in hypoxia there is a significant stress response which leads to an increase in AVP and copeptin (regardless of plasma osmolality). This overrides the suppression of antidiuretic hormones seen at lower altitudes. Studies which demonstrated a rise in osmolality and decrease in AVP had no significant exercise stress (Porchet, Contat et al. 1984, Bestle, Olsen et al. 2002). Fluid retention is a recognised part of AMS and it may be that this stress response is key in leading to fluid retention and AMS.

Chapter 7. Other potential biomarkers – troponin, NGAL and C reactive protein

Summary

Other markers may be of use in diagnosing acute mountain illness based on knowledge of the mechanism behind the illness. Cardiac troponin (hs CTnT) rises with exercise, at HA and with increased PA pressures due to an increase in myocardial stress. Neutrophil gelatinase associated lipocalin (NGAL) is a protein which is expressed by lung, renal tissue and gut during hypoxia. Earlier work by the DMS has shown a rise in NGAL with HA. Highly selective C reactive protein (hs-CRP) is an acute phase protein which rises to reflect inflammation which may be an integral part of the disease process in altitude illness. During the field study in Bolivia assays were carried out for troponin (hs-cTnT), NGAL and hs-CRP. After exertion at sea level, 3833m and 5129m a significant number of subjects had a hs-cTnT above the upper limit of normal (6, 14, 11 respectively). cTnT was significantly higher in those with a PASP >40 mm Hg than those without: (cTnT: 13.8+/-21 vs 7.8+/-6.5, p=0.018). ROC analysis (to investigate the utility of cTnT in detecting a high PASP) revealed an AUC of 0.607 (95% CI 0.520-0.693, p=0.018). There was a statistically significant increase in hs-CRP with increasing altitude but no correlation with AMS. NGAL did not change at any altitude, possibly due to changes in the assay compared to the previous investigation at altitude. Of these the three biomarkers investigated hs-cTnT shows most promise as a biomarker for altitude illness.

7.1 Introduction

Key processes involved in the development of high altitude illness are a high PA pressure (HAPE) and inflammation (AMS). Troponin rises with exercise both of long duration and shorter more intense activity. O'Hanlon found that 11 of 17 athletes completing a marathon run had a rise in troponin above the cut off for myocardial infarction at 6 hours post-race (O'Hanlon, Wilson et al. 2010), whilst other published data show a rise with 30 mins of high intensity exercise (Shave, Ross et al. 2010). This is significant as AMS is thought to occur in association with strenuous exercise. There is little work showing the cTnT response to exercise in hypoxia when PASP will be raised. Recent work has shown a rise in cardiac troponin with maximal exercise (symptom limited) in patients with pulmonary hypertension (Volkers, Rohde et al. 2013). In a different study in patients with raised PASP, hs-cTnT correlated with right ventricular systolic strain and was raised in 90.9% of patients with a PA pressure above 45mmHG

(n=55) (Filusch, Giannitsis et al. 2010). This evidence suggests that hs-cTnT may be useful as a marker for raised PASP (a key mechanism in developing HAPE) and may reflect poor tissue oxygenation warranting further investigation as a biomarker for altitude illness.

NGAL is a 25 kDa peptide, part of the lipocalin family of small soluble proteins. It is produced in a number of human tissues, notably the distal nephron but also in the lung (Cowland and Borregaard 1997, Borregaard N 2006). NGAL rises rapidly in the nephron in response to a renal insult and an NGAL ≥ 150 ng/ml following acute kidney injury (AKI) is predictive of acute renal failure (ARF) well before creatinine has risen (Haase, Bellomo et al. 2010). NGAL is also an acute phase protein (Liu and Nilsen-Hamilton 1995), has a role in inflammation (Borregaard N 2006, Roudkenar, Halabian et al. 2009) and is up-regulated in the lung during inflammation (Cowland and Borregaard 1997, Cowland, Sorensen et al. 2003, Chan, Liu et al. 2009). NGAL is also known to rise in conditions associated with oxidative stress (Roudkenar, Halabian et al. 2011) (Bolignano, Coppolino et al. 2010) and oxidative stress has been implicated in AMS (Bailey, Davies et al. 2001, Bailey, Evans et al. 2009). NGAL may therefore be a useful biomarker increasing at HA secondary to these various stimuli and that the magnitude of any increase might relate to the presence of AMS.

During previous studies we have investigated a cohort of trekkers from 2 expeditions to HA (Mellor, Boos et al. 2013). In order to clarify the relative contribution of AMS, hypoxia or exercise to NGAL levels we also studied a cohort pre- and post-exercise at near sea-level and a further cohort exposed to acute normobaric hypoxia.

The treks involved a total of 52 subjects (32 on the first trek, 20 on the second) in the Khumbu region of Nepal. Blood samples were taken at 3 study altitudes: on day 2 at 3400 m, day 6 at 4270 m and day 10 at 5150 m (following ascent to Everest Base Camp at 5364 m trek 1 and Kala Patthar 5643m trek 2). As in Bolivia samples in this study were collected immediately following a day trekking (“post-exercise”) to the study altitude.

In a separate hypoxic chamber study 14 subjects underwent a 3 hour exposure to normobaric hypoxia (FiO_2 11.6%, equivalent to 4800 m altitude). This exposure included a 5 minute step-test (step height of 25cm, 1 complete step every 2 seconds) at 95 minutes. NGAL was assayed at baseline and after 180 minutes of hypoxic exposure.

As a control a group of 22 subjects performed a trek at near SL with an ascent from sea-level to 1085m over 6 hours (an equivalent gain in altitude and duration of exercise as to that experienced on a trekking day in Nepal).
The results of these studies can be summarized as follows:

- A near sea level ascent produced no significant (p=0.084) rise in NGAL. SL NGAL was 63.9+/-10.6 (ng/ml, mean+/-sd, range 60-104) and post-exercise NGAL was 71.2+/-13.8 (ng/ml, mean+/-sd, range 60-100).
- Ascent to 5150m was made by 46 out of the 52 subjects. SpO_2 (%, mean+/-sem) dropped from 96.7+/-1.6 at 1300 m to 84.4+/-5.4 and 79.2+/-7.3 at 4270 and 5150 m respectively (p<0.001). There was a moderate inverse correlation between NGAL and SpO_2 at 5150 m (r=-0.477, p=0.001) with a weaker inverse correlation between NGAL and SpO_2 at 4270 m (r=-0.340, p=0.019).
- There was a significant change in NGAL (RM ANOVA) with ascent both at rest (p=0.007) and post-exercise (p=0.001) (figure 7.1).
- Although normobaric hypoxia led to a significant fall in oxygen saturations SpO_2, (%, mean+/-sem) from 98.8+/-0.4 at baseline to a nadir of 79.3+/-5.2 (p<0.001), however, NGAL (ng/ml, mean+/-sd, range) showed no change between baseline and 180 minutes: 62.8+/-26 (29-80) vs 66.7+/-25 (27-84), p=0.538.
- There was a significant difference between NGAL depending on the presence or absence of AMS at 5150 m; no AMS (n=23) (NGAL: 123.6+/-11.6); mild AMS (n=16) (NGAL: 171.4+/-18.5, p=0.026 for NGAL versus no AMS); severe AMS (n=7) (NGAL: 200.7+/-33.5, p=0.009 for NGAL versus no AMS).

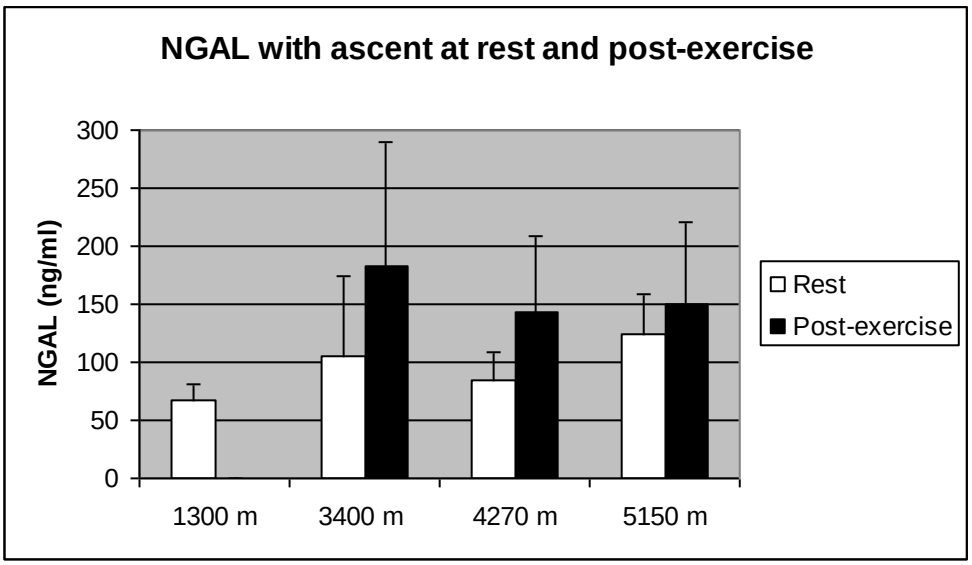


Figure 7.1 NGAL changes with ascent in Nepal (taken from Mellor et al Neutrophil Gelatinase Associated Lipocalin: It's response to Hypoxia and Association with Acute Mountain Sickness; Disease Markers. 35(2013), (5) 537-542.)

NGAL also rises with oxidative stress which is increased by exercise (Bakonyi and Radak 2004) and HA-induced oxidative stress (Huang, Han et al. 2008) has been implicated in AMS (Bailey, Davies et al. 2001). As such it is interesting to note that we found a higher NGAL following exercise and in those with AMS at the highest altitude.

C reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in the blood plasma, the levels of which rise in response to inflammation (i.e., is an acute-phase protein). Acute mountain sickness is more likely to occur in the presence of infection and in itself, may be an inflammatory process (Bailey, Kleger et al. 2004). Unsurprisingly CRP has been shown to increase with altitude, Hartmann found an increase at 3458m and 4559m (with no associated HAPE) (Hartmann, Tschop et al. 2000). Highly selective CRP (hs-CRP) is a more recently developed assay which is more sensitive to show a change within the normal range of values. hs-CRP has shown an association with the risk of a future cardiovascular event in large epidemiological studies (Ridker 2001). There is limited data to suggest hs-CRP may be associated with AMS from a study of 32 climbers ascending to 2900m from sea level (Shintaro, Yuji et al.

2006). The authors found plasma levels of hs-CRP in AMS were significantly higher than those in healthy climbers after climbing (2.433 +/- 0.831 mg/L vs 0.914+/- 0.272 mg/L, P<0.05).

Hypothesis

Inflammation and/or rises in PASP occur with the development of high altitude illness and would lead to a detectable rise in hs-cTnT, NGAL or hs-CRP.

7.2 Methods

The study methodology has been previously explained. Venous blood was sampled from the study group on reaching a new altitude after a trek (exercise) or the following day (rest). All assays were stored at -20 ºC until they could be transferred back to the UK and stored at -80 ºC until assayed.

7.2.1 *Assays*

Highly sensitive cardiac troponin T (hs-cTnT) assay was performed using an electro-chemiluminescence immunoassay (ECLIA) on a Cobas Analyser (Roche Diagnostics, Mannheim, Germany). This assay has a range from 3-10000 ng/L. The upper reference limit (99th percentile) for this assay in healthy volunteers is 14 ng/L (pg/mL).

NGAL was analysed in the field on a Biosite Triage point of care monitor (Alere Ltd, Stockport, UK) using a Triage cardiorenal test kit. The Triage® cardiorenal test is a point-of-care, fluorescence-based immunoassay used which gives a rapid (15 minutes) quantitative measurement of NGAL in a range from 15 to 1,300 ng/ml.

Hs-CRP was assayed using a commercially available, highly-sensitive, immunoturbidimetric assay (Roche diagnostics) was used to measure CRP on samples taken at the same time-points as the NPs. This assay has a measuring range of 0.1-300 mg/L and a between run coefficient of variation between 2.5 and 5.7%.

7.2.2 *Statistical analysis*

For statistical calculations the software package SPSS 21.0 was used. Subjects were excluded who took drugs to aid acclimatization (n=3). Parametric or non-parametric statistical tests were applied after performing the Shapiro-Wilks statistic**.**

Changes in dependent variables between altitudes were initially tested by Student's paired *t* test or Wilcoxon signed ranks test. For independent variables an independent-samples *t* test or Mann Whitney test was used. Either a within subjects ANOVA or a Friedman test was performed to investigate any serial changes in measures (eg PASP or cTnT) with ascent. A correlation analysis between NGAL and SpO_2 was performed using Spearman's rank correlation.

As with the BNP/PASP investigation described in the preceding chapter data were analysed according to a PASP cut-off of 40mmHg. As hs cTnT may rise with cardiac strain a ROC (receiver operating characteristic) analysis was done to investigate the utility of hs-cTnT in identifying a PASP >40 mmHg and AMS/severe AMS.

A p value <0.05 was considered significant throughout.

7.3 Results

All data presented as mean (sd, +/- range where appropriate). The subjects were 35.2±9.1 years old, 175.5±9.5 cm in height and 77.5±13.9 kg in weight. 45 subjects reached the highest study altitude (5129 m).

	Altitude (m)	Sea Level	3833	4450	5129
SpO_2	**Rest**	98 (1.34, 94-99)	88 (4.2, 75–93)	86 (4.96, 62-93)	81 (4.4, 66-90)
	Exercise	97 (1.53, 94-99)	84 (4.95, 71-94)	81 (6.28, 53-90)	79 (4.4, 68-88)
PASP (mmHg)	**Rest**	20.2 (7.0, 10-34.4)	27.2 (10.8, 12-54)	32.5 (8.5, 17-56)	33.2 (9.1, 17-54)
	Exercise	23.1 (6.4, 13.1-38)	29.8 (11.6, 12-58)	31.5 (9.8, 16.7-63)	33.9 (11.6, 17-63)
hs-cTnT (ng/l)	**Rest**	5.0 (2, 3-10.3)	7.3 (5.5, 3-37)	6.1 (3.3, 3-20)	8.2 (7.0, 3-44)
	Exercise	14.4 (17.0, 4-69)	11.7 (8.5, 3-44)	6.9 (3.7, 3-20)	13.7 (22.0 3-120)
NGAL (ng/ml)	**Rest**	42.9 (13.6, (29-80)	27.0 (6.9, 15 – 56)	29.2 (13.6, 15 – 77)	48.7 (30.4, 15 – 147)
	Exercise	46.8 (13.6, 27-84)	34.2 (9.7, 21.2 – 71)	31.0 (10.2, 17– 65)	28 (12.4, 15 – 61)
hsCRP (mg/l)	**Rest**	1.43 (1.7, 0.28-7.29)	3.75 (3.6, 0.57-6.47)	9.1 (15.9, 0.39-62.28)	7.46 (21.3, 0.34-124.02)
	Exercise	1.36 (1.6, 0.26-7.04)	1.71 (2.6, 0.29-17.53)	6.1 (10.6, 0.46-47.62)	7.35 (18.3, 0.28-93.68)

Table 7.1 Biomarkers at each altitude

7.3.1 *hs-cTnT*

Repeated measures analysis showed a significant change in cTnT at HA ($p<0.001$, table 7.1). Peak values were seen after exercise at SL and after trekking at 5129 m. Subjects with a cTnT above the upper limit of normal (ULN, 14 ng/l): SL rest: 0; SL post-exercise: 6; 3833 m post-trek: 14; 3833 m rest: 4; 4450 m post-trek: 2; 4450 m rest: 2; 5129 m post-trek: 11; 5129 m rest: 7. cTnT was significantly higher in those with a PASP >40 mm Hg than those without: (cTnT: 13.8+/-21 vs 7.8+/-6.5, $p=0.018$). ROC analysis (to investigate the utility of cTnT in detecting a high PASP) revealed an AUC of 0.607 (95% CI 0.520-0.693, $p=0.018$), a troponin of 7.13 ng/l had a sensitivity of 60% and a sensitivity of 63.7% of detecting a high PASP. ROC analysis to investigate the utility of cTnT in detecting AMS showed that cTnT was of no value with an AUC of 0.466 (95% CI 0.382 – 0.55).

7.3.2 *NGAL*

Repeated measure analysis showed a significant rise with NGAL with altitude ($p<0.001$), pairwise comparison showed this to be a rise from 4450m to 5129m (mean 30.5ng/mL vs. 37.9 ng/mL). Across all time points there was no correlation between NGAL and oxygenation (as measured as SpO_2) Spearman rho -0.032 $p = 0.604$. In the group with AMS (n=65 LLS>3) median NGAL was 28 (15-147) whereas in the group with no AMS (n=201) median NGAL was 30 (15-98) ($p=0.899$). In those with severe AMS (n= 28) median NGAL was 29 (15 – 60) v's 29 (15 – 147) ($p=0.814$).

7.3.3 *hs-CRP*

Repeated measures analysis showed a significant change in hs-CRP at HA ($p<0.001$), table 6.1. Overall there was a weak correlation between hs-CRP and NT-proBNP (rho=0.367, $p<0.001$) and BNP (rho=0.263, $p<0.001$). However, there was no difference in hs-CRP according to presence or absence of AMS (6.3+/-19 vs. 5.6+/-12 respectively) or severe AMS (6.3+/-15 vs. 5.7+/-14 respectively).

There was correlation between hs cTnT and NTPro-BNP (rho 0.389 $p<0.001$), no correlation between hs-CRP and cTnT but a weak correlation between hs-CRP and NT-proBNP (rho 0.195 $p=0.001$).

7.4 Discussion

This significant cohort at HA demonstrates cTnT are higher in those with a high PASP and exercise, even of short duration at HA, was a significant stimulus to cTnT release.

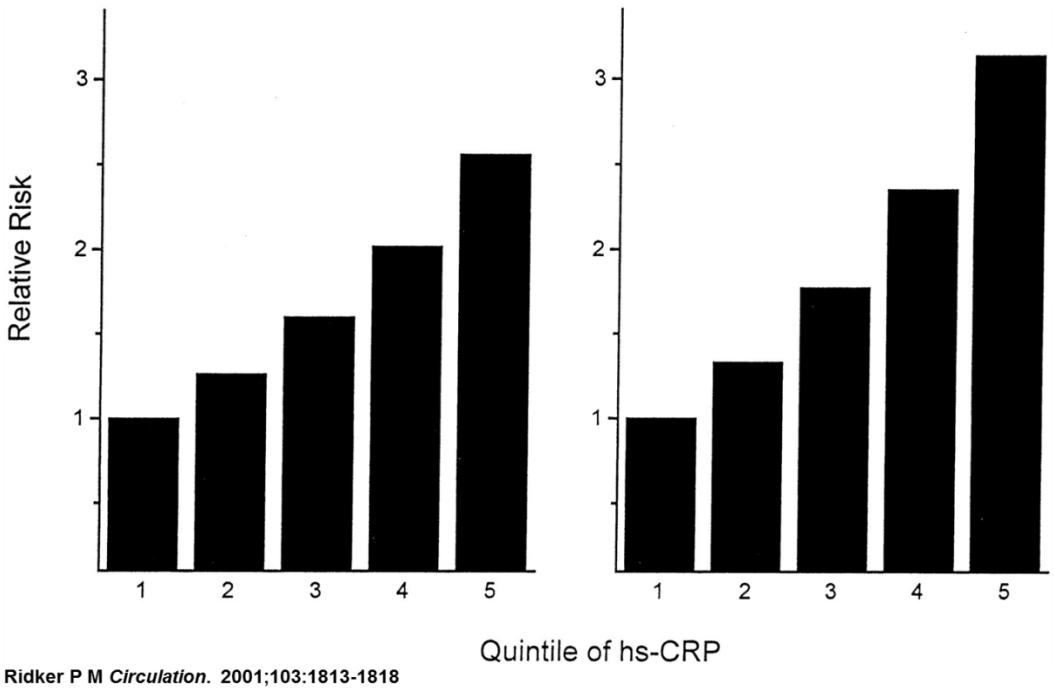
It has previously been reported that at SL exercise stimulates hs-cTnT release (Shave, Ross et al. 2010, Legaz-Arrese, George et al. 2011, Duttaroy, Thorell et al. 2012, Eijsvogels, Veltmeijer et al. 2012). We also found an exercise-induced rise at SL, 3833 m and 5129 m. We did not see a rise in hs-cTnT at 4450 m but the exercise stimulus was perceived to be mild. Although the trek to 5129 m was shorter than that to 4450 m it was of a greater intensity and physically more strenuous. It would seem that exercise intensity, whether over long (30-50 km walking) (Eijsvogels, Veltmeijer et al. 2012) or short duration, (high intensity over 30-60 mins) (Shave, Ross et al. 2010, Duttaroy, Thorell et al. 2012) is key to the stimulation of cTnT release. We also found significantly higher cTnT in those with a high PASP. Similar to the relation between NPs and PASP further work is needed in symptomatic subjects at HA to evaluate the use of cTnT in identifying those with an abnormally high PASP. Whether the cTnT release we have seen is secondary to increased membrane permeability or true myocardial necrosis remains to be elucidated but it is noteworthy that several participants demonstrated a cTnT in the range normally associated with myocardial infarction (ULN 14ng/L) (Thygesen, Alpert et al. 2012).

The hs-CRP results found on this study were consistent with those found at lower altitude by Shintaro. What this data shows is an increase in hs-CRP with increasing altitude and increasing physiological stress. The difficulty is in elucidating what is a normal response and what is pathological.

To use the parallel of hs-CRP in cardiology multiple large epidemiological studies have been used to compile risk based on stratification of hs-CRP results in apparently normal individuals (table 7.2 and figure 7.2).

Quintile	Range hsCRP (mg/dl)	Risk estimate
1	0.01–0.07	Low
2	0.07–0.11	Mild
3	0.12–0.19	Moderate
4	0.20–0.38	High
5	0.38–1.50	Highest

Adjusted relative risks of future myocardial infarction associated with increasing quintiles of HSCRP (hs-CRP) among apparently healthy middle-aged men (left) and women (right).



Ridker P M *Circulation.* 2001;103:1813-1818

Table and figure 7.2. Relative risk of cardiovascular event depending on quintile of hs-CRP (from Ridker, Circulation 2001).

This data is compiled on large population studies of between five and 22 thousand individuals (Ridker 2001). Clearly performing such studies on populations at HA is practically impossible.

One disappointment with this data was the lack of NGAL response to HA. Although there was a statistically significant difference at 5150m this was a very small increase. Our previous data in 2011 had shown a strong rise in NGAL with AMS at 5150m and NGAL values above 100ng/ml with exercise at all altitudes above 1400m. There are good theoretical reasons why NGAL may be an attractive biomarker to use. Investigation with Alere, who make the point of care assay we used showed that they had changed the assay from a competitive assay to a sandwich assay. This has been refined from the first generation to a mammalian (rather than bacterial) antibody system. The new test employs two antibodies that have been selected not to recognize or bind to NGAL complexes (NGAL-MMP-9 heterodimer and NGAL homodimer forms) and is more specific for renally expressed NGAL. As renal failure is not a feature of high altitude illness it is not surprising we saw no change in NGAL. NGAL is also released from lung tissue but this would not be detected with the newer assay. A range of other NGAL antibodies are commercially available and this may be a future route of investigation.

It seems likely that the main benefit of point of care testing of this nature may be to rule out severe illness. As more systems become available a panel of markers may provide greater specificity and sensitivity than one alone and is worthy of future investigation. One finding of interest is that there was correlation between hs cTnT and NTPro-BNP (rho 0.389 $p<0.001$) this suggests a common mechanism leading to increases in both these markers (i.e. increasing myocardial stress). There was no correlation between hs-CRP and cTnT but a weak correlation (rho 0.195 $p=0.001$) with NTPro-BNP (as discussed previously there is increasing evidence that BNP rises in inflammatory conditions). This suggests that there may be value in combining both hs-CRP and either NTPro-BNP or hs-cTnT.

Chapter 8. Physiological data, catecholamines and cortisol at high altitude

8.1 Basic physiological data

8.1.1 *Methods*

8.1.2 *Results*

8.1.3 *Discussion*

8.2 Catecholamines

8.2.1 *Normal physiology*

8.2.2 *Methods*

8.2.3 *Results*

8.2.4 *Discussion*

8.3 Cortisol and adrenocorticotrophic hormone

8.3.1 *Background*

8.3.2 *Methods*

8.3.3 *Results*

8.3.4 *Discussion*

8.4 Conclusions

Summary

Exercise at sea level brings about increases in AVP, cortisol and catecholamines (Melin, Eclache et al. 1980, Zouhal, Jacob et al. 2008). This is an adaptation to improve cardiovascular performance and retain salt and water during prolonged exercise. At high altitude there is an initial excretion of salt and water related to a decrease in aldosterone (Bartsch, Shaw et al. 1988) stimulated by hypoxia. As fluid retention is a key feature of AMS and related to the severity of AMS (Loeppky, Icenogle et al. 2005) it follows that there may be significant interaction between exercise, fluid retention and AMS.

This chapter investigates the changes recorded in physiological variables, catecholamines and cortisol during the field study in Bolivia.

Hypothesis

Changes in stress hormones associated with exercise are key in the pathogenesis of altitude illness

8.1 Basic Physiological data

8.1.1 *Methods*

Data was collected on a daily basis throughout the first 10 days of the trek. This daily data included heart rate, SpO_2, respiratory rate, blood pressure, LLS, AMSc. Blood pressure was monitored on an automated blood pressure cuff (Omron M2 basic, Omron Healthcare, Kyoto, Japan). This had not been validated prior to the expedition although data collected on an earlier expedition using a manual aneroid sphygmomanometer was comparable and the cuff had been used successful on 2 previous expeditions. Recent data has been published validating a very similar automated cuff Omron HEM-7201 (Cho, Tian et al. 2013). Pulse oximetry is a non-invasive measure of arterial oxygen saturation. The device uses the fact that oxygenated and reduced blood absorb different amounts of light at wavelengths between 650 and 950 nm. By switching on and off rapidly the proportion of the signal which changes due to the pulsatile flow of arterial blood can be analysed. The precision of pulse oximeters is within $\pm$ 2-3% when the SaO_2 is 90% or more;

the precision is thus ± 4- 6% if a 95% confidence interval (± 2 SD) is desired. For example, if the SpO_2 is 94%, then the true SaO_2 may be as low as 88% or as high as 100% for 95% of the measurements. Pulse oximeters are also only calibrated to be accurate in the clinical range (70-100%) and at extreme altitude values may fall outside this range giving a greater discrepancy between SpO_2 and arterial oxygen content (SaO_2). The device used for the field study was a Nellcor N-20P pulse oximeter (Nellcor Puritan Bennett Ltd, Coventry.UK), this is designed to operate over the range of saturation 70-100% (+/- 2%) and heart rate range 20-250 bpm. The design specification is to function up to 6300m. In a trial of similar oximeters a mean SpO2 of 86.7+/- 8.6% compares with a SaO_2 of 87.2% +/- 11% at 2240m (Torre-Bouscoulet, Chavez-Plascencia et al. 2006). SpO2 decreases with altitude, this has been shown many times before. This is expected as available inspired oxygen partial pressure (PiO_2) decreases with barometric pressure. The Silver Hut experiments performed in the early 1960's demonstrated that SpO_2 fell from 67% at rest to 63% and 56% with work rates of 300 Kg-m/min and 900 Kg-m/min respectively at 5800m (West, Lahiri et al. 1962). Previous work by the DMS)conducted on Mt Aconcagua in Argentina showed an SpO_2 of 84% at 4250m falling to 77% following a very brief (1 minute) step test (Hooper, Levett et al. 2010). Respiratory rate was observed by one of the other research subjects and measured over a 30 second period (multiplied by 2 to give a rate per minute). LLS and AMSc scores were recorded as reported elsewhere in this thesis.

	Altitude (m)	Sea Level	3833	4450	5129	P (change with altitude)
SPO_2	**Rest**	98 (1.34, 94-99)	88 (4.2, 75–93)	86 (4.96, 62-93)	81 (4.4, 66-90)	<0.001
	Exercise	97 (1.53, 94-99)	84 (4.95, 71-94)	81 (6.28, 53-90)	79 (4.4, 68-88)	<0.001
Heart rate (bpm)	**Rest**	60 (6.10, 50-71)	76 (11.57, 51-102)	82 (12.41, 45-120)	88 (12.74, 65-120)	<0.001
	Exercise	68 (12.75, 43-98)	83 (12.43, (56-120)	88 (13.25, 58-110)	95 (15.27, 66-129)	<0.001
Systolic blood pressure (mmHg)	**Rest**	135 (11.10, 109-150)	136 (11.16, 111-161)	134 (11.43, 117-159)	139 (13.02, 110-168)	0.12
	Exercise	133 (12.84, 107-164)	134 (13.59, 110-174)	135 (14.3, 103-171)	131 (12.66, 88-159)	0.651
Diastolic BP (mmHg)	**Rest**	73 (8.04, 56-90)	83 (9.13, 64-109)	86 (9.11, 69-111)	86 (11.20, 58-113)	<0.001
	Exercise	72 (6.59, 62-83)	83 (8.26, 67-104)	88 (10.26, 71-112)	83 (8.82, 52-109)	<0.001
Respiratory rate (bpm)	**Rest**	15 (2.44, 12-20)	17 (3.40, 12-28)	17 (3.12, 12-28)	19 (3.64, 12-28)	0.001
	Exercise	15 (2.62, 12-20)	18 (3.21, 12-26)	18 (3.03, 14-30)	21 (5.45, 12-40)	<0.001

Table 8.1: Physiological changes at rest and post exercise with altitude (Bolivia field study)

8.1.2 *Results*

The changes in basic physiological variables are presented in table 8.1.
The change in SpO_2 with altitude was significant ($p < 0.001$ across all altitudes at rest and after exercise Kruskal-Wallis). When tested between levels (Rest, Exercise 3833 v 4450 $p=0.001$ and $p= 0.005$ 4450 v's 5129 $p<0.001$ and $p=0.001$) and significant for the change with exercise 3833m $p<0.001$, 4450m $p<0.001$ and 5129m $p=0.011$.

The change of heart rate with altitude was significant with altitude at all times and between rest values and exercise values with increasing altitude ($p<0.001$ for all). Systolic blood pressure did not change significantly whereas diastolic blood pressure changed significantly with altitude in both the resting and post exercise states ($p<0.001$).

The changes in respiratory rate increased significantly with altitude $p<0.001$ in all conditions and for rest values $p=0.001$ and exercise values $p<0.001$.

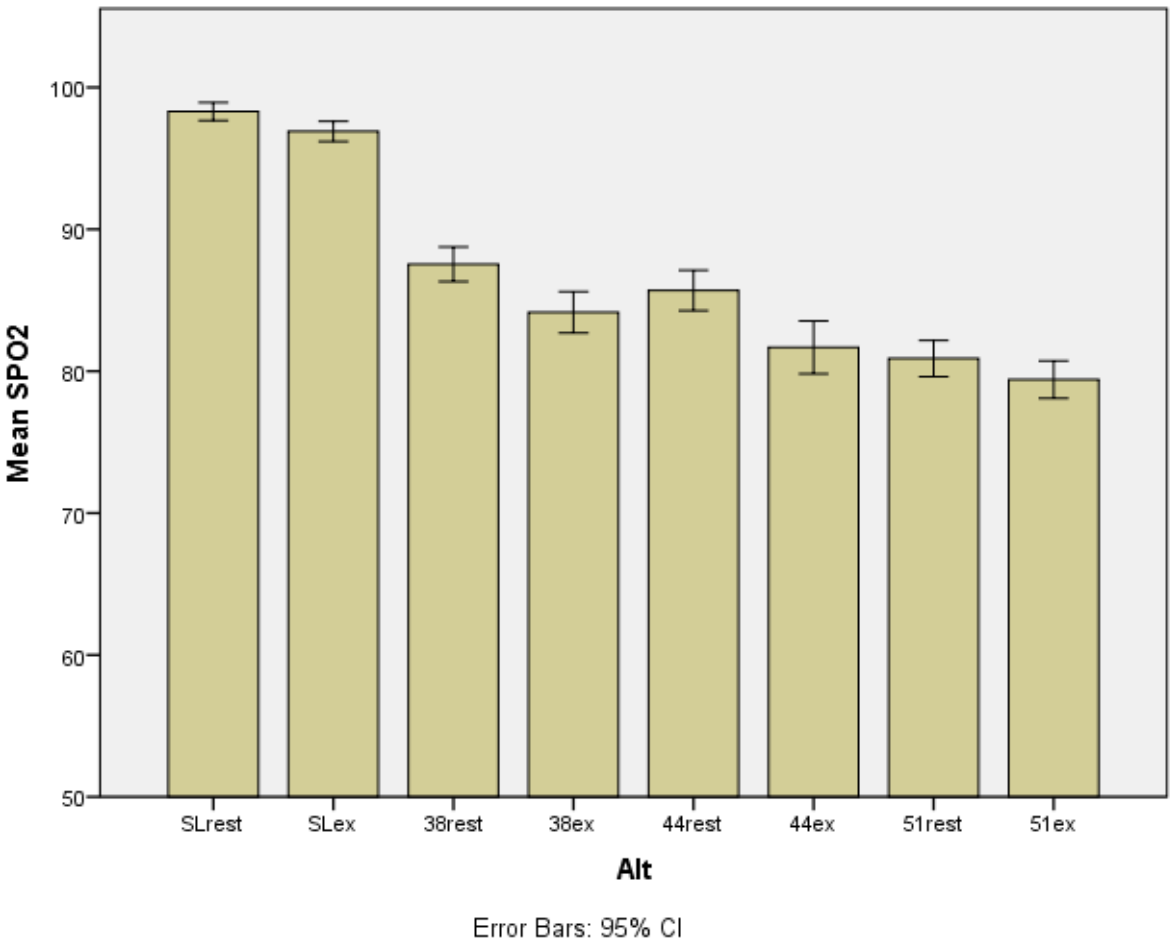


Figure 8.1 Changes in SpO_2 with changes in altitude.

Overall in the data recorded in Bolivia there was a very weak, although statistically significant correlation between SpO_2 and LLS recorded at all time points (n=273 rho = -0.135 p=0.026). The same very weak correlation was evident between the SpO_2 after the exercise stimulus with LLS at rest the following day (n=135 Spearman's rho = -0.176 p=0.041) and between the LLS and SpO_2 at rest the following day (n=139, Spearman's rho -0.187 p = 0.027).

8.1.3 *Discussion*

An increase in minute ventilation is an early response to hypoxia and a failure to increase ventilation has been implicated in AMS (King and Robinson 1972, Hackett, Rennie et al. 1982). Measuring respiration was performed by observation post exercise or at rest and so extremes of ventilatory effort with exertion would

have been missed. The limited observations within a narrow range of results may explain the lack of statistical significance.

It is worth noting that the heart rate and SpO_2 recorded for exercise was actually taken at the end exercise so the nadir of saturation and peak heart rate would have been quite different. The rest values were taken the following day after a night at the new altitude so improvement will be in part recovery from the exercise of the ascent the previous day and in part related to adaptation to the new altitude.

The data on whether or not a low oxygen saturation correlates with AMS or success on a climb is mixed with a number of authors finding a predictive value (Roeggla, Roeggla et al. 1996, Roach, Greene et al. 1998, Basnyat, Lemaster et al. 1999, Burtscher, Flatz et al. 2004, Koehle, Guenette et al. 2009, Karinen, Peltonen et al. 2010, Lazio, Van Roo et al. 2010, Major, Hogan et al 2012) and some finding no relationship between a low SpO_2 and altitude illness (Roach, Houston et al. 1995, O'Connor, Dubowitz et al. 2004, Davies, Kalson et al. 2009). This is not surprising given the potential differences between SpO_2 and SaO_2 (see above) and the fact that most of these studies have been observational and not blinded. If a low SpO_2 reading is regarded as dangerous and subjects advised to rest or turn back then the result becomes self-fulfilling. Interestingly, at the extreme, Grocott reported a climber returning from Everest summit with an SaO_2 of 34.4% who felt well and was not suffering from altitude illness (Grocott, Martin et al. 2009). Those with better oxygenation the following morning could be assumed to be adapting better and therefore not suffering AMS.

8.2 Catecholamines

8.2.1 *Normal physiology*

The adrenal glands produce hormones involved in the body's "stress response". Catecholamines (epinephrine and norepinephrine) are secreted by the medulla, whilst the cortex secretes cortisol. Catecholamines act rapidly to alter heart rate, blood pressure and respiration, whilst cortisol has more prolonged actions on

metabolism (glucose metabolism and fluid balance). The actions of these hormones as part of the body's response to stress and fluid balance suggests that they are likely to be important in understanding the response to the dual stresses of hypoxia and exercise during mountaineering activities.

Metanephrines are the 3-methoxy metabolites of epinephrine and norepinephrine, respectively. The metanephrines are stable metabolites and are co-secreted directly with catecholamines from the adrenal medulla and the sympathetic chain. Due to their stability, assay of these metabolites is preferable to direct assay of epinephrine and norepinephrine, which are unstable and have short plasma half-lives. Metanephrine and normetanephrine are both further metabolized to conjugated metanephrines and vanillylmandelic acid.

Sea level normal values are; Normetanephrine: 120 – 1180 pmol/l, Metanephrine: 80 – 510 pmol/l

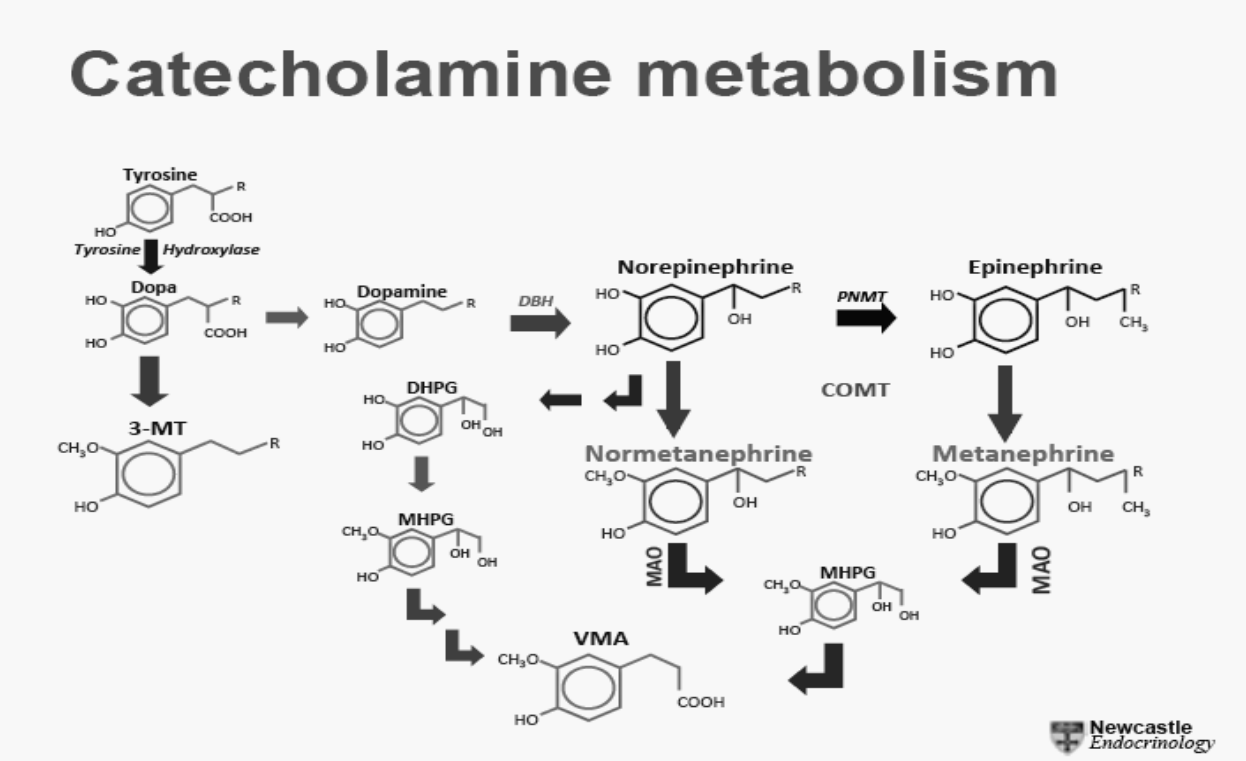


Figure 8.2 Catecholamine synthesis and metabolism (with thanks to Dr S Ball)

8.2.2 *Study methods*

Samples were drawn for catecholamine assay at the three study altitudes into EDTA tubes as previously described. The samples were immediately centrifuged and plasma frozen at -20 ºC prior to transport back to the UK and storage at -80 ºC whilst awaiting assay.

Catecholamine assays are performed at the Royal Victoria Infirmary, Newcastle. Free catecholamines in plasma were quantitated in a four-step procedure: the catecholamines were adsorbed onto alumina at a pH of 8.6, washed and then eluted with a dilute acid. This elutate was analysed by high performance liquid chromatography with electrochemical detection (HPLC-ED) (Anton and Sayre 1962). The estimated lowest operating detection levels of metanephrine and normetanephrine have been quantified as 4.2 $f.mol^{-l}$ and 3.0 $f.mol^{-l}$ respectively. Blood samples once obtained were analysed using Oasis WCX u-elution plates 30um (Waters part no.186002499), the plate was first activated by drawing through 200ul MEOH followed by 200ul 0.01M ammonium phosphate buffer, under vacuum conditions. 100ul sample, QC and standard were then added to the plate, followed by 25ul, internal standard and 100ul 0.01M ammonium phosphate buffer. This mixture was drawn through the vacuum system at approximately 25psi until all wells were empty. A series of washes then occurred starting with 200ul water, which was drawn to waste, followed by 200ul methanol drawn to waste, and 200ul acetonitrile/0.2% formic acid drawn to waste. Between each wash step, the vacuum psi was increased to 40-50psi to ensure each well on the plate is dry before the next wash. The retained samples were then collected into a 1ml elution plate (Waters 186002643) by placing the plate directly below the WCX elution plate and eluting with 3 x 25ul volumes of elution solution (9.8ml 95% ACN/200ul conc formic acid). The plate was then heat sealed ready for analysis. Standards and QC were made in house (by the analyzing biochemist) by spiking Human Albumin Solution 50g/L (for calibrators) and pooled patient plasma (for QC) with stock standards purchased from sigma aldrich and QMX labs.

Normetanephrine and metanephrine levels were investigated according to the present of absence of AMS and the perceived intensity of exercise.

Data distribution was tested using the Shapiro Wilk statistic to determine whether or not it was normally distributed. Differences across altitudes or rest vs. exercise where then tested using Kruskal Wallis test. Data is presented as mean (SD, range) pmol/L.

8.2.3 *Study results*

Number of subjects studied (metanephrine) SL rest 19, SL exercise 20, 3833m 48, 4450m rest 48, exercise 44 – 3 subjects chose to journey by road vehicle due to ill health (gastrointestinal illness and one case HAPE) and 1 inadequate sample, 5129m 47 (one subject failed to climb above BC).

The data for metanephrine and normetanephrine was not normally distributed (Shapiro Wilk statistic 0.942 and 0.808 respectively $p<0.001$).

	Altitude (m)	Sea Level	3833	4450	5129	P value for change with altitude
Metanephrine (pmol/L)	Rest	163.5 (61, 40-288)	187.73 (44.7, 96-301)	203.56 (47.3, 119 – 289)	195.60 (50.0, 113 – 338)	0.078
	Exercise	193.1 (99.2, 40-504)	197.90 (47.7, 126-316)	231.45 (64.3, 115 – 392)	204.30 (85.7, 82 – 415)	0.012
Normetanephrine (pmol/L)	Rest	272.7 (117.2, 109-461)	518.42 (183.3, 191 – 996)	679.60 (231.2, 144 – 1237)	1093.68 (548.5, 433 – 3880)	<0.001
	Exercise	402.5 (158.1, 104 – 709)	571.79 (246.3, 126 – 1359)	953.11 (406.6, 303 - 2006)	1423.72 (786.0, 355 – 4159)	<0.001

Data presented as mean (SD, range)

Table 8.2 Changes in metanephrine and normetanephrine with altitude and exercise.

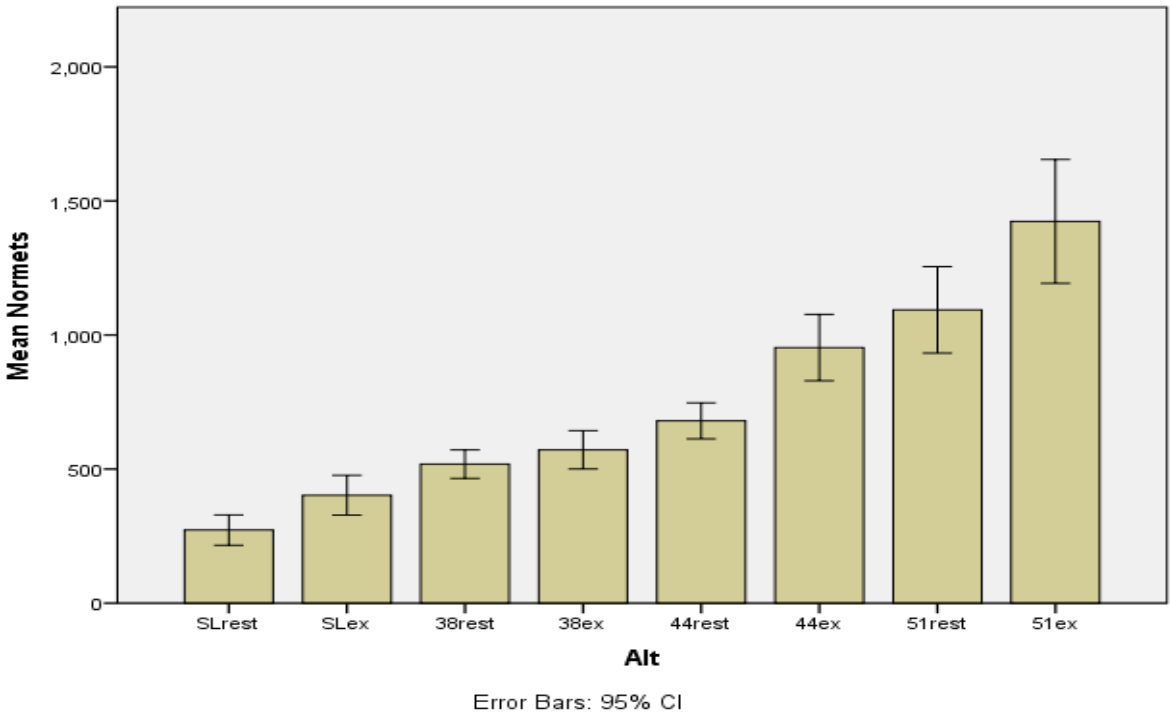


Fig 8.3. Mean normetanephrine at rest and exercise with increasing altitude (pmol/mL)

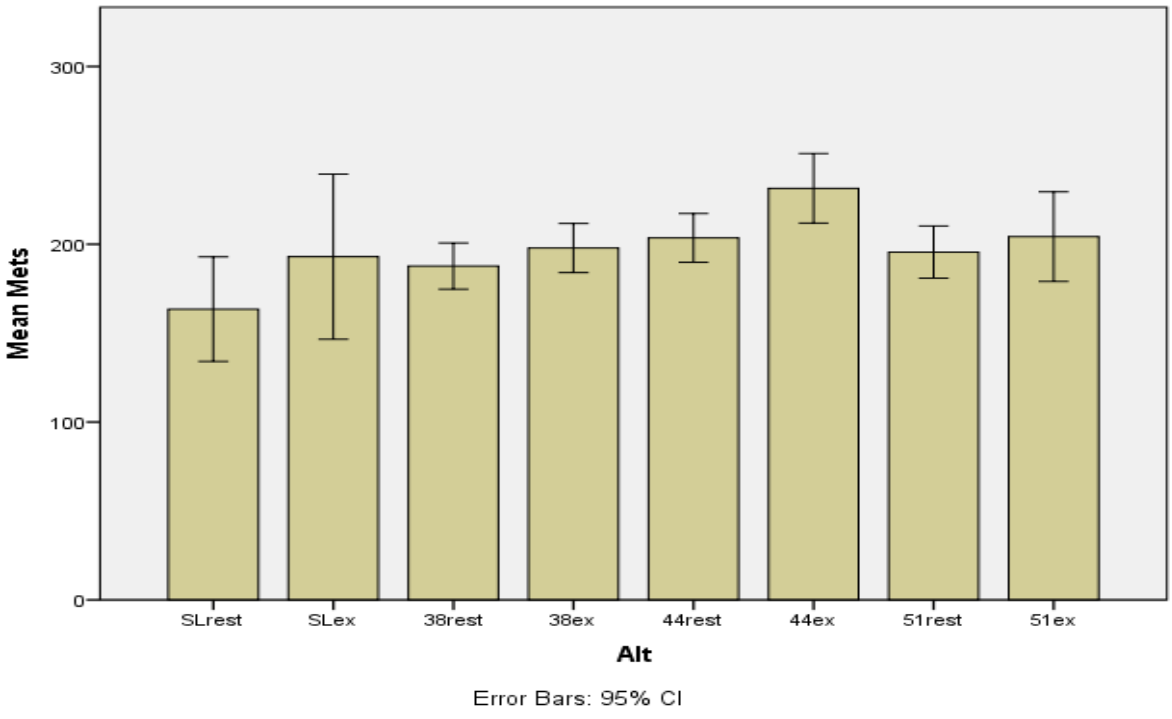


Fig 8.4. Mean normetanephrine at rest and exercise with increasing altitude (pmol/mL)

The change with exercise vs. rest was significant for normetanephrine (p=0.004) but not metanephrine (p=0.12) with a Kruskal Wallis test. There was a significant change in normetanephrine with altitude both rest and with exercise (p<0.001). Metanephrine increased significantly with exercise at each altitude (p=0.012) but not at rest p=0.078. The highest value of metanephrine was recorded at 4450m whereas normetanephrine increased with increasing altitude.

Normetanephrine levels (pmol/L) changed significantly with more intense exercise. When Borg < 15 (N = 95), normetanephrine values were 873 (490.0, 149 – 2490) and with Borg RPE $\geq$ 15 (N=38), 1246 (855.5, 126 – 4159) (p=0.014, Independent samples Mann Whitney U test). This is also supported by the fact that heart rate increased with increasing normetanephrine Spearman's rho 0.303 p<0.001 (figure 8.5).

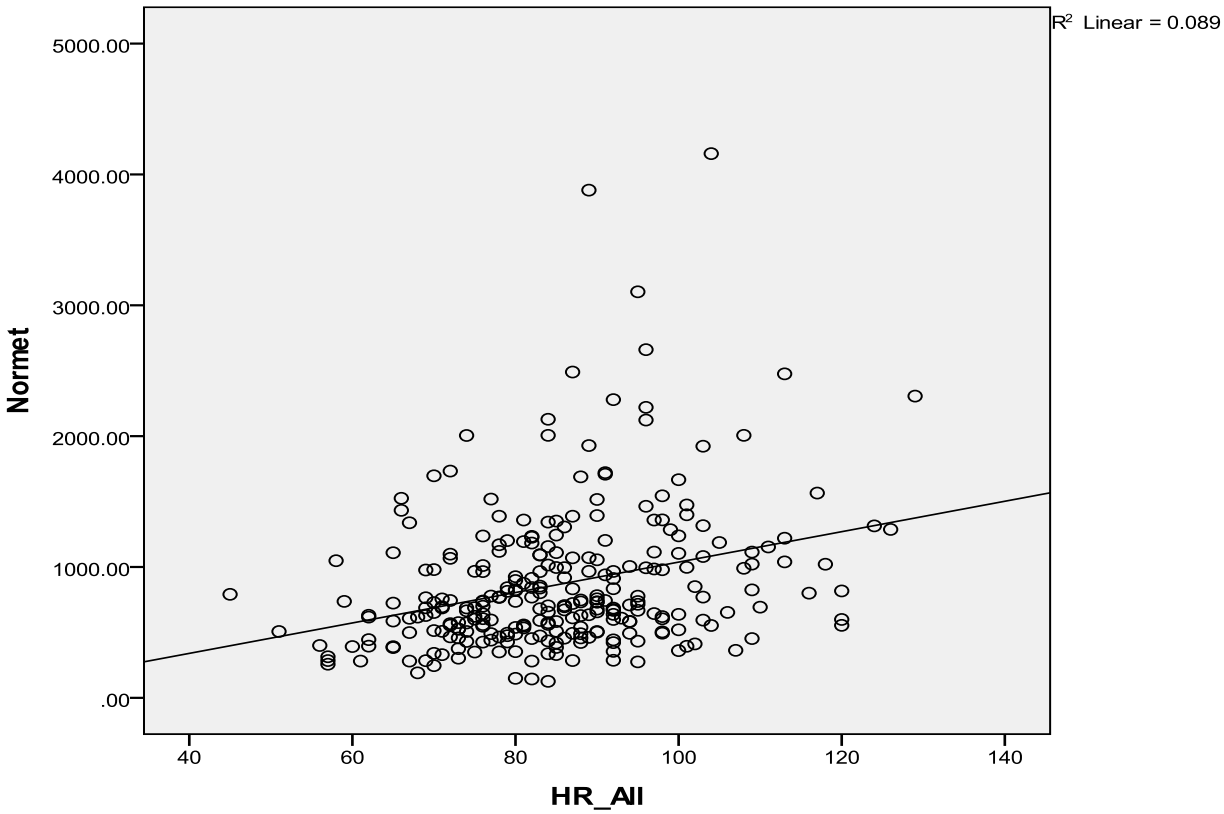


Figure 8.5 Normetanephrine changes with heart rate

Metanephrine levels did not change in the same way with exercise intensity Borg < 15 metanephrine value 217 (72.3, 112 – 415) vs. Borg ≥ 15 195 (59.5, 82 – 392) (p=0.069). There was an inverse correlation between normetanephrine and SpO2 (rho=-0.363, p<0.001).

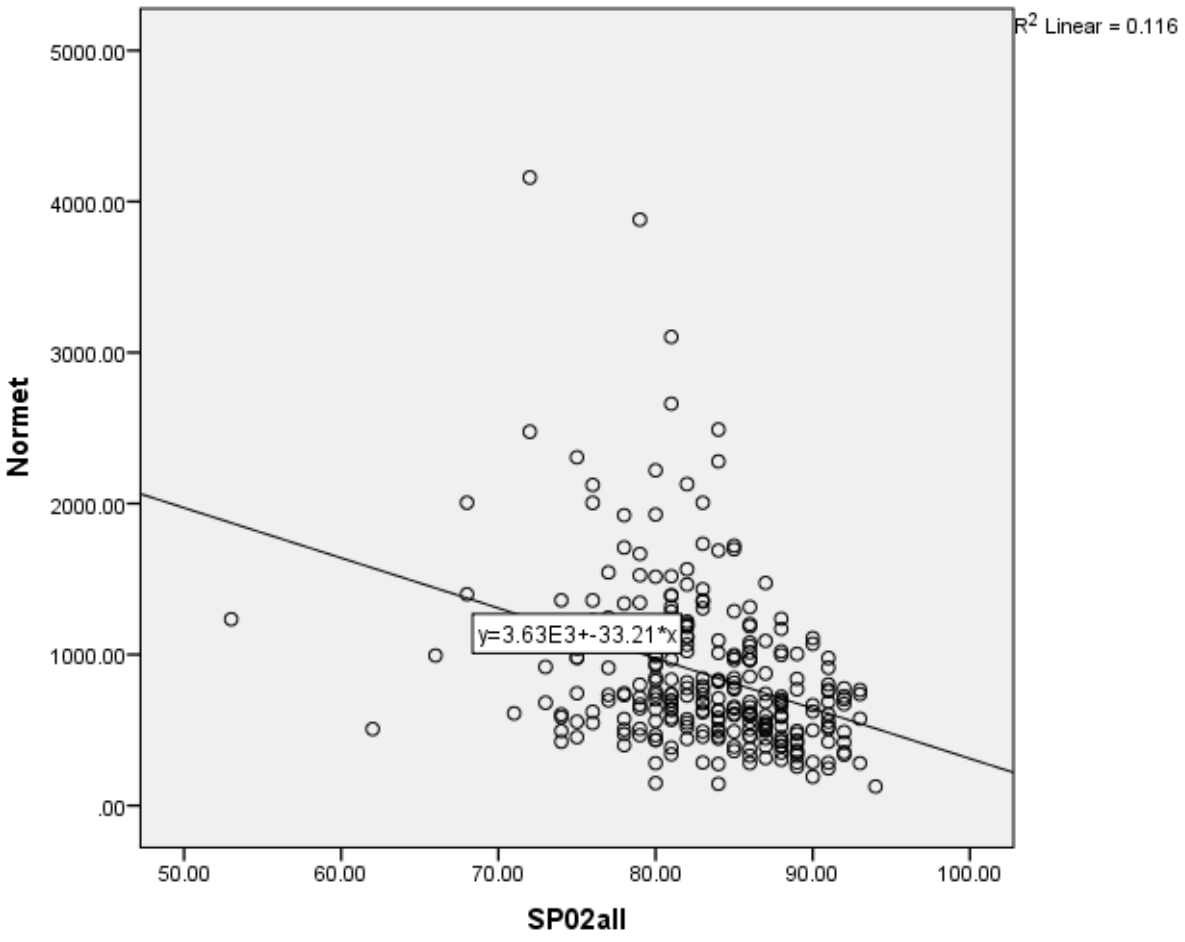


Figure 8.6 Relationship of normetanephrine to SpO_2

There was no correlation between respiratory rate and normetanephrine (Spearman's rho 0.108, p=0.84).

There was no significant difference in the levels of normetanephrine or metanephrine between those without AMS or with AMS or severe AMS.

8.2.4 *Discussion*

The fact that metanephrines changed only very slightly at all altitudes vs. the 5 fold change seen in normetanephrine is interesting. Anand found a 3 fold rise in norepinephrine in Indian soldiers at altitudes above 6000m (albeit over a longer time period) but found no rise in epinephrine (Anand, Chandrashekhar et al. 1993). A correlation between norepinephrine and SpO_2 ($r=0.65$ $p<0.05$) has previously been reported (Rostrup 1998). The actions of catecholamines include increasing respiratory rate and this could be beneficial as part of the response to the increased physiological stress of increasing hypoxia. The data collected during the field study may have shown a weaker correlation as a result of the fact that only SpO_2 at the end of exercise was recorded and the nadir of the fall in SpO_2 may have been different. In fact one of the weaknesses in the study was that heart rate, oxygen saturations and respiratory rate were only measured at the end of exercise and not continually throughout the exercise period.

8.3 Cortisol and adrenocorticotrophic hormone changes at HA and with exercise

8.3.1 *Background*

There is good reason why the cortisol response at HA might be an important part of the generation of high altitude illness. There is a strong correlation between aldosterone and cortisol at HA (McLean, Booth et al. 1989), excess cortisol may contribute to fluid retention, and exercise at SL is also known to cause a rapid rise in serum and salivary cortisol (McLean, Booth et al. 1989, Di Luigi, Baldari et al. 2008, Nunes, Crewther et al. 2011, Usui, Yoshikawa et al. 2011, VanBruggen, Hackney et al. 2011). There have been numerous investigations regarding the cortisol response to HA over the last 30 years, the majority of which have reported a rise in cortisol at rest at HA (Sutton, Viol et al. 1977, Humpeler, Skrabal et al. 1980, Richalet, Rutgers et al. 1989, Sawhney, Malhotra et al. 1991, Anand,

Chandrashekhar et al. 1993, Martignoni, Appenzeller et al. 1997, Zaccaria, Rocco et al. 1998, Barnholt, Hoffman et al. 2006). However, not all studies have found an elevated cortisol at rest (Maher, Jones et al. 1975, McLean, Booth et al. 1989, Savourey, Garcia et al. 1996, Basu, Pal et al. 1997, Benso, Broglio et al. 2007) and even in those that did the numbers studied have usually been small, between 6 (McLean, Booth et al. 1989) and 15 (Sawhney, Malhotra et al. 1991). Furthermore, while exercise is a central feature of most ascents to HA, studies of cortisol at HA have only occasionally included an exercise stimulus and usually one of relatively short duration (<1 hour) (Maher, Jones et al. 1975, McLean, Booth et al. 1989, Zaccaria, Rocco et al. 1998, Chen, Wang et al. 2006).

There is also an apparent paradox in that elevated cortisol at HA has been associated with AMS (Sutton, Viol et al. 1977, Richalet, Rutgers et al. 1989) and yet dexamethasone and prednisolone are beneficial both as a prophylactic against AMS (Ellsworth, Larson et al. 1987, Basu, Sawhney et al. 2002) and as a treatment for AMS and HACE (Hackett, Roach et al. 1988, Levine, Yoshimura et al. 1989). The latter effect reflects dexamethasone's generalised efficacy in combating cerebral oedema, anti-emetic effects and ability to induce euphoria (Zafren 2012). Nevertheless, very few studies have examined any relationship between resting or post-exercise cortisol at HA and AMS.

In a 2009 study of trekkers in Nepal we have previously published data to show that morning cortisol (measured on a salivary swab nmol/l, mean+/-sd) at rest went from 5.5+/-2.9 to 4.7+/-6.8; 3.5+/-2.1; 14.5+/-30.3 at 1300m, 3400m, 4270 m and 5150 m respectively. Afternoon post-exercise cortisol went from 8.5+/-7 to 7+/-6; 4.2+/-4.8; 9.2+/-10.2 at 1300m, 3400 m, 4270 m and 5150 m respectively.

The statistically significant findings at rest were a rise in cortisol from 4270 m to 5150 m (p=0.002). The statistically significant findings post-exercise were an initial drop in salivary cortisol between SL and 4270 m (p=0.01) and 3400 m and 4270 m

(p=0.001) followed by a significant rise in post-exercise cortisol between 4270 m and 5150 m (p<0.001).

From this work it appears that the post-exercise response in cortisol was intact at 3400 m but an apparent subdued response at 4270 m before a restoration in the response at 5150 m. There are similarities between the subdued exercise response to cortisol at 4270 m and that previously found regarding aldosterone. Bouissou et al. (Bouissou, Richalet et al. 1989) found a reduction in the exercise-induced aldosterone rise following graded maximal exercise at 4350 m compared to SL. McLean et al. (McLean, Booth et al. 1989) also found a subdued rise in aldosterone compared to SL at 4450 m following 25 minutes of exercise. We found a restoration in the cortisol response after a day trekking at 5150 m whereas Zaccaria et al. (Zaccaria, Rocco et al. 1998) found no aldosterone response to exercise (albeit after a brief stimulus with a graded exercise test) at 5050 m. Interestingly Bouissou et al. (Bouissou, Fiet et al. 1988) found that in a hypobaric chamber at a simulated 3000 m the ACTH rise in response to 60 minutes exercise was unchanged compared to SL but that the cortisol response was subdued. They found ACTH and cortisol post-exercise correlated strongly at SL but not at HA, suggesting ACTH-driven steroidogenesis at SL but some disconnect at HA with apparent reduced cortisol sensitivity to ACTH. This is similar to the known dissociated response of aldosterone to increased plasma renin activity (PRA) that occurs during hypoxaemic exercise (Shigeoka, Colice et al. 1985) that is thought to be partly due to direct inhibition of aldosterone synthesis in the adrenal gland by hypoxia (Raff, Jankowski et al. 1996). A reduced sensitivity of adrenal cortisol production to ACTH stimulation may, like that of PRA and aldosterone, be an adaptation to reduce potential fluid retention from excess cortisol post-exercise. Any damping effect in the cortisol response to exercise around 4270 m is then over-ridden by the greater physiological stress at 5150 m. Unfortunately in this study there was no recording of Rating of Perceived Exertion or other measure of intensity which might have delineated whether the cortisol response was due to a harder exercise stimulus.

The fact cortisol is a stress hormone and the environment of HA creates a significant physiological stress is the most likely explanation for the rise in cortisol at HA. Part of this stimulus may relate to hypoxia as evidenced by the association between lower SpO_2 and higher cortisol.

Hypothesis

The physiological stress of HA would lead to a rise in ACTH and cortisol. This would lead to fluid retention and be linked to the development of AMS.

8.3.2 *Methods*

In the study in Bolivia we measured plasma rather than salivary cortisol. This was drawn from an antecubital fossa vein into a serum separator tube before being centrifuged, and stored frozen at -20°C until movement back to the UK after which it was stored at -80°C. Assays were performed at Newcastle's Royal Victoria Infirmary (RVI). The Roche Cortisol assay (used at RVI) is a competitive immunoassay using electrochemiluminescence technology. Serum is the preferred sample but heparin (Li-, Na-, NH4+-) or EDTA plasma is acceptable. A minimum sample size of 20µL (+100µL for dead volume) is required. The test is fully automated and run on the Roche Modular E unit (Roche Diagnostics, Burgess Hill, UK). The assays take 18 minutes to run. A calibration sample must be performed once per reagent lot using fresh reagent.

During the first incubation step Cortisol, which has been liberated from binding protein with Danazol, competes with a ruthenium labeled cortisol derivative for binding sites on a cortisol-specific biotinylated antibody. During the second incubation, after addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
The reaction mixture is aspirated in to the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are removed using Procell. Application of a voltage to the electrode induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined by measuring the electrochemiluminescence signal obtained from the reaction product of the sample against a calibration curve generated by 2-point calibration and a master curve provided via the reagent barcode. The analytical range is 0.5 –1750 nmol/L (From pack insert). Samples with concentrations >1750 nmol/L can be checked via the re-run function and the results corrected for the dilution factor of 10. Routine samples are reported as >1750 nmol/L. Low levels are reported as 'less than 20 nmol/L'.

Analytical sensitivity (lower detection limit): 0.5 nmol/L (From pack insert) The detection limit represents the lowest measurable analyte level that can be distinguished from zero.

Functional sensitivity <8.5 nmol/L (From pack insert) The functional sensitivity of the method is defined as the concentration at which the total coefficient of variation is ≤20%.

ACTH is measure on the DPC Immulite machine (Siemens Healthcare, Germany). The assay uses a chemiluminescent detection technique in combination with an immunometric (i.e. a 'sandwich') assay method. Two antibodies to ACTH are employed. The capture antibody is a murine monoclonal anti-ACTH immobilised on to polystyrene beads. The second antibody is a polyclonal rabbit anti-ACTH, labelled with alkaline phosphatase (AlkP) to facilitate quantitation. A 200µL sample of EDTA plasma is required.

Antibody-coated bead, sample and AlkP-labelled antibody are incubated for two 30 minute cycles at 37°C followed by a centrifugal wash step. To the antibody sandwich complex is then added the chemiluminescence substrate, in excess. This phosphate ester of adamantyl dioxetane is hydrolysed by the alkaline phosphatase resulting in an unstable intermediary compound. Continuous production of this results in a prolonged glow. This allows multiple photon readings by the luminometer thus enhancing precision. The signal is directly proportion to the

concentration of alkaline phosphatase and therefore ACTH in the specimen. i.e. as for any immunometric assay the signal is directly proportional to the concentration of the analyte being measured.

The labs' quoted imprecision data are;

Control Material: Bio-Rad Trilevel Control

Within Batch

	N	Mean (ng/L)	SD	CV%
Low	20	12.7	0.65	5.1
Med	20	125.6	3.53	2.8
High	20	446.6	11.8	2.6

Between Batch

	N	Mean (ng/L)	SD	CV%
Low	20	13.0	1.53	11.7
Med	20	122.8	9.73	7.9
High	20	436.2	7.93	9.3

Results below 5 ng/L are reported as <5 ng/L.

The upper limit of the calibration range is 1250 ng/L. Samples with results which are above 1250ng/l are diluted 1 in five with ACTH diluent

Data distribution was tested using the Shapiro Wilk statistic to determine whether or not it was normally distributed. Values for cortisol were compared for rest and exercise with increasing altitude (Krukal-Wallis test) and compared for cohorts experiencing AMS (LLS$\geq$3) or severe AMS (LLS$\geq$6) and those reporting high vs. low perceived exertion (Mann-Whitney U test). Correlations were performed using Spearman's rank correlation coefficient. Data is presented as mean (SD, range).

8.3.3 *Study results*

Number of subjects studied was 20 at sea level, 3833m 48 at rest, 47 exercise; 4450m rest 48, exercise 45 – 2 subjects chose to journey by road vehicle due to ill health (gastrointestinal illness and one case HAPE), 5129m 45 at rest and 46 post exercise due to sampling difficulties and the fact that one subject failed to climb above BC.

The data for cortisol and ACTH was not normally distributed (Shapiro Wilk statistic 0.95 $p<0.001$ and 0.755 $p<0.001$). .

Cortisol changes with altitude are shown below;

	Altitude (m)	**Sea Level**	**3833**	**4450**	**5129**	**P value with increasing altitude**
Cortisol (nmol/L)	Rest	368.7 (133.6, 132-636)	459.1 (201.7,151 – 959)	407 (183.2, 44 – 937)	522.8 (183.5, 223 – 1198)	0.02
	Exercise	262.5 (131.6, 103-659)	369.2 (261.2, 97 – 1591)	364.8 (206.5, 78 – 1049)	632 (182.6, 337 – 1198)	<0.001
ACTH (pmol/L)	Rest	15.9 (6.9, 7-31)	19.7 (12.5, 6-57)	19.6 (9.2, 5-46)	18.6 (8.6, 7-46)	0.494
	Exercise	10.7 (5.6, 5-29)	25.6 (27.4, 6-151)	23.1 (10.3, 8-49)	30.1 (16.2, 5-85)	<0.001

Data presented as mean (SD, range).

Table 8.3 Cortisol and ACTH with changing altitude at rest an exercise.

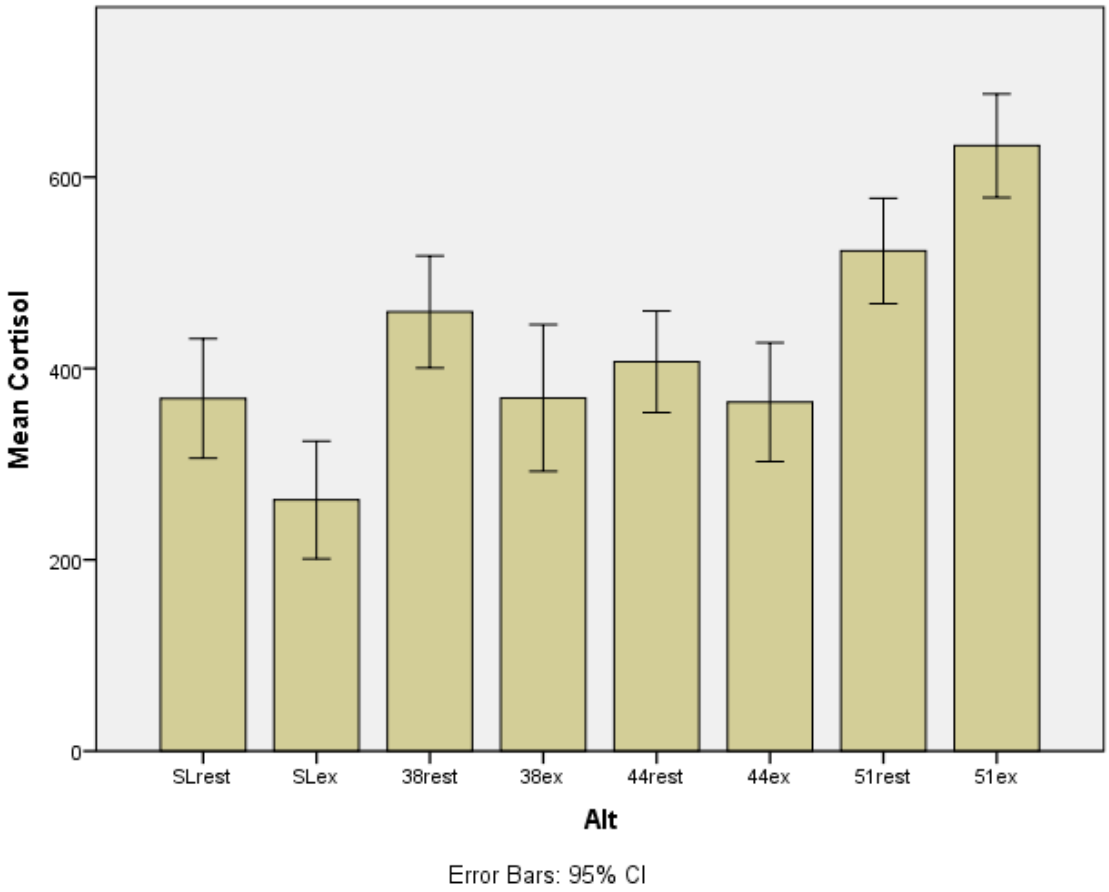


Fig 8.7. Mean cortisol at rest and exercise with increasing altitude (nmol/mL)

Cortisol increased with increasing altitude ($p<0.001$). Values were higher at 3833m than at 4450m but peaked at 5129m. Overall there was no significant change of cortisol with exercise vs. rest ($p=0.097$). Individual Mann Whitney U tests showed a significant reduction in cortisol with exercise at 3833m ($p=0.08$), no change at 4450m and an increase at 5129m ($p<0.001$).

Cortisol was increased in those with severe AMS mean 444.8 vs. 650.4 ($p=0.033$). For those with a LLS$\geq$3 cortisol was increased mean 439.9 vs. 517.5 but this did not reach significance ($p=0.064$).

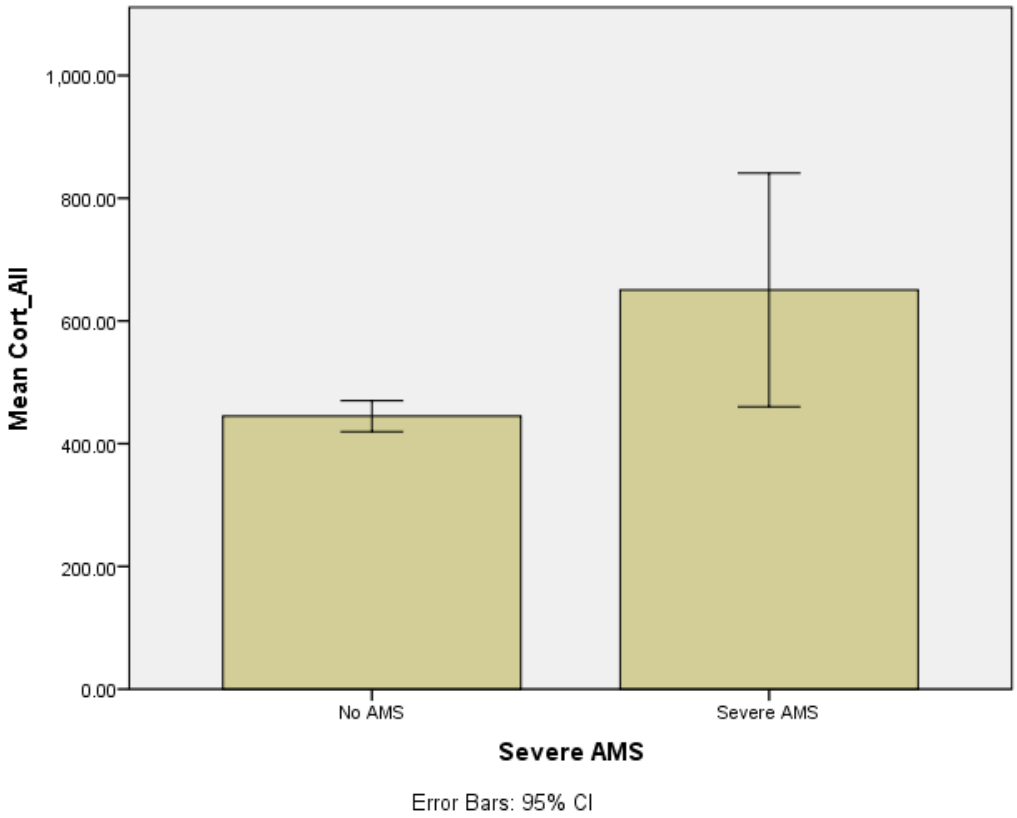


Fig 8.8. Mean cortisol in subjects with severe AMS (LLS≥6) vs. those without (nmol/mL)

There was a very weak but significant inverse correlation between cortisol and SpO2 (Spearman's rho -0.188, p=0.002) but not between cortisol and heart rate (p=0.170).

Adrenocorticotrophic hormone showed significant change with altitude after exercise (p<0.001) but no change in the resting state (p=0.494). ACTH was higher after exercise than at rest (p=0.002). There was no correlation between ACTH and cortisol at 3833m or 4450m at rest but a good correlation with rest at 5129m (rho 0.436 p=0.003). With exercise ACTH and Cortisol correlated at 4450m (Spearman's rho 0.415, p=0.005) and at 5129m (Spearman's rho 0.436, p=0.003).

If the groups were separated by a Borg score ≥ 15 then the Borg <15 group (n=95, mean cortisol 386.4) had a significantly different cortisol to the high Borg group (n=38, mean 603.4 p = <0.001). ACTH in the Borg <15 group was a

mean of 20.88 whereas in the Borg ≥15 group the mean was 24.6, this difference was not significant (p=0.308).

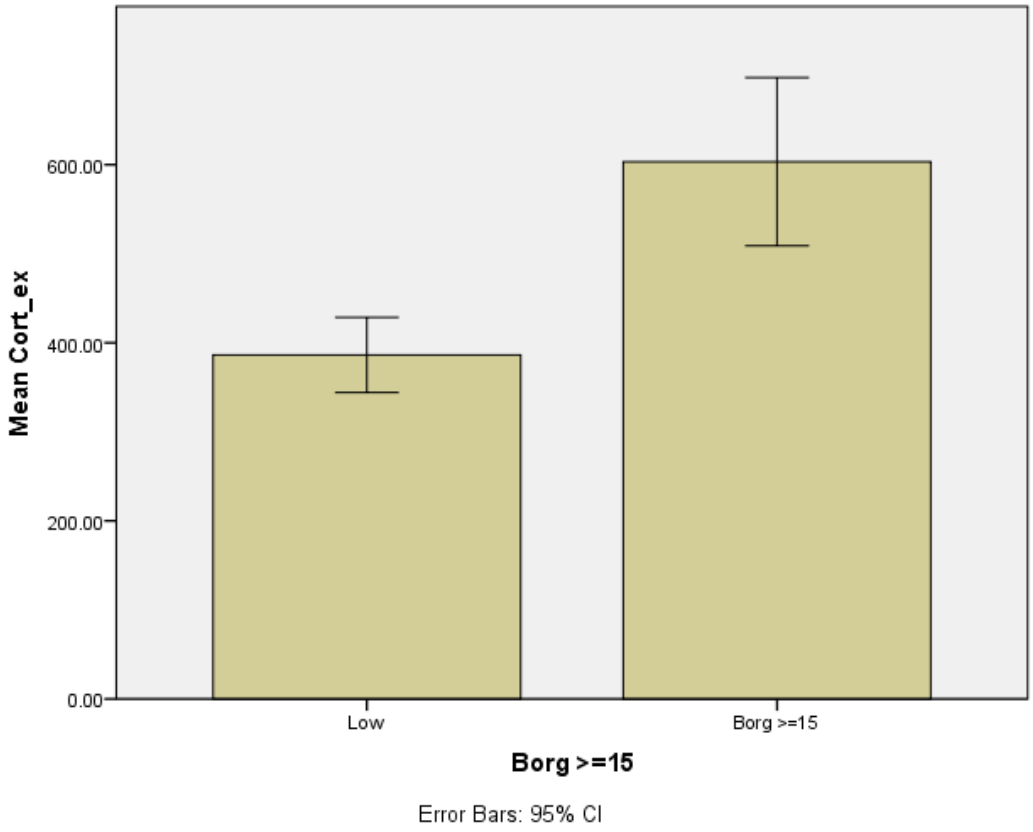


Fig 8.9. Plasma cortisol following exercise in the groups with Borg < 15 vs. ≥15

8.3.4 *Discussion*

The fact that cortisol falls after exercise at sea level, 3833m and 4450m is surprising, however as described earlier the cortisol response is very dependent on the exercise intensity with exercise even up to 62% VO_2 max producing no rise in cortisol (Jacks, Sowash et al. 2002). Cortisol secretion also shows diurnal variation with a peak on waking. Our samples were taken at around this time on rest days with exercise samples being taken after lunchtime when there is a marked decrease in cortisol. This mechanism probably explains the fall at SL, 3833m and 4450m when the exercise stress was not sufficient to provoke a significant response. At 5129m the physiological stress of the strenuous trek and hypoxia was sufficient to increase cortisol. It is significant that there appears to be a disconnect between ACTH and cortisol at low altitudes (even in our data at near sea level exercise) with correlation only apparent at rest at 5129m and with exercise at 4450m and 5129m. Whilst this is an intriguing

finding the data can only be viewed as pilot data and future investigation will have to control timing of the ACTH response with the use of synthetic ACTH as in a short Synacthen test.

Once again the exertion involved during the exercise period seems to be a significant stressor leading to a rise in cortisol. There was an increase in cortisol with a high Borg RPE score and an increased cortisol in those with severe AMS. Whether cortisol rises as a response to exercise in hypoxia, leading to fluid retention and exacerbates AMS symptoms or whether the acute illness of AMS leads to stress and a rise in cortisol cannot be further elucidated from this study.

8.4 Conclusions

A summary of the physiological effects of high altitude ascents are a decrease in SpO_2, a variable effect on blood pressure and an increase in respiratory rate. The novel exploration in this study has been into the relationship of cortisol and catecholamines to exercise intensity by measuring perceived exertion during a trek. This differs both in terms of the large numbers of subjects studied and the methods used in previous studies and shows a highly significant change in plasma cortisol and normetanephrine for those who perceived the trek as "hard". The increase in cortisol was approximately 60% and this was associated with higher cortisol in those reporting severe AMS. The physiological changes seen in terms of the correlation of heart rate to normetanephrine and inverse correlation of SpO_2 to normetanephrine and cortisol support the fact that physiological stress related to exercise is the key stimulus for this rise. The lack of correlation between ACTH and cortisol and relative suppression of cortisol response to exercise until 5129m is a fascinating finding and worthy of further investigation. The time course of all these changes relative to the development of altitude illness is a key feature that cannot be determined from the current data.

Chapter 9. Assessing Exercise Intensity/3 Way Comparison

Summary

The conclusion developed from earlier work in this MD thesis is that exercise intensity plays a key role in development of the symptoms of Acute Mountain Sickness (AMS). Whilst the aim has to be to understand AMS during field studies (i.e. the real world situation) performing standardised exercise testing in the field is extremely difficult. It is important to be able to quantify exertion with a simpler method such as a rating of perceived exertion (RPE) scale. Likewise standardising exercise protocols, rest and diet can only practically be performed in laboratory based studies but studies in hypobaric chambers are difficult to perform and there is no consensus on whether normobaric and hypobaric hypoxia generate the same stimulus.

The data from the Bolivia field study was analysed to investigate the relationship between RPE, physiological variables and AMS scores. The finding was that there is a significant difference between subjects recording high and low RPE and AMS supported by some physiological differences. In a second study the physiological response to standardised exercise in normobaric and hypobaric hypoxia, as well as in the real world environment, was examined. This study showed no difference in heart rate, oxygen uptake or lactate across the three environments and further supported the use of a RPE scale.

Hypothesis

1. A higher rating of perceived exertion will lead to an increase in reported AMS symptoms.
2. There will be no difference in the response to hypoxia in normobaric, hypobaric or “real world” environments.

9.1 Background

The fact that exercise has a major impact on physiological changes during acclimatisation is made in earlier chapters. To briefly recap subjects with a high rating of perceived exertion had a higher level of copeptin, cortisol and normetanephrine, all suggesting a link between exercise intensity and physiological stress. This neuroendocrine response is an adaptation to preserve salt and water, useful if the stressor is hypovolaemia from haemorrhage but potentially detrimental in high altitude environments.

Regardless of any neuroendocrine response exercise will have the added effect or worsening hypoxia due to increased oxygen demands. This could have beneficial effects of stimulating a greater adaptation but is also likely to increase the risk of altitude illness. The guidance frequently given to mountaineers is that some exertion is beneficial whilst hard effort should be avoided. This is based on custom and practice rather than any evidence and is an empirical solution to balance the benefits and risks of greater hypoxic stimulus.

9.1.1 *Methods of Measuring Exercise Intensity*

In an important study using heart rate to assess exercise intensity Bircher and colleagues (Bircher, Eichenberger et al. 1994) investigated whether susceptibility to acute mountain sickness (AMS) and high-altitude pulmonary edema (HAPE) is related to differences of physical fitness at the outset of an expedition and /or the level of exercise during ascent. Heart rate was continuously registered during the ascent from 3610 to 4559 m in 41 mountaineers. During the subsequent stay of 3 days at 4559 m, 12 subjects developed AMS, 13 subjects showed radiographic evidence of HAPE, and 16 subjects remained without significant illness. The level of baseline fitness (as measured by work done at heart rate of 170 bpm) and heart rate during ascent did not significantly differ between these groups. The authors conclude “these results indicate that physical fitness or exercise intensity during ascent were of minor importance for the development of AMS and HAPE in our subjects”.
The findings of 3 recent chamber studies are summarised below;

	N	NH or HH	%VO2 max	Duration	Exercise protocols	Conclusion
Roach (Roach, Maes et al. 2000)	7	HH (4800m)	50% alt adjusted	10 hours	1. 4x30 mins 2. Rest	4 fold ↑LLS with exercise
Schommer (Schommer, Hammer et al. 2012)	16	NH (FiO2 12%)	50% alt adjusted	18 hours	1. 3x45 mins 2. Rest	No change AMS with exercise
Rupp (Rupp, Jubeau et al. 2013)	12	NH (FiO2 12%)	45% alt adjusted	11 hours	1. 4 hours exercise 2. 4 hours ↓FiO2 3. Normoxia	No difference between exercise and rest LLS

Table 9.1 Previous published studies investigating AMS and exercise

In laboratory settings, work can be performed at a fixed proportion of maximal oxygen uptake (VO_2 max). This is a technically difficult process requiring a bicycle ergometer and expired gas analysis. As VO_2 max falls with HA an adjustment must be made with sea level values to make comparison. This requires a voluntary maximum test in a hypoxic environment prior to investigation. The studies summarised above (Roach, Maes et al. 2000, Schommer, Hammer et al. 2012, Rupp, Jubeau et al. 2013) use this technique to reach conflicting results.

The Borg rating of perceived exertion score (chapter 3)(Borg 1970, Borg 1973) is an attractive way to measure exercise intensity during a trek. Although not part of Borg's rating scale local and central RPE can also be recorded (Pandolf 1982). Local RPE relates to sensations or feelings of strain from the exercising muscles and/or joints and a central factor relates primarily to cardiopulmonary sensations. A literature search for perceived exertion and high altitude found two relevant publications. In 1982 Young (Young, Cymerman et al. 1982) studied 8 subjects during acute (2 hours) and chronic (18 days) in the setting of a hypobaric chamber for the acute exposure and a sojourn at HA (4300m in both environments). He measured local muscular RPE, central cardiopulmonary RPE and overall RPE. Central RPE was the highest during chronic HA exposure suggesting that perceptual cues sensed as effort change with HA exposure. Maresh (Maresh, Deschenes et al. 1993) looked at 6 low altitude natives on decompression to 447mmHg (4270m) for 2 days. At low altitude local RPE exceeded central ratings whereas at high altitude central RPE were greater than local. Interestingly he reports a correlation between cardiorespiratory AMSc scores and central RPE on the morning before the exercise (day 2 at HA) (rho = 0.875). However this study is compromised by very low numbers (n=6).

Laboratory based research can solve many of the problems of HA field studies by carefully controlling environmental conditions, oral intake and exercise intensity. Unfortunately the solution is not a simple one. HA can be modelled by either field studies, exposure to hypobaric hypoxia or exposure to normobaric hypoxia where the fraction of inspired oxygen is reduced to simulate a specific altitude. There is, however, debate about whether or not these two

entities are similar. This depends, in part, on what the model is being used for be it sports science, altitude medicine or aviation medicine. Rapid ascent in a hypobaric chamber does seem to be associated with more pronounced respiratory changes than the equivalent FiO_2 (SaO_2 62.3% v's 69.5%) with a 5 minute exposure to 7500m equivalent (Self, Mandella et al. 2011). However in this study the reported symptoms were similar prompting the authors to conclude that normobaric hypoxia was a useful model (for aviation training). This pattern of more pronounced hypoxaemia, hypocapnia and alkalosis (with hypobaric hypoxia) has been found in other studies and may be due to changes in dead space ventilation (Savourey, Launay et al. 2003). One other factor is the calculation of the correct FiO_2 which is often not clear from descriptions of research protocols (Girard, Koehle et al. 2012). The saturated vapour pressure of water (SVP_{H2O}) is constant at any altitude (pressure) and amounts to a value of 47mmHg. The ambient PO_2 must be reduced by 47mmHg as gases are saturated with water vapour on inspiration. In hypobaric environments this leads to a greater reduction of inspired oxygen content than in normobaric environments. As an example a reduction of pressure to 430mmHg in a hypobaric chamber with a normal FiO_2 (0.209) leads to an ambient oxygen tension of 90mmHg (430 x 0.209), the same oxygen tension can be generated with an FiO_2 of 0.119 in normobaricity (760mmHg x 0.119). However to calculate the inspired oxygen tension the SVP of water must be taken into account using the equation:

$$PiO_2 = (P_{ATM} - SVP_{H2O}) \times FiO_2$$

Were PiO_2 is Partial pressure of inspired oxygen, P_{ATM} is atmospheric pressure, SVP_{H2O} saturated vapour pressure of water and FiO_2 is the fractional inspired percentage of oxygen.

Hence in normobaricity the PiO_2 is 85mmHg whereas in hypobaricity it is 80mmHg and the FiO_2 in normobaricity must be set lower to get an equivalent stimulus.

Interestingly no studies have yet been published comparing normobaric and hypobaric chambers with a field study at high altitude.

There are also physical issues with decompression such as changes in air filled spaces such as the middle ear and sinuses. Decompression illness (DCI) can also manifest itself with exposure to hypobaricity. Worrying long term decline in neurocognition associated with white matter hyperintensities on MRI has been noted in U2 pilots frequently exposed to very high altitudes and low pressures (McGuire, Sherman et al. 2012). DCI and worries about neurocognitive damage limit the exposure allowed within the RAF's hypobaric chamber at RAF Henlow, limiting exposure to 3 hours in every 72 hours. This has a major impact on the ability to run studies and is not a sufficient duration to make meaningful observations of AMS.

Two studies were carried out as part of this MD to assess the value of the Borg RPE during exercise at HA. One was a pilot study during the Bolivia field study to collect the highest perceived exertion during the days trek. Study 2 was part of a more complex study comparing hypobaric, normobaric and the "real world". The aims of these studies were therefore to:

1. Assess the practicality of using the Borg score during a field study.
2. To compare the Borg score to heart rate and lactate across hypobaric, normobaric and mountain environments.

9.2 Study One - Perceived Exertion, Physiological Variables and Acute Mountain Sickness

9.2.1 *Methods*

The study was approved by the Ministry of Defence Research Ethics Committee and complied with the standards set in the Declaration of Helsinki. All subjects gave written informed consent.

Forty-eight subjects were recruited for the study. All subjects were members of the UK Military taking part in a dual purpose adventurous training and research expedition.

Studies were performed at three altitudes. This was on day 2 at 3833 m following exercise (a trek of 6 hrs. 600m ascent and 16.8 km), day 3 at 3833m

at rest, day 5 at 4450m following exercise (4 hours 200m ascent, 5.6 km), day 6 at 4450m at rest, day 9 at 5129m following exercise (4 hrs. 400m ascent, 1.59 km) and day 10 at 5129m at rest. The night was spent at the new altitude and data collected at rest the following day. The trekking took place in the Cordillera Real region of Bolivia.

Physiological variables (SpO_2, heart rate, respiratory rate, blood pressure) at rest or within 15 minutes of cessation of exercise were recorded by other, medically trained, expedition members or members of the research team. Heart rate and SpO_2 were recorded on a Nellcor N-20P pulse oximeter (Nellcor Puritan Bennett Ltd, Coventry.UK). Three measurements were made on warm hands and an average recorded.

Perceived Exertion

The Borg Rating of Perceived Exertion (RPE) was recorded (Borg 1970) at the end of each day to record the hardest perceived exertion experienced during the day. This is a 15 point scale from 6 – 20, with values of 6 representing the resting state and 20, exhaustive exercise. RPE using the Borg 15 point scale is related to HR (score is approximately 10x HR) and over 2500 exercise tests Scherr found a more precise relationship to HR defined by the equation HR = 69.34 + (6.23xRPE) (Scherr, Wolfarth et al. 2013). As the European Society of Cardiology recommend exercising at a perceived exertion of 12- 14 (Graham, Atar et al. 2007), a value of 15 or greater was taken as the cut off for a high Borg score. A RPE of 15 or greater is perceived as “hard exertion” by the subject, relates to a heart rate of around 150 bpm at sea level and is the level at which anaerobic metabolism starts to become a significant part of energy generation.

AMS scores

AMS scores were assessed using the Lake Louise score (LLS) (Roach, Bartsch et al. 1993). The LLS is a self-assessment questionnaire that allocates a score of 0 to 3 (symptom not present to severe) for symptoms of AMS (headache, gastrointestinal symptoms, fatigue/weakness, dizzy/light-headedness, difficulty sleeping). A score of 3 or more in the presence of headache is consistent with AMS.

Statistical analysis

For statistical calculations the software package SPSS 21.0 was used. Parametric or non-parametric statistical tests were applied after performing the Kolmogorov-Smirnov statistic**.**

For independent variables an independent-samples *t* test or Mann Whitney test was used. For relationships between categorical variables a Chi-square test was used. Significance was assumed at the p=0.05 level.

9.2.2 *Results*

The subjects were of a mean age of 35 years (range 22-54), mean height 175.6cm (range 155-194) and mean weight 77.8 Kg (range 47-108). There were 34 males and 14 females. All were physically fit, mean 1.5 mile run time 9.85 minutes (range 8.07 – 13.5). All 48 subjects were investigated at 3833m and 4450m, 47 out of 48 subjects reached the highest study altitude.

Compliance with recording the scores was good; there were a possible 143 scores to be collected (48 at 3833m and 4450m and 47 at 5129m), overall there were matched Borg and LLS for 136 (post exercise) and 138 (rest) study points. The mean Borg score for the trek to 3833m was 13.15 (SD 1.92), for the trek to 4450m it was 12 (SD 1.8) and for the trek to 5129m it was 14.98 (SD 1.98). The incidence of AMS at rest the day after the exercise reflected the RPE from the trek. At 3833m the incidence was 27.1% (n=13), 4450m 8.7% (n=4) and at 5129m 37.8% (n=17).

Overall there were 97 observations of a Borg < 15 and 39 of Borg $\geq$ 15 following exercise and 99 of a Borg $\leq$ 15 at rest. This difference is explained by the fact that at 3833m 2 subjects travelled to the high camp by vehicle due to illness and did not record a score. Lake Louise scores differed significantly between the groups with a low (Borg<15 or high Borg >=15) RPE score (table 12.2).

		Borg ≤ 15		Borg ≥ 15		
		N		N		P
Heart Rate	Post exercise	98	87(13.7, 56-120)	39	94 (15.3, 60–129)	0.007
	Following morning	99	80 (11.8, 45-120)	39	87 (14.4, 57–120)	0.003
SpO2	Post exercise	97	82 (5.1, 68-92)	39	81 (4.9, 68-94)	0.371
	Following morning	96	85 (4.7, 74-91)	38	83 (5.1, 66-93)	0.021
LLS	Post exercise	97	1.65 (1.95, 0-9)	39	2.62 (2.3, 0-10)	0.008
	Following morning	99	1.7 (1.83, 0-9)	39	2.67 (2.19, 0-8)	0.012

Table 9.2 Heart rate (bpm), SpO_2 (%) and Lake Louise Score (LLS) in high and low Borg RPE groups. Data presented as mean (SD, range)

Results are presented in tabular form and graphical comparisons (table 9.2 and figure 9.1).

The incidence of AMS at rest the day after the exercise reflected the RPE pattern of response from the trek. At 3833m the incidence was 27% (n=13), 4450m 9% (n=4) and at 5129m 38% (n=17).

Lake Louise scores differed significantly between the groups with a low (Borg<15 or high Borg≥15) RPE score. Immediately post-trek the group with a high RPE LLS was 2.6 +/- 2.3 (0-10) vs. 1.7 +/- 2.0 (0-9) whilst at rest the following day the high RPE LLS was 2.7 +/- 2.2 (0-8) vs. 1.7 +/- 1.8 (0-9) (fig 9.1).

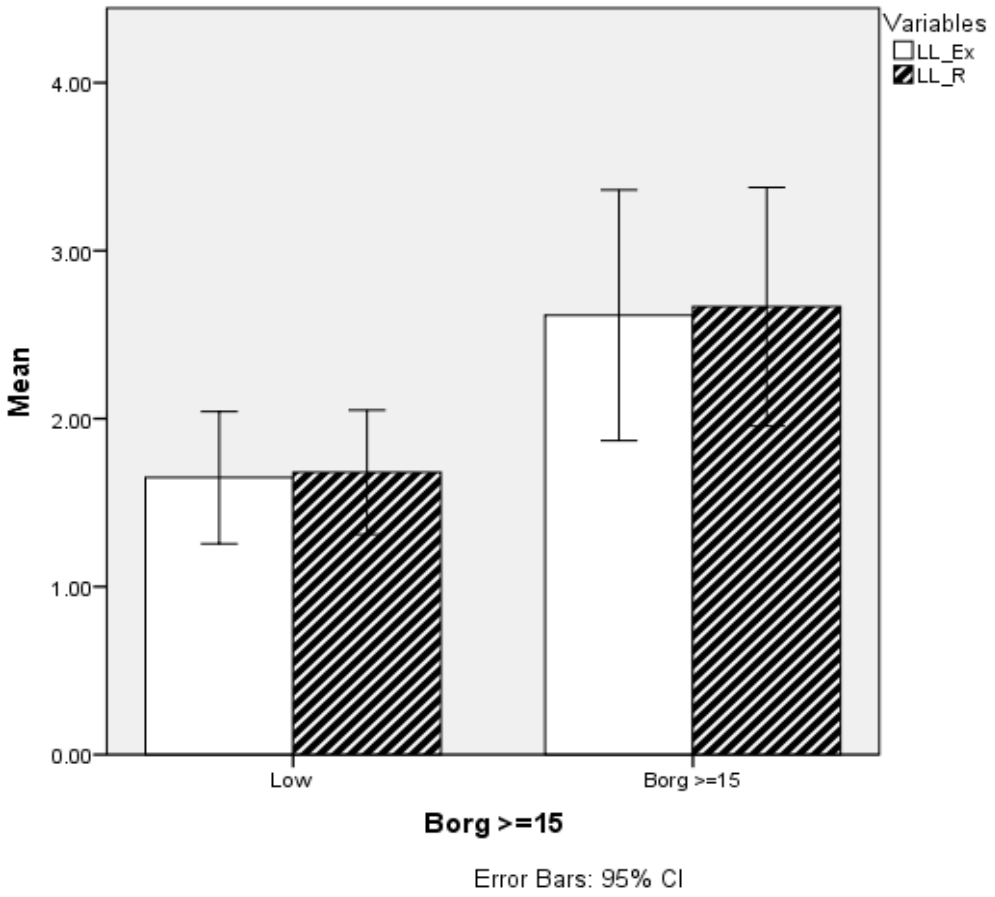


Fig 9.1 Mean LLS scores across the groups with Borg < 15 vs. ≥15

There were 25 recorded Borg RPE scores of 11 or less during the trek; these low scores were associated with an AMS rate of 8% (n=2). This was significantly different to the 110 occasions on which Borg RPE scores of 12 or greater were recorded when the incidence of AMS was 31% (n=34). SpO_2 was significantly lower in those with a high Borg score the morning after the trek 83% +/- 5 (74-91) vs. 86% +/- 5 (66-93) (p=0.022).

The heart rate was significantly higher both on completion of exercise, 94 bpm +/-15 (60-129) vs. 87 bpm +/- 13 (56-120) and at rest 87 bpm +/-14 (57-120) vs. 80 bpm +/- 11 (45 -120) in the group with a high Borg score. Borg score correlated weakly with heart rate both at the completion of exercise (rho 0.181,

p=0.034) and at rest (rho 0.213, p=0.12) across all values.

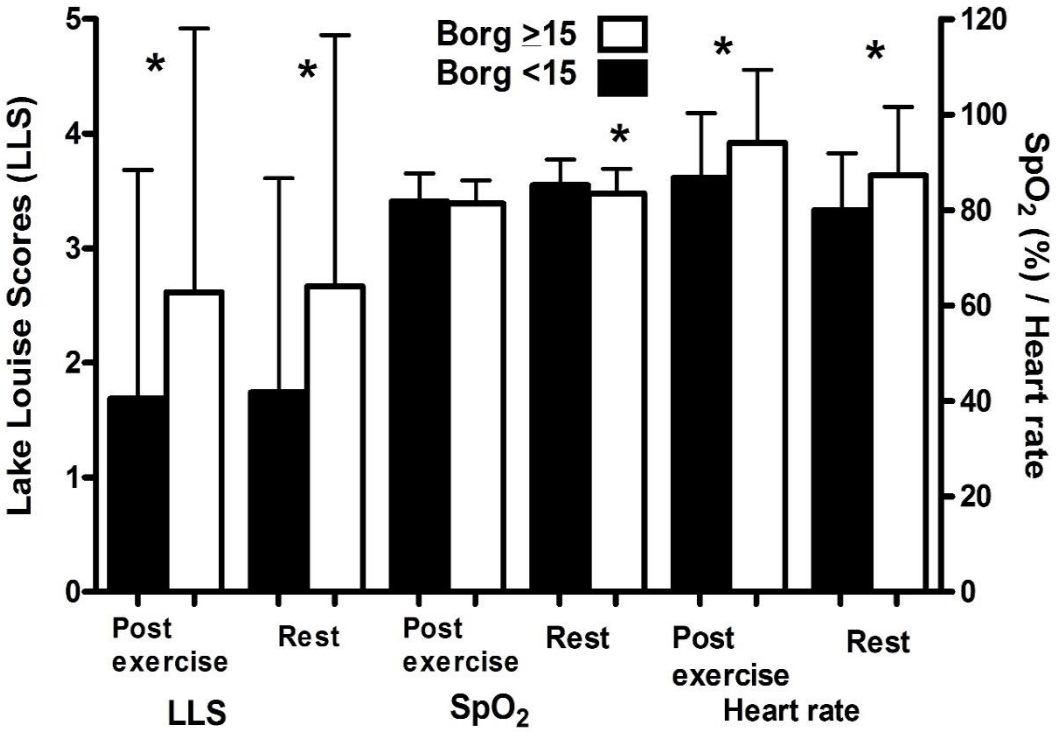


Figure 9.2 values of LLS, HR and Borg RPE score

9.2.3 *Discussion*

This data is the first field study to show a link between the subjects RPE and development of AMS.

One reasons why our data may differ from other studies is that the level of exercise intensity of a “real world” trek was perceived to be high compared to chamber studies. During the field study in Bolivia the mean Borg score for the trek to 3833m was 13.15 (SD 1.92), for the trek to 4450m it was 12 (SD 1.8) and for the trek to 5129m it was 14.98 (SD 1.98). Exercise at 45% altitude adjusted VO_2 max would be expected to correspond to an RPE of 9 - 10. Schommer and Roach’s studies used an exercise intensity of 50% altitude adjusted VO_2 max (Roach, Maes et al. 2000, Schommer, Hammer et al. 2012) whilst Rupp’s subjects cycled at 45% altitude adjusted VO_2 max (Rupp, Jubeau et al. 2013). In our study the number of subjects with a Borg score of 11 or less (which approximates to the exercise intensity’s used in these studies) developing AMS was a third that of those reporting higher scores.

It is interesting that in the study by Roach et al (Roach, Maes et al. 2000), rest in a hypobaric environment produced less AMS than Schommer or Rupp found at rest in normobaric hypoxia despite similar altitude levels and hypoxia (SpO_2 in Rupp's study 83.5 +/- 4.9% at rest and 73.7 +/- 5.7 % at exhaustion, in Roach's 81.4 +/- 1.8% at rest and 76.3 +/- 1.7% with exercise). Equally short bouts of exercise followed by rest, rehydration and a familiar secure environment is very different to the real world where trekking to a new altitude takes all day. Roach's 10 hour chamber study may not have been long enough for AMS to develop and may explain the increase at rest in Schommer's study where the study was carried out over 18 hours. In contrast our study took place during a 10 day trek and some of the changes may have been as a result of the significantly longer period of hypoxia.

The physiological data suggest that the Borg score is meaningful at altitude as those with high Borg had a high heart rate and lower SpO_2 at the end of a period of exercise. Unfortunately we had no way to track changes in heart rate and RPE during exercise. For this reason the correlation of Borg score with HR is lower than would be anticipated although the fact that HR (at the end of exercise) did correlate weakly with the RPE suggests that there was a physiological basis for the higher reported. The association between high RPE and AMS at the end of the trekking day could be anticipated as the effects of exercise may well generate symptoms consistent with a high LLS, however, it is more significant the LLS remained elevated the following morning with associated higher heart rate and lower oxygen saturation.

Using the Borg RPE score during a "real world" exercise stimulus is a logical and attractive proposition. Firstly it requires no specialist equipment and is highly reproducible, at least at sea level (Graham, Atar et al. 2007, Scherr, Wolfarth et al. 2013), secondly, and importantly, multiple other factors can have an impact on perceived exertion during a trek (such as AMS, poor sleep, viral illness etc). This would not alter the work rate required to perform a test at fixed proportion of VO_2 max (even if altitude specific) but would alter perceived exertion and these other conditions may predispose to AMS.

One potential criticism of our data is that the study was not designed specifically to look at the relationship of exercise intensity to AMS and no effort was made to control the pace of individual subjects. The suggestion that increased perceived exertion leads to AMS is intriguing and opens the possibility that controlling the exercise intensity could reduce AMS rates. It could also be postulated that efforts to reduce the perception of exertion per se, through better education, fitness training or reducing anxiety may lead to reductions in AMS, at least as assessed by LLS.

In summary this large field study is the first to link exertion, as recorded using a RPE scale to AMS. Whilst the data should be viewed as pilot data it raises the possibility for using perceived exertion scales as a way to influence behaviour and reduce the incidence of AMS. These results lend support to the view that hard exercise during acclimatisation is detrimental to acclimatisation.

9.3 Study 2 – Comparison of Exercise in 4 Different Environments

Study aims:
The aim of the study was to compare the physiological response to submaximal exercise in the “real world” ie Alpine environment, normobaric and hypobaric hypoxia.

9.3.1 *Methods*

The study was designed as a cross over trial involving 16 subjects recruited from the Defence Medical Services. Subjects were military personnel recruited by word of mouth from medical units based around Catterick.

Environmental modelling:
The following sequence of exercise tests was performed:

1. Sea level maximum exercise test
2. Hypoxic maximum exercise test (NH)
3. Sea level sub-maximal test at 60% max power output (1)
4. Alpine hypoxic sub-maximal test (Torino hut, Italy alt 3370m)
5. Normobaric hypoxic sub-maximal test (see FiO_2 calculation below)

6. Hypobaric hypoxic sub-maximal test (chamber pressure to replicate pressure at Torino hut)

All sub-maximal tests were performed at 60% of power output from hypoxic maximum test (2)

The FiO_2 for the normobaric chamber was calculated by allowing for the saturated vapour pressure of water as follows:

$PiO_2 = (PATM – PH_2O) \times FiO_2$

Participants were screened in accordance with the ACSM guidelines (ACSM)(ASCM's Guidelines for Exercise Testing and Prescription) and were classified as 'low' risk prior to undertaking the study. Each particpant was assessed for Sickle Cell Trait, where haemoglobin carying capacity may be imparied. However, all the participants tested negative. An additional medical examination was required for hypobaric chamber participants in accordance with Royal Air Force policy.

Participants completed two maximal incremental cycle tests to volitional exhaustion to determine their individual maximal workload (W_{max} (Kuipers, Keizer et al. 1987)) and maximal oxygen uptake on a bicycle affixed to a bicycle trainer (Compu Trainer Pro Lab, Racer Mate, USA). The cycle ergometer was calibrated following the manufacturer's instructions. The first test was performed at sea level (FiO_2: 20.9%) with the second test performed a week later during acute exposure to normobaric hypoxia (PiO_2: 95.25 ± 0.28, ~FiO_2: 13.4%, ~3375m). Participants cycled at an initial intensity of 100 watts (W) for 5 minutes, after which the workload was increased by 50 W every 2.5 minutes until a heart rate of 160 beats per minute, after which the work load increased by 25 W every 2.5 minutes to volitional exhaustion. W_{max} was calculated from $W_{max} = W_{out} + (t/150) \times 25$ W in which W_{out} is the highest power output (W) that the participant completed, and *t* the number of seconds the final uncompleted power output was sustained (Kuipers, Keizer et al. 1987). W_{max} was used to

determine the relative exercise intensities to be undertaken by each participant during the experimental trials (viz., power output (W) at a given % W_{max}). Participants started their trials at the same time of day (between 9 am and 2pm) to avoid any influence of circadian variance, following a 12-h fast. After 20 minutes of acute exposure to each environmental condition, oxygen saturation (Nellcor N-20, Covidien, Dublin, Ireland), was recorded. Over the next 10 minutes resting oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) measurements were made using an online gas analysis system (Metalyser, Cortex, Germany). The digital Triple V volume transducer was calibrated using a 3-litre syringe (Hans Rudolph Inc, USA) and the gas analyzers were calibrated using an ambient standard gas mixture of oxygen and carbon dioxide (20.9% O_2 and 0.04% CO_2) and a mass standard gas mixture (Alpha Gravimetric standard, BOC gases, Guildford, UK) of oxygen and carbon dioxide in nitrogen equivalent to expired air (15% O_2 and 5% CO_2). The test-retest reliability for $\dot{V}O_2$ and $\dot{V}CO_2$ ($l.min^{-1}$) had coefficients of variation of 1.3% and 2.4%, respectively.

After a standardized warm up, which included the calibration of the cycle ergometer (Compu Trainer Pro Lab, Racer Mate, USA) to the manufacturer's instructions, participants completed 120 minutes of cycling; 5 minutes at 40% W_{max}, 5 minutes at 45% W_{max}, 5 minutes at 50% W_{max}, 105 minutes at 55% W_{max}. These workloads were calculated from each individuals' sea level and hypoxic W_{max} for the sea level and hypoxic environments, respectively.
Borg RPE score was reported every 15 minutes along with oxygen saturation (Nellcor N-20, Covidien, Dublin, Ireland) and heart rate (Polar® heart rate monitor). The investigator held a card with the 6-20 RPE scale printed on it in front of the subject who pointed to the appropriate value. As some subjects were close to exhaustion by the end of the test (120 minutes) and the test was not always completed for the full duration the RPE at 90 minutes was taken as representative as maximum effort. The decision to use the Borg score at 90 minutes was taken as this is indicative of the maximum exertion before exhaustion and initial investigation showed this as the plateau of the HR values shown in figure 9.3.

Venous lactate was taken from an antecubital fossa vein every 15 minutes. These samples were analysed in house, using enzymatic determination of lactate (Randox Laboratories Ltd) on a semi-automated analyser (ILab 300 plus, Instrumentation Laboratories, UK). The precision of the assay is) is as followed; Within runs 0.81 CV% (13.1 mg/dl) and 0.86 (53.3 mg/dl) and total precision is 5.72% (13.1 mg/dl) and 3.62% (53.3 mg/dl)(from the manufacturers information).

Statistical analysis was carried out using SPSS v 21. The statistical test used is reported with individual values throughout the analysis.

Ethical approval was granted by the MoDREC protocol number 417.

9.3.2 Results

Sixteen subjects were recruited. Two of these did not complete the study due to a low haemoglobin level (1 case) and superficial thrombophlebitis (1 case). The subjects' demographics are shown below:

Age	26 years (21-35)
Height	174.9 cm (157.6 – 189.5)
Weight	72 Kg (157.6 – 86)
Sex	M:F 8:6

Table 9.3 Participant demographics

Only 8 subjects completed testing in the hypobaric chamber due to failure to pass medicals, lack of chamber medical officer and lack of chamber availability.

The FiO_2 set for the normobaric chamber was calculated as shown (table 9.4)

	Alps barometric pressure(mbar)	Alps barometric pressure (mmHg)	**Calculated FiO2**
Participant 1	677.0	507.79	**0.1393**
Participant 2	677.0	507.79	**0.1393**
Participant 3	677.9	508.47	**0.1395**
Participant 4	677.9	508.47	**0.1395**
Participant 5	678.0	508.54	**0.1395**
Participant 6	675.0	506.29	**0.1388**
Participant 7	675.2	506.44	**0.1389**
Participant 8	675.4	506.59	**0.1389**
Participant 9	675.4	506.59	**0.1389**
Participant 10	674.1	505.62	**0.1386**
Participant 11	674.0	505.54	**0.1386**
Participant 12	671.9	503.97	**0.1381**
Participant 14	672.0	504.04	**0.1382**
Participant 16	672.0	504.04	**0.1382**

Table 9.4 Required FiO_2 to provide desired PiO_2 and simulate breathing air at target altitude

Heart rate in different environments

Heart rate data were not normally distributed when a Shapiro-Wilk test was applied and appropriate non-parametric tests for significance were used.

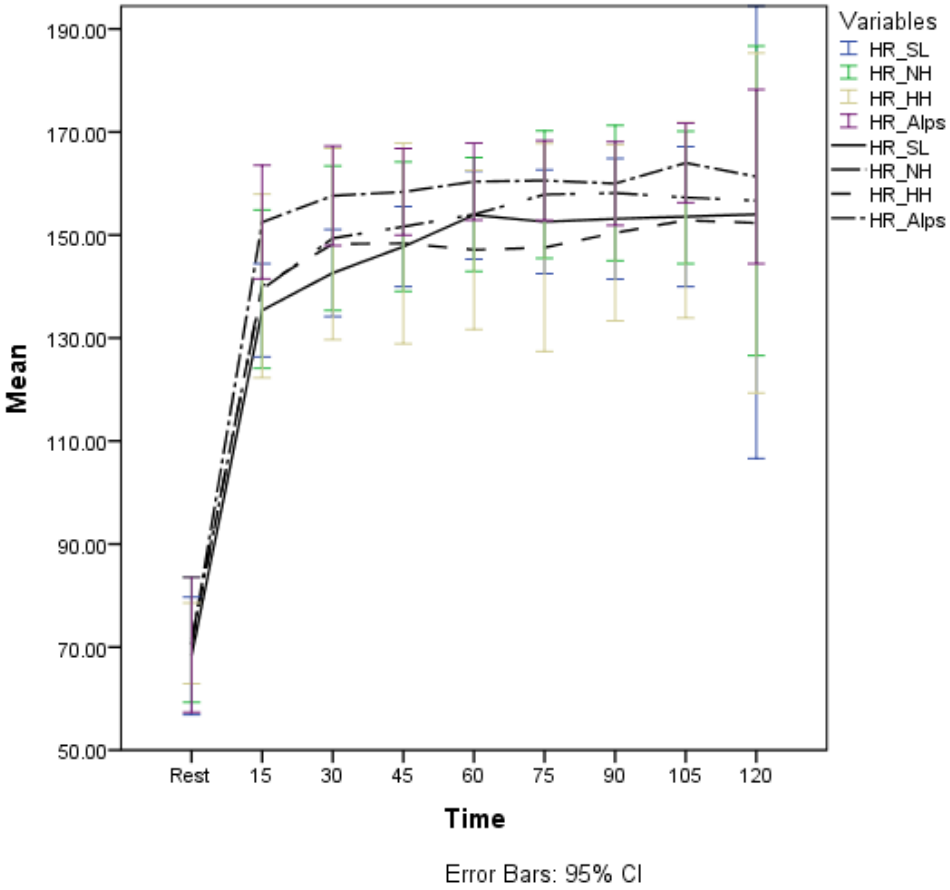


Figure 9.3 Heart rate changes across the 4 environments

Heart rate increased from rest with exercise in all environments, with the exception of HH where the heart rate change at 120 minutes was non-significant, however there were only 3 matched data points for HR120 in HH.

	SL		NH		HH		Alps		P value*
	N		N		N		N		
HR rest	13	67 (11, 51-83)	7	73 (11, 48-91)	8	71 (8, 58-84)	13	74 (13, 52-89)	0.70
HR 60	13	155 (11, 135-175	13	158 (13, 138-174)	8	147 (18, 106-165)	14	162 (9, 146-175)	0.16
HR 90	14	161 (10, 142-174	13	160 (13, 140-176)	7	150 (18, 114-166)	13	154 (14, 129-174)	0.20
HR 120	12	163 (11, 132-178	13	161 (9, 143-175)	4	142 (23, 111-160)	8	164 (11, 144-178)	0.14

Data presented as mean (SD, range) *Kruskal-Wallis test for significance across SL, NH, HH, Alps

Table 9.5 Heart rate values across the different environments

RPE

RPE scores were not normally distributed when the Kolmogorov-Smirnov statistic was applied (statistic 0.113, $p<0.001$).

	SL		NH		HH		Alps	
	N		N		N		N	
RPE	104	12.5 (3.4, 6-20)	104	12.9 (3.4, 6-20)	62	12.2 (4.0, 7-20)	108	13.9 (2.4, 8-18)

Data presented as mean (SD, absolute minimum and maximum reported values)

Table 9.6 Mean RPE scores across all time points in the different environments.

There were no significant differences between the groups (Kruskal-Wallis p=0.538)

Borg correlated with heart rate across all time points (Spearman's rho = 0.419, p=<0.001). This relationship held consistently for the different environments (Alps rho 0.319 p=0.001, NH rho 0.375 p<0.001, HH rho 0.619 p<0.001, SL 0.389 p<0.001).

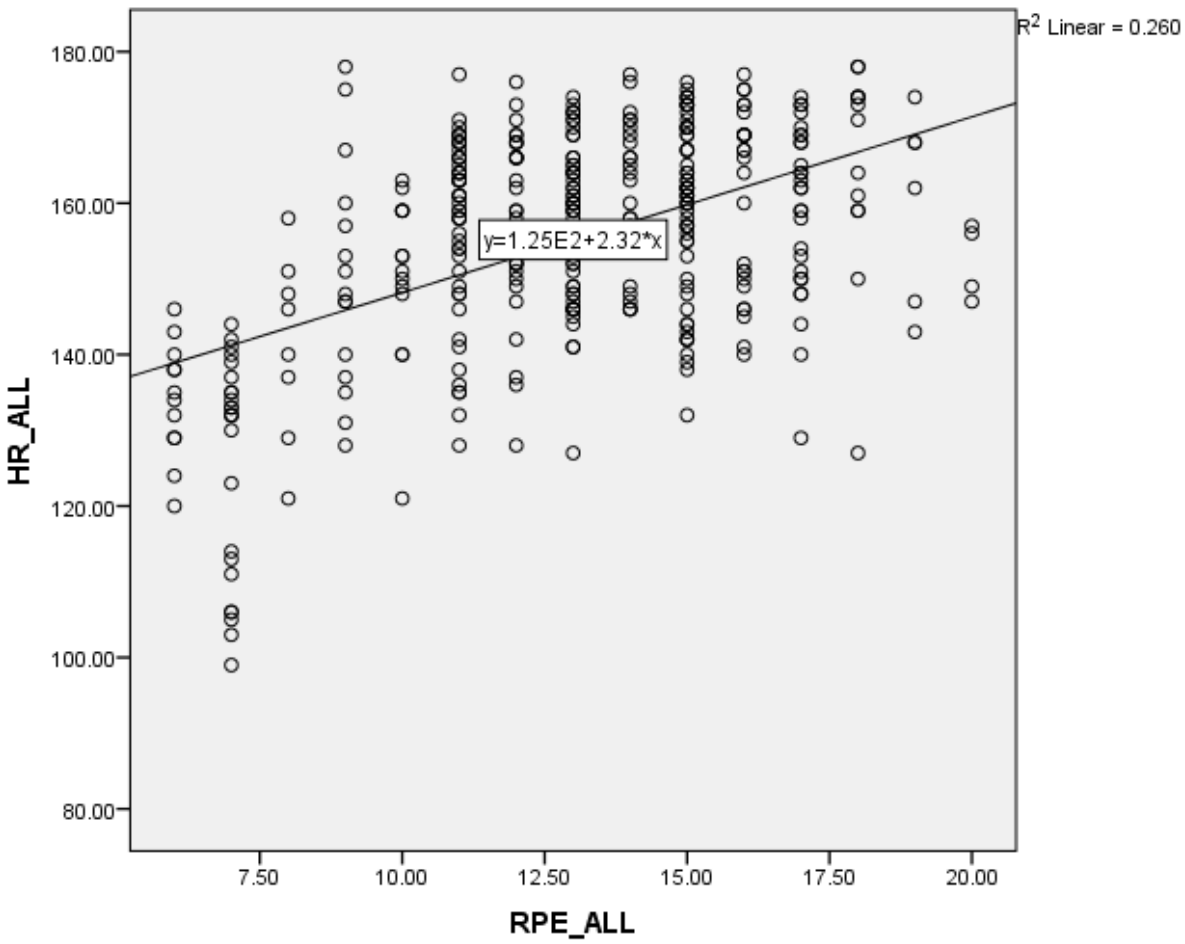


Fig 9.4. Heart rate at all time points vs. Borg RPE (rho 0.419, p<0.001)

There is good correlation between the subject's RPE recorded during a submaximal test at sea level and in each of the other environments (Alps rho = 0.682 p<0.001, NH rho 0.909 p<0.001 and HH rho = 0.861 p<0.001).

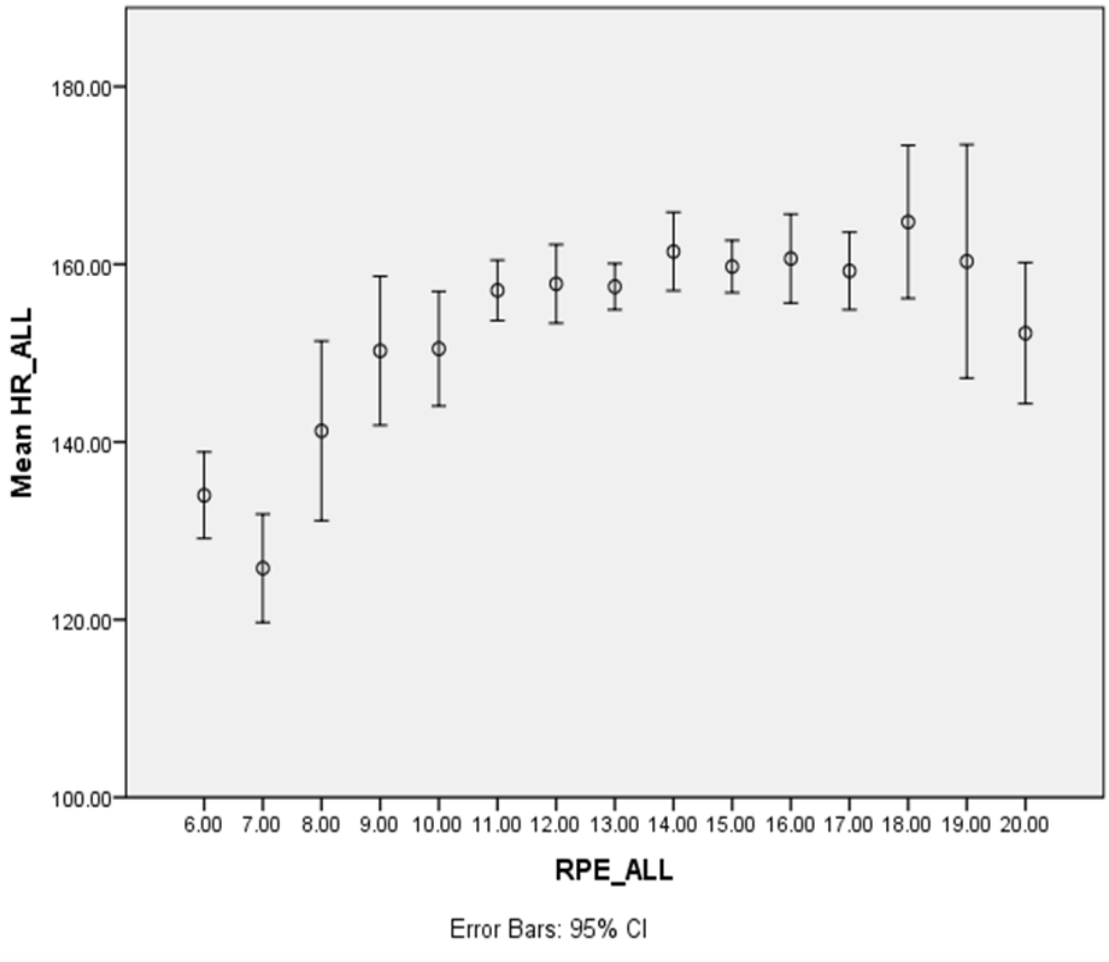


Figure 9.5 Mean heart rate for each recording of RPE.

Using the formula HR = 69.34 + (6.23xRPE) the correlation between actual heart rate and calculated heart rate was rho 0.419, $p<0.001$.

Oxygen Uptake

The oxygen uptake data was normally distributed when tested with the Shapiro-Wilk test and parametric tests were applied.

	SL		NH		HH		Alps		P vs. SL
	N		N		N		N		
VO2 rest	13	0.49 (0.07, 0.35-0.59)	13	0.53 (0.09, 0.39-0.69)	7	0.5 (0.05, 0.42-0.55)	13	0.49 (0.13, 0.33-0.77)	0.564
VO2 60 mins	13	2.50 (0.26 , 2.0-2.86)	13	2.12 (0.27, 1.75-2.47)	7	2.06 (0.27, 1.68-2.51)	14	2.2 (0.35, 1.74-2.88)	0.002
VO2 90 mins	13	2.58, (0.33, 1.88-3.16)	13	2.14 (0.23, 1.71-2.45)	7	2.04 (0.26, 1.67-2.51)	13	2.29 (0.34, 1.81-2.93)	0.001
VO2 120 mins	13	2.78 (0.48, 1.94-3.91)	13	2.14 (0.24, 1.74-2.5)	6	2.0 (0.23, 1.66-2.31)	13	2.29 (0.34, 1.81-2.93)	<0.001

Data presented as mean (SD, range). Note that in the hypoxic environments workload was set at 60% of a hypoxic max test which explains the VO_2 reduction.

Table 9.7 VO_2 changes in the 4 environments

Post hoc analysis (Tukey) showed that the significant difference was between SL and all hypoxic measurements with the exception of $V0_2$ at 90 mins in the Alps (p=0.84).

There was a significant drop in maximum VO_2 achieved in hypoxia when compared to SL with exercise mean+/- sd 46.48 +/- 5.86 vs. 39.28 +/-3.95 mls/Kg/min (Kruskal-Wallis). Work rate was reduced 230.6 +/- 33.8 vs. 205.3 +/- 27.4 Watts but this was not significant (p=0.064)

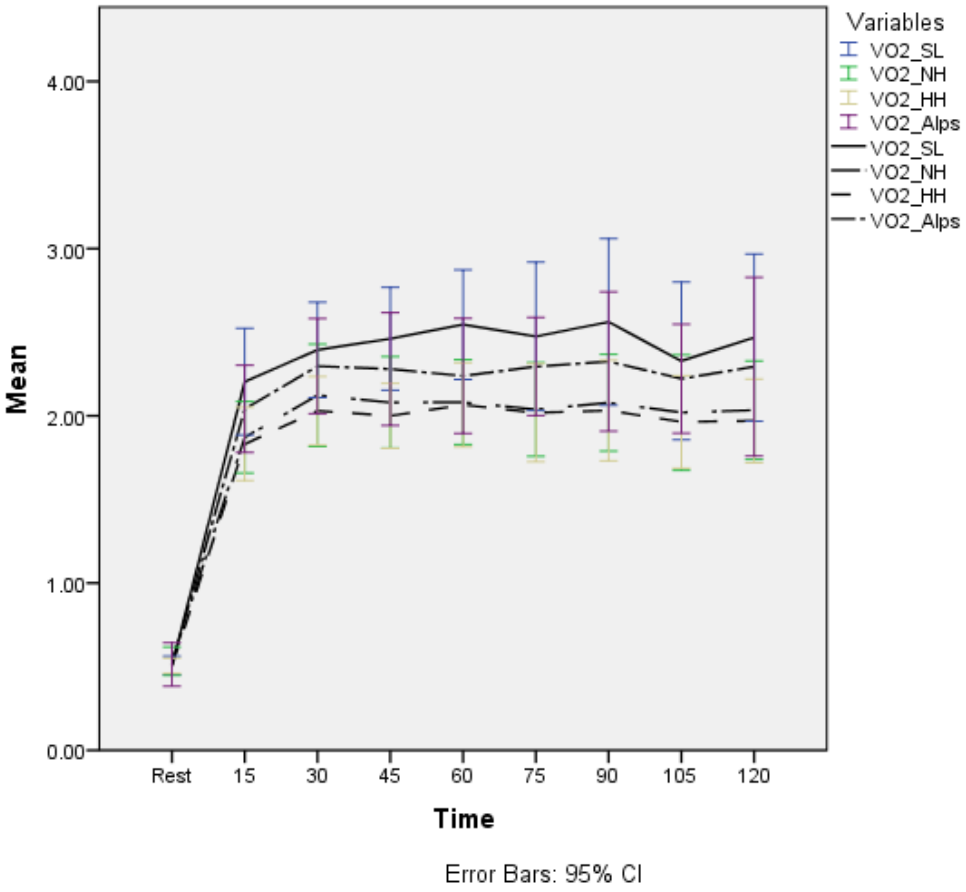


Figure 9.6 Oxygen uptake in the 4 different environments.

Lactate values

Lactate values were not normally distributed at any time point when the Kolmogorov-Smirnov statistic was applied.

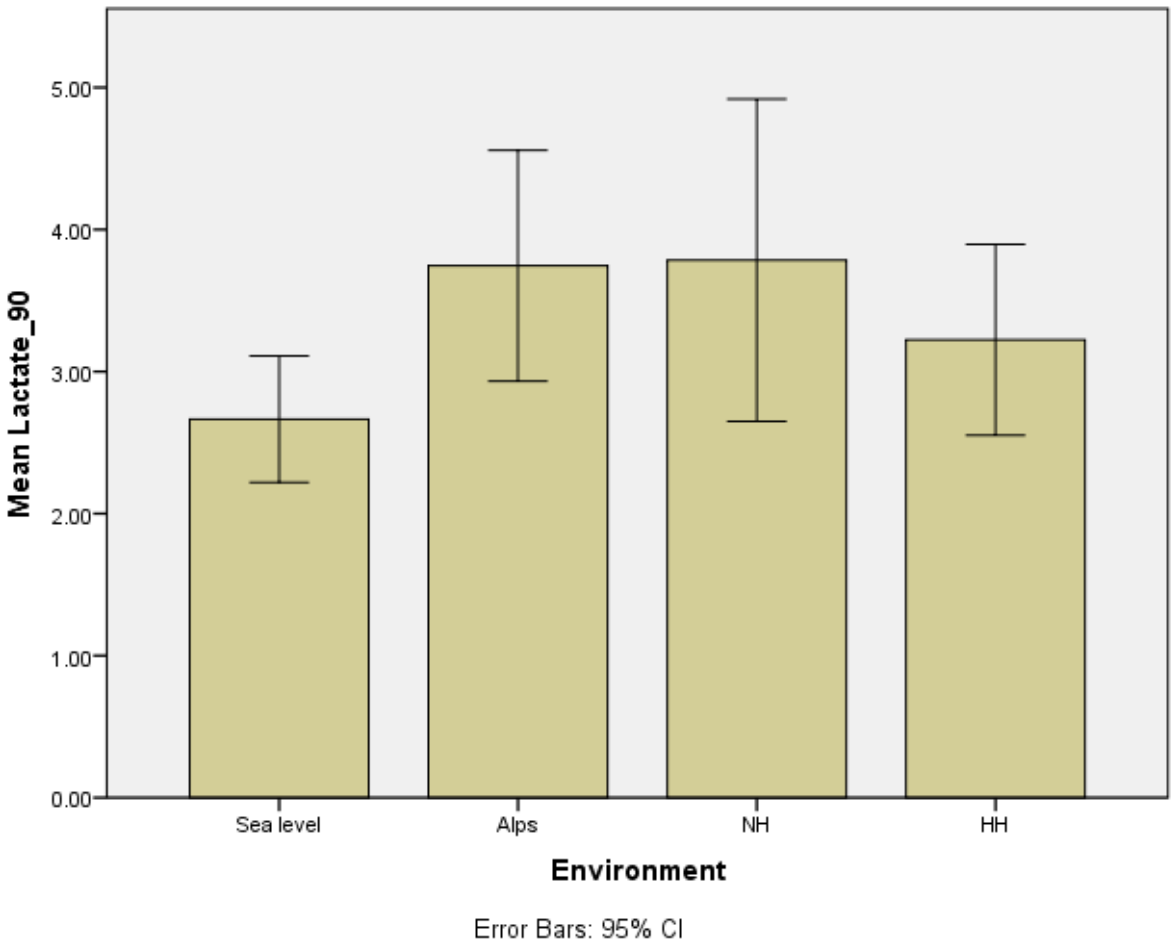


Figure 9.7 Mean lactate values (with 95% confidence intervals shown)

Lactate values at 90 minutes (mean (SD, range) were lower in the sea level environment (mean value at 90 minutes 2.66 mmol/L , 0.71, 1.9-4.0) than in the Alps 3.74 (1.34, 2.0-6.3 p=0.011), NH 3.78 (1.88, 2.18-9.51 p= 0.006) and HH 3.22 (0.80, 2.42-4.77 p=0.069 NS). There were no significant differences between lactate in the NH, HH and Alps environments (p=0.731 Kruskal-Wallis). There was no correlation between lactate and RPE in any environment.

	SL	NH	HH	Alps
Correlation	0.160 (0.139)	0.119 (0.266)	0.274 (0.101)	0.187 (0.071)

Data presented as Spearman's rho (p)

Table 9.8 Correlation between lactate and RPE

Acute Mountain sickness

Lake Louise scores are shown below;

	SL	NH	HH	Alps
Observations	14	14	8	14
Pre-test	0.5 (0.9, 0-3)	1.4 (2.3, 0-9)	0.8 (1.2, 0-3)	1.1 (1.1, 0-3)
15 mins post exercise	0.9 (1.4, 0-4)	2.3 (2.3,0-6)	3.6 (1.2, 0-11)	2.7 (3.1, 0-8)
2 hrs post exercise	0.7 (1.2, 0-4)	1.9 (2.1, 0-6)	2.75 (3.0, 0-8)	2.3 (2.2, 0-7)
22 hrs post exercise				1 (1.6, 0-5)

Data presented as mean (sd, range)

Table 9.9 LLS in each environment

Despite the trend towards higher mean values in the hypoxic environments there was no significant difference in the LLS across the 4 environments (pre-test p=0.407, 15 mins post p=0.170, 2 hours post p=0.112 (Kruskall Wallis). Individual Man Whitney tests showed a significant difference between sea level and the 3 hypoxic environments at both 15 mins post exercise and 2 hours.

Environment	15 mins (p value vs. SL)	2 hrs (p value vs. SL)
Alps	0.048	0.003
NH	0.007	0.017
HH	0.027	0.038

Table 9.10 P value for LLS in hypoxic environments vs. SL (Mann Whitney)

.

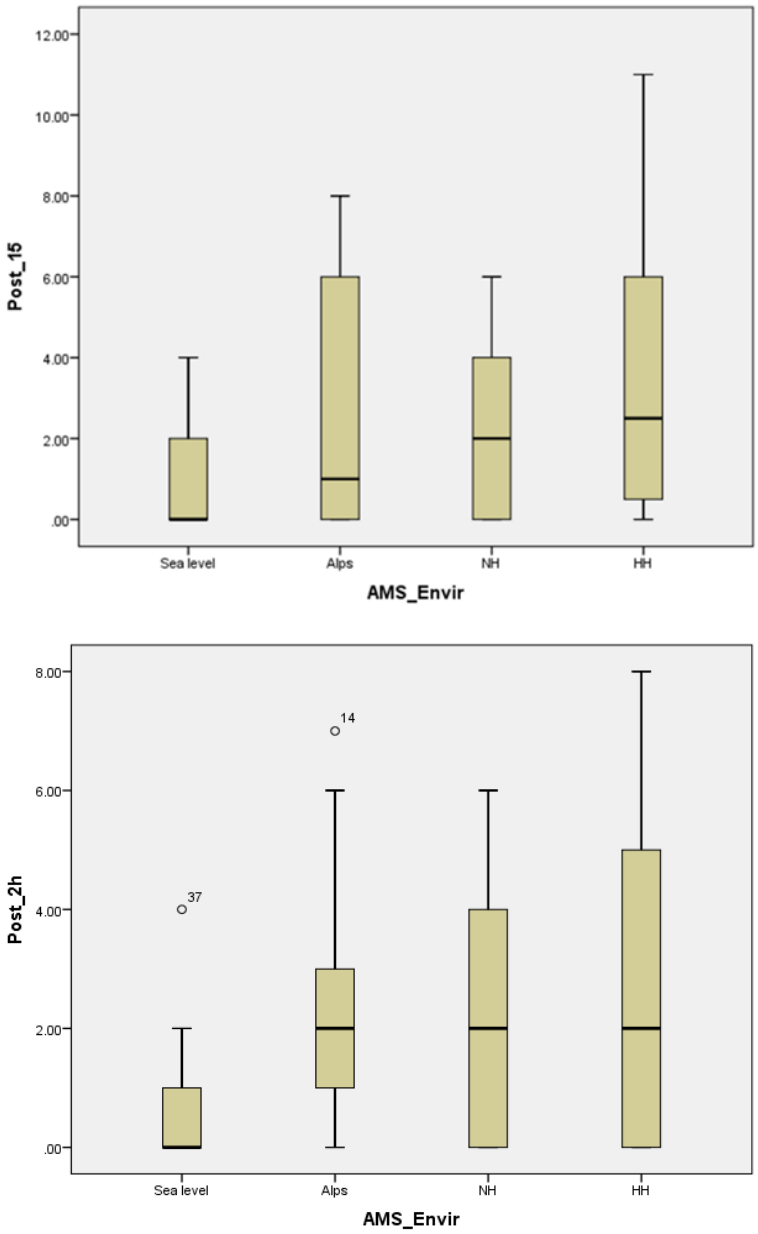
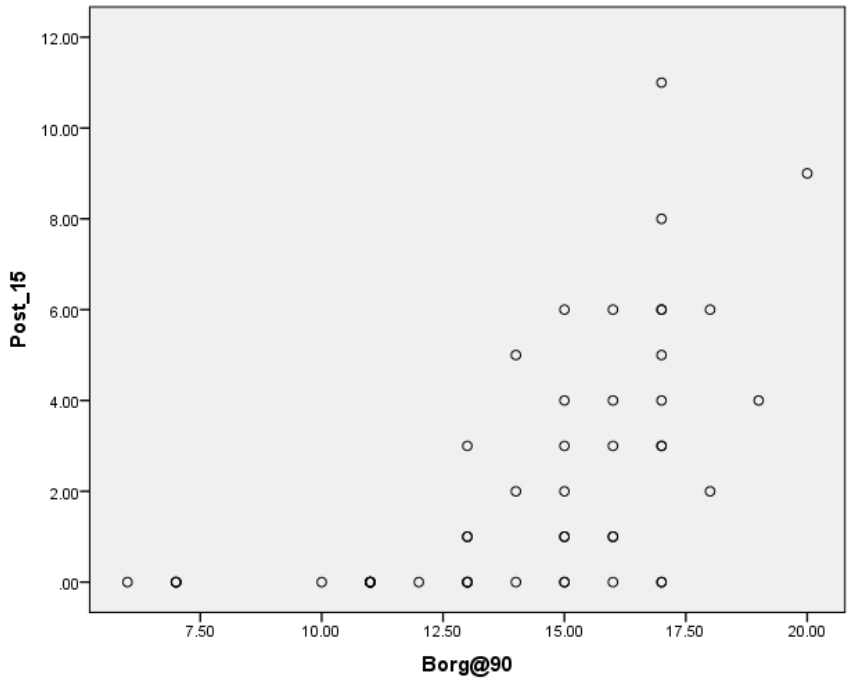


Figure 9.8. Lake Louise scores across the 4 environments at 15 minutes and 2 hours after exercise. The line represents the median, box 95% CI, whisker range

LLS increased significantly over the first 2 hours (Friedmann's Two way analysis of variance by ranks p<0.001), however there was no significant difference between the LLS pre-test and at 22 hours in the alps (p=0.675 Wilcoxon signed rank test). There was a significant difference in LLS at 2 hours when compared to pre-test (p=0.003, Wilcoxon signed rank test).

As there were no significant differences across the 4 environments the Borg scores and LLS were investigated together (n=50).

The Borg score at 90 minutes correlated with the LLS at 15 minutes and 2 hours post exercise (Spearman's rho 0.689, p<0.001 and 0.672, p<0.001 respectively). After 22 hours in the Alps Borg score no longer correlated although numbers were much smaller (n=14) (Spearman's rho 0.098, p=0.738).



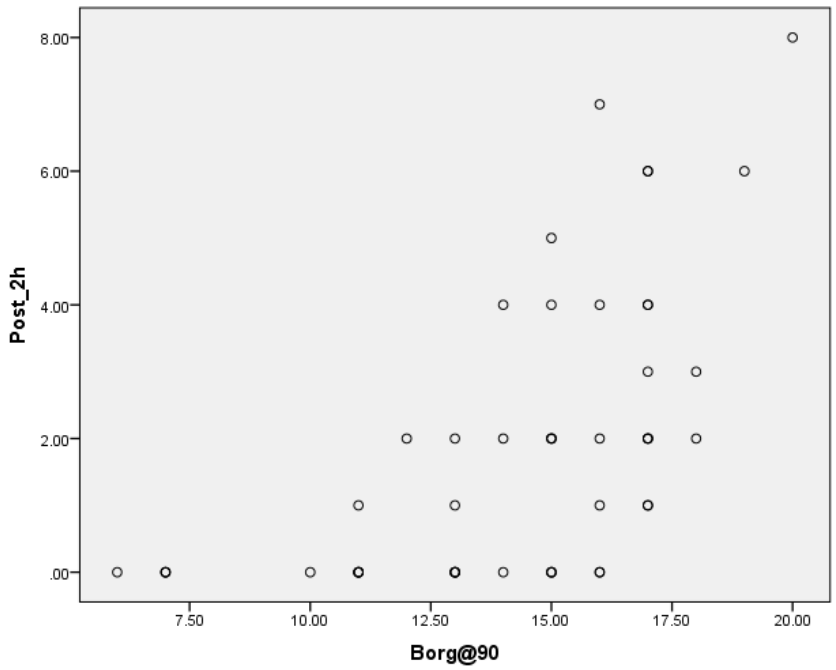


Figure 9.9 Borg scores plotted against LLS at 15 mins and 2 hours post exercise

If Borg scores were split according to the previously used criteria of an RPE ≥ 15 there was an increased LLS in the high Borg group at 15 minutes and 2 hours post exercise ($p<0.001$, Independent samples Mann Whitney U test) (table 9.11).

	Low Borg	High Borg
Observations	20	28
15 mins post exercise	0.6 (1.3, 0-5)	3.5 (2.9, 0-11)
2 hrs post exercise	0.6 (1.1, 0-4)	2.8 (2.3, 0-8)
	4	10
22 hrs post exercise	0.25 (0.5, 0-1)	1.3 (1.8, 0-5)

Table 9.11 LLS divided by Borg RPE post exercise

There were no significant differences in AMS rates amongst the groups. (Pearson Chi squared p=0.441) (Table 9.12)

	2 hrs post exercise				22 hrs post
	SL	NH	HH	Alps	Alps
No AMS	13 (93%)	10 (70%)	5 (36%)	9 (64%)	11 (79%)
AMS	1 (7%)	4 (28%)	3 (21%)	5 (36%)	3 (21%)
Severe AMS	0	1 (7%)	2 (14%)	2 (14%)	2 (14%)
Total	14	14	8	14	14

Table 9.12 Summary of LLS consistent with AMS (3-5) and Severe AMS (>6)

If the rates of AMS and severe AMS at 2 hours post exercise were examined according to the Borg RPE at 90 minutes then there was a significant increase in AMS and severe AMS with increasing Borg score (p=0.001 Kruskall Wallis).

	No AMS	AMS	Sev AMS
Borg@90minutes	13.3 (3.2, 6-18)	16.1 (1.35, 14-18)	17.8 (1.64, 16-20)

Data presented as mean (SD, range)

Table 9.13 Incidence of AMS according to RPE at 90 mins

9.3.3 Discussion

The most important finding of this study is that there was no change in HR, RPE or lactate across 3 environments at well controlled equivalent exercise intensity. Furthermore there were no differences in LLS and a consistent difference in LLS between those reporting a Borg score $\geq$ 15 vs. those reporting lower scores.

Borg RPE seemed to relate to heart rate when looked at in the four environments. Overall numbers were low with a total number of 378 observations in 14 subjects across the 4 different environments (SL plus 3 hypoxic environments) but the relationship between RPE and HR as measured by Spearman correlation was consistent. This is a small number of subjects when compared to Scherr's paper (Scherr, Wolfarth et al. 2013) which comprehensively reviewed 2500 exercise tests, however it confirms the validity of the RPE as reflecting effort. Larger numbers would be required to confirm Scherr's relationship of RPE to HR (HR = 69.34x(6.23xRPE) although the correlation found was statistically significant using that formula.

Decrease in HR at 120 minutes in HH is reported elsewhere with maximal exercise or may just be a function of small numbers (n=4). Lactate values were higher in hypoxic environments (although only significant in NH and Alps (due to increased contribution from anaerobic pathways) . Lactate showed no correlation with RPE which is to be expected in a submaximal exercise test. The mean RPE recorded in this study was around 13 in all 4 environments, this seems higher than the figures used in the Borg score in which 60% maximum effort should equate to an RPE of 11. One reason for this disparity may be that our subjects were not trained cyclists and some of the rating was exercise specific or related to discomfort of being in the saddle for 2 hours. Other authors have suggested that exercise modality does have a bearing on the RPE recorded (Chen, Fan et al. 2002). Lactate showed a marked rise in the hypoxic environments due to a greater contribution from anaerobic metabolism. Although it is generally accepted that lactate levels do not rise in acclimatised individuals at extreme altitude, the "lactate paradox" (West 2007), this is not the case in this relatively short term hypoxic stimulus.

VO2 max fell in hypoxia by 15.5% this is in keeping with other literature and has been recorded since studies as early as 1906 (Ward, Milledge et al. 2000) (Chapter 11, section 9). Although there was a trend towards a fall in work rate this was not statistically significant and HR remained the same. This suggests that work rate remains the same for a given heart rate but consumes less oxygen at HA. This finding has been noted before by Pugh in 1964 who found that oxygen consumption at maximal heart rate at altitude was reduced compared to sea level values, suggesting for the given work rate much was achieved using anaerobic respiration (Pugh, Gill et al. 1964).

LLS did not differ between the 4 environments is surprising. This study was not designed to investigate AMS in isolation as the numbers where small and exposure an inadequate length of time, however, interesting conclusions can be drawn. The decision to include sea level data in the analysis is based on investigation whether exercise can bring on the symptoms of acute mountain sickness, as it can be argued that the study duration was too short to provoke true AMS. One of the obvious problems is using the LLS which has 20% of the score allocated towards previous nights' sleep. Our research is not unique in using the score for studies that do not involve an overnight sojourn at HA and it is an obvious criticism. The other explanation is that whilst there was a clear trend towards higher LLS scores in hypoxia numbers where insufficient to make a statistically significant result. The LLS did increase over the first two hours post exercise however, by 22 hours there was no significant difference in LLS when compared to pre-test values (although 3 subjects reported LLS scores consistent with AMS vs. no AMS in pre-test scoring). Part of this may be the small numbers involved in that LLS could only be measured after 22 hours hypoxia in the Alps. These finding have important implications for the timing of LLS in future studies and may be one reason why the study of Roach found an increase in AMS after exercise (over 10 hours) which contrasted with the results of Rupp and Schommer (11 and 18 hours respectively).

As in the Bolivia field study an increased Borg score correlated with increased AMS. The decision to use the Borg score at 90 minutes was taken as this is indicative of the maximum exertion before exhaustion and is on the plateau of the HR values shown in figure 12.1. The RPE recorded at this point correlated

with the LLS at 15 minutes and 2 hours post exercise. This suggests that the recording of a high LLS is not just reflecting the exertion of the submaximal test as by 2 hours recovery could be expected to have taken place. Overall the reporting of LLS consistent with AMS were the same across the 3 environments and consistent with other published data (13% at 3050m and 34% at 3650m (Barry and Pollard 2003)).

There are a number of weaknesses in the study. Firstly the numbers were lower in hypobaricity. This reflects the difficulties of performing studies in this environment which was a major driver to conduct the study in the first instance. Reasons for lower numbers included failure of subjects (and the independent medical officer) to pass the fitness criteria due to an inability to clear ears and subject availability for what became a prolonged trial. Neither of these factors could have been avoided. Secondly, as part of a parallel study, fluid balance was measured and fluid (in the form of a carbohydrate drink) administered. A total of 2.5 litres of this drink was ingested during the cycle which may have led to transient nausea although this is likely to have subsided by the time LLS were recorded 15 minutes and 2 hours post exercise.

9.4 Conclusions

The field study in Bolivia suggested exercise as measured by the Borg RPE score seems to be predictive of those developing AMS.

Chamber studies showed no major physiological difference noted between 3 hypoxic environments (NH, HH, alps) environments in terms of lactate, heart rate or oxygen uptake.

Across all four environments LLS and AMS are rates the same which suggests it is the exercise stimulus that cause the symptoms of AMS rather than altitude per se over these short periods of time in laboratory studies. For this reason timing of LLS recording is critical and a lack of recovery from bouts of exercise may be pivotal in generating altitude illness.

Chapter 10 – Discussion on significance of findings

10.1 Introduction

During a climbing trip in the Khumbu region of Nepal in 2014 I climbed Island Peak, guiding a group of novices with a very experienced military coleague, SM. SM had acclimatised well, despite a chest infection earlier on in the trip and we both felt strong whilst leading our groups from base camp at 5100m up to the summit of the peak at 6189m. After a brief period on the summit we returned to base camp (a 12 hour day) to be well fed and drink plenty of fluids. SM exhibited no signs of altitude illness or undue exhaustion. The following day SM was well but I noticed a significant amount of periorbital oedema. That morning we descended and over the first 400m of the descent (taking about 90 minutes) SM and I each stopped to pass urine on six different occasions passing far in excess of the half dozen cups of tea we had drunk that morning. Within a two hour period his periorbital oedema had cleared. This acedote highlights my hypothesis and motivation for the initial part of this MD work. Perceived wisdom is that a "climb high sleep low" strategy is the best for acclimatisation. Had we been climbing a higher mountain an ascent to 6000m, as on Island Peak, would have been seen as good acclimatisation and been followed (usually the following day) by an ascent to a similar altitude to sleep. What I observed was that, in the climb of Island Peak, physiological stress caused SM to overide his altitude induced suppression of AVP, a process which required the recovery of returning to less stressful altitudes and diuresis to restore "normality". Had he reascended the following day, despite being well, he may well have started the viscious cycle of stress, fluid retention and formation of tissue oedema and high altitude pathology. This is of course conjecture but is in part borne out by the research. I recorded supression of AVP and copeptin with exercise at HA relative to SL, a change in copeptin and AVP with more strenuous exercise and a change in LLS with strenuous exercise. The missing link was to show a link between AVP levels and AMS or fluid retention, potential weaknesses in this part of the study will be considered later in this chapter.

In a recently book reviewing the contribution of the physiologist Griff Pugh to the success of the 1953 Everest expedition the author, his daughter Harriet Tuckey, refers to Pugh's ascertion that recovery is important during high altitude expeditions (Tuckey 2013) and the, then novel, guidelines he put in place during the expeditions he was involved in. None of the quotes from Pugh's work

mention acclimatisation per se but rather recovery. I think this is an important distinction – I would argue that SM had acclimatised to the 6000m altitude but the following day had not recovered placing him at risk had he ascended further. There are many other interesting insights into the previous history of high altitude medicine and physiology in this book including Pugh's belief that climbers should drink liberally during ascent. I have often wondered where the evidence comes from for this as contraction of plasma volume is an important part of adaptation. During the early expeditions to high mountains stove technology was practically incapable of melting significant amounts of snow for water at high altitudes, Pugh pushed hydration as many mountaineers came down from summit attempts severely dehydrated exacerbating frostbite and exhaustion such as Moreshead and Somervell during the 1922 Everest expedition or Tenzing and Lambert on Annapurna melting snow with a candle! I believe maintaining normal hydration was Pugh's goal. Before the Second World War any application of science was seen as at odds with the gentlemanly nature of mountaineering as "sport" and began to smack of professionalism (training was equally frowned upon). In a presentation to the Alpine Club in 1924 Leslie Stephen (the Club's President) stated "true alpine traveller's loved the mountains for their own sakes and considered scientific intruders with their barometers and theorising to be a simple nuisance".

10.2 Personal Reflection

This MD has been 3 years in completion but really started with my first field study in 2007. Each study has seen a step up in complexity through basic physiological data collection, to point of care monitoring systems through to generators, echo machines and freezers. This has given me great insights into data collection and the pros and cons of this field study is considered later. Even throughout the writing period, preparation of manuscripts for submission has made me consider data presentation and the format of tables and graphs throughout the thesis. Some I have made changes whilst some remain in their orginal form and as I think the evolution is interesting to see. I am indebted to my colleagues Dave Woods and Chris Boos for their help and advice in this. Overall I have benefited hugely in terms of my grasp of academic concepts, ability to critically appraise other work and communicate with others.

Field studies of this nature are a challenge, not just in the reseach methods but in maintaining collaborations, relationships with other investigators and, perhaps most importantly, with the subjects themselves who had to endure some pretty gruelling regimens.

To date six papers have been published as a result of this work with more in the pipeline (Boos, Holdsworth et al. 2013, Boos, Holdsworth et al. 2014, Mellor, Boos et al. 2014, Mellor and Woods 2014, Mellor, Boos et al. 2014, Mellor, Woods et al. 2014). I have also had the oportunity to present findings within the military and to wider audiences at national meetings.

10.3 Study design

Field studies of this nature are complex to design and difficult to control. There are a number of potential short comings with the data collected in Bolivia. These are discussed below;

10.3.1 *Baseline data collection*

Data collection during the near sea level trek in Wales could only be collected on a small sub group of subjects. The main reason for this is that the main field study required participation over a three week period and many of the subjects did not feel able to commit a futher weekend to the study. This meant that data for baseline AVP, copeptin, BNP, catecholamines and cortisol was only available for 20 (2 of whom did not take part in any further analysis). For this reason repeat measures statistics could not be used on some of the results and group means or other appropriate tests had to be used to investigate the significance of changes.

10.3.2 *Lack of control of activity*

There was a lack of control of individuals activity on the trek. Whilst trekking distances etc were common to the whole group, some individuals made other treks to surrounding view points for acclimatisation purposes or photography. It was not considered ethical to restrict this when an individual's financial contribution was key to financing the expedition. The impact of this on the overall results would have been minimal although it may have resulted in improved acclimsatisation in some individuals.

10.3.3 *Period of time at new altitude*

The only way to perform this study was judged to be using the trek as the exercise component. This created a fundamental problem in that the “rest” day values reflected an increase in time at the new altitude as well as rest. The only way around this would have been to have performed a predetermined exercise test at the new altitude (such as a step test or further trek) during the “rest” day. This would have been difficult for a number of reasons. Most importantly subjects are unlikely to have tolerated several hours of exercise which was the intended duration i.e. a real world trekking stimulus. Effectively removing the rest day would have had an impact on the acclimatisation profile of the expedition and would have been ethically challenging. In this respect field studies cannot be as provocative for AMS as laboratory studies due to the potential risk of both the disease and the hazards of rescue. Performing a calibrated exercise test may have meant more complex equipment and added to the logistic burden. The impact of this would be small overall and the results reflect what happens in the “real world” scenario which was always the intention.

10.3.4 *Physiological Data Collection*

The majority of the team were medical (doctors, nurses and allied health professionals). The team members collected data on each other to assist the research team. This may have led to some inaccuracy and ideally data collection should have been blinded from the subjects. In particular, resiratory rate was not well collected.

10.3.5 *Borg Score Was Collected Retrospectively*

The Borg RPE was collected retrospectively at the end of the trekking day. This was done on completion of the activity and had the advantage of giving one value to define the perceived exertion of the trek. It would have been interesting to have a peak heart rate or nadir of saturation during the trek to correlate with and validate the Borg score. There is also the possibility that those individuals who finished the trek and began to feel unwell would automatically record a higher RPE than those feeling well which would lead to

higher RPE scores in those with AMS without, necessarily, demonstrating effect.

10.3.6 *Three way environment study*

This study, by nature of design, was easier to control and make direct comparisons. The main short coming of this study is that 2 hours of cycling a static bike is a significantly difficult activity, regardless of intensity, for those not used to racing bike saddles. One cannot rule out a learning effect or perhaps an anticipatory effect. This may have influenced Borg RPE scores or heart rates. During the study in the Alps the group were camped at around 1400m, this could have an an unknown effect on the physiology of those later in the study.

10.3.7 *Lake Louise Score*

As mentioned early in the thesis no “gold standard” exists for the diagnosis of AMS. Recent work published has looked at the evolution of symptoms for subjects in a 10 hour chamber study at 4800m (Burtscher, Wille et al. 2014). This paper tends to suggest that there are two distinct clusters of symptoms which is further supported by some retrospective analysis from Hall and the Edinburgh study group (Hall, Maccormick et al. 2014). The chamber study may provide a blueprint for future work and would be a particularly useful way to investigate the role of AVP/copeptin and relationship to the development of symptoms of AMS.

10.4 Important Conclusions For High Altitude Medicine And Research

Perhaps the most important conclusion from the Boliva field study is that exercise has a profound influence on physiological parameters with Norepinephrine, Cortisol and Copeptin showing a split between those with a low perceived exertion and those individuals who recorded a higher RPE. This helps to explain some of the inconsistencies in other published work.
One interesting finding was that two similar ascent profiles in two different countries can lead to different rates of AMS (figure 10.1).

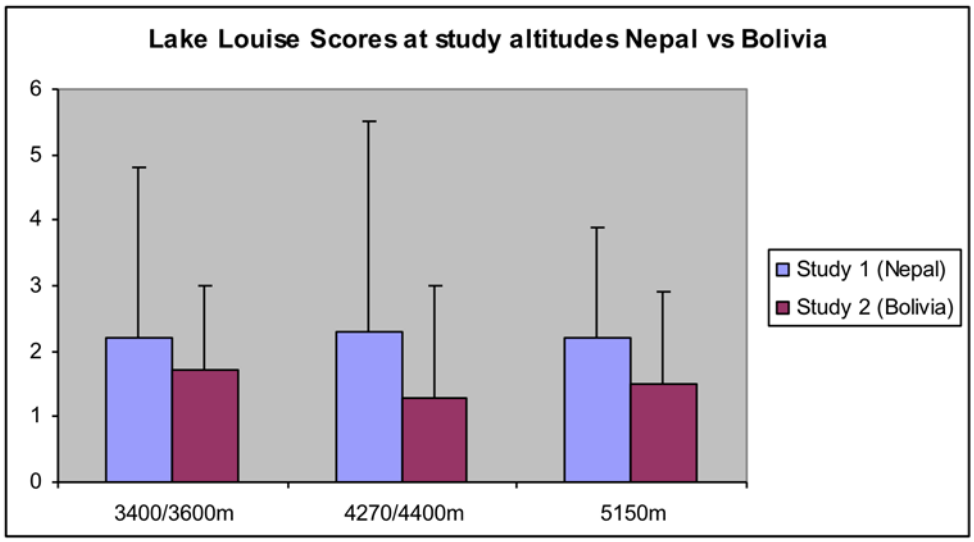


Figure 3 Lake Louise scores at the main study altitudes in study 1 vs study 2. RM ANOVA revealed a significant rise in LL score with ascent ($p<0.001$) with LL scores higher overall in study 1 than 2 ($p=0.047$).

Figure 10.1 Differences in LLS in Bolivia and Nepal

This may also explain differences in the data from other field studies. On the face of it the ascent profile in Bolivia (flying in to 4061m) would be judged to be more provocative for AMS than the trek to 3500m in Nepal, but this does not seem to have been the case. Exercise has been considered above as a potential difference. One other difference, not considered in other studies, is the potential difference in anxiety. On arrival in La Paz one is in a comfortable hotel, eating in restaurants and familiar surroundings. At a corresponding altitude in Nepal the expedition had had 2 nights under canvas and was eating in a cold mess tent. Anxiety in itself is a very important consideration for those planning future studies and expeditions. Missoum reported that trait-anxiety and anxiety prior to an ascent is associated with higher AMS rates on an expedition to the Dhaulagiri area in 1992 (Missoum, Rosnet et al. 1992). Many anxiety scores exhibit symptoms in common with the LLS. This has two important implications. Firstly, future studies should record anxiety concurrently with AMS scores and secondly if anxiety can be controlled and manipulated can self reported AMS be reduced? It also stands to reason that studies of climbers on technical routes cannot be compared to those exposed to HA for the first time or those with experience on a non-demanding trek. Pre-existing anxiety

traits may have an impact on catecholamine levels at higher exercise intensities (Peronnet, Blier et al. 1986) and in turn, catecholamine levels may influence respiratory rate, heart rate and therefore SpO_2 and potentially BNP, cortisol or AVP.

One other possible confounder is that some of the LLS score difference could be accounted for by caffeine withdrawal headache. This is more likely to have been the case in Nepal rather than Bolivia where good coffee was available until a later stage in the expedition.

Many studies cited in this thesis are less than 24 hour duration notably (the exercise chamber studies, some AVP ones etc). These studies all quote LLS as a diagnostic test for AMS. None of the authors account for how subjects answer the question about sleep. The assumption is that it will be the same throughout but clearly sleep the night before is not influenced by hypoxia in a chamber the following day and the sleep component for the LLS accounts for 20% of the numerical score. Thus a subject who had slept poorly the night before could easily be diagnosed with mild AMS with only a slight headache. Once again this highlights the need for a biomarker as a diagnostic test for AMS.

10.5 Implications For The Military

There are a number of potential considerations for the military from the findings of this research.

10.5.1 Setting Strategies For Pre-acclimatisation

Future military work will focus on strategies to “pre-acclimatise” troops prior to high altitude deployment. Part of this will involve a period of pre-acclimatisation in a chamber environment. The results of the three environment study suggest that normobaricity can provide as great a physiological stress as hypobaric hypoxia at least when exercise is conducted. Monitoring of the exercise stress can also be carried out using Borg RPE, avoiding pushing the subject beyond a Borg RPE of 15 to allow physiological stress but adequate recovery. The use of BNP as a marker also enables the degree of exercise and hypoxia to be

individually assessed to create a response but allow resolution between exposures.

10.5.2 *Consideration Of Anxiety*

Mental strategies to deal with the anticpated effects of anxiety could be useful to reduce any cross over between AMS and anxiety. This would be improved by exercise in hypoxia but could be augmented with visualisation techniques in line with military research into virtual environments for analgesia.

10.5.3 Future Collaborations

A key part of this work has been to develop and cement collaboration with Leeds Beckett University with their expertise in sports science and access to a wide range of cardiopulmonary testing and environmental chamber.

10.6 Future Research

This work has led to a range of ongoing projects to investigate links between AMS and anxiety, strategies for pre-acclimatisation and the relationship between Borg score and exercise during hypoxia.

Bibliography

Publications as a direct result of work for this thesis;

Boos, C. J., D. A. Holdsworth, D. P. Hall, A. Mellor, J. O'Hara and D. R. Woods (2014). "Comparison of two methods of assessing total body water at sea level and increasing high altitude." Clin Physiol Funct Imaging 34(6): 478-484.

Boos, C. J., D. A. Holdsworth, D. R. Woods, K. Green, J. Naylor and A. Mellor (2013). "Cardiac biomarkers and high altitude pulmonary edema." Int J Cardiol 167(3): e65-66.

Mellor, A., C. Boos, D. Holdsworth, J. Begley, D. Hall, A. Lumley, A. Burnett, A. Hawkins, J. O'Hara, S. Ball and D. Woods (2014). "Cardiac Biomarkers at High Altitude." High Alt Med Biol.

Mellor, A. and D. Woods (2014). "Physiology studies at high altitude; why and how." J R Army Med Corps 160(2): 131-134.

Mellor, A. J., C. J. Boos, S. Ball, A. Burnett, S. Pattman, M. Redpath and D. R. Woods (2014). "Copeptin and arginine vasopressin at high altitude: relationship to plasma osmolality and perceived exertion." Eur J Appl Physiol.

Mellor, A. J., D. R. Woods, J. O'Hara, M. Howley, J. Watchorn and C. Boos (2014). "Rating of Perceived Exertion and Acute Mountain Sickness During a High-Altitude Trek." Aviat Space Environ Med 85(12): 1214-1216.

References cited

American College for Sports Medicine Guidelines for Exercise Testing and Prescription, 9th Edition 2013.

Anand, I. S., Y. Chandrashekhar, S. K. Rao, R. M. Malhotra, R. Ferrari, J. Chandana, B. Ramesh, K. J. Shetty and M. S. Boparai (1993). "Body fluid compartments, renal blood flow, and hormones at 6,000 m in normal subjects." J Appl Physiol (1985) **74**(3): 1234-1239.

Anton, A. H. and D. F. Sayre (1962). "A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines." J Pharmacol Exp Ther **138**: 360-375.

Appenzeller, O. and S. C. Wood (1992). "Peptides and exercise at high and low altitudes." Int J Sports Med **13 Suppl 1**: S135-140.

Armstrong, L. E. (2007). "Assessing hydration status: the elusive gold standard." J Am Coll Nutr **26**(5 Suppl): 575S-584S.

Baggish, A. L., R. R. van Kimmenade and J. L. Januzzi, Jr. (2008). "Amino-terminal pro-B-type natriuretic peptide testing and prognosis in patients with acute dyspnea, including those with acute heart failure." Am J Cardiol **101**(3A): 49-55.

Bailey, D. M., B. Davies, I. S. Young, D. A. Hullin and P. S. Seddon (2001). "A potential role for free radical-mediated skeletal muscle soreness in the pathophysiology of acute mountain sickness." Aviat Space Environ Med **72**(6): 513-521.

Bailey, D. M., K. A. Evans, P. E. James, J. McEneny, I. S. Young, L. Fall, M. Gutowski, E. Kewley, J. M. McCord, K. Moller and P. N. Ainslie (2009). "Altered free radical metabolism in acute mountain sickness: implications for dynamic cerebral autoregulation and blood-brain barrier function." J Physiol **587**(Pt 1): 73-85.

Bailey, D. M., G. R. Kleger, M. Holzgraefe, P. E. Ballmer and P. Bartsch (2004). "Pathophysiological significance of peroxidative stress, neuronal damage, and membrane permeability in acute mountain sickness." J Appl Physiol (1985) **96**(4): 1459-1463.

Bailey, D. M., G. R. Kleger, M. Holzgraefe, P. E. Ballmer and P. Bartsch (2004). "Pathophysiological significance of peroxidative stress, neuronal damage, and membrane permeability in acute mountain sickness." J Appl Physiol **96**(4): 1459-1463.

Baker, L. B., J. A. Lang and W. L. Kenney (2009). "Change in body mass accurately and reliably predicts change in body water after endurance exercise." Eur J Appl Physiol **105**(6): 959-967.
Bakonyi, T. and Z. Radak (2004). "High Altitude and Free Radicals." J Sports Sci Med **3**(2): 64-69.
Balanescu, S., P. Kopp, M. B. Gaskill, N. G. Morgenthaler, C. Schindler and J. Rutishauser (2011). "Correlation of plasma copeptin and vasopressin concentrations in hypo-, iso-, and hyperosmolar States." J Clin Endocrinol Metab **96**(4): 1046-1052.
Barnholt, K. E., A. R. Hoffman, P. B. Rock, S. R. Muza, C. S. Fulco, B. Braun, L. Holloway, R. S. Mazzeo, A. Cymerman and A. L. Friedlander (2006). "Endocrine responses to acute and chronic high-altitude exposure (4,300 meters): modulating effects of caloric restriction." Am J Physiol Endocrinol Metab **290**(6): E1078-1088.
Barry, P. W. and A. J. Pollard (2003). "Altitude Illness." BMJ **326**(7395): 915-919.
Bartsch, P., D. M. Bailey, M. M. Berger, M. Knauth and R. W. Baumgartner (2004). "Acute mountain sickness: controversies and advances." High Alt Med Biol **5**(2): 110-124.
Bartsch, P., M. Maggiorini, M. Ritter, C. Noti, P. Vock and O. Oelz (1991). "Prevention of high-altitude pulmonary edema by nifedipine." N Engl J Med **325**(18): 1284-1289.
Bartsch, P., M. Maggiorini, W. Schobersberger, S. Shaw, W. Rascher, J. Girard, P. Weidmann and O. Oelz (1991). "Enhanced exercise-induced rise of aldosterone and vasopressin preceding mountain sickness." J Appl Physiol **71**(1): 136-143.
Bartsch, P., H. Mairbaurl, M. Maggiorini and E. R. Swenson (2005). "Physiological aspects of high-altitude pulmonary edema." J Appl Physiol (1985) **98**(3): 1101-1110.
Bartsch, P., N. Pfluger, M. Audetat, S. Shaw, P. Weidmann, P. Vock, W. Vetter, D. Rennie and O. Oelz (1991). "Effects of slow ascent to 4559 M on fluid homeostasis." Aviat Space Environ Med **62**(2): 105-110.
Bartsch, P., S. Shaw, M. Franciolli, M. P. Gnadinger and P. Weidmann (1988). "Atrial natriuretic peptide in acute mountain sickness." J Appl Physiol **65**(5): 1929-1937.

Basnyat, B., J. Lemaster and J. A. Litch (1999). "Everest or bust: a cross sectional, epidemiological study of acute mountain sickness at 4243 meters in the Himalayas." Aviat Space Environ Med **70**(9): 867-873.
Basu, M., K. Pal, R. Prasad, A. S. Malhotra, K. S. Rao and R. C. Sawhney (1997). "Pituitary, gonadal and adrenal hormones after prolonged residence at extreme altitude in man." Int J Androl **20**(3): 153-158.
Basu, M., R. C. Sawhney, S. Kumar, K. Pal, R. Prasad and W. Selvamurthy (2002). "Hypothalamic-pituitary-adrenal axis following glucocorticoid prophylaxis against acute mountain sickness." Horm Metab Res **34**(6): 318-324.
Behn, C., O. F. Araneda, A. J. Llanos, G. Celedon and G. Gonzalez (2007). "Hypoxia-related lipid peroxidation: evidences, implications and approaches." Respir Physiol Neurobiol **158**(2-3): 143-150.
Benso, A., F. Broglio, G. Aimaretti, B. Lucatello, F. Lanfranco, E. Ghigo and S. Grottoli (2007). "Endocrine and metabolic responses to extreme altitude and physical exercise in climbers." Eur J Endocrinol **157**(6): 733-740.
Bert, P. (1878). La Pression Barometrique: Recherches de physiologie experimentale. Paris, Masson.
Bestle, M. H., N. V. Olsen, T. D. Poulsen, R. Roach, N. Fogh-Andersen and P. Bie (2002). "Prolonged hypobaric hypoxemia attenuates vasopressin secretion and renal response to osmostimulation in men." J Appl Physiol **92**(5): 1911-1922.
Bhandari, S. S., I. Loke, J. E. Davies, I. B. Squire, J. Struck and L. L. Ng (2009). "Gender and renal function influence plasma levels of copeptin in healthy individuals." Clin Sci (Lond) **116**(3): 257-263.
Bircher, H., U. Eichenberger, M. Maggiorini, O. Oelz and P. Bärtsch (1994). "Relationship of mountain sickness to physical fitness and exercise intensity during ascent." J Wilderness Med **5**: 302-311.
Bland, J. M. and D. G. Altman (1986). "Statistical methods for assessing agreement between two methods of clinical measurement." Lancet **1**(8476): 307-310.
Blume, F. D., S. J. Boyer, L. E. Braverman, A. Cohen, J. Dirkse and J. P. Mordes (1984). "Impaired osmoregulation at high altitude. Studies on Mt Everest." JAMA **252**(4): 524-526.
Boldanova, T., M. Noveanu, T. Breidthardt, M. Potocki, T. Reichlin, A. Taegtmeyer, M. Christ, K. Laule, C. Stelzig and C. Mueller (2010). "Impact of

history of heart failure on diagnostic and prognostic value of BNP: results from the B-type Natriuretic Peptide for Acute Shortness of Breath Evaluation (BASEL) study." Int J Cardiol **142**(3): 265-272.
Bolignano, D., G. Coppolino, V. Donato, A. Lacquaniti, C. Bono and M. Buemi (2010). "Neutrophil gelatinase-associated lipocalin (NGAL): a new piece of the anemia puzzle?" Med Sci Monit **16**(6): RA131-135.
Bonen, A. (1976). "Effects of exercise on excretion rates of urinary free cortisol." J Appl Physiol **40**(2): 155-158.
Boos, C. J., D. A. Holdsworth, D. P. Hall, A. Mellor, J. O'Hara and D. R. Woods (2014). "Comparison of two methods of assessing total body water at sea level and increasing high altitude." Clin Physiol Funct Imaging **34**(6): 478-484.
Boos, C. J., D. A. Holdsworth, D. R. Woods, K. Green, J. Naylor and A. Mellor (2013). "Cardiac biomarkers and high altitude pulmonary edema." Int J Cardiol **167**(3): e65-66.
Borg, G. (1970). "Perceived exertion as an indicator of somatic stress." Scand J Rehabil Med **2**(2): 92-98.
Borg, G. A. (1973). "Perceived exertion: a note on "history" and methods." Med Sci Sports **5**(2): 90-93.
Borregaard N, C. J. (2006). "Neutrophil gelatinaseassociated lipocalin, a siderophore-binding eukaryotic protein." Biometals **19**: 211-215.

Bouissou, P., J. Fiet, C. Y. Guezennec and P. C. Pesquies (1988). "Plasma adrenocorticotrophin and cortisol responses to acute hypoxia at rest and during exercise." Eur J Appl Physiol Occup Physiol **57**(1): 110-113.

Bouissou, P., J. P. Richalet, F. X. Galen, M. Lartigue, P. Larmignat, F. Devaux, C. Dubray and A. Keromes (1989). "Effect of beta-adrenoceptor blockade on renin-aldosterone and alpha-ANF during exercise at altitude." J Appl Physiol (1985) **67**(1): 141-146.
Brandenberger, G. and M. Follenius (1975). "Influence of timing and intensity of musclar exercise on temporal patterns of plasma cortisol levels." J Clin Endocrinol Metab **40**(5): 845-849.
Brooks, S., J. Burrin, M. E. Cheetham, G. M. Hall, T. Yeo and C. Williams (1988). "The responses of the catecholamines and beta-endorphin to brief maximal exercise in man." Eur J Appl Physiol Occup Physiol **57**(2): 230-234.

Buono, M. J. and J. E. Yeager (1991). "Increases in aldosterone precede those of cortisol during graded exercise." J Sports Med Phys Fitness **31**(1): 48-51.
Burd, J., D. R. Weightman, B. A. Spruce and P. H. Baylis (1984). "A solid phase radioimmunoassay for human plasma arginine vasopressin." Clin Chim Acta **136**(2-3): 251-256.
Burtscher, M., M. Flatz and M. Faulhaber (2004). "Prediction of susceptibility to acute mountain sickness by SaO2 values during short-term exposure to hypoxia." High Alt Med Biol **5**(3): 335-340.
Burtscher, M., M. Wille, V. Menz, M. Faulhaber and H. Gatterer (2014). "Symptom Progression in Acute Mountain Sickness During a 12-Hour Exposure to Normobaric Hypoxia Equivalent to 4500 M." High Alt Med Biol.
Calbet, J. A. and C. Lundby (2009). "Air to muscle O2 delivery during exercise at altitude." High Alt Med Biol **10**(2): 123-134.
Cargill, R. I. and B. J. Lipworth (1995). "Acute effects of ANP and BNP on hypoxic pulmonary vasoconstriction in humans." Br J Clin Pharmacol **40**(6): 585-590.
Cargill, R. I., L. C. McFarlane, W. J. Coutie and B. J. Lipworth (1996). "Acute neurohormonal responses to hypoxaemia in man." Eur J Appl Physiol Occup Physiol **72**(3): 256-260.
Carlsson, E., I. Bosaeus and S. Nordgren (2004). "Body composition in patients with short bowel syndrome: an assessment by bioelectric impedance spectroscopy (BIS) and dual-energy absorptiometry (DXA)." Eur J Clin Nutr **58**(6): 853-859.
Casals, G., J. Ros, A. Sionis, M. M. Davidson, M. Morales-Ruiz and W. Jimenez (2009). "Hypoxia induces B-type natriuretic peptide release in cell lines derived from human cardiomyocytes." Am J Physiol Heart Circ Physiol **297**(2): H550-555.
Castillo Martinez, L., E. Colin Ramirez, A. Orea Tejeda, E. Asensio Lafuente, L. P. Bernal Rosales, V. Rebollar Gonzalez, R. Narvaez David and J. Dorantes Garcia (2007). "Bioelectrical impedance and strength measurements in patients with heart failure: comparison with functional class." Nutrition **23**(5): 412-418.
Chan, Y. R., J. S. Liu, D. A. Pociask, M. Zheng, T. A. Mietzner, T. Berger, T. W. Mak, M. C. Clifton, R. K. Strong, P. Ray and J. K. Kolls (2009). "Lipocalin 2 is required for pulmonary host defense against Klebsiella infection." J Immunol **182**(8): 4947-4956.

Chen, M. J., X. Fan and S. T. Moe (2002). "Criterion-related validity of the Borg ratings of perceived exertion scale in healthy individuals: a meta-analysis." J Sports Sci **20**(11): 873-899.

Chen, S. M., J. S. Wang, W. C. Lee, C. W. Hou, C. Y. Chen, Y. H. Laio, C. H. Lin and C. H. Kuo (2006). "Validity of the 3 min step test in moderate altitude: environmental temperature as a confounder." Appl Physiol Nutr Metab **31**(6): 726-730.

Chiurchiu, V., V. Izzi, F. D'Aquilio, F. Carotenuto, P. Di Nardo and P. M. Baldini (2008). "Brain Natriuretic Peptide (BNP) regulates the production of inflammatory mediators in human THP-1 macrophages." Regul Pept **148**(1-3): 26-32.

Cho, K., M. Tian, Y. Lan, X. Zhao and L. L. Yan (2013). "Validation of the Omron HEM-7201 upper arm blood pressure monitor, for self-measurement in a high-altitude environment, according to the European Society of Hypertension International Protocol revision 2010." J Hum Hypertens **27**(8): 487-491.

Christ-Crain, M., T. Breidthardt, D. Stolz, K. Zobrist, R. Bingisser, D. Miedinger, J. Leuppi, M. Tamm, B. Mueller and C. Mueller (2008). "Use of B-type natriuretic peptide in the risk stratification of community-acquired pneumonia." J Intern Med **264**(2): 166-176.

Cibella, F., G. Cuttitta, S. Romano, B. Grassi, G. Bonsignore and J. Milic-Emili (1999). "Respiratory energetics during exercise at high altitude." J Appl Physiol **86**(6): 1785-1792.

Clancy, L. J., J. A. Critchley, A. G. Leitch, B. J. Kirby, A. Ungar and D. C. Flenley (1975). "Arterial catecholamines in hypoxic exercise in man." Clin Sci Mol Med **49**(5): 503-506.

Clark, A. L., S. Galloway, N. MacFarlane, E. Henderson, T. Aitchison and J. J. McMurray (1997). "Catecholamines contribute to exertional dyspnoea and to the ventilatory response to exercise in normal humans." Eur Heart J **18**(11): 1829-1833.

Claybaugh, J. R., C. E. Wade, A. K. Sato, S. A. Cucinell, J. C. Lane and J. T. Maher (1982). "Antidiuretic hormone responses to eucapnic and hypocapnic hypoxia in humans." J Appl Physiol Respir Environ Exerc Physiol **53**(4): 815-823.

Coiro, V., R. Volpi, E. Volta, A. Melani, M. L. Maffei and P. Chiodera (2011). "Inhibitory effect of dexamethasone on arginine-vasopressin release induced by physical exercise in man." J Investig Med **59**(3): 599-601.
Corte, T. J., S. J. Wort, M. A. Gatzoulis, R. Engel, G. Giannakoulas, P. M. Macdonald and A. U. Wells (2010). "Elevated brain natriuretic peptide predicts mortality in interstitial lung disease." Eur Respir J **36**(4): 819-825.
Cosby, R. L., A. M. Sophocles, J. A. Durr, C. L. Perrinjaquet, B. Yee and R. W. Schrier (1988). "Elevated plasma atrial natriuretic factor and vasopressin in high-altitude pulmonary edema." Ann Intern Med **109**(10): 796-799.
Cowland, J. B. and N. Borregaard (1997). "Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans." Genomics **45**(1): 17-23.
Cowland, J. B., O. E. Sorensen, M. Sehested and N. Borregaard (2003). "Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alpha." J Immunol **171**(12): 6630-6639.
Davidson, N. C. and A. D. Struthers (1994). "Brain natriuretic peptide." J Hypertens **12**(4): 329-336.
Davies, A. J., N. S. Kalson, S. Stokes, M. D. Earl, A. G. Whitehead, H. Frost, I. Tyrell-Marsh and J. Naylor (2009). "Determinants of summiting success and acute mountain sickness on Mt Kilimanjaro (5895 m)." Wilderness Environ Med **20**(4): 311-317.
De Angelis, C., C. Ferri, L. Urbani and S. Farrace (1996). "Effect of acute exposure to hypoxia on electrolytes and water metabolism regulatory hormones." Aviat Space Environ Med **67**(8): 746-750.
De Geer, L., M. Fredrikson and A. Oscarsson (2012). "Amino-terminal pro-brain natriuretic peptide as a predictor of outcome in patients admitted to intensive care. A prospective observational study." Eur J Anaesthesiol **29**(6): 275-279.
Dellasanta, P., S. Gaillard, L. Loutan and B. Kayser (2007). "Comparing questionnaires for the assessment of acute mountain sickness." High Alt Med Biol **8**(3): 184-191.
Di Luigi, L., C. Baldari, P. Sgro, G. P. Emerenziani, M. C. Gallotta, S. Bianchini, F. Romanelli, F. Pigozzi, A. Lenzi and L. Guidetti (2008). "The type 5 phosphodiesterase inhibitor tadalafil influences salivary cortisol, testosterone, and dehydroepiandrosterone sulphate responses to maximal exercise in healthy men." J Clin Endocrinol Metab **93**(9): 3510-3514.

Dimitroulas, T., G. Giannakoulas, H. Karvounis, A. Garyfallos, L. Settas and G. Kitas (2012). "B-type natriuretic peptide in rheumatic diseases: a cardiac biomarker or a sophisticated acute phase reactant?" Autoimmun Rev **11**(12): 837-843.

Dimsdale, J. E., L. H. Hartley, T. Guiney, J. N. Ruskin and D. Greenblatt (1984). "Postexercise peril. Plasma catecholamines and exercise." JAMA **251**(5): 630-632.

Donadio, C., C. Consani, M. Ardini, G. Bernabini, F. Caprio, G. Grassi, A. Lucchesi and B. Nerucci (2005). "Estimate of body water compartments and of body composition in maintenance hemodialysis patients: comparison of single and multifrequency bioimpedance analysis." J Ren Nutr **15**(3): 332-344.

Due-Andersen, R., U. Pedersen-Bjergaard, T. Hoi-Hansen, N. V. Olsen, C. Kistorp, J. Faber, F. Boomsma and B. Thorsteinsson (2008). "NT-pro-BNP during hypoglycemia and hypoxemia in normal subjects: impact of renin-angiotensin system activity." J Appl Physiol (1985) **104**(4): 1080-1085.

Eide, R. P., 3rd and C. A. Asplund (2012). "Altitude illness: update on prevention and treatment." Curr Sports Med Rep **11**(3): 124-130.

Eliasdottir, S. B., G. Klemenzson, B. Torfason and F. Valsson (2008). "Brain natriuretic peptide is a good predictor for outcome in cardiac surgery." Acta Anaesthesiol Scand **52**(2): 182-187.

Ellis, K. J., S. J. Bell, G. M. Chertow, W. C. Chumlea, T. A. Knox, D. P. Kotler, H. C. Lukaski and D. A. Schoeller (1999). "Bioelectrical impedance methods in clinical research: a follow-up to the NIH Technology Assessment Conference." Nutrition **15**(11-12): 874-880.

Ellsworth, A. J., E. B. Larson and D. Strickland (1987). "A randomized trial of dexamethasone and acetazolamide for acute mountain sickness prophylaxis." Am J Med **83**(6): 1024-1030.

Elstein, D., A. Nir, M. Klutstein, B. Rudensky and A. Zimran (2005). "C-reactive protein and NT-proBNP as surrogate markers for pulmonary hypertension in Gaucher disease." Blood Cells Mol Dis **34**(3): 201-205.

Engell, D. B., O. Maller, M. N. Sawka, R. N. Francesconi, L. Drolet and A. J. Young (1987). "Thirst and fluid intake following graded hypohydration levels in humans." Physiol Behav **40**(2): 229-236.

Espiner, E. A. (1994). "Physiology of natriuretic peptides." J Intern Med **235**(6): 527-541.

Fazlinezhad, A., M. K. Rezaeian, H. Yousefzadeh, K. Ghaffarzadegan and M. Khajedaluee (2011). "Plasma Brain Natriuretic Peptide (BNP) as an Indicator of Left Ventricular Function, Early Outcome and Mechanical Complications after Acute Myocardial Infarction." Clin Med Insights Cardiol **5**: 77-83.

Feddersen, B., H. Ausserer, B. Haditsch, H. Frisch, S. Noachtar and A. Straube (2009). "Brain natriuretic peptide at altitude: relationship to diuresis, natriuresis, and mountain sickness." Aviat Space Environ Med **80**(2): 108-111.

Felker, G. M., J. W. Petersen and D. B. Mark (2006). "Natriuretic peptides in the diagnosis and management of heart failure." CMAJ **175**(6): 611-617.

Fennymann, R. (1967). The character of physical law. Cambridge, MA, MIT Press.

Filusch, A., E. Giannitsis, H. A. Katus and F. J. Meyer (2010). "High-sensitive troponin T: a novel biomarker for prognosis and disease severity in patients with pulmonary arterial hypertension." Clin Sci (Lond) **119**(5): 207-213.

Galasko, G. I., A. Lahiri, S. C. Barnes, P. Collinson and R. Senior (2005). "What is the normal range for N-terminal pro-brain natriuretic peptide? How well does this normal range screen for cardiovascular disease?" Eur Heart J **26**(21): 2269-2276.

Gao, M., R. Wang, Z. Jiayong, Y. Liu and G. Sun (2013). "NT-ProBNP levels are moderately increased in acute high-altitude pulmonary edema." Exp Ther Med **5**(5): 1434-1438.

Gertsch, J. H., B. Corbett, P. S. Holck, A. Mulcahy, M. Watts, N. T. Stillwagon, A. M. Casto, C. H. Abramson, C. P. Vaughan, C. Macguire, N. N. Farzan, B. N. Vo, R. J. Norvelle, K. May, J. E. Holly, H. Irons, A. M. Stutz, P. Chapagain, S. Yadav, M. Pun, J. Farrar and B. Basnyat (2012). "Altitude Sickness in Climbers and Efficacy of NSAIDs Trial (ASCENT): randomized, controlled trial of ibuprofen versus placebo for prevention of altitude illness." Wilderness Environ Med **23**(4): 307-315.

Gertsch, J. H., P. S. Holck, B. Basnyat and B. M. Corbett (2013). "In reply to "ibuprofen for prevention of acute mountain sickness-is bigger really better?"." Wilderness Environ Med **24**(2): 178-179.

Gertsch, J. H., G. S. Lipman, P. S. Holck, A. Merritt, A. Mulcahy, R. S. Fisher, B. Basnyat, E. Allison, K. Hanzelka, A. Hazan, Z. Meyers, J. Odegaard, B. Pook, M. Thompson, B. Slomovic, H. Wahlberg, V. Wilshaw, E. A. Weiss and K. Zafren (2010). "Prospective, double-blind, randomized, placebo-controlled

comparison of acetazolamide versus ibuprofen for prophylaxis against high altitude headache: the Headache Evaluation at Altitude Trial (HEAT)." Wilderness Environ Med **21**(3): 236-243.

Girard, O., M. S. Koehle, M. J. MacInnis, J. A. Guenette, S. Verges, T. Rupp, M. Jubeau, S. Perrey, G. Y. Millet, R. F. Chapman, B. D. Levine, J. Conkin, J. H. Wessel, 3rd, H. Nespoulet, B. Wuyam, R. Tamisier, P. Levy, D. P. Casey, B. J. Taylor, E. M. Snyder, B. D. Johnson, A. S. Laymon, J. L. Stickford, J. C. Weavil, J. A. Loeppky, M. Pun, K. Schommer, P. Bartsch, M. C. Vagula and C. F. Nelatury (2012). "Comments on Point:Counterpoint: Hypobaric hypoxia induces/does not induce different responses from normobaric hypoxia." J Appl Physiol **112**(10): 1788-1794.

Goetze, J. P., R. Mogelvang, L. Maage, H. Scharling, P. Schnohr, P. Sogaard, J. F. Rehfeld and J. S. Jensen (2006). "Plasma pro-B-type natriuretic peptide in the general population: screening for left ventricular hypertrophy and systolic dysfunction." Eur Heart J **27**(24): 3004-3010.

Goldsmith, S. R., C. Iber, C. D. McArthur and S. F. Davies (1990). "Influence of acid-base status on plasma catecholamines during exercise in normal humans." Am J Physiol **258**(6 Pt 2): R1411-1416.

Graham, I., D. Atar, K. Borch-Johnsen, G. Boysen, G. Burell, R. Cifkova, J. Dallongeville, G. De Backer, S. Ebrahim, B. Gjelsvik, C. Herrmann-Lingen, A. Hoes, S. Humphries, M. Knapton, J. Perk, S. G. Priori, K. Pyorala, Z. Reiner, L. Ruilope, S. Sans-Menendez, W. S. Op Reimer, P. Weissberg, D. Wood, J. Yarnell, J. L. Zamorano, E. Walma, T. Fitzgerald, M. T. Cooney, A. Dudina, A. Vahanian, J. Camm, R. De Caterina, V. Dean, K. Dickstein, C. Funck-Brentano, G. Filippatos, I. Hellemans, S. D. Kristensen, K. McGregor, U. Sechtem, S. Silber, M. Tendera, P. Widimsky, A. Altiner, E. Bonora, P. N. Durrington, R. Fagard, S. Giampaoli, H. Hemingway, J. Hakansson, S. E. Kjeldsen, M. L. Larsen, G. Mancia, A. J. Manolis, K. Orth-Gomer, T. Pedersen, M. Rayner, L. Ryden, M. Sammut, N. Schneiderman, A. F. Stalenhoef, L. Tokgozoglu, O. Wiklund and A. Zampelas (2007). "European guidelines on cardiovascular disease prevention in clinical practice: executive summary. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts)." Eur J Cardiovasc Prev Rehabil **14 Suppl 2**: E1-40.

Greenfield, S. M., G. J. Webster, A. S. Brar, K. Ah Mun, E. R. Beck and F. R. Vicary (1996). "Assessment of residual gastric volume and thirst in patients who drink before gastroscopy." Gut **39**(3): 360-362.
Grissom, C. K., G. A. Zimmerman and R. E. Whatley (1997). "Endothelial selectins in acute mountain sickness and high-altitude pulmonary edema." Chest **112**(6): 1572-1578.
Grocott, M., A. Richardson, H. Montgomery and M. Mythen (2007). "Caudwell Xtreme Everest: a field study of human adaptation to hypoxia." Crit Care **11**(4): 151.
Grocott, M. P., D. S. Martin, D. Z. Levett, R. McMorrow, J. Windsor and H. E. Montgomery (2009). "Arterial blood gases and oxygen content in climbers on Mount Everest." N Engl J Med **360**(2): 140-149.
Grocott, M. P., D. S. Martin, M. H. Wilson, K. Mitchell, S. Dhillon, M. G. Mythen, H. E. Montgomery and D. Z. Levett (2010). "Caudwell xtreme Everest expedition." High Alt Med Biol **11**(2): 133-137.
Groves, B. M., J. T. Reeves, J. R. Sutton, P. D. Wagner, A. Cymerman, M. K. Malconian, P. B. Rock, P. M. Young and C. S. Houston (1987). "Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen." J Appl Physiol **63**(2): 521-530.
Grunig, E., D. Mereles, W. Hildebrandt, E. R. Swenson, W. Kubler, H. Kuecherer and P. Bartsch (2000). "Stress Doppler echocardiography for identification of susceptibility to high altitude pulmonary edema." J Am Coll Cardiol **35**(4): 980-987.
Guo, Q., P. Barany, A. R. Qureshi, S. Snaedal, O. Heimburger, P. Stenvinkel, B. Lindholm and J. Axelsson (2009). "N-terminal pro-brain natriuretic peptide independently predicts protein energy wasting and is associated with all-cause mortality in prevalent HD patients." Am J Nephrol **29**(6): 516-523.
Haas, V., T. Schutz, S. Engeli, C. Schroder, K. Westerterp and M. Boschmann (2012). "Comparing single-frequency bioelectrical impedance analysis against deuterium dilution to assess total body water." Eur J Clin Nutr **66**(9): 994-997.
Haase, M., R. Bellomo and A. Haase-Fielitz (2010). "Neutrophil gelatinase-associated lipocalin." Curr Opin Crit Care.
Hackett, P. H., M. L. Forsling, J. Milledge and D. Rennie (1978). "Release of vasopressin in man at altitude." Horm Metab Res **10**(6): 571.

Hackett, P. H. and O. Oelz (1992). The Lake Louise consensus on the quantification of altitude illness. Hypoxia and Mountain Medicine. Sutton JR, Houston CS and C. G. Burlington, VT, Queen City Printers**:** 327-330.

Hackett, P. H., D. Rennie, S. E. Hofmeister, R. F. Grover, E. B. Grover and J. T. Reeves (1982). "Fluid retention and relative hypoventilation in acute mountain sickness." Respiration **43**(5): 321-329.

Hackett, P. H. and R. C. Roach (2004). "High altitude cerebral edema." High Alt Med Biol **5**(2): 136-146.

Hackett, P. H., R. C. Roach, R. A. Wood, R. G. Foutch, R. T. Meehan, D. Rennie and W. J. Mills, Jr. (1988). "Dexamethasone for prevention and treatment of acute mountain sickness." Aviat Space Environ Med **59**(10): 950-954.

Hall, C. (2005). "NT-ProBNP: the mechanism behind the marker." J Card Fail **11**(5 Suppl): S81-83.

Hall, D. P., K. Duncan and J. K. Baillie (2011). "High altitude pulmonary oedema." J R Army Med Corps **157**(1): 68-72.

Hall, D. P., I. J. Maccormick, A. T. Phythian-Adams, N. M. Rzechorzek, D. Hope-Jones, S. Cosens, S. Jackson, M. G. Bates, D. J. Collier, D. A. Hume, T. Freeman, A. A. Thompson and J. K. Baillie (2014). "Network analysis reveals distinct clinical syndromes underlying acute mountain sickness." PLoS One **9**(1): e81229.

Hamilton, A. J., A. Cymmerman and P. M. Black (1986). "High altitude cerebral edema." Neurosurgery **19**(5): 841-849.

Harris, N. S., R. P. Wenzel and S. H. Thomas (2003). "High altitude headache: efficacy of acetaminophen vs. ibuprofen in a randomized, controlled trial." J Emerg Med **24**(4): 383-387.

Hartmann, G., M. Tschop, R. Fischer, C. Bidlingmaier, R. Riepl, K. Tschop, H. Hautmann, S. Endres and M. Toepfer (2000). "High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein." Cytokine **12**(3): 246-252.

Haugen, E., J. Chen, J. Wikstrom, J. Gronros, L. M. Gan and L. X. Fu (2007). "Parallel gene expressions of IL-6 and BNP during cardiac hypertrophy complicated with diastolic dysfunction in spontaneously hypertensive rats." Int J Cardiol **115**(1): 24-28.

Hew-Butler, T., M. D. Hoffman, K. J. Stuempfle, I. R. Rogers, N. G. Morgenthaler and J. G. Verbalis (2011). "Changes in copeptin and bioactive vasopressin in runners with and without hyponatremia." Clin J Sport Med **21**(3): 211-217.

Hew-Butler, T., E. Jordaan, K. J. Stuempfle, D. B. Speedy, A. J. Siegel, T. D. Noakes, S. J. Soldin and J. G. Verbalis (2008). "Osmotic and nonosmotic regulation of arginine vasopressin during prolonged endurance exercise." J Clin Endocrinol Metab **93**(6): 2072-2078.

Hew-Butler, T., T. D. Noakes, S. J. Soldin and J. G. Verbalis (2008). "Acute changes in endocrine and fluid balance markers during high-intensity, steady-state, and prolonged endurance running: unexpected increases in oxytocin and brain natriuretic peptide during exercise." Eur J Endocrinol **159**(6): 729-737.

Hildebrandt, W., A. Ottenbacher, M. Schuster, E. R. Swenson and P. Bartsch (2000). "Diuretic effect of hypoxia, hypocapnia, and hyperpnea in humans: relation to hormones and O(2) chemosensitivity." J Appl Physiol **88**(2): 599-610.

Hill, E. E., E. Zack, C. Battaglini, M. Viru, A. Viru and A. C. Hackney (2008). "Exercise and circulating cortisol levels: the intensity threshold effect." J Endocrinol Invest **31**(7): 587-591.

Holm, J., M. Vidlund, F. Vanky, O. Friberg, E. Hakanson and R. Svedjeholm (2013). "Preoperative NT-proBNP independently predicts outcome in patients with acute coronary syndrome undergoing CABG." Scand Cardiovasc J Suppl **47**(1): 28-35.

Holwerda, D. A. (1972). "A glycopeptide from the posterior lobe of pig pituitaries. 2. Primary structure." Eur J Biochem **28**(3): 340-346.

Hooper, T. J., D. Z. Levett, A. J. Mellor and M. P. Grocott (2010). "Resting and exercising cardiorespiratory variables and acute mountain sickness." J R Nav Med Serv **96**(1): 6-12.

Huang, H. H., C. L. Han, H. C. Yan, W. Y. Kao, C. D. Tsai, D. H. Yen, C. I. Huang and W. T. Chen (2008). "Oxidative stress and erythropoietin response in altitude exposure." Clin Invest Med **31**(6): E380-385.

Humpeler, E., F. Skrabal and G. Bartsch (1980). "Influence of exposure to moderate altitude on the plasma concentraton of cortisol, aldosterone, renin, testosterone, and gonadotropins." Eur J Appl Physiol Occup Physiol **45**(2-3): 167-176.

Inder, W. J., J. Hellemans, M. P. Swanney, T. C. Prickett and R. A. Donald (1998). "Prolonged exercise increases peripheral plasma ACTH, CRH, and AVP in male athletes." J Appl Physiol (1985) **85**(3): 835-841.

Inoue, T., M. Kawai, T. Nakane, A. Nojiri, K. Minai, K. Komukai, T. Ogawa, K. Hongo, M. Matsushima and M. Yoshimura (2010). "Influence of low-grade inflammation on plasma B-type natriuretic peptide levels." Intern Med **49**(24): 2659-2668.

Jacks, D. E., J. Sowash, J. Anning, T. McGloughlin and F. Andres (2002). "Effect of exercise at three exercise intensities on salivary cortisol." J Strength Cond Res **16**(2): 286-289.

Jensen, J., L. P. Ma, M. L. Fu, D. Svaninger, P. A. Lundberg and O. Hammarsten (2010). "Inflammation increases NT-proBNP and the NT-proBNP/BNP ratio." Clin Res Cardiol **99**(7): 445-452.

Jochberger, S., J. Dorler, G. Luckner, V. D. Mayr, V. Wenzel, H. Ulmer, N. G. Morgenthaler, W. R. Hasibeder and M. W. Dunser (2009). "The vasopressin and copeptin response to infection, severe sepsis, and septic shock." Crit Care Med **37**(2): 476-482.

Jochberger, S., N. G. Morgenthaler, V. D. Mayr, G. Luckner, V. Wenzel, H. Ulmer, S. Schwarz, W. R. Hasibeder, B. E. Friesenecker and M. W. Dunser (2006). "Copeptin and arginine vasopressin concentrations in critically ill patients." J Clin Endocrinol Metab **91**(11): 4381-4386.

Jochberger, S., C. Velik-Salchner, V. D. Mayr, G. Luckner, V. Wenzel, G. Falkensammer, H. Ulmer, N. Morgenthaler, W. Hasibeder and M. W. Dunser (2009). "The vasopressin and copeptin response in patients with vasodilatory shock after cardiac surgery: a prospective, controlled study." Intensive Care Med **35**(3): 489-497.

Julian, C. G., A. W. Subudhi, M. J. Wilson, A. C. Dimmen, T. Pecha and R. C. Roach (2011). "Acute mountain sickness, inflammation, and permeability: new insights from a blood biomarker study." J Appl Physiol (1985) **111**(2): 392-399.

Karadag, O., M. Calguneri, B. Yavuz, E. Atalar, A. Akdogan, U. Kalyoncu, S. Kiraz, S. Aksoyek, F. Ozmen and A. I. Ertenli (2007). "B-type natriuretic peptide (BNP) levels in female systemic lupus erythematosus patients: what is the clinical significance?" Clin Rheumatol **26**(10): 1701-1704.

Karinen, H., J. Peltonen and H. Tikkanen (2008). "Prevalence of acute mountain sickness among Finnish trekkers on Mount Kilimanjaro, Tanzania: an observational study." High Alt Med Biol **9**(4): 301-306.
Karinen, H. M., J. E. Peltonen, M. Kahonen and H. O. Tikkanen (2010). "Prediction of acute mountain sickness by monitoring arterial oxygen saturation during ascent." High Alt Med Biol **11**(4): 325-332.
Karlstrom, P., U. Alehagen, K. Boman and U. Dahlstrom (2011). "Brain natriuretic peptide-guided treatment does not improve morbidity and mortality in extensively treated patients with chronic heart failure: responders to treatment have a significantly better outcome." Eur J Heart Fail **13**(10): 1096-1103.
Kellog, R. (1963). The Regulation of Human Respiration. D. Cunningham and B. Lloyd. Oxford, Blackwell Scientific Publications**:** 379-394.
Kerbaul, F., R. Giorgi, C. Oddoze, F. Collart, C. Guidon, P. J. Lejeune, J. Villacorta and F. Gouin (2004). "High concentrations of N-BNP are related to non-infectious severe SIRS associated with cardiovascular dysfunction occurring after off-pump coronary artery surgery." Br J Anaesth **93**(5): 639-644.
King, A. B. and S. M. Robinson (1972). "Ventilation response to hypoxia and acute mountain sickness." Aerosp Med **43**(4): 419-421.
Klausen, T., N. V. Olsen, T. D. Poulsen, J. P. Richalet and B. K. Pedersen (1997). "Hypoxemia increases serum interleukin-6 in humans." Eur J Appl Physiol Occup Physiol **76**(5): 480-482.
Kleger, G. R., P. Bartsch, P. Vock, B. Heilig, L. J. Roberts, 2nd and P. E. Ballmer (1996). "Evidence against an increase in capillary permeability in subjects exposed to high altitude." J Appl Physiol (1985) **81**(5): 1917-1923.
Kleindienst, A., G. Brabant, N. G. Morgenthaler, K. C. Dixit, H. Parsch and M. Buchfelder (2010). "Following brain trauma, copeptin, a stable peptide derived from the AVP precusor, does not reflect osmoregulation but correlates with injury severity." Acta Neurochir Suppl **106**: 221-224.
Knoepfelmacher, M., M. J. Pradal, R. D. Dio, L. R. Salgado, M. Semer, B. L. Wajchenberg and B. Liberman (1997). "Resistance to vasopressin action on the kidney in patients with Cushing's disease." Eur J Endocrinol **137**(2): 162-166.
Koehle, M. S., J. A. Guenette and D. E. Warburton (2009). "Oximetry, heart rate variability, and the diagnosis of mild-to-moderate acute mountain sickness." Eur J Emerg Med.

Kuipers, H., H. A. Keizer, F. Brouns and W. H. Saris (1987). "Carbohydrate feeding and glycogen synthesis during exercise in man." Pflugers Arch **410**(6): 652-656.

Kushner, R. F., D. A. Schoeller, C. R. Fjeld and L. Danford (1992). "Is the impedance index (ht2/R) significant in predicting total body water?" Am J Clin Nutr **56**(5): 835-839.

Kyle, U. G., I. Bosaeus, A. D. De Lorenzo, P. Deurenberg, M. Elia, J. M. Gomez, B. L. Heitmann, L. Kent-Smith, J. C. Melchior, M. Pirlich, H. Scharfetter, A. M. Schols, C. Pichard and E. W. G. Composition of the (2004). "Bioelectrical impedance analysis--part I: review of principles and methods." Clin Nutr **23**(5): 1226-1243.

Lazio, M. P., J. D. Van Roo, C. Pesce, S. Malik and D. M. Courtney (2010). "Postexercise peripheral oxygen saturation after completion of the 6-minute walk test predicts successfully reaching the summit of Aconcagua." Wilderness Environ Med **21**(4): 309-317.

Levine, B. D., K. Yoshimura, T. Kobayashi, M. Fukushima, T. Shibamoto and G. Ueda (1989). "Dexamethasone in the treatment of acute mountain sickness." N Engl J Med **321**(25): 1707-1713.

Liang, F., A. M. Kapoun, A. Lam, D. L. Damm, D. Quan, M. O'Connell and A. A. Protter (2007). "B-Type natriuretic peptide inhibited angiotensin II-stimulated cholesterol biosynthesis, cholesterol transfer, and steroidogenesis in primary human adrenocortical cells." Endocrinology **148**(8): 3722-3729.

Lipman, G. S. and N. C. Kanaan (2013). "Ibuprofen for prevention of acute mountain sickness: is bigger really better?" Wilderness Environ Med **24**(2): 177-178.

Lipman, G. S., N. C. Kanaan, P. S. Holck, B. B. Constance and J. H. Gertsch (2012). "Ibuprofen prevents altitude illness: a randomized controlled trial for prevention of altitude illness with nonsteroidal anti-inflammatories." Ann Emerg Med **59**(6): 484-490.

Liu, Q. and M. Nilsen-Hamilton (1995). "Identification of a new acute phase protein." J Biol Chem **270**(38): 22565-22570.

Loeppky, J. A., M. V. Icenogle, D. Maes, K. Riboni, H. Hinghofer-Szalkay and R. C. Roach (2005). "Early fluid retention and severe acute mountain sickness." J Appl Physiol **98**(2): 591-597.

Lukaski, H. C., P. E. Johnson, W. W. Bolonchuk and G. I. Lykken (1985). "Assessment of fat-free mass using bioelectrical impedance measurements of the human body." Am J Clin Nutr **41**(4): 810-817.
Lundby, C., H. Pilegaard, J. L. Andersen, G. van Hall, M. Sander and J. A. Calbet (2004). "Acclimatization to 4100 m does not change capillary density or mRNA expression of potential angiogenesis regulatory factors in human skeletal muscle." J Exp Biol **207**(Pt 22): 3865-3871.
Macinnis, M. J., S. C. Lanting, J. L. Rupert and M. S. Koehle (2013). "Is poor sleep quality at high altitude separate from acute mountain sickness? Factor structure and internal consistency of the lake louise score questionnaire." High Alt Med Biol **14**(4): 334-337.
Maeder, M. T., D. Staub, M. H. Brutsche, N. Arenja, T. Socrates, M. Reiter, J. Meissner, N. G. Morgenthaler, A. Bergmann, J. Struck and C. Mueller (2010). "Copeptin response to clinical maximal exercise tests." Clin Chem **56**(4): 674-676.
Maggiorini, M., C. Melot, S. Pierre, F. Pfeiffer, I. Greve, C. Sartori, M. Lepori, M. Hauser, U. Scherrer and R. Naeije (2001). "High-altitude pulmonary edema is initially caused by an increase in capillary pressure." Circulation **103**(16): 2078-2083.
Maggiorini, M., A. Muller, D. Hofstetter, P. Bartsch and O. Oelz (1998). "Assessment of acute mountain sickness by different score protocols in the Swiss Alps." Aviat Space Environ Med **69**(12): 1186-1192.
Maher, J. T., L. G. Jones, L. H. Hartley, G. H. Williams and L. I. Rose (1975). "Aldosterone dynamics during graded exercise at sea level and high altitude." J Appl Physiol **39**(1): 18-22.
Maresh, C. M., M. R. Deschenes, R. L. Seip, L. E. Armstrong, K. L. Robertson and B. J. Noble (1993). "Perceived exertion during hypobaric hypoxia in low- and moderate-altitude natives." Med Sci Sports Exerc **25**(8): 945-951.
Maresh, C. M., W. J. Kraemer, D. A. Judelson, J. L. VanHeest, L. Trad, J. M. Kulikowich, K. L. Goetz, A. Cymerman and A. J. Hamilton (2004). "Effects of high altitude and water deprivation on arginine vasopressin release in men." Am J Physiol Endocrinol Metab **286**(1): E20-24.
Major, S A, Hogan, RJK, Yeates, E, Imray CHE. Peripheral arterial desaturation is further exacerbated by exercise in adolescents with acute mountain sickness. Wilderness and Environmental Medicine **23**(1), 15-23.

Maresh, C. M., B. J. Noble, K. L. Robertson and J. S. Harvey, Jr. (1985). "Aldosterone, cortisol, and electrolyte responses to hypobaric hypoxia in moderate-altitude natives." Aviat Space Environ Med **56**(11): 1078-1084.

Marticorena E, R. L., Severino J, Galvez J, Penaloza D. (1969). "Systemic blood pressure in white men born at sea level: Changes after long residence in high altitudes." Am J Cardiol **23**: 364-368.

Martignoni, E., O. Appenzeller, R. E. Nappi, G. Sances, A. Costa and G. Nappi (1997). "The effects of physical exercise at high altitude on adrenocortical function in humans." Funct Neurol **12**(6): 339-344.

Marumoto, K., M. Hamada and K. Hiwada (1995). "Increased secretion of atrial and brain natriuretic peptides during acute myocardial ischaemia induced by dynamic exercise in patients with angina pectoris." Clin Sci (Lond) **88**(5): 551-556.

McGregor, A., M. Richards, E. Espiner, T. Yandle and H. Ikram (1990). "Brain natriuretic peptide administered to man: actions and metabolism." J Clin Endocrinol Metab **70**(4): 1103-1107.

McGuire, S. A., P. M. Sherman, A. C. Brown, A. Y. Robinson, D. F. Tate, P. T. Fox and P. V. Kochunov (2012). "Hyperintense white matter lesions in 50 high-altitude pilots with neurologic decompression sickness." Aviat Space Environ Med **83**(12): 1117-1122.

McLean, C. J., C. W. Booth, T. Tattersall and J. D. Few (1989). "The effect of high altitude on saliva aldosterone and glucocorticoid concentrations." Eur J Appl Physiol Occup Physiol **58**(4): 341-347.

Mehra, M. R., P. A. Uber, D. Walther, M. Vesely, J. G. Wohlgemuth, J. Prentice, D. Tayama and M. Billingham (2006). "Gene expression profiles and B-type natriuretic peptide elevation in heart transplantation: more than a hemodynamic marker." Circulation **114**(1 Suppl): I21-26.

Melin, B., J. P. Eclache, G. Geelen, G. Annat, A. M. Allevard, E. Jarsaillon, A. Zebidi, J. J. Legros and C. Gharib (1980). "Plasma AVP, neurophysin, renin activity, and aldosterone during submaximal exercise performed until exhaustion in trained and untrained men." Eur J Appl Physiol Occup Physiol **44**(2): 141-151.

Mellor, A., C. Boos, D. Holdsworth, J. Begley, D. Hall, A. Lumley, A. Burnett, A. Hawkins, J. O'Hara, S. Ball and D. Woods (2014). "Cardiac Biomarkers at High Altitude." High Alt Med Biol.

Mellor, A., C. Boos, M. Stacey, T. Hooper, C. Smith, J. Begley, J. Yarker, R. Piper, J. O'Hara, R. King, S. Turner and D. R. Woods (2013). "Neutrophil gelatinase-associated lipocalin: its response to hypoxia and association with acute mountain sickness." Dis Markers **35**(5): 537-542.
Mellor, A. and D. Woods (2014). "Physiology studies at high altitude; why and how." J R Army Med Corps **160**(2): 131-134.
Mellor, A. J., C. J. Boos, S. Ball, A. Burnett, S. Pattman, M. Redpath and D. R. Woods (2014). "Copeptin and arginine vasopressin at high altitude: relationship to plasma osmolality and perceived exertion." Eur J Appl Physiol.
Mellor, A. J. and D. Woods (2012). "Serum neutrophil gelatinase-associated lipocalin in ballistic injuries: a comparison between blast injuries and gunshot wounds." J Crit Care **27**(4): 419 e411-415.
Mellor, A. J., D. R. Woods, J. O'Hara, M. Howley, J. Watchorn and C. Boos (2014). "Rating of Perceived Exertion and Acute Mountain Sickness During a High-Altitude Trek." Aviat Space Environ Med **85**(12): 1214-1216.
Milledge, J. S. (2010). "The Silver Hut expedition, 1960-1961." High Alt Med Biol **11**(2): 93-101.
Milledge, J. S., J. M. Beeley, S. McArthur and A. H. Morice (1989). "Atrial natriuretic peptide, altitude and acute mountain sickness." Clin Sci (Lond) **77**(5): 509-514.
Milledge, J. S., J. R. Broome and J. M. Beeley (1988). "Microvascular fragility and acute mountain sickness." Br Med J (Clin Res Ed) **296**(6622): 610.
Milledge, J. S., P. S. Thomas, J. M. Beeley and J. S. English (1988). "Hypoxic ventilatory response and acute mountain sickness." Eur Respir J **1**(10): 948-951.
Missoum, G., E. Rosnet and J. P. Richalet (1992). "Control of anxiety and acute mountain sickness in Himalayan mountaineers." Int J Sports Med **13 Suppl 1**: S37-39.
Mizuno, M., G. K. Savard, N. H. Areskog, C. Lundby and B. Saltin (2008). "Skeletal muscle adaptations to prolonged exposure to extreme altitude: a role of physical activity?" High Alt Med Biol **9**(4): 311-317.
Montgomery, A. B., J. Mills and J. M. Luce (1989). "Incidence of acute mountain sickness at intermediate altitude." JAMA **261**(5): 732-734.
Moraes, J. C., A. C. Ribeiro, C. G. Saad, A. C. Lianza, C. A. Silva and E. Bonfa (2013). "NT-proBNP levels may be influenced by inflammation in active

ankylosing spondylitis receiving TNF blockers: a pilot study." Clin Rheumatol **32**(6): 879-883.
Morgenthaler, N. G. (2010). "Copeptin: a biomarker of cardiovascular and renal function." Congest Heart Fail **16 Suppl 1**: S37-44.
Morgenthaler, N. G., B. Muller, J. Struck, A. Bergmann, H. Redl and M. Christ-Crain (2007). "Copeptin, a stable peptide of the arginine vasopressin precursor, is elevated in hemorrhagic and septic shock." Shock **28**(2): 219-226.
Morgenthaler, N. G., J. Struck, C. Alonso and A. Bergmann (2006). "Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin." Clin Chem **52**(1): 112-119.
Muller, B., N. Morgenthaler, D. Stolz, P. Schuetz, C. Muller, R. Bingisser, A. Bergmann, M. Tamm and M. Christ-Crain (2007). "Circulating levels of copeptin, a novel biomarker, in lower respiratory tract infections." Eur J Clin Invest **37**(2): 145-152.
Nagaya, N., T. Nishikimi, Y. Okano, M. Uematsu, T. Satoh, S. Kyotani, S. Kuribayashi, S. Hamada, M. Kakishita, N. Nakanishi, M. Takamiya, T. Kunieda, H. Matsuo and K. Kangawa (1998). "Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension." J Am Coll Cardiol **31**(1): 202-208.
Nagaya, N., T. Nishikimi, M. Uematsu, T. Satoh, S. Kyotani, F. Sakamaki, M. Kakishita, K. Fukushima, Y. Okano, N. Nakanishi, K. Miyatake and K. Kangawa (2000). "Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension." Circulation **102**(8): 865-870.
Nakanishi, K., F. Tajima, H. Itoh, Y. Nakata, H. Osada, N. Hama, O. Nakagawa, K. Nakao, T. Kawai, K. Takishima, T. Aurues and T. Ikeda (2001). "Changes in atrial natriuretic peptide and brain natriuretic peptide associated with hypobaric hypoxia-induced pulmonary hypertension in rats." Virchows Arch **439**(6): 808-817.
Nawata, H., M. Ohashi, M. Haji, R. Takayanagi, K. Higuchi, N. Fujio, T. Hashiguchi, A. Ogo, R. Nakao, K. Ohnaka and et al. (1991). "Atrial and brain natriuretic peptide in adrenal steroidogenesis." J Steroid Biochem Mol Biol **40**(1-3): 367-379.
Nicholson, S., M. Richards, E. Espiner, G. Nicholls and T. Yandle (1993). "Atrial and brain natriuretic peptide response to exercise in patients with ischaemic heart disease." Clin Exp Pharmacol Physiol **20**(7-8): 535-540.

Noble, B. J., G. A. Borg, I. Jacobs, R. Ceci and P. Kaiser (1983). "A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate." Med Sci Sports Exerc **15**(6): 523-528.

Nunes, J. A., B. T. Crewther, C. Ugrinowitsch, V. Tricoli, L. Viveiros, D. de Rose, Jr. and M. S. Aoki (2011). "Salivary hormone and immune responses to three resistance exercise schemes in elite female athletes." J Strength Cond Res **25**(8): 2322-2327.

Nussbaumer-Ochsner, Y., N. Schuepfer, J. Ursprung, C. Siebenmann, M. Maggiorini and K. E. Bloch (2012). "Sleep and breathing in high altitude pulmonary edema susceptible subjects at 4,559 meters." Sleep **35**(10): 1413-1421.

O'Brien, C., C. J. Baker-Fulco, A. J. Young and M. N. Sawka (1999). "Bioimpedance assessment of hypohydration." Med Sci Sports Exerc **31**(10): 1466-1471.

O'Connor, T., G. Dubowitz and P. E. Bickler (2004). "Pulse oximetry in the diagnosis of acute mountain sickness." High Alt Med Biol **5**(3): 341-348.

O'Hanlon, R., M. Wilson, R. Wage, G. Smith, F. D. Alpendurada, J. Wong, A. Dahl, D. Oxborough, R. Godfrey, S. Sharma, M. Roughton, K. George, D. J. Pennell, G. Whyte and S. K. Prasad (2010). "Troponin release following endurance exercise: is inflammation the cause? a cardiovascular magnetic resonance study." J Cardiovasc Magn Reson **12**: 38.

Ogawa, T. and A. J. de Bold (2012). "Brain natriuretic Peptide production and secretion in inflammation." J Transplant **2012**: 962347.

Okazaki, S., Y. Tamura, T. Hatano and N. Matsui (1984). "Hormonal disturbances of fluid-electrolyte metabolism under altitude exposure in man." Aviat Space Environ Med **55**(3): 200-205.

Olsen, N. V., I. L. Kanstrup, J. P. Richalet, J. M. Hansen, G. Plazen and F. X. Galen (1992). "Effects of acute hypoxia on renal and endocrine function at rest and during graded exercise in hydrated subjects." J Appl Physiol (1985) **73**(5): 2036-2043.

Pandolf, K. B. (1982). "Differentiated ratings of perceived exertion during physical exercise." Med Sci Sports Exerc **14**(5): 397-405.

Paredes, O. L., J. Shite, T. Shinke, S. Watanabe, H. Otake, D. Matsumoto, Y. Imuro, D. Ogasawara, T. Sawada and M. Yokoyama (2006). "Impedance

cardiography for cardiac output estimation: reliability of wrist-to-ankle electrode configuration." Circ J **70**(9): 1164-1168.

Park, H. J., S. H. Baek, S. W. Jang, D. B. Kim, D. I. Shin, W. S. Shin, P. J. Kim, H. B. Jung, H. O. Jung, K. B. Seung and K. B. Choi (2010). "Direct comparison of B-type natriuretic peptide and N-terminal pro-BNP for assessment of cardiac function in a large population of symptomatic patients." Int J Cardiol **140**(3): 336-343.

Peoples, G. E., T. Gerlinger, R. Craig and B. Burlingame (2005). "The 274th Forward Surgical Team experience during Operation Enduring Freedom." Mil Med **170**(6): 451-459.

Peronnet, F., P. Blier, G. Brisson, P. Diamond, M. Ledoux and M. Volle (1986). "Plasma catecholamines at rest and exercise in subjects with high- and low-trait anxiety." Psychosom Med **48**(1-2): 52-58.

Pieralli, F., I. Olivotto, S. Vanni, A. Conti, A. Camaiti, G. Targioni, S. Grifoni and G. Berni (2006). "Usefulness of bedside testing for brain natriuretic peptide to identify right ventricular dysfunction and outcome in normotensive patients with acute pulmonary embolism." Am J Cardiol **97**(9): 1386-1390.

Porchet, M., H. Contat, B. Waeber, J. Nussberger and H. R. Brunner (1984). "Response of plasma arginine vasopressin levels to rapid changes in altitude." Clin Physiol **4**(5): 435-438.

Powers, J. S., L. Choi, R. Bitting, N. Gupta and M. Buchowski (2009). "Rapid measurement of total body water to facilitate clinical decision making in hospitalized elderly patients." J Gerontol A Biol Sci Med Sci **64**(6): 664-669.

Provan, S. A., K. Angel, S. Odegard, P. Mowinckel, D. Atar and T. K. Kvien (2008). "The association between disease activity and NT-proBNP in 238 patients with rheumatoid arthritis: a 10-year longitudinal study." Arthritis Res Ther **10**(3): R70.

Pugh, L. G., M. B. Gill, S. Lahiri, J. S. Milledge, M. P. Ward and J. B. West (1964). "Muscular Exercise at Great Altitudes." J Appl Physiol **19**: 431-440.

Raff, H., B. M. Jankowski, W. C. Engeland and M. K. Oaks (1996). "Hypoxia in vivo inhibits aldosterone synthesis and aldosterone synthase mRNA in rats." J Appl Physiol (1985) **81**(2): 604-610.

Ramirez, G., P. A. Bittle, M. Hammond, C. W. Ayers, J. R. Dietz and G. L. Colice (1988). "Regulation of aldosterone secretion during hypoxemia at sea level and moderately high altitude." J Clin Endocrinol Metab **67**(6): 1162-1165.

Ramirez, G., D. Pineda, P. A. Bittle, H. Rabb, R. Rosen, D. Vesely and S. Sasaki (1998). "Partial renal resistance to arginine vasopressin as an adaptation to high altitude living." Aviat Space Environ Med **69**(1): 58-65.
Rhodes, H. L., K. Chesterman, C. W. Chan, P. Collins, E. Kewley, K. T. Pattinson, S. Myers, C. H. Imray and A. D. Wright (2011). "Systemic blood pressure, arterial stiffness and pulse waveform analysis at altitude." J R Army Med Corps **157**(1): 110-113.
Richalet, J. P. (2001). "The scientific observatories on Mont Blanc." High Alt Med Biol **2**(1): 57-68.
Richalet, J. P., A. Hornych, C. Rathat, J. Aumont, P. Larmignat and P. Remy (1991). "Plasma prostaglandins, leukotrienes and thromboxane in acute high altitude hypoxia." Respir Physiol **85**(2): 205-215.
Richalet, J. P., P. Merlet, M. Bourguignon, J. L. Le-Trong, A. Keromes, C. Rathat, B. Jouve, M. A. Hot, A. Castaigne and A. Syrota (1990). "MIBG scintigraphic assessment of cardiac adrenergic activity in response to altitude hypoxia." J Nucl Med **31**(1): 34-37.
Richalet, J. P., V. Rutgers, P. Bouchet, J. C. Rymer, A. Keromes, G. Duval-Arnould and C. Rathat (1989). "Diurnal variations of acute mountain sickness, colour vision, and plasma cortisol and ACTH at high altitude." Aviat Space Environ Med **60**(2): 105-111.
Ridker, P. M. (2001). "High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease." Circulation **103**(13): 1813-1818.
Roach, R., B. Kayser and P. Hackett (2011). "Pro: Headache should be a required symptom for the diagnosis of acute mountain sickness." High Alt Med Biol **12**(1): 21-22; discussion 29.
Roach, R. C., P. Bartsch, O. Oelz, P. H. Hackett and L. L. A. S. C. Committee (1993). The Lake Louise acute mountain sickness scoring system. Hypoxia and molecular medicine. H. C. Sutton JR, Coates G. Burlington, Vt, Charles S. Houston**:** 272-274.
Roach, R. C., E. R. Greene, R. B. Schoene and P. H. Hackett (1998). "Arterial oxygen saturation for prediction of acute mountain sickness." Aviat Space Environ Med **69**(12): 1182-1185.

Roach, R. C., C. S. Houston, B. Honigman, R. A. Nicholas, M. Yaron, C. K. Grissom, J. K. Alexander and H. N. Hultgren (1995). "How well do older persons tolerate moderate altitude?" West J Med **162**(1): 32-36.

Roach, R. C., D. Maes, D. Sandoval, R. A. Robergs, M. Icenogle, H. Hinghofer-Szalkay, D. Lium and J. A. Loeppky (2000). "Exercise exacerbates acute mountain sickness at simulated high altitude." J Appl Physiol (1985) **88**(2): 581-585.

Rock, P. B., W. J. Kraemer, C. S. Fulco, L. A. Trad, M. K. Malconian, M. S. Rose, P. M. Young and A. Cymerman (1993). "Effects of altitude acclimatization on fluid regulatory hormone response to submaximal exercise." J Appl Physiol (1985) **75**(3): 1208-1215.

Roeggla, G., M. Roeggla, A. Podolsky, A. Wagner and A. N. Laggner (1996). "How can acute mountain sickness be quantified at moderate altitude?" J R Soc Med **89**(3): 141-143.

Roggla, G., B. Moser, A. Wagner and M. Roggla (2000). "Correlation between raised body temperature and acute mountain sickness score at moderate altitude." Wien Klin Wochenschr **112**(6): 290-292.

Rolls, B. J., R. J. Wood, E. T. Rolls, H. Lind, W. Lind and J. G. Ledingham (1980). "Thirst following water deprivation in humans." Am J Physiol **239**(5): R476-482.

Rostrup, M. (1998). "Catecholamines, hypoxia and high altitude." Acta Physiol Scand **162**(3): 389-399.

Roudkenar, M. H., R. Halabian, P. Bahmani, A. M. Roushandeh, Y. Kuwahara and M. Fukumoto (2011). "Neutrophil gelatinase-associated lipocalin: a new antioxidant that exerts its cytoprotective effect independent on Heme Oxygenase-1." Free Radic Res **45**(7): 810-819.

Roudkenar, M. H., R. Halabian, A. M. Roushandeh, M. R. Nourani, N. Masroori, M. Ebrahimi, M. Nikogoftar, M. Rouhbakhsh, P. Bahmani, A. J. Najafabadi and M. A. Shokrgozar (2009). "Lipocalin 2 regulation by thermal stresses: protective role of Lcn2/NGAL against cold and heat stresses." Exp Cell Res **315**(18): 3140-3151.

Rupp, T., M. Jubeau, G. Y. Millet, S. Perrey, F. Esteve, B. Wuyam, P. Levy and S. Verges (2013). "The effect of hypoxemia and exercise on acute mountain sickness symptoms." J Appl Physiol (1985) **114**(2): 180-185.

Sampson, J. B., A. Cymerman, R. L. Burse, J. T. Maher and P. B. Rock (1983). "Procedures for the measurement of acute mountain sickness." Aviat Space Environ Med **54**(12 Pt 1): 1063-1073.

Sartori, C., L. Vollenweider, B. M. Loffler, A. Delabays, P. Nicod, P. Bartsch and U. Scherrer (1999). "Exaggerated endothelin release in high-altitude pulmonary edema." Circulation **99**(20): 2665-2668.

Savourey, G., N. Garcia, Y. Besnard, A. Guinet, A. M. Hanniquet and J. Bittel (1996). "Pre-adaptation, adaptation and de-adaptation to high altitude in humans: cardio-ventilatory and haematological changes." Eur J Appl Physiol Occup Physiol **73**(6): 529-535.

Savourey, G., J. C. Launay, Y. Besnard, A. Guinet and S. Travers (2003). "Normo- and hypobaric hypoxia: are there any physiological differences?" Eur J Appl Physiol **89**(2): 122-126.

Sawhney, R. C., A. S. Malhotra and T. Singh (1991). "Glucoregulatory hormones in man at high altitude." Eur J Appl Physiol Occup Physiol **62**(4): 286-291.

Scharhag, J., M. Herrmann, A. Urhausen, M. Haschke, W. Herrmann and W. Kindermann (2005). "Independent elevations of N-terminal pro-brain natriuretic peptide and cardiac troponins in endurance athletes after prolonged strenuous exercise." Am Heart J **150**(6): 1128-1134.

Scharhag, J., T. Meyer, M. Auracher, M. Muller, M. Herrmann, H. Gabriel, W. Herrmann and W. Kindermann (2008). "Exercise-induced increases in NT-proBNP are not related to the exercise-induced immune response." Br J Sports Med **42**(5): 383-385.

Scherr, J., B. Wolfarth, J. W. Christle, A. Pressler, S. Wagenpfeil and M. Halle (2013). "Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity." Eur J Appl Physiol **113**(1): 147-155.

Schoene, R. B. (2008). "Illnesses at high altitude." Chest **134**(2): 402-416.

Schommer, K., M. Hammer, L. Hotz, E. Menold, P. Bartsch and M. M. Berger (2012). "Exercise intensity typical of mountain climbing does not exacerbate acute mountain sickness in normobaric hypoxia." J Appl Physiol (1985) **113**(7): 1068-1074.

Self, D. A., J. G. Mandella, O. V. Prinzo, E. M. Forster and R. M. Shaffstall (2011). "Physiological equivalence of normobaric and hypobaric exposures of humans to 25,000 feet (7620 m)." Aviat Space Environ Med **82**(2): 97-103.
Shave, R., P. Ross, D. Low, K. George and D. Gaze (2010). "Cardiac troponin I is released following high-intensity short-duration exercise in healthy humans." Int J Cardiol **145**(2): 337-339.
Shigeoka, J. W., G. L. Colice and G. Ramirez (1985). "Effect of normoxemic and hypoxemic exercise on renin and aldosterone." J Appl Physiol (1985) **59**(1): 142-148.
Shintaro, S., K. Yuji, N. Tetsuya, K. Kenta and O. Hiedekazu (2006). "Change of plasma High Sensitive C-reactive protein in climbers." Jpn Med Assoc J **49**: 358-364.
Singh, I., C. C. Kapila, P. K. Khanna, R. B. Nanda and B. D. Rao (1965). "High-Altitude Pulmonary Oedema." Lancet **1**(7379): 229-234.
Singh, I., P. K. Khanna, M. C. Srivastava, M. Lal, S. B. Roy and C. S. Subramanyam (1969). "Acute mountain sickness." N Engl J Med **280**(4): 175-184.
Strobel, G., M. Neureither and P. Bartsch (1996). "Effect of acute mild hypoxia during exercise on plasma free and sulphoconjugated catecholamines." Eur J Appl Physiol Occup Physiol **73**(1-2): 82-87.
Sudoh, T., K. Kangawa, N. Minamino and H. Matsuo (1988). "A new natriuretic peptide in porcine brain." Nature **332**(6159): 78-81.
Sutton, J. R., J. T. Maher and C. S. Houston (1983). "Operation Everest II." Prog Clin Biol Res **136**: 221-233.
Sutton, J. R., G. W. Viol, G. W. Gray, M. McFadden and P. M. Keane (1977). "Renin, aldosterone, electrolyte, and cortisol responses to hypoxic decompression." J Appl Physiol Respir Environ Exerc Physiol **43**(3): 421-424.
Sviri, G. E., J. F. Soustiel and M. Zaaroor (2006). "Alteration in brain natriuretic peptide (BNP) plasma concentration following severe traumatic brain injury." Acta Neurochir (Wien) **148**(5): 529-533; discussion 533.
Tanino, Y., J. Shite, O. L. Paredes, T. Shinke, D. Ogasawara, T. Sawada, H. Kawamori, N. Miyoshi, H. Kato, N. Yoshino and K. Hirata (2009). "Whole body bioimpedance monitoring for outpatient chronic heart failure follow up." Circ J **73**(6): 1074-1079.

Tissot van Patot, M. C., G. Leadbetter, L. E. Keyes, J. Bendrick-Peart, V. E. Beckey, U. Christians and P. Hackett (2005). "Greater free plasma VEGF and lower soluble VEGF receptor-1 in acute mountain sickness." J Appl Physiol **98**(5): 1626-1629.

Torre-Bouscoulet, L., E. Chavez-Plascencia, J. C. Vazquez-Garcia and R. Perez-Padilla (2006). "[Precision and accuracy of "a pocket" pulse oximeter in Mexico City]." Rev Invest Clin **58**(1): 28-33.

Toshner, M. R., A. A. Thompson, J. B. Irving, J. K. Baillie, J. J. Morton and A. J. Peacock (2008). "NT-proBNP does not rise on acute ascent to high altitude." High Alt Med Biol **9**(4): 307-310.

Toth, M., K. H. Vuorinen, O. Vuolteenaho, I. E. Hassinen, P. A. Uusimaa, J. Leppaluoto and H. Ruskoaho (1994). "Hypoxia stimulates release of ANP and BNP from perfused rat ventricular myocardium." Am J Physiol **266**(4 Pt 2): H1572-1580.

Tuckey, H. (2013). Everest the first ascent. The untold story of Griffith Pugh, the man who made it possible., Randomhouse, London.

Usui, T., T. Yoshikawa, K. Orita, S. Y. Ueda, Y. Katsura, S. Fujimoto and M. Yoshimura (2011). "Changes in salivary antimicrobial peptides, immunoglobulin A and cortisol after prolonged strenuous exercise." Eur J Appl Physiol **111**(9): 2005-2014.

VanBruggen, M. D., A. C. Hackney, R. G. McMurray and K. S. Ondrak (2011). "The relationship between serum and salivary cortisol levels in response to different intensities of exercise." Int J Sports Physiol Perform **6**(3): 396-407.

Vasan, R. S., E. J. Benjamin, M. G. Larson, E. P. Leip, T. J. Wang, P. W. Wilson and D. Levy (2002). "Plasma natriuretic peptides for community screening for left ventricular hypertrophy and systolic dysfunction: the Framingham heart study." JAMA **288**(10): 1252-1259.

Vaz Perez, A., W. Doehner, S. von Haehling, H. Schmidt, A. V. Zimmermann, H. D. Volk, S. D. Anker and M. Rauchhaus (2010). "The relationship between tumor necrosis factor-alpha, brain natriuretic peptide and atrial natriuretic peptide in patients with chronic heart failure." Int J Cardiol **141**(1): 39-43.

Vila, G., M. Resl, D. Stelzeneder, J. Struck, C. Maier, M. Riedl, M. Hulsmann, R. Pacher, A. Luger and M. Clodi (2008). "Plasma NT-proBNP increases in response to LPS administration in healthy men." J Appl Physiol **105**(6): 1741-1745.

Volkers, M., D. Rohde, T. Zelniker, C. S. Weiss, E. Giannitsis, H. A. Katus and F. J. Meyer (2013). "High-sensitive Troponin T increase after exercise in patients with pulmonary arterial hypertension." BMC Pulm Med **13**: 28.

Ward, M., W., J. Milledge and W. JB. (2000). High Altitude Medicine and Phsyiology. London, Arnold.

Warner, M. M. and G. S. Mitchell (1991). "Role of catecholamines and beta-receptors in ventilatory response during hypoxic exercise." Respir Physiol **85**(1): 41-53.

Weidemann, A., B. Klanke, M. Wagner, T. Volk, C. Willam, M. S. Wiesener, K. U. Eckardt and C. Warnecke (2008). "Hypoxia, via stabilization of the hypoxia-inducible factor HIF-1alpha, is a direct and sufficient stimulus for brain-type natriuretic peptide induction." Biochem J **409**(1): 233-242.

West, J. B. (1982). "Diffusion at high altitude." Fed Proc **41**(6): 2128-2130.

West, J. B. (2007). "Point: the lactate paradox does/does not occur during exercise at high altitude." J Appl Physiol (1985) **102**(6): 2398-2399.

West, J. B. (2010). "American medical research expedition to Everest." High Alt Med Biol **11**(2): 103-110.

West, J. B. (2011). "Con: Headache should not be a required symptom for the diagnosis of acute mountain sickness." High Alt Med Biol **12**(1): 23-25; discussion 27.

West, J. B., S. Lahiri, M. B. Gill, J. S. Milledge, L. G. Pugh and M. P. Ward (1962). "Arterial oxygen saturation during exercise at high altitude." J Appl Physiol **17**: 617-621.

West JB, S. R., Milledge JS. (2007). High Altitude Medicine and Physiology. London, Arnold.

Westermann, I., M. W. Dunser, T. Haas, S. Jochberger, G. Luckner, V. D. Mayr, V. Wenzel, K. H. Stadlbauer, P. Innerhofer, N. Morgenthaler, W. R. Hasibeder and W. G. Voelckel (2007). "Endogenous vasopressin and copeptin response in multiple trauma patients." Shock **28**(6): 644-649.

Wolff, B., D. Haase, P. Lazarus, K. Machill, B. Graf, H. G. Lestin and D. Werner (2007). "Severe septic inflammation as a strong stimulus of myocardial NT-pro brain natriuretic peptide release." Int J Cardiol **122**(2): 131-136.

Woods, D., T. Hooper, P. Hodkinson, S. Ball, R. Wakeford, B. Peaston, C. Bairsto, N. Green and A. Mellor (2011). "Effects of altitude exposure on brain natriuretic peptide in humans." Eur J Appl Physiol **111**(11): 2687-2693.

Woods, D., T. Hooper, A. Mellor, P. Hodkinson, R. Wakeford, B. Peaston, S. Ball and N. Green (2011). "Brain natriuretic peptide and acute hypobaric hypoxia in humans." J Physiol Sci **61**(3): 217-220.

Woods, D. R., J. Begley, M. Stacey, C. Smith, C. J. Boos, T. Hooper, A. Hawkins, P. Hodkinson, N. Green and A. Mellor (2012). "Severe acute mountain sickness, brain natriuretic peptide and NT-proBNP in humans." Acta Physiol (Oxf) **205**(3): 349-355.

Woods, D. R., A. Davison, M. Stacey, C. Smith, T. Hooper, D. Neely, S. Turner, R. Peaston and A. Mellor (2012). "The cortisol response to hypobaric hypoxia at rest and post-exercise." Horm Metab Res **44**(4): 302-305.

Woods, D. R., A. Mellor, J. Begley, M. Stacey, J. O'Hara, A. Hawkins, J. Yarker, S. Foxen, C. Smith and C. Boos (2013). "Brain natriuretic peptide and NT-proBNP levels reflect pulmonary artery systolic pressure in trekkers at high altitude." Physiol Res **62**(6): 597-603.

Yalow, R. S. and S. A. Berson (1959). "Assay of plasma insulin in human subjects by immunological methods." Nature **184 (Suppl 21)**: 1648-1649.

Yock, P. G. and R. L. Popp (1984). "Noninvasive estimation of right ventricular systolic pressure by Doppler ultrasound in patients with tricuspid regurgitation." Circulation **70**(4): 657-662.

Young, A. J., A. Cymerman and K. B. Pandolf (1982). "Differentiated ratings of perceived exertion are influenced by high altitude exposure." Med Sci Sports Exerc **14**(3): 223-228.

Zaccaria, M., S. Rocco, D. Noventa, M. Varnier and G. Opocher (1998). "Sodium regulating hormones at high altitude: basal and post-exercise levels." J Clin Endocrinol Metab **83**(2): 570-574.

Zafren, K. (2012). "Does ibuprofen prevent acute mountain sickness?" Wilderness Environ Med **23**(4): 297-299.

Zouhal, H., C. Jacob, P. Delamarche and A. Gratas-Delamarche (2008). "Catecholamines and the effects of exercise, training and gender." Sports Med **38**(5): 401-423.