The Brain at Altitude:
The Cerebral Vasculature, Hypoxia and Headache

Mark Howard Wilson

Department of Medicine

UCL

PhD Thesis

I, Mark Howard Wilson, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Prologue

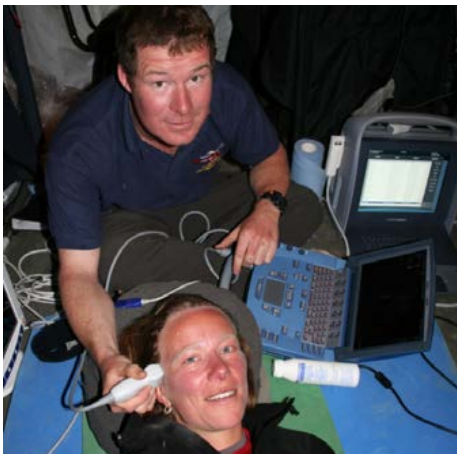
For many years I have been fascinated by the neuro(patho)physiology of high altitude, hypoxia and microgravity. This interest has led me to question some of the tenets that are currently taught as 'fact'. At best, some appear oversimplifications. By way of example, I have questioned and explored the Monro-Kellie doctrine. This describes the skull as a closed box within which the addition of mass leads, after a period of compliance, to a rise in intracranial pressure (ICP). However, "mass" added in response to hypoxia largely represents that of blood (as cerebral blood flow changes). As a result, pressure changes dynamically.

Some 60% of astronauts suffer space motion sickness (space adaptation syndrome), the development of which has been linked to rising intracranial pressure. My BSc project investigated the use of ultrasound to measure changes in transcranial distance as an index of intracranial pressure in astronauts. Subsequently, I worked in Pre-Hospital Care (with HEMS, London's Air Ambulance) before becoming a neurosurgeon- both situations in which changes in ICP can be matters of life and death. Meanwhile, a brief spell working for the Himalayan Rescue Service had allowed me to observe subjects with presumed raised ICP in response to the hypoxia of altitude.

These experiences led me to first consider the key factors that regulate ICP. At that time, research was largely focussed on changes in arterial inflow, which rises substantially in response to hypoxia (just as it can in cases of cerebral trauma). However, I was struck by the similarity between the headaches described by my patients with venous hypertension, and those experienced by high-altitude mountaineers. I thus postulated that differences in venous efferent structure, or venous engorgement, might play an important role in regulating ICP when cerebral arterial inflow rises. I began to explore this concept further. Shortly after UCL's Centre for Altitude, Space and Extreme Environment (CASE) was born, I became the lead for Neuroscience Research, allowing me to pursue this issue further. Expeditions with the Birmingham Medical Research Expeditionary Society (BMRES) to Ladakh, India were followed by a UCL pilot expedition to Cho Oyu, an 8201m peak in China, in 2006.

This thesis is the culmination of (or perhaps one major stop upon) this journey. The climb has been hard. But I hope you find the view as exciting and worthwhile as I do.

Transcranial Ultrasound with NASA
1994



…and again on Everest
2007

Dedication

To my wife Kelly and my children Katharine and Oscar.

Acknowledgements

A huge number of people have been involved in the research in parallel with this thesis.

Caudwell Xtreme Everest: Mike Grocott, Denny Levett, Dan Martin, Monty Mythen, Kay Mitchell, Sundeep Dhillon and especially Chris Imray and Hugh Montgomery who have helped me in so many ways over many years.
Investigators who have helped in this and other data capture include: V. Ahuja, G. Aref-Adib, R. Burnham, A.Chisholm, K. Clarke, D. Coates, M. Coates, D. Cook, M. Cox, S. Dhillon, C. Dougall, P. Doyle, P. Duncan, M. Edsell, L. Edwards, L.Evans, P. Gardiner, M. Grocott, P. Gunning, N. Hart, J. Harrington, J. Harvey, C. Holloway, D. Howard, D. Hurlbut, C. Ince, M. Jonas, J. van der Kaaij, M. Khosravi, N. Kolfschoten, D. Levett, H. Luery, A. Luks, D. Martin, R. McMorrow, P. Meale, K. Mitchell, H. Montgomery, G. Morgan, J. Morgan, A. Murray, M. Mythen, S. Newman, M. O'Dwyer, J. Pate, T. Plant, M. Pun, P. Richards, A.Richardson, G. Rodway, J. Simpson, C. Stroud, M. Stroud, J. Stygal, B. Symons, P. Szawarski, A. Van Tulleken, C. Van Tulleken, A. Vercueil, L. Wandrag, J. Windsor;
Scientific Advisory Group for Xtreme Everest– B. Basnyat, C. Clarke, T. Hornbein, J. Milledge, J. West.
Birmingham Medical Research Expeditionary Society: Chris Imray, Alex Wright, Jo Bradwell
Retinal Imaging: Graeme Holland and Derek Styles
MRI and Sea Level Studies / Analysis: Indran Davagnanam, John Thornton, Xavier Golay, Cameron Holloway, Lisa Strycharczuk, Cathy Green, Alexander Tamm, Nadia Griva, Jan Stygall, Raj Dattani
Neurosurgical colleagues: Neil Kitchen, Fergus Robertson, Stefan Brew and Laurence Watkins.
NASA: Prof Alan Hargens – without whom none of this would have happened.
Statistics: Shashivadan Hirani
Proof Reading: Juliet Solomon
I must also thank the Guoman "Tower Thistle" Hotel – the majority of this PhD was written in their foyer.

Supervisors: Prof Hugh Montgomery and Prof Stan Newman
Examiners: Prof Martin Smith and Peter Robbins

Funding:

This PhD is self funded, however, sponsorship was sought for individual studies:

Caudwell Xtreme Everest: Mr John Caudwell, BOC Medical (now part of Linde Gas Therapeutics), Eli Lilly Critical Care, The London Clinic, Smiths Medical, Deltex Medical and The Rolex Foundation (unrestricted grants). Peer reviewed research grants were awarded by the Association of Anaesthetists of Great Britain and Ireland (AAGBI), the UK Intensive Care Foundation and the Sir Halley Stewart Trust. The CXE volunteers who trekked to Everest base camp also kindly donated to support the research. Some of this work was undertaken at University College Hospitals - University College London Comprehensive Biomedical Research Centre which received a portion of funding from the UK Department of Health Research Biomedical Research Centres funding scheme.

MRI Studies: The JABBS fund (through BMRES) kindly sponsored the Venous MRI study.

Abbreviations in this thesis:

ACE	Angiotensin Converting Enzyme
ADC	Apparent Diffusion Coefficient
AHS	Ascent Headache Score
AMS	Acute Mountain Sickness
AOE	Arterial Oxygen Extraction
AT	Anaerobic Threshold
CCS	Combined Conduit Score
CBF	Cerebral Blood Flow
CXE	Caudwell Xtreme Everest
CPET	Cardiopulmonary Exercise Testing
CSF	Cerebro-Spinal Fluid
DFI	Dynamic Flow Index
EBC	Everest Base Camp
$EtCO_2$	End Tidal Carbon Dioxide
EtO_2	End Tidal Oxygen
EPO	Erythropoietin
FiO_2	Partial Pressure of Inspired Oxygen
HACE	High Altitude Cerebral (O)edema
HAPE	High Altitude Pulmonary (O)edema
HD	Headache Duration
HAH	High Altitude Headache
HIF	Hypoxia Inducible Factor
HS	Headache Severity (grade 0-4)
HSI	Headache Severity Index (HS x HD)
ICP	Intracranial Pressure
LL	Lake Louise
MAP	Mean Arterial Blood Pressure (/mmHg)
MCA	Middle Cerebral Artery
MCAv	Middle Cerebral Artery Velocity
MCA_D	Middle Cerebral Artery Diameter
MRI (A)	Magnetic Resonance Imaging (Angiography)
NIRS	Near Infrared Spectroscopy
NO	Nitric Oxide

ONSD	Optic Nerve Sheath Diameter
PI	Pulsitility Index
PVI	Pressure Volume Index
RI	Resistivity Index
rSO_2	Regional Brain Oxygen Saturation (/%)
SaO_2	Peripheral Arterial Oxygen Saturation (/%)
TCD	Transcranial Doppler
TIA	Transient Ischaemic Attack
THS	Total Headache Score (sum of all HS)
TMD	Tympanic Membrane Displacement
VEGF	Vascular Endothelial Growth Factor
VO_2Max	Maximal rate of Oxygen Consumption

Brief (300 word) Abstract:

This thesis studies the effect of hypoxia (at rest and during exercise) on the arterial and venous cerebral circulation, investigating the venous system role in high altitude headache.

Methods: 1) **Hypobaric hypoxic studies** investigated 198 trekkers and 24 Investigators to 5300m, 14 to 6400m and 8 to 8848m. 2) **Normobaric hypoxic studies** used Magnetic Resonance Imaging (MRI)) at sea-level. Four domains were addressed:

i. Arterial*: Hypobaric hypoxia:* (n=24) Transcranial Doppler (TCD) measured middle cerebral artery diameter (MCA_D) and blood velocity (MCA_v). *Sea-Level normobaric hypoxia:* (n=7) A hypoxicator (FiO_2 = 11%) for 3 hours with a 3Tesla MRI scan measured MCA_D and MCA_v.
ii. Brain Oxygenation: Near Infrared Spectroscopy (NIRS) monitored Regional Brain Oxygenation (rSO_2).
iii. Venous: Retinal imaging at altitude and MRI at sea-level assessed the venous system.
iv. Headache: A daily diary recorded headache burden.

Results: Arterial: Hypobaric and normobaric hypoxia induced MCA dilatation. Mean (±(SEM)) MCA_D increased in hypoxia (from 5.23(±0.23)mm (at 5300m) to 9.34(±0.88)mm (at 7950m)($p<0.001$) (TCD). At sea-level, (after 3 hours FiO_2 = 11%) MCA_D increased from 3.04(±0.13)mm to 3.27(±0.13)mm (MRI).

Brain Oxygenation: rSO_2 decreased more than peripheral arterial saturation (SaO_2), especially during exercise. The relative percentage reduction in resting SaO_2 and rSO_2 from 75m to 5300m was -22.23 ±0.56% and -30.61 ±1.28% ($p<0.001$) respectively.

Venous: Hypoxia induced retinal and cerebral venous distension. Twenty-three of 24 subjects exhibited retinal venous distension (range 5 to 44%). Degree of distension correlated with headache ($r = 0.553$, $p=0.005$). Possession of a narrow transverse sinus strongly related to retinal and cerebral venous distension and headache.

Headache: Headache Severity Index (HSI) (headache score x duration) correlated inversely to both lateral and third ventricular volumes summed ($r = -0.5$, $p = 0.005$) and pericerebellar CSF volume ($r = -0.56$, $p = 0.03$).

Conclusions: Large cerebral arteries dilate and veins distend with hypoxia. This suggests an important influence of cerebral venous anatomy and physiology on headache, with implications for pathophysiological states and their management.

Full Abstract

Aims

In this thesis, I aim to investigate the effect of hypoxia on the arterial and venous cerebral circulation. I seeks to characterise the response of cerebral arterial calibre (as a regulator of flow) to hypoxia, to investigate changes in cerebral oxygenation both at rest and with exercise during systemic hypoxia and, uniquely, to investigate whether the venous system might have a role in the pathogenesis of high altitude headache.

Methods

The studies within this thesis fall into two categories. **Hypobaric hypoxic studies** were conducted as part of the Caudwell Xtreme Everest Expedition (CXE) of 2007, the partial pressure of inspired oxygen falling with ascent to altitude. This studied 198 trekkers and 24 Investigators in London (75m), Kathmandu (1300m), Namche Bazaar (3500m) and Everest Base Camp (5300m). Fourteen of the investigators were also studied in the Western Cwm (6400m), and 8 of these at the summit (8848m). Meanwhile, **normobaric hypoxic studies** (with Magnetic Resonance Imaging (MRI)) were performed at The National Hospital for Neurology and Neurosurgery, Queen Square, University College London. Across these, four domains were addressed:

v. Arterial Studies*: Hypobaric hypoxic studies* were performed using Transcranial Doppler (TCD) at rest on the 24 investigators. Right Middle Cerebral Artery Velocity (MCA_v) and right Middle Cerebral Artery Diameter (MCA_D) were measured at each altitude and cross sectional area and blood flow subsequently calculated. *Sea-Level normobaric hypoxic studies* (n=7) were performed using a hypoxicator (FiO_2 = 11%) for 3 hours ending concurrently with a 3Tesla Magnetic Resonance Imaging scan. Similarly MCA_v and MCA_D were measured using this technique.

vi. Brain Oxygenation Studies: Regional Brain Oxygenation (rSO_2) was monitored at rest and during exercise in both the CXE trekker and investigator groups using Near Infrared Spectroscopy (NIRS). Cluster analysis was performed to investigate if desaturation at sea level predicted desaturation at altitude.

vii. Venous Studies: The venous system was investigated dynamically with two types of study: *Retinal Imaging:* All CXE investigators had retinal imaging performed at sea level and again at Everest Base Camp with arterial and venous measurements made. *Magnetic Resonance Imaging:* Static (anthropomorphic) T1 MRIs and Dynamic (Susceptibility Weighted and Gadolinium Enhanced MR Venograms) were performed in a number of normobaric normoxic and hypoxic experiments.

viii. Headache Assessment: Headache burden was monitored in CXE subjects by daily diary recording of headache score and duration to create a number of different scores including headache severity index.

These domains were related to one another, in a manner determined *a priori*.

Results

Arterial Studies: Mean (±(SEM)) middle cerebral artery (MCA) diameter increased in hypoxia (from 5.23(±0.23)mm (at 5300m) to 6.66(±0.32)mm (at 6400m) to 9.34(±0.88)mm at 7950m as measured using TCD ($p<0.001$). At sea level, after 3 hours of hypoxia (FiO_2 = 11%) MCA increased from 3.04(±0.13)mm to 3.27(±0.13)mm, when measured using MRI. At altitude this dilatation was found to increase estimated MCA flow (from 13.30(±0.97)ml/sec at 75m to 41.15(±8.5)ml/sec at 7950m ($p<0.01$)), which increased estimated oxygen delivery (from 2.47(±0.19)mlO_2/sec to 6.98(±1.39)mlO_2/sec ($p<0.01$)). Using MRI at sea-level it was found that dilatation increased estimated MCA flow (from 2.33(±0.33)mls/sec vs 3.23 (±0.48)mls/sec ($p=0.01$) and maintained oxygen delivery (0.44mls(±0.06)O_2/sec vs 0.45(±0.07)mlsO_2/sec $p=0.58$).

Brain Oxygenation Studies: rSO_2 decreased more rapidly relative to peripheral arterial saturation (SaO_2), especially during exercise. The relative percentage reduction in resting SaO_2 from 75m to 5300m was -22.23 ± 0.56%. The relative percentage reduction in resting rSO_2 from 75m to 5300m was -30.61 ± 1.28% (paired t test $p<0.001$). During exercise at 5300m, relative SaO_2 fell by 9.2 + 0.59% between rest and VO_2Max while relative rSO_2 fell 15.6 +0.97% between rest and VO_2Max (paired t-test $p=0.002$). Cluster analysis demonstrated that those who cerebrally desaturate during exercise at sea level desaturate more at altitude ($p<0.05$).

Venous Studies: Retinal and cerebral venous distension occurred in response to hypoxia. Twenty-three of 24 subjects exhibited retinal venous distension ranging from 5 to 44%. The degree of this distension correlated with ascent headache score ($r = 0.553$, $p=0.005$). A correlation was also demonstrated between peripheral saturation at 5300m and the change in venous retinal vessel diameter ($r = -0.55$, $p = 0.005$). Similarly, $ETCO_2$ at 5300m also correlated with retinal venous vessel diameter ($r =-0.4$, $p = 0.05$)

Similarly, transverse sinus morphology (the narrowing of one or both transverse sinuses) was strongly related to the degree of both retinal and cerebral venous distension and with headache burden. A hypoxic Magnetic Resonance Venogram study demonstrated that cerebral and retinal vein engorgement correlated ($r = 0.598$, $p=0.05$), and rose as Combined Conduit Score (CCS - a measure of venous outflow restriction) fell ($r=-0.75$, $p<0.05$).

Headache Assessment: Headache Severity Index (HSI) was calculated as headache score multiplied by the duration of headache each day as a measure of headache burden at altitude. In addition to the venous relations above, HSI correlated inversely to both lateral and third ventricular volumes summed ($r = -0.5$, $p = 0.005$) and pericerebellar CSF volume ($r = -0.56$, $p = 0.03$). HSI only related to rSO_2 when preceded by an acute drop in FiO_2 (increase in altitude).

Conclusions

An increase in cerebral arterial blood flow occurs in response to hypoxia, to which an increase in cerebral arterial diameter contributes. Until now, this had been thought to remain unchanged. Increased cerebral blood flow can lead to venous engorgement when outflow is compromised, and this correlates with headache. This suggests an important influence of cerebral venous anatomy and physiology on ICP regulation, with possible significant implications for pathophysiological states and their management.

Publications:

First Author Publications resulting from this thesis (See Appendix – Cits = Citations)

Title	Authors	Journal	Date	Cits	IF
The cerebral effects of ascent to high altitudes	Wilson MH, Newman S, Imray CH	Lancet Neurology	2009	126	23.5
Direct measurement of ICP at High Altitude and Correlation of ventricular Size with AMS	Wilson MH, Milledge J	Neurosurgery	2008	24	2.79
Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia	Wilson MH, Edsell, M *et al*	Journal of Cerebral Blood Flow and Metabolism	2011	47	5.01
Stroke at high altitude diagnosed in the field using portable ultrasound	Wilson MH, Levett DZ *et al*	Wilderness Medicine	2011	6	0.94
The headache of high altitude and microgravity – Similarities with clinical syndromes of cerebral venous hypertension	Wilson MH, Imray CHE and Hargens AR	High Altitude Medicine and Biology	2011	10	1.77
The cerebral venous system and anatomical predisposition to high altitude headache	Wilson M, Davaganam I, Holland G *et al*	Annals of Neurology	2013	6	11.1
Brain oxygenation at rest and during exercise at altitude	Wilson MH *et al*	Extreme Physiology and Medicine	Being Submitted		
Neurosciences on Everest	Wilson MH, Kitchen, N	World Neurosurgery	2010		0.68

Publications as co-author resulting from this thesis and other first author related publications (See Appendix)

Title	Authors	Journal	Date	Cits	IF
Changes in pupil dynamics at altitude	Wilson MH, Edsell M, Imray C, Wright A	High Altitude Medicine and Biology	2008	9	1.77
Design and conduct of Caudwell Xtreme Everest	Levett DZH, Martin DS, Wilson MH *et al*	BMC Medical Research Methodology	2010	12	2.67
Caudwell Xtreme Everest Expedition	Grocott, M, Martin D, Wilson MH *et al*	High Altitude Medicine and Biology	2010	11	1.77
High Altitude Ataxia – Its assessment and Relevance	Bird B, Wright A, Wilson MH *et al*	Wilderness and Environmental medicine	2011	3	0.94

Letters

Title	Authors	Journal	Date	Cits	IF
High-altitude cerebral effects: risks and mechanisms	Wilson MH, Imray C	Lancet Neurology	2009		23.5
High altitude is / is not for the birds!	Imray C, Wilson MH,	Journal of Applied Physiology	2011	4	3.75
Has anyone seen my executive function recently?	Imray C, Kelly A, Wilson, M	Wilderness Environmental Medicine	2013		0.94

Book Chapters

Chapter Title	Authors	Book	Editor	Date
Space Medicine	Fong K and Wilson MH	Oxford Textbook of Travel Medicine	Zuckerman, J	2013
The Cerebral Circulation and Brain at High Altitude	Ainslie P, Wilson MH and Imray C	High Altitude	Swenson E and Bärtsh P	In press

Table of Contents

Chapter 1: INTRODUCTION AND BACKGROUND

See "The Brain at Altitude – The cerebral effects of ascent to high altitude" in Lancet Neurology (Wilson, Newman et al. 2009) for a shorter paper derived from this literature review, and "Direct measurement of intracranial pressure at high altitude and correlation of ventricular size with acute mountain sickness" in Neurosurgery (Wilson and Milledge 2008) which reported the results of a preliminary investigation.

1.1 Abstract

Cellular hypoxia is the final common mechanism of brain injury, not just in asphyxia, but also in the diverse conditions in which cerebral perfusion is impaired directly (for instance, by embolic stroke) or indirectly (by raised intracranial pressure, such as that which occurs after head injury). Exposure to altitude (and thus hypobaric hypoxia) appears to offer a reproducible model for the study of cerebral cellular hypoxia in normal individuals. This chapter reviews the accepted understanding, at the time of this thesis commencement, of neurological clinical syndromes that occur upon such exposure, and the physiology, genetics and molecular mechanisms that underpin them. High Altitude Illness has been considered a spectrum of diseases and symptoms, from High Altitude Headache (HAH) through to Acute Mountain Sickness (AMS) and then High Altitude Cerebral (O)Edema (HACE). All represent clinical syndromes rather than defined illnesses: indeed, evidence for the existence of oedema in HACE is sparse. Most investigations of the aetiology of HAH (and HACE in particular) have, to date, focused on parameters that are relatively easy to study. These include arterial blood flow (measured using transcranial Doppler, TCD) and circulating or cellular "factors" (such as Hypoxia Inducible factor, HIF; Vascular Endothelial Derived Growth Factor, VEGF; and Nitric Oxide, NO). The venous system has received far less attention, largely because the tools to study it (in the field or laboratory) are far less well developed. In addition, because venous vessels lack muscle within their walls, they have been considered 'mere passive conduits' of no relevance to hypoxia. In this introduction, I shall demonstrate that the formation of retinal haemorrhages is associated with that of microhaemorrhages in the brain, and that both are caused by venous hypertension. This, and similarities with clinical syndromes of

venous hypertension, underpin my hypothesis that the venous system is involved in high altitude headache.

1.2 Introduction

With air travel providing easier mountain access, and increasing demand for adventurous holidays, millions of people now travel to altitude each year to ski, trek, climb and work (as soldiers, astronomers, miners and guides). Consequently, Acute Mountain Sickness (AMS) has become a common complaint in such travellers. As a result of differences in definition and ascent profiles between studies, the reported prevalence of AMS varies widely, but approximately half of trekkers ascending to 5000m are affected (Vardy and Judge 2006). In recent years, AMS and HACE have become occupational hazards for the workers on the Qinghai-Tibetan Railway with an overall incidence of 45-95% and 0.49% respectively (Wu, Ding et al. 2006; Wu, Ding et al. 2007) and for the rapidly expanding populations of native lowland Chinese in Tibet. An awareness of the presentations, pathogenesis and treatment of altitude-related illness is thus increasingly important to the medical profession.

Existing reviews have described well the epidemiology and management of high altitude illness (Hackett and Roach 2001; Basnyat and Murdoch 2003). This chapter reviews the current understanding of neurological clinical syndromes and the underlying pathophysiological changes in cerebral perfusion and oedema formation that occur on ascent to altitude. It also explores the possible molecular and genetic mechanisms involved and the neuropsychological sequelae.

Search Strategy and selection criteria:
An extensive literature review of articles listed within PubMed from 1969 using MESH terms [Brain] and combinations of [Altitude], [Hypoxia], [Cerebral Blood Flow], [Acute Mountain Sickness], [High Altitude Cerebral (O)Edema] and [Neuropsychology] form the basis of this review. In addition, abstracts from recent international scientific meetings were considered. As requested for the Lancet Neurology review, priority was given to more recent publications, and especially to those published since 2005.

1.2.1 Clinical Relevance and Translation of Research

The findings from this thesis can translate into the physiological changes that occur in some forms of brain injury. However, probably of greater translational significance are the cerebral physiological changes that occur with extreme systemic hypoxia to brain (and non-brain) injured patients in the critical care environment.

The controlled study of restrictions in cerebral oxygenation resulting from brain injury is difficult because injuries vary by mechanism (e.g. gunshot, subarachnoid haemorrhage, ischemia) and location (e.g. frontal, parietal), and due to heterogeny in patient characteristics (such as age). Because most effects of short duration hypobaric hypoxia are reversible, the study of the brain at altitude offers a clean, repeatable, controlled, prospective, ethical model of brain responses to hypoxia with few confounding variables (Grocott, Montgomery et al. 2007). Whilst elements of the pathophysiology of hypoxic brain injury will differ from (for example) traumatic brain injury, there may well be more mechanisms in common than might be initially considered. Cellular hypoxia can be the result of failure in any stage of oxygen delivery to a tissue bed. Ischemia refers to lack of blood flow which itself results in tissue hypoxia although other mechanisms relating to the presence of coagulated blood and failure to remove waste products may be superimposed. Carbon monoxide and cyanide poisoning, and anoxia associated with cardiopulmonary arrest, all result in cerebral oedema, loss of grey-white differentiation and then selective damage starting with watershed areas and areas with high metabolic rate (such as the basal ganglia) (Varnell, Stimac et al. 1987; James 1988; Kasamo, Okuhata et al. 1993; Mills, Gunasekar et al. 1999). Traumatic brain injury and brain tumours often result in cerebral oedema that, if ICP rises, can compromise perfusion and, especially in trauma, cause subsequent ischaemic injury. Similar pathophysiological processes are thought to occur in HACE. Obstructive Sleep Apnoea with periodic breathing and COPD result in chronic hypoxia with intermittent exacerbations of acute hypoxia in a similar manner to the effects of altitude.

In the critical care environment, hypoxia is commonplace. Hypoxaemia can occur with hypoventilation, ventilation/perfusion mismatch, right-to-left shunting or limitation of diffusion across the alveolar-capillary membrane. Tissue (and cerebral) hypoxia may arise as a consequence of hypoxaemia or as a result of reduced oxygen delivery due to decreased cardiac output or decreased circulating haemoglobin concentration (anaemia). It may also occur with the systemic inflammatory response syndrome, with microcirculatory dysfunction or alterations in cellular and mitochondrial function (Brealey, Brand et al. 2002). Moreover, neuronal ischaemia is a common result of a number of specific disease processes from direct obstruction of cerebral arterial flow (e.g. embolic infarction) or from reduced perfusion (e.g. from raised intracranial pressure).

Failure to adapt to hypoxia therefore has a high price. The mechanisms of such cellular adaption (for example, the sequelae resulting from the non-degradation of factors such as hypoxia-inducible factor 1α [HIF 1α], and its potential roles in cancer, cardiac and neuronal pathologies) have been extensively studied (Ogunshola and Al-Ahmad 2012; Ong and Hausenloy 2012; Rohwer, Zasada et al. 2012; Hu, Liu et al. 2013). This can be achieved in standard laboratory conditions. The study of macrocellular changes is more difficult. Rodent brains are very different to those of humans. Size and adaptation to upright posture mean that physiological changes in one may not necessarily translate to the other. Studying patients is difficult because of the heterogeneity of disease (injury type), location (within the brain) and constitutional factors of patients. However, exploration of the mechanisms underlying differences in susceptibility to hypoxia-induced injury (be they physiological pathways such as those regulating compensatory oxygen delivery, pathophysiological pathways influencing oedema formation, or anatomical factors affecting cerebral or cranial compliance) may suggest novel prophylactic or therapeutic targets of broad clinical relevance. A means by which to study the cerebral response to sustained hypoxia is required in order to achieve this.

1.2.2 Changes in Atmosphere with Increasing Altitude

Whilst barometric pressure decreases exponentially as altitude is gained, the percentage of each gas component of air remains the same up to 12,000m.

Hence, although the percentage of oxygen remains constant at 20.93%, increasing altitude results in a lower inspired partial pressure of oxygen (figure 1-1). This reduction in driving gradient on the oxygen cascade may compromise the supply of adequate oxygen to the tissues (figure 1-2).

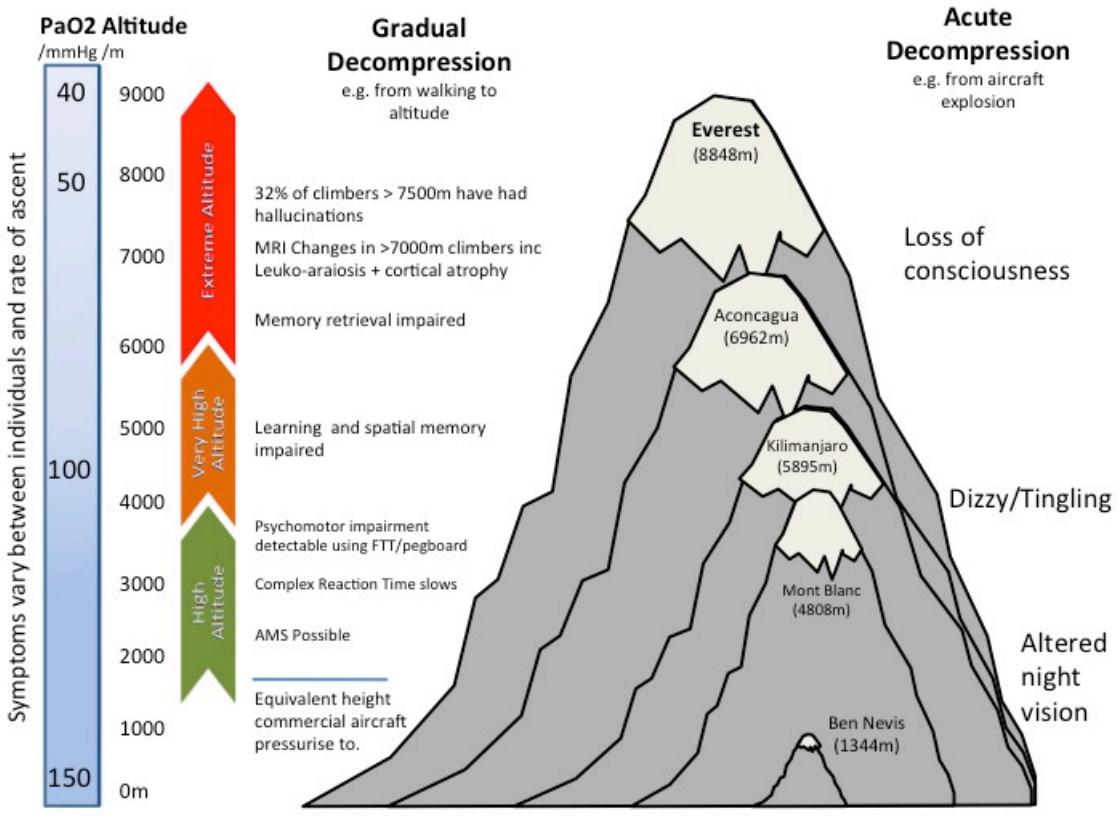


Figure 1-1 The relationship between altitude (classified as high (1500-3500m), very high (3500-5500m) and extreme (>5500m)) (Dietz 2008), the partial pressure of inspired oxygen (PiO_2), and some of the neurological consequences of acute and gradual exposure to these pressure changes. Note – the neurological consequences vary greatly from person to person and with rate of ascent. HACE is far more common at higher altitudes although there are case reports at 2,500m. Data are derived as follows: Hallucinations (Garrido, Javierre et al. 2000), MRI changes (Garrido, Castello et al. 1993) , Memory Retrieval (Kramer, Coyne et al. 1993), Learning (Kramer, Coyne et al. 1993) Spatial (Nelson 1982) , Psychomotor (Berry, McConnell et al. 1989) , CRT(Fowler, Elcombe et al. 1987), West and Milledge (West, Schoene et al. 2007))

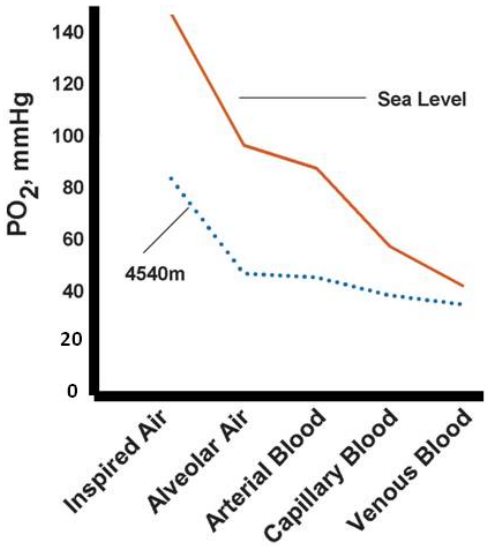


Figure 1-2 The oxygen transport cascade at sea level (red line) and 4540m (blue line) illustrating oxygen partial pressure at the major stages of oxygen delivery, suggesting potential points of functional adaptation. Adapted from Beall, 2007 (Beall 2007).

Whilst compensatory hyperventilation, tachycardia, erythropoietin-induced

polycythaemia and increased cerebral blood flow partially maintain cerebral oxygen delivery at altitude (West, Schoene et al. 2007), the brain remains exquisitely sensitive to hypoxia and consequently is the first organ to be compromised when these mechanisms are inadequate.

1.2.3 The History of the Effects of Acute Hypobaric Hypoxia

The neurological effects of altitude exposure have long been recognised. Plutarch (326 BC) described several features of mountain sickness during Alexander's march over India (Plutarch 1912) and Rustinian (1298 AD) recorded similar symptoms suffered by Marco Polo during his exploration of Tibet (Castelló-Roca 1993). Jose de Acosta, a Spanish Jesuit of the sixteenth century described headaches sickness and vomiting while crossing the Andes.

On 5th September 1862 two British balloonists, Glaisher and Coxwell, ascended to over 8800m (Glaisher 1862; West 2004; Rodway 2007). Glaisher reported paralysis of his arms and legs and sudden loss of vision before losing consciousness (figure 1-3). Coxwell lost the use of his hands and could only open the valve to initiate balloon descent by pulling the cord with his teeth. After landing, they walked seven miles to the nearest village with no residual neurological deficits. Thirteen years later, the first deaths attributable to acute high altitude hypoxia exposure were reported when three Frenchmen lost consciousness ascending through 7000m and two died (Bert 1943).

Figure 1-3 James Glaisher (unconscious) and Henry Coxwell (pulling the valve chord with his teeth having lost use of his hands) above 8,800m (1862) (West 2004).

These early accounts relate to the acute neurological effects of hypobaric hypoxia. The first detailed clinical descriptions of the consequences of slower ascent were given by Thomas Ravenhill in 1913 whilst a medical officer at the Collahuasi and Poderosa mines in northern Chile at altitudes between 4690m and 4940m (Ravenhill 1913; West 1996). He provided a classification of high

altitude illness and described the features of both high altitude cerebral and pulmonary oedema.

1.3 Clinical Syndromes at Altitude

1.3.1 Classification of Neurological Effects of Hypobaric Hypoxia in Adults

Neurological Effects of Hypobaric Hypoxia
High Altitude Headache
Acute Mountain Sickness
High Altitude Cerebral Edema
Other neurological "events" reported at altitude
Transient ischaemic attacks
Cerebral infarction and haemorrhage
Migraine
Cerebral venous thrombosis
Seizures
High altitude syncope
Cranial nerve palsies
Ophthalmological disturbances: retinal haemorrhages; amaurosis fujax; cortical blindness
Acute cerebral dysfunction of extreme altitude (related to additional acute cerebral hypoxia e.g. from HAPE – rapidly reverses with oxygen)
Neuropsychological effects:
Déjà vu
Emotional lability
Cognitive slowing/inaccuracies
Hallucinations/third man

Hypobaric hypoxia has a spectrum of pathophysiological effects on the brain (Box 1-1). Slowing the ascent rate can reduce the occurrence of most altitude specific syndromes. Too rapid ascent is thought to cause (in increasing severity) High Altitude Headache, Acute Mountain Sickness and then potentially life threatening, High Altitude Cerebral Oedema. Current recommendations are to sleep no higher than 300m above the previous night with a rest day for every 1000m climbed. "Climb high, sleep low" is a common adage.

Box 1-1 Neurological effects of ascent to high altitude

1.3.2 High Altitude Headache (HAH)

The International Headache Society (2004) define the diagnostic criteria for HAH as A) headache exhibiting at least two of the following characteristics: frontal or fronto-temporal, dull or pressing, mild or moderate and aggravated by exertion, movement, straining, coughing or bending, and associated with; B) ascent to altitude over 2,500m, and which; C) has developed in the last 24 hours and; D) resolves within 8 hours of descent.

HAH occurs in up to 80% of visitors to altitude and can be difficult to distinguish from headache secondary to dehydration. HAH should resolve with use of

simple analgesics (paracetamol / ibuprofen) but further management consists of rehydration, stopping ascent, and descending if there is no improvement.

1.3.3 Acute Mountain Sickness (AMS)

AMS is commonly considered to represent progression of HAH. The Lake Louise Consensus Group defined AMS as the presence of headache in an unacclimatised person who has recently arrived at an altitude above 2500m, plus the presence of one or more of the following: gastrointestinal symptoms; anorexia, nausea or vomiting; insomnia; dizziness; and lassitude or fatigue (Roach, Bartsch et al. 1993). The group also established a scoring system to provide a quantitative element to AMS severity. A less commonly used (though more detailed) severity scoring system is the Environmental Symptom Questionnaire (Sampson, Cymerman et al. 1983). Box 1-2 lists possible risk factors for AMS.

Risk Factors for AMS:
Rapid ascent
Exertion
Past history of altitude illness
Young adults
Genetic predisposition

Box 1-2 Possible risk factors for AMS. Despite these being known, it is not currently possible to predict who, in a group of similar aged people travelling together to altitude for the first time, will develop AMS.

Management of AMS consists of simple analgesia and descent to a lower altitude. For more severe cases acetazolamide, dexamethasone (see drugs at altitude Box 1-3), supplemental oxygen or use of a portable hyperbaric chamber may be required.

The two main aims of pharmacological treatment for high altitude illness are to increase ventilation (e.g. with carbonic anhydrase (CA) inhibition) and to reduce inflammatory and cytokine responses (e.g. by steroids/antioxidants). In addition, descent and supplementary oxygen must be considered for increasingly severe symptoms. Medications that improve High Altitude Pulmonary Edema (HAPE) can also improve concurrent AMS and HACE by improving oxygenation.

Acetazolomide (125-250mg bd): Is used in AMS prophylaxis and therapy (Bradwell, Wright et al. 1992; Grissom, Roach et al. 1992). It blocks CA in red blood cells, renal tubules, chemoreceptors, the brain and pulmonary/systemic blood vessels. Its mechanism of action is thought to principally be through renal CA inhibition causing a bicarbonate diuresis and metabolic acidosis that increases minute ventilation. CSF production is also reduced. The numbers needed to treat to prevent one case of AMS are between 8 (Basnyat, Gertsch et al. 2003) and 4.5 (Bartsch and Schneider 2001). No benefit of 375mg bd over 125mg bd was demonstrated in a randomised trial (Basnyat, Gertsch et al. 2006). 250mg tds has been shown to improve cerebral oxygenation during exercise at 5700m but this effect is nullified with acclimatisation (Vuyk, Van Den Bos et al. 2006). Serious side effects are rare but an unpleasant taste to carbonated drinks, an initial diuresis and peripheral and perioral tingling that increases with dosage are commonly reported (Basnyat, Gertsch et al. 2006).

Dexamethasone (For prophylaxis: up to 8mg/day; for acute treatment of HACE: 8mg by any route stat and 4mg 6 hourly (orally if tolerated) subsequently): Is thought to act by reducing cytokine release and capillary permeability. Dexamethasone 8mg/day in divided doses can prevent AMS (Johnson, Rock et al. 1984; Fowler, Elcombe et al. 1987; Hackett, Roach et al. 1988; West, Schoene et al. 2007) but potential side effects have limited its use to those in whom acetazolamide is contraindicated, and to rescue workers in whom rapid ascent is mandated and unavoidable. The benefits of steroid prophylaxis occur without altering physiological variables such as peripheral arterial oxygen saturation (Basu, Sawhney et al. 2002). It is normally used to treat rather than prevent AMS/HACE.

Ginko Biloba and anti-oxidants: Ginko biloba is a Chinese medicine that scavenges free radicals. Its benefits are unclear. Early studies suggested benefit (Roncin, Schwartz et al. 1996) however a recent (though poorly designed) large trial, failed to show evidence of AMS prevention (Gertsch, Basnyat et al. 2004). Antioxidant supplementation with ascorbic acid, tocopherol acetate and α-lipoic acid reduced AMS score and improved arterial oxygen saturations in a 10 day ascent to 5180m (Bailey and Davies 2001), however antioxidants may interfere with acetazolamide's action.

Pharmacotherapy for HAPE:
By improving arterial oxygen saturation, these measures may aid concurrent HACE, but alone do not prevent AMS.

Calcium Channel Blockers – e.g. Nifedipine (20mg slow release tds) Inhibit hypoxia induced pulmonary artery vasoconstriction. Slow release Nifedipine 20mg tds reduces HAPE in susceptible individuals (Bartsch, Maggiorini et al. 1991) and given orally every 6 hours can be used (with oxygen and rapid descent) to treat HAPE (Oelz, Maggiorini et al. 1989). Caution must be used as significant hypotension can result

Phosphodiesterase Inhibitors – e.g. Sildenafil (50mg) increase cerebral and peripheral oxygenation by reducing pulmonary hypertension through inactivation of secondary messengers (Chan, Hoar et al. 2005). Tadalafil (10mg) has been shown to reduce HAPE in HAPE-prone subjects (Maggiorini, Brunner-La Rocca et al. 2006) but does not prevent AMS.

Box 1-3 Pharmacotherapy to prevent and treat High Altitude Illness

1.3.4 High Altitude Cerebral (O)edema (HACE)

If the cerebral effects of altitude are considered to occur as a spectrum of disease, HACE is the final life-threatening stage. It is diagnosed clinically in

persons who have recently arrived at high altitude, most of whom will have features of AMS or HAPE. It is characterised by psychiatric changes of varying degree, confusion, ataxia of gait, and disturbances of consciousness that may progress to deep coma (Hackett and Roach 2004). The prevalence in trekkers at around 4500m is thought to be 0.5 - 1% (Hackett, Rennie et al. 1976). Management consists of urgent descent, and administration of oxygen and dexamethasone (8-10mg iv, im or PO then 4mg 6 hourly). If descent is not possible, and the airway can be protected, a portable hyperbaric chamber can be used.

As I shall discuss later in this chapter, this clinical syndrome has the pathological descriptor of "oedema" in its title. Whilst limited data suggest that oedema has occurred in some (Hackett, Yarnell et al. 1998), this has not been shown to be universal. It may well be that there is another cause of the above symptoms and oedema is the final, pre-terminal result.

1.3.5 High Altitude Illness in Children

Many thousands of children are exposed to high altitude when travelling with their parents but the paediatric population has not been studied extensively (Pollard, Niermeyer et al. 2001). The incidence of AMS seems to be similar in children and young adults (Yaron, Waldman et al. 1998); however, diagnosis and distinguishing it from other pathologies may be more difficult in this group (Theis, Honigman et al. 1993). As in adults, no clear correlation between physiological changes and AMS symptoms has been demonstrated (Yaron, Niermeyer et al. 2003). The Lake Louise Scoring System can underestimate AMS prevalence in children because of difficulties in articulating symptoms (Southard, Niermeyer et al. 2007). A Children's Lake Louise Score assessing parameters of unexplained fussiness, feeding, playfulness and sleeping may be of use in pre-verbal children (Pollard, Niermeyer et al. 2001). A slow ascent to prevent, and an early descent to treat AMS in children are imperative.

1.3.6 Sleep at High Altitude

Sixty percent of individuals ascending rapidly to 3500m describe sleep disturbance, of which recurrent awaking is the most common complaint

(Jafarian, Gorouhi et al. 2008). Periodic breathing (reflecting alternating respiratory stimulation by hypoxia and subsequent inhibition by hyperventilation-induced hypocapnia) is frequently observed and the resulting cyclical fall in arterial oxygen saturation is thought to significantly contribute to the pathogenesis of AMS (Burgess, Johnson et al. 2004). In addition, periodic breathing disrupts sleep in fellow tent sharers and has significant neuropsychological effects (Virues-Ortega, Buela-Casal et al. 2004). Temazepam, Zopiclone and Acetazolamide are all considered safe at altitude, maintain oxygen saturations and reduce sleep disturbance, although it is not yet clear if combination therapy is advantageous (Luks 2008).

1.3.7 Other High Altitude Related Neurological Conditions

A number of usually focal "events" that also occur at sea-level (and fall outside the usual definition of altitude sickness) appear to have an increased incidence at altitude (Basnyat, Wu et al. 2004).

Transient Ischaemic Attacks (TIAs) tend to occur in a younger population at altitude implying that aetiology does not reflect atherosclerosis and may be related to vasospasm, hypocapneic vasoconstriction or putative prothrombotic effects of hypoxia. Right to left shunts increase with both exercise and altitude, meaning that embolisation via a patent foramen ovale (PFO) may account for some focal neurological events, or episodes of migraine (Imray, Pattinson et al. 2008). Such effects may also contribute to rare cases of **cerebral infarction**, to which dehydration and hypoxia-induced polycythaemia may also contribute (Clarke 2006). **Transient global amnesia** is reported and may occur through similar mechanisms in the limbic cortex.

Migraine can be difficult to distinguish from AMS, but suspicion should be raised if it is associated with focal neurology.

Cerebral venous thrombosis has been a common finding at autopsy of patients who die at altitude (Dickinson, Heath et al. 1983; Song, Asaji et al. 1986) and may be related to volume depletion and polycythaemia (Zhou 1984). Altitude may also be a trigger in those genetically predisposed through familial thrombophilia (Boulos, Kouroukis et al. 1999; Baumgartner, Siegel et al. 2007; Nair, Mohapatro et al. 2008).

Seizures are relatively common even at sea level. It is not known if seizure frequency increases at altitude, although hypoxia and hyperventilatory hypocapnia are thought to be triggers (Hackett 2001).

High altitude syncope is thought to be a vasovagal phenomenon related to hypoxaemia although increased frequency of arrhythmias may be contributory (Woods, Allen et al. 2008). It usually occurs at moderate altitude and rapidly resolves (Nicholas, O'Meara et al. 1992).

Cranial nerve palsies are well recognised both in the presence and absence of AMS/HACE. Most commonly, the 6th cranial nerve is affected, perhaps due to trunk compression from adjacent brain swelling. Facial and Hypoglossal cranial nerve palsies have also been described (Basnyat, Wu et al. 2004).

Ophthalmological disturbances: **Retinal haemorrhages** are a common occurrence with or without AMS symptoms. Unless the macula is involved, they are relatively benign and resolve spontaneously. Their pathogenesis is unknown, but may relate to increased blood flow, or breakdown in the blood-retina barrier (Mader and Tabin 2003). The monocular blindness of **amaurosis fujax** may result from compromised blood supply to the retina and hence the aetiology may be similar to the TIAs described above. Similarly, **cortical blindness** may represent vascular compromise to the visual cortex.

Obviously, many other neurological conditions that occur at sea level still occur at altitude (e.g. subarachnoid haemorrhage), and mild hypoxia can unmask more indolent conditions (e.g. cerebral tumours) (Zrinzo, Crocker et al. 2006).

1.3.8 Chronic Mountain Sickness (CMS)

CMS (also known as Monge's disease after Carlos Monge who described it in 1925) can develop after living at altitude for an extended time. Its central feature is an excessive erythrocytosis that drives polycythaemia, to which (through associated hyperviscocity) many of its associated symptoms can be related (headache, dizziness, tinnitus, sleep disturbance, fatigue and mental confusion). Pulmonary hypertension and cor pulmonale are common.

1.4 Current Pathophysiological theories

Other than the occurrence of past altitude illness, there are no obvious predisposing factors that identify one individual as being at higher risk of developing AMS than another when ascending to the same altitude at the same rate. The indiscriminate nature of AMS led Ross in 1985 to write of “the random nature of cerebral mountain sickness” (Ross 1985). This is based on the Monro-Kellie doctrine(Monro 1823; Kellie 1824) of the skull being a “closed box” and hence if a mass (tumour/blood/oedema) increases in volume within the skull, something else has to be displaced or pressure will rise. Ross’s “Tight Fit” hypothesis proposed that inter-individual variation in neuroaxis compliance (i.e. the inability of some to be able to accept brain swelling compared to others) accounted for similar variability in AMS susceptibility (figure 1-4). A number of factors influence such compliance: brain volume compared to skull volume (the atrophy of aging causes an increase in ventricular and sulcal size); and the volume of the spinal canal when compared to that of the spinal cord. A Pressure-Volume Index (PVI) can be calculated (the volume of fluid that needs to be added to CSF to raise the CSF pressure by a factor of 10) and this varies widely between individuals. For some 20 years the “tight fit” hypothesis (in which, because the skull is a closed box, oedematous brain increases intracranial pressure once compliance is reached) has been considered key to explaining differences in susceptibility to AMS, despite paucity of supporting data. If it is a key factor, then increases in cerebral volume (and thus pressure) need also to be explained. Whilst these may occur due to the occurrence of oedema (see above), an increase in cerebral blood flow, or obstruction to venous outflow, might also be postulated to play a role and this forms the basis of a core hypothesis studied in this thesis (see end of this chapter).

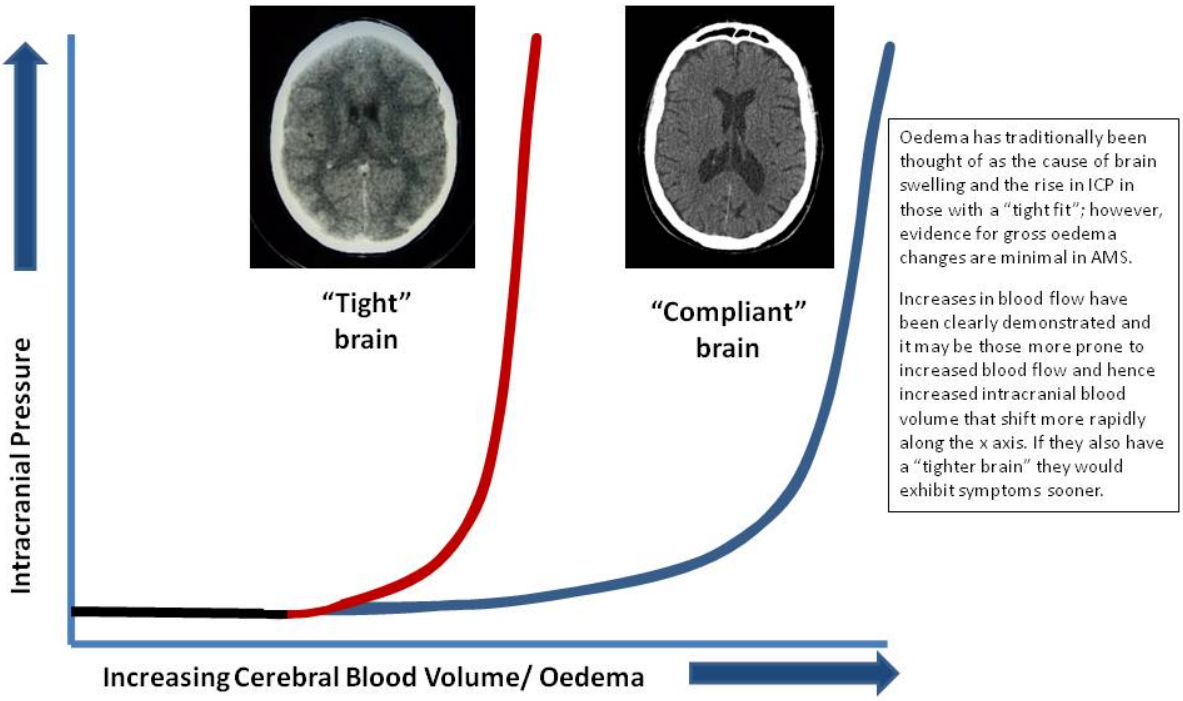


Figure 1-4 Schematic graph of the "Tight-Fit" hypothesis demonstrating that lack of CSF buffer results in a predisposition to a rise in ICP with oedema formation or increased blood volume.

In addition to this anatomical predisposition, it has been suggested that there is a physiological predisposition to the formation of cerebral oedema itself. This may relate in part to an individual's ability to maintain arterial oxygen saturation at altitude, especially during exercise (Roach and Hackett 2001). The classic adaptations to hypoxia (tachypnoea, tachycardia, and erythropoeisis to aid tissue oxygen delivery) are well known. Variations in acute hypoxic ventilatory response may well be associated with differences in cerebral blood flow and hence oedema formation. Recently, an individual's ability to alter oxygen utilisation efficiency at the mitochondrial level has also been suggested to account for some of the variations seen (Gore, Clark et al. 2007).

1.4.1 Investigating the Tight Fit Hypothesis

Assuming the "Tight Fit" hypothesis to be true, an elevated ICP should underpin the development of AMS. Evaluation of this hypothesis therefore requires measurement of ICP or its surrogates.

Indirect Measures of ICP

- *Lumbar CSF Pressures:* There is little evidence that CSF pressures as measured by lumbar puncture are increased in AMS. Singh *et al.* (Singh, Khanna et al. 1969) demonstrated elevated lumbar spinal fluid pressures in 34 Indian soldiers who were rapidly transported from sea level to 5867m. Lumbar CSF pressures were 6-21cm H_2O higher than pressures after recovery. This may however reflect acute decompression effects. It is unclear whether these soldiers had AMS or HACE. While Hartig and Hackett demonstrated, in three subjects, that acute hypoxic gas inhalation resulted in a rise in lumbar CSF pressure, more gradual decompression to a simulated ascent of 5000m was not associate with any change, even in those in whom AMS developed (Hartig and Hackett 1992). Similarly, Bailey *et al* found lumbar CSF pressures to be normal after exposure to 16 hours of 12% O_2 and identified no difference in pressures between AMS and non AMS groups (Bartsch, Bailey et al. 2004).
- *Tympanic Membrane Displacement (TMD):* Wright *et al.* used a TMD technique to study 24 subjects before and during ascent to 5200m (Wright, Imray et al. 1995). Changes suggesting a rise in ICP were found with acute hypoxic exposure (ascending rapidly to 3400m), but there was no additional rise in ICP in those who developed mild to moderate AMS. They thus concluded that a rise in ICP, if it occurs, is a late phenomenon in AMS/HACE.
- *Optic Nerve Sheath Diameter (ONSD):* Recently, ONSD has been used as a surrogate for the measurement of ICP at altitude and in those with AMS (Fagenholz, Gutman et al. 2007; Sutherland, Morris et al. 2008). In a study of 13 mountaineers, ONSD was measured ultrasonically with regression analysis used to explore correlation with a number of variables. ONSD was positively associated with increasing altitude (0.1mm increase per 1000m 95% CI 0.05-0.14) and AMS score (0.12mm per Lake Louise Score (CI 0.06 to 0.18). Associations were also found with resting heart rate and arterial oxygen saturation (0.2mm increase per 10% $Sa0_2$ decrease) (Sutherland, Morris et al. 2008). A study of 287 subjects similarly demonstrated a larger mean optic nerve sheath

diameter (ONSD: 5.34mm, 95%CI 5.18-5.51mm) in AMS sufferers compared to non-sufferers (4.46mm, 95%CI 4.39-4.54mm) (Fagenholz, Gutman et al. 2009). However a more recent study of 23 subjects has failed to show any difference in ONSD between AMS sufferers and non-sufferers (Lawley, Oliver et al. 2012), once again providing conflicting data regarding the association of rising ICP with AMS.

- *Pulistility Index (PI)*: As explained below, PI (measured using transcranial Doppler of ratios of flow velocities) may give an indication of ICP, but its reliability remains uncertain.

Direct ICP Measurements

Brian Cummins, a neurosurgeon from Bristol, is the only investigator to have directly monitored ICP in humans at altitude (Wilson and Milledge 2008)[†]. He first investigated intracranial pressure in three climbers using a telemetric ICP monitoring device inserted prior to departure. Only the youngest subject developed AMS. All of them had normal ICPs at rest at all altitudes. However the youngest subject suffered a dramatic rise in ICP at 4725m during any form of mild exertion. Mild exertion and neck turning can be simple manoeuvres that reduce venous outflow and hence tip an individual from the "compensating" part of the cerebral compliance curve into the decompensated part which results in a steep rise in ICP (figure 1-4). Such non-invasive manoeuvres in future may be used as tests of compliance.

Field conditions limit the application of many technologies (such as magnetic resonance imaging) to the study of the brain at altitude. Two tools however, are portable and particularly useful at investigating brain oxygenation and brain blood flow: Cerebral Near Infrared Spectroscopy (NIRS) and Transcranial Doppler (TCD) respectively. Since they are used extensively during the studies described in this thesis, a background to them is given here although further technical details are provided in the methodology chapter (chapter 2) and the chapters concentrating on TCD (chapter 3) and NIRS (chapter 4).

[†] His data from his expedition to Hagshu in Pakistan was thought to have been lost in a car fire. However, I asked his wife Anne if I could write a historical piece and she found the data in a box in the loft. Sadly Brian died in 2004 and hence I wrote it up for him.

1.4.2 Cerebral Oxygenation at Altitude

Cerebral NIRS is a non-invasive technique utilising the differential absorptive properties of oxy- and de-oxyhaemoglobin for near infrared light (700-1100nm). The technique provides continuous monitoring of regional cerebral oxygenation (rSO_2 – a mixed arterial, venous, capillary and tissue oxygenation measure of the area interrogated by the near infrared light, approximately 4cm deep to the light source/detector). NIRS has been used extensively for research purposes, but its clinical use is still in its infancy. Its use to optimise cerebral oxygenation during coronary artery bypass surgery results in a significantly lower prevalence of major organ dysfunction and shorter length of stay (Murkin, Adams et al. 2007). Cerebral NIRS measurements have been shown to correlate with jugular venous bulb saturations in healthy volunteers undergoing isocapnic hypoxia (Henson, Calalang et al. 1998). The technique has also been validated with PET scanning (Rostrup, Law et al. 2002) and ^{133}Xe washout methods (Boushel, Langberg et al. 2000). Most studies using NIRS have investigated frontal cortex rSO_2.

There is a progressive fall in resting rSO_2 with ascent to altitude (Imray, Barnett et al. 1998), (Hadolt and Litscher 2003) but the fall does not correlate with AMS severity (Imray, Barnett et al. 1998). Supplemental CO_2 increases rSO_2 (presumably by reducing hypocapnia-induced vasoconstriction) (Imray, Walsh et al. 2003) and acetazolomide helps to maintain cerebral oxygenation up to 5700m (Vuyk, Van Den Bos et al. 2006).

During exercise on a recumbent bicycle at sea level, rSO_2 increases (from 68.4% to 70.9% at submaximal exercise and to 69.8% at VO_2Max, n=9) (Imray, Myers et al. 2005). However, at altitude (studied up to 5,260m), rSO_2 decreases with increasing exercise. Similarly, Subudi *et al.* studied 13 cyclists exercising to VO_2Max under normoxic and acute hypoxic (12% FiO_2) conditions (Subudhi, Dimmen et al. 2007). In normoxia, frontal cortex rSO_2 increased as workload increased from 25 to 75% of VO2 max then fell as workload increased further to 100% of VO2 max. During hypoxia, however, cerebral rSO_2 dropped across all work levels. It has therefore been suggested that cerebral oxygen delivery may contribute to the limit of exercise at altitude.

Brain Oxygen Consumption

There are few published data relating to brain oxygen consumption or extraction at altitude as such field studies require both arterial and (jugular) venous measurements. Moller *et al.* studied cerebral metabolic rates of oxygen and glucose, and cerebral blood flow using the Kety-Schmidt technique in 9 acclimatised subjects at rest and during exercise at sea level and 5,260m. Despite marked changes in breathing, no changes in cerebral blood flow or oxidative metabolism were demonstrated (Moller, Paulson et al. 2002).

Positron Emission Tomography has demonstrated that Quechuas (high altitude natives of the Andes) have lower glucose metabolic rates in brain regions classically associated with higher cortical function (e.g. the frontal cortex) compared to low-landers. This could provide a defence mechanism to protect the brain from chronic hypoxia (Hochachka, Clark et al. 1994; Hochachka and Monge 2000).

There is also evidence to suggest that animals that adapt well to hypoxia can reduce their brain oxygen consumption at high altitude (Curran-Everett, Iwamoto et al. 1991). Curran-Everett demonstrated that 7 out of 9 sheep exposed to 72 hours of hypoxia were able to reduce their cerebral oxygen extraction (Curran-Everett, Iwamoto et al. 1991). The “sick” sheep (defined as those that ate less and lay down more) all had the lowest oxygen extraction fractions during normoxia. Subsequently they also had a greater cerebral blood flow per unit of cerebral O_2 consumption both during normoxia and hypoxia. These different cerebral responses to hypoxia may be related to individual variations to AMS susceptibility.

1.4.3 Changes in Cerebral Blood Flow at Altitude

One of the proposed mechanisms for the development of HACE is vasogenic oedema initiated by an increase in cerebral blood flow (CBF). The major determinants of CBF in normoxia are blood pressure, and the partial pressures of arterial oxygen and carbon dioxide. On ascent to altitude, hypoxia tends to

increase CBF, while hypocapnia decreases CBF, and the balance of these two effects is crucial in determining the overall CBF.

Severinghaus *et al.* (Severinghaus, Chiodi et al. 1966) used the Kety-Schmidt nitrous oxide washout method (Wolff 2000) to describe the CBF response to ascent to 3810m. CBF had increased by 24% within 6- 12 hours, and was still 13% higher at 3-5 days. Jensen *et al.* used radiolabelled Xenon to measure CBF in 12 subjects ascending from 150 to 3475m, and found that it increased by 24% (Jensen, Wright et al. 1990). Ascending from 3200m to 4785-5430m was associated with a further rise in CBF, to 53% above sea level values. However, no difference was seen between subjects with and without AMS.

Transcranial Doppler (TCD) has been used extensively to assess relative changes in cerebral blood flow velocity at altitude. It requires an experienced sonographer using a consistent insonation window, angle and depth and a suitable temporal bone window. TCD measures flow velocity which has been assumed to represent overall flow volume. It has been hypothesised that the headache of AMS could be secondary to an increase in blood flow (Jensen, Wright et al. 1990; Jansen, Krins et al. 1999). Baumgartner *et al.* studied 10 subjects immediately, 3 and 6 hours after decompression to 4,559m and found no significant changes in middle cerebral artery flow velocity (MCAv) and no correlation with the development of AMS (Baumgartner, Spyridopoulos et al. 1999). The lack of very early change in MCAv was corroborated by carotid and vertebral artery flow studies (Reeves, Moore et al. 1985; Huang, Moore et al. 1987) but there were substantial inter-individual differences in these studies. In contrast, 12-24 hours after arrival at altitudes ranging between 3475 and 4559m, several Doppler studies reported MCAv increases of 20% to 27% (Huang, Moore et al. 1987; Otis, Rossman et al. 1989; Baumgartner, Bartsch et al. 1994). These ultrasound findings are in accordance with the results of Severinghaus (Severinghaus, Chiodi et al. 1966) and Jensen (Jensen, Wright et al. 1990). The delayed increase in MCAv could explain the delay in the development of AMS/HACE.

Others have suggested that it is not the change in CBF, but the loss of autoregulation that is important in the development of AMS. Van Osta *et al.*

studied MCAv in 35 volunteers ascending to 4559m. MCAv did not change, although a rise in the dynamic cerebral autoregulation index (ARI – calculated from recordings of MCAv and blood pressure during transient induced hypotension) correlated with the headache component of the AMS score (Van Osta, Moraine et al. 2005).

It has been suggested that hypoxia may have a direct vasodilatatory effect on the large basal arteries of the Circle of Willis. This would lead to a underestimate of flow variation by MCAv and could explain the unchanging values of MCAv which Ter Minassian reported in the Operation Everest III study (Ter Minassian, Beydon et al. 2001). These authors ruled this out, quoting a paper written in 1930 to state that the vasodilatory effects of hypoxia act mainly on small pial and cortical arteries downstream of the Circle of Willis (Wolff and Lennox 1930). They further discount the possibility of hypoxia-induced MCA dilatation by stating that the increase in MCAv is of the same order of magnitude as the increase in CBF shown by nitrous oxide inhalation (Severinghaus, Chiodi et al. 1966) and the ^{133}Xenon technique. Giller has suggested that the lack of knowledge of changes in vessel diameter is a major handicap to the interpretation of TCD data (Giller 2003), and hypoxic-induced hyperventilation and subsequent reduction in $PaCO_2$ may result in vasoconstriction. In the context of subarachnoid haemorrhage, neurosurgeons / neurointensivists interpret an increase in MCAv to mean vasoconstriction or vasospasm. Another core aim of this thesis is to investigate cerebral artery vasodilatation in response to hypoxia.

Pulsitility Index: Gosling's Pulsatility Index (PI), is calculated from Transcranial Doppler measurements of the middle cerebral artery (MCA): PI = systolic velocity - diastolic velocity/mean velocity; a normal value being less than 1). In recent years, a good correlation between PI and ICP has been found in the context of non-specific intracranial pathologies (Bellner, Romner et al. 2004), trauma (Moreno, Mesalles et al. 2000; Tan, Feng et al. 2001; Voulgaris, Partheni et al. 2005; Bor-Seng-Shu, Hirsch et al. 2006), cerebral mass lesions (including haematomas)(Cardoso and Kupchak 1992; Harada, Hayashi et al. 1993; Czosnyka, Richards et al. 1996), hydrocephalus (Norelle, Fischer et al. 1989; Quinn and Pople 1992; Nadvi, Du Trevou et al. 1994; Goh and Minns

1995; Hanlo, Gooskens et al. 1995; Iacopino, Zaccone et al. 1995; Vajda, Buki et al. 1999; Rainov, Weise et al. 2000) and subarachnoid haemorrhage (Soehle, Chatfield et al. 2007). In general, correlation is strongest when ICP is over 20, meaning that PI may be a poor index of ICP in the normal or only slightly elevated range. The correlation between ICP and PI across such a range of pathologies implies that the technique may be of use in noninvasively assessing ICP at altitude.

Ter Minassian measured PI during a simulated ascent of Mount Everest (Operation Everest III) in 2001 (Ter Minassian, Beydon et al. 2001). Eight subjects were studied in a hypobaric chamber decompressed to altitudes of 5000, 6000, 7000 and 8000m. All measurements were done on day 3 after "arrival" at the altitude bar those of the 8000m altitude which were done after 4 hours. They calculated both PI and Resistivity Index (RI): RI = (systolic velocity – diastolic velocity)/ systolic velocity). Their demonstration of a very clear reduction in PI at each altitude gain seems to conflict with the proposal that PI should rise with ICP (if one assumes that ICP is rising). Of note however, the $PaCO_2$ values also fell dramatically and this hyperventilatory response to hypobaric hypoxia may alter the usefulness of PI since the fall in $PaCO_2$ causes cerebral vasoconstriction. Variations in $PaCO_2$ have previously been shown to alter PI independently (Homburg, Jakobsen et al. 1993; Czosnyka, Richards et al. 1996).

Palma *et al.* (Palma, Macedonia et al. 2006) studied 9 individuals ascending to 4300m. They assessed PI and Dynamic Flow Index (DFI, Mean Flow Velocity / PI). They found that DFI was increased in subjects with AMS at 4300m when compared to asymptomatic subjects. Whether this relates to changes in cerebral haemodynamics or intracranial pressure cannot be evaluated.

There is a widespread view that exercise at altitude increases the likelihood of an individual developing AMS and HACE. Imray *et al.* assessed cerebral perfusion during exercise in 9 individuals ascending to 5,260m (Imray, Myers et al. 2005). They demonstrated an increase in resting MCAv with increasing altitude and a further increase during exercise up to 50% of VO_2Max, beyond which MCAv declined. Marked rises in blood pressure and an elevated MCAv

could stress the blood brain barrier, possibly initiating vasogenic oedema. Subudi *et al.* have similar results and both groups suggest that cerebral blood flow and hypoxia may limit exercise performance (Subudhi, Lorenz et al. 2008).

1.4.4 Animal Studies of Relevance

Krasney and co-workers designed the first conscious animal model of HACE in 1990 (Krasney, Curran-Everett et al. 1990; Curran-Everett, Iwamoto et al. 1991). In addition to measuring ICP invasively in the lateral ventricle of sheep (n=20), they also calculated cerebral oxygen extraction and investigated changes in wet-to-dry brain weight (n=9) with 72 hours of normobaric hypoxia (arterial oxygen tension of 40mmHg giving an arterial oxygen saturation of 50%). Whilst ICP did not change with the 72 hours of hypoxia, wet-dry brain weight ratios increased in all regions, but especially in the white matter, caudate nuclei and thalamus. The authors hypothesised that the lack of a rise in ICP with the increase in brain volume was due to reciprocal loss of volume from the ventricles. They noted that, in normoxia, it is possible to withdraw 3-4 mls of CSF from the ventricular catheter with ease. However, after 7 hours of hypoxia, it was often impossible to withdraw more than 0.5-1.0mls. In addition, this study demonstrated that cerebral blood flow per unit cerebral O_2 consumption doubled and the cerebral O_2 extraction fraction decreased in seven of nine sheep.

Yang *et al.* (Yang, Sun et al. 1993) studied ICP in goats exposed to a PaO_2 of 40Torr (=40mmHg, equivalent to an altitude of 4000m). Although there were methodological issues, ICP and cerebral blood flow increased, and intracranial compliance decreased with 2 hours of hypoxia. Exposed to the same PaO_2, ICP rose (and cerebral oedema occurred) in sheep which exhibited AMS behaviour (off food/water), but not in those behaving normally (Yang, Bergo et al. 1994). In New Zealand white rabbits, ICP did not rise with exposure to simulated hypobaric hypoxia of 5000m for 6 hours, whether or not steroids were administered (Pendon and King 2003). Meanwhile, bar-headed geese (*Anser indicus)* make an annual migration across the Himalayas flying between 5000 and 9000m. The partial pressure of oxygen at 9000m is 30% of that at sea level hence it is amazing they can undertake the energy expensive process of

flapping flight. They have a number of adaptations to achieve this (Butler 2010). Firstly their haemoglobin has a higher affinity for oxygen than lowland birds. They are also able to hyperventilate to a greater extent. Most importantly, they do not suffer a reduction in cerebral blood flow as a result of the low partial pressures of CO_2 that accompany their hyperventilation (Casey, Imray et al. 2011). Thus, an ability to continue to deliver oxygen to the brain without hyperventilation-induced vasoconstriction may well be a very advantageous adaptation.

It must be remembered that cerebral anatomy of other mammals and birds is very different to that of humans. The postural differences also dramatically influence cerebral fluid pressures, the above studies must all be interpreted with extreme caution.

1.4.5 Brain Imaging Investigations

1.4.5.1 Imaging Changes with AMS

CT scans performed on climbers with HAPE and neurological dysfunction have demonstrated the presence of small ventricles and cisterns, and the disappearance of cerebral sulci (Koyama, Kobayashi et al. 1988). Magnetic Resonance Imaging (MRI) allows improved assessment of oedema and, while not measuring ICP, can infer changes in ICP from changes in brain volume (e.g. loss of ventricular space, sulci effacement). It has been used in a number of studies where subjects have developed AMS, and there are clinical reports of MRIs of patients who have suffered with HACE. Overall, the results raise the question as to whether oedema has a significant role in AMS.

Cytotoxic (intracellular) (Houston and Dickinson 1975) and vasogenic (extracellular water accumulation due to increased blood brain barrier permeability) (Hackett, Yarnell et al. 1998) oedema have both been postulated to be core mechanisms in HACE pathogenesis. A particularly useful MRI technique, diffusion weighting, differentiates between these two forms of oedema.

Amongst 9 subjects exposed to a simulated sudden ascent to 4572m, a 2.77% (36.2ml) increase in brain matter volume was found at 32 hours (Morocz, Zientara et al. 2001). These volume changes only occurred in grey (not white) matter, regardless of whether AMS symptoms were present or not. Meanwhile, in 10 subjects who had MRIs after 10 hours of exposure to a simulated altitude of 4500m (eight of whom suffered AMS), none had cerebral oedema (Fischer, Vollmar et al. 2004). They demonstrated that ventricular CSF volume decreased in all subjects, more so in those with severe AMS. This again implies that that the brain parenchyma or other intracerebral components expand in hypoxia. Meanwhile, in 22 subjects (half of whom suffered AMS, of which seven received metoclopramide and paracetamol), cerebral swelling of the order of 7 +/-4.8ml (approximately 0.5% of total brain volume) was observed after 16 hours of exposure to 12% O_2 (equivalent to 4500m) (Kallenberg, Bailey et al. 2007). In addition they studied T_2 Relaxation Time (T_2rT – a technique that reflects changes in parenchymal water content) and the Apparent Diffusion Coefficient (ADC – which reflects changes in the diffusibility of water molecules). ADC helps differentiate vasogenic oedema (as water moves intracellularly its diffusibility, and hence ADC, falls) from cytotoxic oedema (as water moves extracellularly its diffusibility and ADC increases). Specific regions of interest within the brain (white matter, basal ganglia, genu and splenium of corpus callosum and cerebellar white matter) were studied. Hypoxia resulted in a general increase in T_2rT representing an increase in parenchymal oedema. ADC values were consistently lower in those who developed AMS symptoms. This suggested that hypoxia causes a generalised vasogenic oedema, but that with AMS may be associated with an additional cytotoxic (intracellular) component. This may occur through a reduction in the Na^+/K^+ ATPase pump (see below). This study also demonstrated that those developing AMS had a greater brain:intracranial volume ratio, supporting the “tight fit” hypothesis.

Schoonman *et al.* (Schoonman, Sandor et al. 2008) studied 9 students exposed to isobaric hypoxia (N_2 enriched air) to obtain arterial oxygen saturations of 75-80% for 6 hours. Seven of the 9 developed AMS. Visual inspection of the MRIs failed to show any oedema. However, there was a general increase in ADC with hypoxia while ADC values negatively correlated with severity of cerebral symptoms. Hence, similarly to Kallenberg, the authors concluded that

vasogenic oedema occurs in isobaric hypoxia irrespective of AMS, while severe AMS is associated with an additional mild cytotoxic component. Fischer *et al.* demonstrated that, whilst exposure to a simulated altitude of 4500m did not induce demonstrable oedema, a mean 10% reduction in intracranial CSF volume occurred at 10-12 hours of exposure. This may well reflect cerebral swelling caused by increased cerebral blood flow even if oedema does not form (Fischer, Vollmar et al. 2004).

Although studies using ADC thus suggest the development of vasogenic oedema in response to hypoxia, and possibly of cytotoxic oedema in those developing severe AMS, a more recent hypoxic MRI study (Dubowitz, Dyer et al. 2009) demonstrates that hypoxia causes brain swelling without oedema formation. This study of 12 subjects found cerebral swelling and compression of ventricular CSF spaces after only 40 minutes of hypoxia.

Matsuzama *et al.* (Matsuzawa 1992) reported slightly increased T_2 signal intensity in 4 of 7 subjects with AMS in a 24 hour simulated altitude experiment.

In summary, MRI studies of simulated ascents to “very high altitude” in hypobaric chambers demonstrate that acute hypoxia causes brain parenchymal enlargement. This may be a combination of vasogenic oedema with hypoxia and cytotoxic oedema when in the context of AMS, but this is by no means conclusive and another “volume” could be contributing to this apparent parenchymal enlargement.

1.4.5.2 Imaging and Autopsy Changes with HACE

Autopsy evidence from climbers and soldiers dying of HACE has confirmed the presence of gross cerebral oedema (Singh, Khanna et al. 1969; Dickinson, Heath et al. 1983). Animal experiments of severe hypoxia show similar oedema (Krasney 1994) as do imaging studies of patients with hypoxic/anoxic injury secondary to cardiac arrest, cyanide or carbon monoxide poisoning (Varnell, Stimac et al. 1987; James 1988; Kasamo, Okuhata et al. 1993; Mills, Gunasekar et al. 1999).

Hackett *et al.* (Hackett, Yarnell et al. 1998) reported a series of 9 subjects who had brain MRIs from 16 to 132 hours after the onset of HACE. Eight of these subjects had severe concomitant HAPE. Seven of the 9 had increased T2 signal intensity especially in the corpus callosum and centrum semiovale with no grey matter abnormalities. White matter is thought to be more prone to vasogenic oedema because of its orderly structure. They concluded that HACE is characterised by reversible white matter oedema suggestive of a vasogenic mechanism. Interestingly, the images from this paper (see figure 1-5) demonstrate that these subjects had large sulci and hence would not be expected to suffer with AMS if a purely tight fit hypothesis was true. Further, a number of studies have shown that the corpus callosum develops restricted diffusion on MRI following death in hypoxic neonates (Takenouchi, Heier et al. 2010) and following cardiac arrest in adults (Bianchi and Sims 2008).

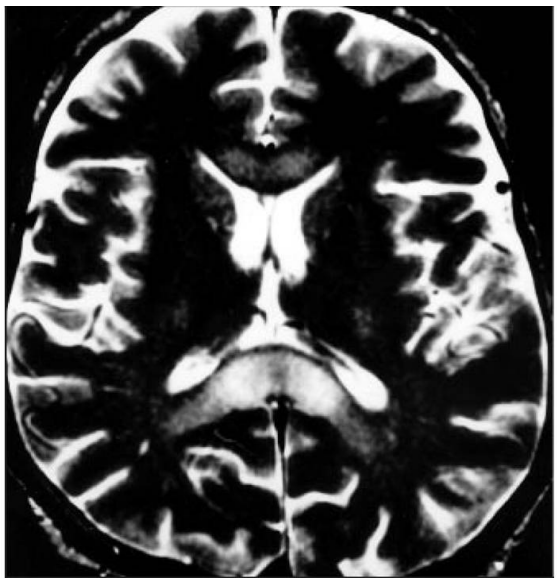


Figure 1-5 MRI of HACE patient after descending showing oedema in the corpus callosum. The large sulci argue against the "tight fit" hypothesis being integral to HACE. (From Hackett *et al.* 1998 (Hackett, Yarnell et al. 1998)).

If HACE was simply a continuation of AMS, then one might expect to find some mild white matter changes (increased T_2 signal intensity in the corpus callosum) in subjects with AMS during chamber studies of hypobaric hypoxia. This has not been the case (Morocz, Zientara et al. 2001). Hence, these changes appear to be specific to HACE, not altitude exposure or AMS.

In addition to white matter oedema, autopsies of victims of HACE have commonly demonstrated ring haemorrhages (figure 1-6).

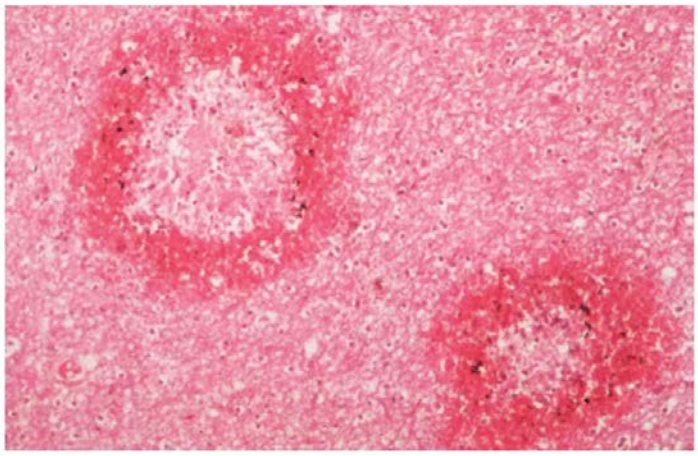
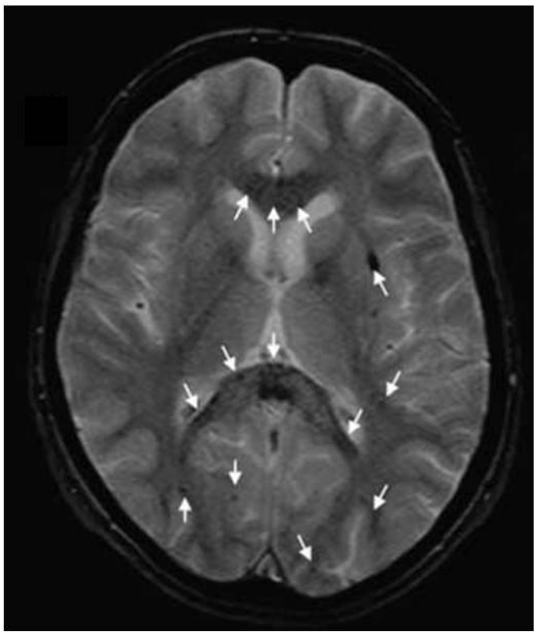


Figure 1-6 Ring haemorrhages seen at autopsy in HACE sufferers (Clarke 2006)

Using MRI, Kallenberg *et al.* have demonstrated that these ring microhaemorrhages (as evidenced by multiple haemosiderin deposits) occur in those with nonfatal HACE but not AMS (Kallenberg, Dehnert et al. 2008). These are found predominantly in the corpus callosum and persist at least many months after the episode of HACE (figure 1-7).

Figure 1-7 Microhaemorrhages on T2* weighted sequence MRI (Kallenberg, Dehnert et al. 2008).



The reason for the predilection for the corpus callosum is unknown, although it is supplied by small, short perforating arteries lacking adrenergic tone, which might thus be more susceptible to hypoxic vasodilatation, autoregulatory failure and hence over perfusion (Hackett 1999).

Microhaemorrhages are, however, a hallmark of venous outflow obstruction (Tsai, Wang et al. 1995; Kim 2004) which is thus implicated in HACE pathogenesis on this basis. Of note, the superficial veins lie in the (pain sensitive) subarachnoid space (Kilic and Akakin 2008) and hence distension due to distal obstruction could prove to be a mechanism for headache even in AMS. Exploring this hypothesis is a main focus of this thesis.

Retinal venous dilatation is almost universal on rapid ascent to altitude and retinal haemorrhages are common even in those without AMS (figure 1-8).

Approximately 59% of those with HACE and a third of AMS sufferers develop them (Hackett and Roach 2004). This adds further weight to a venous hypertensive mechanism. Interestingly, the haemorrhages occur in the superficial and occasionally deep intraretinal layers of the retina in the same manner as haemorrhages from central retinal vein occlusion (a more complete and acute venous obstruction).

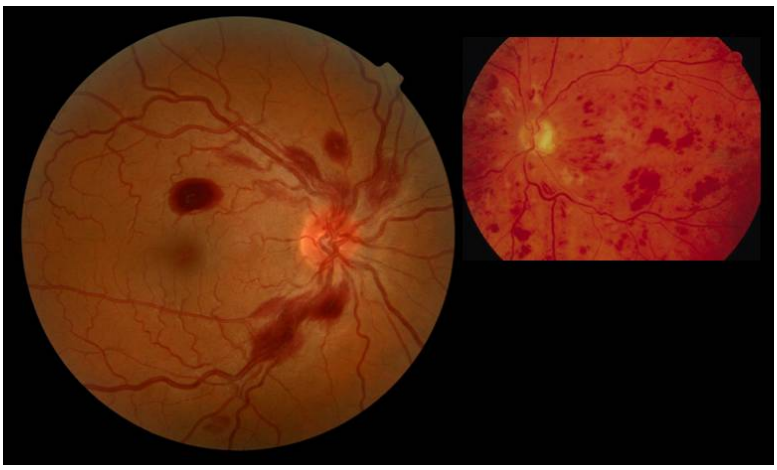


Figure 1-8 Retinal haemorrhages resulting from a) ascent to altitude and b) central retinal vein occlusion

1.4.6 AMS and HACE at a Vessel Level

The ICP, cerebrovascular and MRI studies described above have given us a greater understanding of gross changes resulting from exposure to hypobaric hypoxia, but there are still many questions as to the mechanisms of these changes on a vascular and cellular level.

1.4.7 Factors Affecting Vessel Tone

Oxygen and Carbon Dioxide

The relative importance of oxygen and carbon dioxide in regulating cerebral blood flow with exposure to altitude has been debated since the time of Bert (Bert 1878) and Mosso (Mosso 1898) over 100 years ago. Hypoxia is thought to cause vasodilatation and an increase in CBF (Jensen, Sperling et al. 1996). However, this is countered by a falling $PaCO_2$ (due to the hypoxic ventilatory response) causing vasoconstriction (Brugniaux, Hodges et al. 2007). The CBF response to isocapnic hypoxia is therefore greater than the response to poikilocapnic hypoxia (Ainslie and Poulin 2004). If an individual can accept hypoxaemia without mounting a significant hypoxic ventilatory response, they

will remain hypoxaemic and normocapnic. If, however, an individual develops a significant hypoxic ventilatory response they will maintain (or even increase) oxygenation, but also develop hypocarbia (although paradoxical behaviour is occasionally seen (Zubieta-Calleja, Zubieta-Castillo et al. 1994)). Supporting oxygenation may well confer advantage at altitude. Whilst the acute addition of 3-5% CO_2 to inspired air induces a feeling of wellbeing during high altitude exposure (Harvey, Raichle et al. 1988) and improves brain oxygenation (Imray, Clarke et al. 2001; Imray, Walsh et al. 2003), possibly through a hypercarbic ventilatory drive mechanism, chronic exposure to raised CO_2 may worsen symptoms (Maher, Cymerman et al. 1975). The exact mechanism by which CO_2 causes vasodilatation is not known. However, it may be that the altered pH activates potassium channels in the vascular smooth muscle wall (Nelson and Quayle 1995). Whatever the mechanism, it is faster than previously thought, with vessel diameter changes occurring within 6 seconds of altered pH / pCO_2 (Poulin, Liang et al. 1998).

The hyperventilatory-induced hypocapnia of acute hypoxia results in respiratory alkalosis. Over a few days of acclimatisation the pH and $PaCO_2$ relationship in central brainstem chemoreceptors is reset through an exchange of extracellular and cerebrospinal fluid bicarbonate.

Autoregulation and Sympathetic Nervous System:

Cerebral autoregulation refers to the changing of vessel calibre to ensure that CBF is matched to metabolic needs. Cerebral autoregulation can be divided into static (keeping CBF constant over gradual, progressive changes in cerebral perfusion) and dynamic (the rapid regulation in response to changes in arterial blood pressures that occur over seconds) (Zhang, Zuckerman et al. 2002). The sympathetic nervous system, which densely innervates the cerebral circulation, also appears to influence CBF though both systemic (e.g. cardiac output) and local actions.

Adenosine

Brain adenosine levels increase rapidly within 30 seconds to 5 minutes of exposure to exposure, and mirror the increase in CBF (Winn, Rubio et al. 1981). This mechanism is thought to be involved in the very fast arteriole distension of hypoxic vasodilatation and it is thought that the adenosine expression must be close to arteriolar smooth muscle, e.g. from glial end feet.

Potassium (K^+)

The hypoxia-induced local rise in K^+ is also thought to be involved in vasodilatation. Endothelial K^+ channel activation results in increased concentration of intracellular calcium which in turn results in release of Nitric Oxide (Faraci and Heistad 1998).

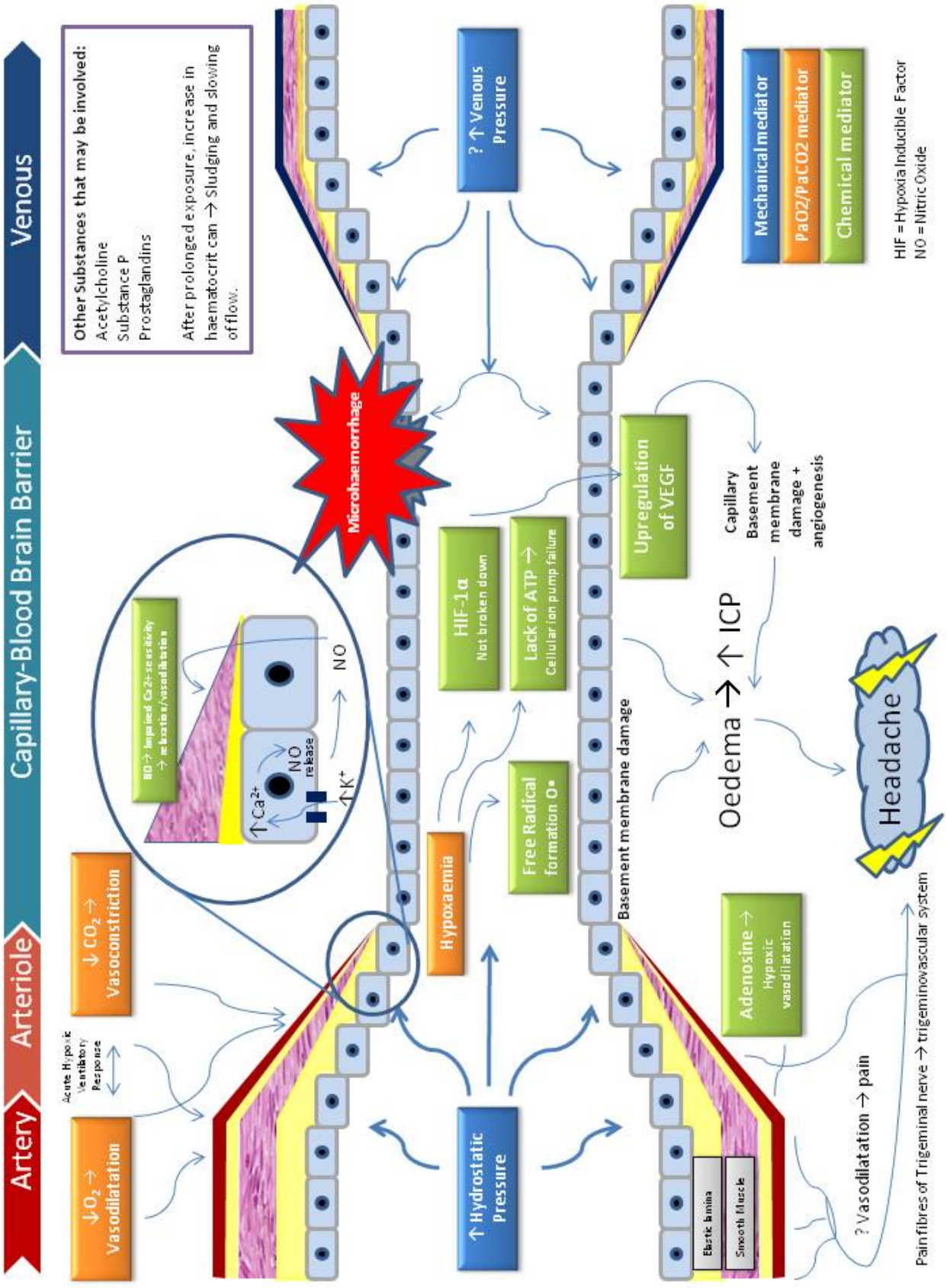
Nitric Oxide (NO)

Nitric Oxide Synthase III (endothelial Nitric Oxide Synthase – eNOS) produces NO or Endothelial Derived Relaxing Factor (Sanders, Kelley et al. 2000). NO has a short half-life and rapidly diffuses to vascular smooth muscle where it interacts with Ca^{2+} modulation mediated by cGMP. This results in vasodilatation. Inappropriate NO release with hypoxia has been implicated in altitude maladaptation syndromes. Appenzeller *et al.* (Appenzeller, Claydon et al. 2006) used TCD changes in response to an exogenous NO donor in 9 altitude–native Ethiopians and 9 altitude-native Peruvians (who as a race suffer considerably more with the maladaptation syndrome, chronic mountain sickness) to assess how adapted to altitude each race is. The circulatory response to NO was minimal in Ethiopians at low altitude, while Peruvians had a large response. In contrast, at high altitude, Ethiopians had a large response, whilst that in Peruvians was minimal. They concluded that Peruvians were well-adapted lowlanders while Ethiopians were highlanders adapted to that life.

1.4.8 Underlying Mechanisms of Brain Oedema

The use of steroids in the prevention and treatment of AMS and HACE perhaps offers indirect evidence that oedema is a component of their pathogenesis (Klatzo 1967; Fishman 1975). Multiple factors influence cerebrovascular tone, flow and permeability. Figure 1-9 provides an overview.

Figure 1-9 Mechanisms thought to underlie AMS and HACE demonstrated through a schematic vessel progressing from artery to vein form left to right. Mechanical factors (blue) increase intravascular pressure and hence can cause vasogenic oedema and vessel wall damage. These pressures can be arterial (increased hydrostatic pressure - associated with increased flow) or venous (if there is an element of venous outflow obstruction). The partial pressures of oxygen and carbon dioxide (orange) are thought to have direct vasoactive properties with hypoxaemia causing vasodilatation and hypocarbia causing vasoconstriction. A balance between these is found by the hypoxic ventilator response. Cytotoxic oedema may result from direct hypoxia induced Na^+/K^+ ATPase failure. Many chemical mediators have been implicated. Free radical formation could directly damage vessel basement membranes causing vasogenic oedema. Hypoxia Inducible Factor 1α accumulation and subsequent Vascular Endothelial Growth Factor upregulation could contribute to further basement membrane damage and oedema formation. Local hyperkalaemia could trigger calcium mediated nitric oxide release that in turn can act on vessel smooth muscle to cause vasodilatation. Neuronally mediated adenosine release could also cause vasodilatation. Vessel dilatation has been implicated in mediating pain via the trigeminovascular system and hence headache. The key element of HACE is microhaemorrhage formation that may relate to vessel damage from chemical mediators/cytokines or damage through increased hydrostatic pressure.


Artery
Arteriole
Capillary-Blood Brain Barrier
Venous
Other Substances that may be involved:
Acetylcholine
Substance P
Prostaglandins
After prolonged exposure, increase in haematocrit can → Sludging and slowing of flow.
↓O2 → Vasodilatation
↓ CO2 → Vasoconstriction
Acute Hypoxic Ventilatory Response
↑ Hydrostatic Pressure
Hypoxaemia
Free Radical formation O•
HIF-1α
Not broken down
Lack of ATP →
Cellular ion pump failure
? ↑ Venous Pressure
Microhaemorrhage
Upregulation of VEGF
Capillary Basement membrane damage + angiogenesis
Mechanical mediator
PaO2/PaCO2 mediator
Chemical mediator
HIF = Hypoxia Inducible Factor
NO = Nitric Oxide
Oedema → ↑ ICP
Headache
Basement membrane damage
Adenosine → Hypoxic vasodilatation
Elastic lamina
Smooth Muscle
? Vasodilatation → pain
Pain fibres of Trigeminal nerve → trigeminovascular system
NO → Impaired Ca2+-sensitivity → arteriolar vasodilatation
↑Ca2+
NO release
↑K+
NO

1.4.9 Factors Affecting Vessel Permeability

Hydrostatic Pressure

A number of studies have demonstrated increased CBF at altitude (Severinghaus, Chiodi et al. 1966; Jensen, Wright et al. 1990; Jansen, Krins et al. 1999). This is thought to result in an increase in hydrostatic pressure. Such increases occur in other clinical conditions such as hypertensive encephalopathy and toxaemia of pregnancy. These conditions also cause a reversible increase in white matter T2 signal (Na, Hong et al. 2004). Impaired autoregulation may also result in greater hydrostatic pressure.

Venous Hypertension

Idiopathic (or Benign) Intracranial Hypertension (also known as pseudotumor cerebri) has recently been demonstrated to be closely related to sinovenous outflow obstruction (Farb, Vanek et al. 2003; Owler, Parker et al. 2005). This obstruction would directly increase hydrostatic pressures. Such anatomical variations that cause this clinically may only become apparent under the stressor of hypoxia and hence account in part for the predisposition some have for AMS/HACE. HAPE itself may increase central venous and hence jugular venous pressures which in turn could contribute to HACE if an underlying venous hydrostatic mechanism is true. The presence of retinal and cerebral haemorrhages also suggests this as a mechanism.

Direct Effects of Hypoxia:

Hypoxia itself can damage basal membrane structures (Miserocchi, Passi et al. 2001). Houston proposed that hypoxia suppresses the sodium-potassium pump in cell membranes leading to cell swelling (Houston 1989). This was felt to be of relevance to acute but not gradual hypoxia. However, this theory has been rekindled by Kallenberg's finding of cytotoxic oedema. A reduction in cellular PO_2 decreases the expression and activity of the Na^+/K^+ ATPase in various cell types such as alveolar epithelial cells, endothelial cells and neuronal cells. This may represent a mechanism that cells (including possibly neurons) use to reduce energy expenditure in hypoxia.

Chemical Mediators of Permeability

Hypoxia Inducible Factor (HIF)

HIF-1 is a heterodimeric factor composed of HIF-1α and HIF-1β protein subunits. HIF-1α is constantly being made in large quantities in most cell types and, in normoxia, it is just as rapidly destroyed by HIF prolyl-hydroxylase. In hypoxia, HIF prolyl-hydroxylase is inhibited and HIF-1α therefore accumulates, is transported into the nucleus, binds to HIF1beta, and (as a heterodeimer) binds to promoter/enhancer elements causing increased transcription of classic hypoxia-inducible target genes. Such genes are involved in mediating a wide variety of responses, amongst them angiogenesis (Vascular Endothelial Growth Factor, VEGF (Yamakawa, Liu et al. 2003)) and erythropoesis (erythropoietin (Sanchez-Elsner, Ramirez et al. 2004)). Atrial naturetic peptide and nitric oxide synthase are also induced (Brzecka 2005).

Vascular Endothelial Growth Factor (VEGF):

Severinghaus first proposed an increase in VEGF expression as an explanation for increased vascular permeability in hypoxia (Severinghaus 1995). Hypoxia induces VEGF expression, whilst VEGF-specific antibodies prevent cerebral vascular leakage. Dexamethasone appears to block VEGF expression and hence reverse hypoxia-induced brain oedema (Schoch, Fischer et al. 2002). Early studies had shown no correlation between VEGF levels and AMS (Dorward, Thompson et al. 2007). Recently, however, the soluble VEGF receptor (sFlt-1) which can bind VEGF in the circulation and was not accounted for in earlier work, has been studied (Tissot van Patot, Leadbetter et al. 2005). In this study of 20 subjects who were driven to 4,300m to have blood samples taken, subjects who developed AMS had lower sFlt-1 and hence significantly higher levels of free plasma VEGF on ascent than well subjects.

Free Radicals

The study of free radicals is difficult due to their very short half-life. A number of studies however imply that neuroxidation and the subsequent inflammatory response may damage cerebrovascular endothelium (Bailey and Davies 2001; Bailey 2003; Bailey 2004; Bailey, Kleger et al. 2004; Bailey, Roukens et al. 2006) (Chan, Schmidley et al. 1984). In a recent study, Bailey *et al.* found that

there was a progressive increase in blood and CSF concentrations of free radicals (lipid derived alkoxyl and alkyl species) and IL-6 during a 16 to 18 hour simulated exposure to 4600m (12% O_2) (Bailey, Roukens et al. 2006). Although this induced a mild (0.6% or 7ml) increase in brain volume, no underlying morphological changes (e.g. oedema) were seen on MRI. No correlation between free radical formation and AMS was observed.

1.5 Genetic Predisposition to AMS

The study of genetophysiology, in particular the genetics behind hypoxic adaptation, is one of the most rapidly developing areas in high altitude research (Grocott and Montgomery 2008). Because of the polygenic nature of the human response to hypobaric hypoxia, it is likely that several genetic loci, each with a small but significant contribution, determine phenotypic outcome (Stobdan, Karar et al. 2008). Studies can be divided into those that investigate a population's adaptation to high altitude and those that correlate performance at altitude with polymorphic variations.

Erythropoietin (EPO): The rise in serum EPO concentrations with hypoxia was the first example of hypoxia induced increase in gene expression to be identified. A dinucleotide marker *DS7S477* is associated with variation in this response (Stobdan, Karar et al. 2008). EPO and the subsequent rise in haemoglobin are fundamental to acclimatisation and maintaining brain oxygenation.

Hypoxia-inducible Factor 1 (HIF1): Polymorphic differences in HIF1α have been found between Sherpas and Japanese subjects (Suzuki, Kizaki et al. 2003). Variations have also been associated with differences in maximal exertional oxygen consumption (VO_2max)(Prior, Hagberg et al. 2003).

Angiotensin-1 converting enzyme (ACE): ACE is a key enzymatic regulator of circulatory homeostasis, being responsible for the synthesis of angiotensin II, a vasoconstrictor that also provokes aldosterone release and thus sodium and water retention. It also degrades vasodilator kinins. The absence (deletion, D allele) of a 287 base pair fragment is associated with higher ACE tissue activity

than its presence (Insertion, I allele) and hence may enhance vasoconstriction and fluid retention through increased levels of angiotensin II and aldosterone. An excess in prevalence of the I allele has been identified amongst elite mountaineers and Sherpas (Woods and Montgomery 2001; Woods, Pollard et al. 2002; Droma, Hanaoka et al. 2008). Such associations do not seem mediated through differences in AMS susceptibility: Koehle *et al.* found no association between ACE and Angiotensin II Receptor 1 gene polymorphisms and AMS in Nepalese Pilgrims ascending to 4380m (Koehle, Wang et al. 2006; Rupert and Koehle 2006). Similarly, in mountaineers ascending to 4559m, those of DD genotype did not appear to suffer more AMS (Dehnert, Weymann et al. 2002). Instead, such associations may be driven by differences in tissue ACE expression, regulating hypoxic ventilatory drive (Patel, Woods et al. 2003) and metabolic efficiency (Williams, Rayson et al. 2000).

Aldosterone synthase: Elevated aldosterone levels have been associated with AMS (617±116 with vs 233±42 pmol/l without AMS) (Bartsch, Maggiorini et al. 1991) and wild type polymorphisms of CYP11B2 which confer lower aldosterone levels are considerably more common in Himalayan highlanders compared to lowlanders (Rajput, Arif et al. 2006).

Nitric Oxide Synthase 3 (NOS3): Higher levels of exhaled NO in Tibetans and Bolivian Aymara (Beall, Laskowski et al. 2001; Erzurum, Ghosh et al. 2007), and the success of inhaled NO in treating HAPE suggested NOS3 as a candidate gene. Polymorphic variations can render NOS3 more susceptible to intracellular proteases, reducing NO production and thereby impairing vasodilatation. An overrepresentation of Glu and 4b alleles in intron 4 of NOS3 (G894T) in Sherpas and Ladakhi suggest it may have a role in evolutionary adaptation to high altitude (Stobdan, Karar et al. 2008).

Polymorphisms of endothelin 1 (involved in pulmonary hypertension in hypoxia), β2-adrenergic receptor (which activates the Na^+/K^+ ATPase pump), fibrinogen (altering pro-coagulable properties), phosphodiesterase type 5A (involved in cGMP breakdown which in turn effects vascular smooth muscle tone) and of genes that may increase susceptibility to AMS/HACE are also being investigated (Stobdan, Karar et al. 2008). The results of these studies may

enable us to better understand the genetics of brain injury (Wilson and Montgomery 2007).

Zhou *et al* investigated alterations in gene expression in rat brain with chronic constant and chronic intermittent hypoxia (Zhou, Saidel et al. 2008). They found that in rat cortex, the expression of 80 genes was altered by chronic intermittent hypoxia (16 up- and 64 down-regulated) and constant hypoxia increased this to 137 genes (34 up- and 103 down-regulated). Expression of a similar number of genes was altered in the hippocampal region, although these were mostly upregulated. There are clearly many more genes to investigate.

1.6 Neuropsychological Effects of Hypobaric Hypoxia

A number of studies have shown impairment of arithmetic ability (Wu, Li et al. 1998), memory and metamemory (Du, Li et al. 1999; Pelamatti, Pascotto et al. 2003), language, perception, learning, cognitive flexibility and psychomotor skills (Bouquet, Gardette et al. 1999; Virues-Ortega, Buela-Casal et al. 2004) with ascent to altitude. Increases in reaction time (Kramer, Coyne et al. 1993; Bolmont, Bouquet et al. 2001) and auditory evoked potential P300 latency (Wesensten, Crowley et al. 1993), and a slowing of pupil constriction (Wilson 2008) have also been observed, and indicate a fundamental slowing of neuronal processing. As with other symptoms, the neuropsychological changes relate to the rate of ascent and the altitude (Virues-Ortega, Buela-Casal et al. 2004). As a result there are large differences between studies where different ascent protocols have been applied. In addition neuropsychological performance is susceptible to the influence of fatigue and also anxiety (Bolmont, Thullier et al. 2000). Sleep disturbance, either through periodic breathing and waking between periods of apnoea (Reite, Jackson et al. 1975) or secondary to disturbance by tent companions, is common and may contribute to daytime neuropsychological impairment.

It is also important to distinguish between neuropsychological changes due to hypobaric hypoxia and changes due to AMS. The literature suggests that AMS has little effect on short-term memory, but is associated with significant

impairment in performance of conceptual tasks. Without AMS, conceptual tasks are scarcely effected (Forster 1985; Kramer, Coyne et al. 1993).

Perception: Whilst no threshold change has been found for auditory stimuli, there is a rise in threshold for detecting visual stimuli when dark adapted (Kobrick and Appleton 1971). There are mixed results regarding changes in colour perception, but any change, if present, is of minimal significance (Virues-Ortega, Buela-Casal et al. 2004).

Memory: Short-term memory has been shown to decline at 4500m, an effect especially noticeable above 6000m, while long-term memory appears to be preserved (Berry, McConnell et al. 1989; Virues-Ortega, Buela-Casal et al. 2004). Both animal (Nelson, Dunlosky et al. 1990) and human (Kramer, Coyne et al. 1993) studies have compared controlled and automatic processing tasks and the results imply that moderately rapid exposure to hypobaric hypoxia causes a reduced capacity to learn rather than to retrieve. Spatial memory has been found to become impaired between 3800 and 5000m (Nelson 1982).

Cognitive flexibility has been assessed using Stroop Color and Word test or Wisconsin Card Sorting Test™. It has been shown to be significantly impaired in world class mountain climbers even months after their last ascent (Regard, Oelz et al. 1989).

Motor Skills: Motor speed and precision are reduced compared to sea level (Berry, McConnell et al. 1989; Hornbein, Townes et al. 1989). The finger taping test (FTT) and Purdue pegboard tests have been used to assess psychomotor changes but confounding variables in the field include fatigue and the cold. This may explain the wide variation in altitudes between different studies where dysfunction is detected (Berry *et al.* at 3500m (Berry, McConnell et al. 1989), Bolmont *et al.* at 8000m (Bolmont, Thullier et al. 2000)). The American Medical Research Everest Expedition (AMREE-1981) demonstrated that 15 of the 16 climbers had impaired FTT immediately after the expedition and 13 still had impairment a year on (West 1984) implying that some damage may be long term.

Alterations in balance have been quantitatively examined using a wobble board (Johnson, Simmons et al. 2005). A positive test (done on arrival at altitude) gave a predictive value for AMS of 66.7% at 4650m and 100% at 5005m. Brain oxygenation was also found to correlate with stability on the wobble board (while peripheral saturations did not).

Psychological changes: New onset anxiety disorders are relatively common in trekkers to altitude (Fagenholz, Murray et al. 2007). These are often focused on health concerns. A heightened state of anxiety has been shown to offset some of the reduction in reaction time and psychomotor ability (Bolmont, Thullier et al. 2000).

Auditory and visual hallucinations (e.g. a "third man") are common in climbers at very high altitude (Garrido, Javierre et al. 2000). It has been suggested that altitude-related hypoxia may account for the fundamental revelations contributing to the three monotheistic religions (Arzy, Idel et al. 2005) although Mount Hermon (2,814m) and Mount Sinai (2,285m) are not high and a better argument could probably be made for Buddhism or Hinduism.

Evidence of long-term brain injury: Anooshiravani *et al.* did not detect any functional or structural alterations (using MRI) in a group of 8 climbers who had climbed a 6000m peak (Anooshiravani, Dumont et al. 1999). However, Paola *et al.* have demonstrated that ascents to extreme altitude are associated with reduced white matter density and volume in areas related to the left motor cortex (Paola, Bozzali et al. 2008). Garrido *et al.* have performed studies demonstrating increased signal intensity in periventricular, posterior parietal, and occipital cortex in five of nine climbers who had ascended over 7000m (Garrido, Segura et al. 1995). Vichow-Robin spaces (CSF spaces around vessels) tend to be enlarged in regular climbers suggesting that chronic hypoxia induced brain atrophy (Garrido, Castello et al. 1993).

1.7 Conclusions

The brain is the most oxygen dependent organ in the body and many pathophysiological processes either cause and result from an interruption to its

oxygen supply. Insights into the causes and consequences of cerebral cellular hypoxia will help with the management of many acute neurological conditions. The last 25 years has seen a number of advances of our understanding of the neuropathophysiology induced by hypobaric hypoxia. It is also evident that the original “Tight Fit” hypothesis is oversimplified. Evidence is accumulating that while HACE may represent a continuation of AMS, there are key pathological differences (such as the formation of microhaemorrhages). It may well be that the “oedema” component of its name is distracting from the underlying pathological mechanism.

1.8 Core Hypotheses Investigated in This Thesis

This thesis sets out to investigate in greater depth some core cerebral physiological changes that occur in response to hypoxia. Specifically, these relate to cerebral blood flow into and out from the cranium, and to associated differences in brain oxygenation. The null hypotheses are:

1) **Cerebral arteries do not vasodilate or constrict with hypobaric hypoxia**

   TCD has been used in many high altitude studies. The velocity of blood is invariably interpreted as "flow" and, as explained in the introduction, all previous high altitude TCD studies have assumed that vessel calibre remains constant. On the other hand, the common teaching is that hypoxia causes vasodilatation and hypocarbia vasoconstriction at a microcirculatory level. I aimed to explore whether Middle Cerebral Artery calibre changes with hypoxia.

2) **Brain oxygenation changes reflect peripheral oxygenation saturation changes**

   High Altitude Headache does not appear to simply reflect a fall in brain oxygenation, given that no correlation between brain oxygenation and headache has previously been reported. By assessing brain oxygenation in a large cohort of subjects, a correlation between rSO_2 changes (both at rest and during exercise) and headache score was sought.

3) **The venous system adequately drains the increased cerebral blood flow of hypoxia**

   As explained above, the venous system appears to be involved in the formation of microhaemorrhages both in the retina and brain in hypoxia. A core hypothesis is that venous distension secondary to an inability to adequately drain the increased cerebral blood flow with hypoxia causes high altitude headache. Figure 1-10 demonstrates this "modified tight-fit" hypothesis in graphical format.

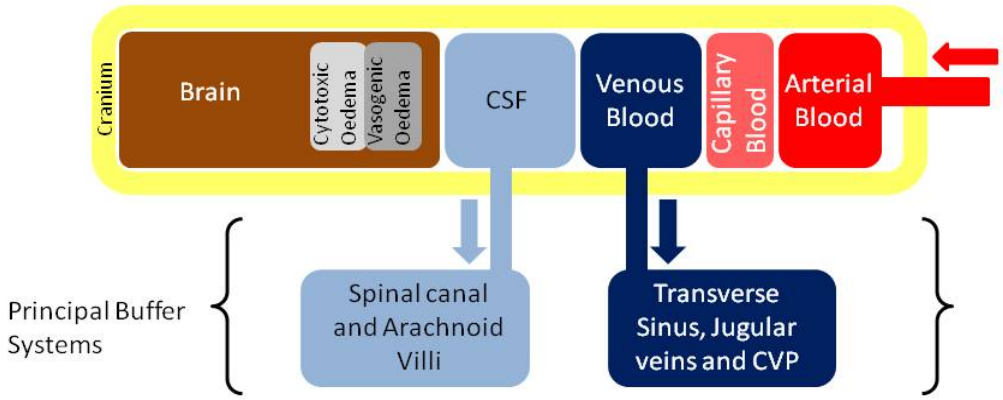


Figure 1-10 Schematic diagram of the modified "Tight Fit" hypothesis with venous hypertension/outflow obstruction. ICP will rise if contents increase with a concurrent failure to buffer. Factors that could therefore alter ICP and hence predispose to AMS include:
An increase in volume of:
Blood (arterial and capillary) from increased flow
Oedema – both vasogenic and cytotoxic from hypoxia
A failure in the buffer systems of:
CSF – less CSF buffer resulting in earlier ICP rise ("Tight Fit")
Venous outflow – for example with a predisposition to obstruction (as in Benign Intracranial Hypertension or Sagittal/Transverse Sinus Thrombosis)

2 Chapter 2: METHODOLOGY

See "Design and conduct of Caudwell Xtreme Everest: an observational cohort study of variation in human adaptation to progressive environmental hypoxia" BMC Medical Research Methodology 2010, 10:98(Levett, Martin et al. 2010) and
"Caudwell Xtreme Everest Expedition" High Altitude Medicine and Biology 2010, 11, 2(Grocott, Martin et al. 2010) in the Appendix for Papers relating to the field study of this work.

2.1 Introduction

This thesis studies the effects of hypoxia on the brain using many different modalities. Broadly, the research can be divided into:

a) HIGH ALTIUDE HYPOBARIC HYPOXIA STUDIES – These were performed during an expedition to Cho Oyu (8201m), mounted to test equipment durability and research methodologies to be deployed on a subsequent high altitude observational cohort study (the "Caudwell Xtreme Everest Expedition"). Only data from the later expedition is presented in this thesis.
b) SEA LEVEL NORMOBARIC HYPOXIA STUDIES – These comprised sea level Magnetic Resonance Imaging Studies prior to and following the field studies.

By far the largest component of this thesis relates to data from high altitude field studies, and comprises a number of anatomical, physiological and functional studies within it. These areas will be explained individually below. Firstly, however, an overview of the subjects, ascent profile and general aspects of the main field study (the Caudwell Xtreme Everest Expedition) will be provided.

The nature of the neuroscience studies can be grouped as demonstrated in table 2-1.

Anatomical	Physiological	Functional
High Altitude Studies		
Anthropomorphic Ultrasound Vessel Analysis Retinal Vessel Analysis	Cerebral Blood Flow (Transcranial Doppler) Cerebral Oxygenation (Near Infrared Spectroscopy)	Pupillometry Neuropsychology Headache Monitoring
Sea Level Studies		
MRI Vessels and Compartment Volumes Morphology Studies	MCA flow analysis Venous flow analysis	Headache Monitoring

Table 2-1 The main focus of each study can be classified as anatomical, physiological or functional. By collecting these data on all subjects, it is possible to cross-reference and correlate changes, although this was not the aim of this thesis.

The specific methodology of each study is outlined in the chapter relevant to it. Following the generic description of the high altitude and sea level studies below, specific information regarding the equipment and considerations to each sub-study is given.

2.1.1 Background

This work is a continuation of earlier work investigating intracranial pressure (ICP) in astronauts (Torikoshi, Wilson et al. 1995) and attempts, amongst other things, to establish whether ICP increases at high attitude in mountaineers (Wilson 2008; Wilson and Milledge 2008).

In 2003 the Centre for Aviation (now Altitude), Space and Extreme Environment Medicine at UCL planned an Expedition to Everest in 2007. The purpose of this expedition was to investigate human physiology in hypoxia (Grocott, Richardson et al. 2007) with the more specific aim of improving our understanding of human adaptation to cellular hypoxia, a fundamental mechanism of injury in critical illness.

Prior to this expedition, I joined the Birmingham Medical Research Expeditionary Society (BMRES) on an expedition to Ladakh. In addition to the cardiovascular work this group undertook, I carried out a simple experiment investigating the effects of hypoxia on pupil size and reaction speed (Wilson 2008).

In the two years prior to the Everest expedition, a great deal of planning was carried out. This included designing the experiments, resourcing equipment, obtaining ethical permission and then testing the equipment and protocols. An expedition to Cho Oyu in 2006 did this successfully (although personally, a rapid ascent following caring for another climber who had suffered a stroke, meant that I suffered from severe mountain sickness and retinal haemorrhages and hence did not summit).

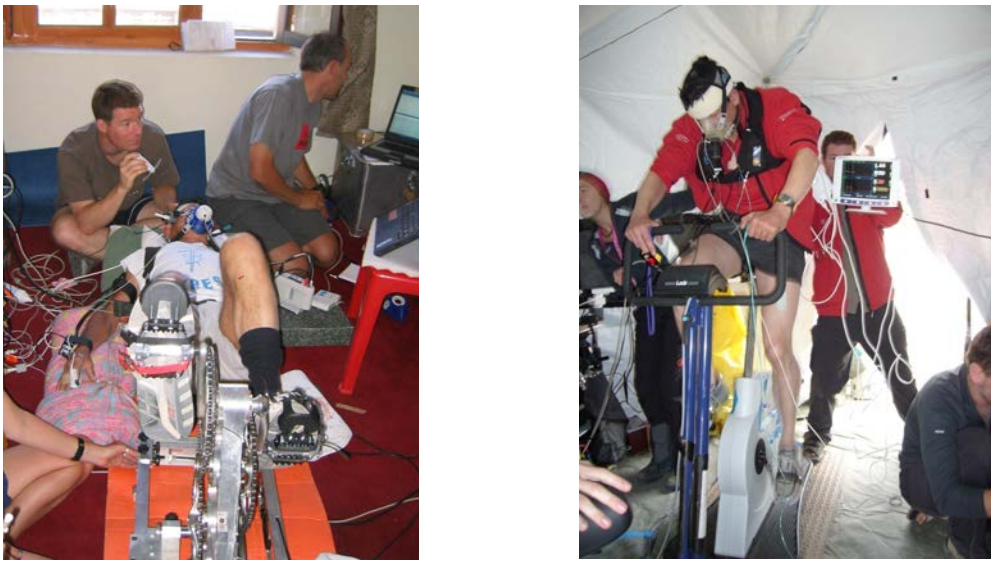


Figure 2-1 Measuring brain oxygenation (left) during recumbent exercise in Ladakh (BMRES 2005) and (right) on an upright bike on Cho Oyu (CASE 2006).

2.1.2 Clinical Link

Studying brain injury in patients is difficult. Brain injury is not a single disease entity, but multiple (extradural, subdural, contusion, haematoma, diffuse axonal injury etc.) that can affect different parts of the brain (frontal cortex, motor strip, brain stem) in different people (young, old), hence establishing physiological changes with these confounding variables is extremely difficult. Cellular hypoxia is a common final pathway for a number of these types of injuries. Mild hypoxia is also a reversible and ethical brain insult, hence our use of it to greater understand basic human physiology.

By studying a large group of people, and because of recent developments in molecular genetics, a better understanding of individual variability and the effects of hypoxaemia/cellular hypoxia should be achieved. These translational findings are now evident.

2.1.3 Ethics and Consent

The trekker and investigator studies were all approved through the UCL Ethics committee (Codes 0292/005, 0292/007, 0292/008, 0292/009, 0292/010, 0292/011, 0292/015 0292/016, 0292/017, 0292/018, 0292/022). The later sea level hypoxic MRI studies were also approved by UCL (2901/001). All subjects received documentation and verbal explanations of the studies and were free to withdraw at any time. Informed consent was obtained from all subjects. Following each study a report has been submitted back to the UCL ethics committee.

2.2 High Altitude Hypobaric Hypoxia Studies:

The Caudwell Xtreme Everest (CXE) Study:

This observational cohort study of progressive incremental exposure to hypobaric hypoxia was designed to describe the spectrum of adaptive responses in humans exposed to graded environmental hypobaric hypoxia and identify factors (physiological and genetic) associated with individual variation in these responses.

Many other body systems were investigated (e.g. gastrointestinal, musculoskeletal, cardiovascular, respiratory), however, the methodology of this

thesis will describe generic methods and then only those specific to the neuroscience work.

The overall study design, risk management plan and each individual protocol were approved by the University College London Research Ethics Committee. The studies were conducted between January and June 2007. Verbal and written informed consent was obtained from all subjects. All funding was unrestricted.

2.2.1 Subjects, Settings and Ascent Profile

Subject Selection, Health Assessment and Exclusion

All subjects were adults (over 18 years, no upper age limit) and underwent health screening at two stages prior to the study. Pregnant females, subjects with diabetes mellitus, significant cardiac or respiratory disease, and subjects who would normally be excluded from cardiopulmonary exercise testing (as based on the American Thoracic Society and American College of Chest Physicians guidelines(2003)) were excluded. Mountain medicine qualified doctors employed by the company arranging the logistics of the trek itself performed the initial screening. The second stage of the screening was performed within CXE to confirm fitness to travel and, in addition, fitness to undertake the research.

Two groups of healthy volunteers were studied (figure 2-2):

1) Group 1 (trekkers) – members of the public recruited by word of mouth and publicity.
2) Group 2 (investigators) – selected individuals from the investigating team (a group of 60 doctors, scientists, health professionals and students). This group was further subdivided into base-camp laboratory staff and a climbing team. Requirements for group 2 included previous event free ascent above 4000m. Requirements for the climbing team included previous event free ascents over 6500m, and for those summiting, an event free ascent over 8000m.

A breakdown of subject variables and the numbers of subjects completing the trek / climb in each group is given later in this chapter.

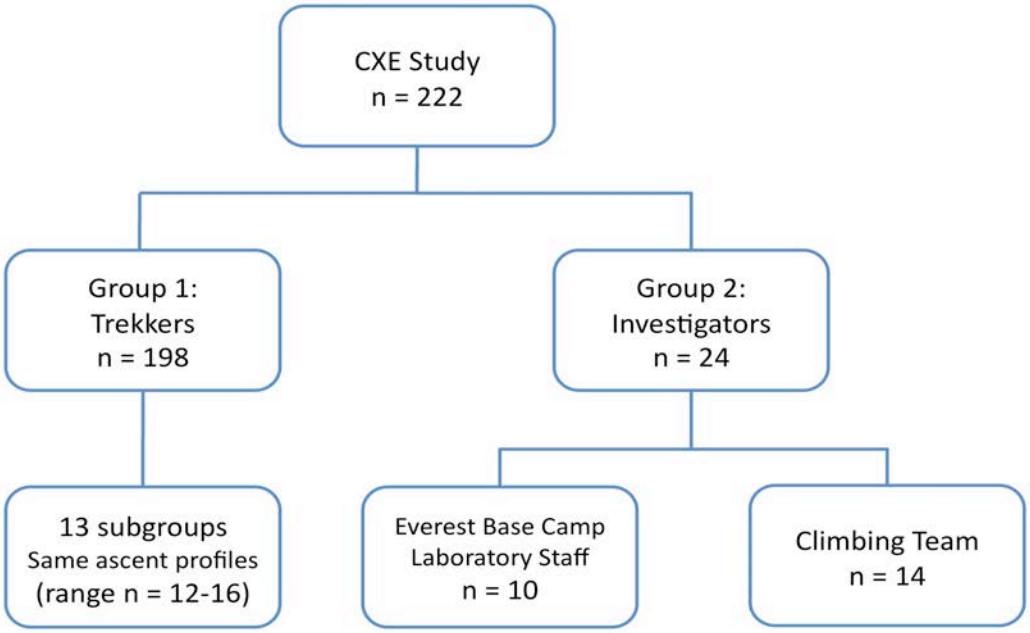


Figure 2-2 Diagram demonstrating the division of the 222 subjects of the Caudwell Xtreme Everest (CXE) Expedition.

Setting:

2.2.2 Baseline Study

Baseline measurements for all studies were performed at UCL (75m) between January 4th and February 26th 2007. As part of the baseline study, **anthropomorphic measurements** were made to assess all subjects' head volumes. This was not subsequently repeated during the field study.

2.2.3 Field Study

Field studies were completed between 31st March and 6th June 2007. All studies were carried out in specially built laboratories manned by the trained investigator teams. The laboratories were at: Kathmandu (1300m), Namche Bazaar (3500m), Pheriche (4250m), Everest Base Camp (5300m), Western Cwm (6400m), South Col (7950m) and the Balcony (8400m). Laboratory altitudes, barometric pressures and inspired partial pressures of oxygen are summarised in table 2-2.

Laboratory	*Approx Altitude metres*	*Ambient Temperature ˚C*	*Barometric Pressure millibar*	*Barometric Pressure mmHg*	*Barometric Pressure Kpa*	*PiO_2 mmHg*	*PiO_2 Kpa*
LONDON	75	24.1 (1)	1005	754 (10)	100.5 (1.3)	148.0	19.7
KATHMANDU	1300	26.1 (1.5)	867	650 (3)	86.7 (0.4)	126.2	16.8
NAMCHE	3500	19.6 (2.6)	670	505(3)	67.3 (0.4)	95.4	12.7
PHERICHE	4250	13.1 (1.7)	615	461 (2)	61.5 (0.3)	86.7	11.6
EBC	5300	21.5 (5.6)	538	404 (3)	53.8 (0.3)	74.7	9.9
WCWM	6400	12.7 (3.9)	467	350 (0.9)	46.7 (0.1)	63.4	8.5
SOUTH COL	7950	15 (8.9)	389	292 (2.3)	38.9 (0.3)	51.3	6.8
BALCONY	8400	Not recorded	363	272	36.3	47.1	6.3

Table 2-2 Laboratory altitude, mean barometric pressure, mean laboratory temperature and inspired partial pressure of oxygen. Data are presented as mean (standard deviation).

Ascent profile: All subjects flew from London to Kathmandu (overnight). They then flew to Lukla (2800m) in the Khumbu region and trekked to Everest Base Camp (EBC, 5300m) (see figure 2-3). All subjects were sequentially tested at laboratories in Kathmandu, Namche Bazaar, Pheriche and Everest Base Camp. The time course and altitude ascent profiles for Group 1 and 2 are summarised in figure 2-3 and 2-4. Expedition day 1 was defined as the day of departure from Kathmandu. The ascent rate was chosen to minimise the incidence of high altitude illness and hence maximise the number of subjects able to contribute data at the highest laboratory. As such, this is a study of hypoxia, NOT altitude illness specifically.

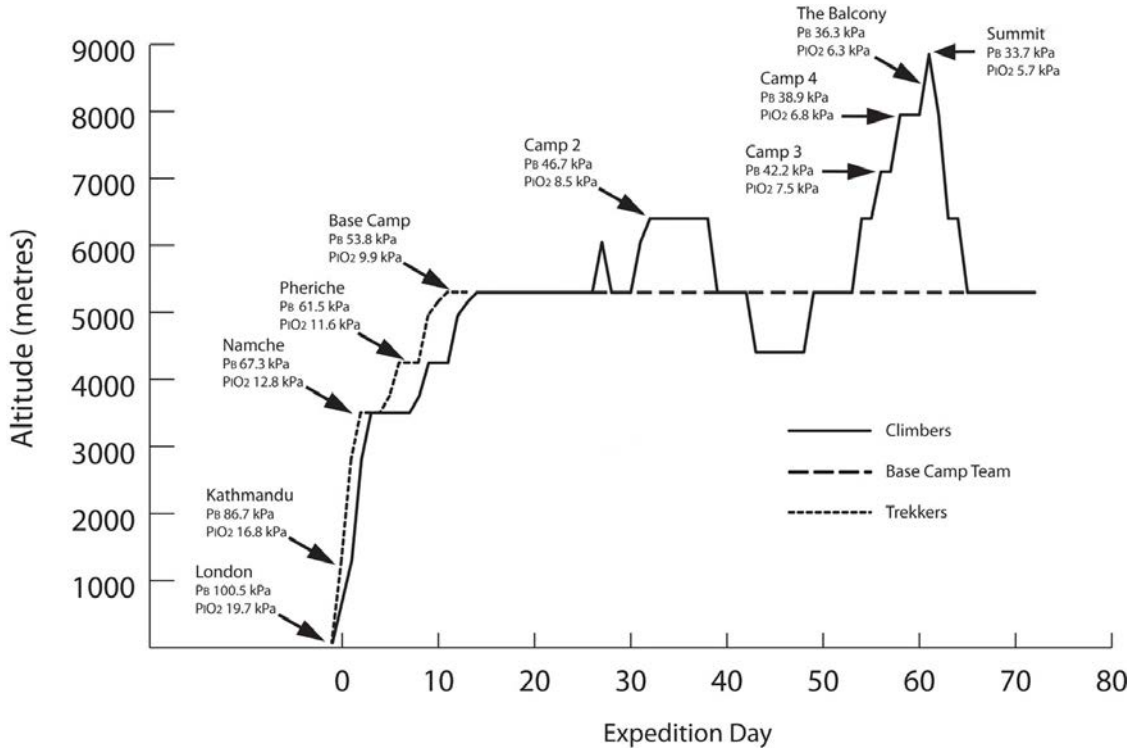


Figure 2-3 Ascent profile, mean barometric pressure and mean PiO_2 for group 1 (Trekkers) and group 2 (Investigators: Climbers and Base Camp team).

Expedition Day	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Location of Group 1: Trekkers					UK	K	K		N	N	N		P	P	P			E	E	E	P		N		K	
Location of Group 2: Investigators	UK	K	K	K	K	K	K		N	N	N	N	N		P	P	P			E	E	E	E	E	E	E

Figure 2-4 Schedule of testing for group 1 (Trekkers) and group 2 (Investigators). Expedition day 1 was defined as the day of departure from Kathmandu. UK: United Kingdom; K: Kathmandu 1300 m; N: Namche 3500 m; P: Pheriche 4250 m; E: Everest Base Camp 5300 m. Shaded boxes: Testing days Unshaded boxes Group 1: arrival day at laboratory Unshaded boxes Group 2: arrival day at laboratory and/or laboratory set up day.

Group 1 (trekkers) was divided into 13 smaller groups of a maximum of 16 subjects. Two groups left the UK each week for the duration of the expedition. All Group 1 subjects followed an identical ascent profile arriving at EBC on day 11. All Group 2 (Investigator) subjects followed an identical ascent profile to EBC on day 13 which was a modified version of group 1's ascent necessary for the logistical demands during the set-up phase of the laboratories (additional time was spent in Kathmandu and two additional days were spent at Namche Bazaar, 3450m). On rest days during the ascent to EBC, excursions were strictly limited such that all subjects remained within 300 vertical metres of the laboratory altitude at all times in order to guarantee an identical pattern of hypoxic exposure.

The ten laboratory staff subsequently remained at EBC for the duration of the expedition (68 days). For these investigators, excursions were limited to within 500 vertical metres of EBC altitude for the duration of the expedition. The climbing team (n=14) followed an identical ascent profile until the completion of all testing at Camp 2 (Western Cwm), including identical acclimatisation outings (figure 2-3). Subjects were not exposed to any supplemental oxygen until the completion of core testing at Camp 2.

All summit team climbers used supplemental oxygen at flow rates of 2-4l/min for the summit climb and 0.5l/min while asleep above Camp 3. Testing was repeated at the end of the expedition (immediately prior to departure) for all group 2 subjects at EBC (days 66 to 71).

Subjects in group 1 (trekker) were tested on either the day after arrival at any given altitude, or on the following day (Day 1 subjects, or Day 2 subjects). Group 2 (investigator) subjects were tested on Day 1, 2 or 3 after arrival at a given altitude. For each subject, the timing of such studies was kept constant to control for the effects of continued adaptation at the laboratory altitude. Furthermore, subjects were tested at the same time of day at all laboratories to control for diurnal variations in physiological responses.

To minimise the confounding effects of hypoxic adaptation prior to the study period, all subjects refrained from any form of hypoxic training (hypoxic tents etc.) and did not travel above 3000 metres for 3 months prior to departure. Subjects did not take prophylactic medication (e.g. acetazolamide) to prevent acute mountain sickness. Any individuals diagnosed with mountain sickness were treated appropriately using specific guidelines and a record of all medication taken during the expedition was kept.

Cardiopulmonary Exercise Testing (CPET): CPET was an integral part of the study used to investigate oxygen consumption and efficiency during aerobic, anaerobic and maximal exercising. As part of the CPET test, I also monitored brain oxygenation (see chapter 4). Any subject diagnosed by the expedition medical team with altitude illness was excluded from CPET. Prior to CPET, each subject was monitored. Specific altitude dependent symptom and

physiological criteria were used to trigger referral to the expedition medical officer for consideration of exclusion from CPET testing at that laboratory (table 2-3).

Exclusion criteria for testing at field laboratories
• Resting blood pressure >200 mmHg Systolic, and or >110 mmHg Diastolic • Acute systemic infection (discuss with medical officer) • AMS requiring treatment with acetazolamide, dexamethasone or nifedipine • Acute chest pain • New arrhythmias or ECG changes • Resting arterial Oxygen saturations <90% at sea level; <85% Kathmandu, <80% Namche <75% Pheriche, <70% at Everest Base Camp
Criteria for stopping test
• Excessive rise in blood pressure: ○ >250 mmHg Systolic; >115 mmHg Diastolic • Drop in systolic blood pressure of >10 mmHg from baseline, with other indications of ischaemia (see below) • >2 mm ST depression or >1 mm ST elevation • Onset of angina or angina-like symptoms • Onset of new arrhythmia other than ventricular ectopics • Nervous system symptoms - ataxia, dizziness or near syncope • Subject requests termination of test

Table 2-3 Criteria for exclusion from exercise testing at field laboratories and for stopping CPET

2.2.4 Specific Neurosciences Studies

A number of neuroscience studies were completed. All are explained here in the methods, but not all the results are presented in this thesis. Their inclusion here is because some are mentioned later in the thesis, but also to demonstrate that beyond this thesis, combining studies may draw further conclusions.

Core Studies:
All subjects from group 1 and 2 underwent all of the following neuroscience studies:

1) Diary Monitoring: - A daily diary of physiological measurements and headache assessment
2) Resting and exercise assessment of brain oxygenation
3) Retinal Imaging
4) Anthropomorphic skull assessment (baseline study only)
5) Neurocognitive assessment (not reported in this thesis)
6) Pupillometry (not reported in this thesis)

In addition, group 2 underwent additional studies investigating:

1) Cerebral blood flow and resting supine brain oxygenation
2) Intraocular pressure

Twelve subjects pooled from both groups 1 and 2 also underwent a volumetric MRI scan (see below).

2.2.4.1 Daily Diary - Physiological and Headache Assessments

All subjects completed a physiological and symptoms scoring diary on each day of the expedition. The Lake Louise Score (Roach, Bartsch et al. 1993), Environmental Symptoms Questionnaire (Sampson, Cymerman et al. 1983) and a “Headache Severity Index” were recorded each morning. The headache severity index was calculated by grading the headache of the previous 24 hours (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = excruciating) and multiplying it by the number of hours the headache persisted. In addition, location of headache was recorded using a skull diagram and the time of day the headache occurred (00:00 to 06:00; 06:00 to 12:00; 12:00 to 18:00; 18:00 to 24:00 could be circled). Resting heart rate, blood pressure, respiratory rate and

peripheral saturations were also recorded after 10 minutes rest prior to breakfast each morning.

2.2.4.2 Brain Oxygenation / Near Infrared Spectroscopy (NIRS)

All subjects (Group 1 [n=198] and 2 [n=24]) had assessment of regional brain oxygenation (rSO_2) at rest and during exercise using a Cerebral Near Infrared Oximeter (NIRS - Invos Model B or C, Somanetics, Pennsylvania, figure 2-5).

Background to NIRS: Jobsis (Jobsis 1977) reported that transillumination of brain and myocardium with near infra-red light could be used to evaluate tissue oxygen saturation. NIRS is a technique that uses the differential absorption of near infrared light by oxy and deoxyhaemoglobin (Owen-Reece, Smith et al. 1999). This was first used to study human brain in 1985, and by 1993 the first commercial monitor was available. By comparing the ratio of oxy to total haemoglobin, a regional saturation (rSO_2) value is generated. The INVOS system (Model B or C, Somanetics, Pennsylvania) utilises a single emitting diode and two detecting diodes, one 3cm and one 4cm from the emitter. The closer detects blood that has been through scalp and skull, the more distant one through scalp, skull and brain (figure 2-6). By subtracting the differences in absorption between these two readings, a more “pure” brain rSO_2 is generated. A brief description of principals of NIRS is given below. Greater detail with regards to the physics underlying the principals can be found in a number of reviews (Owen-Reece, Smith et al. 1999; Murkin and Arango 2009).

Normal resting cerebral oxygen consumption is 3.5ml/100g brain/min (Rowell 1993) and therefore the rate of oxygen consumption of the entire (on average 1,400g) brain is 50ml O_2/min. Hence, although the brain only weighs 2% of the total body weight of a 70Kg male, its oxygen consumption represents ~20% of the 250ml O2/min average metabolic rate for the entire body.

The measurement of tissue oxygenation and haemoglobin content is determined by the difference in intensity between a transmitted and received light delivered at specific wavelengths as described by the Beer-Lambert law. Above a wavelength of 1300nm, water absorbs most light, below 700nm, light is scattered too much. Between this is the near infrared range and between 700

and 850nm the absorption spectra of Haemoglobin (Hb) and Oxyhaemoglobin (HbO_2) are maximally separated. The isobestic point (wavelength at which oxy and deoxyHb species have the same molar absorptivity) is 810nm.

The Beer Lambert law states that:

$\Delta A = L \times \mu$

where ΔA is the amount of light attenuation, L the differential photon path length through tissue and μ the absorption coefficient of chromophore X (which can be expressed as the tissue concentration of X multiplied by the extinction coefficient of chromophore X) hence the light attenuation is directly proportional to the concentration of X. If path length is known (estimated by computer modelling), absolute changes in chromophore concentration can be calculated.

There are two computer interpretation systems from this stage. The first is a non-quantitative cerebral oximetry technique such as the INVOS system. This measures the ratio of light absorption by oxygenated and total haemoglobin, hence does not need to quantify haemoglobin concentration. The second system provides quantitative concentration measurements (such as the Hamamatsu (Hamamatsu City, Japan) and Critikon (Ascot, Berkshire, UK) systems).

Figure 2-5 A subject demonstrating two NIRS forehead pads connected to a NIIS (Invos Model C) Niroscope.

The INVOS system used in this study emits light at 730nm and 805nm from a single LED and this light is detected by optodes at 3 and 4 centimetres distal. Only rSO_2 is calculated and recorded. They do not publish their algorithm for the calculation of rSO_2 hence it is not possible to work back and calculate ratios of oxygenated to deoxygenated haemoglobin. The advantage of the INVOS system (and reason for its use in this study) was that the machines are rugged, relatively compact and can run on batteries. This makes them more suitable to the expedition environment. We also required two machines to run concurrently in Namche, Everest Base Camp and one on Everest itself. The free loan of the total 5 INVOS machines made this possible. However, in retrospect, the greater

analysis that would be possible with other machines would have yielded much more interesting and interpretable results.

Note, our group has also studied skeletal muscle oxygenation during exercise at altitude which has been reported separately (Martin, Levett et al. 2009).

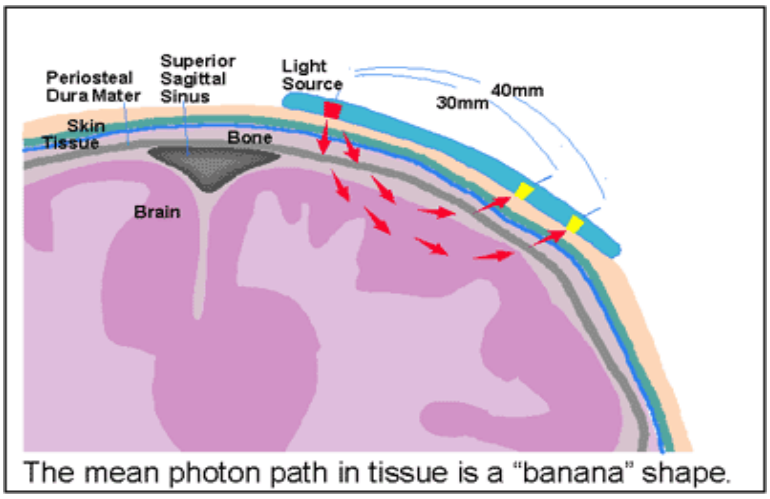


Figure 2-6 Schematic demonstrating the patented Invos system of utilising the differential pathways of near infrared light through scalp, skull and brain to generate a more reliable brain rSO_2.

The NIRS readings were obtained while sitting upright on the cycle ergometer before and during CPET testing. An Infrared emitter/receiver pad was placed on each side of the subject's forehead and secured with a headband. The subject rested for 5 minutes prior to CPET testing, after which 3 bilateral NIRS measurements were taken. Brain oxygenation data were collected continuously at four-second intervals from rest throughout the CPET. Three simultaneous data values were averaged for both left and right rSO_2 values at rest, after 3 minutes of unloaded cycling, at anaerobic threshold and at VO_2Max. This enabled a graphical demonstration of each subjects change in rSO_2 during the exercise test. (See the specific study regarding analysis of this data).

Group 2 underwent an additional NIRS study as part of the Cerebral Blood Flow protocol (see below). As part of this study, resting rSO_2 was recorded as above but while recumbent having rested for 5 minutes.

All of these studies were performed at: baseline, 1300m, 3500m, 4250m, 5300m and in the climbing group at 6400m and 7950m.

2.2.4.3 Retinal Imaging

All subjects underwent retinal imaging (8TRC NW200 Non-Mydriatic Digital Ophthalmoscope; TopCon, Tokyo, Japan) in London and again on arrival at Everest Base Camp. Group 2 also underwent reimaging prior to descent from Base Camp. Prior to imaging, all subjects were asked to stare at an Amsler chart with the right, then left eye, to assess for any schotoma (*see* Appendix figure 2-A). If found, they were asked to draw the "blind spot" onto the Amsler chart.

The TopCon 8MegaPixel Digital retinal camera (8TRC NW200 Non-Mydriatic Digital Ophthalmoscope; TopCon, Tokyo, Japan figure 2-7) enabled high quality retinal imaging (figure 2-8) without the need for pupil dilatation. Subsequent analysis included assessment of: retinal artery calibre (superior retinal artery), retinal vein calibre (superior and inferior retinal vein), optic disc margins, optic disc diameter and the presence of retinal haemorrhages.

Figure 2-7 The TopCon 8MegaPixel Non-Dilating retinal camera in use in the research tent at Everest Base Camp (5300m).

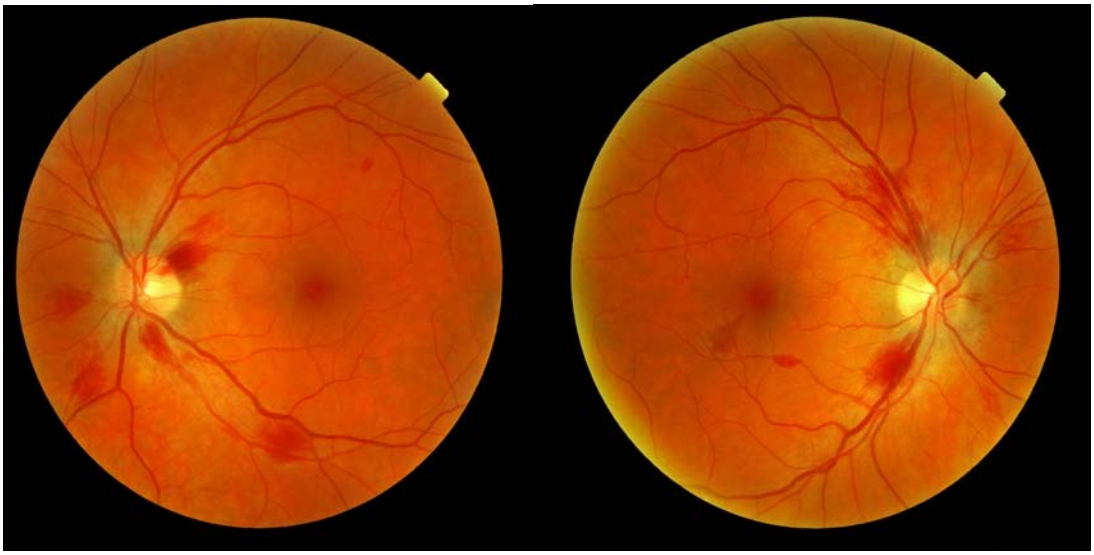


Figure 2-8 Right and left retinal images demonstrating typical retinal haemorrhages.

2.2.4.4 Neurocognitive Assessment

All subjects underwent neurocognitive assessment. This comprised a series of studies that in total took 40 minutes to perform. These included: trail making, letter cancellation, word finding, Symbol Digit, Rey, Stroop, Grooved Pegboard and block design tests (see chapter 9 and appendix B). In addition State Trait Anxiety Inventory (STAI) and Center for Epidemiologic Studies – Depression Score (CED–D) were assessed. Investigators performing these tests were trained to ask the questions in the same manner to attempt to standardise measurements. Subsequent sea level control studies were also performed.

The neurocognitive studies were performed at: baseline, 1300m, 3500m, 4250m, 5300m and in the climbing group at 6400m and 7950m. Only a brief outline of results is reported in chapter 9.

2.2.4.5 Anthropomorphic Study

The “Tight Fit” Hypothesis implies that subjects with more compliant CSF systems will be less prone to a rise in ICP and hence high altitude headache, if that is the cause. It could therefore be hypothesised that subjects with relatively larger skull volumes would be protected from high altitude headache. Using specially designed callipers (figure 2-9), all subjects underwent the following skull measurements (figure 2-10):

- Maximum head length (glabela to inion: L)
- Maximum head breadth (between the two parietal eminences: W)
- Auricular height (external acoustic meatus to bregma: H)
- Head Circumference (from glabela to ionion)

Figure 2-9 Callipers specifically designed for obtaining measurements described in Figure 2-10. a) measured length and width. b) with the base plate removed, height was measured.

The first three measurements were then used in the Lee-Pearson Formula to calculate intracranial volume (Sahin, Acer et al. 2007).

Male ICV: 0.000337 (L–11) × (B–11) x (H–11) + 406.01
Female ICV: 0.0004 (L–11) × (B–11) × (H–11) + 206.60

These calculations were subsequently compared with summations of daily Lake Louise scores.

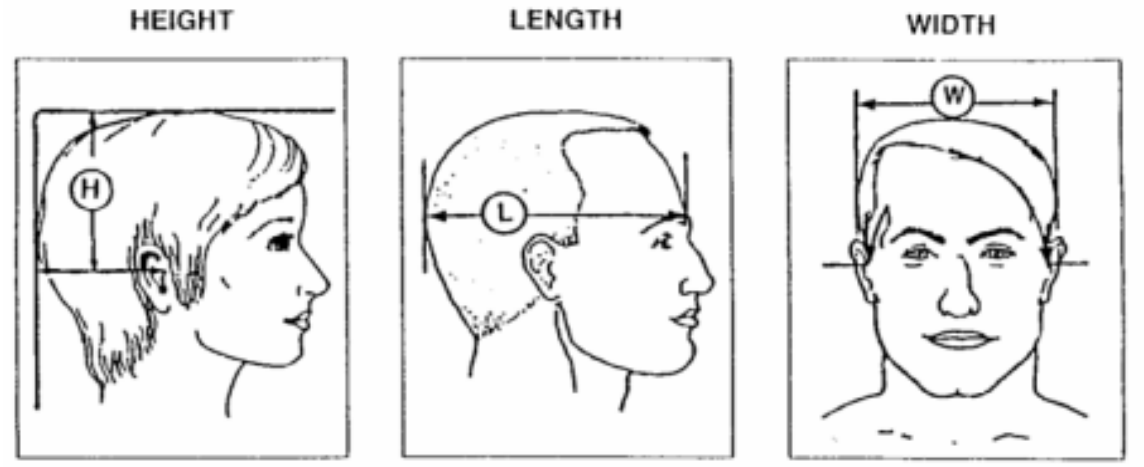


Figure 2-10 Illustrations showing the measurement of cranial height, length and width.

2.2.4.6 Pupillometry

All subjects underwent pupillometry studies using a ForSite Digital Pupilometer (Neuroptics Inc, Irvine CA – figure 2-11). This is a battery operated, hand-held, portable device that incorporates a white light flash emitter and an infrared digital video camera to record the responding pupil changes. The device emits a constant infrared light, the wavelength of which (850 nm) is beyond the normal response of the human eye. To stimulate the pupil, it then emits a flash of white light of fixed intensity for a duration of 0.8 seconds. Simultaneously, an integrated video camera records the changes in pupil size at 40 frames per second. From this, the following dynamic pupillary variables are calculated: maximum and minimum pupillary diameters (Max A and Min A, respectively, measured to 0.1 mm), the percentage change in diameter before and after constriction (Max A - MinA/MaxA] / 100), constriction latency as well as the velocities of constriction (CV), and dilation (DV). Results are displayed on an LCD screen with a graphical representation and numerical values (figure 12).

Prior to performing pupillometry, all subjects remained in darkness for 2 minutes under a black veil. The right eye was then assessed. In the case of failure to obtain data, the pupillometry was repeated after a further minute. The study was then repeated in the opposite eye.

This study was performed on all subjects at baseline, 1300m, 3500m, 4250m, 5300m and as many as possible at 6400m and 7950m.

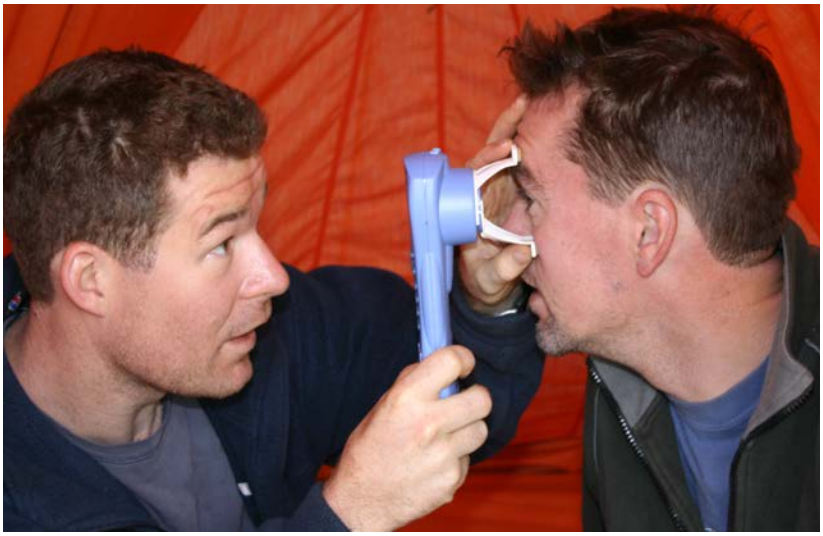


Figure 2-11 ForSite Digital Pupilometer (Neuroptics Inc, Irvine CA) being used without light excluding black cover.

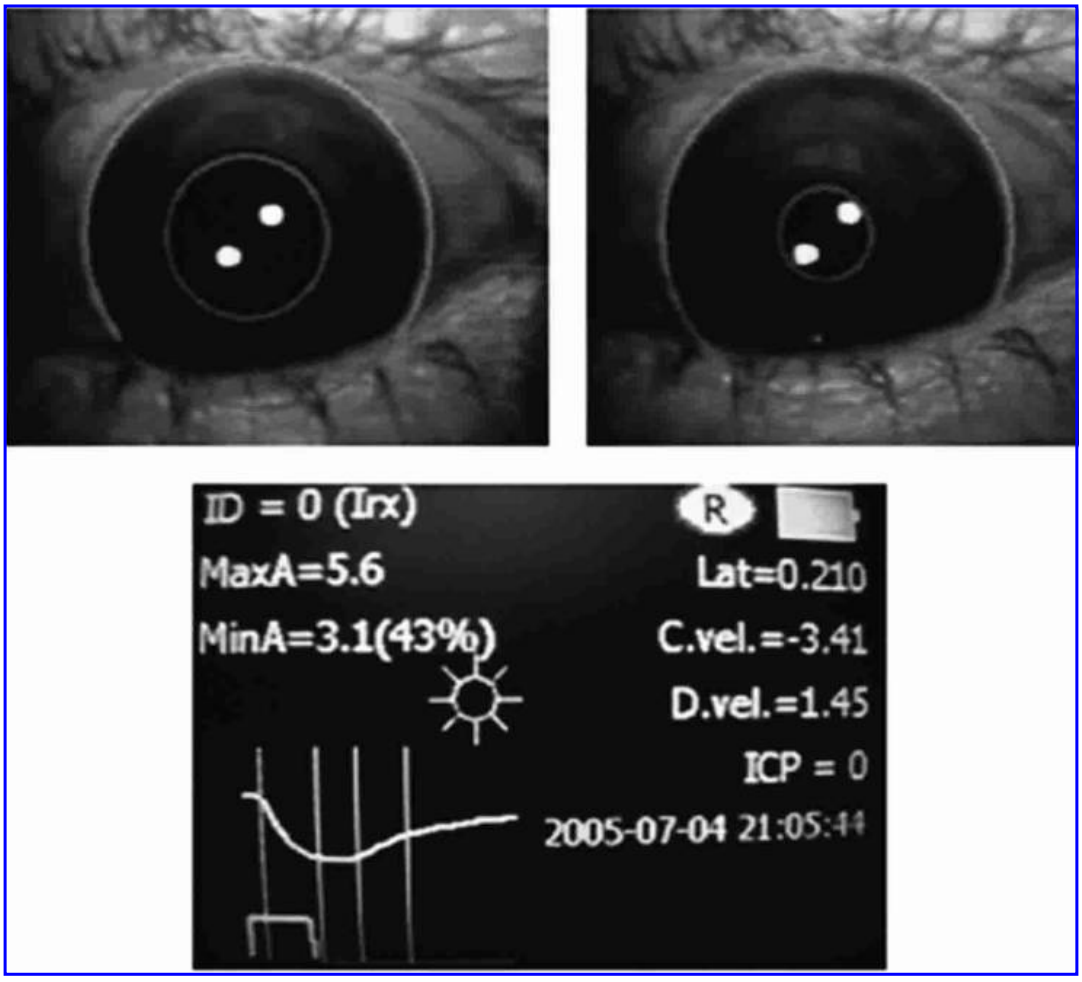


Figure 2-12 Pupil images at maximum and minimum dilatation. Below is the digital readout.

2.2.4.7 Additional Group 2 (Investigator Group) Studies

In addition to the above, group 2 also underwent the following assessments:

Resting Cerebrovascular Studies

2.2.4.8 Transcranial Doppler (TCD)

The aim of this TCD study was to assess the velocity of blood in, and the calibre of, the right Middle Cerebral Artery (MCA). This assessment was performed in all subjects in group 2. The subject rested supine for 5 minutes prior to the start. The right MCA was insonated via the temporal bone window (figure 2-13), by one of two skilled observers (MW/CI), using a 5-1 MHz Transducer MicroMaxx^{TM}, (Sonosite, Bothell, WA, USA).

Figure 2-13 MW insonating right temporal window of an investigator.

The clinoid process of the sphenoid bone, the Circle of Willis and the distal internal carotid artery were initially identified, and then the M1 segment of the MCA was identified (characterised by flow towards the transducer). The Doppler gain was set in a standard fashion (Martinoli and Derchi 1997). An optimal portion of the MCA without branches and with laminar flow was then selected and the depth recorded. Once identified, the centre of the artery was insonated and MCA blood velocity (MCA_{Vel}), Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV), Pulsatility Index (PI) and Resistively Index (RI) calculated by the inbuilt software (figure 2-14).

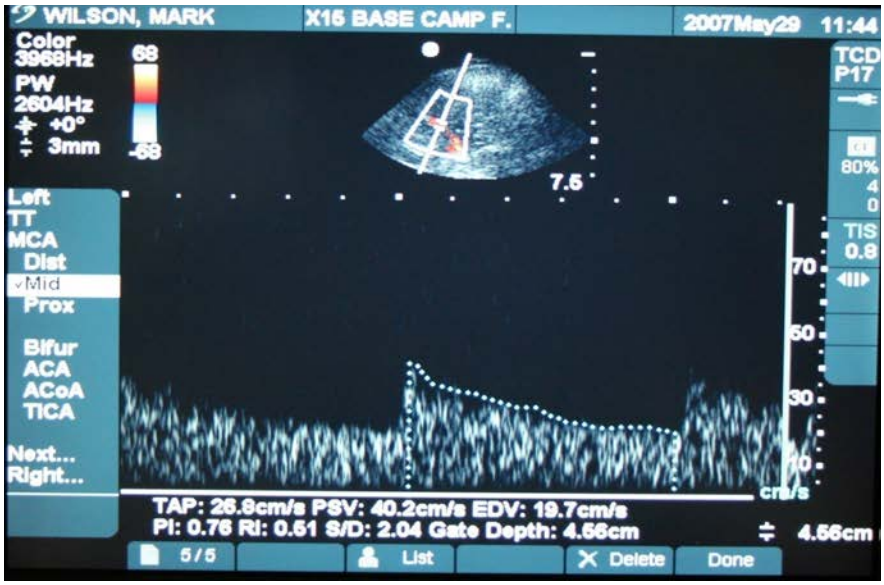


Figure 2-14 Display of Sonosite Micromax demonstrating graph and calculations.

On subsequent studies (at other altitudes), every effort was made to insonate the same depth (to within 1 mm). After 3-5 minutes of insonation, the 2D image movie sequence was saved, and the frame with the maximum vessel diameter (systole) studied. Using the on screen calliper tool, the width of the vessel at the point of insonation was measured and recorded. The angle of insonation was constant for each individual, since the position of the probe on the temporal

bone window and the position on the interrogated section of the MCA, were fixed.

All measurements were performed without supplemental oxygen up to 6400m. At 7950m, subjects were off supplementary oxygen (2lmin^{-1}) for at least 30 minutes prior to the NIRS and TCD measurements being made. The NIRS and TCD studies were then repeated with the subjects receiving 2lmin^{-1} supplemental oxygen via a TopOut re-breath regulator system (Topout Mask Mk 2, Topout Oxygeneering Ltd, Cotgrave, UK) to assess reversibility of the initial measurements. At 7950m, the investigating clinician used supplementary oxygen (2lmin^{-1}).

Flow and Oxygen Delivery Calculations:

Blood Oxygen content was calculated using the formula:

Blood Oxygen content = 1.39 x Hb x SaO_2/100

The small quantity of dissolved Oxygen (decreasing further at altitude) was not included in the estimation.

Middle Cerebral Artery Blood Flow was calculated using the formula:

Flow = $\pi(MCA_{diam}/2)^2$ x MCA_{vel}

This estimation does not take account of vessel wall resistance or changes due to any turbulent flow. Oxygen delivery was calculated by multiplying blood oxygen content with flow.

All variables were analysed and statistical significance sought using single factor ANOVAs within the groups n=24 to 5300m, n = 14 to 6400m and n=5 to 7950m. Microsoft Excel™ and SPSS™ version 14 (Michigan, USA) statistical packages were used.

2.2.4.9 Resting Near Infrared Spectroscopy (NIRS)

Regional Brain Oxygen Saturation (rSO_2) measurements were made immediately prior to TCD insonation (Invos Cerebral Oximeter 5100C, Somanetics, MI, USA – see above). After the skin was cleaned, probes were placed over the right and left frontal lobes avoiding both the sagittal and frontal

sinuses and left in situ during the TCD analysis. After this, three consecutive readings were taken from each side, from which means were calculated.

2.2.4.10 Intraocular Pressure (IOP)

Group 2 subjects who did not wear contact lenses had intraocular pressure measurements made in both eyes using a hand held Icare™ tonometer (TA01i, Espoo, Finland). This direct contact technique is different to air puff techniques previously used at altitude as it negates confounding problems with changes in air pressure. The device uses the average of 7 measurements to calculate IOP. This was performed with the subject resting (sitting) looking directly ahead in each eye at baseline, 1300m, 3500m, 4250m, 5300m and in some of the climbing group at 6400m and 7950m.

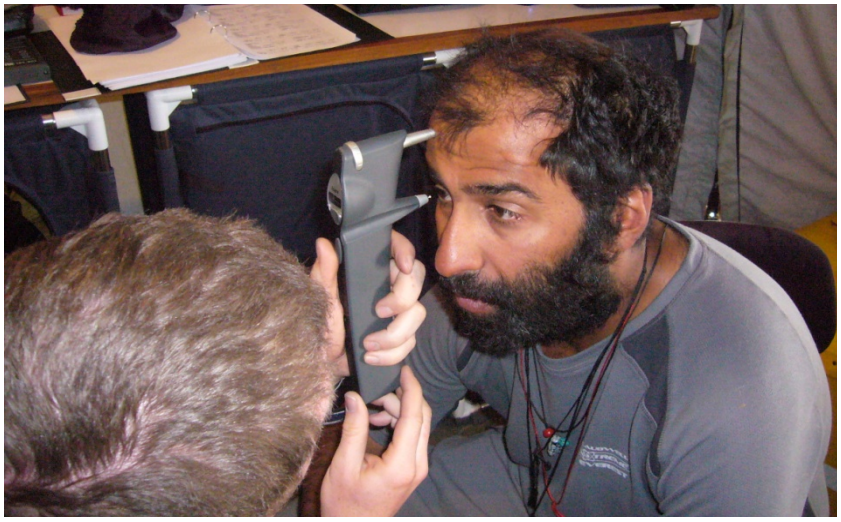


Figure 2-15 An ICare tonometer measuring intraocular pressure.

2.2.5 Non-Neuroscience Studies

A number of non-neuroscience studies were performed and are not reported within this thesis. These include Maximum Exercise Capacity and Metabolic Efficiency (although NIRS was used to assess brain oxygenation during these studies), Spirometry, Systemic Oxygen Content, Plasma Biomarkers and Genes Associated with Hypoxia. Details of these studies can be found in the group methodology paper (Levett, Martin et al. 2010).

2.3 Numbers of Subjects Completing Neurosciences Studies

Two hundred and eight volunteers applied to join the expedition as trekkers, and four withdrew prior to baseline sea level testing for personal reasons. One volunteer was advised not to trek as a result of medical screening. Two hundred and three volunteers were tested at sea level and five of these withdrew prior to departure (one because of a back injury and four for personal reasons). In the light of findings at baseline exercise testing, six subjects were withdrawn from subsequent maximum CPET testing, and three of these were also withdrawn from the steady state CPET testing. Sixty-two applicants applied to join the investigator group of whom 60 were selected and able to participate. Twenty-four were selected to be investigator subjects (investigators, group 2) at EBC of whom 14 met criteria to become part of the climbing team, 10 of whom became the summit team. One hundred and ninety eight trekkers (Group 1) and 24 investigators (Group 2) who had been tested in the UK commenced the trek. The baseline characteristics of the study groups are summarized in Table 2-4.

	Group 1 (Trekkers) number (%)	**Group 2 (Investigators) number (%)**
Total	198 (100)	24 (100)
Male	125 (63)	18 (75)
Previous Altitude Exposure (>3500m)	85 (43)	23 (96)
Previous Extreme Altitude Exposure (>5000m)	37 (19)	21 (88)
Smoker	13 (7)	0 (0)
Race - white	191 (97)	22 (92)
Mean Age (SD)	44.7 (13.7) (range: 18-73)	35.2 (9.3) (range: 19-59)

Table 2-4 Baseline characteristics of the CXE study population.

Of 198 trekkers who left the UK, 190 (96%) reached Everest Base Camp. Of the eight subjects who did not arrive at EBC, the reason for this was acute mountain sickness in three subjects (1.5% of total), and non-altitude specific medical conditions in five (2.5% of total) (table 2-5). In the investigators group

(group 2), all 24 subjects reached Everest Base Camp. Of the climbing team, all 14 subjects reached camp 2. Eight of ten summit climbers successfully reached the summit of Mount Everest. One member of the climbing team descended because of altitude illness at camp 3. One member of the climbing team turned back during a summit attempt with no altitude illness. One member of the laboratory staff was evacuated prior to the completion of the expedition with non-altitude related illness (table 2-6).

	Kathmandu (1300m)	**Namche (3500m)**	**Phericheche (4250m)**	**Everest Base Camp (5300m)**
Subjects at Laboratory	198	197	195	190
Reason for Absence	• n/a	• Respiratory Tract infection	• Abscess • AMS	• AMS • Angina • Respiratory tract Infection (2) • Diarrhoea and vomiting

Table 2- 5 GROUP 1 (Trekkers) - Number of subjects arriving at each laboratory and reasons for absence from laboratory. AMS = Acute Mountain Sickness.

	Kathmandu (1300m)	Namche (3500m)	Phericheche (4250m)	EBC (5300m)	WCwm (6400m)	SCol (7950m)	EBCend (5300m)
Subjects at laboratory	24	24	24	24	14	12	23
Reason for absence	n/a	n/a	n/a	n/a	n/a	• HACE • AMS	• Septic Shock

Table 2-6 Group 2 (Investigators) – Number of subjects arriving at each laboratory and reasons for absence from the laboratory.

The numbers of subjects tested in each neuroscience protocol at each laboratory for group 1 and group 2 are summarized in tables 2-5 and 2-6 respectively. Mean laboratory pressures and laboratory temperatures are recorded in table 2-1.

	Sea Level	**Kathmandu 1300m**	**Namche 3500m**	**Pheriche 4250m**	**EBC 5300m**
Subjects at laboratory	198	198	197	195	190
CORE STUDIES					
Daily diary	198	195	196	194	190
CPX Ramp (+NIRS)	190*	189	184	183	153
CPX ME	195**	n/a	191	n/a	164
Neurocognitive	198	160 (on return)	195	n/a	185
Pupillometry	198	n/a	191	n/a	186
Cranial measurements	198	n/a	n/a	n/a	n/a
Retinal photography	183	n/a	n/a	n/a	183
ADDITIONAL STUDIES					
Structural and volumetric MRI study	7	n/a	n/a	n/a	n/a

Table 2-5 Group1 (trekkers n=198) – Testing performed at each laboratory. *6 subjects withdrawn from incremental CPET prior to departure and 2 because of poor baseline data quality. 3 subjects withdrawn from efficiency CPET prior to departure.**

	Archway 75m	Kathmandu 1300m	Namche 3500m	Pheriche 4280m	EBC (I) 5300m	EBC (II) 5300m	Camp 2 6400m	South Col 7950m
Subjects at laboratory	24	24	24	24	24	23	14	12
CORE STUDIES								
Daily diary	24	24	24	24	24	23	14	5
CPX Ramp (+muscle and brain NIRS)	24	24	22	24	23	22	14	5
Neurocognitive	21	21*	21	21	21	n/a	13	6
Pupillometry	24	24	24	20	24	23	14	0
Cranial measurement	24	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Retinal photography	24	n/a	n/a	n/a	24	23	n/a	n/a
ADDITIONAL STUDIES								
Cerebral Doppler	24	24	24	24	24	24	13	5
MR brain volumetric studies	15	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 2-6 Group 2 (Investigators) (n=24) – Testing performed at Each Laboratory, * = on return.

2.4 Sea Level MRI Studies

A total of three MRI studies are described in this thesis. While each investigated a specific question, they also contained elements that acted as pilot studies for the subsequent study. Full details of each MRI study and technique is provided within the respective chapter.

2.4.1 Anthropomorphic (Normoxic Study)

The purpose of this study was to assess cerebral compartment volumes and attempt to correlate these volumes (brain parenchyma, CSF and venous volumes) with headache scores from the Everest expedition.

Subjects: Twelve subjects (all male) recruited from Xtreme Everest volunteers and investigators underwent cranial MRI scans prior to and following the Everest expedition. These studies were performed in Oxford, UK using a 1.5 Tesla MRI and included standard T1 and T2 volumetric protocols.

Analysis: Only the T1, post-Everest images were used for this anthropomorphic component of the study. Each subject's study was imported into a volumetric analysis software package (Analyze 9.0, Analyzedirect, KS, USA). A semi-automated technique was used to demarcate and calculate the surface area of each structure in each slice. These were coded and the automated software then used the Calvari technique to sum the surface areas from each slice to calculate a volume. The following volumes were rendered and calculated by a single, blinded observer: total intracranial volume, supratentorial volume, infratentorial volume, total brain parenchyma volume, supratentorial CSF volume (excluding ventricles), infratentorial CSF volume (excluding ventricles), total non-ventricular CSF volume, lateral and 3rd ventricular volumes, aqueduct and 4th ventricular volumes, total ventricular volume (lateral + 3rd + aqueduct + 4th ventricle), total supratentoiral CSF volume, total infratentorial CSF volume, total CSF volume. In addition the following were also calculated: the tentorial angle, the petrous angle, the tentorial-clival angle, the sagital and occipital sinus venous volumes, the left transverse sinus and jugular bulb volume, the right transverse sinus and jugular bulb and total venous volumes. Figure 2-16 demonstrates examples of the volumetric images generated.

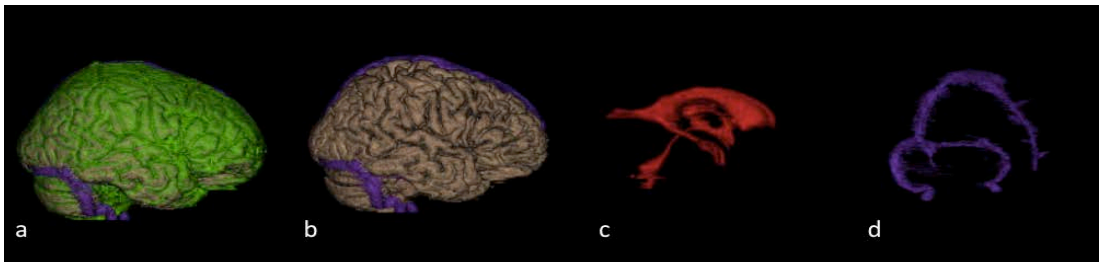


Figure 2-16: Exemplar images of a) total intracranial volume, b)brain and venous volume, 2) lateral and third ventricular volumes and d) venous volumes, generated during the volumetric analysis study.

2.4.2 Arterial (Hypoxic Study)

The high altitude cerebral Doppler study demonstrated that the extreme hypobaric hypoxia experienced in the climbers higher on the mountain in the Western Cwm and on the South Col, resulted in significant increases in the diameter of the right Middle Cerebral Artery (see chapter 3). This result has implications not just for high altitude medicine but for the clinical use of transcranial Doppler. We therefore undertook a hypoxic MRI study to attempt to confirm our findings with another modality.

Subjects: Seven subjects (5 male, 2 female) took part in this study. Subjects underwent resting NIRS, TCD and basic physiology (heart rate, blood pressure, end-tidal CO_2) studies as outlined above. They had a normoxic MRI study (as outlined below). Following this, they were then connected to a hypoxicator (Everest Summit Hypoxic Generator, Hypoxic Systems, New York, NY, USA) exposing them to an FiO_2 of 12% for three hours. Following this, further NIRS, TCD and basic physiological parameters were recorded at 90 minute and 3 hours. Then, whilst still connected to the hypoxicator, the subjects underwent the MRI protocol again.

MRI Protocol: A 3 Tesla MRI scanner (TIM Trio, Siemens AG, Eriangen, Germany) was used to obtain Magnetic Resonance Angiography (MRA) images at baseline and again after 3 hours exposure to hypoxia. A consistent section of the right proximal MCA (approximately 1 cm from the bifurcation to correspond to the TCD area of investigation) was studied to provide estimates of vessel diameter and blood flow velocity. Subsequent analysis measuring the diameter of both MCAs at three points along their lengths was also performed. Full methodological details are provided in chapter 3.

As part of this study, a pilot MRI sequence was performed to investigate venous changes. These susceptibility-weighted images were analysed qualitatively and are described within chapter 6.

2.4.3 Venous (Hypoxic Study)

The retinal imagining study, the anthropomorphic work and the venous pilot study component of the arterial MRI added strength to my belief that venous system was important in the pathogenesis of high altitude headache. A final hypoxic MRI study specifically designed to investigate the venous system was therefore undertaken.

Subjects: Fourteen subjects (12 male, 2 female) were enrolled. All had full blood count and urea and electrolyte blood tests prior to their study to record haemoglobin and to ensure a normal estimated glomerular filtration rate prior to the administration of contrast. All completed the study. In a random order, the subjects had a normoxic MRI and a hypoxic MRI study separated by a minimum of 24 hours (to ensure no effect of hypoxia and no residual contrast). Subjects also underwent both normoxic and hypoxic retinal imaging. The hypoxic study comprised baseline physiological measurements followed by 1 hour of hypoxia delivered through a hypoxicator (Everest Summit Hypoxic Generator, Hypoxic Systems, New York, NY, USA). The subjects had continuous peripheral arterial oxygen saturation and end-tidal carbon dioxide measurements, while heart rate and 5 minute blood pressure recordings were also made. Symptoms of headache were enquired about at 30 minutes and 1 hour.

MRI Protocol: A 3 Tesla MRI (TIM Trio, Siemens AG, Eriangen, Germany) was used. Standard T1 and T2 volumetric studies were performed followed by phase contrast and SWI sequences. To eliminate the effects that may be caused by the altered susceptibility of blood in hypoxia, gadolinium contrast as part of a timed venous phase MRI scan was administered to clearly delineate the venous structures. Velocities of blood flow in the sagittal and transverse sinuses were recorded.

2.5 Statistical Analysis of Field and MRI Studies

Statistical tools appropriate to each data set were applied using a combination of SPSS (version 17) and Excel. The statistical analysis for each experiment is

described in the corresponding chapter. Statistical support was provided by Shashi Hirani (UCL/City University) and by Professor Stanton Newman (UCL/City University). The power for individual studies is discussed in each chapter, however, many of the studies used the numbers of subjects they did for logistical reasons rather than specific power calculations.

Overall Statistical Analysis

Completeness of data, reasons for data loss (including subject drop out) are explained in each chapter. Only within the brain oxygenation chapter was a small volume of data imputed. Statistical supervision was provided by an independent statistician Shashi Hirani. Frequency plots were performed to confirm that data had a normal distribution prior to statistical analysis. Relationships between variables were examined using Pearson's r correlation when data were interval and parametric. Spearman's Rho was used when data were non-parametric. When single correlations were sort, significance was set at $p<0.05$. To reduce the risk of falsely identifying significance, when multiple correlations were investigated from a single data source, correlations were only considered significant when $p<0.01$. The coefficient of determination (r^2) is also reported as an indicator of the correlation effect size (this is especially so in the correlation of headache and physiological variables).

2.6 Special Note on Headache Assessment

The assessment of headache is a subjective and difficult measurement to make in comparison with most of the other physiological measurements within this thesis. The summation of "grades" of headache may not be a valid tool for comparing "headache burden" between subjects. This section explains the background to and the different headache assessment mechanisms used.

Headache analysis and the use of Headache Severity Index for the temporal quantification of headache at altitude:

2.6.1 Abstract

The ability to assess the subjective symptom of headache in a quantifiable way is key to the subsequent interpretation of physiological and anatomical

differences that may contribute to high altitude headache. This short report discusses the use of the headache component of the Lake Louise Score and the calculation of a headache severity index in 24 subjects ascending to 5300m.

2.6.2 Introduction

Headache is a discomfort within the cranium, the subjective nature of which makes quantifying and comparing headaches across a group of people or across a time period difficult. It is, however, necessary to attempt to do this, to see how a patients headache varies over time, to assess if a treatment modality is effective and for high altitude research, to compare individuals who suffer with headaches to those that do not. More importantly, a good assessment tool should be able to discriminate the different severities of headache experienced. First, clear definitions of high altitude headache and acute mountain sickness are required.

High Altitude Headache (HAH): The International Headache Society (2004) define the diagnostic criteria for HAH as A) exhibiting at least two of the following characteristics: frontal or fronto-temporal, dull or pressing, mild or moderate and aggravated by exertion, movement, straining, coughing or bending, and associated with; B) ascent to altitude over 2,500m, and which; C) have developed in the last 24 hours and; D) resolve within 8 hours of descent.

Acute Mountain Sickness (AMS): AMS is commonly considered to represent progression of HAH. The Lake Louise Consensus Group defined AMS as the presence of headache in an unacclimatised person who has recently arrived at an altitude above 2500m plus the presence of one or more of the following: gastrointestinal symptoms: anorexia, nausea or vomiting; insomnia; dizziness; and lassitude or fatigue (Roach, Bartsch et al. 1993). The group also established a scoring system to provide a quantitative element to AMS severity. A less commonly used, though more detailed severity scoring system is the Environmental Symptom Questionnaire (Sampson, Cymerman et al. 1983).

The Lake Louise Assessment for headache comprises the following subjective scoring system (table 2-7).

Headache severity	Score
None	0
Mild	1
Moderate	2
Severe	3

Table 2-7 The grades of headache used with the Lake Louise Scoring system.

This is a good tool for an immediate assessment of a patient's headache. Visual analogue scales can also be used (Lundqvist, Benth et al. 2009).

The Environmental Symptom Questionnaire (ESQ-III) comprises 67 questions. A weighted factor score (AMS-C) is calculated for answers to 11 of the 67 items. If the AMS-C score is >0.7, the individual is classified as having AMS. There are a number of problems with the administration of the ESQ-III: 1) the length of time to complete; 2) inaccurate answers due to boredom; 3) intentional or unintentional skipping of questions and 4) multiple answers to the same question due to stray pencil marks (Beidleman, Muza et al. 2007). The Lake Louise Scoring system tends to overestimate AMS when compared to the ESQ-III.

Unfortunately, for the purposes of our studies, none of these systems accurately allow the cumulative assessment of headache over time (Beidleman, Muza et al. 2007).

Compare the following examples using the Lake Louise score as a component within a diary. A subject records that yesterday he had a headache of severity 3 out of 3. Another had a headache of 1 out of 3. Just analysing this suggests the first subject had the worst headache. However, subject 1's headache only lasted half an hour on waking while the second subjects lasted all day. Now who has the worst headache?

The Headache Severity Index is a tool used by the pharmaceutical industry to compare effectiveness of medications (Schrader, Stovner et al. 2001). It not only ascribes a momentary severity of headache score, but also a duration to each episode measured in hours. The scoring system is as follows:
Each day a subject records the headache severity the previous day (0-4, table 2-8) and the number of hours that headache was present.

Headache severity	Score
None	0
Mild	1
Moderate	2
Severe	3
Excruciating	4

Table 2-8 Grading system (0-4) for the Headache Severity Index

Using such a method, a headache of grade 3 out of 4 for an hour has the same value as a headache of grade 1 out of 4 for three hours.
For this study, we compared Lake Louise and Headache Severity Scores for 24 subjects ascending to 5300m.

2.6.3 Methods

Twenty-four subjects (18 male) recruited from the investigators within the Caudwell Xtreme Everest Investigators team, ascended to 5300m (Everest Base Camp). The baseline studies, ascent profile and general methodology have been described above.
Each subject had a diary to be completed each morning. This comprised a series of didactic questions relating to many aspects of their wellbeing. They recorded their Lake Louise score (and the individual components and grade of headache) daily. One section specifically asked questions regarding headache. Within this section the questions in table 2-8 were asked.

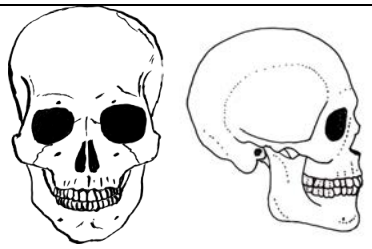
Grade of headache experienced in previous 24 hours (1-4)		Duration of headache (/hrs)	
Time of day of headache (please circle)	00:00 – 06:00 06:00 – 12:00 12:00 – 18:00 18:00 – 24:00	Locations of headache (please mark on skull)	
Exacerbating factors			

Table 2-8 Table with the Ascent Headache Score (sum of each headache score for previous 24 hours after arrival at a new altitude), the Total headache Score (the sum of all daily headache scores irrespective of ascent) and the Headache Severity Index (the sum of headache score x duration/hours for each day).

Using these latter data, a Daily Headache Score (grade x number of hours) was calculated for each subject and this value was summed for the period of arrival at altitude (3,300m) to three days after arrival at the destination (5,300m).

Three scores will be compared. Firstly the sum of Lake Louise Scores only recorded on the morning after arrival at a new altitude (Ascent Headache Score). Since AMS tends to be worst at such time (and since few people drank alcohol while ascending in the previous 24 hours) this technique should remove most other causes of headache (e.g. alcohol that might contribute on day 2 or 3 at a specific altitude). Secondly, the sum of Lake Louise scores for each individual on every day for the period of study (whether ascent had occurred or not) was calculated (the Total Headache Score). Finally, the Headache Severity Index was calculated for each subject daily and summed for the entire duration of study.

If data points were missing from the diary, an attempt was made to establish a reason (e.g. illness). The following days' figures were imputed.

Subject No	Ascent Headache Score	Total Headache Score	Headache Severity Index
X01	1	2	39
X02	4	5	41
X03	0	2	1.5
X04	0	1	2
X05	1	3	6
X06	0	1	1
X07	2	3	27.5
X08	0	0	0
X09	0	0	0
X10	2	4	4
X11	0	0	0
X12	2	8	153
X13	8	12	90
X14	4	5	152.5
X15	3	2	24
X16	8	15	141.5
X17	1	2	9
X18	0	1	1
X19	0	1	4
X20	1	1	4
X21	0	0	2
X22	1	4	25
X23	1	4	64
X24	2	5	68

Table 2-9 demonstrates these results for the entire investigator group.

2.6.4 Results

Diary records were well maintained. The total (daily) headache score generates 312 data points (24 subjects x 13 days of trekking). Of these, only 4 data points were missing. The Headache Severity Index generates twice the number of data points (as daily score and duration need to be recorded). Of these, 9 data points were missing. For these points, imputation using the mean duration of other headaches the individual had suffered was used.

For the Investigators, it became clear from studying the diaries that duration of headache was very poorly documented. This may have been because of lack of understanding or because the diary is quite extensive with other data (such as heart rate, peripheral saturations, blood pressure) and this field was not considered important. Because of this, although we have good data for the investigator group, the trekker group rely on total and ascent headache score only.

2.6.5 Conclusions

By incorporating a duration component into the scoring system, a broader range of headache scores is produced. On broad inspection, this also appears to correlate well with the headaches reported to me as the Expedition Doctor at Base Camp. In future studies the importance of the duration field must be emphasised by trek leaders to their groups.

3 Chapter 3: ARTERIAL EFFECTS OF HYPOXAEMIA

Please see (Wilson, Edsell et al. 2011) Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia – an ultrasound and MRI study. Journal of Cerebral Blood Flow and Metabolism June 2011, 31, 2019-2029 in Appendix

3.1 Abstract

To understand cerebrovascular physiology in hypoxia, both arterial inflow and venous outflow must be studied. Transcranial Doppler is a widely used non-invasive technique for assessing cerebral artery blood flow. All previous high altitude studies assessing cerebral blood flow in the field have used Doppler to measure arterial blood velocity and have assumed vessel diameter to not alter. *Methods:* This chapter reports two studies that demonstrate that this is not the case. Firstly, I report the highest recorded study of cerebral blood flow (7950m on Everest) and demonstrate that above 5300m, middle cerebral artery (MCA) diameter increases (n=24 at 5300m, 14 at 6400m and 5 at 7950m). Secondly I performed normobaric hypoxic (FiO_2 = 12%) Magnetic resonance angiography (MRA) studies using a 3 Tesla MRI scanner to accurately measure MCA diameter (n=7).

Results: Mean MCA diameter at sea level was 5.30mm, at 5300m 5.23 mm, at 6400m 6.66mm and at 7950m 9.34mm ($p<0.001$ for change between 5300 and 7950m). The dilatation that occurred at 7950m reversed with oxygen. The normobaric MRA also demonstrated significant dilatation of the MCA diameter and this correlated with MCA changes as measured by ultrasound.

Conclusion: I thus conclude that that cerebral artery diameter is not constant, but can respond to alterations in inspired oxygen partial pressure. It also appears that transcranial 2D ultrasound can be used at the bedside or in the remote setting to assess middle cerebral artery calibre.

3.2 Introduction

Normal cerebral function is dependent on an adequate and continuous supply of oxygen. With increasing altitude, barometric pressure falls, and with it the partial pressure of atmospheric and inspired oxygen. Acclimatisation to such an environmental hypobaric hypoxic stress involves a number of adaptive processes (including hyperventilation and a rise in haematocrit (Ward, Milledge et al. 2000)) which serve to restore arterial oxygen content towards sea level values. In addition, increased cerebral blood flow is believed to be one compensatory mechanism serving to maintain normal oxygen flux to the brain in the face of arterial hypoxaemia. Such hypoxaemia is common in critically ill patients and is thought to occur locally in ischaemic stroke, the third commonest cause of death in the UK (1995). A greater understanding of the cerebrovascular response to hypoxia is thus of broad interest, as would be the validation of clinically relevant techniques used in the assessment of flow in intracranial vessels.

Transcranial Doppler (TCD) measurement of flow velocity in the Middle Cerebral Artery (MCA) has been used to assess cerebral blood flow (CBF) dynamics both at rest and during exercise at altitude (Otis, Rossman et al. 1989; Baumgartner, Bartsch et al. 1994; Baumgartner, Spyridopoulos et al. 1999; Jansen, Krins et al. 2000; Ter Minassian, Beydon et al. 2001; Jansen, Kagenaar et al. 2002; Appenzeller, Passino et al. 2004; Lysakowski, Von Elm et al. 2004; Imray, Myers et al. 2005; Norcliffe, Rivera-Ch et al. 2005; Van Osta, Moraine et al. 2005; Palma, Macedonia et al. 2006; Ainslie, Burgess et al. 2007; Feddersen, Ausserer et al. 2007; Subudhi, Dimmen et al. 2007). Assuming that cerebral arterial diameter remains constant in the face of sustained hypoxia, investigators have inferred changes in cerebral blood flow from changes in the velocity of blood in the MCA. This assumption is, however, disputed (Giller 2003). Further, the opposite assumption is made in many clinical situations: in the management of subarachnoid haemorrhage, for example, changes in TCD-derived blood velocity are assumed to represent changes in vessel diameter (vasospasm).

The profound hypoxaemia experienced by climbers at extreme altitude (>5500m) (Grocott, Martin et al. 2009) is known to be associated with cerebral dysfunction (Ward, Milledge et al. 2000; Virues-Ortega, Buela-Casal et al. 2004), which is thought to account for approximately 70% of deaths over 8000m on Everest (Firth, Zheng et al. 2008). Such data suggest that cerebral oxygenation may not be fully maintained through adaptive responses, which may include changes in cerebral blood flow. However, these blood flow responses remain poorly documented. Indeed, the only studies of CBF using TCD velocity under conditions of comparable hypoxaemia (performed in a hypobaric chamber) did not record vessel diameter (Ter Minassian, Beydon et al. 2001).

In the past, the measurement of MCA diameter (MCA_{Diam}) has only been possible by direct vision at surgery (Giller, Bowman et al. 1993), by use of contrast angiography (Du Boulay and Symon 1971) or magnetic resonance angiography – techniques inappropriate for remote extreme altitude field studies. Transcranial Colour Doppler Power signal has previously been used to indirectly infer MCA cross-sectional area (MCA_{csa}) in a laboratory setting (Poulin and Robbins 1996; Poulin et al 2002). Under conditions of mild hypobaric hypoxia, no significant change in MCA_{csa} was noted. The recent development of portable ultrasound devices that incorporate both 2D colour flow mapping and concurrent pulse wave Doppler ultrasonography permits measurement of both vessel diameter and the velocity of the blood within it. The 2D ultrasound ensures that the same segment of the artery can be reliably visualised and assessed.

I thus aimed to use such ultrasound imaging and Doppler measurements to characterise the contribution of altered vessel diameter to changes in MCA flow (MCA_{Flow}) and calculated oxygen delivery (MCA_{OD}) seen in response to hypobaric hypoxia. In addition, a sea level MRA study was performed in normoxia and 12% hypoxia to determine whether acute hypoxia caused MCA vessel dilatation and to assess the level of correlation between TCD and MRA methodologies.

3.3 Methods

Ethical approval for this study was provided by University College London (UCL) (Code 0292/015). Written informed consent was obtained from all participants. Please see chapter 2 for generic details of the CXE expedition.

3.3.1 High Altitude TCD Study

Twenty-four subjects, the investigators of the Caudwell Xtreme Everest Research Expedition (18 male, mean age 35.2, range 19-59, (Grocott, Martin et al. 2010)), were studied over 71 days. In brief, and as described in chapter 2, all subjects trekked to 5300m (group 1, n = 24), of whom 14 subsequently continued to 6400m (group 2) and 5 to 7950m (group 3). Each subject was studied between 1 and 3 days after arrival at each new altitude. The study day was constant for each subject, the sole exception being at 7950m, where all subjects (n=5) were investigated on the second day after arrival. No caffeine or alcohol, or medications that could affect cerebral blood flow were consumed prior to the measurement on the study day. Immediately before each study, subjects rested in a horizontal position for 15 minutes. Climbers were not exposed to any supplemental oxygen until 7100m. At 7950m, subjects were off supplementary oxygen (2 $lmin^{-1}$) for at least 30 minutes prior to the Near Infrared Spectroscopy (NIRS) and TCD measurements being made. The NIRS and TCD studies were then repeated with the subjects receiving 2$lmin^{-1}$ supplemental oxygen via a TopOut re-breath regulator system (Topout Mask Mk 2, Topout Oxygeneering Ltd, Cotgrave, UK) to assess reversibility of the initial measurements. At 7950m, the investigating clinician (CHEI) used supplementary oxygen (2 $lmin^{-1}$).

Measurements:

Blood pressure was recorded (mean of three non-invasive recordings) using an automated cuff (Omron M7, IL, USA); arterial oxygen saturation (SaO_2) by near infrared finger pulse oximetry probe (Nonin, Onyx Model 9500, Plymouth, MN USA); haemoglobin concentration of whole venous blood by photometry (Hemocue Whole Blood haemoglobin System, Hemocue AB, Angelhoim,

Sweden); and resting end-tidal CO_2 ($ETCO_2$) by infrared capnometer (Cortex Metamax 3b, Leipzig, Germany).

Near Infrared Spectroscopy (NIRS): Regional Brain Oxygen Saturation (rSO_2) measurements were made immediately prior to TCD insonation (Invos Cerebral Oximeter 5100C, Somanetics, MI, USA). The skin was cleaned and probes were placed over the right and left frontal lobes avoiding both the sagittal and frontal sinuses, and left *in situ* during the TCD analysis. Three consecutive readings were taken from each side, from which means were calculated. NOTE: although resting brain oxygen was monitored during this study, the main study of brain oxygenation at rest and during exercise whilst hypoxic is described in chapter 4.

Transcranial Doppler: In a supine subject (figure 3-1), the right MCA was insonated via the temporal bone window, by one of two skilled observers, using a 5-1 MHz Transducer MicroMaxx^{TM} (Sonosite, Bothell, WA, USA). The clinoid process of the sphenoid bone, the Circle of Willis and the distal internal carotid artery were initially found, and then the M1 segment of the MCA identified (characterised by flow towards the transducer). Doppler gain was set in a standard fashion (Martinoli and Derchi 1997). An optimal portion of the MCA without branches and with near laminar flow was then selected and the depth recorded. Once identified, the centre of the artery was insonated and MCA blood velocity (MCA_{Vel}), Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV), Pulsatility Index (PI) and Resistivity Index (RI) calculated by the inbuilt software (Figure 3-2a). In subsequent studies, every effort was made to insonate the same depth (to within 1 mm). After 3-5 minutes of insonation, the 2D image movie sequence was saved, and the frame with the maximum vessel diameter (systole) studied. Using the on screen calliper tool, the width of the vessel at the point of insonation was measured and recorded (Figure 3-2b). The angle of insonation was constant for each individual, since the position of the probe on the temporal bone window and the position on the interrogated section of the MCA were fixed.

Figure 3-1 In a supine subject, the right MCA was insonated as described in the methods.

3.3.2 Sea Level Hypoxic MRI Study

Seven subjects (5 male; mean age: 34.4 range 22-48) were recruited from the Caudwell Xtreme Everest investigators. None had ascended above 1000m in the preceding 6 months. Physiological, TCD and NIRS measurement techniques were identical to the field study and performed in normoxia, and with 90 minutes and 180 minutes of hypoxic exposure. All TCD measurements were performed three times by both investigators.

Hypoxia: After baseline measurements, subjects were subjected to 3 hours of normobaric hypoxia (FiO_2 = 12%; approximately equivalent to an altitude of 4,400m) using a tight fitting mask and hypoxicator (Everest Summit Hypoxic Generator, Hypoxic Systems, New York, NY). Inspired oxygen concentration was regularly checked (Class R-17D Oxygen Sensor, Oxycheq, Florida, USA). Extended MRI-compatible tubing enabled the subjects to remain hypoxic during the MRI and TCD studies at 3 hours.

MRI: A 3 Tesla MRI (TIM Trio, Siemens AG, Eriangen, Germany) was performed at baseline and at 3 hours of hypoxia. At both time points, 3-dimensional time of flight (TOF) Magnetic Resonance Angiography (MRA) was performed (TR=8.6ms; TE 4ms; FA 20^o; 3 acquisition slabs; matrix 256 x256 x 15; voxel dimensions 1.2 x 1.0 x 7.0mm), principally to permit estimation of MCA diameter. To measure MCA blood flow velocity, a single-slice 2-dimensional ECG-triggered segmented phase-contrast acquisition (TR 30.3ms; TE 5.5ms; FA 30^o; matrix 384x384; voxel dimensions 0.5 x 0.5 x 6.0mm) was performed with through-plane flow-sensitization with velocity-encoding factor of

150 cm/s. An 800ms ECG-synchronized acquisition window was sampled with 25 phases proving velocity sensitive images with an effective temporal resolution of 32ms. A consistent section of the right proximal MCA (approximately 1 cm from the bifurcation to correspond to the TCD area of investigation) was studied to provide estimates of blood velocity, the imaging plane being prescribed orthogonally to the main axis of the MCA.

In order to estimate MCA diameter, TOF MRA data were post-processed using the maximum intensity projection (MIP) function on a calibrated Siemens Leonardo workstation (Siemens AG, Erlangen, Germany). An independent consultant neuroradiologist, blinded to the pre- or post- hypoxia induced status of the subjects, assessed the maximum and minimum diameters, the circumference and cross sectional area of both MCAs on 2data sets in all subjects. A semi-automated vessel tracing technique utilising the In-Space vessel analysis program (Syngo MMWP Software, version VE36A with service pack SP03) was performed to analyse the length of MCA on the post-processed 3-dimensional rendered MIP images. This was done by manually entering 2 data points; proximally at the A1/M1 bifurcation of the terminal internal carotid artery and distally at the distal M1 segment of the MCA at the bi/tri-furcation. Multiplanar views of the segmented length for analysis of the M1 segment were then automatically generated by the program, which included a true cross-sectional view (Figure 2c). This automatically generated length of the M1 segment of the MCA was then divided equally into 5 data points, which were replicated and were therefore consistent in both pre- and post- hypoxia studies for the respective lateralised M1 segment. The window width and level were standardised on the true cross sectional display panel at 200:100. A semi-automated calculation of the cross-sectional area and circumference was performed by the program using a 'best-fit' algorithm with minimal refinement of the threshold levels and individual plotted data points. The maximum and minimum diameters were also determined at the same data point. This process was repeated at all 5 data points for each side (left and right M1 segments) in pre- and post- hypoxia studies in all patients. Similar vessel analysis techniques have been previously utilised by other researchers to interrogate TOF MRA acquisitions (Reese, Bochelen et al. 1999; Beckmann 2000; Besselmann, Liu et al. 2001; Choy, Ganesan et al. 2006).

MCA flow velocities were obtained from the phase-contrast imaging data also using software provided by Siemens (Argus Flow tool). The extent of the MCA margins was determined by manually defining an enclosing region of interest (ROI), which was adjusted for each phase to account for changes through the cardiac cycle. The software then automatically determined the average flow velocity for each subject.

Figure 3-3 demonstrates the experimental protocol within the National Hospital for Neurology and Neurosurgery's MRI facilities.

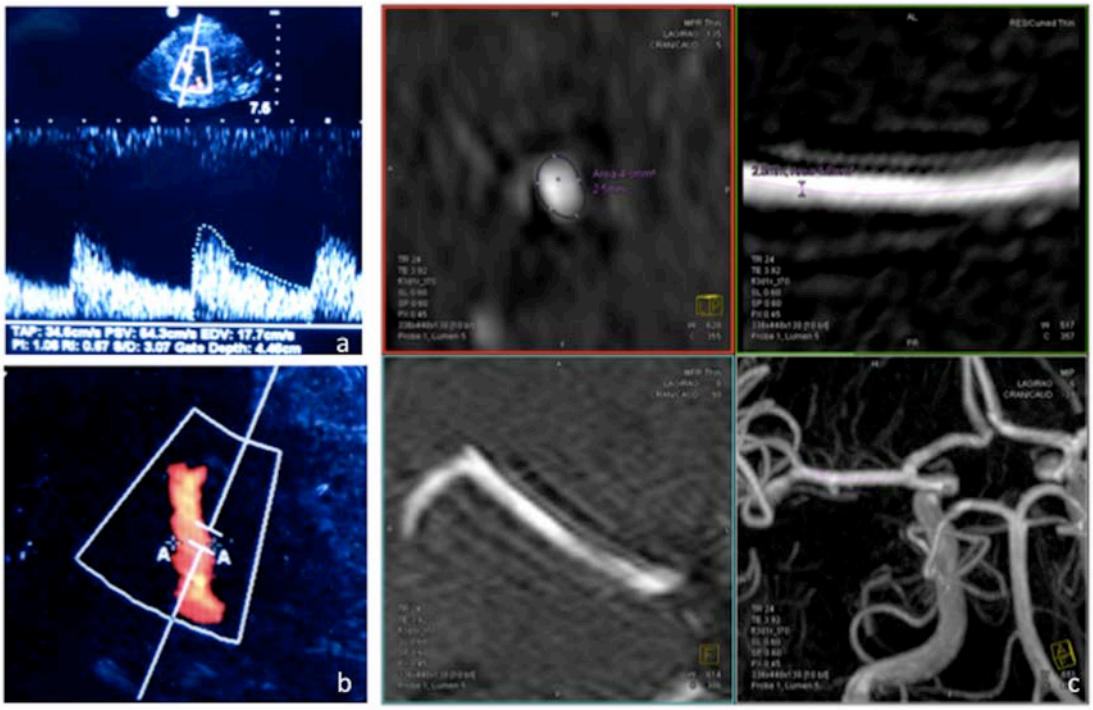


Figure 3-2 Ultrasound images demonstrating a) MCA velocity and b) vessel diameter measurement; c) composite of 4 MRI Images demonstrating MCA multiplanar reconstruction and analysis

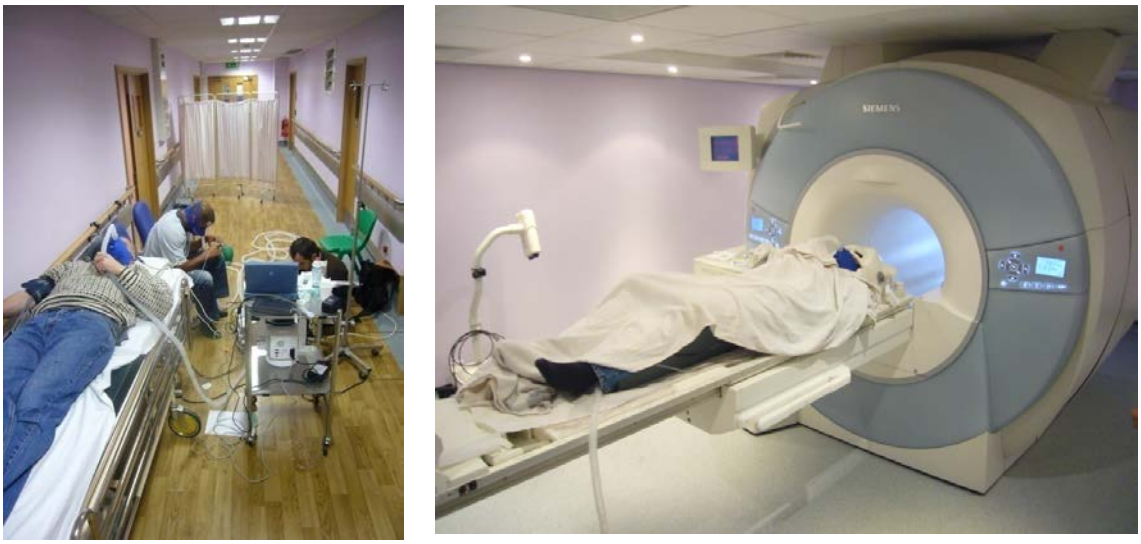


Figure 3-3 Images of hypoxic subjects and undergoing MRI imaging while still hypoxic

Flow and Oxygen delivery calculations: Blood Oxygen content was calculated using the formula: *Blood Oxygen content = 1.36 x Hb x* $SaO_2/100$. The small quantity of dissolved oxygen (decreasing further at altitude) was not included in the estimation.

Middle Cerebral Artery Blood Flow was calculated using the formula: *Flow* = $\pi(MCA_{diam}/2)^2 \times MCA_{vel}$. This estimation does not take account of vessel wall resistance or changes due to any turbulent flow. Oxygen delivery was calculated as the product of blood flow and oxygen content.

3.3.3 Statistics

High Altitude Study: For each measure, differences in scores between altitudes were examined using the linear mixed models procedure in SPSS™ version 18 (IBM, Michigan, USA) in order to maximize the utilisation of the data collected. The method of restricted maximum likelihood was used to estimate model parameters, and variance / covariance structures were modelled as heterogeneous Toeplitz. Pairwise comparisons within each analysis were conducted using estimated marginal means using Sidak's adjustment to compensate for multiple comparisons. For all tests, significance was set to <0.05.

Sea Level Hypoxic MRI Study: Differences were again examined using the linear mixed models procedure in SPSS™ for consistency. The same means

and the same variables were found to reach significance when checked with general linear models. Relationships between variables were examined using Pearson's correlation. Correlations were considered significant when $p<0.05$. The coefficient of determination (r^2) is also reported as an indicator of the correlation effect size.

Inter-rater reproducibility for the MRI/TCD ratings was examined using intra-class correlations (averaged measures) to examine the agreement between raters over the range of measures taken at different time points.

3.4 Results

3.4.1 High Altitude TCD Study:

There were no technical problems encountered with the TCD and NIRS devices. Data were not available on one subject at 3500m (non-altitude related gastrointestinal disturbance) and one subject at 5300m (severe Acute Mountain Sickness). These missing data were accounted for as part of the multi-level modelling technique. Subject characteristics and basic physiological variables for the different groups at each altitude are presented in table 3-1.

	75m	1300m	3500m	4250m	5300m	6400m	7950m	7950m + 2l	Numerator df	Denominator df	F	Sig
Systolic BP (mmHg)	129.85 a,b	129.25 a,b	128.32 a	132.89 a,b	139.72 b	136.03 a,b	126.33 a,b	-	6	15.05	2.84	0.047
CI	(124.21-135.50)	(122.66-135.84)	(122.42-134.21)	(127.90-137.88)	(133.45-145.99)	(130.18-141.88)	(108.52-133.14)					
Diastolic BP (mmHg)	77.85 a	81.25 a,c	84.94 a,b,c	86.68 b,c	90.90 b	91.48 b	84.96 a,b,c	-	6	22.69	6.50	<0.01
CI	(73.86-81.84)	(77.22-85.29)	(80.52-89.36)	(83.14-90.23)	(86.81-95.00)	(86.67-96.29)	(74.08-95.83)					
Mean BP (mmHg)	95.18	97.25	99.40	102.08	107.17	106.29	98.76	-	6	18.82	5.02	0.003
CI	(91.10-99.26)	(92.62-101.88)	(95.09-103.70)	(98.36-105.80)	(102.74-111.61)	(101.56-111.02)	(85.96-111.56)					
Peripheral Sats (%)	97.63 a	95.67 b	89.75 c	85.88 d	79.63 e	75.13 e	65.90 f	95.03 a,b	7	13.91	215.18	<0.001
CI	(97.24-98.02)	(95.01-96.32)	(88.39-91.11)	(84.01-87.74)	(77.68-81.60)	(72.35-77.91)	(63.74-68.07)	(92.78-97.27)				
End Tidal CO_2 (mmHg)	35.53 a	32.27 b	27.36 c	25.65 d	20.62 e	16.75 f	13.00 g	-	6	37.89	205.64	<0.001
CI	(34.23-36.82)	(30.97-33.57)	(26.12-28.60)	(24.42-26.88)	(19.54-21.70)	(15.72-17.78)	(11.99-14.01)					
Haemoglobin (mg/dl)	13.97	14.53	15.43	15.52	17.63	19.09	18.83	-	6	24.19	52.69	<0.001
CI	(13.61-14.34)	(14.20-14.87)	(14.97-15.89)	(15.11-15.93)	(17.00-18.27)	(17.96-20.23)	(18.17-19.49)					

Table 3-1 Subject characteristics and basic physiological variables with estimated marginal means, significance of change and confidence intervals (CI) using multilevel modelling. Note: altitudes with the same superscript letter do not differ significantly (i.e. they belong to a homogenous subset).

Table 3-2 summarises the means of measured variables, the confidence intervals and the significance of changes with increasing altitude.

	75m	1300m	3500m	4250m	5300m	6400m	7950m	7950m + 2l	Numerator df	Denominator df	F	Sig
Left rSO_2 (%)	68.77 a	66.00 a	62.62 b	58.94 c	54.16 c,d	49.27 d	41.95 e	62.57 a,b,c,e	7	10.01	80.27	<0.001
CI	(65.91-71.63)	(64.30-69.70)	(59.87-65.37)	(55.89-61.99)	(51.07-57.25)	(44.84-53.70)	(39.76-44.15)	(57.02-68.12)				
Right rSO_2 (%)	69.47 a	67.75 a	61.36 b	58.57 b,c	53.98 c,d	50.95 d	39.55 e	61.75 b	7	12.43	82.87	<0.001
CI	(66.74-72.20)	(64.57-70.93)	(58.88-63.84)	(55.90-61.24)	(51.11-56.85)	(47.23-54.67)	(36.14-42.96)	(61.42-62.08)				
O_2 Content (mls/100mls)	18.55 a, b	18.91 a	18.83 a	18.12 a, b	19.02 a	19.29 a	16.81 b	24.27 c	7	14.65	80.14	<0.001
CI	(18.08-19.02)	(18.44-19.38)	(18.25-19.41)	(17.54-18.70)	(18.16-19.89)	(17.70-20.87)	(15.87-17.76)	(23.18-25.37)				
MCAv (cm/sec)	59.66	56.08	62.63	60.14	66.97	66.42	62.92	49.03	7	7.64	2.11	0.163
CI	(53.25-66.07)	(51.32-60.84)	(54.89-70.38)	(53.22-67.07)	(59.17-74.76)	(59.78-73.06)	(42.73-83.11)	(27.17-70.88)				
PSV (cm/sec)	91.32	84.34	91.73	92.39	102.75	107.10	96.15	66.70	7	3.91	3.67	0.116
CI	(82.12-100.51)	(76.89-91.80)	(82.21-103.24)	(81.81-102.96)	(91.74-113.75)	(96.71-117.48)	(63.46-128.84)	(15.68-117.73)				
EDV (cm/sec)	41.25	40.55	43.48	42.98	48.38	49.10	46.42	37.09	7	7.90	2.06	0.167
CI	(36.96-45.55)	(37.42-43.67)	(38.34-48.61)	(37.62-48.35)	(42.37-54.39)	(43.45-54.75)	(26.56-66.28)	(23.25-50.93)				

	75m	1300m	3500m	4250m	5300m	6400m	7950m	7950m + 2l	Numerator df	Denominator df	F	Sig
Pulsitility Index	0.85	0.81	0.78	0.82	0.82	0.87	0.83	0.71	7	7.98	1.81	0.213
CI	(0.79-0.90)	(0.76-0.86)	(0.71-0.84)	(0.72-0.92)	(0.75-0.89)	(0.87-0.97)	(0.45-1.2)	(0.50-0.91)				
Resistivity Index	0.54	0.54	0.52	0.53	0.53	0.54	0.52	0.47	7	8.44	1.16	0.410
CI	(0.51-0.57)	(0.52-0.56)	(0.49-0.55)	(0.50-0.56)	(0.50-0.56)	(0.50-0.57)	(0.41-0.64)	(0.37-0.57)				
MCA Diameter (mm)	5.30 a	5.70 a,b	5.51 a,c	5.40 a	5.23 a	6.66 b,c,d	9.34 b,e	0.65 a,d,e	7	8.02	8.84	0.003
CI	(5.01-5.59)	(5.38-6.02)	(5.05-5.97)	(5.07-5.73)	(4.78-5.68)	(6.03-7.30)	(7.62-11.06)	(5.03-8.02)				
MCA Flow (ml/sec)	13.30 a	14.54 a	15.62 a,b	14.42 a	15.04 a,b	23.68 b	41.16 a,b	15.27 a,b	7	5.44	6.18	0.026
CI	(11.38-15.21)	(12.46-16.61)	(12.53-18.71)	(11.75-17.09)	(11.74-18.34)	(18.93-28.43)	(24.51-57.82)	(0.64-29.89)				
O_2 Delivery (ml/sec)	2.47 a	2.74 a,b	2.94 a,b	2.61 a	2.87 a,b	4.69 b	6.98 a,b	3.68 a,b	7	3.20	4.86	0.101
CI	(2.10-2.85)	(2.36-3.11)	(2.34-3.55)	(2.12-3.10)	(2.25-3.50)	(3.61-5.78)	(4.25-9.70)	(-0.75-8.11)				

Table 3-2 Estimated Marginal Means, significance of change and confidence intervals (CI) for each variable using multilevel modelling. Note: altitudes with the same superscript letter do not differ significantly (i.e. they belong to a homogenous subset).

Regional cerebral oxygenation (rSO_2) values (derived from NIRS), peripheral saturations (SaO_2) and end-tidal CO_2 ($EtCO_2$) decreased with each increase in altitude ($P<0.05$). MCA_{vel} did not change at any altitude. MCA_{Diam} remained constant until extreme altitude (6400 and 7950m) where a marked increase was observed (5.3mm at sea level, 6.66mm at 6400, 9.34mm at 7950m, $p<0.002$).

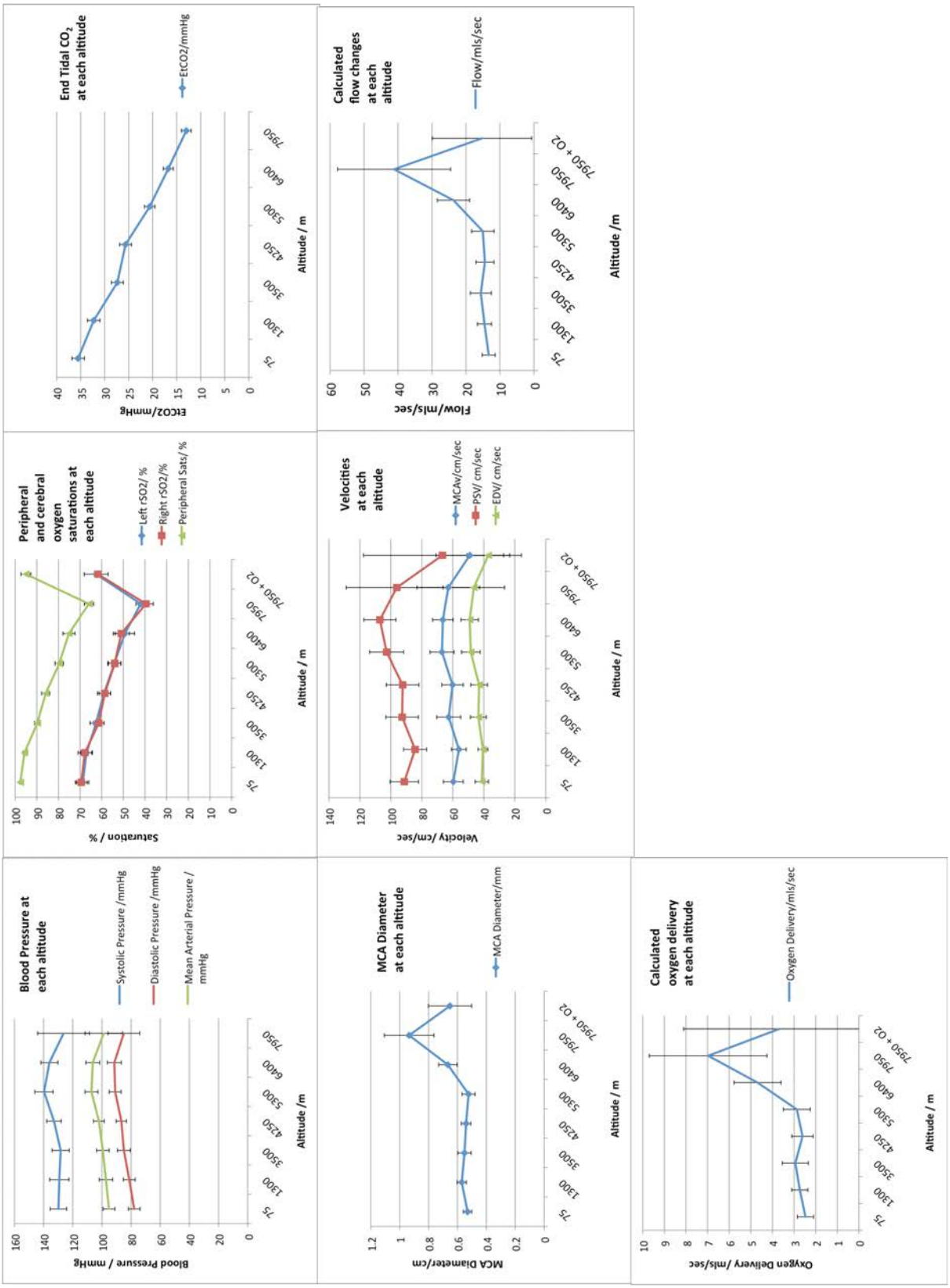


Figure 3-4 Composite of 7 graphs demonstrating changes in blood pressure, SaO_2, rSO_2, end-tidal CO_2, peak systolic, end diastolic and mean velocities, MCA_{Diam}, calculated MCA_{Flow} and Oxygen delivery (note, blood pressure and $EtCO_2$ were not reassessed after oxygen administration at 7950m).

Sea Similarly, calculated MCA flow and MCA oxygen delivery markedly increased at 6400m and above (MCA flow from 13.3ml/sec at sea level to 23.7ml/sec and 6400 and 41.2ml/sec at 7950; Oxygen delivery from 2.5ml/sec at sea level to 4.7ml/sec at 6400 and 7.0 ml/sec at 7950m; $p<0.01$ for all). Figure 3-4 demonstrates these changes in graphical format.

3.4.2 Level Hypoxic MRI Study

All 7 subjects completed 3 hours of hypoxia and underwent the complete study. Because of technical difficulties, one observer was unable to adequately measure the MCA values utilising TCD in one subject in normoxia. This data set was otherwise complete. Tables 3 displays the changes in mean blood pressure, pulse, SO_2, rSO_2, end-tidal CO_2, Oxygen content, ultrasound and MRA-measured vessel diameters, and blood velocity and calculated blood flow and oxygen delivery.

	Normoxia	90min Hypoxia	180min Hypoxia	Numerator df	Denominator df	F	Sig
Pulse (beats/min)	58.00	62.71	64.14	2	6.39	1.62	0.27
CI	(46.99–69.01)	(53.39–72.035)	(55.03–73.26)				
Systolic BP (mmHg)	115.90	113.86	114.71	2	6.04	0.42	0.67
CI	(109.32–122.40)	(105.36–122.36)	(97.13–132.30)				
Diastolic BP (mmHg)	68.00	62.86	66.57	2	6.00	2.44	0.167
CI	(58.95-77.05)	(59.30-66.42)	(55.54-77.61)				
SaO_2 (%)	98.29 [a]	76.00 [b]	74.86 [b]	2	7.05	34.03	<0.001
CI	(96.06-100.51)	(67.66-84.34)	(67.55-82.16)				
rSO2 (mean R&L) (%)	71.10 [a]	50.47 [b]	50.33 [b]	2	5.70	65.71	<0.001
CI	(63.58-78.61)	(45.92-55.03)	(44.62-56.04)				
$ETCO_2$ (kPa)	5.23 [a]	2.64 [b]	2.63 [b]	2	6.20	44.08	<0.001
CI	(4.43-6.03)	(1.95-3.33)	(1.97-3.30)				

	Normoxia	90min Hypoxia	180min Hypoxia	Numerator df	Denominator df	F	Sig
Oxygen Content (ml per 100mls)	18.78 [a]	14.57 [b]	14.34 [b]	2	6.37	29.20	0.001
CI	(17.84-19.71)	(12.51-16.63)	(12.53-16.15)				
TCD measured MCAv (cm/sec)	65.23 [a]	74.29 [b]	71.60 [a,b]	2	6.15	15.08	0.004
CI	(48.36-82.10)	(55.60-92.98)	(51.51-91.68)				
MRA measured MCAv (cm/sec) [§]	32.80	-	38.75	1	6.00	17.56	0.006
CI	(21.61-43.99)	-	(27.25-50.35)				
TCD measured Diameter (mm)	5.44 [a]	6.23 [b]	6.28 [a,b]	2	4.16	11.28	0.021
CI	(5.17-5.70)	(5.67-6.78)	(5.61-6.95)				
MRA measured Diameter(mean R&L) (mm) [§]	3.04 [a]	-	3.27 [b]	1	6.00	17.56	0.006
CI	(2.79-3.29)		(3.01-3.53)				
TCD Calculated Flow (ml/sec)	14.83 [a]	22.07 [b]	21.87 [b]	2	6.54	19.45	0.002
CI	(12.34-17.31)	(18.31-25.84)	(15.90-27.84)				
MRA Calculated Flow (ml/sec) [§]	2.33 [a]	-	3.23 [b]	1	6	12.40	0.013
CI	(1.65-2.99)		(2.28-4.18)				
TCD Calculated O_2 delivery (mlsO2/sec)	2.77	3.18	3.15	2	6.35	2.14	0.184
CI	(2.38-3.15)	(2.63-3.74)	(2.15-4.14)				
MRA Calculated O_2 delivery (mlsO2/sec)[§]	0.44	-	0.45	1	6.00	0.33	0.586
CI	(0.32-0.55)		(0.32-0.59)				

Table 3-3 Estimated Marginal Means, F values and Confidence Intervals (CI) of heart rate, blood pressure, periphral and regional brain saturations, End Tidal CO2, calculated oxygen content, transcranial Doppler, MRA measured velocities and Ultrasound and MRI measured diameters, calculated flows and calculated oxygen delivery (note, there was no MRI study at 90 minutes of hypoxia).

Middle Cerebral Artery diameter increased after three hours exposure to 12% hypoxia, when measured using ultrasound or MRI (TCD: 5.44mm to 6.28mm; MRI: 3.04mm to 3.27mm P=<0.05 for both). Cerebral blood velocity did not

significantly increase when assessed with either method (TCD: 65.2cm/sec to 71.6cm/sec; MRI: 32.8cm/sec to 38.8cm/sec (p=0.13)).

Cerebral blood flow, calculated with either methodology, increased (TCD: 14.8ml/sec to 21.9ml/sec; MRI: 2.3ml/sec to 3.2ml/sec p<0.01). Calculated Oxygen delivery was maintained whether measured using ultrasound or MRI.

3.4.3 Correlation of TCD and MRI

TCD and MRI measured vessel diameters correlate (r = 0.82 (Pearson's). r^2 =0.67 (figure 3-4)). However, although there was a strong correlation, a sizeable, though constant, difference between TCD and MRI values was identified: for example, normoxia TCD measured MCA_{Diam} =5.44mm; normoxia MRA measured MCA_{Diam} = 3.04mm. This results in marked differences in calculated flow and oxygen delivery since the square of the radius has a large contribution to these calculations - see discussion).

The interclass correlation between the two TCD observers was 0.76.

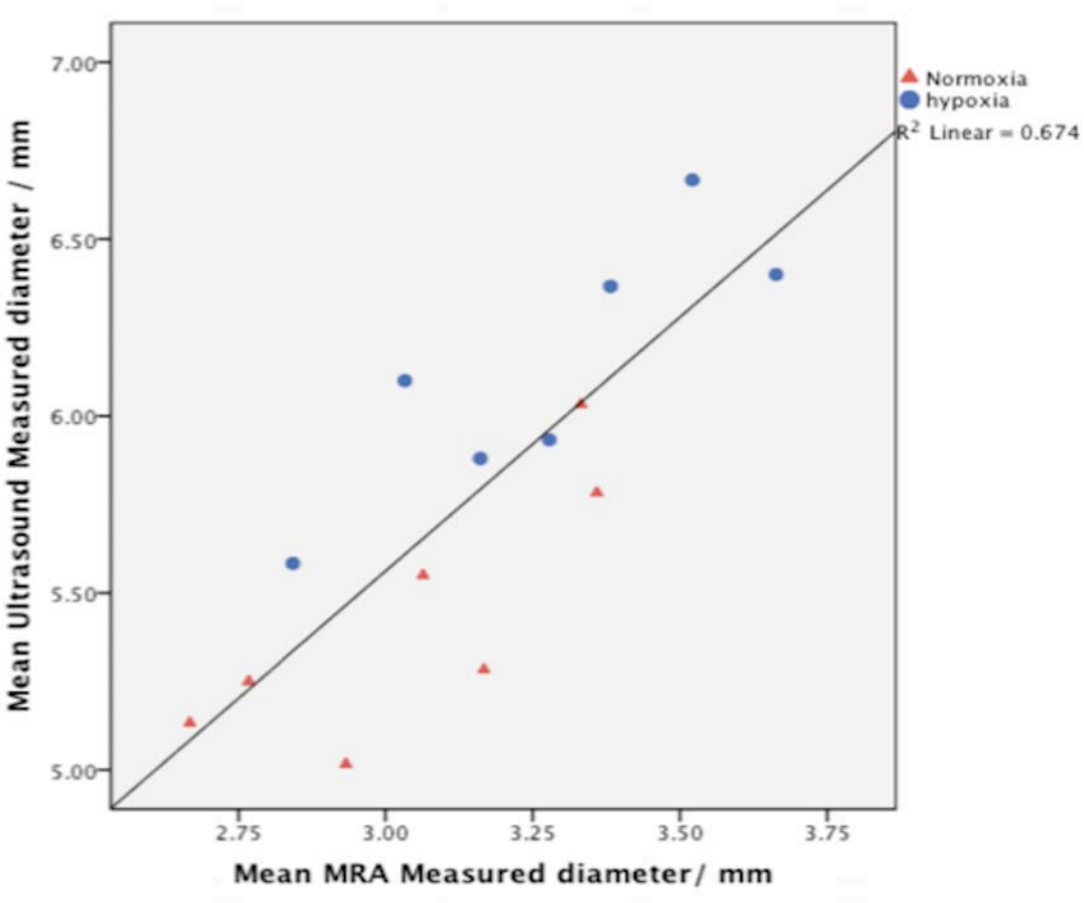


Figure 3-4 Correlation between TCD and MRI measurements of MCA diameter

3.5 Discussion

This is the first field study to assess cerebral perfusion over 5500m. The technical advance demonstrated in this study is that transcranial ultrasound can be used to measure changes in cerebral vessel diameters and the changes detected using such a technique correlate with MRI measurements. I have shown, for the first time, that exposure to hypoxia is associated with an increase in middle cerebral artery diameter and that this is a consistent finding in both normobaric and hypobaric hypoxia. Thus, the measurement of velocity alone is likely to be unreliable in evaluating middle cerebral artery blood flow**.** In acclimatised subjects ascending to extreme altitude, the vessel calibre change appears to be of greater importance to increasing flow than changes in the velocity of the blood within it. Oxygen supplementation at 7950m rapidly reversed the observed MCA dilatation. Such dilatation and its rapid reversal through administration of supplemental oxygen have not previously been described, and challenge currently accepted concepts relating to adaptive mechanisms.

The main strength of this study is that two differing techniques (MRA and ultrasound) have demonstrated that MCA diameter increases with hypoxia and that these techniques are well correlated. I have demonstrated the same phenomenon in normobaric and hypobaric hypoxia and that vasodilatation is reversed by the administration of supplemental oxygen at 7950m. However, my studies do have inherent weaknesses which relate to subject selection, technical and logistical limitations. Firstly, subjects were all experienced high altitude climbers, whose physiological responses may, in some way, have been 'selected for'. Many were young (which may account for some of the differences in MCA diameter compared to angiographic/cadaver studies – see below). Thus, these findings require confirmation in those of different ages, sex and ethnic group as well as in non-mountaineers.

Secondly, combining assessments of vessel anatomy (from colour mapping) and flow velocity (from pulse-wave Doppler) allows vessel flow to be estimated. Such calculations do, however, assume frictionless laminar flow. Further, only maximal MCA_{Diam} is used. True measures of flow would thus have to integrate

flow velocity with changes in vessel diameter across the cardiac cycle. Caution should thus be applied when interpreting absolute values. However, I am confident about the observed trends and relative changes. In support, the marked increases in vessel diameter observed at extreme altitude (6400m and 7950m) were rapidly reversed with supplemental oxygen. Further, in the high altitude study, the ascent profile of 17 days to 5300m was relatively gentle and all subjects were partly acclimatized when studied, having been at the study altitude for 1 to 3 days. The lack of increase in MCA_{Vel} which I observed is thus consistent with other studies of MCA velocity measured 24-72 hours after arrival at altitude(Chan, Hoar et al. 2005; Van Osta, Moraine et al. 2005; Brugniaux, Hodges et al. 2007; Ainslie, Ogoh et al. 2008). There was no change in MCA_{Diam} up to 5300m, suggesting repeated measurements of MCA_{Diam} using this technique are reliable and repeatable.

Thirdly, the measurements at 7950m demonstrating the largest increase in MCA_{Diam} were performed within 36-48 hours of arrival, and all subjects had used supplementary oxygen to climb from 7100m to that altitude. It may be that these larger observed changes were a more acute effect. Further studies during exercise and with acute exposure, both of which may accentuate the changes, are advocated.

Although changes in relative measurements of ultrasound and MRA-measured middle cerebral artery diameters correlated well, actual values were significantly different (for example, TCD diameter measurements in normoxia and hypoxia were 5.44mm and 6.28mm while corresponding MRA diameter measurements were 3.04mm and 3.27mm). Such disparity has been previously reported, ultrasound (both Colour Doppler as we used and Power Doppler) yielding larger diameter measurements than MRI (Table 3-4). Since my ultrasound and MRA measurements correlate well, this implies that although the ultrasound measured diameter may not be a true diameter, it reliably reflects changes in diameter. It may be that the plane of the ultrasound, although consistent, is not truly tangential to the vessel and hence the cross-sectional area may be more eliptiform. Alternatively, the increase in velocity of blood at the vessel wall edges may make it appear more visible on Doppler ultrasound and give the impression of a widened vessel. I note Poulin and Robbins previous work

(Poulin and Robbins 1996; Poulin *et al* 2002) that did not demonstrate a change in the Power signal (implying no change in vessel cross-sectional area). It may be that the actual cross-sectional diameter change is closer in value to that detected by MRI, in which case, Power signal may not change significantly.

Measurement Modality	Mean MCA diameter (mm)	Number of subjects	*Notes
Cadaver	2.5-4mm (mean =3.35mm) (Pai, Varma et al. 2005)	5	
MRA (all 1.5Tesla)	2.9mm (Serrador, Picot et al. 2000) 2.73mm (Schreiber, Gottschalk et al. 2000) 2.23mm (Tarasow, Abdulwahed Saleh Ali et al. 2007) 2.95mm (Hansen, Pedersen et al. 2007) 3.4mm (Valdueza, Balzer et al. 1997)	12 8 36 12 6	
Angiography	2.38mm (Tarasow, Abdulwahed Saleh Ali et al. 2007)	36	
Power Doppler Proximal MCA: Distal MCA:	 5.2mm 4.3mm(Muller, Schwerdtfeger et al. 2000)	 17	 Subjects suspected of having vasospasm
TCCS Proximal MCA: Distal MCA:	 5.9mm 4.9mm(Muller, Schwerdtfeger et al. 2000)	 17	 Subjects suspected of having vasospasm

Table 3-4 Results of various studies measuring mean MCA diameters using direct vision (in cadaver studies), MRA, angiography and Doppler. The differences between MRA- and Doppler-measured diameters are similar to my data. TCCS = Transcranial Colour-Coded Sonography

My data imply that rSO_2 decreases in the face of increased cerebral oxygen delivery at 7950m. An increased delivery of de-oxygenated blood would however not be expected to result in an increase in regional oxygen saturation. rSO_2 measures the ratio of oxygenated to deoxygenated blood in the interrogated region and does not reflect the flux of blood (or flux of oxygen) passing through. In addition, many other factors (such as alterations in the contribution of arterial and venous compartments due to changes in intra-vessel volume) will affect rSO_2 (Wolff, Richardson et al. 2007).

There have not been many studies validating TCD- estimated changes in cerebral blood flow against other measures of cerebral perfusion. It is

interesting to note that some of the few studies that have attempted to correlate cerebral perfusion as measured using the Kety-Schmidt technique have found very poor correlations with TCD velocity measurements (Weyland, Stephan et al. 1994; Nuttall, Cook et al. 1996). Giller's group investigated further the use of TCD during exercise and concluded that, because of probable vessel diameter change, the use of TCD velocities to interpret cerebral blood flow during exercise might be invalid (Giller, Giller et al. 2000). Our data supports the view that caution must be used when using TCD velocity data to imply changes in cerebral perfusion.

3.5.1 Possible Mechanisms of Vasodilatation:

In order to maintain cerebral oxygen delivery (COD) in an increasingly hypoxic environment, one would expect to see an increase in cerebral blood flow (CBF). This can be influenced by alterations in vessel diameter and the velocity of blood within it (which in turn is determined by blood pressure and blood viscosity - Poiseuille's law). In this study, the first to measure both diameter and velocity with two techniques, vasodilatation appears to be the principal factor affecting flow. It may be that with increasing viscosity of blood, vasodilatation becomes the most important mechanism.

A number of mechanisms could be proposed to underlie vasodilatation:

Hypoxia: Hypoxia-induced increases in adenosine and nitric oxide, previously thought to mediate vasodilatation at an arteriolar level, might cause arterial vasodilatation. Other factors (such as those mediated by Hypoxia Inducible Factor and the cascade it induces) are thought to occur over a longer time period (Wilson, Newman et al. 2009). The rapid reversal of arterial dilatation with oxygen suggests a direct hypoxic effect.

Hypocarbia: A paradoxical phenomenon of hypocarbic vasodilatation has previously been observed in forced hyperventilation (Wollman, Smith et al. 1968; Du Boulay and Symon 1971). Du Boulay and Symon noted vasodilatation angiographically with $PaCO_2$ values of 20-25mmHg (2.6-3.33kPa). Whilst such a mechanism was not thought physiologically relevant, the extreme

hyperventilation and consequent hypocapnea that occurred at 7950m (mean $ETCO_2$ = 12.8mmHg = 1.7KPa) might be inducing this paradoxical effect.

3.5.2 Implications in High Altitude Illness

The arterial oxygen content (CaO_2) at rest in a sub-group of my subjects has previously been reported and is maintained at sea-level values up to and above 7100m(Grocott, Martin et al. 2009). The reduction in CaO_2 above that altitude coincides with the marked MCA arterial dilatation observed. Exercise at altitude is known to decrease CaO_2 and increase blood pressure (Imray, Myers et al. 2005). Similarly, Moller reported that CaO_2 was not only maintained, but increased at rest at high altitude. Opposite to sea level observation, CaO_2 decreased slightly at the altitude of 5260m (Moller, Paulson et al. 2002).
Although the increase in MCA_{Diam} only occurred above 5300m, a similar change may also occur at lower altitudes as a response to acute hypoxia, or during exercise at altitude (both of which are known to be potential triggers for AMS and HACE). According to LaPlace's Law (vessel wall tension= blood pressure x radius), the observed increase in MCA_{Diam} will result in an increase in vessel wall tension.

The trigeminovascular system has been implicated in the genesis of both high altitude headache and Acute Mountain Sickness (AMS) (Jansen, Krins et al. 2000; Van Osta, Moraine et al. 2005). The observed cerebral vessel dilatation may act as a direct mechanical trigger for this system. Alternatively, failure to dilate might result in increased MCA velocities and raised arterial pressures in an attempt to maintain an adequate cerebral oxygen delivery, which could also have implications in the development of high altitude illness.

Deaths above 8000m on Everest have been associated with cognitive impairment, ataxia, profound fatigue, late summit times and a tendency to fall behind (Firth, Zheng et al. 2008). Our group's recent study with blood gas analysis at 8400m (n=4) demonstrated that mean PaO_2 was 3.28kPa and $PaCO_2$ was 1.77kPa (Grocott, Martin et al. 2009). It is therefore suggested that some climbers suffer an acute hypoxic cerebral dysfunction and it may be that

they are reaching the limits of the adaptive mechanisms for maintaining CaO_2 and Cerebral Oxygen Delivery.

3.5.3 Clinical Implications

Giller, in his editorial “The Emperor has no clothes”, challenged the long held assumption that any changes in cerebral artery diameter that might occur are of no significance (Giller 2003). Other studies have highlighted the need to obtain quantitative measures of cerebral blood flow if there is reason to suspect that the diameter of the MCA might not remain constant, for example, when drugs such as nitroglycerin are used (Zuj, Greaves et al. 2007).

The two studies reported here confirm that marked cerebral hypoxia *is* associated with significant increases in cerebral artery diameter. The wider implication from this study is that any future investigations measuring cerebral vessel blood velocity must also consider potential changes in vessel diameter. Vasospasm is known to occur following subarachnoid haemorrhage (Gonzalez, Boscardin et al. 2007). This study demonstrates that hypoxia also affects vessel calibre. Other conditions and factors such as sepsis, inflammatory mediators, drugs and alterations in blood pH may have similar effects.

These findings offer new insights into the possible underlying pathophysiology of AMS and HACE, and highlight the importance of concurrent measurement of vessel calibre when using Doppler velocities to infer flow. The correlation of ultrasound measurements with MRA measurements implies that ultrasound may enable repeated assessments of cerebral artery size and flow at the bedside, during hospital transfer or in the field (Wilson, Levett et al. 2011).

3.6 Conclusions:

This is the first field study of cerebral perfusion above 5500m, and the first to show that exposure to extreme hypobaric hypoxia is associated with an increase in MCA diameter that is rapidly reversed by inhaled supplemental oxygen. These field TCD findings have been replicated and confirmed using MRA in acute hypoxia at sea level. This has uniquely demonstrated that ultrasound and MRA MCA measurements correlate. The increased diameter, as

opposed to increased blood velocity, is the major factor increasing cerebral blood flow and maintaining oxygen delivery. This may have implications for the pathogenesis of cerebral high altitude illness and the acclimatization process. Future studies inferring cerebral blood flow from transcranial Doppler velocity measurements at altitude and clinical studies where oxygenation may change, must take vessel calibre into account.

4 Chapter 4: BRAIN OXYGENATION AT REST AND DURING EXERCISE TO VO_2MAX AT ALTITUDE

4.1 Abstract

The cerebral hypoxia occurring on ascent to high altitude is thought to be central to a number of physiological processes and clinical conditions (such as Acute Mountain Sickness and High Altitude Cerebral Oedema). Cerebral hypoxia is exacerbated by exercise during exposure to systemic hypoxia. I sought to (i) better define this relationship (ii) characterise its inter-individual variation, and (iii) explore the extent to which sea-level variables might predict this response.

Methods: Subjects were drawn from the Caudwell Xtreme Everest Expedition, as described in past chapters. Changes in regional cerebral oxygenation (rSO_2) at rest and with exercise to VO_2Max were sought amongst 171 individuals of the 'trekker' cohort, ascending to 5300m. A smaller group ("investigators" n=24) ascending to 5300m and the resting cerebral oxygenation of a subgroup (n=7) ascending to 7950m were also studied.

Results: Trekkers: At sea level, mean absolute rSO_2 rose with increasing work intensity from rest to anaerobic threshold ([mean $\pm$ SEM] 68.9 $\pm$ 0.54% vs 71.3 $\pm$ 0.55%, p <0.001), but fell when VO_2Max was reached (67.4 $\pm$ 0.71%% ; p <0.001 from anaerobic peak). Values of rSO_2 at rest and at VO_2Max both fell with increasing altitude (resting values 59.3 $\pm$ 0.53% vs 53.6 $\pm$ 0.62%, and at VO_2Max 50.3 $\pm$ 0.68% vs 45.11 $\pm$ 0.62% for 3500m and 5300m respectively). Proportionally the relative reduction in rSO_2 was greater than the relative reduction in SaO_2, both at rest with increasing altitude and with exercise at altitude. The relative percentage reduction in resting SaO_2 from 75m to 5300m was -22.23 ± 0.56%. The relative percentage reduction in resting rSO_2 from 75m to 5300m was -30.61 ± 1.28% (paired t test p<0.001). During exercise at 5300m, SaO_2 fell 9.2 + 0.59% between rest and VO_2Max while rSO_2 fell 15.6 +0.97% between rest and VO_2Max (expressed as a percentage of the respective resting $Sa0_2/rSO_2$ value at that altitude) (paired t-test p=0.002). Cluster analysis demonstrated that those who had a greater cerebral desaturation at 75m during exercise have a greater desaturation at 5300m (p<0.05). The desaturation both at rest and during exercise in relatively acute hypoxia (arrival at 3500m) appeared to be greater in the right than left cerebral

hemispheres (p=0.005). At increasing altitude, a positive correlation between resting rSO_2 and height and male gender was demonstrated, as was a negative correlation between age and female gender. No correlation was found between rSO_2 and headache score.

Conclusions: Subjects who cerebrally desaturate during exercise at sea level tend to desaturate more at altitude. Although subjects who cerebrally desaturate more (both resting measures and with exercise) recorded a higher headache severity index this difference is not statistically significant. Further analysis (e.g. of neurocognitive data) may demonstrate a correlation between desaturation and mental performance, but that is beyond the scope of this chapter.

4.2 Introduction

Exercise-induced hypoxaemia of high altitude contributes to the pathogenesis of AMS (Hackett and Roach 2001) and also to impaired mental performance which, in extreme, may be significant factor in high altitude deaths (Firth, Zheng et al. 2008).

Past studies of cerebral oxygenation (rSO_2) at altitude have involved small numbers of highly selected subjects. I sought to extend these observations to a much larger cohort, and also to explore the hypotheses that:

1) Changes in cerebral oxygenation during exercise at sea level are indicative of changes during exercise at altitude.
2) Changes in cerebral oxygenation correlate with headache at altitude.

4.3 Methods

General methodology and exercise protocols of the Caudwell Xtreme Everest expedition have been described elsewhere (Grocott, Martin et al. 2010; Levett, Martin et al. 2010), being summarised in chapter 2. Ethical approval was granted by University College London.

Subjects: The study was divided into two groups.

Trekker Group: A group of 198 trekkers (125 male, age range 18.3 to 70.3; mean = 44.7 years) who ascended to 5300m over 11 days.

Investigator Group: A group of Base Camp investigators and climbers (n=24, 18 male, mean age 35.2 years [range 19-59]) who ascended to 5300m over 13 days. Fourteen of these ("Climbers") ascended higher on the mountain with 7 being studied at 7950m (the South Col of Everest).

Ascent: Baseline studies were performed in London. Further studies were performed at 3500 (Namche Bazaar) and 5300m (Everest Base Camp). The ascent profile is demonstrated in figure 2-3.

Subjects were either studied on day 1 or day 2 after arrival at the new altitude and this study day was constant for each subject. Subjects were required to sit on an upright cycle ergometer (figure 4-1) whilst being prepared for the study. Pulse and peripheral arterial oxygen saturations (SaO_2) were monitored throughout the study. Regional brain oxygenation (rSO_2) was monitored using a cerebral oximeter (INVOS C NIRS device, Invos, Somanetics, MI, USA) with measurements being taken from both left and right frontal regions at 7-second intervals (figure 4-2). The probes had self-adhesive properties to stick to the skin but were, in addition, held with a black sweatband to help prevent movement and the incursion of extraneous light. rSO2 data were recorded internally within the NIRS devices and subsequently converted to excel format for analysis. After 3 minutes of rest, and a further 3 minutes of unloaded exercise, subjects underwent a graded exercise, with maximal exercise capacity assessed using an incremental cardiopulmonary exercise testing (CPET) standardised ramp with breath-by-breath expired gas analysis (below). The time point of AT and VO_2Max was retrospectively calculated from CPET data (below). VO_2 was calculated as the average oxygen consumption for the individual breaths taken during 20 second increments of the exercise test. The time for VO_2Max was reported as the middle time point of the 20-second time interval with maximal VO_2. Data were plotted on graphs of SaO_2 versus time and were visually assessed for quality and completeness.

Figure 4-2 is the display of the cerebral oximeter at the end of a study at 5300m. It has been annotated to demonstrate the time points subsequently studied.

Figure 4-1 Exercise testing on two upright cycle ergometers at Everest Base Camp. The NIRS forehead probes are secured under a sweatband.

Exercise Protocol: The subjects performed an incremental ramp test to the limit of tolerance using an electromagnetically braked cycle ergometer (Lode Corival; Lode, Groningen, the Netherlands) and a breath-by-breath cardiopulmonary exercise testing system (Metamax 3b; Cortex, Leipzig, Germany). A full calibration of the breath-by-breath system was performed before each test. Prior to the incremental exercise test, subjects warmed up with a low- intensity 30-minute constant work rate protocol. A ramp slope of 20 to 35 W/minute was chosen depending on the sex, age and physical fitness of the subjects in order to obtain a predicted test duration of approximately 10 to 15 minutes. The ramp slope was kept constant throughout each study. Readings were taken during 3 minutes of complete rest, 3 minutes of unloaded cycling then during exercise to Anaerobic Threshold (AT - time variable per individual) and to maximal oxygen consumption (VO_2Max – time variable per individual). NIRS readings continued into the recovery phase for a further 2 minutes.

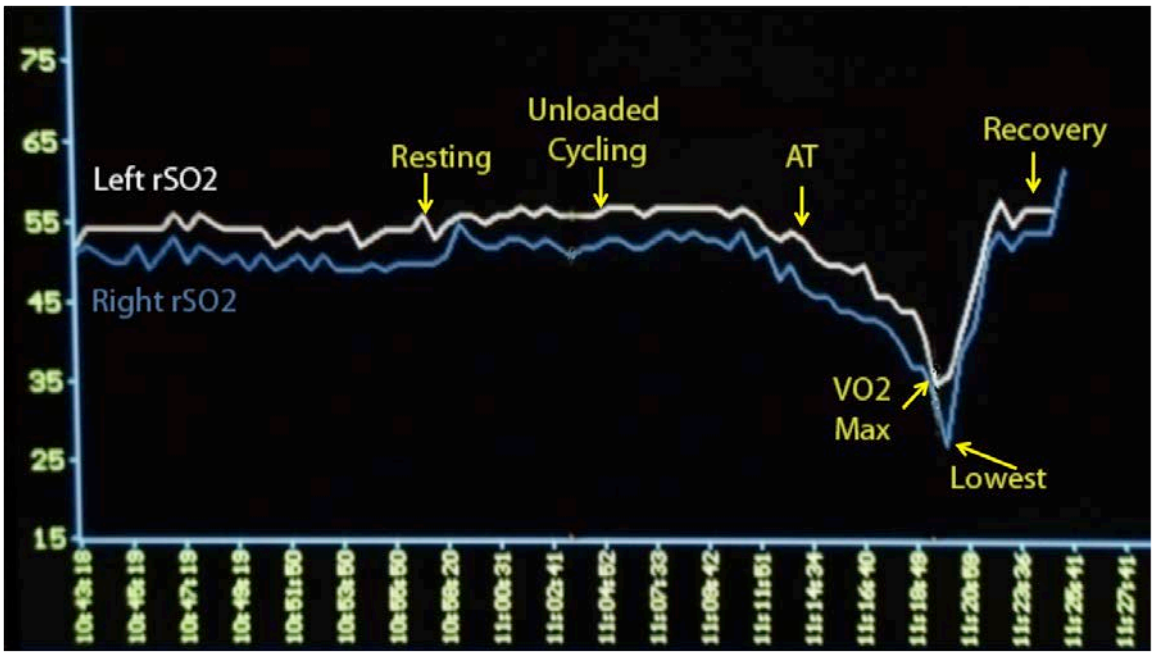


Figure 4-2 Labelled raw output of time vs rSO_2 (regional brain oxygen saturation) displayed on the INVOS C. This demonstrates the left (white) and right (blue) values and the typical changes that occur at each stage of the cycle ergometer exercise to VO_2Max at Everest Base Camp (5300m).

Variables: Basic physiological variables were recorded in addition to rSO_2 at key time points. These included: peripheral arterial oxygen saturation (SaO_2) measured on the subjects' right index finger, blood pressure (only the mean arterial pressure MAP is reported here) and end-tidal CO_2 ($EtCO_2$). Details on the equipment used for these and the cycle ergometer is available separately (Levett, Martin et al. 2010), and summarised in chapter 2.

Additional variables were calculated from those recorded:
Percentage Changes: Because SaO_2 and rSO_2 are not directly comparable (virtually all subjects will have a normoxic resting peripheral saturation of 95 to 99%, while the resting rSO_2 values have greater variability and start lower). Hence two terms are used for clarity in reporting – Absolute percentage changes (of raw SaO_2 and rSO_2 values) and relative percentage changes (relative to baseline values). The relative baseline for resting values at each altitude uses the value at rest at sea level. For the exercise experiments, the relative changes are expressed as a percentage of the resting value at the corresponding altitude.

Arterial Oxygen Extraction: Arterial Oxygen Extraction (AOE) is calculated as:

$$AOE = (p+1)(1-rSO_2/SaO_2) \approx 1.39 \times (1-rSO_2/SaO_2)$$

This assumes the ratio (p) of arterial blood (Va) to venous blood (Vv) within the area of investigation is constant (the ratio being Va/Vv = 0.39) (Wolff, Richardson et al. 2007). As explained in the introduction, I believe that the venous component of intracranial blood volume increases. AOE is however commonly calculated hence I have included it in the analysis but will interpret it cautiously.

4.3.1 Study Power:

The number of subjects in the CXE groups overall were largely determined by logistic constraints. However, with α=p=0.05 and power (ß) = 0.8, detecting an effect size of 0.5 between two populations who respond differently would need N=64 in the two populations. Assuming the populations are of equal size this

would require N = 128 (Cohen 1992). Allowing for attrition of subjects, the expedition aimed to study 200 subjects. In reality, this specific study analyses 171data sets.

Cluster and Statistical Analysis:

The technique of cluster analysis is explained within the section on cluster analysis below. For comparative analyses, SaO_2 or rSO_2 values were expressed as a percentage either of the resting value at 75m (when studying changes with altitude) or as a percentage of the resting value at the start of an exercise protocol (when studying changes with exercise).

No basic data from the trekking group were imputed (subjects with such missing data were excluded). For the core group (n=24), two subjects missed exercising testing at 3500m (Namche) and their NIRS data was imputed using linear modelling.

Multilevel modelling was used to maximise the use of all available data. Hence, the number of subjects at each altitude varies (see results). Estimated marginal means were calculated for each variable. Post-hoc tests within multi-level modelling were used to compare means. Additional comparisons were performed with either independent or paired T-tests. For correlations between demographic features (e.g. age, smoking, height, sex) Pearson's 2 tailed correlations were used, correlations between dichotomas and serial variables were point biserial. Statistical significance was set at 0.05, but when large numbers of correlations were analysed, was set at 0.01 to avoid capitalising on chance.

4.4 Results

Note – because of the large amount of data collected, a brief summary and explanation of some results is given within the results section. A more detailed discussion of relevant results is given within the Discussion.

4.4.1 Trekker Group:

Of 198 starting, 190 subjects reached Everest Base Camp (5300m) and had data recorded (6 subjects withdrew from incremental CPET prior to departure and 2 were removed because of poor baseline data quality). Missing data

principally resulted from: 1) subjects being unwell and unable to ascend/ complete exercise or 2) technical error during the study.

Of the 190 data sets, 16 had studies done on incorrect days, hence they were excluded (leaving 174 studies). Three studies were missing more than one altitudes data set and were also removed. Of the remaining 171 subjects, 6 were missing London (75m) data, 3 were missing Namche (3500m) data and 27 were missing data from Everest Base Camp (3500m); hence the number of data sets at each altitude was 165, 168 and 144 respectively. Note, of the 27 missing data at Everest Base Camp, 14 had acute mountain sickness, 6 diarrhoea and vomiting, 4 respiratory tract infections, 1 hypertension and 2 arrhythmias.

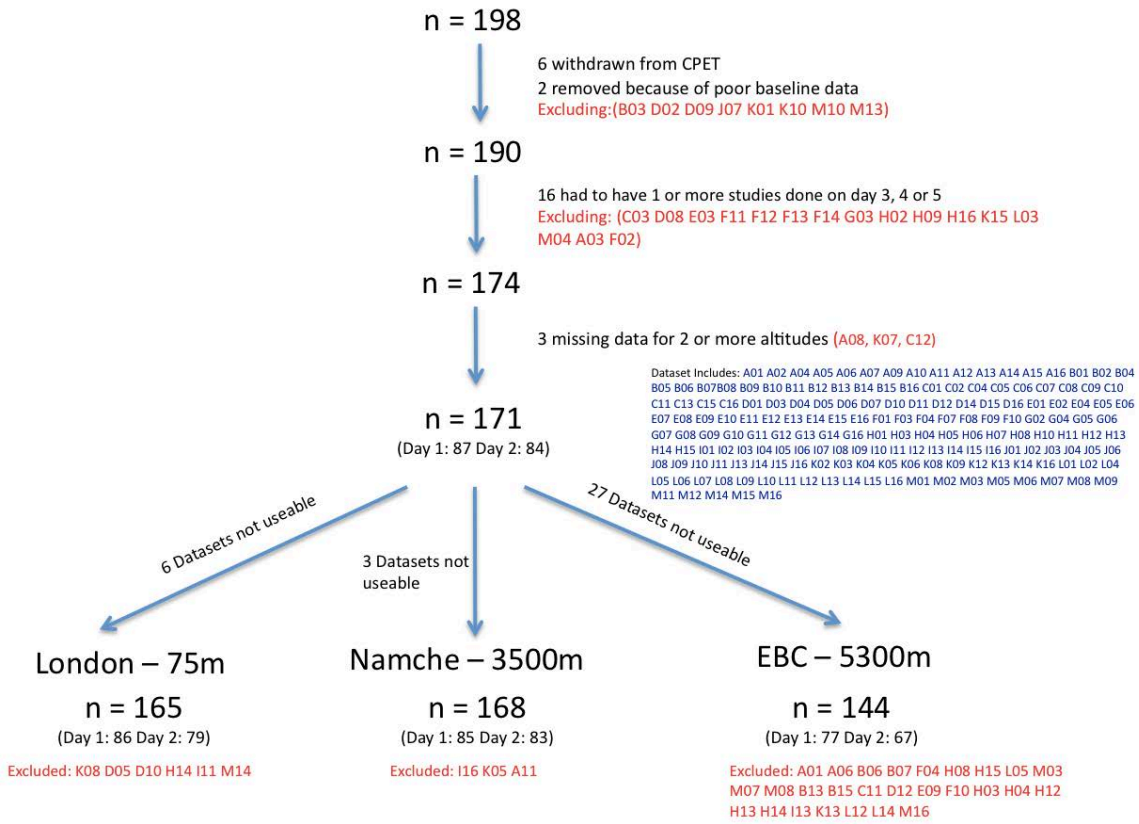


Figure 4.3: Explanation of subject and data attrition.

Descriptive Results

4.4.1.1 General Results

Regional Cerebral Oxygenation (rSO_2):

Table 1 shows the estimated marginal mean rSO_2 using a mixed model analysis for left, right and mean rSO_2 during rest, unloaded cycling, at anaerobic threshold (AT) and maximal oxygen consumption (VO_2Max) at each of the altitudes studied (75m, 3500m and 5300m). In addition it displays the estimated marginal mean of the lowest rSO_2 values recorded in each rSO_2 variable (i.e. the point of maximal brain desaturation which was usually in the order of 18-24 seconds after VO_2Max (see below)). Finally, the estimated marginal mean arterial oxygen extraction during rest, unloaded cycling, at AT, at VO_2Max and at the point of maximum desaturation is displayed. Superscript letters demonstrate significant differences between groups i.e. a is significantly different to b, which is significantly different to c. n=171, except for AOE at AT, VO_2Max and Lowest where some SaO_2 data was missing – for these data resting AOE n = 171, unloaded AOE n= 171, AT AOE n = 167, VO_2Max AOE n= 165, the lowest AOE n = 164.

Table 4-1 Table of rSO_2, AOE and percentage change values at each altitude during each stage of exercise. *See text for further details. Groups with different superscript letters are significantly different from each other.

	Mean	75m	3500m	5300m	dF	F	Sig
Resting	Mean Left	69.03[a]	59.69[b]	53.55[c]	184.53	391.38	<0.005
	99% CI	67.59–70.47	58.37-61.01	51.91-55.18			
	Mean Right	68.89[a]	58.57[b]	53.40[c]	180.67	371.67	<0.005
	99% CI	67.43-70.35	57.17-59.97	51.70-55.09			
	Mean rSO2	68.96[a]	59.13[b]	53.45[c]	178.54	506.62	<0.005
	99% CI	67.60-70.32	57.88-60.38	51.90-55.01			
	Mean AOE	0.401[a]	0.459[b]	0.452[b]	169.63	44.16	<0.005
	99% CI	0.38-0.42	0.44-0.48	0.43-0.48			n=171
Unloaded	Mean Left	69.17[a]	59.10[b]	52.10[c]	179.97	425.28	<0.005
	99% CI	67.74-70.61	57.73-60.47	50.41-53.79			
	Mean Right	69.26[a]	58.07[b]	52.00[c]	182.70	419.86	<0.005
	99% CI	67.81-70.72	56.63-59.52	50.30-53.71			
	Mean rSO2	69.21[a]	58.59[b]	52.02[c]	175.77	552.67	<0.005
	99% CI	67.85-70.57	57.29-59.88	50.43-53.62			
	Mean AOE	0.397[a]	0.467[b]	0.478[b]	170.65	68.88	<0.005
	99% CI	0.38-0.42	0.45-0.49	0.45-0.51			n=171
	rSO2 %age	+0.43[a]	-0.94[b]	-2.81[c]	177.83	41.04	<0.005
	99% CI	-0.53to+0.91	-1.52 to-0.35	-3.62to-2.08			
AT	Mean Left	71.29[a]	55.52[b]	48.86[c]	174.26	758.82	<0.005
	99% CI	69.78-72.80	54.15-56.89	47.16-50.55			
	Mean Right	71.38[a]	54.74[b]	48.44[c]	179.99	808.83	<0.005
	99% CI	69.86-72.90	53.30-56.18	46.84-50.05			
	Mean rSO2	71.33[a]	55.14[b]	48.63[c]	174.12	1029.6	<0.005
	99% CI	69.91-72.75	53.82-56.45	47.08-50.17			
	rSO2 %age	+3.55[a]	-6.79[b]	-9.04[c]	181.70	429.19	<0.005
	99% CI	+2.73to+4.37	-7.82to-5.76	-10.12to-7.95			*
	Mean AOE	0.38[a]	0.45[b]	0.42[b]	138.58	20.41	<0.005
	99% CI	0.34-0.41	0.43-0.47	0.39-0.45			
VO_2 Max	Mean Left	67.64[a]	50.66[b]	45.28[c]	184.59	545.12	<0.005
	99% CI	65.64-69.63	48.95-52.37	43.48-47.09			
	Mean Right	67.13[a]	49.81[b]	45.10[c]	198.72	495.32	<0.005
	99% CI	65.17-69.09	47.98-51.64	43.48-46.71			
	Mean rSO2	67.38[a]	50.24[b]	45.17[c]	192.79	681.61	<0.005
	99% CI	65.53-69.23	48.57-51.91	43.59-46.76			
	Mean AOE	0.41 [a]	0.52 [b]	0.49 [b]	149.98	54.63	<0.005
	99% CI	0.38-0.44	0.49-0.55	0.46-0.53			*
	rSO2 %age	-2.29[a]	-15.12[b]	-15.67[b]	217.34	200.66	<0.005
	99% CI	-4.06to-0.54	-17.25to-12.98	-17.66to-13.68			
Lowest	Mean Left	64.89[a]	48.53[b]	43.32[c]	181.78	445.81	<0.005
	99% CI	62.86-66.92	46.80-50.26	41.52-45.12			
	Mean Right	64.87[a]	48.02[b]	43.97[c]	205.17	450.63	<0.005
	99% CI	62.91-66.83	46.18-49.85	42.31-4562			
	Mean rSO2	64.87[a]	48.28[b]	43.64[c]	193.97	584.39	<0.005
	99% CI	63.01-66.74	46.59-49.96	42.05-45.23			
	Mean AOE	0.45[a]	0.55[b]	0.52[b]	147.10	44.45	<0.005
	99% CI	0.42-0.48	0.52-0.58	0.49-0.55			*
	rSO2 %age	-5.96[a]	-18.41[b]	-18.54[b]	222.30	171.51	<0.005
	99% CI	-7.74to-4.17	-20.61to-16.22	-20.51to-16.58			
Recovery	Mean Left	70.93 [a]	60.54 [b]	52.41 [c]	192.58	403.82	<0.005
	99% CI	69.26 – 72.59	59.07 – 62.01	50.73 – 54.09			
	Mean Right	70.04 [a]	59.28 [b]	51.94 [c]	196.48	321.18	<0.005
	99% CI	68.38 – 71.71	57.66 – 60.91	50.23 – 53.65			
	Mean rSO2	70.49 [a]	59.92 [b]	52.16 [c]	193.44	491.60	<0.005
	99% CI	68.97 -72.01	58.48 – 61.36	50.58 – 53.74			
	rSO2 %age	2.33 [a]	1.58 [b]	-2.37 [c]	254.51	28.61	<0.005
	99%CI	1.03 to 3.64	-0.16 to 3.32	-3.87 to -0.86			

4.4.1.2 Summary of Basic Data and Spilt by Day of Study

Table 4-2 reports the changes of basic variables that are important in interpreting brain oxygenation changes at each altitude during the exercise protocol (HR, SaO_2, Hb, MAP, $EtCO_2$, average Headache Score (HA) and total Headache Score (Tot HA). The mean rSO_2s and arterial oxygen extractions are also shown:

N	Males / Females	Mean Age	Weight (75m)	Weight (5300m)
171	106/65	44.57 (SE 1.03)	74.34 (SE = 0.98)	72.25 (SE = 1.02)

No of Smokers = 12

Altitude	75m		n= 165		3500m		n= 168		5300m		n= 144	
	Rest	AT	VO_2 Max	Rec	Rest	AT	VO_2 Max	Rec	Rest	AT	VO_2 Max	Rec
HR	78.1	129.3	170.4	-	84.1	127.2	159.6	-	90.9	118.7	138.4	-
SEM	0.92	1.13	1.05	-	1.11	1.11	1.30	-	1.13	1.12	1.53	-
SaO_2	96.9	96.5	96.3	-	88.4	81.7	80.0	-	79.4	72.6	72.1	-
SEM	0.08	0.08	0.10	-	0.23	0.32	0.31	-	0.35	0.52	0.49	-
Hb	14.48				14.72				15.77			
SEM	0.09				0.10				0.11			
MAP	96.6	-	131.2	119.4	97.4	-	122.8	114.3	98.9	-	121.5	118.4
SEM	0.94	-	1.88	2.12	0.82	-	1.25	1.23	0.94	-	1.35	1.44
$PEtCO_2$	33.78	42.13	36.06	-	27.02	28.70	24.12	-	20.46	21.04	17.67	-
	0.23	0.33	0.40	-	0.20	0.22	0.23	-	0.17	0.17	0.16	-
HA	0.06				0.17				0.38			
Total HA	8				25				43			
Mean rSO_2	68.94	71.34	67.40	70.43	59.11	55.07	50.11	59.82	53.68	48.85	45.33	52.27
SEM	0.54	0.55	0.71	0.59	0.48	0.51	0.64	0.56	0.61	0.62	0.63	0.62
AOE	0.40	0.38	0.41	0.45	0.46	0.45	0.52	0.55	0.45	0.42	0.49	0.52
SEM	0.008	0.013	0.012	0.012	0.008	0.009	0.011	0.012	0.01	0.012	0.014	0.014

Table 4-2 Mean (+/-SEM) figures for the group as a whole (combining both day 1 and day 2 subjects). HR = Heart Rate (/beats per minute), SaO_2 = Peripheral oxygen saturation (/%), Hb = Haemoglobin concentration (/mg/dl), MAP = Mean Arterial Pressure (/mmHg), PEtCO2 = end tidal CO_2 partial pressure (/mmHg). HA = Headache Score, rSO_2 = brain oxygenation (/%), AOE = Arterial Oxygen Extraction (/%).

Comparison of subjects studied on Day 1 and Day 2:

Table 3 demonstrates the same data, divided by day of exercise testing: on the day after arrival at the altitude (Day 1 – table 4-3a) or on the second day after arrival (Day 2- table 4-3b).

Day	N	Males/Females	Mean Age	Weight (75m)	Weight (5300m)
1	87	56/31	45.98 (SE 1.40)	76.45 (SE 1.52)	73.55 (SE1.57)

Altitude	75m		n=86		3500m		n= 85		5300m		n=77	
	Rest	AT	VO_2 Max	Rec	Rest	AT	VO_2 Max	Rec	Rest	AT	VO2 Max	Rec
HR	77.8	128.5	169.2	-	85.5	126.2	158.6	-	89.7	116.1	135.8	-
SEM	1.2	1.6	1.4	-	1.2	1.5	1.7	-	1.4	1.5	2.1	-
SaO_2	96.9	96.5	96.4	-	88.3	81.6	80.4	-	79.69	72.5	71.8	-
SEM	0.11	0.11	0.14	-	0.34	0.47	0.47	-	0.48	0.69	0.62	-
Hb	14.51				14.63				15.72			
SEM	0.133				0.135				0.15			
MAP	96.54	-	131.4	121.2	96.5	-	122.9	113.7	99.3	-	122.7	119.6
SEM	1.37	-	2.39	2.39	1.17	-	1.64	1.60	1.27	-	1.7	2.03
$PetCO_2$	33.98	42.30	35.43	-	27.22	28.60	23.86	-	20.53	21.1	17.6	-
	0.30	0.46	0.53	-	0.26	0.31	0.31	-	0.21	0.23	0.23	-
HA	0.04				0.15				0.32			
Tot HA	3				13				20			
Mean rSO_2	68.40	70.75	66.56	69.70	58.57	54.55	49.83	59.25	53.36	48.71	44.80	51.84
SEM	0.69	0.74	0.99	0.79	0.65	0.67	0.88	0.77	0.74	0.76	0.88	0.75
AOE	0.41	0.37	0.42	0.45	0.47	0.45	0.535	0.57	0.45	0.43	0.51	0.53
SEM	0.01	0.12	0.016	0.016	0.01	0.13	0.156	0.02	0.13	0.01	0.02	0.02

Table 3a

Day	N	Males / Females	Mean Age	Weight (75m)	Weight (5300m)
2	84	50/34	43.1 (1.51)	72.17 (SE 1.2)	70.74 (SE 1.25)

Altitude	75m		n=79		3500m		n= 83		5300m		n=67	
	Rest	AT	VO_2 Max	Rec	Rest	AT	VO_2 Max	Rec	Rest	AT	VO_2 Max	Rec
HR	78.4	130.0	171.7	-	82.7	128.3	160.7	-	92.3	121.8	141.7	-
SEM	1.4	1.64	1.55	-	1.86	1.68	1.95	-	1.8	1.6	2.2	-
SaO_2	96.9	96.5	96.2	-	88.4	81.9	79.6	-	79.1	72.7	72.5	-
SEM	0.11	0.13	0.13	-	0.30	0.44	0.42	-	0.50	0.82	0.80	-
Hb	14.4				14.8				15.82			
SEM	0.138				0.15				0.15			
MAP	96.6	-	131.0	117.6	98.3	-	122.7	115.0	98.4	-	119.8	117.0
SEM	1.3	-	2.9	3.2	1.2	-	1.9	1.9	1.4	-	2.2	2.0
$PetCO_2$	33.57	41.95	36.74	-	26.8	28.80	24.40	-	20.37	20.95	17.71	-
SEM	0.36	0.46	0.59	-	0.30	0.31	0.33	-	0.27	0.26	0.23	-
HA	0.7				0.19				0.45			
Tot HA	5				12				23			
Mean rSO_2	69.54	71.98	68.31	71.21	59.66	55.62	50.40	60.43	54.04	49.01	45.94	52.77
SEM	0.83	0.81	1.02	0.88	0.72	0.77	0.94	0.80	1.00	1.01	0.89	1.01
AOE	0.39	0.39	0.40	0.44	0.45	0.44	0.50	0.53	0.44	0.40	0.47	0.50
SEM	0.01	0.1	0.02	0.02	0.01	0.01	0.1	0.02	0.1	0.01	0.02	0.02

Table 3b

Table 4-3 a and b respectively display results for basic variables and for brain oxygenation on subjects studied on day 1 and day 2. HR = heart rate, SaO_2 = peripheral oxygen saturation, Hb = haemoglobin, MAP = mean arterial pressure, HA = Headache square, $PetCO_2$ = end tidal CO_2, rSO_2 = regional brain oxygenation, AOE = arterial oxygen extraction.

4.4.1.3 Other variables – a summary of these results

Note – graphs for some of the following variables appear in chapter 5.

Heart Rate: Resting heart rate (HR) increases with altitude (mean resting HR at 75m (Mean ± SEM) = 78.1 ±0.92 bpm. Mean resting HR at 5300m = 90.9 ±1.1bpm; p = <0.001). Heart rate clearly increases with exercise (from resting 78.1 ±0.92 bpm to VO_2Max 170.4 ± 1.0bpm) at sea level, but this increase is reduced at 5300m (from resting 91.1 ±1.1 to VO_2Max 138.4 ±1.5; p<0.001).

SaO_2: Although significant, the decrease in peripheral saturation (SaO_2) during exercise at sea level is considerably less than during exercise at altitude (at 75m resting SaO_2 = 96.9 ±0.09%, VO_2Max SaO_2 = 96.3 ±0.096 p = 0.001; at 5300m resting SaO_2 = 80.0 ±0.40%, VO2Max SaO_2 = 72.1 ±0.49% p <0.001).

Haemoglobin: The mean haemoglobin increased from 14.5 ±0.98g/dl to 15.8 ±1.1g/dl over the 11 days of ascent (p<0.001).

Mean Arterial Pressure: Resting mean arterial blood pressure (MAP) increased from 96.9±1.1mmHg at 75m to 98.9±0.9mmHg at 5300m (p<0.001). MAP increased to a greater extent with exercise (from rest at 75m 96.9±1.1mmHg to VO_2Max at 75m 131.2±1.9mmHg; p<0.001) however at altitude, this increase is less (from rest at 5300m 98.9±0.9mmHg to 121.6±1.5mmHg at VO_2Max; p <0.001) Note - no blood pressure was recorded at Anaerobic Threshold as the time of this was calculated retrospectively.

Headaches: The Average Headache Score (HA) on the day of study (out of a total of 4) was consistently low, being 0.06 ±0.02 at 75m, 0.17 ±0.3 at 3500m and 0.38 ±0.05 at 5300m. Although the differences are statistically significant (p<0.001), the low values probably reflect the slow ascent profile.

Mean rSO_2: The mean rSO_2 increases at AT when not hypoxic (75m), and returns to a normal or slightly sub-normal at VO_2Max (Mean rSO_2 at 75m at rest 68.9 ±0.5%, at AT 71.3±0.6, at VO_2 Max = 67.4±0.7; all values are significantly different from their previous value p<0.001). At altitude, there is a consistent

decrease in mean rSO_2 with exercise. (Mean rSO_2 at 5300m at rest = 53.7 ±0.6% at AT = 48.84±0.6% and at VO_2Max = 45.3 ±0.6% (p=<0.001 between these). The mean rSO_2 (at rest and during exercise) is consistently higher on the second day after ascent than on the first day (e.g. at rest on Day 1 mean rSO_2 at 5300m = 53.36±0.74%, on day 2 = 54.04±1; at VO_2Max day 1 = 44.8±0.9%, day 2 = 45.9±0.9% (p<0.001). This implies that an element of acclimatisation has occurred.

Arterial Oxygen Extraction: As can be seen from table 4-1, AOE does not significantly change during exercise when normoxic (75m) (AOE at 75m at rest = 0.41±0.01%, at VO_2 Max = 0.41±0.01% (p=0.44). However, on ascent to altitude, the AOE significantly increases both at rest (AOE at 75m at rest =0.40±0.01%, at 5300m at rest = 0.45±0.1%, p <0.001) and during exercise (AOE at 5300m at rest = 0.43 ±0.01%, at VO_2Max = 0.49±0.01%, p<0.001). It should also be noted that these results must be interpreted with extreme caution as they may actually represent a change in arterial:venous compartment ratio rather than a genuine increase in arterial oxygen extraction.

4.4.1.4 rSO_2 at VO_2Max and Lowest rSO_2

At altitude, many subjects exhibited a further drop in rSO_2 after they had reached VO_2Max. Hence, rSO_2 was recorded at the time of VO_2Max, but the lowest three values following this were also recorded. At 75m, the value of rSO_2 at VO_2Max usually did not have any areas with a significantly different rSO_2 value around it (before or after), however an attempt was made to find a lower value to be consistent. The values of lowest rSO_2 and the time to the lowest rSO_2 at sea level are probably meaningless. The values at 3500m and 5300m are real.

The mean (and SEM) rSO_2 values at VO_2Max, the lowest values and the mean time between them at each altitude are displayed in table 4-4.

	75m				3500m				5300m			
	Resting	VO_2Max	Lowest	Time diff	Resting	VO_2Max	Lowest	Time diff	Resting	VO_2Max	Lowest	Time diff
rSO_2	68.94	68.31	64.89	29.2	59.11	50.11	48.17	18.4	53.68	45.33	43.54	24.2
SE	0.54	1.02	0.71	3.75	0.48	0.64	0.65	4.6	0.61	0.63	0.64	3.4

Table 4-4 The differences between rSO_2 at VO_2Max and the lowest rSO_2 reading at each altitude with the mean time difference (time diff) between the readings.

The further reduction of rSO_2 after VO_2Max could be technical (e.g. a delay in the cerebral oximeter analysing and displaying the result) or biological (e.g. there is a period of further cerebral desaturation before restoration of normal cerebral oxygenation occurs). This study is not designed to investigate this although it is discussed in greater depth in the discussion.

There is close correlation (figure 4-4) between VO_2Max and the lowest rSO_2 (at both 3500m and 5300m, the correlation is 0.97 ($p<0.005$)). Hence, since it is not clear what the lowest rSO_2 values mean, only rSO_2 at VO_2Max is reported beyond this point.

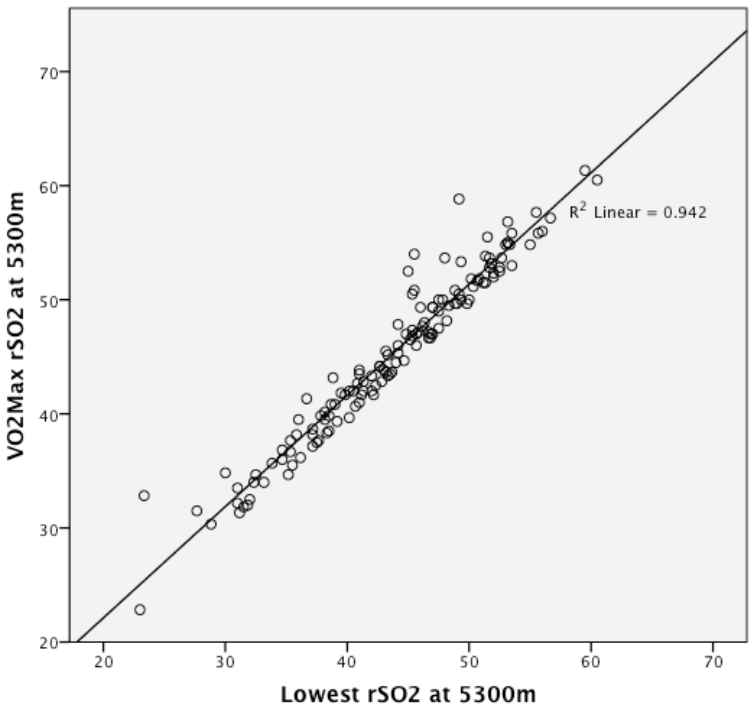


Figure 4-4: The correlation between rSO_2 (/%) at VO_2max and the lowest recorded rSO_2 at 5300m.

CORRELATIONS:

4.415 – Correlation Within rSO_2 Readings

Correlation between Left and Right rSO_2 Values:

Paired sample correlations and t-tests were used to compare mean left and right rSO_2 values at each altitude and at rest, unloaded cycling, AT and VO_2Max. Table 4-5 reports the mean values for left and right rSO_2, the number of subjects, standard deviation and standard error of the mean, at each altitude at rest, unloaded, AT and VO_2Max.

		Descriptive data				Correlation		Paired Differences							
											95% CI of Difference				
Pair	Left / Right Location and Stage of Exercise	Mean	N	Std. Deviation	S.E.M.	Correlation	Sig.	Mean difference	Std. Deviation	S.E.M.	Lower	Upper	t	df	Sig. (2 tailed)
1	L rSO2 L Rest	68.9	165	7.18	.56	.78	.000	0.08	4.80	0.37	-0.66	0.82	0.22	164	.829
	R rSO2 L Rest	68.9	165	7.38	.57										
2	L rSO2 N Rest	59.6	168	6.61	.51	.70	.000	1.15	5.26	0.41	0.35	1.96	2.84	167	.005
	R rSO2 N Rest	58.5	168	7.00	.54										
3	L rSO2 BC Rest	53.7	144	7.79	.65	.75	.000	0.12	5.51	0.46	-0.78	1.03	0.27	143	.790
	R rSO2 BC Rest	53.6	144	7.86	.66										
4	L rSO2 L Unload	69.1	165	7.20	.56	.79	.000	-0.15	4.75	0.37	-0.88	0.59	-0.39	164	.695
	R rSO2 L Unload	69.3	165	7.31	.57										
5	L rSO2 N Unload	59.1	168	6.86	.53	.70	.000	1.06	5.47	0.42	0.23	1.89	2.52	167	.013
	R rSO2 N Unload	58.0	168	7.24	.56										
6	L rSO2 BC Unload	52.2	144	8.09	.67	.76	.000	0.07	5.56	0.46	-0.84	0.99	0.15	143	.877
	R rSO2 BC Unload	52.1	144	8.03	.67										
7	L rSO2 L AT	71.2	165	7.41	.58	.78	.000	-0.14	4.95	0.39	-0.90	0.62	-0.36	164	.722
	R rSO2 L AT	71.4	165	7.52	.59										
8	L rSO2 N AT	55.4	168	6.86	.53	.75	.000	0.82	4.94	0.38	0.07	1.57	2.15	167	.033
	R rSO2 N AT	54.6	168	7.21	.56										
9	L rSO2 BC AT	49.0	142	8.18	.69	.76	.000	0.33	5.47	0.46	-0.58	1.24	0.72	141	.475
	R rSO2 BC AT	48.6	142	7.61	.64										
10	L rSO2 L VO2Max	67.6	165	9.88	.77	.76	.000	0.42	6.76	0.53	-0.62	1.46	0.80	164	.426
	R rSO2 L VO2Max	67.1	165	9.62	.75										
11	L rSO2 N VO2Max	50.5	168	8.51	.66	.78	.000	0.90	5.88	0.45	0.00	1.79	1.98	167	.049
	R rSO2 N VO2Max	49.6	168	9.08	.70										
12	L rSO2 BC	45.3	141	8.53	.72	.72	.000	0.13	6.05	0.51	-0.88	1.13	0.25	140	.806
	R rSO2 BC	45.2	141	7.53	.63										

Table 4-5 Comparison of left and right rSO_2 values at each altitude and at each stage of the exercise protocol (Key: L/R = Left/Right; rSO_2 = regional saturation; L = London 150m, N = Namche 3500m, BC = Everest Base Camp 5300m; Rest = Resting, Unload = Unloaded cycling, AT = Anaerobic Threshold, VO_2Max = VO_2Max). A significant difference was found between right and left rSO_2 values throughout the exercise protocol at 3500m (Namche, underlined).

The left and right rSO_2 values were consistently and highly correlated (>0.7, and $p < 0.001$). There was only one location where there was a significant difference ($p<0.01$) between left and right rSO_2 values: at Namche (3500m),

where the mean resting left rSO_2 exceeded that on the right at rest (by 1.15%, p=0.005) and throughout the exercise protocol (underlined in Table 4-5).

4.4.1.5 Correlation between rSO_2 and SaO_2

Figure 4.5 demonstrate a graph of resting peripheral saturations and mean rSO_2 in London (75m), Namche (3500m) and Everest base Camp (5300m).

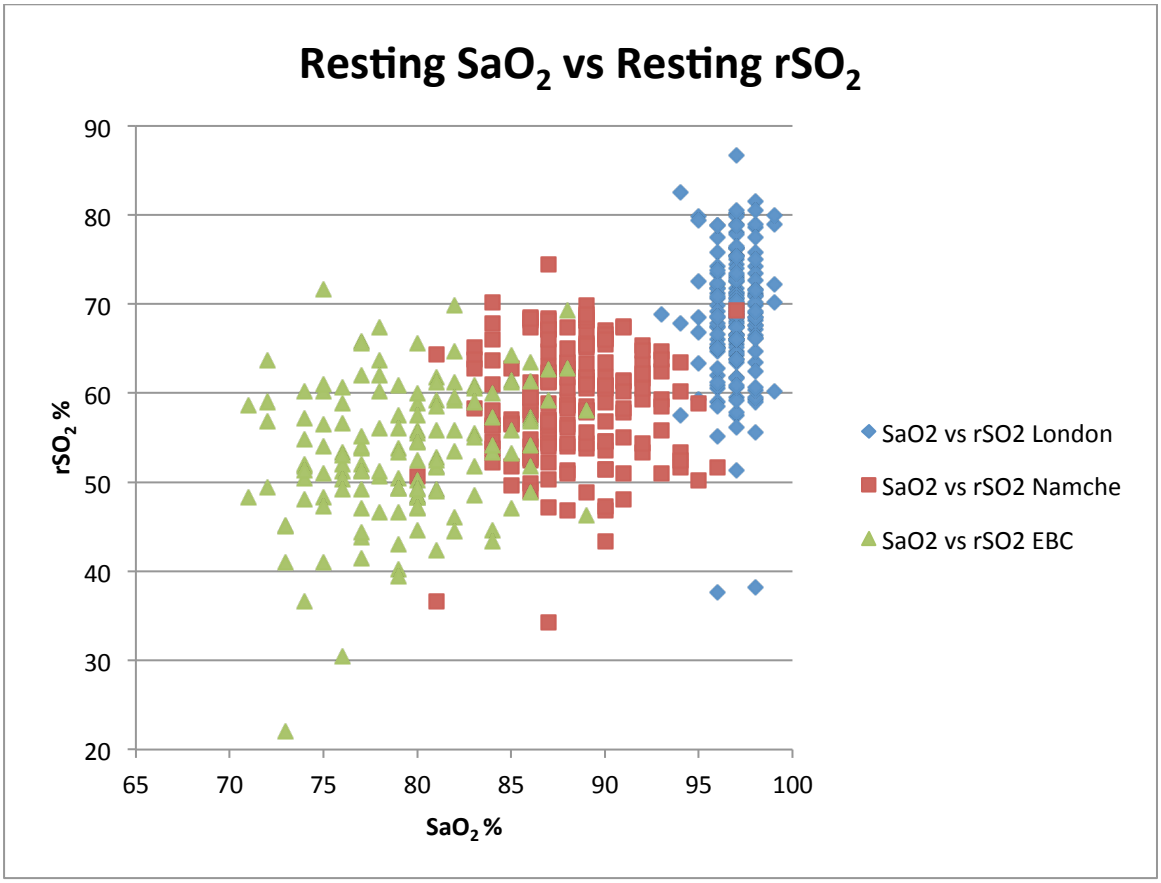


Figure 4-5 A plot of all resting SaO_2 values against corresponding rSO_2 values. The different symbols specify location.

Using a Pearson 2 tailed correlation (Table 4-A in Appendix), there is only a correlation between resting SaO_2 and rSO_2 at Everest Base Camp. The correlation at Namche does not achieve significance.

Relative Percentage changes in SaO_2 and rSO_2 at rest compared to 75m

Expressing the resting SaO_2 and rSO_2 values at 3500m and 5300m as a percentage of their values at 75m (baseline) demonstrates how these variables alter in relation to one another.

Table 4-6 demonstrates absolute resting SaO_2 and rSO_2 values and their relative percentage of baseline values.

	Absolute value at 75m	Absolute value at 3500m	3500m value expressed as a percentage of 75m value	Absolute value at 5300m	5300m value expressed as a percentage of 75m value
SaO_2	96.9	88.4	91.2	79.4	81.9
SEM	0.1	0.2	0.26	0.3	0.36
rSO_2	68.9	59.1	86.0	53.7	77.5
SEM	0.5	0.5	0.5	0.6	0.7

Table 4-6 The fall in resting SaO_2 and resting rSO_2 at altitude expressed as a percentage of their original value.

When expressed as a percentage of the original value, rSO_2 decreased by 4.4%% more than SaO_2 (paired t-test $p<0.001$).

Percentage changes in SaO_2 and rSO_2 during exercise at each altitude

	75m					3500m					5300m				
				As a %age of resting value					As a %age of resting value					As a %age of resting value	
	Rest	AT	VO_2max	AT	VO_2Max	Rest	AT	VO_2Max	AT	VO_2Max	Rest	AT	VO_2Max	AT	VO_2Max
SaO_2	96.9	96.5	96.3	99.6	99.4	88.4	81.7	80.0	92.6	90.6	79.4	72.6	72.1	90.9	90.2
SEM	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.3	0.3	0.3	0.3	0.5	0.5	0.6	0.6
rSO_2	68.9	71.3	67.4	103.6	97.7	59.1	55.1	50.1	93.2	84.8	53.7	48.8	45.3	91.0	84.2
SEM	0.5	0.6	0.7	0.3	0.7	0.5	0.5	0.6	0.4	0.8	0.6	0.6	0.6	0.4	0.8

Table 4-7 Changes in SaO_2 and rSO_2 with exercise at each altitude expressed as a percentage of their value when at rest at that altitude

From this it can be seen that at altitude (both 3500m and 5300m), exercise to AT and VO_2Max is associated with a fall in SaO_2 to approximately 90% of their starting values. Cerebral oxygenation (expressed as a percentage of the rSO_2 at rest at the corresponding altitude) falls to 93.2 ±0.4% and 91.0 ±0.4% of its starting values at AT (at 3500m and 5300m respectively $p<0.001$), but falls considerably further (to 84.8 ±0.8% and 84.2 ±0.8% at 3500m and 5300m respectively $p<0.001$) when exercising harder to VO_2Max. This continuous desaturation to VO_2Max does not occur in the peripheral circulation (expressed as a percentage of the resting SaO_2 value at that altitude) at 3500m at AT = 92.6 ±0.3 and at VO_2Max = 90.6 ±0.3% ($p = 0.06$) and at 5300m is AT = 90.9 ±0.6% and at VO_2Max = 90.2 ±0.6% ($p = 0.2$).

4.4.1.6 Correlations between rSO_2 and Demographics and time to VO_2Max

Age

There is a significant negative correlation between subject age and rSO_2 at AT (Pearson correlation = -0.19 p=<0.05) and VO_2Max (Pearson correlation -0.26 p=<0.05) when normoxic (75m) (table 4.8). Similarly, at 3500m, this negative correlation between subject age and rSO_2 continues (figure 4.6). Only at VO_2Max at 5300m did this not quite achieve significance (see table 4.8). Although age seems associated with rSO_2, the spread of data is large.

	75m			3500m			5300m		
	Rest	AT	VO_2Max	Rest	AT	VO_2Max	Rest	AT	VO_2Max
Pearson Corr	-0.13	-0.19*	-0.26*	-1.5*	-0.21*	-0.16*	-0.19*	-0.22*	-1.6
Sig (2-tailed)	0.10	0.02	0.01	0.04	0.01	0.04	0.02	0.01	0.05

Table 4-8 Pearson's correlation results and their significance for correlations between rSO_2 and age during exercise. * = Significant (p<0.05)

Figure 4-6 demonstrates graphically the correlation between age and rSO_2 during exercise where this correlation is strongest, at 3500m (Namche).

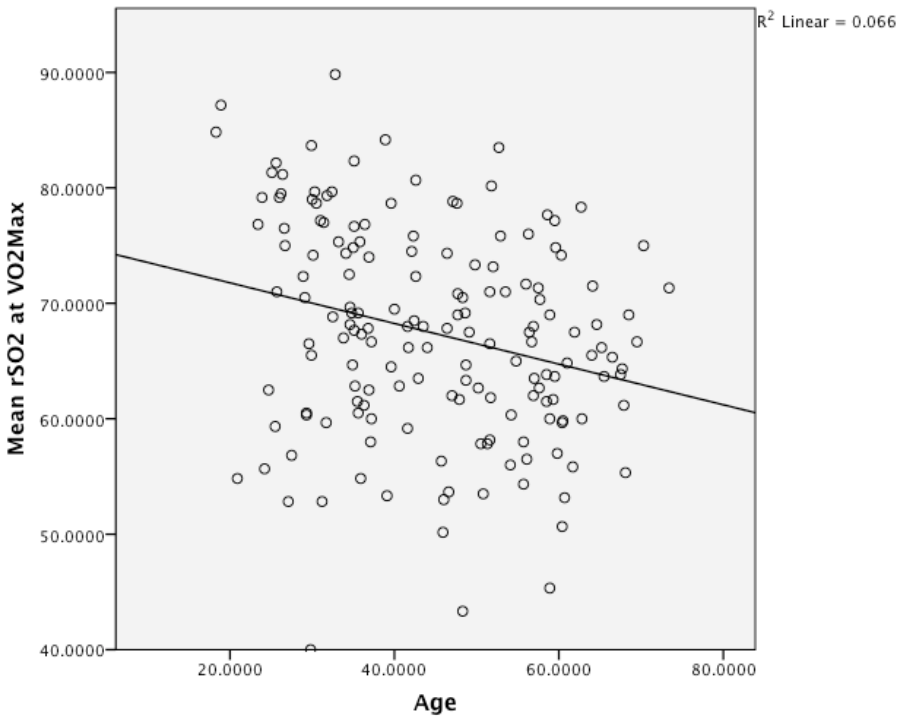


Figure 4-6 Relationship between rSO_2 and age at VO_2Max at 3500m. (p=0.04)

Smoking

No correlation was found at any altitude or at any stage of the exercise protocol between smoking and rSO_2. This may reflect the small numbers; only 12 people were recorded as regular smokers.

Height

There was a consistent significant positive correlation between height and rSO_2.

	75m			3500m			5300m		
	Rest	AT	VO2Max	Rest	AT	VO2Max	Rest	AT	VO2Max
Pearson Corr	0.23*	0.32*	0.37*	0.36*	0.38*	0.28*	0.33*	0.34*	0.25*
Sig (2-tailed)	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002

Table 4-9 Pearson's correlation between exertional rSO_2 and subject height.

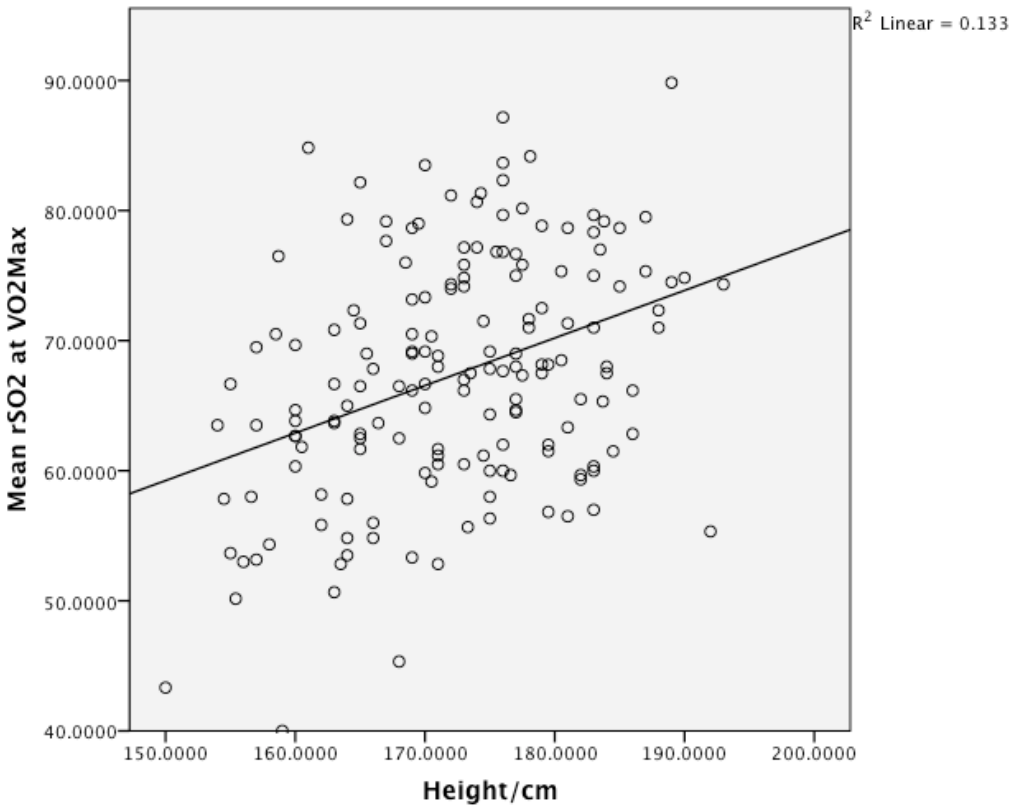
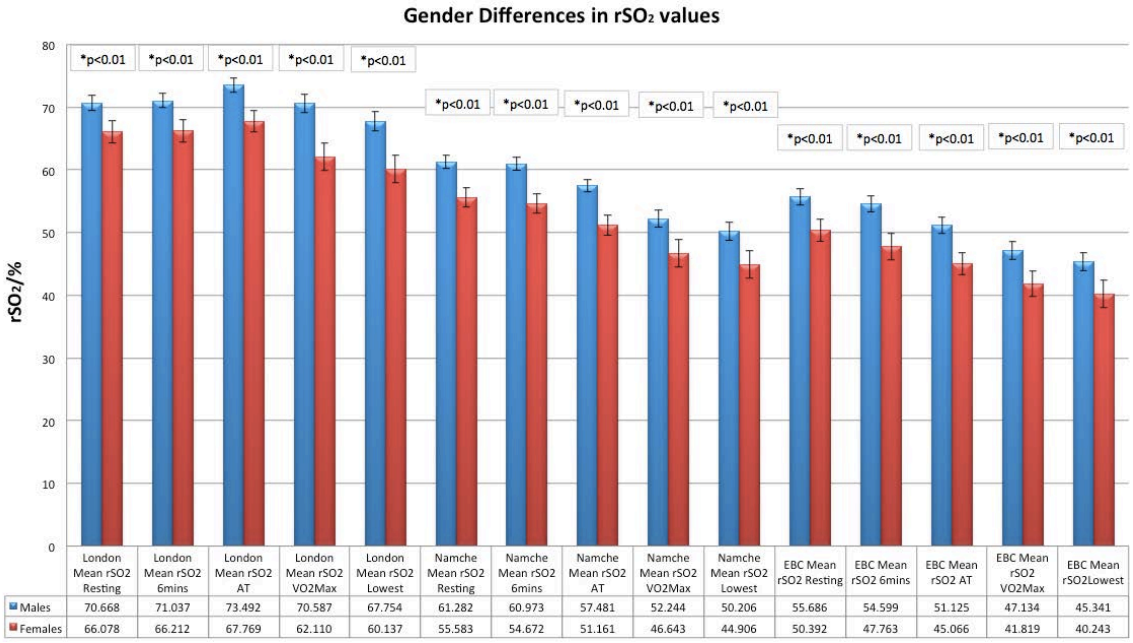


Figure 4-7 Correlation between subject height and rSO_2 at VO_2Max at 3500m ($p<0.001$).

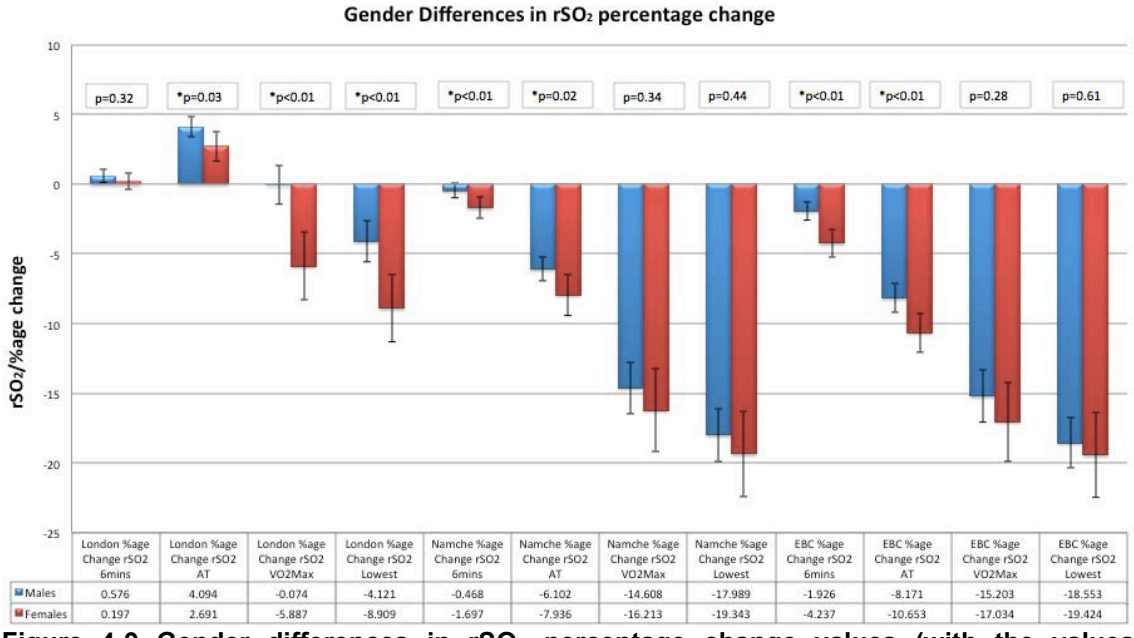
Gender

Figures 4-8 and 4-9 demonstrate the rSO_2 values and changes in rSO_2 by gender. Actual rSO_2 values were consistently lower in females than in males (ANOVA p=0.000 at all points in Figure 4-8).



	London Mean rSO2 Resting	London Mean rSO2 6mins	London Mean rSO2 AT	London Mean rSO2 VO2Max	London Mean rSO2 Lowest	Namche Mean rSO2 Resting	Namche Mean rSO2 6mins	Namche Mean rSO2 AT	Namche Mean rSO2 VO2Max	Namche Mean rSO2 Lowest	EBC Mean rSO2 Resting	EBC Mean rSO2 6mins	EBC Mean rSO2 AT	EBC Mean rSO2 VO2Max	EBC Mean rSO2Lowest
Males	70.668	71.037	73.492	70.587	67.754	61.282	60.973	57.481	52.244	50.206	55.686	54.599	51.125	47.134	45.341
Females	66.078	66.212	67.769	62.110	60.137	55.583	54.672	51.161	46.643	44.906	50.392	47.763	45.066	41.819	40.243

Figure 4-8 Gender differences in rSO_2 values at each altitude (London 75m, Namche 150m, EBC 5300m) at each level of exercise (rest, 6 minutes unloaded cycling, AT = Anaerobic Threshold, VO2Max and the Lowest rSO2 reading post-VO2Max) with 95%CT and p values demonstrated.



	London %age Change rSO2 6mins	London %age Change rSO2 AT	London %age Change rSO2 VO2Max	London %age Change rSO2 Lowest	Namche %age Change rSO2 6mins	Namche %age Change rSO2 AT	Namche %age Change rSO2 VO2Max	Namche %age Change rSO2 Lowest	EBC %age Change rSO2 6mins	EBC %age Change rSO2 AT	EBC %age Change rSO2 VO2Max	EBC %age Change rSO2 Lowest
Males	0.576	4.094	-0.074	-4.121	-0.468	-6.102	-14.608	-17.989	-1.926	-8.171	-15.203	-18.553
Females	0.197	2.691	-5.887	-8.909	-1.697	-7.936	-16.213	-19.343	-4.237	-10.653	-17.034	-19.424

Figure 4-9 Gender differences in rSO_2 percentage change values (with the values expressed as a percentage of the resting rSO_2 value at that altitude). (London 75m, Namche 150m, EBC 5300m) at each level of exercise (6 minutes unloaded cycling, AT = Anaerobic Threshold, VO2Max and the Lowest rSO2 reading post-VO2Max) with 95%CT and p values demonstrated.

Other Physiological variables:

$EtCO_2$

See chapter 5 for correlations between rSO_2 and $ETCO_2$.

Time to VO_2Max

It could be hypothesised that a correlation would exist between the time to reach VO_2Max and the percentage fall in rSO_2 at VO_2Max. Table 4-B in the appendix demonstrates that such a correlation does not exist.

4.4.1.7 Correlation between rSO2 and Basic Headache Data

Table 4-C in the appendix uses a 2-tailed Pearson correlation of all rSO_2 variables (including absolute figures and percentage change) to explore their relationship with headache presence and headache score (as recorded at the time of exercise). Correlation reached significance between rSO_2 values at 3500m and headache at Everest Base Camp. This may be because the ascent to 3500m was actually the greatest relative increase in altitude and hence the greatest reduction in inspired oxygen partial pressure. However, the lack of correlation with other variables does not provide good evidence for rSO_2 changes being responsible for headache. Correlations analysis was also performed using rSO_2 absolute and percentage values with arterial oxygen extraction and headache scores (recorded from diary data). No significant correlation was demonstrated. In chapter 5, I analyse headaches in greater detail using additional scoring systems that demonstrate minor correlations.

4.4.1.8 Cluster Analysis

One aim of the brain oxygenation study was to attempt to demonstrate if changes in brain oxygenation at sea level during exercise could predict brain oxygen desaturation at altitude. The process of cluster analysis is a tool to group people into similar clusters (in terms of cerebral desaturation at sea level) and differences in how these clusters perform at altitude can then be studied. Four methods of hierarchical cluster analysis (Between Groups, Furthest Neighbour, Within Groups, and Ward's methods) were employed to ascertain

the optimal number of clusters in each analysis. The number of clusters to seek was indicated on the plot of the agglomeration coefficients against the number of clusters formed, where there was a sharp jump in the size of adjacent coefficients (i.e. the measure of similarity or dissimilarity). For the cluster procedure, squared Euclidean distance was employed and item scores were standardised. The FocalPoint function within the ClustanGraphics8 programme was utilised to ascertain that the cluster solution was not destabilised by case-order. The 'top solution' of the FocalPoint analysis (based on a k-means iterative analysis) was employed to allocate subjects to the clusters. The 'top solution' is defined as unique classifications corresponding to the lowest Euclidian sum of squares (ESS) criterion value for a given number of clusters). Stability of the solution is indicated by its reproducibility, calculated as the percentage of 1000 randomly ordered samples producing the same solution and the percentage of overlapping cases with alternative cluster membership classifications.

The initial attempt to group subjects at sea level was based on raw resting rSO_2 values. This resulted in 3 clusters, broadly described as those with high rSO_2 values, those with medium and those with low rSO_2 values. This however is not a useful clustering as Near Infrared Spectroscopy is designed to be a trend monitor and absolute rSO_2 values have many confounding variables when trying to compare individuals. For example, differences in skin and skull thickness and blood supply will cause different results between people. Table 4-D in the appendix demonstrates this.

It is therefore more useful to cluster according to the pattern of rSO_2 changes. For example, at sea level, some people appear to increase the rSO_2 value as they exercise while others lower their rSO_2 value. This was therefore used to cluster subjects.

A cluster analysis was performed on the entire trekker data set (unlike the earlier NIRS work, not excluding those who did not complete at Base Camp). This was done by running the London (75m) percentage desaturations through Clustan software (Clustan Graphics 8, Edinburgh, Scotland). Plots of the agglomeration coefficients from the four methods of clustering (Between

Groups, Furthest Neighbour, Within Groups, Ward's) suggested that the best solution was reflected in a two-cluster solution. The dendogram in figure 4-10 demonstrates the spilt between clusters 1 and 2.

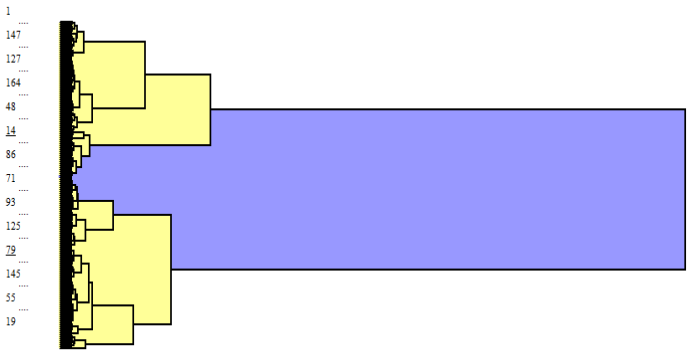


Figure 4-10 Dendogram demonstrating 2 cluster solution which was the most stable solution found.

The Agglomeration Schedule for this cluster analysis is in Appendix Figure 4A Table 4-E in the Appendix lists of subjects in each cluster at each altitude. Cluster 1 comprised 78 people cluster 2 had 87 (6 were not assigned).

Figure 4-11 demonstrates the percentage change in rSO2 values during the exercise protocol for both clusters. The original cluster designation was based entirely on London data. In the London data it can be clearly seen that cluster 1 desaturate at VO_2Max while cluster 2 either maintain or increase their rSO_2.

At Namche and Everest Base Camp, both clusters desaturate with exercise, but cluster 1 desaturates further. Hence, desaturating at sea level tends to predict greater desaturation at altitude.

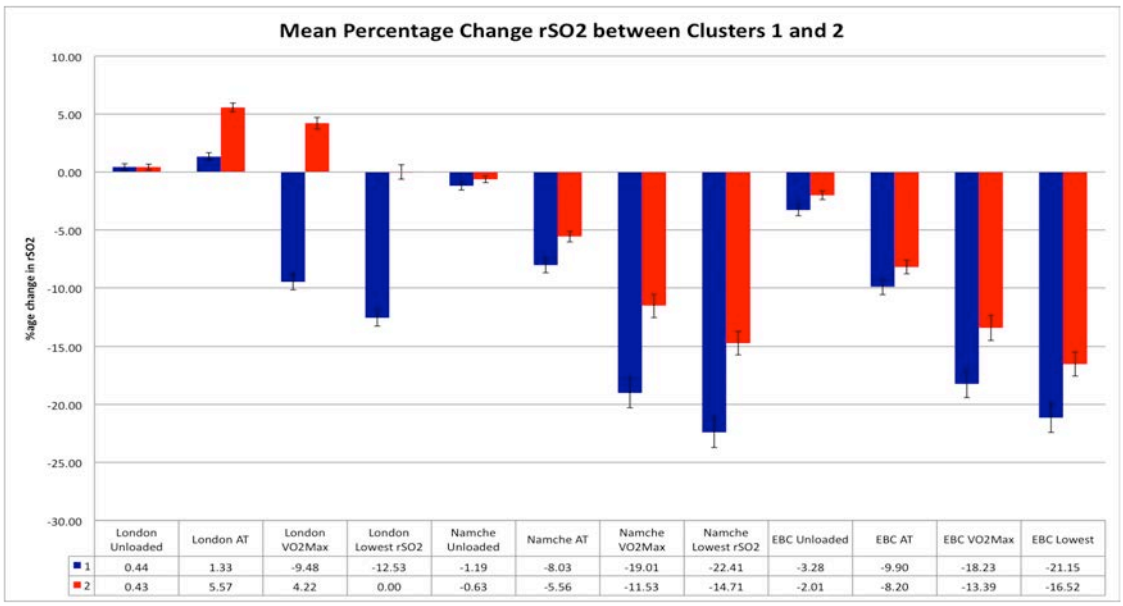


Figure 4-11 The mean percentage desaturation in rSO_2 for Clusters 1 and 2.

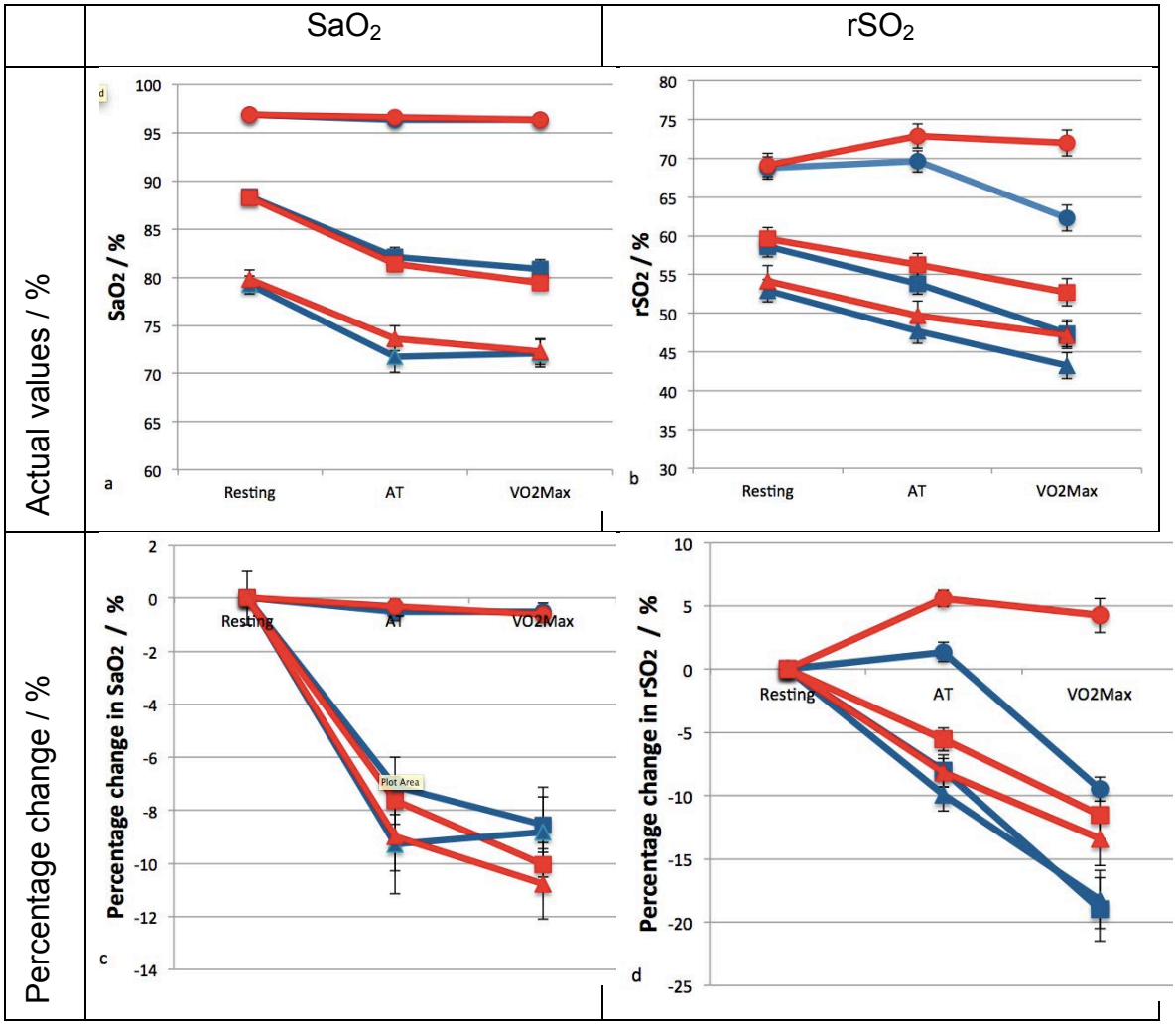


Figure 4-12 Graphs of changes in SaO_2 and rSO_2 at different exercise intensities at 3 different altitudes (□London = 150m, ■Namche = 3500m and ▲Everest Base Camp (EBC) = 5300m) for each cluster. a) Actual changes in SaO2 , b) actual changes in rSO_2, c) percentage changes in SaO2 from rest (with rest being 100%), d) percentage changes in rSO_2 from rest (with rest being 100%). All graphs have 95%CI bars.

Figure 4-12 demonstrates the actual and percentage changes in SaO_2 and rSO_2 with exercise at each altitude for each cluster. The mean SaO_2 value decreased at rest with gain in altitude. This decrease is virtually identical for Cluster 1 and Cluster 2 (hence peripheral arterial oxygen saturation at rest would not discriminate the clusters). With exercise at each altitude, the peripheral saturations are not significantly different between the two clusters. The rSO_2 values provide greater discrimination. Figure 4-11b shows that cluster 1 had cerebral desaturation at VO_2Max at sea level while cluster 2 increased their cerebral oxygenation slightly. This was probably the basis of the automated clustering. At 3,500m and 5,300m, both cluster 1 and 2 have very similar resting rSO_2 values, implying that at rest, the two clusters cannot be

discriminated. With exercise, cluster 1 appears to consistently desaturate more than cluster 2 at both 3500m and 5300m. Figures 4-11c and 4-11d demonstrate the same data but as a percentage change from the resting values. Again, there is no difference of SaO_2 between the clusters. There are two things to note from Figure 4-11c and d. Firstly, the percentage desaturation is considerably greater for rSO_2 than it is for SaO_2 (figure 4-11c). This suggests either greater oxygen extraction or an increased cerebral venous volume. Secondly, this phenomenon is consistently greater for Cluster 1 implying that Cluster 1 either have a higher arterial oxygen extraction, or they develop a greater venous compartment than cluster 2 (see discussion).

The implication of this cluster analysis is that there is something anatomically or physiologically different between the two groups that, with the same peripheral saturations, cause one group to have a greater fall in cerebral oxygenation. It is unlikely that one group has a greater cerebral metabolic use of oxygen and hence an increase in venous volume in one cluster relative to the other (causing a cerebral desaturation) should be considered.

Headache Correlation for the Clusters:

I hypothesised that the cluster with the greatest desaturation would have the greatest headache score.
Figure 4-13 demonstrates the mean Headache Severity Index (HSI) (a) and the components that make up the HSI (Headache score (b) and headache duration (c)).

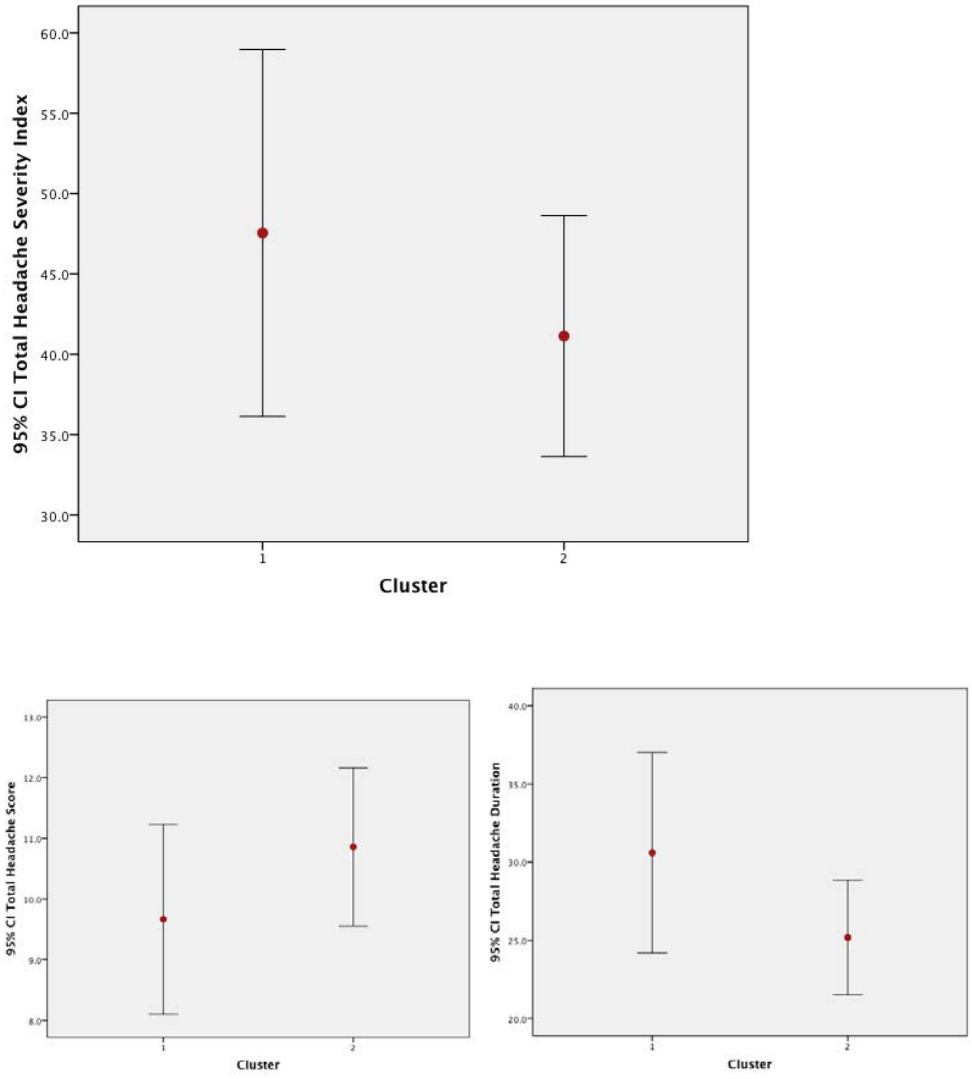


Figure 4-13 The mean Headache Severity Index (a) and the components that make up HIS (Headache score (b) and headache duration (c)) for each cluster.

On average, cluster 1 subjects had a greater headache severity index (M=47.5, SE = 5.3) than cluster 2 (M=41.1, SE = 3.77). This difference was not however significant $t(131) = 0.94$, $p>0.05$. Note, there were significant differences in variance between the two groups (Levene's test for Equality of Variances p= 0.02). The failure to achieve significance may relate to the large spread of headache scores between the two groups. Although not significant, it is noted that it is headache duration that is greater (and has wider error bars) in cluster 1. This cluster analysis had 165 subjects. A post hoc power calculation confirmed that this was more than the 134 subjects required to detect a medium effect size (0.3) with $\alpha=0.05$ and power $(1-\beta) = 0.95$. However, if the effect size is small (0.1) this would need to be 1289 subjects.

Headaches when grouping Trekkers into those who have greater and less than average peripheral and cerebral desaturations at rest and during exercise

Using independent t-tests and grouping the trekker groups into those who have above and below mean saturation changes may demonstrate a difference in headache severity between the two groups:

Changes in mean rSO_2 at rest between 75m and 5300m:
Mean percentage change in rSO_2 (at rest) between 75 and 5300m = -22.50%. The mean headache severity index of those with >-22.50% change = 47.25 (SD = 46.9 SEM =5.6). The mean headache severity index of those with <-22.50% change = 41.22 (SD = 36.6 SEM = 4.4). t (-.85 df 137) p=0.4.

Changes in mean rSO_2 at rest to VO_2max at 5300m:
Mean percentage change in rSO_2 from rest to VO_2Max at 5300m was -15.9%. The mean headache severity index of those that had a >-15.9% change was 47.84 (SD = 47.8 SEM=5.5). The mean headache severity index of those with <-15.9% change = 33.2 (SD = 33.2 SEM = 4.2) p = 0.16.

Hence, in summary, both the cluster analysis and techniques to split the trekker group into those that desaturate with altitude and with exercise at altitude reveals that there is not a significant difference between any of the groups. In all cases the groups with greater desaturation had more headaches but this never achieved significance. The large spread of headache data (see large standard deviations) may be a factor in this.

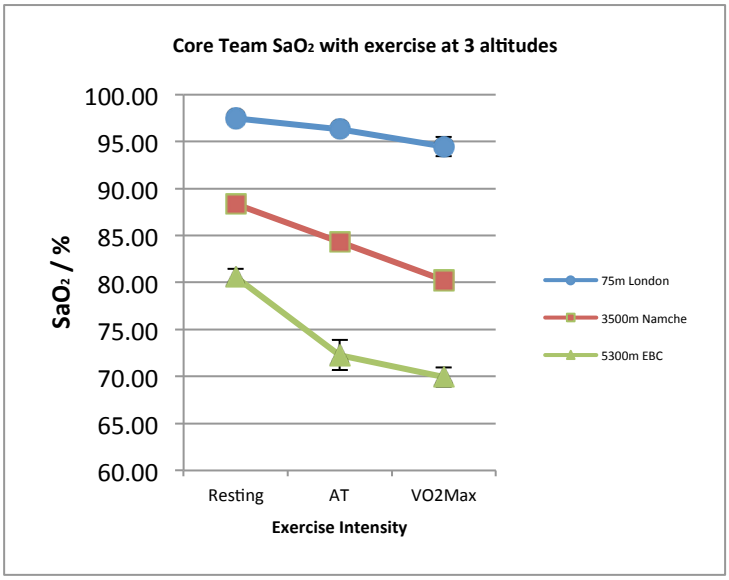
Note: In the following chapter, as part of the multiple variables at each altitude studied in relation to headache burden, one rSO_2 value group consistently correlated significantly. This was right-sided rSO_2 values (at rest and during exercise) at Namche (3500m) with total headache score. This may be because in our study this was the most acute hypoxic “jump” subjects were exposed to. See next chapter for more details.

CORE GROUP:

4.4.2 Core Group Ascent to Base Camp

All 24 Subjects ascended to 5300m. Two subjects did not undergo exercise testing at 3500m. Because $rS0_2$ was not recordable on one subject at any altitude, this study reports n=23 with the two missingdata sets at 3450m being imputed.

Figure 4-13 demonstrates the mean changes in rSO_2 at rest, after 3 minutes of unloaded cycling, at AT and at VO_2Max.



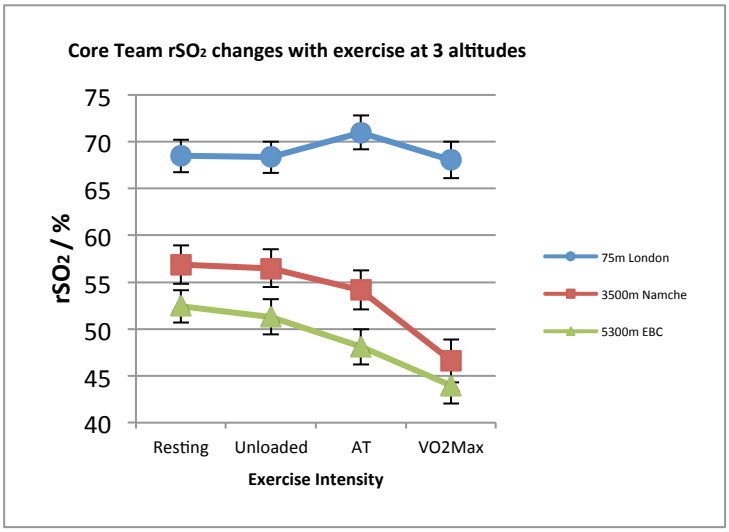


Figure 13-a Mean peripheral arterial oxygen saturation (SaO_2) at rest, AT and VO_2Max at each altitude (75, 3500 and 5300m). Figure 13-b demonstrates the mean rSO_2 values during rest, unloaded cycling, at AT and VO_2Max at the same three altitudes. Note: Although monitored throughout, SaO2 was not specifically recorded during unloaded cycling.

Individual Core Member Changes:

In the appendix, Figures 4-B (i, ii and iii) demonstrate rSO_2 for each of the 23 subjects at 75m, 3500m and 5300m respectively. From this it can be seen that individuals tend to retain their ranking.

No correlation could be demonstrated for resting rSO_2 at 5300m, rSO_2 at VO_2Max or percentage change in rSO_2 at VO_2Max and headache score.

The number of subjects in the core group was too small for cluster analysis.

Resting rSO_2 in Climbing Group to 7950m (n=7)

Table 11 demonstrates changes in SaO_2, $ETCO_2$ and left and right rSO_2 for a subgroup of 7 subjects studied up to 7950m (the South Col).

	75m	1300m	3500m	4250m	5300m	6400m	7950m	7950m + 2l Oxygen	Numerator df	Denominator df	F	Sig
Peripheral Sats (%)	97.63 [a]	95.67 [b]	89.75 [c]	85.88 [d]	79.63 [e]	75.13 [e]	65.90 [f]	95.03 [a,b]	7	13.91	215.18	<0.001
CI	(97.24-98.02)	(95.01-96.32)	(88.39-91.11)	(84.01-87.74)	(77.68-81.60)	(72.35-77.91)	(63.74-68.07)	(92.78-97.27)				
End Tidal CO_2 (mmHg)	35.53 [a]	32.27 [b]	27.36 [c]	25.65 [d]	20.62 [e]	16.75 [f]	13.00 [g]	-	6	37.89	205.64	<0.001
CI	(34.23-36.82)	(30.97-33.57)	(26.12-28.60)	(24.42-26.88)	(19.54-21.70)	(15.72-17.78)	(11.99-14.01)					
Left rSO_2 (%)	68.77 [a]	66.00 [a]	62.62 [b]	58.94 [c]	54.16 [c,d]	49.27 [d]	41.95 [e]	62.57 [a,b,c,e]	7	10.01	80.27	<0.001
CI	(65.91-71.63)	(64.30-69.70)	(59.87-65.37)	(55.89-61.99)	(51.07-57.25)	(44.84-53.70	(39.76-44.15)	(57.02-68.12)				
Right rSO2 (%)	69.47 [a]	67.75 [a]	61.36 [b]	58.57 [b,c]	53.98 [c,d]	50.95 [d]	39.55 [e]	61.75 [b]	7	12.43	82.87	<0.001
CI	(66.74-72.20)	(64.57-70.93)	(58.88-63.84)	(55.90-61.24)	(51.11-56.85)	(47.23-54.67)	(36.14-42.96)	(61.42-62.08)				

Table 4-11 Peripheral arterial oxygen saturations, end-tidal CO_2 and left and right rSO_2 for a subgroup of 7 subjects studied up to 7950m (the South Col).

4.5 Discussion

This study has demonstrated in a large number of people who ascended to 5300m, that there is a fall in brain oxygenation and this is exacerbated during exercise. This cerebral desaturation is greater than peripheral desaturation and varies for individuals. Those who desaturate more at sea level, also desaturate more at altitude.

Key Findings from this study:

1) At rest rSO_2 falls to a greater extent (of its percentage baseline) than SaO_2.
2) During exercise rSO_2 again decreases to a greater extent than SaO_2.
3) Those who desaturate during exercise at sea level appear to desaturate more at altitude.
4) The left hemisphere desaturates less in acute hypoxia.
5) On average males, those who are younger and taller people have higher rSO_2 values.
6) Although there is a trend for those with greater cerebral desaturation to have more headaches, this does not achieve significance.

One principal hypothesis studied was that those with greater cerebral desaturation would have a greater headache burden. Although in each group (when clustered based on desaturation during exercise at sea-level, and when subdivided based on desaturation at rest and during exercise at altitude) those who desaturated had a greater mean headache severity index, at no point did this achieve significance.

Box 1: lists the key findings of this research.

4.5.1 Explanation of Brain Oxygenation Results

Please see the methods chapter for a general background of NIRS. Note, since rSO_2 starts as a smaller percentage compared to SaO_2, if could be argued that the relative percentage fall in rSO_2 will be greater simply because I am comparing relatives values. However, this would not explain the significantly greater relative fall with exercise between 3500m and 5300m.

Inter-individual differences:
Unlike peripheral saturation (which at sea level are similar in most people), cerebral oxygenation (as measured using NIRS) appears to have a much wider

range of values (London resting rSO_2 ranged from 37.6 to 86.6%). Because of this, NIRS (especially the INVOS system) is generally considered a trend monitor. I used it as a trend monitor within individuals at different altitudes and during exercise. I also studied the changes in these trends between individuals. Many factors contribute to the rSO_2 value which could account for the variation. In anatomical and physiological terms, these differences can be extracerebral (skin/scalp/skull) and arterio-venous partitioning. I shall explain these prior to further analysis of the study findings.

Extracerebral Tissue / Spatial resolution: The mean depth of photon penetration is approximately 1/3rd of the transmitter/receiver separation (hence a 5cm separation gives a 1.7cm depth penetration). The INVOS system uses one near infra-red source and two optodes 3 and 4 cm from the source hence the closer detects superficial tissues while the 4cm separated optode monitors superficial and deeper tissues. A subtraction algorithm gives a measure of deeper "cortical" saturation. It has been estimated, using computer modelling, that a typical volume interrogated by NIRS is approximately 30% brain and 70% non-cerebral (skin and scalp) (Hiraoka, Firbank et al. 1993). With the INVOS system it has been estimated that 85% of cerebral rSO_2 is derived from cortical tissue with the remaining 15% derived from overlying extracerebral tissue (Murkin and Arango 2009). A study using a pneumatic tourniquet to produce scalp ischaemia resulted in a reduction in rSO_2. This demonstrates that the INVOS system does not eliminate the contribution of skin and scalp completely (Germon, Kane et al. 1994). This may be one reason for inter-individual differences and may also account for the gender differences noted.

Cerebral arterial / venous (A/V) blood partitioning: Cerebral NIRS measures mean tissue oxygenation and hence incorporates arterial venous and capillary blood. The cerebral cortex average tissue haemoglobin is classically thought to be distributed approximately 30% arterial, 70% venous (some authors use 25% arterial: 75% venous) (Watzman, Kurth et al. 2000; Ohmae, Ouchi et al. 2006). There appears to be however, a considerable biological variation in A/V rations between individuals (Watzman, Kurth et al. 2000). Watzman *et al* demonstrated (using NIRS and jugular bulb saturation monitoring) that, in 20 children, the mean A/V ratio was 16:84, but this varied from 40:60 to 0:100.

The INVOS system does not allow access to their algorithms or other recorded values other than rSO_2. It is therefore not possible to get any other ratios (e.g. of oxygenated haemoglobin and total haemoglobin) hence calculation of arterial: venous ratios are not possible. Wolff *et al* have previously published demonstrating that arterial oxygen extraction (E calculated as 1.39 (1 – rSO_2/SaO_2)) remains constant for brain at a low altitude but decreases at altitudes of around 5000m (Wolff, Richardson et al. 2007). They also stated that the ratio (p) of arterial to venous blood in the area of investigation can be calculated from $rSO_2 = (SaO_2.p+SaO_2(1-E)/p+1))$. However, we cannot assume that oxygen extraction is constant and without knowing the jugular venous saturation or the ratios as outlined above, it is not possible to calculate A:V ratios. We note in our study that rSO_2 values fall to a much greater extent than SaO_2 values at altitude. This either reflects a greater oxygen extraction, or more likely, an increase in the venous component of cerebral blood volume.

In our study there was little change in SaO_2 during exercise at sea level. This is in contrast to the initial rise then fall of rSO_2. This is demonstrated especially well in the Cluster analysis (figure 4.12). In this, it can be seen that Cluster 1 has a considerably more precipitous fall in rSO_2, even though SaO_2 is the same as cluster 2. Either the first cluster has a higher oxygen extraction or this group develops venous engorgement during the exercise.

Actual meaning of rSO_2: The demonstration that rSO_2 is a trend monitor was made very eloquently by Schwartz *et al (Schwarz, Litscher et al. 1996)*. They found that the mean value of rSO_2 in 18 dead subjects was 51+/- 27% compared with 68+-/5% in healthy adults. Six of the 18 dead subjects had a value greater than the lowest values of the healthy adults. In our study, some subjects (one from the core group and one from the trekker group) had consistently low rSO_2 values that did not alter with hypoxia or exercise (e.g. 15%). These subjects were excluded. This may represent a very large frontal sinus or persistent obstruction to the infra-red light. Such interpretation of NIRS studies probably reflects the limited data that can be gained from machines that just give a single rSO_2 value without any explanation of how it was derived.

More detailed oxygenation ratios would almost certainly enable more robust conclusions.

4.5.2 Gender

No study has previously been large enough to clearly demonstrate a significant difference in brain oxygenation between the sexes at altitude. Jausovec has reported a significantly higher oxygenated haemoglobin level in males compared to females (n=155) (Jausovec and Jausovec 2010) at baseline. Others have noted a generally higher brain oxygenation in males than females during verbal fluency tasks (Kameyama, Fukuda et al. 2004). Very large numbers of subjects are required to show these differences. This may be a genuine physiological difference (for example, females are far more at risk of idiopathic intracranial hypertension, and hence there may be an element of predisposition to venous insufficiency and hence venous congestion), or they may simply be artefact e.g. related to an extracranial / skin / scalp/frontal sinus phenomenon.

4.5.3 Age

During hypoxic exercise, greater cerebral desaturation appears to occur with advancing age. This has not been demonstrated before. It may be the underlying factor as to why the two clusters separated out since there was a significant difference of mean age between the two.

4.5.4 Comparisons with previous studies using NIRS at altitude

To place my results in context, I shall outline a number of studies that have used NIRS at altitude, both to investigate cerebral oxygenation at rest and during exercise.

4.5.4.1 Experiments investigating rSO_2 changes with altitude

Hadolt and Litscher (using an INVOS 3100 machine) gave an account in 2003 of the use of NIRS on 17 volunteers at altitude. 2 had AMS and they thought that this might correlate to an acute drop in rSO_2 (Hadolt and Litscher 2003). However, they had a faster ascent rate and studied people within 6 hours of

arrival at altitude (not at 2-3 days as in the current study). In a similar manner to our results, they also found that cerebral saturations decreased to a greater extent than peripheral.

In contrast, Imray *et al* (with a Critikon 2020 system) demonstrated that cerebral oxygenation fell less than peripheral saturations up to 4680m and that medroxyprogesterone resulted in higher peripheral and cerebral saturations (Imray, Barnett et al. 1998). This may reflect the more acute ascent of this group, or it may represent a difference in cerebral oximetry technique. Imray *et al* have also demonstrated that increasing inhaled CO_2 at altitude increases cerebral oxygenation (Imray, Brearey et al. 2000; Imray, Clarke et al. 2001; Imray, Walsh et al. 2003).

4.5.4.2 Experiments investigating the temporal change in rSO2 during exercise

Saito *et al* were the first to report on the effects of exercise at altitude on cerebral oximetry in 1999. Using an INVOS 3100 system, they found that rSO_2 fell during exercise at altitude while it was maintained at sea level (Saito, Nishihara et al. 1999). Again, the reduction of rSO_2 was greater than SpO_2.

Our group has also studied exercising skeletal muscle at altitude and found that the pattern of absolute oxygenation remains the same at altitude and at sea level (Martin, Levett et al. 2009). This is different to our findings that at sea level, rSO_2 tends to increase at AT and approach baseline again at VO_2Max, while at altitude there is a progressive desaturation to VO_2Max.

In a small study (n=6) Shibuya and colleagues investigated brain oxygenation at sea level at supramaximal intensities (150% of VO_2Max). This (like our study) demonstrated an increase in cerebral oxygenation initially but at maximal and supramaximal exercise, then cerebral oxygenation fell (Shibuya, Tanaka et al. 2004). This is the earliest recording of a rise in rSO_2 with exercise at sea-level. Other studies have also demonstrated that cerebral rSO_2 can increase especially during lower work rates in normoxia (Hiura, Mizuno et al. ; Ekkekakis

2009) and that changes in rSO_2 in hypoxia are larger than in normoxia(Subudhi, Dimmen et al. 2007).

Imray *et al* studied brain oxygenation during supine exercise up to 5,260m (n=9) (Imray, Myers et al. 2005). They also demonstrated that at sea level, brain oxygenation was maintained during exercise, but at altitude, above 30% of VO_2Max, rSO_2 fell.

Rooks *et al* have compiled a meta-analysis of NIRS studies during exercise at sea level (Rooks, Thom et al. 2010). Their meta-analysis is difficult to interpret as they included different NIRS techniques, however, with “hard” and “very hard” (= VO_2Max and above) exercise, they demonstrated an increase in total haemoglobin concurrent with a fall in brain oxygenation. This implicates an increase in the venous component of the intracerebral mixture.

There are a number of neuropsychological studies correlating brain oxygenation with function. For example, Ando *et al* demonstrated that cerebral oxygenation decreases with exercise in hypoxia and this correlates with an increased reaction time to peripheral visual stimuli (Ando, Yamada et al. 2010). At the time of writing, the neuropsychology data from our study has not been completely analysed, hence we cannot comment on this.

Some studies have suggested that the fall in rSO_2 is due to a fall in cerebral blood flow secondary to hypocapnia induced vasoconstriction (Bhambhani, Malik et al. 2007). Others have implied it may well be that an increase in the venous component of NIRS may contribute (Heine, Subudhi et al. 2009).

4.5.4.3 Experiments investigating rSO_2 changes both with exercise and altitude

With acute isocapnic hypoxia, the desaturation of brain oxygenation is greater than that of muscle NIRS (Peltonen, Kowalchuk et al. 2007). Subudhi and colleagues (Subudhi, Dimmen et al. 2007) studied 13 male cyclists in normoxia and hypoxia (FiO_2 = 12%) during incremental exercise up to a maximum of 25 Watts/min using an Oxymom (Artinis, The Netherlands) oximiter. As reported above, in normoxia there was an initial rise in brain oxygenation up to 75%

maximum, then a fall. In hypoxia, brain oxygenation fell progressively with exercise. They also studied muscle oxygenation and demonstrated that in both normoxia and hypoxia, there was a progressive desaturation with exercise. Rupp and Perrey demonstrated very similar results with sustained contraction exercise (Rupp and Perrey 2009). Perry has also written a comprehensive review of NIRS in exercise (Perrey 2008).

Subudhi has shown that during exercise, prefrontal areas desaturate more than premotor and motor regions, suggesting that this cortical desaturation may contribute to an integrative decision to stop exercising (Subudhi, Miramon et al. 2009). A number of other authors have suggested that it is cerebral desaturation rather than skeletal muscle impairment that limits exercise capacity in hypoxia (Smith and Billaut 2010). More recently, we have performed "Rush and Rest" studies at altitude and although not published yet, the results imply that the limits of exercise capacity in hypoxia (be that cerebral or skeletal muscle) vary between individuals.

4.5.5 Brain Oxygenation and Headache

I had believed that with the large numbers of subjects in this study, we would be able to demonstrate a difference in cerebral oxygenation between those who suffered headaches and those who did not. Although an independent t-test of headache severity between clusters and the group split into those above and below average desaturation at rest while ascending and desaturation with exercise at altitude demonstrated that those who desaturated more had a greater headache burden, this was not statistically significant with the number of subjects in this study. Other studies of headache and NIRS are rare. Previously Vuyk *et al* investigated the response of rSO_2 to acetazolamide treatment (Vuyk, Van Den Bos et al. 2006) in 16 subjects ascending Cho Oyu. At 3700m, the 8 taking acetazolomide had a significantly lower Lake Louise Score (LLS – 0.75+/-1) compared to those that were not (2.9+/-2 $p<0.05$). High LLS were associated with low rSO_2 both at rest and during exercise.

No other studies have attempted to correlate headache with rSO_2 at altitude. The study described above by Vuyk *et* al used the Lake Louise Score and

demonstrated a significantly higher score in those that desaturated more, however, the effect size is small and the other variables that comprise the LLS (e.g. difficulty sleeping, nausea) may be more relevant. The subjects (n=16) were also exposed to a much greater altitude (Cho Oyu summit 8201), hence the lower altitude and slower ascent profile of our study might account for some differences.

4.5.6 What Causes the Reduction in rSO_2 at Altitude and During Exercise?

I have shown that, at sea level, one group of people at least (cluster 1) have a fall in rSO_2 when exercising despite unchanged SaO_2. This might be explained by hyperventilation-induced vasoconstriction, a substantial increase in brain oxygen extraction, or the presence of cerebral venous congestion. The latter might be postulated to occur with thoracic pressure increases (lip pursing) during exercise. At altitude, rSO_2 decreases at a greater rate again than SaO_2. Again, this implies either hyperventilation induced vasoconstriction, greater oxygen extraction or venous congestion.

Heine *et al* used canonical correlation analysis (CCA) with an Oxymon MKIII (Artnis Medical Systems, The Netherlands) system to investigate this in 23 subjects(Heine, Subudhi et al. 2009). They found that cerebral blood flow velocity shared the least amount of variance with NIRS measurements and the reduction in CBFv was not accompanied by a reduction in cerebral blood volume. The venous contribution to NIRS appeared to explain a larger amount of variation in cerebral oxygenation than hypocapnia-induced reduction in CBFv.

A broader review providing evidence for venous congestion at altitude is provided in the appendix (Wilson, Imray et al. 2011).

4.5.7 Future Study

To demonstrate that a fall in rSO_2 is associated with a higher headache score, a much harsher ascent profile is required.

To conclusively demonstrate using NIRS that there is an increase in the venous component of cerebral blood volume, either a NIRS system that provides a ratio of oxygenated to total haemoglobin must be used, or concurrent jugular venous

bulb sampling is required. However, I have gone on to use magnetic resonance imaging and retinal analysis (chapters 5 and 6) to investigate the presence of venous engorgement at altitude.

4.6 Conclusion

Changes in cerebral oxygenation during exercise at sea level can give an indication of the changes that occur at altitude. Males, younger and taller subjects appear to preserve brain oxygenation and, in relatively acute exposure, the dominant left cerebral hemisphere maintains a very slightly (but significantly) higher rSO_2.

Regional brain oxygenation decreases at rest and during exercise at altitude at a faster rate than peripheral arterial oxygen saturation. This supports the contention that a component of the reduction in rSO_2 might relate to changes in cerebral venous engorgement. This issue is dealt with in chapters 5 and 6.

5 Chapter 5: HIGH ALTITUDE HEADACHE ASSESSMENT AND CORRELATION WITH BASIC ANTHROPOMORPHIC AND PHYSIOLOGICAL DATA

5.1 Abstract

Aim: To document headache burden during ascent to 5300m and attempt to correlate headache with anatomical (anthropomorphic) and physiological variables.

Methods: Twenty-four core team and 198 trekkers from the CXE expedition kept daily diaries of headache severity (providing 4 different scoring systems) and basic physiological measures. Prior to departure, skull measurements were performed to calculate intracranial volumes. In London (75m), Namche (3500m) and at Everest Base Camp (EBC, 5300m) additional physiological variables were recorded as part of an exercise to VO_2Max test. Relationships between these demographic (age, sex, smoking), anatomical (intracranial volume) and physiological (heart rate, peripheral saturations, altitude specific rSO_2, blood pressure, End Tidal O_2 and End Tidal CO_2, and haemoglobin concentration) variables and headache burden were sought.

Results: There was no correlation between headache score derived from any methodology, and calculated intracranial volume or patient sex. Total headache score was greater in the young (Pearson's = -2.47 (p=0.001 n=182)) and increased with lower resting peripheral arterial oxygen saturations at EBC (5300m) (Pearson's = -2.11 (p=0.004, n=182)). At Namche, right sided rSO_2 values (at rest and during exercise) were consistently and significantly correlated with total headache score. EtO_2 and $EtCO_2$ values consistently correlated with total headache score, from rest through to maximal exercise at all altitudes, from sea-level to 5300m. There was no correlation between headache burden and haemoglobin concentration.

Conclusion: There is no relationship between calculated intracranial volume and high altitude headache and no difference in headache burden between sexes. EtO_2 and $EtCO_2$ appear closely related to headache burden. With the greater hypoxic challenge of ascent to Namche (3500m) it appears that left sided cerebral oxygenation is protected and right-sided cerebral oxygenation values correlate with headache.

5.2 Introduction

For the past 20 years the two main pillars that have been accepted as the cause of high altitude headache have been the anatomical “tight fit” hypothesis put forward by Ross (Ross 1985) and the physiological “hypoxaemia” hypothesis described by Hackett and Roach (Hackett and Roach 2001).

The Caudwell Xtreme Everest Study (Grocott, Martin et al. 2010) was principally designed to investigate the effects of hypoxia. The slow ascent rate with multiple stops at the same altitude meant the study was not ideal for the analysis of high altitude illness or headache, which the ascent profile had been designed to avoid. However, with the large number of people ascending, it offered an opportunity to document the headaches that occurred and to attempt to correlate them with anatomical and physiological variables.

Headache Burden: The quantitative assessment of the subjective symptom of headache burden has not been well validated. Ascribing a headache a score within a range or on a visual-analogue scale (Rupp, Jubeau et al. 2012) is straightforward, but the validity of calculating a “burden” over a period of time by summing these semi-categorical results has not been demonstrated. The Lake Louise Score classifies headache as: Not present (0), mild (1), moderate (2) or severe (3). The headache pharmaceutical industry uses a “Headache Severity Index” which comprises a headache score (none (0), mild (1), moderate (2), severe (3), excruciating (4)) multiplied by the number of hours that headache is experienced to give a more accurate idea of burden (Schrader, Stovner et al. 2001). Within this study I used a number of these headache assessment systems.

Anatomical Cranial Measurements: A study of 10 subjects in 1985 (Wilson and Milledge 2008) implied that subjects with greater ventricular volume and relative cerebral parenchymal atrophy suffered less with headaches. Whilst it would not have been possible to perform magnetic resonance imaging (MRI) on all of the subjects on the Caudwell Xtreme Everest project, it was possible to measure cranial dimensions and estimate intracranial volume. Whilst clearly this does not

reflect intracranial compliance, it provides a large number of subjects to investigate any protective effect a large cranium may have in headache susceptibility.

Physiological Measurements: In addition to daily headache assessment, the large cohort of subjects recorded daily measurements of basic physiological variables (pulse, resting peripheral arterial oxygen saturation, blood pressure). Further measurements were made as part of the VO_2Max test (see chapter 2 and (Levett, Martin et al. 2010)). Brain oxygenation was also monitored at rest and during exercise, with data being previously reported in chapter 4. Correlations of these basic physiological variables and rSO_2 values at specific altitudes with headache were sought.

5.3 Methods

One hundred and ninety-eight trekkers (see chapter 2) were studied during ascent to 5300m over 11 days. Twenty-four investigators were studied during ascent to 5300m over 13 days. Because of the larger cohort size and more acute ascent, these results focus on the 198 trekkers although the investigator group is discussed at the end of the chapter.

5.3.1 Headache Assessment

On waking and prior to breakfast, each subject completed a personal diary each morning. The classic high altitude assessment tools, the Environmental Symptoms Questionnaire and Lake Louise assessment which included a "headache" score, grades severity from 0-3 (none, mild, moderate and severe). In addition to this component of the diary, a table (figure 5-1) was provided for more detailed headache assessment. The Environmental Symptoms and Lake Louise assessments do not quantify duration of headache (which may be an important consideration in evaluating "headache burden"). In this table the grade of headache over the preceding 24 hours ranging from 0-4 (none, mild, moderate, severe, excruciating) and the duration of that headache (in hours) was reported. This meant that a headache severity index (HSI = grade x duration) could be calculated on a daily basis. By adding the daily HSI scores, a cumulative headache burden was calculated. Boxes were also available to

indicate the time of day at which the headache was most severe, the location of headache and exacerbating factors. Also within the diary were sections for medication taken and alcohol consumption.

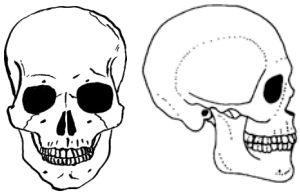
Grade of headache experienced in previous 24 hours (1-4)		Duration of headache (/hrs)	
Time of day of headache (please circle)	00:00 – 06:00 06:00 – 12:00 12:00 – 18:00 18:00 – 24:00	Locations of headache (please mark on skull)	
Exacerbating factors			

Figure 5-1 The diary table in which headache symptoms and duration were reported by each subject on a daily basis.

The main headache scores studied were:

Lake Louise Headache Score (LL) – Headache Score (from 0-3 none, mild, moderate and severe). **The Total LL** = the cumulative (sum) of daily LL headache scores.

Ascent Headache Score (AHS) – This was the sum of the LL scores upon days following ascent only (total = 7 days of ascent). This attempted to remove other causes of headache (especially exertion from exercise testing and alcohol).

Headache Severity Score (HSS) – The headache score (from 0-4 none, mild, moderate, severe, excruciating). The Total HSS = the cumulative (sum) of daily HSS.

Headache Duration (HD) – the duration of headache each day. **Total Headache Duration** = the sum of each headache duration a subject recorded irrespective of grade

Headache Severity Index (HSI) – headache severity score (0-4) x duration each day. **Total Headache Severity Index** = the sum of the daily HSIs.

5.3.2 Anthropomorphic Assessment

One hundred and ninety-eight subjects (125 male, mean age 44.6 (range 18-73) underwent cranial measurement using specially designed callipers (chapter

2). The following skull measurements were measured (see chapter 2 for images).

The following were measured:

- Maximum head length (glabella to inion: L)
- Maximum head breadth (between the two parietal eminences: W)
- Auricular height (external acoustic meatus to bregma: H)
- Head circumference (from glabella to ionion)

For height we measured the distance from the right external auditory meatus to the bregma, removing the calliper arm nearest the handle to allow close apposition to the skull, and keeping the distal calliper arm horizontal using the spirit level in the callipers arm.

All measurements were calculated in millimetres, in the normal anatomical position. All measurements were repeated three times by the same investigator, the average of the three measurements was then used to calculate ICV. To reduce inter-observer bias, all measurements were performed by one of two investigators.

Cranial volumes were calculated using the Lee-Pearson formula (Manjunath 2002; Golalipour, Jahanshaei et al. 2005) .

Male ICV: 0.000337 (L–11) × (B–11) x (H–11) + 406.01
Female ICV: 0.0004 (L–11) × (B–11) × (H–11) + 206.60

These formulae have a reported mean error of 3-4% (Haack and Meihoff 1971) and have been shown to be reliable for calculating intracranial volume (Sahin, Acer et al. 2007). In addition, a non-gender specific formula (the Dekaban formula) was also compared (Dekaban 1977). This is:

0.523 x (L=2t) x B (Bx2t) x (Ht –t)

where t is the thickness of the cranial vault and soft tissues (for simplicity considered to be 7.5mm for this study).

5.3.3 Physiological Assessment

Each subject recorded the following with their headache scores: resting heart rate, resting peripheral arterial oxygen saturations, and three resting blood pressure readings. Subjects also underwent an upright cycle ergometer test to VO_2Max (full details chapter 4). Brain oxygenation was recorded during this in London (75m), Namche Bazaar (3450m) and Everest Base camp (5300m). The brain oxygenation components are reported in chapter 4. The following physiological parameters were also reported: pulse, blood pressure, peripheral saturations (SaO_2), end tidal Oxygen (EtO_2) and Carbon Dioxide ($EtCO_2$); and oxygen consumption at rest, at anaerobic threshold (AT) and at maximal oxygen consumption (VO_2Max). Haemoglobin concentration was also measured at each altitude.

An association of headache scores with all of these anatomical and physiological measurements was sought.

5.4 Results

5.4.1 Trekker Results

Of the 198 trekkers, 190 reached Everest Base Camp. Two suffered with acute mountain sickness, the other 6 with non-altitude related illness.

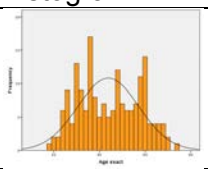
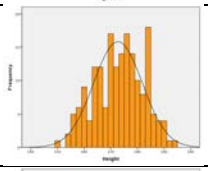
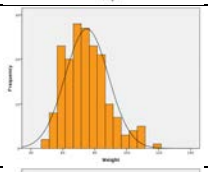
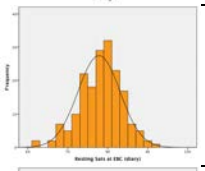
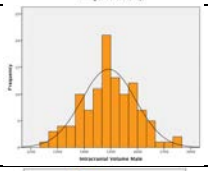
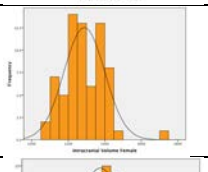
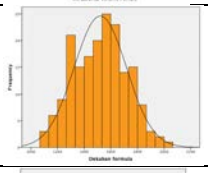
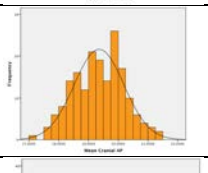
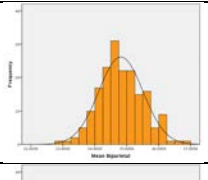
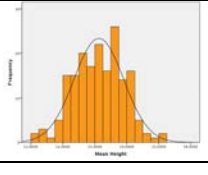
Of the 190 subjects, 182 had full anthropomorphic and basic diary data. None of the 8 subjects in whom data sets were incomplete were reported to be suffering from high altitude illness. One hundred and sixty-nine subjects had complete VO_2Max data in London, 167 in Namche and 144 at Everest base camp.

Descriptive Data:

Table 5-1 provides means, standard deviations, maxima, minima, interquartile range values and histograms for the basic demographic and anthropomorphic data demonstrating that continuous data mostly had a normal distribution. Headache measures (the sum of ordinal measures) were not normally distributed as large numbers of subjects report no or minimal headache.

5.4.1.1 Demographic and Anthropomorphic Variables:

Frequency for Parametric Data:

	n	Mean	SD	Min/Max	IQR	Histogram
Age/years	182	43.91	13.46	18 / 73	32.00 / 56.00	
Height/cm	182	172.68	9.18	150 / 193	165.75 / 180.00	
Weight/Kg	182	74.99	13.52	49 / 117	64 / 83.25	
Resting Sats EBC	182	77.79	5.30	62 / 91	74.00 / 81.00	
Male ICV / mls	112	1491.90	102.09	1235 / 1751	1429.25 / 1569.75	
Female ICV / mls	70	1287.87	111.58	1235 / 1751	1212.25 / 1373.25	
Dekaban Formula	182	1520.92	196.83	1088 / 2019	1363.75 / 1656.25	
Cranial AP Distance / cm	182	19.35	0.84	17.23 / 21.43	18.70 / 19.97	
BiparietalDistance / cm	182	14.81	0.70	12.83 / 16.77	14.33 / 15.27	
Cranial Height / cm	182	13.12	0.78	11.20 / 15.13	12.53 / 13.63	

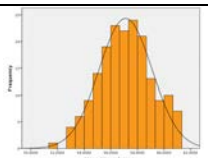
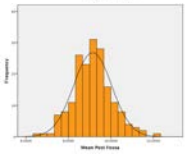
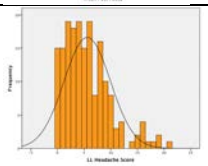
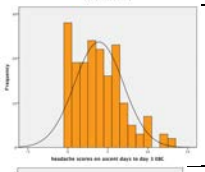
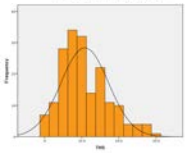
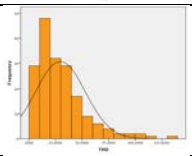
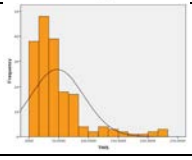
Cranial Circumference /cm	182	57.11	1.99	51.90 / 61.27	55.63 / 58.45	
Post Fossa Length	182	9.14	0.90	6.6 / 12.1	8.59 / 9.65	
LL HS	182	5.57	4.35	0 / 21	2.00 / 8.00	
Ascent HS	182	3.89	3.05	0 / 13	1.00 / 6.00	
THS	182	10.82	6.41	0 / 30	6.00 / 14.00	
THD	182	29.57	23.66	0.00 / 139.00	12.95 / 39.50	
THSI	182	47.58	45.30	0.00 / 230.0	18.87 / 60.63	

Table 5-1 Table of descriptive data for basic demographic / diary data, anthropomorphic measurements and headache scores. Resting Sats EBC = resting peripheral saturations (SaO_2) at Everest Base Camp (5300m). Male ICV = male intracranial volume calculated using the Lee Paterson formula. Female ICV = female intracranial volume calculated using the Lee-Paterson formula. Dekaban Formula = intracranial volume (both sexes) calculated using the Dekaban formula. Cranial APD = cranial anterior-posterior distance, Biparietal Distance = cranial width, Cranial Height = height from external auditory meatus to vertex. Post fossa length = distance form external auditory meatus to occipital prominence. LLHS = the sum of each days headache component of the Lake Louise Score. Ascent headache Score = the sum of the headache scores on days following an increase in altitude only. THS = Total Headache Score (the sum of all of the headache scores that went up to make the headache severity index). THD = Total Headache Duration (the sum of all the hours of headache for each individual). THIS = Total Headache Severity Index = Sum of headache severity scores x duration for each day.

5.4.1.2 Physiological Variables

Multiple physiological variables were studied at each altitude, all of which follow a normal distribution. These have been included in Tables 5-A, B and C in the Appendix. Means of some of these variables are displayed in Table 5-2.

	London (75m)	Namche (3450m)	EBC (5300m)
Hb/g/dl	14.48	14.72	15.77
Resting Sys BP	125.2	125.93	124.86
Resting Dia BP	81.9	83.61	85.82
Resting SaO_2	96.91	88.36	79.4
Resting HR	78.12	84.12	90.91
Resting EtO_2	108.30	62.38	48.88
Resting $EtCO_2$	33.78	27.02	20.46
Resting Mean rSO2	68.94	59.11	53.68
AT SaO_2	96.49	81.73	72.6
AT HR	129.25	127.21	118.68
AT EtO_2	101.15	62.46	50.08
AT $EtCO_2$	42.13	28.7	21.04
AT Mean rSO_2	71.34	55.07	48.85
VO_2Max SaO_2	96.34	80.01	72.1
VO_2 Max HR	170.41	159.63	138.42
VO_2 Max EtO_2	115.90	73.13	57.63
VO_2 Max $EtCO_2$	36.06	24.13	17.67
VO_2 Max Mean rSO_2	67.40	50.11	45.33

Table 5-2 Means of some physiological data at 75m, 3450m and 5300m. Further details with numbers of subjects, SD, interquartile ranges and frequency histograms are displayed in appendix tables A, B and C.

5.4.1.3 Assessment of Headache Scoring Systems

The mean results for the different headache scoring systems are shown in table 5-3

	LL headache Score	Ascent Headache Score	Total Headache Score	Total Headache Duration	Total Headache Severity Index
Mean	5.57	3.89	10.82	29.57	47.58
SD	4.35	3.05	6.41	23.66	45.30
SEM	0.32	0.23	0.48	1.75	3.36
Max	21	13	30	139	230
Min	0	0	0	0	0
Quartile	2	1	6.25	13	19.25
Quartile 3	8	6	14	39.5	60
IQR	6	5	7.75	26.5	40.75
Numbers	181	181	182	182	182

Table 5-3 The Headache Scores for 182 subjects who ascended to EBC (5300m) and had adequate diary records. Where LL Headache Score is the sum of the headaches recorded as part of the daily dairy Lake Louise survey. (Headaches scored 0 Nil, 1 Mild, 2 Moderate, 3 Severe). Ascent Headache Score is the sum of the individual LL Headache Scores on days after an ascent. Total Headache Score is the sum of all headaches recorded each day within the Headache Severity Index part of the diary. (Headaches scored 0 Nil, 1 Mild, 2 Moderate, 3 Severe, 4 Excruciating). Total Headache Duration is the sum of the number of hours each individual headache was endured (part of HSI calculation). Total Headache Severity Index (HSI) is the sum of all individual HSIs (Headache Score x Duration).

The Lake Louise Headache Score (and its derivative Ascent Headache Score) were reported separately to the Headache Severity Index (with its components being the headache score and duration). One would expect all of these measures to correlate and table 5-4 confirms this.

	LL headache Score	Ascent Headache Score	Total Headache Score	Total Headache Duration	Total Headache Severity Index
LL headache Score	1	.938**	.701**	.594**	.652**
Sig (2 tailed)		0	0	0	0
n	181	181	181	181	181
Ascent Headache Score	.938**	1	.653**	.567**	.616**
Sig (2 tailed)	0		0	0	0
n	181	181	181	181	181
Total Headache Score	.701**	.653**	1	.651**	.726**
Sig (2 tailed)	0	0		0	0
n	181	181	182	182	182
Total Headache Duration	.594**	.567**	.651**	1	.934**
Sig (2 tailed)	0	0	0		0
n	181	181	182	182	182
Total HSI	.652**	.616**	.726**	.934**	1
Sig (2 tailed)	0	0	0	0	
n	181	181	182	182	182

a) Pearson's Correlation** = Correlation is significant at the 0.01 level

	LL headache Score	Ascent Headache Score	Total Headache Score	Total Headache Duration	Total Headache Severity Index
LL headache Score	1	.931**	.697**	.612**	.639**
Sig (2 tailed)	.	0	0	0	0
n	181	181	181	181	181
Ascent Headache Score	.931**	1	.632**	.553**	.578**
Sig (2 tailed)	0	.	0	0	0
n	181	181	181	181	181
Total Headache Score	.697**	.632**	1	.680**	.760**
Sig (2 tailed)	0	0	.	0	0
n	181	181	182	182	182
Total Headache Duration	.612**	.553**	.680**	1	.953**
Sig (2 tailed)	0	0	0	.	0
n	181	181	182	182	182
Total HSI	.639**	.578**	.760**	.953**	1
Sig (2 tailed)	0	0	0	0	.
n	181	181	182	182	182

b) Spearman's Correlation ** = Correlation is significant at the 0.01 level

Table 5-4 Tables demonstrating good correlations between all headache scoring systems; a using Pearson's Correlation and b using Spearman's. Total HIS = Total headache Severity Index.

From the above it could be assumed that all headache-measuring systems are comparable. The graphs in figure 5-2 demonstrate “headache score” (mean = green line, total = red) with each ascent profile (blue line). There is a very large spread of data and hence the error bars have intentionally been left off of these graphs.

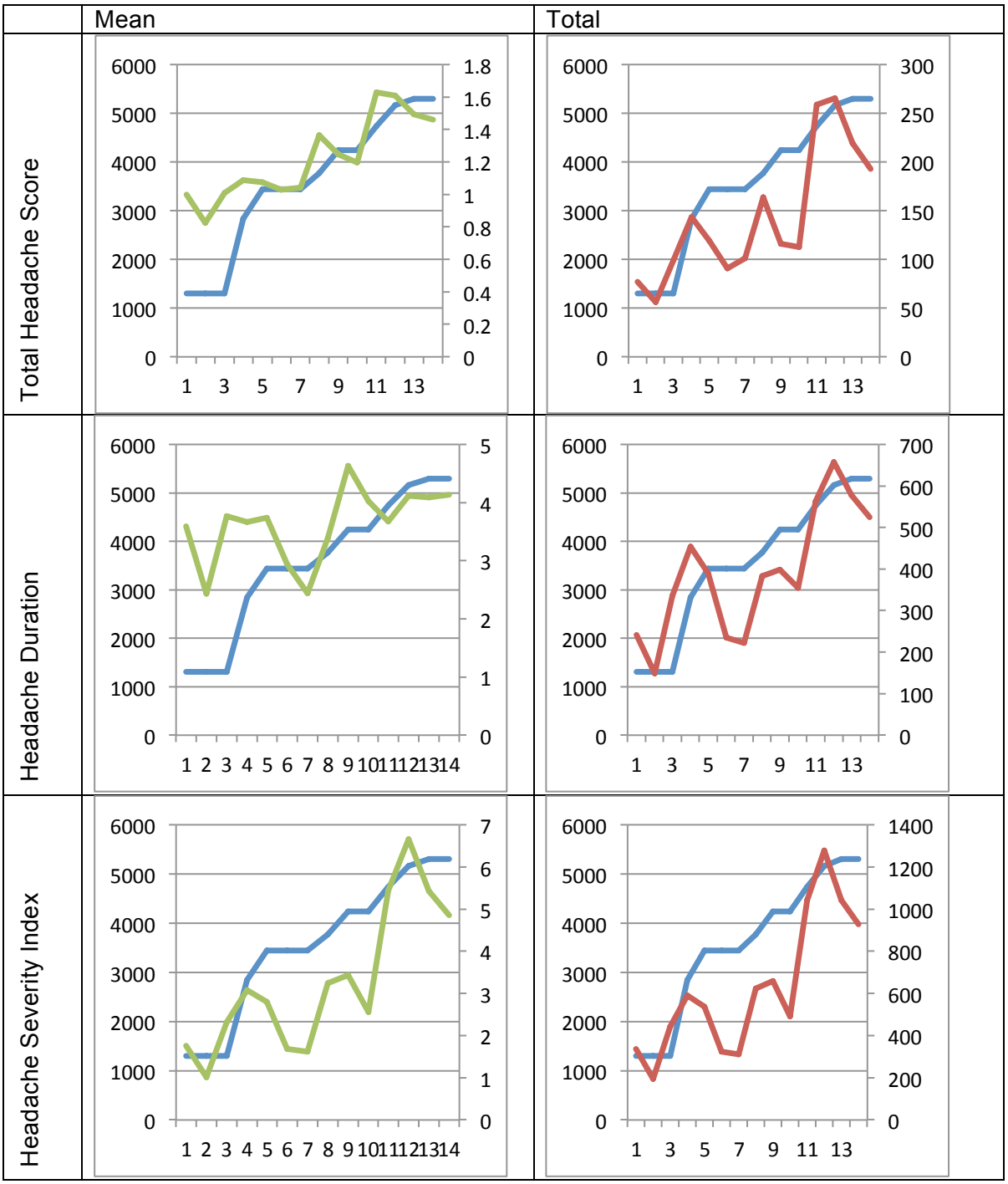


Figure 5-2 Graphs demonstrating mean (green) and total (red) Headache Scores (the sum of every subjects Lake Louise Score on each of the 13 days), the mean and total headache duration (the sum of each of the subjects headache duration on each of the 13 days) and the mean and total headache severity Index (the sum of each subjects HIS on each of the 13 days) compared with altitude (blue).

Correlations:

5.4.1.4 Correlations between Headache Score, basic demographics and gender

Basic demographic data are demonstrated in Table 5-5. Mean headache data for males and females are demonstrated in Tables 5-6a and b respectively. There was no significant correlation for subject sex except when using a Spearman's correlation (Table 5-7b) with Total Headache Score when a significant difference (p=0.049) of females having more headaches was just achieved. However, with multiple-analyses, a p value of 0.01 should probably be required. Independent t-tests demonstrated no significant differences between males and females for any headache scores.

No significant correlations were demonstrable between any of the headache scoring systems and height, weight or smoking habits (Table 5-7 and b). Similarly, no significant differences (anatomical, or headache scoring) were demonstrated between smokers and non-smokers.

	Male/Female	Age	Height	Weight	Resting Sats at EBC/ % (diary)
Mean	112/70	43.87	172.67	74.99	77.79
SD		13.44	9.17	13.51	5.29
SEM		1.00	0.68	1.00	0.39
Max		73.4	193	117	91
Min		18.3	150	49	62
Quartile 1		32.575	165.625	64.125	74
Quartile 3		56.25	179.5	83	81
IQR		23.675	13.875	18.875	7
Numbers		182	182	182	182

Table 5-5 Basic demographics for all those arriving at EBC (5300m)

Mean Headaches for Males/Females:

	LL	AHS	THS	THD	THIS
Mean	5.42	3.90	10.72	28.89	44.70
SD	4.08	2.98	6.51	22.69	38.70
SEM	0.30	0.22	0.48	1.68	2.87
Max	21	13	30	139	203
Min	0	0	0	0	0
Quartile 1	2	1	6.75	11.63	17.23
Quartile 3	8	6	14	40.44	61
IQR	6	5	7.25	28.81	43.78
Numbers	111	111	112	112	112

Table 6a Headache Data for Males

	LL	AHS	THS	THD	THIS
Mean	5.81	3.87	10.97	30.67	52.19
SD	4.77	3.18	6.30	25.27	54.21
SEM	0.35	0.24	0.47	1.87	4.02
Max	19	12	26.5	113.5	230
Min	0	0	0	0	0
Quartile 1	2	2	6.25	13.6875	22.43525
Quartile 3	8	5	14.75	38.375	53.77
IQR	6	3	8.5	24.6875	31.33475
Numbers	70	70	70	70	70

Table 6b Headache Data for Females

Table 5-6 Headache data for a) males and b) females

	LL headache Score	Ascent Headache Score	Total Headache Score	Total Headache Duration	Total Headache Severity Index
Smoking	-0.057	-0.066	0.02	0.065	0.043
Sig (2-tailed)	0.447	0.375	0.784	0.383	0.569
n	181	181	182	182	182
Height	0.04	0.064	0.12	0.054	0.022
Sig (2-tailed)	0.594	0.394	0.107	0.473	0.77
n	181	181	182	182	182
Weight	0.044	-0.005	0.019	0.037	0.081
Sig (2-tailed)	0.557	0.95	0.8	0.624	0.28
n	181	181	182	182	182
Sex	0.066	0.092	0.124	-0.024	-0.017
Sig (2-tailed)	0.376	0.217	0.097	0.746	0.817
n	181	181	182	182	182

Table 7a Correlation between smoking, height, weight or sex and headache score using Pearson's Correlation. None achieve significance.

	LL headache Score	Ascent Headache Score	Total Headache Score	Total Headache Duration	Total Headache Severity Index
Smoking	-0.061	-0.074	0.034	0.112	0.106
Sig (2-tailed)	0.418	0.321	0.645	0.132	0.153
n	181	181	182	182	182
Height	0.085	0.09	0.13	0.106	0.106
Sig (2-tailed)	0.256	0.231	0.081	0.154	0.156
n	181	181	182	182	182
Weight	0.008	-0.028	0.009	0.01	0.023
Sig (2-tailed)	0.92	0.711	0.904	0.897	0.754
n	181	181	182	182	182
Sex	0.116	0.138	.146*	0.021	0.031
Sig (2-tailed)	0.119	0.064	0.049	0.774	0.673
n	181	181	182	182	182

Table 7b Correlations demonstrable between smoking, height, weight and headache score using Spearman's correlation. None achieve significance at p <0.01. Females have significantly more headaches when p = 0.05.

5.4.1.5 Correlation With Age

Only the Total Headache Score correlated with age (Pearson's = -2.47 (p=0.001, n=182); Spearman's = -2.25 (p=0.002, n=182). LL, AHS, THD and HSI did not correlate. The effect size of this is however small (r = 0.24).

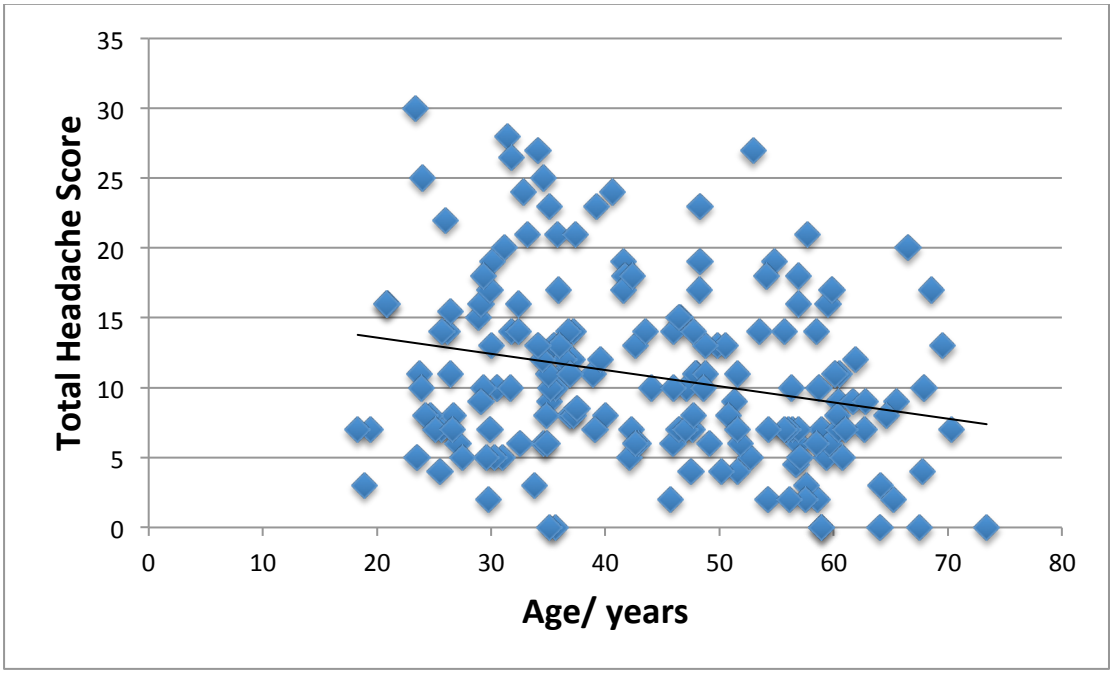


Figure 5-3 Correlation of age and total headache score. (Pearson's = -2.47 (p=0.001, n=182); Spearman's = -2.25 (p=0.002, n=182).

Correlations of Headache with Anatomy - Anthropomorphic Data:

5.4.1.6 Calibration with MRI and Correlations between Intracranial Volume measurement techniques

Chapter 6 explains the use of MRI to calculate multiple intracranial volumes. Since 11 of the subjects also had calliper measurements, I used this as a reference tool to check the validity of the calliper technique (figure 5-4).

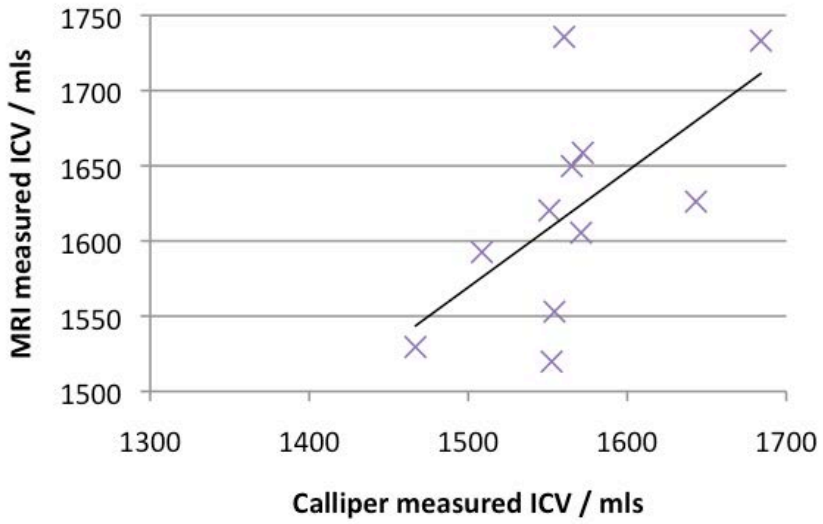


Figure 5-4 Relationship between ICV calculations from calliper tools and MRI measured intracranial volume. A good correlation was demonstrated (r = 0.62). The mean calliper measured ICV was 1547.47mls (SD 85.39 using the Lee-Paterson formula)) while the mean MRI measured ICV was 1619.60mls (SD 66.66) r = 0.62.

The mean calliper measured ICV was 1547.47mls (SD 85.39) while the mean MRI measured ICV was 1619.60mls (SD 66.66).

As can be seen a good correlation between calliper and MRI measured intracranial capacity was found.

Consistent differences were shown between intracranial volumes (for male and female versions of the Lee-Patterson formula and for the Dekaban formula as well as the individual measurements (AP, bipatertal diameter, head height and circumference) that make them ($p<0.01$ in all cases) (Table 5-8). It would be expected that males and females have different intracranial volumes and this was confirmed.

		Intracranial Volume Male	Intracranial Volume Female	Dekaban formula	Mean Cranial AP	Mean Biparietal	Mean Height	Mean Circumference	Mean Post Fossa
ICV Male	PearsCor.	1	.b	1.000**	.581**	.662**	.775**	.710**	0.058
	Sig.		.	0	0	0	0	0	0.544
	N	113	0	113	113	113	113	113	113
ICV Female	PearsCor.	.b	1	1.000**	.737**	.710**	.834**	.822**	0.21
	Sig.	.		0	0	0	0	0	0.083
	N	0	69	69	69	69	69	69	69
Dekaban formula	PearsCor.	1.000**	1.000**	1	.763**	.780**	.843**	.854**	.156*
	Sig.	0	0		0	0	0	0	0.035
	N	113	69	182	182	182	182	182	182
Mean Cranial AP	PearsCor.	.581**	.737**	.763**	1	.421**	.487**	.866**	.344**
	Sig.	0	0	0		0	0	0	0
	N	113	69	182	182	182	182	182	182
Mean Biparietal	PearsCor.	.662**	.710**	.780**	.421**	1	.459**	.667**	0.017
	Sig.	0	0	0	0		0	0	0.821
	N	113	69	182	182	182	182	182	182
Mean Height	PearsCor.	.775**	.834**	.843**	.487**	.459**	1	.568**	0.05
	Sig.	0	0	0	0	0		0	0.503
	N	113	69	182	182	182	182	182	182
Mean Circumference	PearsCor.	.710**	.822**	.854**	.866**	.667**	.568**	1	.197**
	Sig.	0	0	0	0	0	0		0.008
	N	113	69	182	182	182	182	182	182
Mean Post Fossa	PearsCor.	0.058	0.21	.156*	.344**	0.017	0.05	.197**	1
	Sig.	0.544	0.083	0.035	0	0.821	0.503	0.008	
	N	113	69	182	182	182	182	182	182

Table 5-8 Correlation (Pearson's 2 tailed) between the different calculations of Intracranial Volume (ICV) and the measurements that contribute to those calculations.

5.4.1.7 Correlation between ICV and Headache Score

However, no correlation was demonstrated between any of the headache scoring systems and any of the intracranial volume measuring systems (Table 5-9).

	LL headache Score	Ascent Headache Score	Total Headache Score	Total Headache Duration	Total Headache Severity Index
Intracranial Volume Male	-0.014	0.039	-0.027	-0.097	-0.072
Sig (2-tailed)	0.888	0.685	0.773	0.305	0.447
N	112	112	113	113	113
Intracranial Volume Female	0.051	0.103	0.053	0.144	0.061
Sig (2 tailed)	0.68	0.398	0.666	0.237	0.621
N	69	69	69	69	69
Dekaban Formula	-0.002	0.064	0.001	-0.01	-0.04
Sig (2 tailed)	0.981	0.391	0.99	0.895	0.592
N	181	181	182	182	182

Table 5-9a Pearson's correlations between intracranial volume measurements and headache scoring systems. No correlations reach significance.

	LL headache Score	Ascent Headache Score	Total Headache Score	Total Headache Duration	Total Headache Severity Index
Intracranial Volume Male	-0.023	0.033	-0.044	-0.081	-0.061
Sig (2-tailed)	0.813	0.731	0.643	0.396	0.519
N	112	112	113	113	113
Intracranial Volume Female	0.053	0.111	0.04	0.127	0.089
Sig (2 tailed)	0.666	0.366	0.746	0.297	0.467
N	69	69	69	69	69
Dekaban Formula	0.008	0.069	-0.016	-0.005	-0.028
Sig (2 tailed)	0.917	0.354	0.827	0.942	0.712
N	181	181	182	182	182

Table 5-9b Spearman's correlation between intracranial volume measurements and headache scoring systems. No correlations reach significance.

5.4.1.8 Correlation between Headache Score and Basic Physiology

Resting Saturations at EBC (as measured by subjects in diary):

Total headache Score correlated with Resting peripheral oxygen saturations (SaO_2) at EBC (Pearson's = -2.11 (p=0.004, n=182). It also achieved significance with ascent headache score (-0.195, p=0.008, n=181). The effect size is small (r= 0.21).

A correlation between resting peripheral arterial oxygen saturation at 5300m and headache was found with all headache measurement systems: (Spearman's correlation: with THS = -2.30 (p=0.02, n=182); LL score = -1.74 (p=0.019, n=181); AHS = -2.16 (p=0.04, n=181); THD = -0.157 (p=0.0034, n=182); THIS =-1.87 (p=0.012, n=182).

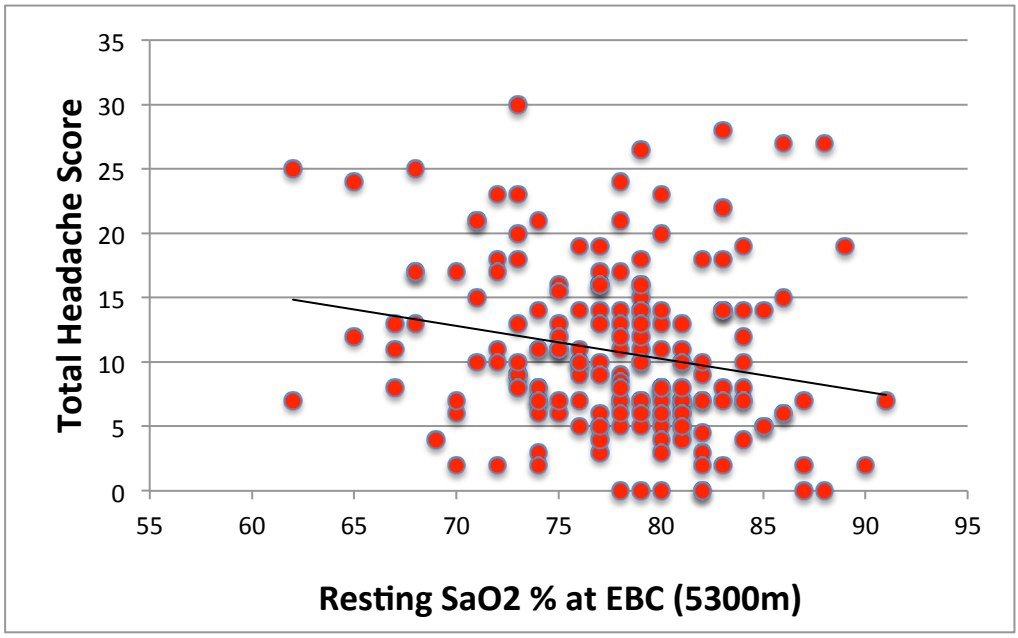


Figure 5-5 Correlation between resting SaO_2 and headache score. Although this achieves significance, the effect size is small (r = 0.21)

Correlations with other physiological variables:

5.4.1.9 Correlations in London (75m)

No significant correlation was demonstrated between any of the headache measuring systems and:

SaO_2 - Resting, AT or VO_2Max
Heart Rate - Resting, AT or VO_2Max
Systolic, Diastolic or Mean Blood Pressure at rest or VO_2Max
Time to AT, VO_2Max or recovery time
Arterial Oxygen Extraction
Haemoglobin concentration

Although resting EtO_2 ad $EtCO_2$ did not correlate with headache, they did at AT (with THS) and at VO_2Max (with THD and THIS) as demonstrated in table 5-10.

There was similarly a significant correlation between right (and hence mean) rSO_2 values and THD and THIS at AT and VO_2Max, though not THS.

Table 5.10 is a table of significant correlations between Headache Scores and Physiological Variables in London (Spearman's Rho). The reason for including the rSO_2 values is to demonstrate the persistent small correlation with right rSO_2 values.

	Total Headache Score	Total Headache Duration	Total Headache Severity Index
EtO_2 at AT Correlation coefficient	-.176*	-0.127	-0.94
Sig (2 –tailed)	0.022	0.101	0.22
n	169	169	169
$EtCO_2$ at AT Correlation coefficient	0.203**	0.182*	0.151
Sig (2 –tailed)	0.008	0.018	0.050
n	169	169	169
EtO_2 at VO_2Max Correlation coefficient	-0.135	-0.211**	-0.176*
Sig (2 –tailed)	0.79	0.006	0.022
n	169	169	169
$EtCO_2$ at VO2Max Correlation coefficient	0.128	0.210**	0.171**
Sig (2 –tailed)	0.098	0.006	0.387
n	169	169	127
Left rSO_2 at AT	0.062	0.147	0.125
Sig (2 –tailed)	0.433	0.061	0.113
n	163	163	163
Right rSO_2 at AT	0.062	0.159*	0.141
Sig (2 –tailed)	0.429	0.042	0.073
n	163	163	163
Mean rSO_2 at AT	0.077	0.17*	0.152
Sig (2 –tailed)	0.33	0.03	0.053
n	163	163	163
Left rSO_2 at VO_2Max	0.129	0.159	0.136
Sig (2 –tailed)	0.101	0.043	0.083
n	163	163	163
Right rSO_2 at VO_2Max	0.093	0.187*	0.169*
Sig (2 –tailed)	0.239	0.017	0.031
n	163	163	163
Mean rSO_2 at VO_2Max	0.115	0.178*	0.156*
Sig (2 –tailed)	0.144	0.023	0.046
n	163	163	163

- = significant at the 0.05 level, ** = significant at the 0.01 level

Table 5-10 Significant correlations between headache scores and physiological variables measured during VO_2max testing in London (75m).

5.4.1.10 Correlations at Namche (3450m)

No significant correlation was demonstrated between any of the headache measuring systems and:
Heart Rate - Resting, or at AT or VO_2Max
Systolic, Diastolic or Mean Blood Pressure at rest or VO_2Max
Time to AT, VO_2Max or recovery time
AT or VO_2max/litres or Kg
Haemoglobin concentration

Again, significant correlations were shown between EtO_2 and $EtCO_2$ at rest, AT and VO_2Max, as were correlations with right rSO_2 (table 5-11).

Total Headache Score (though not THD or THIS) correlated with SaO_2 at rest (Spearman's =-0.173, p =0.026, n=166), AT (Spearman's = -0.27, p = 0.001 n=151) though not at VO_2Max.

Again, right rSO_2 values appear to correlate with headache considerably more often than left. Arterial Oxygen Extraction correlated at rest (Spearman's = -0.194, p = 0.012, n=165) and VO_2Max (Spearman's = -0.194, p=0.017, n=150) with Total Headache Scores.

	Total Headache Score	Total Headache Duration	Total Headache Severity Index
EtO_2 at Rest Correlation coefficient	-0.222**	-0.167*	-0.176*
Sig (2 –tailed)	0.004	0.031	0.023
n	167	167	167
$EtCO_2$ at Rest Correlation coefficient	0.259**	0.204**	0.189*
Sig (2 –tailed)	0.001	0.008	0.015
n	167	167	167
EtO_2 at AT Correlation coefficient	-0.25**	-0.254**	-0.254**
Sig (2 –tailed)	0.001	0.001	0.001
n	167	167	167
$EtCO_2$ at AT Correlation coefficient	0.245**	0.236**	0.23**
Sig (2 –tailed)	0.001	0.002	0.003
n	167	167	167
EtO_2 at VO2Max Correlation coefficient	-0.152	-0.223**	-0.204**
Sig (2 –tailed)	0.051	0.004	0.008
n	166	166	166
$EtCO_2$ at VO2Max Correlation coefficient	0.183*	0.243**	0.211**
Sig (2 –tailed)	0.018	0.002	0.006
n	166	166	166
Left rSO_2 at Res	0.081	0.089	0.066
Sig (2 –tailed)	0.298	0.254	0.396
n	166	166	166
Right rSO_2 at Rest	0.192*	0.235**	0.239**
Sig (2 –tailed)	0.013	0.002	0.002
n	166	166	166
Mean rSO_2 at Rest	0.147	0.175*	0.166*
Sig (2 –tailed)	0.058	0.024	0.033
n	166	166	166
Left rSO_2 at AT	0.061	0.07	0.057
Sig (2 –tailed)	0.435	0.371	0.462
n	166	166	166
Right rSO_2 at AT	0.146	0.194*	0.199*
Sig (2 –tailed)	0.061	0.012	0.01
n	166	166	166
Mean rSO_2 at AT	0.115	0.14	0.138
Sig (2 –tailed)	0.139	0.073	0.077
n	166	166	166
Left rSO_2 at VO_2Max	0.044	0.044	0.044
Sig (2 –tailed)	0.575	0.574	0.573
n	166	166	166
Right rSO_2 at VO_2Max	0.128	0.182*	0.186*
Sig (2 –tailed)	0.102	0.019	0.016
n	166	166	166
Mean rSO_2 at VO_2Max	0.092	0.126	0.13
Sig (2 –tailed)	0.24	0.106	0.095
n	166	166	166

- = significant at the 0.05 level, ** = significant at the 0.01 level

Table 5-11 Significant correlations between headache scores and physiological variables measured during VO2max testing at Namche (3450m).

5.4.1.11 Correlations at Everest Base Camp (5300m)

Again, no significant correlation was demonstrated between any of the headache measuring systems and:

Heart Rate - Resting, or at AT or VO_2Max
Systolic, Diastolic or Mean Blood Pressure at rest or VO_2Max
Time to AT, VO_2Max or recovery time
AT or VO_2Max/litres or Kg
Haemoglobin concentration

Again, significant correlations were shown between EtO_2 and $EtCO_2$ at rest, AT and VO_2Max (Table 5-12). However, the weak association with right rSO_2 values that occurred at Namche was no longer present. Similarly, any correlation with Arterial Oxygen Extraction was lost also.

Total Headache Score (though not THD or THIS) correlated with SaO_2 at rest (Spearman's =-0.223, p =0.007, n=144), AT (Spearman's = -0.313, p = 0.001 n=143) and VO_2Max (Spearman's = -0.248, p = 0.012, n=101).

	Total Headache Score	Total Headache Duration	Total Headache Severity Index
EtO_2 at Rest Correlation coefficient	-0.356**	-0.268**	-0.287**
Sig (2 –tailed)	0	0.001	0
n	145	145	145
$EtCO_2$ at Rest Correlation coefficient	0.327**	0.316**	0.332**
Sig (2 –tailed)	0	0	0
n	144	144	144
EtO_2 at AT Correlation coefficient	-0.325**	-0.283**	-0.291**
Sig (2 –tailed)	0	0.001	0
n	143	143	143
$EtCO_2$ at AT Correlation coefficient	0.268**	0.285**	0.291**
Sig (2 –tailed)	0.001	0.001	0
n	143	143	143
EtO_2 at VO_2Max Correlation coefficient	-0.189*	-0.231**	-0.227**
Sig (2 –tailed)	0.024	0.006	0.007
n	142	142	142
$EtCO_2$ at VO_2Max Correlation coefficient	0.225**	0.249**	0.255**
Sig (2 –tailed)	0.007	0.003	0.002
n	142	142	142
Left rSO_2 at Res	-0.086	0.034	-0.024
Sig (2 –tailed)	0.305	0.681	0.777
n	144	144	144
Right rSO_2 at Rest	0.006	0.149	0.109
Sig (2 –tailed)	0.947	0.074	0.195
n	144	144	144
Mean rSO_2 at Rest	-0.025	0.118	0.066
Sig (2 –tailed)	0.767	0.159	0.432
n	144	144	144
Left rSO_2 at AT	-0.11	-0.018	-0.075
Sig (2 –tailed)	0.192	0.836	0.373
n	142	142	142
Right rSO_2 at AT	-0.038	0.091	0.05
Sig (2 –tailed)	0.652	0.282	0.551
n	142	142	142
Mean rSO_2 at AT	-0.061	0.058	0.007
Sig (2 –tailed)	0.474	0.491	0.931
n	142	142	142
Left rSO_2 at VO_2Max	-0.041	0.023	-0.011
Sig (2 –tailed)	0.63	0.784	0.901
n	141	141	141
Right rSO_2 at VO_2Max	0.008	0.117	0.105
Sig (2 –tailed)	0.927	0.168	0.217
n	141	141	141
Mean rSO_2 at VO_2Max	-0.016	0.078	0.054
Sig (2 –tailed)	0.854	0.356	0.522
n	141	141	141

- = significant at the 0.05 level, ** = significant at the 0.01 level

Table 5-12: Significant correlations between headache scores and physiological variables measured during VO_2Max testing at Everest Base Camp.

5.4.1.12 Correlations between Headache and End Tidal Gas Analyses

EtO_2 and Total Headache Score

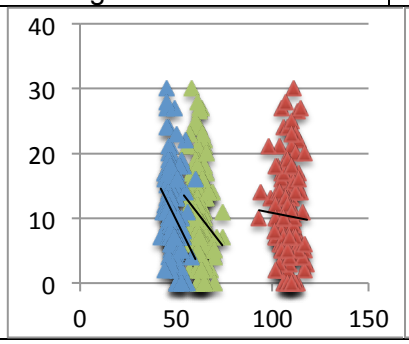
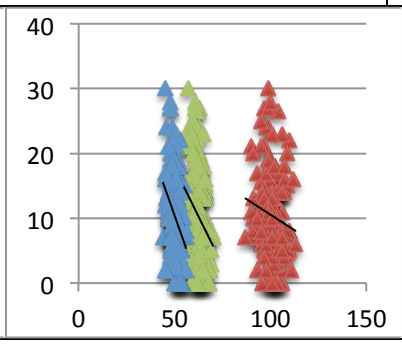
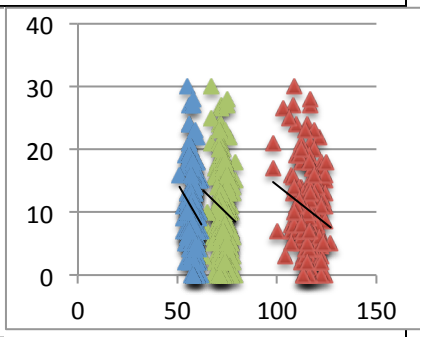
	Resting	AT	VO2 Max
Total Headache Score			
	EtO2/mmHg		
	r in London = 0.036	r in London = 0.144	r in London = 0.198
	r in Namche = 0.175	r in Namche = 0.266	r in Namche = 0.134
	r in EBC = 0.294	r in EBC = 0.328	r in EBC = 0.186

Table 5-13 Table of EtO2 readings with headache score at sea level (red), Namche bazaar (green) and at Everest Base camp (blue)

$EtCO_2$ and Total Headache Score

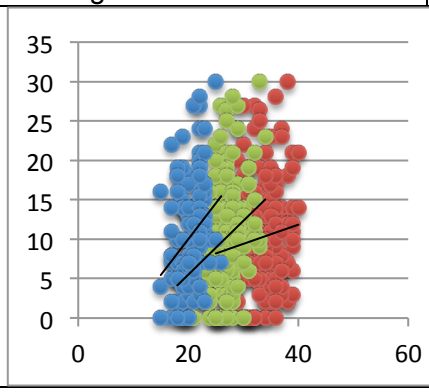
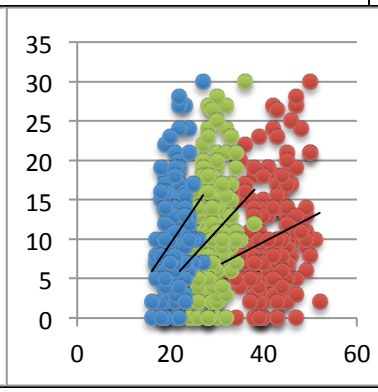
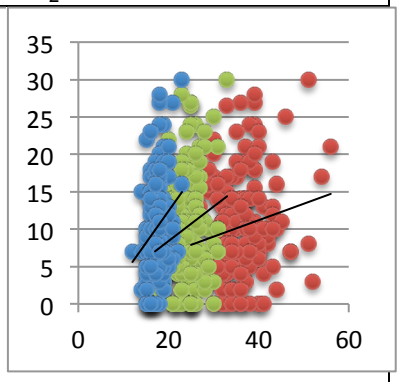
	Resting	AT	VO_2 Max
Total headache Score			
	$EtCO_2$/mmHg		
	r in London = 0.114	r in London = 0.199	r in London = 0.175
	r in Namche = 0.267	r in Namche = 0.28	r in Namche = 0.208
	r in EBC = 0.295	r in EBC = 0.286	r in EBC = 0.262

Table 5-14 Table of $EtCO_2$ readings with headache score at sea level (red), Namche bazaar (green) and at Everest Base camp (blue)

Although the r-values are not large, EtO_2 and $EtCO_2$ consistently correlate with headache, from rest through to maximal exercise at all altitudes, from sea-level to 5300m.

5.4.1.13 Correlation of rSO_2 and $EtCO_2$

Because $EtCO_2$ correlated with headache, a correlation was sought between $EtCO_2$ and rSO_2. Although (like right rSO_2 and Total Headache Score) a

significant correlation was demonstrable at rest, AT and VO_2Max at sea level, this correlation (Table 5-15) was diminished by Namche and had disappeared at EBC (figure 5-16)

	London			Namche			EBC		
	Rest	AT	VO2 Max	Rest	AT	VO2 Max	Rest	AT	VO2 Max
Cor Co	0.35 **	0.47 **	0.34 **	0.18 *	0.21 **	-0.025	-0.095	-0.015	-0.116
Sig	0.00	0.00	0.00	0.20	0.005	0.752	0.258	0.856	0.172
m	165	165	165	168	168	167	143	142	141

Table 5-15: Correlation coefficients and Significance for respective EtCO2 and rSO2 at rest, AT and VO2Max and each altitude. ** = Significant at the 0.01 level. * = Significant at the 0.05 level. The correlation is lost with increasing altitude.

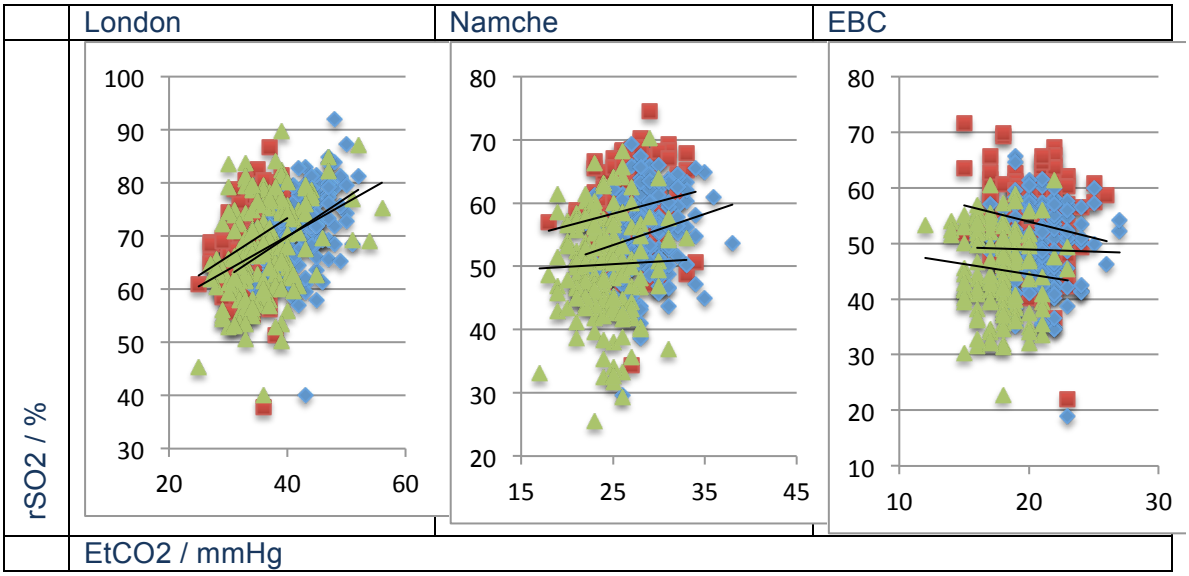


Figure 5-16: Graphs demonstrating the differences in $EtCO_2$ and the correlating rSO_2 at the three work rates and the three altitudes. Red square = at rest, blue diamond = at AT, green triangle = at VO_2Max.

5.4.2 Investigator results

Headache Scores for Investigator group (n=24):

Subject No	Ascent Headache Score	Total Headache Score	Headache Severity Index
X01	1	2	39
X02	4	5	41
X03	0	2	1.5
X04	0	1	2
X05	1	3	6
X06	0	1	1
X07	2	3	27.5
X08	0	0	0
X09	0	0	0
X10	2	4	4
X11	0	0	0
X12	2	8	153
X13	8	12	90
X14	4	5	152.5
X15	3	2	24
X16	8	15	141.5
X17	1	2	9
X18	0	1	1
X19	0	1	4
X20	1	1	4
X21	0	0	2
X22	1	4	25
X23	1	4	64
X24	2	5	68

Table 5-16 A table demonstrating the various headache scores for individual members of the core investigator team. This demonstrates that headache severity index spreads headache score, however, because of the small numbers further correlations are not studied in this text.

Further analysis of the investigator headaches is not reported here as more inferences can be made from the larger trekker data set.

5.5 Discussion

The main aim of this study was to look for an underlying correlation between basic anthropomorphic of physiological variables and the headache burden experienced by subjects ascending a relatively gentle ascent to 5300m. In doing so it also assessed a number of techniques for quantifying headache burden and for measuring intracranial volume.

5.5.1 Headache Assessment

All the headache assessment techniques used in this study (summations of Lake Louise Headache Scores, summation of scores only days immediately after ascent, summation of headache scores multiplied by duration (headache severity index)) correlated. This is to be expected as some are derived from components of the others. However, it confirms that any of these headache assessment tools can be used for future study. The main problems I had were with data collection itself.

Problems with headache data collection:

1) *It is a subjective measurement* – the self-assessment of headache, an ordinal/categorical variable, suffers because some people report pain more easily than others, while others try to cover pain up, especially when in a group to ensure that they are not held back. Other than keeping reporting anonymous (which we did through personalised diaries rather than having to report headache to a third person), and having clear definitions of categories of headache, there is little that can be done to improve this.
2) *Recording of headache* - In hindsight the headache self-assessment box (table 5.1) could have been better designed. I planned that subjects would circle the period that they had their headache (morning, afternoon, evening, night time) and then write in the duration of the headache (in hours). Unfortunately, many just circled the hours (e.g. 06:00 – 12:00). By recording headache severity more regularly (for example hourly through the use of a phone App), data collection could be much improved.

3) *Influence of other variables* – Exercise is thought to exacerbate high altitude headache. As a result, our subjects' headache experiences could have been influenced by the exercise tests they also underwent. More importantly, the large quantity of alcohol consumed by some core staff and trekkers could also account for some headaches experienced. Interestingly, Group G consumed more alcohol than all other trek groups combined, but the headache burden in this group did not differ from that in other groups. By having a score that just assessed the day immediately after ascent (when few people had drunk the night before – ascent headache score), this gave us the opportunity to see how much this influenced the result. The total headache scores still correlated with the ascent headache score hence this method of headache assessment is probably not necessary.
4) *There are many causes of headache* - Dehydration and fatigue are often quoted to be common causes of headaches and some of the headaches reported in this study will relate to this rather than altitude. A large number of subjects had a headache on arrival in Kathmandu. Clearly this is not altitude related but due to the stress of getting away / the flight.

As a community, it would be worth developing a tool that included duration as well as severity of pain for high altitude headache assessment and monitoring. The Headache Severity Index introduced here helps spread the data although it has no clear advantage in this study.

5.5.2 Anthropomorphic Assessment

This study has demonstrated a good correlation between Lee-Pearson and Dekaban estimations of intracranial volume. Previous studies comparing anthropometry with water volume of cadaveric skulls have suggested that anthropometry overestimates actual skull volume (Sahin, Acer et al. 2007). In my study, it would appear that compared to MRI measurements, in most circumstances it slightly underestimates volumes. However, it does demonstrate that there is a correlation between calliper and MRI measurements and hence those with larger skull volumes should be distinguishable from those with smaller volumes. Not surprisingly, this "intracranial volume" does not

correlate with any of the measures of headache burden. Even if a “tight-fit” mechanism was the underlying mechanism of high altitude headache, intracranial volume bears little relationship to compliance (brain atrophy / ventricular size etc are very variable) and hence this study was perhaps unlikely to yield a positive finding. Such a negative result does not refute the tight fit hypothesis, or the possibility that those with less compliance are more susceptible to headaches. On searching the literature, there has previously been very little study into any correlation between cerebral anatomy and headache. Cumming’s work (Wilson and Milledge 2008)(which is discussed in chapter 6) used CT to assess compliance, but little else is published.

5.5.3 Headache, End Tidal O_2 and End Tidal CO_2

It is probably not surprising that subjects with lower end tidal partial pressure of oxygen and higher end tidal partial pressure of carbon dioxide experience greater headaches, although this does not appear to have been previously reported in the literature. The ability to tolerate hypoxaemia without its driving an increase in minute ventilation could theoretically result in higher partial pressures of CO_2, vasodilatation, increased cerebral blood flow and hence increased cerebral volume.

5.5.4 Headache and rSO_2

Whilst I had expected a strong correlation between rSO_2 and headache score, I was unable to demonstrate this in my studies. However, the correlation between right-sided rSO_2 at Namche (3,500m - the place of the most acute drop in FiO_2) is noteworthy. This is also the altitude at which (as discussed in the previous chapter) I found the left cerebral hemisphere to be significantly protected from hypoxia compared to the right. Further acute studies (rather than on day 2 and 3 following arrival at altitude) with a faster ascent profile may demonstrate this relationship with more confidence. It is also interesting to note that (see chapter 7) this group also have greater retinal venous distension.

5.6 Conclusion

This study has demonstrated a number of techniques for assessing headache burden and shown strong correlation between them all. A number of algorithms

to estimate intracranial volumes have also been compared with each other and with MRI analysis. No correlation, however, has been shown between headache volume and intracranial volume. A negative correlation between age, peripheral saturations end tidal Oxygen and headache burden has been found, with a positive correlation between end tidal carbon dioxide and headache burden. The only measure of cerebral oxygenation that appeared to correlate with headache burden was right-sided NIRS measurements up to Namche Bazaar (3,500m). Further studies are needed if the pathogenesis of high altitude headache is to be better understood.

6 Chapter 6: ANTHROPOMORPHIC MRI INVESTIGATION INTO ANATOMICAL PREDISPOSITION TO HIGH ALTITUDE HEADACHE

This study is published as: The Cerebral Venous System and Anatomical Predisposition to High Altitude Headache (Wilson, Davagnanam et al. 2013) (see appendix).

Introduction and Background:

It was apparent that basic anthropomorphic measurements did not demonstrate any correlation with high altitude headache. I therefore undertook a more detailed experiment on a small number of subjects to investigate possible associations of headache with intracranial compartment volumes and intracranial venous volumes. This study and the following chapter (chapter 7) on retinal analysis are grouped together because both focus on the cerebral venous system. Following the results of these studies, I then undertook studies of the venous vasculature under hypoxic conditions (chapter 8).

6.1 Abstract

Introduction: If the “tight-fit” hypothesis is correct, there should be a correlation between cranial volumes and headache. If venous system hypertension contributes to high altitude headache, any increase in outflow resistance could exacerbate such symptoms. This study therefore aimed to evaluate CSF, parenchymal and venous compartment volumes.

Methods: Twelve subjects (all male) who ascended to 5300m underwent a T1 cranial MRI study on return to the UK. From this, volumetric analyses of brain, CSF and vascular compartments within the skull were performed and correlated with headache burden (ascent headache score).

Results: Ascent headache score was found to correlate with: lateral and 3rd ventricular volume (Spearman’s rho =-0.5 p = 0.05); infratentorial CSF volume excluding ventricles (the pericerebellar CSF volume) (Spearman’s rho = -0.56 p = 0.03), and; the volume of the smallest transverse sinus correlated with headache (Pearson’s = -0.7 p=0.006, Spearman’s = -0.557 p = 0.03).

Conclusions: Anatomical differences between individuals may contribute towards susceptibility of high altitude headache. The ability to buffer

parenchymal or vascular changes (through CSF) may provide some protection from headache. Probably of greater importance, a more restricted venous drainage pattern correlates with greater headache burden.

6.2 Introduction

In 1985, Ross put forward the “tight fit” hypothesis to explain the random nature of high altitude sickness (Ross 1985). In simple terms, the hypothesis states that a subject with greater cranial and spinal compliance will be able to accommodate more brain swelling before a rise in intracranial pressure (ICP). Assuming that it is the rise in ICP that causes a headache, those with “tight” intracranial contents will be more susceptible to altitude as a causative trigger for headache. Although the backbone of our current understanding (Hackett 1999), this has been modified to incorporate a physiological component. Exercising at altitude is thought to exacerbate cerebral hypoxemia, exacerbate cerebral oedema and make altitude illness worse (Roach and Hackett 2001).

There is, however, minimal evidence supporting the “tight-fit” hypothesis as it stands (Wilson, Newman et al. 2009). Some studies have not found cerebral oedema even with severe acute mountain sickness (AMS) (Fischer, Vollmar et al. 2004). Others have found that vasogenic oedema occurs in most subjects, but those with AMS have more cytotoxic oedema (Kallenberg, Bailey et al. 2007).

There are some reports (and chapter 5 in the thesis) that support the widely held belief that younger (rather than older) adults are more susceptible to AMS (Serrano-Duenas 2000; Silber, Sonnenberg et al. 2003). It could be hypothesized that this is because the brain atrophies over time, allowing the skulls of older climbers to better accommodate brain swelling. It could also be argued that young climbers tend to ascend rapidly which may also independently make them more vulnerable. Of note, one recent study has shown no difference in AMS between adolescents and adults (Dallimore and Rowbotham 2009).

Brain Cummins investigated headaches in 10 subjects during an expedition in 1985 (Wilson and Milledge 2008). Each had a CT scan prior to departure. An independent neurologist was asked to grade the ventricles as large, normal or small. There appeared to be a correlation with headache and AMS scores, the subjects with the smallest ventricles suffering the worst. Whilst this provides evidence for tight fit, it may also be a secondary correlation e.g. the younger subjects (who might be more prone to headaches at altitude for another reason) have the least atrophic brains.

6.3 Methods

6.3.1 MRI Study

Twelve subjects (all male, mean age 35.78 years [range 24-48.2]) underwent a 1.5T MRI study (T1/T2, 1.5mm interval) both prior to and on return (within 1 month) from the CXE expedition (described in chapter 2). Only the post-climb images were analysed for this study. The T1 images were imported into Analyze 9.0 (AnalyzeDirect, KS, USA) and a single independent blinded observer segmented and calculated the following volumes for each subject: total intracranial volume, supratentorial volume, infratentorial volume, total brain volume, supratentorial CSF volume (=supratentorial volume minus supratentorial brain volume), infratentorial CSF volume (infratentorial volume minus infratentorial brain volume), both lateral ventricle and 3rd ventricle volume, aqueduct of Sylvius and 4th ventricular volume, total ventricular volume, total CSF volume, Sagittal and Occipital Sinus volume and total venous volume. In addition, the angle of the tentorium in relation to the base of skull and the angle between the two petrous bones were measured. Figures 6-1, 6-2 and 6-3 demonstrate the calculation of some of these volumes and angles.

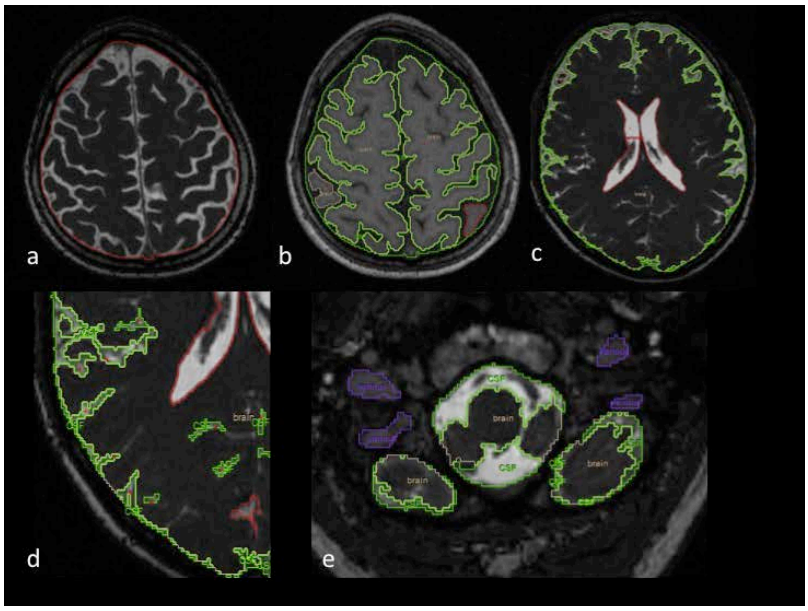


Figure 6-1 Analysis of MRI scans to enable volume calculations. a) the inner table of the skull is demarcated to measure total intracranial volume. b) the brain surface is demarcated using the inbuilt software. c) the ventricles are demarcated. d) where there are small pockets of CSF, for example, in deep sulci that are missed with the software, they are then added. e) other structures, such as venous sinuses are demarcated. Note: for this study, arterial volumes were not calculated.

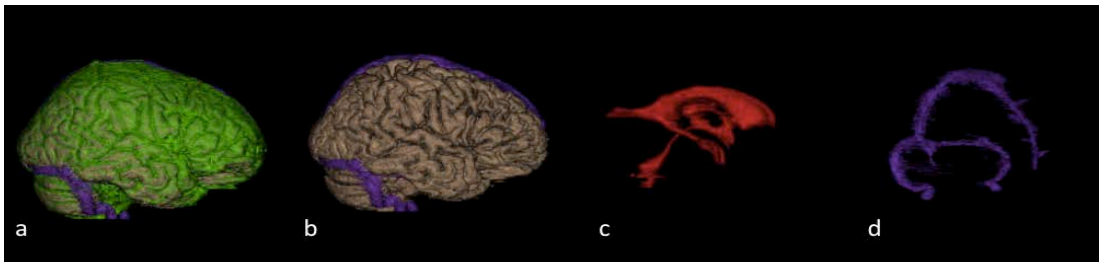


Figure 6-2 Once all structures have been rendered, each can be added and removed and its volume calculated.

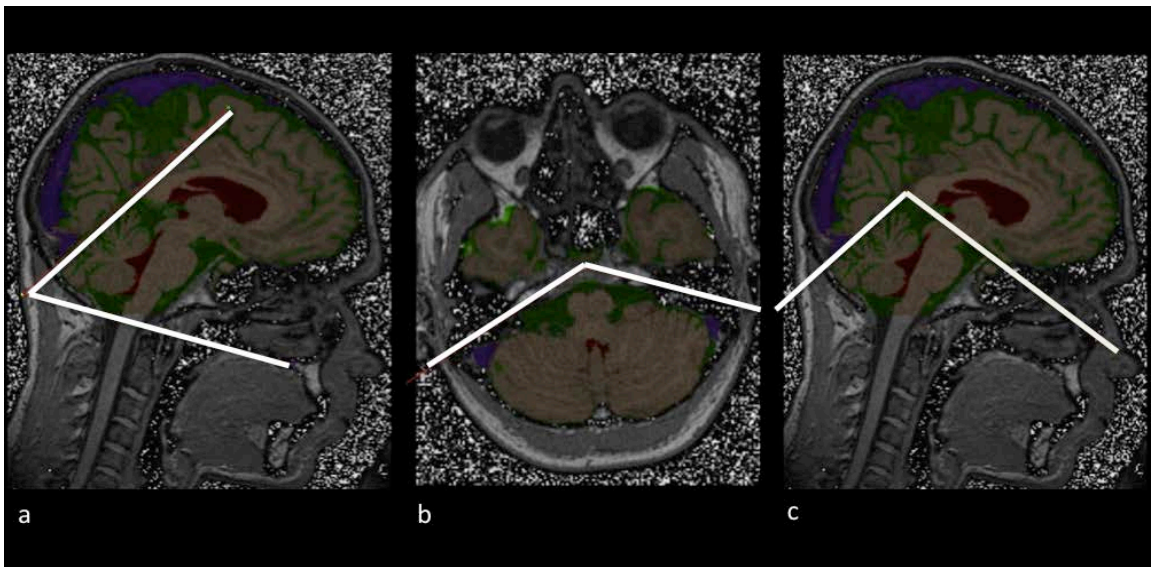


Figure 6-3 In addition to volumes, angles relating to the posterior fossa were also measured. These included: a) the tentorial angle (the angle between the tentorium and a line drawn from the hard palate on mid-sagittal MR); b) the petrous angle (the angle

between the lines drawn along the axis of the petrous bones ventral to the internal auditory canals on axial MR) and c) the tentorial-clival angle (the angle formed between the top of the tentorium and the top of the clivus).

6.3.2 Calibration Study

The intracranial volumes estimated using callipers were compared to the volumes calculated from the MRI studies (see chapter 5 for results).

6.3.3 Headache

Headache scores were calculated from daily diary monitoring in which, at the beginning of each day, each subject was asked to grade their headache (0-3) as part of the Lake Louise assessment. (Please see chapters 2 and 5 for more details.) In this study the Ascent Headache Score (the sum of the headache scores in the 24 hours after the seven ascents) was used. This was used to maximise headaches related to altitude and minimise any contribution from alcohol although I have previously shown (chapter 5) that all headache-scoring systems correlated.

6.4 Results

6.4.1 Compartment Volumes Angles and Headache Correlation

Table 6-1 reports the correlations (Spearman's rho) with ascent headache score for the compartment volumes and angles described.

Volume or angle measured	Volume/mls (or angle/ degrees)	Range / mls	Stand Dev	Spearman's rho Correlation coefficient & ascent headache score	n
Intracranial volume	1619.60	1519.80-1735.69	66.67	0.06	0.42
Supratentorial volume	958.41	897.91-1024.22	57.72	0.15	0.32
Infratentorial volume	327.05	279.34-365.81	30.39	-0.36	0.13
Brain parenchyma volume	1285.49	1179.95-1385.51	64.53	-0.06	0.43
Supratentorial CSF volume (excl. ventricles)	217.54	169.64-181.53	36.45	0.01	0.48
Infratentorial CSF volume (excluding ventricles)	62.40	45.34-85.94	13.09	-0.56*	0.03
Total non ventricular CSF volume	279.95	221.60-33-42	45.82	-0.11	0.37
Lateral and 3rd Ventricle	24.01	13.14-32.95	6.46	-0.5*	0.05
Aqueduct and 4th ventricle	2.20	1.61-3.14	0.5	0.27	0.20
Ventricular volume (= lateral +3rd +aqueduct +4th)	26.29	15.21-35.83	6.57	-0.47	0.06
Total supratentorial CSF	241.63	183.74-305.78	41.36	-0.16	0.31
Total infratentorial CSF	64.60	47.70-88.83	13.32	-0.49	0.05
Total CSF	306.23	244.71-394.61	51.18	-0.16	0.31
Sagittal and occipital sinus venous volume	15.98	12.45-21.44	2.63	-0.15	0.32
Left transverse sinus and jugular bulb	6.14	2.72-10.08	2.02	-0.05+	0.44
Right transverse sinus and jugular bulb	5.80	1.98-8.51	2.34	-0.39+	0.11
Total venous volume	27.91	20.72-35.77	4.18	-0.39	0.10
Tentorial angle	55.26	42.5-61.7	5.71	0.51*	0.05
Petrous angle	101.76	84.3-132.2	14.05	-0.12	0.35
Tentorial-clival angle	106.78	98.5-126.6	6.79	0.24	0.22

Table 6-1: Cranial compartment volumes in 12 male subjects. Correlation coefficients (non-parametric single tailed) and p values demonstrating correlation with ascent headache scores are reported. *=p<0.05 using a single tailed Spearman's Rho. + = not significant when analysed as a whole. However, when the smallest transverse sinus volume is compared to headache score, the result is significant (Pearson's = -.07 p = 0.006, Spearman's = -.0557 p = 0.03).

6.4.2 Transverse Sinus Volumes and Headache

There appeared to be a correlation between transverse sinus volume and headache. Studying this further reveals that those suffering more tend to have one narrow transverse sinus (<3mls) (figure 6-4).

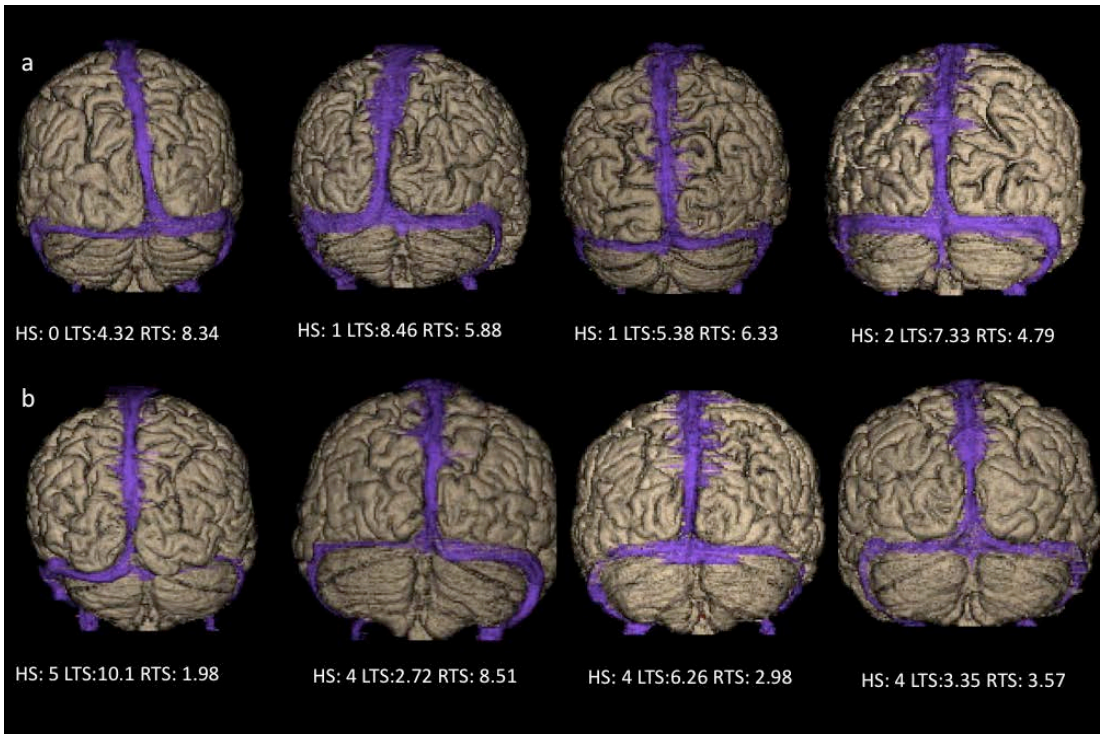


Figure 6-4 Left (LTS, mls) and right (RTS, mls) transverse sinus volume in a) the four subjects with the lowest ascent headache scores (HS) and b) the four subjects with the highest ascent headache scores. Three of four in group b (all bar the last subject) have marked LTS/RTS asymmetry with marked narrowing of the non-dominant sinus to <3 mls in volume. The last subject had bilateral narrowing.

There is a good correlation (Pearson's = -.07 p = 0.006, Spearman's r=-0.56, p = 0.03) between the volume of the smallest transverse sinus and the ascent headache score as demonstrated in figure 6-5.

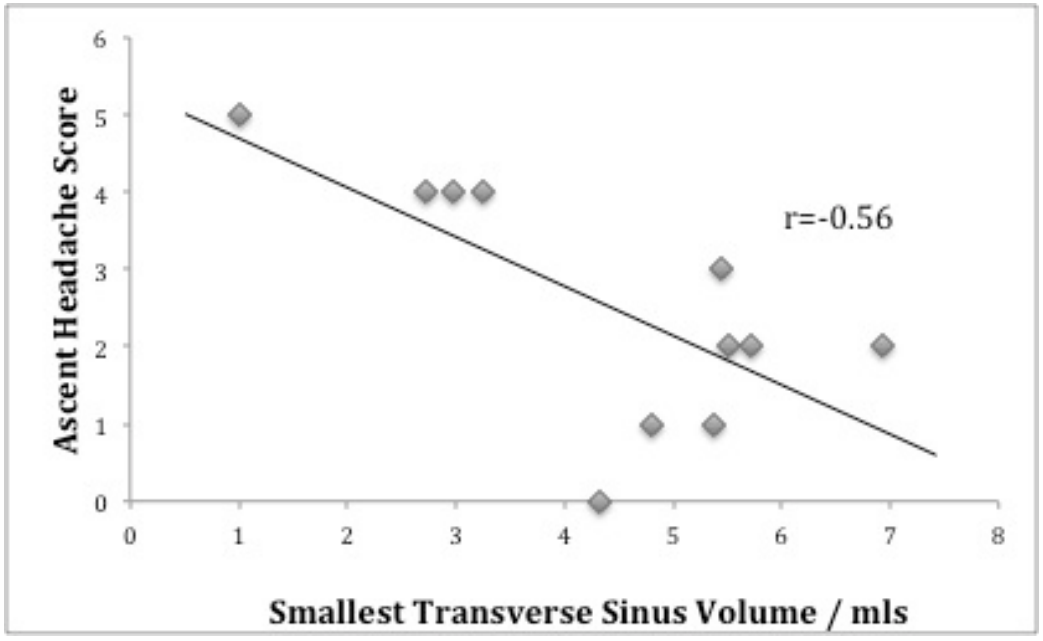


Figure 6-5 Relationship between the volume of the smallest transverse sinus and high-altitude headache score in 12 male subjects (Pearson's = -0.7 p=0.006, Spearman's = -0.557 p = 0.03).

6.4.3 Calibration Study

See Chapter 5 for the calibration of callipers with MRI measurements.

6.5 Discussion

This study has two principal anatomical findings. Firstly, having a small ventricular CSF volume or small pericerebellar CSF volume is associated with greater headache burden. Secondly having a relatively narrow transverse sinus is associated with more severe headaches.

The first finding that those with smaller ventricular CSF volumes and less pericerebellar space have greater headaches is in keeping with Ross's tight fit hypothesis (Ross 1985). It implies that the CSF may buffer cerebral engorgement. This is also in keeping with a study originally performed by Brian Cummings which after his death I subsequently analysed and published (Wilson and Milledge 2008). In this study, ventricular volume (independently graded as large, normal or small) correlated with headache in 10 subjects ascending to 5030m. Other studies have demonstrated headache syndromes in those with a "crowded" posterior fossa (Chen, Lirng et al. 2004). Hence our findings also imply that in those with minimal posterior fossa compliance, hypoxia may induce headache.

My second principal finding, that transverse sinus narrowing was associated with greater high altitude headaches, is consistent with a venous pathogenesis of headache. Clinically, transverse sinus stenosis and venous insufficiency is associated with a syndrome called Idiopathic (or benign) Intracranial Hypertension (IIH) (Rohr, Bindeballe et al. 2012). It has been claimed that all headaches are mediated by the trigeminocervical nucleus (Bogduk 1995). This receives innervations from the dura lining the brain and sinuses. Hence, increases in the cavernous and other sinuses because of relative venous insufficiency would be interpreted as headache. It may be that the increased cerebral blood flow with hypoxia tips people who normally drain their cerebral circulation adequately, over into this IIH like state. This is discussed in more detail in the thesis discussion and in "The Headache of High Altitude and Microgravity – Similarities with Clinical Syndromes of Cerebral Venous Hypertension" (Wilson, Imray et al. 2011).

6.5.1 Headache Score

Headache is a very subjective phenomenon: one individual reporting a "grade" of headache might be quite different to another. However, it is the best assessment we can make. There are also many causes of headache. By restricting headaches that count to the headache score the morning after an ascent, we aimed to maximise the recording of altitude specific related headaches. As explained in chapter 5, all techniques of headache scoring we used correlated.

6.6 Conclusion

This study has implicated both the cerebral compliance (volume of CSF) and the inability to drain venous blood adequately as being components of the pathogenesis of high altitude headache. This is consistent with my hypothesis that increased cerebral blood flow in hypoxia, in some, cannot be drained adequately and those who are unable to buffer this increased cerebral volume develop headaches. Further studies looking at the venous system dynamically were required and have been reported in chapter 8.

7 Chapter 7: HYPOXIA CAUSES RETINAL VENOUS DISTENSION WHICH CORRELATES WITH HEADACHE

This study has been accepted for publication as: The Cerebral Venous System and Anatomical Predisposition to High Altitude Headache, Wilson et al Annals of Neurology (Wilson, Davagnanam et al. 2013).

7.1 Abstract

Background: High Altitude Retinopathy (HAR) is a well-recognised complication of ascent to high altitude. The mechanism of retinal haemorrhage formation with hypoxia is not well understood. With the retinal venous system draining directly intracranially, I hypothesised that retinal venous distension should occur if venous hypertension or outflow restriction contributes to high altitude headache.
Aim: This study was designed to investigate retinal vessel changes during a gradual ascent to 5300m and to correlate such changes with headache symptoms.
Methods: Twenty-four subjects ascended from 1100m (Kathmandu) to 5300m (Everest Base Camp) over 17 days. Digital retinal images were obtained at sea level and at 5300m within 3 days of arrival. During the ascent, a daily diary of headache severity was recorded by each subject. Subjects peripheral saturations and $ETCO_2$ were also recorded as part of the exercise study (see chapter 4).
Results: Twenty-three subjects exhibited retinal venous distension ranging from 5 to 44%. The degree of this distension correlated with ascent headache score (Pearson's = 0.496, p=0.014; Spearman's rho = 0.553, p=0.005). A correlation was also demonstrated between peripheral saturation at 5300m and the change in venous retinal vessel diameter (r = -0.55, p = 0.005). Similarly, $ETCO_2$ at 5300m also correlated with retinal venous vessel diameter (r=-0.4, p = 0.05).
Conclusions: The correlation of retinal venous distension with headache severity supports a venous component to the pathogenesis of high altitude headache. The correlations with peripheral saturations and $ETCO_2$ imply that those with a greater hypoxic ventilatory response (maintaining peripheral saturations and lowering $ETCO_2$) have less venous engorgement and less headache burden.

7.2 Introduction

The current generally accepted theory of high altitude headache is that of cerebral oedema developing to a greater extent in individuals who are more hypoxic (a physiological component) while a relative lack of cranial compliance results in a rise in intracranial pressure in those susceptible to high altitude headache (an anatomical component) (Roach and Hackett 2001). I have already reported the dramatic changes in arterial cerebral blood flow with hypoxia (Wilson, Edsell et al. 2011). It is often forgotten however that the same amount of blood needs to leave the skull vault.

The retina receives its blood supply intracranially via the ophthalmic artery and its venous drainage is via ophthalmic veins intracranially to the cavernous sinus, internal cerebral veins, vein of Galen, and Transverse and Sigmoid sinuses before finally entering the jugular system. Since both the retina's blood supply and drainage originate largely intracranially, changes in these vessels probably reflect cerebral vascular changes.

Singh et al were the first to describe retinal changes at altitude (Singh, Khanna et al. 1969). There have subsequently been many descriptions of retinal haemorrhage at altitude (Lang and Kuba 1997; Wiedman and Tabin 1999) (Mullner-Eidenbock, Rainer et al. 2000) and pathogenic mechanisms proposed including Hypoxia Inducible Factor 1α (HIF-1α) (Arjamaa and Nikinmaa 2006) and increased arterial flow mechanisms (Mullner-Eidenbock, Rainer et al. 2000).

I have hypothesised that cerebral venous congestion plays a central role in high altitude headache (HAH). As such, it would be expected that retinal venous changes should reflect this. Hence, this study investigates retinal arterial and venous changes during a gradual ascent to 5300m and correlates vessel diameter changes with headache severity.

7.3 Methods

This prospective observational cohort study was a component of the Caudwell Xtreme Everest Study (chapter 2) and ethical approval was given by University College London.

Subjects: Twenty-four subjects (the Investigators, 18 male, mean age 35.2 years (range 19-59)) were recruited. None had any known pre-existing ocular disease.

Ascent profile: All 24 subjects ascended as a group to 5300m over 17 days as described in chapter 2.

7.3.1 Retinal Imaging

All subjects underwent bilateral retinal imaging (8TRC NW200 Non-Mydriatic Digital Opthalmoscope; TopCon, Tokyo, Japan) in London and again within 2 days of arrival at Everest Base Camp. Prior to imaging, all subjects were asked to stare at an Amsler chart with the right, then left eye, to assess for any schotoma (*see Appendix Fig 2-A*). If found, they were asked to draw the "blind spot" onto the Amsler chart.

7.3.2 Retinal Analysis

Subsequent retinal image analysis was performed independently by two investigators. Direct exact measurements were not possible (since there is no reference or scale with retinal images) hence the pre- and post-climb images were matched in size and Arbitrary Units (AU) used to enable measurement and percentage change calculations. The diameter of the optic disc was measured and a standard distance (200 AU) between the centre of the optic disc and the relevant vessels was measured to locate the position on the vessel for calibre measurements. As part of multiple measurements, the diameter of the superior and inferior retinal veins and the diameter of the superior temporal retinal artery were measured using Topcon™ (Topcon, Japan) and Sante™ software (Santesoft Ltd, Greece; figure 7-1). This was measured in both eyes by two independent clinicians and mean percentage venous and arterial calibre, and optic disc diameter change was calculated for each subject. The number of retinal haemorrhages and a grade of papilloedema (0-5, based on the optic disc margins using the Modified Frisén scale (Scott, Kardon et al. 2010) (Appendix Fig7-A)) were also recorded.

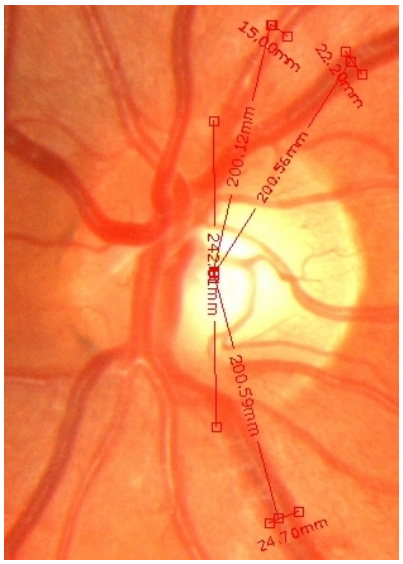


Figure 7-1 Image demonstrating measurement of the optic disc, superior and inferior retinal veins and superior temporal retinal artery (units do not correspond to millimetres).

7.3.3 Headache Severity

Each subject recorded his or her headache score (ranging from 0 (no headache) to 4 (severe)) for the preceding 24 hours each morning. In addition the duration of that headache (in hours) was reported (see chapter 2). The use of any medication was noted. Two headache scores were used for the retinal study. Firstly the Total Headache Severity Index (the sum of each daily headache score multiplied by its duration) was used to correlate retinal vascular changes with headache burden. Secondly, to reduce the contribution of non-altitude related headaches, the ascent headache score (sum of the headache scores following 24 hours after an ascent – 7 ascents in total) were used to group the subjects into those who suffered headaches (score ≥1, n= 15) and those that did not (score = 0, n=9).

7.3.4 Statistics

The results were analysed using Spearman's Rho and Wilcoxon Rank Sum Tests in SPSS 14.0™.

Other Physiological Variables: Pulse, blood pressure and peripheral arterial oxygen saturations were recorded daily. At sea level and at 5300m subjects also had resting end tidal CO_2 ($ETCO_2$) measurements and brain oxygenation (rSO_2) measurements performed as part of the exercise tests as explained in

chapter 5. This enabled correlation of retinal changes with these variables as well.

7.4 Results

All 24 subjects completed the ascent. Cumulative headache scores ranged from 0 to 8 (median = 1). Both venous and arterial calibre increased significantly on ascent (p = <0.01). Figure 7-2 demonstrates the typical increase in vessel diameter.

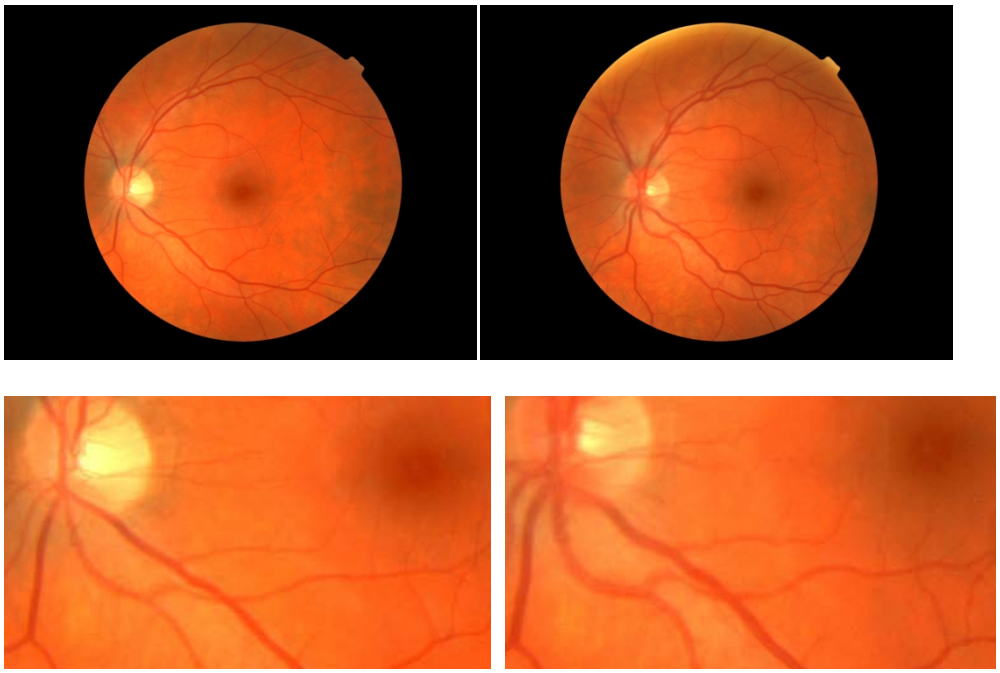


Figure 7-2 Retinal venous distension. Figure 4a – retinal image at sea level; 4b – retinal image at 5300m.

Arterial Changes

Mean arterial distension was 20.6% (SD 14.5%). There was no significant correlation with Headache Severity Index (Pearson's Correlation = -0.11, p=0.61; Spearman's = 0.19 p=-0.38) (Figure 7-3).

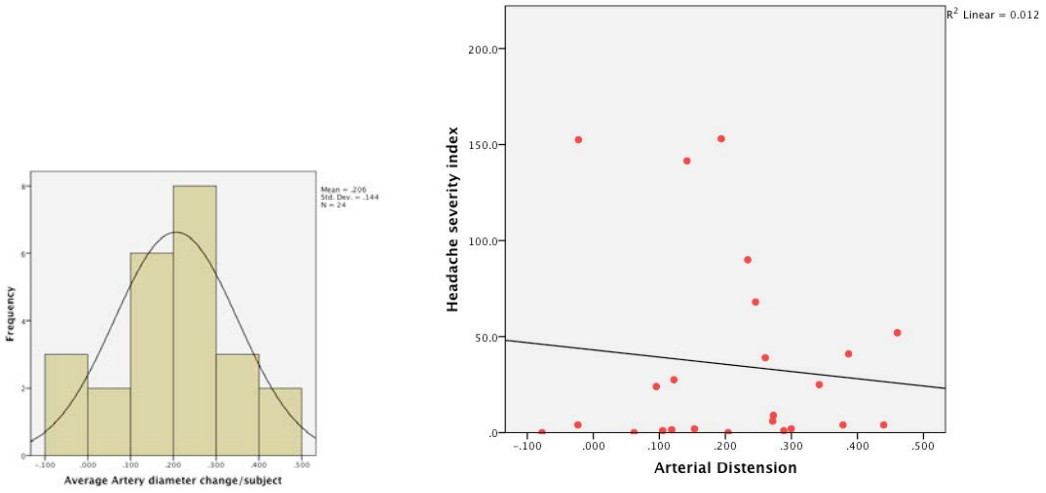


Figure 7.3 a) Arterial Distension (%) vs frequency demonstrating a normal distribution. b) graph of arterial distension (%) vs Headache severity index. There is no relationship.

The mean (±SD) arterial distension in those that suffered headache was 22.8% (±14.8)(n=15) while in those with no headaches it was 17.0%(±13.9)(n=9). Using an independent t-test, there is no significant difference between these groups (t=0.95 p=0.35).

Venous Changes

Mean venous distension was 24.5% (SD 11.2%). There was a significant correlation with Headache Severity Index (Pearson's Correlation = 0.54 p<0.01, Spearman's = 0.67 p<0.001) (Figure 7.4).

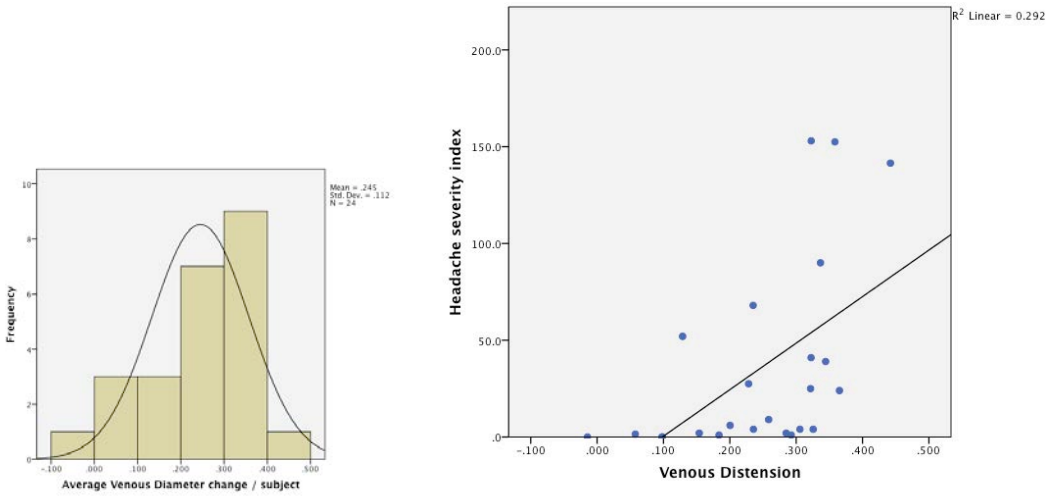


Figure 7.4 a) Venous Distension (%) vs frequency (number of subjects) demonstrating a normal distribution with slight kurtosis b) graph of venous distension (%) vs Headache Severity Index. The correlation between Headache Severity Index and venous distension is significant (p<0.01).

The mean (±SD) venous distension in those that suffered headache was 29.3% (±7.9)(n=15) while in those with no headaches it was 16.4%(±11.7)(n=9). Using

an independent t-test, this difference in venous distension between the groups is significant (t=3.2 p=0.004).

Optic Disc: There was no significant increase in mean disc size (+0.3% (SD 2.3%)). As can be seen from Fig 7.5, the centre of the distribution of changes in disc diameter is 0.0, hence the variability around this point probably reflects variance caused by the measurement technique. There was no significant correlation between optic disc diameter and headache severity index (Pearson's Correlation = 0.21 p = 0.34; Spearman's = 0.38, p = 0.08).

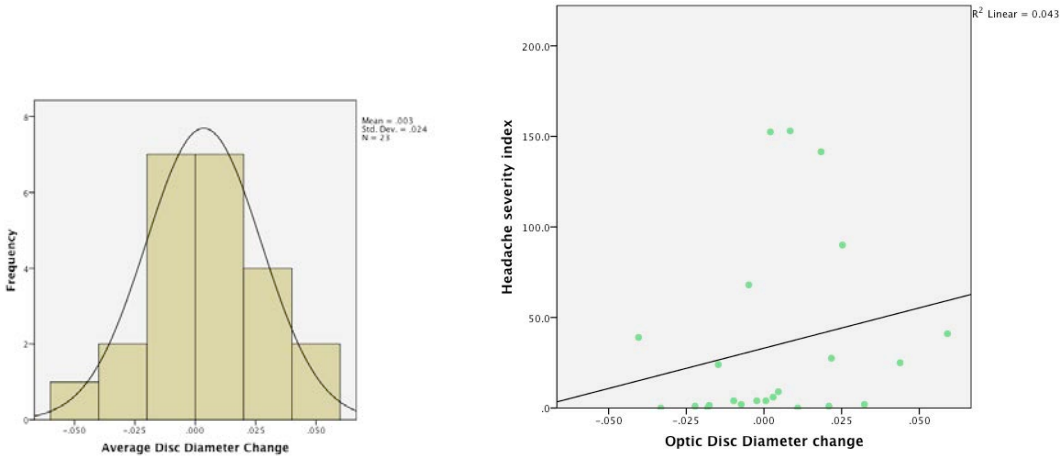


Figure 7.5 a) Optic Disc diameter change (%) vs frequency (number of subjects) demonstrating a normal distribution. b) graph of optic disc diameter change (%) vs Headache severity index. The correlation is not significant (p>0.05).

The mean (±SD) optic disc diameter change in those that suffered headache was 0.8% (±2.4)(n=14) while in those with no headaches it was -0.3%(±2.1)(n=9). Using an independent t-test, this difference in venous distension between the groups is not significant (t=1.2 p=0.25).

Only one subject developed retinal haemorrhages (which were detectable on Amsler chart challenge). Four subjects had the lowest grade of papilloedema at 5300m.

The relationship between changes in arterial diameter and peripheral arterial oxygen saturation at 5300m (Figure 7-6) or $ETCO_2$, (Figure7-7) are shown below. Similarly, the relationship between changes in *venous* diameter and peripheral arterial oxygen saturation at 5300m or $ETCO_2$ are shown in Figures 7-7 and 7-8 respectively.

Arterial changes with other physiological variables:

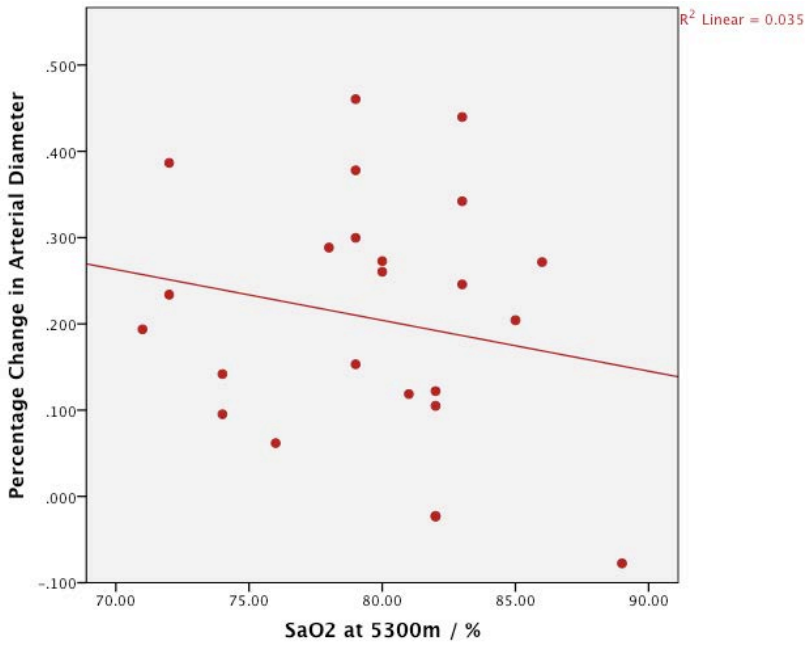


Figure 7-6 Peripheral arterial oxygen saturation at 5300m vs percentage change in arterial diameter. r = -0.18, r^2 = 0.035 (Pearson's correlation = -0.18 p=0.38).

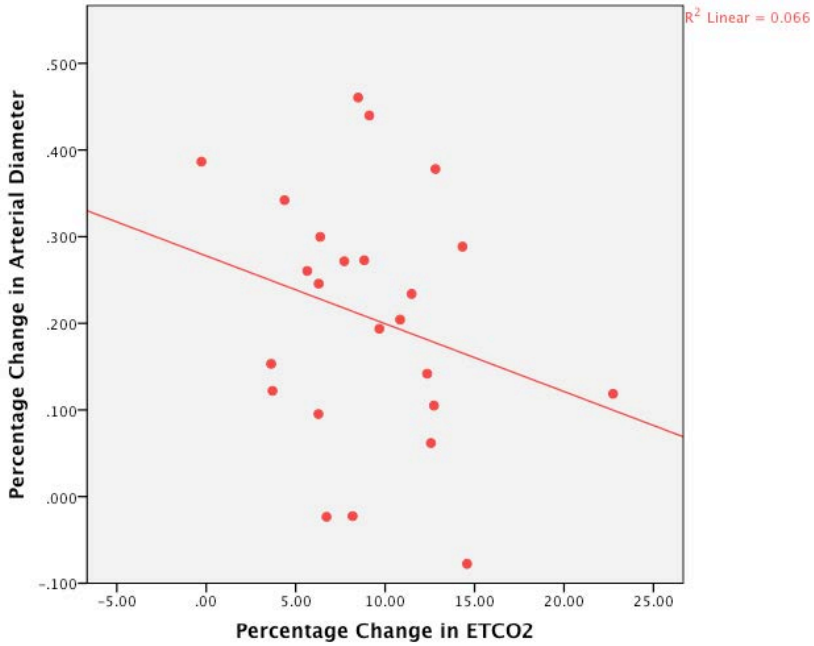


Figure 7-7 Percentage fall in $EtCO_2$ from baseline vs percentage change in arterial diameter. r = 0.26, r^2 = 0.066. (Pearson's correlation = -0.26 p=0.23).

Venous Changes with other physiological variables:

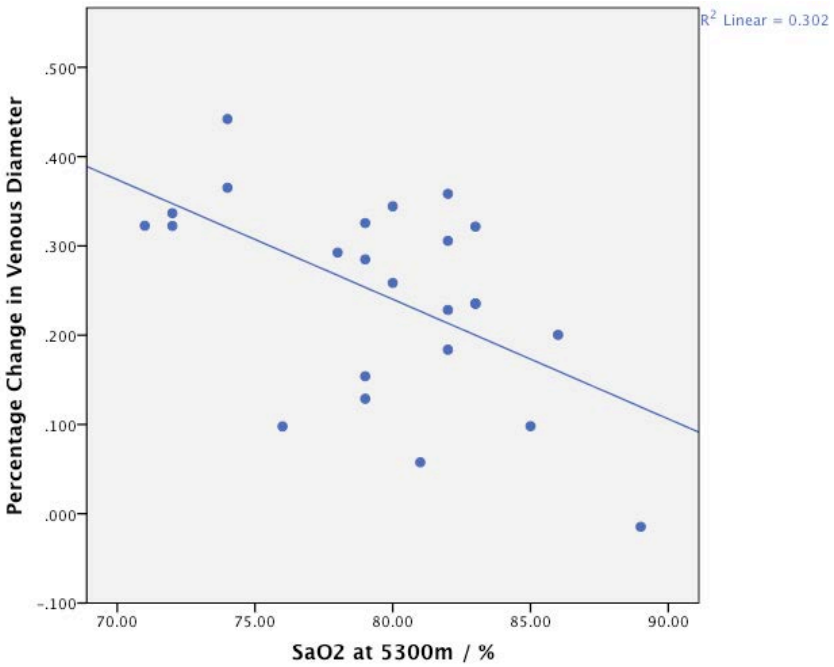


Figure 7-8 Peripheral saturation at 5300m vs percentage change in venous diameter. r = 0.55 r^2 =0.3 (Pearson's correlation = -0.55 p = 0.005).

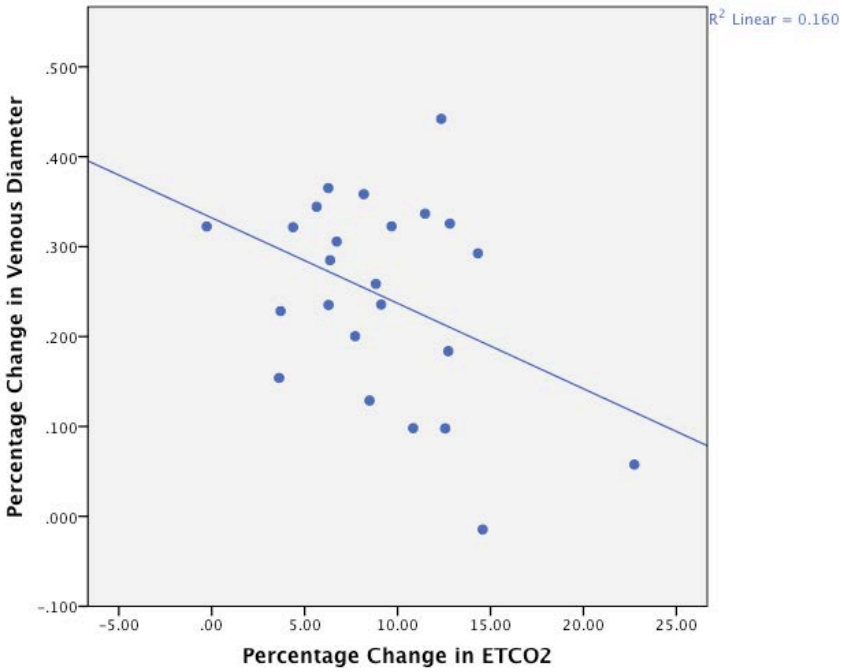


Figure 7-9 Correlations between venous diameter and end tidal CO_2. r = 0.4 r^2 = 0.16 (Pearson's correlation = -0.4 p= 0.05).

Both peripheral arterial oxygen saturation at 5300m and the fall in $EtCO_2$ correlated significantly with venous distension, but not with arterial changes.

7.5 Discussion

The principal finding in this study is that hypobaric hypoxia induces retinal venous and arterial distension, but only venous distension correlates with headache severity. This finding gives considerable strength to the hypothesis that the venous system is implicated in the development of high altitude headache. The correlations with peripheral saturations and $ETCO_2$ imply that those with a greater hypoxic ventilatory response have less venous engorgement and less headache burden.

7.5.1 Strengths and Weakness

As explained earlier in this thesis, headache is a subjective measure. As such, caution is required when treating it as a linear measure and comparing this to a physiological measure. In addition, this study used a gradual ascent and images were taken 24 hours after arrival at altitude by which time, some degree of acclimatisation may have occurred, hence the percentage changes may be smaller than that shown by others (Bosch, Merz et al. 2009).

General Discussion

Brinchmann-Hansen *et al* demonstrated in 1989 that both arteries and veins dilate on exposure to hypobaric hypoxia of altitude (Brinchmann-Hansen, Myhre et al. 1989) . More recently, Bosch *et al* (Bosch, Merz et al. 2009) have demonstrated a correlation between both arterial and venous distension and headache severity. In our study, these results are supported although venous distension appeared to be a greater and more significant component. This may be a reflection of the 24 hours between arrival and imaging.

In a separate study Bosch *et al* (Bosch, Barthelmes et al. 2008) have also demonstrated that 59% of climbers to 6865m developed optic disc swelling and that this was more prevalent in those with higher AMS scores. More recently, work related to the Tuebingen High Altitude Ophthalmology (THAO) study (Willmann, Fischer et al. 2011) has demonstrated that Optic Nerve sheath oedema occurred in 79% of 18 volunteers who ascended to 4559m. The

incidence of AMS was 55% and there was no correlation between quantification of Optic Nerve sheath oedema and AMS or peripheral arterial oxygen saturation or heart rate.

7.5.2 Possible Mechanism of Venous Distension:

A number of theories as to the cause of retinal vascular changes at altitude have previously been suggested. Mechanical distension may occur through the increase in cerebral blood flow velocity that has been demonstrated by transcranial Doppler measurement (Imray, Myers et al. 2005).

Chemical and molecular mediators such as HIF-1α (Arjamaa and Nikinmaa 2006), nitric oxide and VEGF may also contribute. However, while it is clear that such mediators could cause arteriolar and capillary dilatation, they would probably not be the cause of the large venous distension seen.

Significantly in this study, the venous changes also correlate with other physiological variables, namely peripheral arterial oxygen saturation at 5300m and the fall in $EtCO_2$ between 75m and 5300m. Arterial changes do not. This finding might be explained by direct or indirect effects. Venous distension was greater in subjects with lower SaO_2 at 5300m and those who had less reduction in $EtCO_2$. If those with lower hypoxic ventilatory drive (a smaller reduction in $EtCO_2$) also had elevated venous pressures (perhaps due to higher intrathoracic pressures or restriction in downstream venous capacitance), this could explain their increased retinal venous distension. However, other causes are possible, and remain to be explored.

Further analysis of Group 1 (trekker) data (in progress) will hopefully clarify these findings.

7.6 Conclusions

On exposure to hypobaric hypoxia, both retinal arteries and veins dilate. The venous distension correlates with headache but no such correlation exists for arterial changes. Venous distension is greater in those with lower peripheral saturations at 5300m and in those who have less of a fall in $EtCO_2$ between 75m and 5300m, i.e. those who have less hypoxic ventilatory drive. Hence this study supports the venous system being instrumental in the pathogenesis of

high altitude headache and suggests that this may relate to hypoxic ventilatory response.

8 Chapter 8: HYPOXIC VENOUS MRI STUDIES

8.1 Hypoxia Causes Cerebral Venous Distension – a MRI Pilot Study

This pilot study is reported in The Headache of High Altitude and Microgravity – Similarities with Clinical Syndromes of Cerebral venous hypertension (Wilson, Imray et al. 2011).

8.1.1 Abstract

Hypoxia causes retinal venous distension. Clinically, cerebral venous hypertension causes headache. This study aimed to explore whether hypoxia causes cerebral venous distension.

Methods: Seven subjects had susceptibility weighted MRI scans prior to and upon completion of a 3-hour hypoxic (FiO_2 = 12%) episode. This was done as part of a study principally looking at arterial inflow (chapter 3).

Results: Five subjects reported headaches while 2 reported a feeling of "fullness". MRI images appeared to demonstrate cerebral venous distension in all subjects.

Conclusions: The hypothesis that hypoxia causes cerebral venous distension appears to be true. However, further study is required to confirm these findings.

8.1.2 Introduction

My retinal study, and other studies, have demonstrated that hypobaric hypoxia causes retinal venous distension and the severity of headache reflects the degree of distension (Bosch, Merz et al. 2009). Venous hypertension is known to cause headache in a number of different pathologies such as Idiopathic Intracranial Hypertension (IIH) (Sander, Poppert et al. 2011). I have already demonstrated that hypoxia causes a very significant rise in cerebral blood flow (Wilson, Edsell et al. 2011) and hence, I hypothesised that any degree of venous obstruction may cause cerebral venous distension to occur. With the dense innervation of areas such as the cavernous sinus with sympathetic and trigeminal fibres, this could result in the perception of pressure headache.

This study was principally a pilot test performed at the end of the hypoxic MRI study described in chapter 3 (hence subjects had been exposed to 3 hours of hypoxia). Following the arterial study sequences I performed a susceptibility-weighted sequence. Such a sequence is particularly good at demonstrating blood and hence venous vessels can be clearly seen.

8.1.3 Methods

The study was approved by UCL Ethics committee. Volunteers were 7 subjects from the Xtreme Everest group (5 male; mean age 34.4years, range 22-48). Full details of the methods of the study and MRI sequences are explained in chapter 3. Hypobaric hypoxia (FiO_2 = 12%) was achieved using a hypoxicator (Everest Summit Hypoxic Generator, Hypoxic Systems, New York, NY, USA) with 4 metres of elephant tubing to enable maintenance of hypoxia during the MRI acquisition. At the end of the normoxic MRI sequence and again at the end of the hypoxic MRI sequence, a susceptibility-weighted sequence was acquired. Concurrent brain oxygenation (rSO_2) was measured outside the MRI scanner as previously explained. Subjects were also asked if they had a headache or other symptoms to report.

8.1.4 Results

Five of the subjects reported having a headache at the end of the 3 hours of hypoxia. Two described a feeling of head “fullness”.

Mean peripheral saturations fell from (mean (±SEM)) 98.3% (±1.13) in normoxia to 74.9% (±3.72) at 3 hours of hypoxia ($p<0.001$). Mean cerebral oxygenation fell from 71.1% (±3.83) to 50.3% (±2.91) ($p<0.001$). Whilst it was not possible to quantify changes in cerebral venous dimensions (see technical limitations in discussion), all subjects appeared to demonstrate cerebral venous distension (figure 8-1). Despite the small numbers, the two subjects known to suffer with the worst headaches at altitude appeared to have the greatest cerebral venous distension.

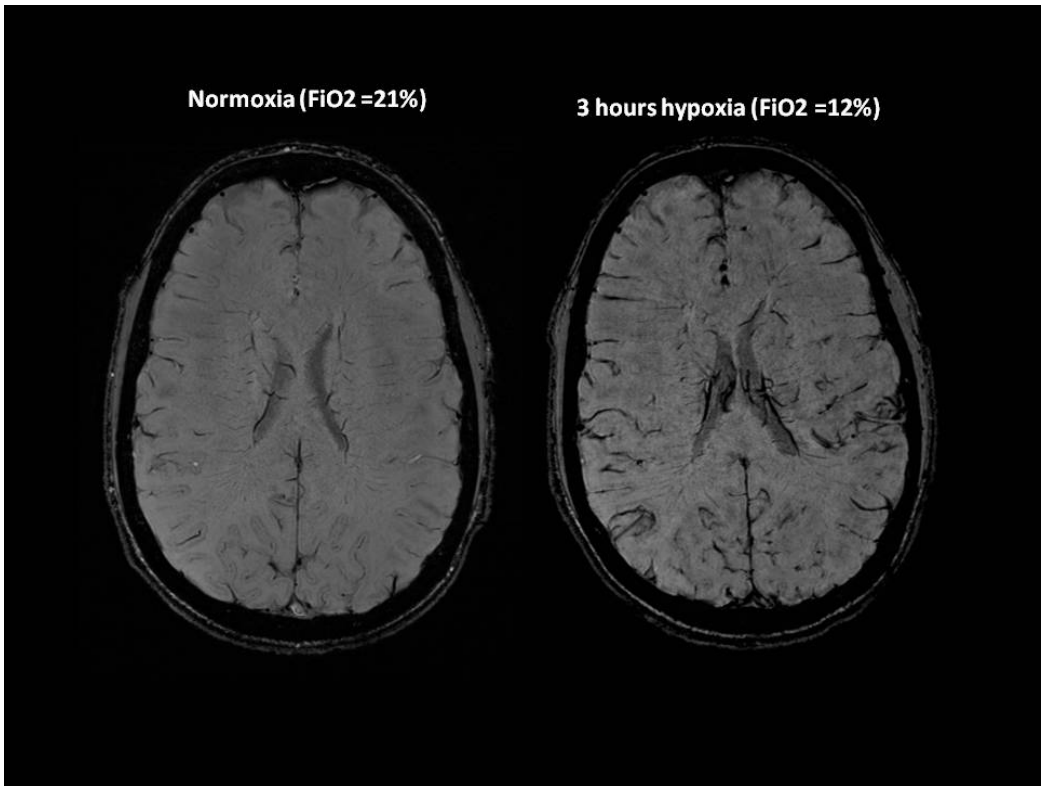


Figure 8-1 Corresponding susceptibility weighted images from the same subject in normoxia and hypoxia (FiO_2 =12%). The cortical and thalamostriate veins appear more prominent and distended in the hypoxic sequence.

8.1.5 Discussion

This study appears to demonstrate that cerebral venous structures distend with hypoxia. However, the small numbers and technical limitations meant that a further definitive study is required.

Technical Limitations

In designing this study, I sought advice regarding the best MRI imaging technique to study venous anatomy. Susceptibility-weighted images demonstrate blood vessels well because of the paramagnetic properties of blood. Because of other sequences that were being aquired concurrently (arterial spin-labelling), our radiologist did not want to use contrast. However, it became apparent after the study that the paramagnetic properties of oxy and deoxygenated-blood are themselves different. Hence, some aspects of the changes visible in the normoxic and hypoxic scans may actually be due to the changes in the paramagnetic properties of the blood as it desaturated rather than actual change to vessel calibre. As such, this precluded further analysis or quantification other than the qualitative findings described.

It was apparent that a more detailed study using a technique to image vessels that does not alter with the oxygenation of blood, would be necessary. I therefore designed another study that would also investigate where venous obstruction might be occurring.

8.1.6 Conclusion

From this initial pilot study it would appear that in hypoxia there is distension of intracerebral veins. However, this study has small numbers and the altered susceptibility weighting of blood means it should be repeated with bigger numbers and gadolinium to confirm my findings.

8.2 Restricted venous drainage causes greater cerebral venous distension in hypoxia

This study has been published as: The Cerebral Venous System and Anatomical Predisposition to High Altitude Headache, (Wilson, Davagnanam et al. 2013).

8.2.1 Abstract

I postulated that high altitude headache (HAH) results when hypoxia-associated increases in cerebral blood flow occur in the context of restricted venous drainage. This study investigated this hypothesis.

Methods: Eleven subjects underwent Gadolinium-enhanced Magnetic Resonance Venography before and during a hypoxic challenge (FiO_2= 0.11, 1 hour). Subsequent images were analysed for degree of cerebral venous engorgement and transverse sinus narrowing.

Results: Cerebral and retinal vein engorgement correlated (Spearman Rho 0.598, p=0.05), and rose as Combined (cerebral venous efferent) Conduit Score (CCS) fell (a measure of venous outflow restriction: r=-0.66, p<0.05 and r=-0.75, p<0.05 respectively).

Conclusion: This study supports the hypothesis that a relative restriction in venous outflow results in venous distension when cerebral blood flow is increased in the context of hypoxia.

8.2.2 Introduction

I have previously shown that retinal venous distension occurs at altitude and this correlates to headache severity. Similarly, a number of clinical headache syndromes relate to venous hypertension and have many characteristics similar to High Altitude Headache. I have reported a pilot Hypoxic MRI study which appeared to demonstrate cerebral venous distension with hypoxia, however, because of technical limitations it was not possible to report this confidently, nor was it possible to quantify this effect. I therefore sought to demonstrate cerebral venous engorgement (and to define the cerebral venous anatomy) using a more robust method.

8.2.3 Methods

Subjects: Eleven subjects (2 women; mean age = 37.2, range 21-74 years) were recruited (experienced trekkers/mountaineers from the Centre for Altitude, Space and Environment and from the Birmingham Medical Research Expeditionary Society). Following retinal imaging in normoxia, an intravenous cannula was inserted. Subjects then lay supine for 10 minutes, before continuous monitoring of peripheral arterial oxygen saturations (Nonin, Onyx Model 9500, Plymouth, MN USA) and brain oxygenation (rSO_2 using NIRS, as described in chapter 2) was commenced. Subjects were then rendered hypoxic for 60 minutes (*vide infra*), at the end of which period (and whilst still hypoxic) cranial magnetic resonance imaging was performed. The mean of three consecutive rSO_2 values was documented at each of three time points (normoxia, and after 30 and 60 minutes of hypoxia). Subjects were asked to describe and grade (0-4) any headache they had at the end of 1 hour of hypoxia. In the same manner, and after a minimum of 10 minutes recumbent rest, magnetic resonance imaging was performed under normoxic conditions at least 24 hours before or after hypoxic exposure.

Retinal Imaging: Each subject underwent bilateral retinal imaging (8TRC NW200 Non-Mydriatic Digital Opthalmoscope ; TopCon, Tokyo, Japan). This was done in normoxia prior to the study, and whilst still hypoxic at the end of the hypoxic MRI study. These images were subsequently analysed by a blinded observer with calibre measurements taken of retinal arteries and retinal veins.

Hypoxia: After baseline measurements, subjects were exposed to 1 hour of normobaric hypoxia (FiO_2 = 11%; approximately equivalent to an altitude of 4,400 m), using a tight fitting mask (Everest Summit Hypoxic Generator, Hypoxic Systems, New York, NY, USA) and extended MRI-compatible tubing. Inspired oxygen concentration was regularly monitored (Class R-17D Oxygen Sensor, Oxycheq, Marianna, FL, USA).

Magnetic Resonance Venography: MR venography was performed (3 tesla TIM TRIO, Siemens, Erlangen, Germany) using a 3D bolus-tracked gadolinium-enhanced MRV sequence. A 0.2ml/kg intravenous bolus of Dotarem (Guerbet,

Villepinte, France) was administered. Bolus tracking (Siemens CARE Bolus, Siemens, Erlangen, Germany) was performed at the posterior aspect of the superior sagittal sinus, with the MRV scan triggered at the first appearance of contrast. Parameters for the 3D MRV sequence were: TR 3.07ms, TE 1.11ms, FOV 300mm, flip angle 18°, 224 slices of 1mm thickness, voxel dimensions 1.0 x 0.8 x 1.0mm, centric phase-encoding order, acquisition time 1.01min. To prevent any residual gadolinium affecting the later imaging, hypoxic and normoxic MRI scans were performed at least 24 hours apart.

Image analysis: To equilibrate the windowing, the imaging window level and width were set respectively to a factor of half and double the value of contrast signal intensity within the sagittal sinus just proximal to the torcula. A single, blinded Consultant Neuroradiologist analysed the resultant images with axial, sagittal and coronal Subtracted Maximal Intensity Projection (MIP) reconstructions. Normoxic and hypoxic images were presented simultaneously, and images graded on a scale of being the same (0), having mild greater prominence of venous structures (1) or having considerably greater prominence of venous structures (2). Attempts to perform this analysis quantitatively (using digital subtraction, for example with FMRIB Software Library (FSL)) were not successful, largely because of small differences in extra-cranial contrast enhancement.

Combined Conduit Score (CCS): As a result of the findings in the high altitude study, the appearances of the transverse and sigmoid sinus were graded using the Combined Conduit Score (CCS) (Farb, Vanek et al. 2003). This system grades left and right drainage systems (using the sagittal sinus as the reference) as follows: 0 (aplastic), 1 (hypoplastic / severe stenosis: <25% of the lumen of the distal superior sagittal sinus), 2 (moderate narrowing: 25-50%), 3 (mild narrowing: 50-75%) and 4 (no significant narrowing: 75-100%). The two figures (out of 4) for each side are summed to give the combined conduit score, with 8 being the maximum signifying no narrowing. A diagram with the technique for calculating this score is in the Appendix (Figure 8-A).

8.2.3.1 Statistical Analysis

The primary endpoints were (i) cerebral venous distension with hypoxia and (ii) its relationship with CCS. SPSS (version 20, IBM, NY, USA) was used for analysis. Bivariate correlations were used (Pearson's for continuous data, Spearman's Rho for ordinal) as appropriate. Statistical significance was set at $p \leq 0.05$.

8.2.4 Results

8.2.4.1 General Result

Cerebral venous engorgement occurred in all subjects in response to hypoxia (Table 8-1).

Subject	$EtCO_2$ @1 hr / kPa	Headache Score at 1 hr	%age change SaO_2	%age change rSO_2	%age change Retinal venous distension	Venous prominence (0,1or 2)	Right TS score	Left TS score	CCS
Mean	2.18	1.00	-27.15	-37.21	16.58	1.36	3.36	2.91	6.27
SD	0.66	0.77	9.81	15.72	8.91	0.50	1.03	1.04	1.27
Upper CI	1.74	0.48	-33.74	-48.45	10.59	1.02	2.67	2.21	5.42
Lower CI	2.63	1.52	-20.57	-25.97	22.57	1.70	4.05	3.61	7.13

Table 8-1 Subject data recorded at the end of 1 hour of exposure to hypoxia (FiO_2 0.11). $EtCO_2$ represents end tidal CO_2. Venous prominence is scored as 0 (the same), 1 (mild) and 2 (considerable). Right and left Transverse Sinus (TS) scores and combined conduit scores (CCS) are described in the methods.

Figure 8-1 illustrates the typical changes that occur in venous drainage with hypoxia.

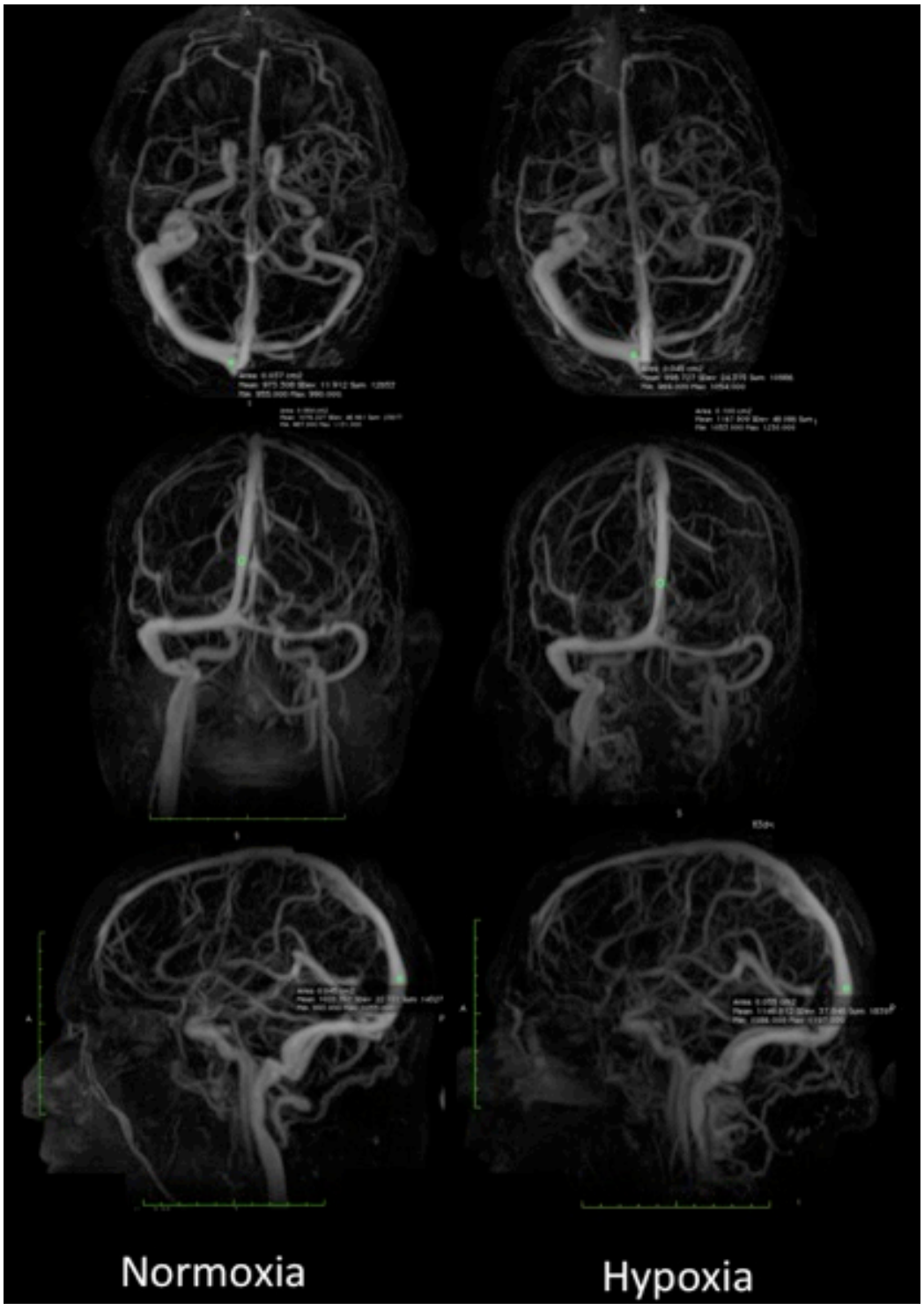


Figure 8-1 Exemplar of the increase in venous prominence noted in response to normobaric hypoxia (FiO_2 =11%). This subject has asymmetry in venous drainage, with relative narrowing of the left transverse sinus (CCS = Right 4; Left = 1. Total = 5).

8.2.4.2 Venous Changes and CCS

Reductions in venous CCS were associated with increasing distension of cerebral veins (Pearson's = -0.637, $p<0.05$; Spearman's Rho correlation = -0.655, $p<0.05$) and retinal veins (mean increase in retinal venous diameter +16.6% (±8.9%); Pearson Correlation = -0.775 $p<0.005$, Spearmen's Rho

correlation =-0.745, p<0.05). Two of the subjects with the smallest CCS also had the worst headaches, however, with the small numbers of this study and the subjective nature of headache after only 1 hour of hypoxia, a correlation between CCS and headache score was not possible.

8.2.4.3 Retinal Changes

Mean retinal venous diameter rose by 16.6% (±8.9%), the magnitude of engorgement correlating with that of the cerebral veins (Spearman Rho 0.598, p=0.05). There was a strong correlation between retinal venous distension and CSS (Pearson Correlation = -0.775 p<0.005, Spearman's Rho correlation =-0.745, p<0.05).

8.2.4.4 Brain Oxygenation

Those with large transverse sinuses (higher CCS) tended to have a smaller reduction in rSO_2 (Figure 8-2) although the correlation did not achieve significance (r = 0.61, p = 0.061).

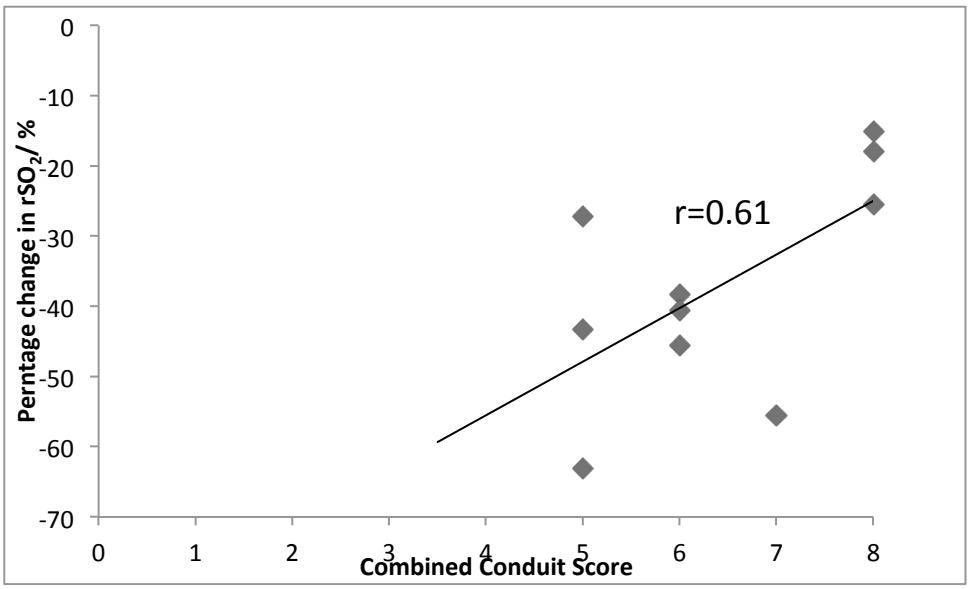


Figure 8-2: Change in rSO_2 with CCS. Although there is a positive correlation (r=0.61) it does not achieve significance (p=0.06)

8.2.5 Discussion

This study supports the hypothesis that venous engorgement (both retinal and cerebral) occurs with hypoxia. It also supports the concept that subjects with a greater restriction in venous outflow (smaller CCS score) have greater distension of cerebral veins.

I have been very cautious interpreting the headache scores in this small study. Headache data in this study were documented for reasons of safety. Only 8 of the 11 subjects actually reported headache, which was self-graded on our reported 1-4 scale (5 grade 1; 3 grade 2). Whilst two of the three subjects with the worst headache scores also had amongst the worst CCS scores (CSS = 5 in both), such data should be considered with extreme caution. High altitude headache may take some time to both develop and become established and maximal after physiological changes (such as those in vascular response) have occurred. Thus, any symptoms recorded with exposure to a single isolated and unsustained degree of normobaric hypoxia may not be expected to mirror those observed upon sustained exposure to a range of altitudes. The generally mild nature of the headaches observed reinforces these issues.

This study did not monitor $EtCO_2$. In view of the correlation between retinal venous distension and $EtCO_2$ we are currently undertaking another MRI study investigating arterial and venous changes concurrently (over 24hours of hypoxia) and $EtCO_2$ is monitored as part of this.

Overall however, this study appears to support my other work demonstrating greater headaches in those with narrower transverse sinuses (smaller CSS scores – chapter 6) and retinal venous distension with hypoxia (chapter 7). It also validated our pilot study.

8.2.6 Conclusion

This small hypoxic MRI study demonstrated venous distension with hypoxia and that this correlates with the degree of transverse sinus narrowing (CCS score). This is the first published study demonstrating cerebral venous distension with hypoxia and supports this thesis' hypothesis of venous system involvement in the development of high altitude headache.

9 Chapter 9: SUMMARY OF ADDITIONAL NEUROSCIENCE STUDIES NOT CORE TO THIS THESIS

9.1 Pupillometry

Pupillometry was performed on all 198 subjects during their ascent to Everest Base Camp. Unlike my previous study in Ladakh (Wilson 2008) where pupil dynamics were investigated within an hour of arrival and immediately the following morning, on the Everest expedition, pupil dynamics were studied 24 hours or 48 hours after arrival. The changes therefore in this study were much smaller presumably because an element of acclimatisation had occurred. Figure 9.1 demonstrates pupil aperture change with peripheral saturations for all trekkers in London (75m), Namche (3500m) and at Everest Base Camp (5300m). There is slightly less percentage change in pupil size following exposure to light as SaO_2 falls. The correlation is significant (Pearson's correlation - r=0.1365, p=0.003)

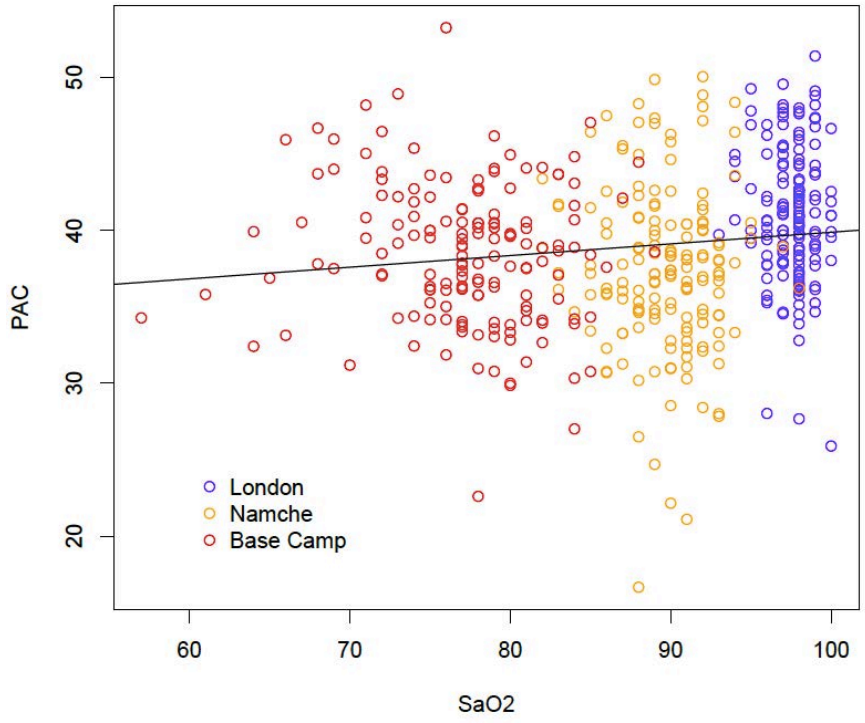


Figure 9.1 Graph of peripheral saturations (SaO_2/%) vs pupil aperture change (/%) for all trekkers (n=198) in London (75m), Namche (3500m) and at Everest base camp (5300m) p=0.003.

9.2 Intraocular Pressure

I measured intraocular pressure in the investigator team during the ascent to Everest Base Camp. No significant changes occurred during ascent and no correlation with headache reporting was noted.

9.3 Neuropsychology

Multiple neuropsychological studies were performed on all of the investigators and trekkers as detailed in chapter 2. Upon return a considerable amount of work was required to create a control group to study the learning effect that occurs with repeated neuropsychological tests. As such, we now have the largest controlled neuropsychological data set at altitude. Correlations of individual neuropsychological changes with physiological variables (e.g. SaO2, rSO2) still needs to be analysed, however group changes have been calculated.

The following tests were undertaken:

Trail Making (A and B) – Trail making A is simply a timed join the numbered circles exercise. Trail making B is join the circles 1, A, 2, B, 3, C etc (see appendix Figure 2Bi).

Controlled Oral Word Association Test (COWA): This tests the timed oral production of spoken words beginning with a designated letter. It consists of three word-naming trials, each lasting 1 minute, and the score is the sum of acceptable words on all three trials.

Stroop Color Word test (SCWT): This determines selective attention and executive functions (see Appendix figure 2-Bii). In part A, the time needed to complete reading 100 colour names is recorded. Part B is the time needed to say the 100 colours of words that are printed in different coloured ink to the colour they spell. The total number of correct/failures were registered.

Letter Cancellation: This is a timed test in which the subject has to cross out all the instances of a designated letter from a printed sheet (see Appendix figure 2-Biii).

Digit Symbol Modalities Test: Subjects are presented with a key containing a list of individual digits and corresponding abstract symbols (Appendix figure 2-Biv).

The subject then has to rapidly translate a long string of symbols into their corresponding digits. Scores reflect the number correct within 90 seconds.

Grooved Pegboard: This test of manual dexterity requires the subject to place 25 pegs into holes on a board varying in orientations using one hand moving from left to right with the right hand and right to left with the left hand. Scores indicate time to completion.

Block Design: This is designed to assess visuospatial reasoning (Appendix figure 2B-vi). The subject is presented with a set of red and white blocks along with a set of patterns presented one at a time. The blocks have to be assembled as rapidly as possible to match the pattern. Responses were scored for speed and accuracy.

Data Analysis: In order to assess change at a group level, cognitive outcomes were compared between baseline and follow-up assessments for the trekker and control groups using a series of mixed factor analyses of variance (ANOVA). This design consists of a within subject variable (neuropsychological test), with two levels (Baseline and each of the follow up assessments across ascent), and one between-subjects variable (group).

The reliable change index (RCI) method (Lewis, Maruff et al. 2007) was used to determine individual differences on test scores controlling for the effect of measurement error, and practice effects. RCIs were determined by subtracting the baseline score (X_1) from the follow up scores (X_2), giving $[DELTA]_X$ for each individual participant for a given task. The mean expected change for the controls, $[DELTA]_{Xc}$, calculated in the same way, was then subtracted from this, removing any practice effect. This score was then divided by the within subject standard deviation for control group (WSD), controlling for the expected variability.

These RCI scores were then used to create individual and combined test score ($Z_{combined}$) using the sum of z RCI scores for each test divided by the standard deviation of this summation in the control group. This technique identifies cognitive decline by comparing the changes in test scores of an individual trekker with changes in the test scores of the control group over the same

interval. The sign is adjusted so that negative z scores indicate deterioration from the baseline test. Confidence intervals were set at 95%.

Results

Complete cognitive data across all four assessments could be obtained in N = 153 trekkers with missing data being N = 13 in Namche; N = 23 in Everest Base Camp and N = 48 in Kathmandu.

Reasons for missing data include being unable to complete testing due to poor health/injury or tiredness (N=8 in Namche; N=12 in EBC; N=13 in Kathmandu); unable to complete due to other reasons (e.g. preoccupied with other concerns; no reading glasses) (N=6 in EBC; N=1 in Kathmandu), failure to administer/score part or whole assessment missing due to logistic difficulties (e.g. weather conditions; early flight departures; malfunction in testing apparatus/ timer) (N=4 in Namche; N = 5 in EBC; N=33 in Kathmandu), not motivated/withdrawal (N=1 in Kathmandu).

Changes in Cognitive Functioning

All participants scored within the reference range (within one standard deviation [SD] of general population test norms) in all neuropsychological testings. Trekkers and control had equivalent NP performance with the exception of REY Total where control outperformed trekkers on all assessments including baseline.

Generally, mean cognitive performance across NP tests was significantly better from baseline to follow-up assessments (in both trekkers and controls participants), albeit not uniformly so for all tests (i.e. TMT-A and COWA in Namche; GPND and REYD in EBC), showing evidence of the expected practice effects with repeated NP administrations over short time intervals (*p*s <.05).

Interaction effects were significant only for TMT-A (Namche p=.024); TMT-B (Namche p=.009); COWA (EBC p=.032; K p=.003); LCT (Namche p=.035; K p=.007); BD (Kathmandu p=.001; EBC p=.003; Kathmandu p=.001); SD (Namche TREND p=.056) and SPCWC (Kathmandu p=.034). Post-hoc tests showed that improvements over repeat assessments were greater in the control

group relative to trekkers where slopes were less steep but still significant. Evidence of a significant decline at the group level was not revealed on any neuropsychological measure for either group.

Individual Differences:

Controlling for practice effects using RCI methodology revealed decline in cognitive performance for the trekkers across ascent. As shown in Table 9-1, mean RCI scores were negative signifying cognitive decline across ascent for all tasks except for the GPD, GPND and AVLT-L, which only deteriorated at the highest point of ascent (i.e. Everest Base Camp).
Decline was more pronounced in tests of verbal ability (e.g. COWA; LCT) and executive function (e.g. Block Design; Trails B).

Incidence Of Cognitive Decline:

The numbers and percentages of declines, improvements, stability on NP tests across ascent as defined by RCI methodology are shown in Table 6-2. 90% RCI confidence intervals (not displayed) were used to classify individual performance – individuals whose RCI scores fell within the 90% CI were classified as cognitively stable on that test whereas scores outside were designated as significantly ‘improved’ or ‘deteriorated’. As can be seen there is considerable heterogeneity with changes in both directions. In comparing baseline performance to the subsequent testing points across ascent, 39.7% to 51.3% displayed decline in Namche, 39.3% to 49.2% in Everest Base Camp and 37.3% to 49.1% in Kathmandu. Notably however an almost equal percentage of individuals performed over 90% CI indicating improved performance.

	Namche		EBC		Kathamandu	
	M (Sd)	95% CI	M (Sd)	95% CI	M (Sd)	95% CI
Trails A*	-.69 (1.38)	-.89, -.50	-.16 (1.30)	-.35, .02	-.41 (.82)	-.54, -.28
Trails B*	-.80 (1.42)	-1.01, -.060	-.63 (.155)	-.86, -.41	-.05 (.79)	-.18, .06
COWA Total+	-.72 (1.76)	-.98, -.47	-.72 (1.45)	-.94, -.51	-.83 (1.17)	-1.02, -.64
LCT Time*	-.75 (1.66)	-.98, -.51	-.54 (1.59)	-.77, -.31	-.95 (1.48)	-1.18, -.71
Stroop Time*	-.17 (1.15)	-.33, -.004	-.24 (.99)	-.38, -.09	-.30 (.90)	-.45, -.16
GPD*	1.13 (2.61)	.76, 1.50	-.15 (2.29)	-.49, .17	.12 (1.74)	-.14, .39
GPND*	.56 (1.36)	.37, .75	-.32 (2.44)	-.68, .03	.24 (1.71)	-.02, .50
AVLT-L+	.06 (1.64)	-.16, .29	-.08 (1.79)	-.34, .17	.14 (1.46)	-.08, .37
AVLT-D+	-.48 (1.42)	-.69, -.28	-.56 (2.39)	-.91, -.21	-.47 (1.91)	-.77, -.17
Symbol Digit+	-.60 (1.32)	-.79, -.42	-.14 (1.29)	-.33, .04	-.43 (1.03)	-.59, -.27
Block Design+			-.87 (1.06)	-1.02, -.71	-.74 (.95)	-.89, -.59
Total RCI Score/no.tests	-.25 (.62)	-.34, -.16	-.36 (.71)	-.47, -.25	-.27 (.49)	-.35, -.18

Table 6.1. Mean Reliable Change Index (RCI)(SD) score and upper and lower boundaries at 95% confidence interval.

NP tests	NAMCHE			EVEREST BASE CAMP			KATHAMANDU		
	Worse n (%)	Stable n (%)	Better n (%)	Worse n (%)	Stable n (%)	Better n (%)	Worse n (%)	Stable n (%)	Better n (%)
TMTA	81 (41.5)	27 (13.8)	87 (44.6)	85 (45.9)	25 (13.5)	75 (40.5)	72 (45)	30 (18.8)	58 (36.3)
TMTB	77 (39.7)	22 (17)	84 (43.3)	75 (40.5)	21 (11.4)	89 (48.1)	74 (46.8)	26 (16.5)	58 (36.7)
LCT	78 (40.2)	21 (10.8)	95 (49)	76 (41.3)	18 (9.8)	90 (48.9)	57 (35.8)	30 (18.9)	72 (45.3)
STROOP	76 (40.9)	30 (16.1)	80 (43)	78 (43.8)	16 (9)	84 (47.2)	59 (39.3)	29 (19.3)	62 (41.3)
GPD	89 (45.6)	21 (10.8)	85 (43.6)	77 (41.6)	23 (12.4)	85 (45.9)	78 (49.1)	13 (8.2)	68 (42.8)
GPND	86 (44.3)	27 (13.9)	81 (41.8)	86 (46.7)	23 (12.5)	75 (40.8)	59 (37.3)	34 (21.5)	65 (41.1)
SD	83 (42.8)	26 (13.4)	85 (43.8)	85 (46.2)	21 (11.4)	78 (42.4)	71 (44.4)	30 (18.8)	59 (36.9)
AVLT-L	79 (40.9)	20 (10.4)	94 (48.7)	80 (43.7)	29 (15.8)	74 (40.4)	71 (44.7)	17 (10.7)	71 (44.7)
AVLT-D	96 (49.7)		97 (50.3)	72 (39.3)	54 (29.5)	57 (31.1)	67 (42.1)	31 (19.5)	61 (38.4)
COWA	97 (51.3)	15 (7.9)	77 (40.7)	77 (42.8)	29 (16.1)	74 (41.1)	62 (39.7)	25 (16)	69 (44.2)
BD				91 (49.2)	19 (10.3)	75 (40.5)	69 (45.1)	15 (9.8)	69 (45.1)
Total NP	75 (42.6)	25 (14.2)	76 (43.2)	72 (42.4)	20 (11.8)	78 (45.9)	63 (45)	19 (13.6)	58 (41.4)
TOTAL PLUS BD				70 (41.2)	25 (14.7)	75 (44.1)	58 (43.3)	21 (15.7)	55 (41)

Table 6-2: Patterns of change (frequency and percentage change) across ascent points compared to baseline (95% CI).
Note: The block design was not performed in Namche so total scores were calculated with and with block design in subsequent assessments.

Discussion:

This is the largest study to prospectively investigate the altitude-related changes in cognitive abilities across a trekking medical research expedition to Mount Everest. At the group level, cognitive performance improved in both trekkers and controls. While decline on individual tests was not evident at the

group level, this is almost certainly because repeat testing obscures deterioration of function because any “loss of ability” (i.e. worse NP performance) may be compensated by the gain made because of practice effect due to short time intervals between assessments.

Adjusting for practice effects using RCI methodology, study results provided clear evidence of overall cognitive impairment related to conditions of hypoxia. Trekkers were found to experience neuropsychological decline (as indexed by negative mean RCI scores) across several domains during the trek to Everest from baseline at sea levels to testing points at high altitude. Cognitive domains with greatest reliable declines across the ascent included visuo-spatial processing, complex attention, and verbal skills. Cognitive performance worsened with higher altitude with declines being uniformly evident in all NP tasks at the highest point of ascent, i.e. Everest Base Camp (5,300 m). Notably, cognitive decline persisted when trekkers descended to lower altitude. Although mean RCI of cognitive performance improved from Everest Base Camp to return to Kathmandu (1,300m) it did not return to baseline levels and was still lower than Namche (3,500m) on ascent, indicating that return to lower attitude does not readily/immediately restore the cognitive effects of exposure to extreme hypoxia.

Futher Analysis:

Further analysis of this data set and correlation with physiological variables (such as peripheral and regional brain saturation) are on going and will be published in due course.

Conclusion:

The initial analysis of the neuropsychological study data demonstrates that hypoxia is associated with a cognitive decline across a number of different modalities. Further analysis with physiological variables is required to study this in greater depth.

10 Chapter 10: DISCUSSION

10.1 Summary of This Thesis

This thesis set out to investigate in greater detail the cerebrovascular physiological changes that occur with hypoxia. The main findings that add to our knowledge are that:

1) Large cerebral arteries (demonstrated with the middle cerebral artery) dilate in response to hypoxia and this is detectable using ultrasound and MRI (chapter 3).
2) Subjects who experience cerebral oxygen desaturation with exercise at sea level tend to face greater desaturation during exercise at altitude. Such desaturation does not correlate with headache (chapter 4).
3) Those with smaller ventricular volumes and less pericerebellar CSF have a greater headache burden on ascent to altitude (chapter 5).
4) Both the retinal and cerebral venous systems distend in response to acute and sustained hypoxia (chapter 6) but only retinal venous distension correlates with headache burden.
5) Retinal venous distension negatively correlates with peripheral saturation at 5300m and with fall in $EtCO_2$ between 75m and 5300m. This implies that retinal venous distension is greater in those with a lower hypoxic ventilatory response.
6) Both retinal and cerebral venous distension correlate with restriction in venous drainage (chapter 6) which in turn correlates with headache severity.

The above findings fundamentally change the current understanding of high altitude headache from being a pure intracranial pressure, “tight-fit” problem to being a mismatch of arterial inflow and ability to drain venous blood. The “tight-fit” element, from the studies reported here, relates to the compliance (in terms of CSF space) that can accommodate this arterial/venous imbalance. Of note, this insufficiency of venous drainage is now thought to be fundamental in the development of “Space Flight Induced Intracranial Hypertension and Vision Alterations” (Alexander, Gibson et al. 2012) (see below) (Wilson, Imray et al. 2011).

Lack of Evidence for Raised ICP at Altitude

In addition to my studies, a number of other studies have occurred concurrently which imply that it is not a rise in intracranial pressure specifically that causes headache. For example, optic disc oedema occurs in a large proportion of subjects but its quantification does not correlate with AMS (Willmann, Fischer et al. 2011). Similarly, optic nerve sheath diameter (possibly the best non-invasive ICP monitoring technique currently available) has also been shown not to relate to high altitude headache (Lawley, Oliver et al. 2012). Henry Querfurth has developed an opthalmodynamometry technique for non-invasively monitoring ICP. This technique uses the measure of retinal vein occlusion pressures to infer intracranial pressure and has been validated with concurrent invasive ICP monitoring on intensive care(Querfurth, Arms et al. 2004). Like Marchbank's tympanic membrane displacement technique (Wright, Imray et al. 1995), opthalmodynamometry does not show any correlation between inferred ICP and AMS symptomatology (Querfurth, Lieberman et al. 2010).

In my initial review (chapter 1), I stated that the evidence for oedema causing high altitude headache syndromes was weak (Kallenberg, Bailey et al. 2007). Since then, other studies have demonstrated that a small amount of cerebral swelling does occur with hypoxia but this does not appear to correlate with the occurrence of AMS (Dubowitz, Dyer et al. 2009).

I shall now briefly review my main findings and the literature that has resulted from them.

10.2 Arterial Inflow

I have demonstrated that hypoxia causes a significant dilatation of the middle cerebral artery. The assumption that only pial vessels control CBF (as put forward by Fog in 1938 (Fog 1938)) no longer appears to be true in extreme hypoxia, and under such conditions, the increase in cerebral blood flow is probably greater than would be appreciated by any increase in velocity alone. This should be taken into account when reviewing early studies using transcranial Doppler during hypoxia. I assume that the changes I have observed in the middle cerebral artery occur across all intracranial vessels.

It can be noted from my study that although the correlation between MRA and ultrasound measurements of MCA diameter correlate well (r^2=0.674), the absolute values were quite different (mean TCD diameter measurements in normoxia and hypoxia were 5.44 and 6.28 mm, while corresponding MRA diameter measurements were 3.04 and 3.27mm). Colino and Binsted (Colino and Binsted 2012) questioned my data and demonstrated that there was a proportional bias in the sample measured by the regression line (y=0.59x +0.01). They noted that a sizeable proportion of my data lay above the upper limit of agreement (which expresses the maximum deviation between any two repeated observations with 95% certainty), the upper limit being 3.23 and the lower being 2.04. To investigate this further they transformed this data, logging the differences in ultrasound and MRA measured diameters and plotting this against the mean log of the ultrasound and MRA measured diameter (figure 10-1). From this they concluded that there was indeed good agreement between ultrasound and MRA measured MCA diameters, despite a systematic bias and therefore only a correction factor need be applied to the data to correct the bias. They also stated that the data points in figure 10-1 are homoscedastic (uniform over a measurement range, parametrically confirmable via Levene's test), thus random error can be estimated using the standard error of the mean, coefficient of variation and standard deviation. The coefficient of variation from the log-transformed data (figure 10-2) is uniform across the measurement range and maintains a value of ~4.1% from which they concluded that the measurement tools were well calibrated.

While it is convenient that Colino and Binsted's work demonstrates good calibration of my TCD and MRI measurements, it should be noted that graphing a difference (TCCD – MRA) of related variables that have previously been shown to have a linear relationship would result in a linear plot. Hence some of their conclusions may actually relate to the technique they have used to analyse the two variables.

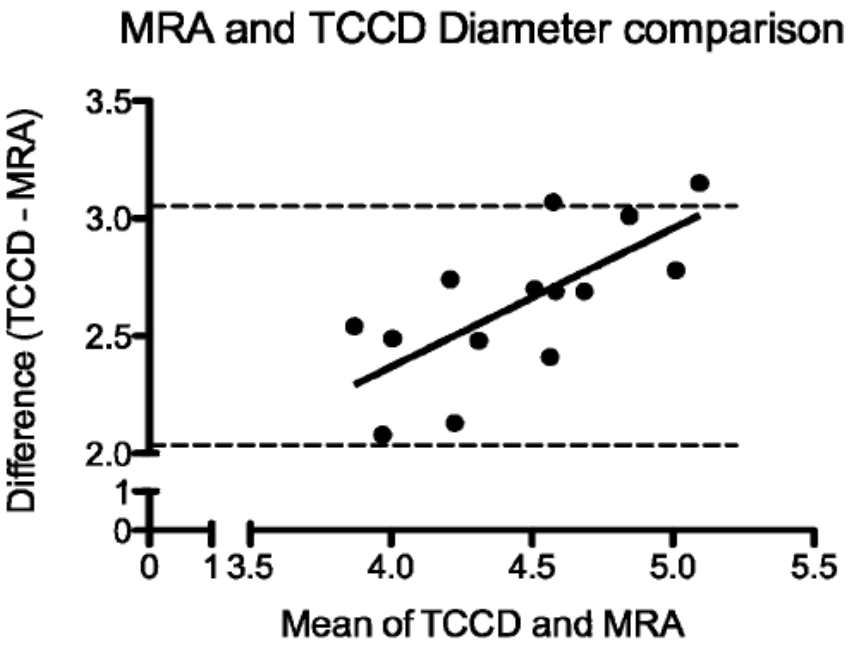


Figure 10-1 MRA and ultrasound MCA vessel diameter comparison. The abscissa depicts the mean of ultrasound and MRA diameter measurement in a participant-wise manner. The ordinate depicts the difference of ultrasound and MRA within each subject. There is a proportional bias in the sample measured by the regression line (*y*= 0.59x +0.01). The limits of agreement (depicted by two dashed lines) cannot be used to measure agreement because it assumes there is no relationship between error and the magnitude of the measured value. Therefore the data needs to be transformed to eliminate proportional bias. This virtually eliminates the proportional bias in the sample. From Colino and Binsted's analysis of my data (Colino and Binsted 2012).

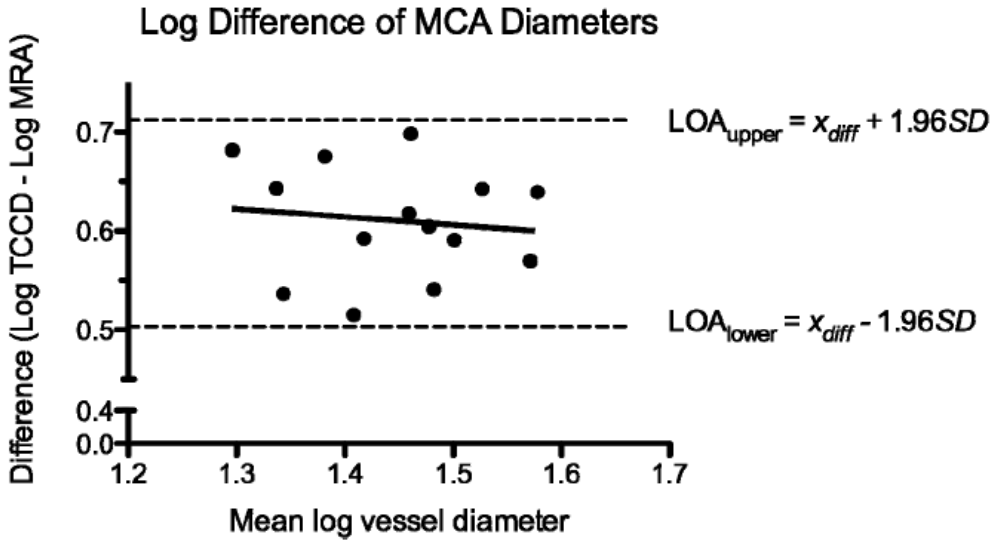


Figure 10-2 MRA and ultrasound log MCA vessel diameter comparison. The abscissa depicts the mean log ultrasound and MRA diameter measurement in a participant-wise manner. The ordinate depicts the difference of the log ultrasound and log MRA within each subject. Logarithmic transformation of the data virtually eliminated the proportional bias in the sample (regression line: -0.08*x* +0.72). The limits of agreement (two dashed lines) is better suited to this transformed data. From a sample of 14 data points, Colino and Binsted conclude that there is good agreement between ultrasound and MRA vessel diameter measurements despite a systematic bias. Therefore a correction factor need only be applied to the data to correct this bias. From Colino and Binsted's analysis of my data (Colino and Binsted 2012).

Following my study, Ogoh *et al.* used a similar technique to investigate velocity and calibre changes in the internal carotid and vertebral arteries during hypoxia and concluded that isocapnic hypoxia (FiO_2 =12%) increased flow in both, while hypocapnic hypoxia only increased flow in the vertebral arteries (Ogoh, Sato et al. 2012). Similarly, Willie *et al* (Willie, Macleod et al. 2012) have studied intra and extra-cranial vessel changes both to hypoxia and hypo/hypercarbia. They demonstrated that $PaCO_2$ has a marked effect on extracranial vessels, the internal carotid artery for example "dilating" from 47mm (when $PaCO_2$ = 15mmHg) to 59mm (when $PaCO_2$ = 65mmHg) (n=12), and similarly an approximate ~25% increase in diameter in the vertebral as well. Although velocities increased in extracranial vessels with hypoxia, there was no change in calibre of the internal carotid artery, although the vertebral artery did increase in size (by 9% with an SaO_2 of 70%). They conclude that, as I have said in my publication, *there is now ample support in the literature to preclude the assumption of constant cerebral vessel diameter at extremes of blood gases.*

In the year following our expedition to Everest, Dubowitz's group reported that, using arterial spin labelling MRI, cerebral blood flow was found to increase in 12 subjects rendered hypoxic for 30 minutes (FiO_2 = 12.5%), but there was no difference between those who were AMS susceptible (n=6) and AMS resistant (n=6) nor was there any difference between grey and white matter blood flow (Dyer, Hopkins et al. 2008). These are small numbers, but again this study points away from arterial blood delivery being the primary cause of high altitude headache.

10.2.1 Clinical Implications of Arterial Findings

The finding that middle cerebral artery calibre is not constant has significant implications for the use of transcranial Doppler in the clinical setting when a patient's oxygenation status may alter. It should therefore become routine that, while measuring velocity, a vessel calibre measurement should also be made, if nothing more than to confirm consistency in location of insonation and no gross changes in calibre.

10.3 Brain Oxygenation

My studies demonstrated that:

1) At sea level rSO_2 rises with exercise to AT and in many (approximately half) is still increased at VO_2Max compared to baseline.
2) There was a degree of acclimatisation with improved rSO_2 in the day 2 group over day 1 group.
3) Following VO_2Max at altitude, there is commonly a further dip in rSO_2 (between 24 and 48 seconds later) where rSO_2 is at its lowest – this could be biological or a technological phenomenon.
4) Left and right NIRS readings correspond at all altitudes except on arrival at Namche (3500m) which is the location of the biggest fall in FiO_2. Hence in acute hypoxia, there may be an element of protection given to the dominant (in most people left) cerebral hemisphere.
5) At rest, rSO_2 falls to a greater extent (of its percentage baseline) compared to SaO_2.
6) During exercise, especially when more hypoxic (at 5300m), rSO_2 falls to a greater extent than SaO_2. This and the previous point could imply a greater cerebral usage of oxygen than in the periphery or it could imply a shift in the arterial:venous compartment volumes.
7) Mean rSO_2 falls with increasing age and increases with increasing height. Although statistically significant both of these are probably clinically insignificant.
8) The mean female rSO_2 is consistently less than the mean male rSO_2.
9) In the cluster analysis there is no differences between Cluster 1 and Cluster 2's peripheral saturations, at rest or during exercise at any altitude; however, cluster 1 consistently (at all altitudes and exercise levels) cerebrally desaturate more than cluster 2. This would imply that cluster 1 either extracts more oxygen or develops a greater venous compartment than cluster 2.
10) Those who cerebrally desaturate more had greater headache scores however this did not achieve significance.

I had hoped to be able to analyse the components of NIRS data that contribute to the rSO_2 algorithm in greater detail. Prior to our trip to Everest, I used a Hamamatsu NIRS device (Hamamatsu Photonics, Tokyo, Japan) and I had wanted to use other systems that would enable greater interpretation of the near infra-red signal beyond just rSO_2. However, I had to balance this with also taking a light-weight, robust, battery powered system that was affordable (we used 2 machines at each lab concurrently, i.e. a total of 8 niroscopes were required). Because of this, I used the Invos system.

Subudhi *et al* have questioned whether cerebral oxygen delivery limits incremental exercise performance (Subudhi, Olin et al. 2011). They hypothesised that raising end tidal pCO_2 during exercise would increase cerebral blood flow, oxygen delivery and hence peak power output on a cycle ergometer. However, manipulating $EtCO_2$ like this was not found to increase power output.

10.3.1 Clinical Implications of Brain Oxygenation Findings

My study, the largest cohort of brain oxygenation monitoring during ascent to altitude, did not reveal any correlation between rSO_2 and headache. Possibly the most useful lesson is that it is very difficult to interpret rSO_2 in isolation. A greater understanding of what the value is derived from would enable greater interpretation. Despite this, it appears that some people are better at maintaining rSO_2 (especially during exercise) in hypoxia than others.

10.4 Venous Outflow

One of the main findings in this thesis is that an imbalance between arterial inflow and venous outflow could be the underlying mechanism in the development of high altitude headache. This may be the initial step (rather than oedema formation) that subsequently leads to an ICP rise (if the later actually occurs).

The venous system tends to be the forgotten component of cerebral circulation. There have been many high altitude studies investigating arterial inflow but

none (until ours) investigating venous outflow. As such, a brief reminder of venous anatomy and physiology is provided below.

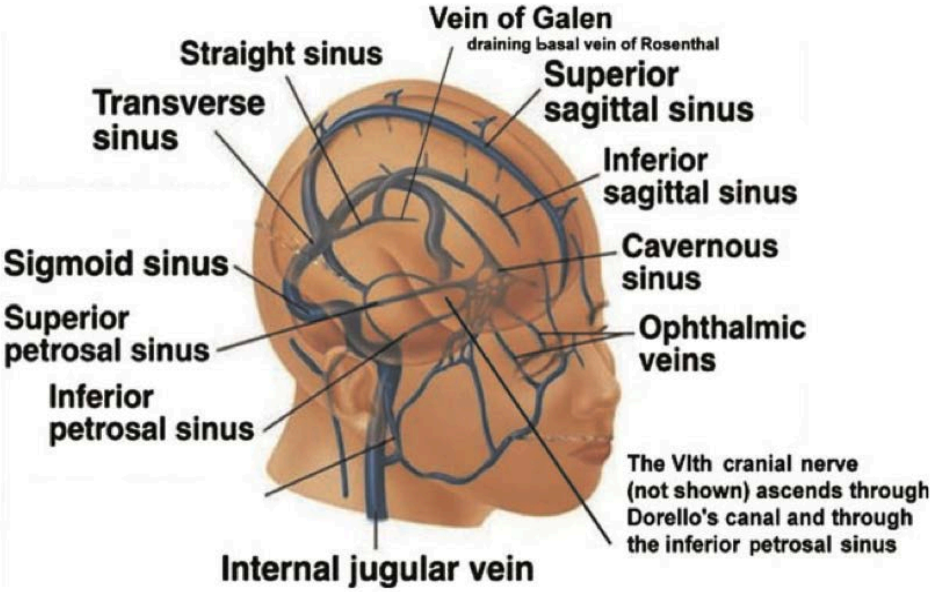


Figure 10-3 A Simplified diagram of basic intracranial venous structures (from an article I wrote for High Altitude Medicine and Biology) (Wilson, Imray et al. 2011).

At rest, the brain receives approximately 14% of the cardiac output, around 700ml per minute (McArdle, Katch et al. 2006). This volume also needs to be drained per minute, a factor often overlooked. The average male intracranial volume is only twice this (1473ml) (Abbott, Netherway et al. 2000). The internal jugular veins are the main venous drainage in humans and hence any obstruction in this exit route will increase venous and subsequently intracranial pressures (as well known to any neurointensivist / neurosurgeon who regularly sees raised intracranial pressure secondary to tight cervical collars).

As bipeds with large brains, humans have developed unique anatomical differences compared to other mammals. Neurosurgeons are distinctly aware that the sagittal sinus has a negative pressure when patients are in the sitting position as this can result in (potentially fatal) air embolism if opened. When supine, bleeding from the sinuses can be torrential. Most other mammals do not have such pressure changes with which to contend.

Three main venous drainage systems converge into the internal jugular veins (figure 10-3):

a) Cortical venous drainage occurs via bridging veins that cover the brain surface. These veins drain into the superior sagittal sinus which flows posteriorly to the torculla (confluence of sinuses) and then to the transverse sinus (in most people, to the right transverse sinus).
b) Deeper (anterior) venous drainage occurs into the cavernous sinuses anteriorly, which in turn drain via superior and inferior petrosal sinuses into the jugular bulbs.
c) Central (thalamic) areas drain via a series of small veins into the internal cerebral veins superiorly and the basal veins of Rosenthal inferiorly. These unite (behind the splenium of the corpus callosum) to form the Great Vein of Galen which then drains via the straight sinus to join the torcula and then to the transverse sinus (in most people, to the left transverse sinus).

The final common venous outlets for all the above tributaries are the two internal jugular veins. Very minor additional venous drainage is provided by orbital veins and vertebral venous plexi (the latter of which are far more important in supine mammals such as swine).

The sinuses themselves comprise dura mater lined with endothelium hence are very susceptible to external compression and distension. The cavernous sinus has extensive sympathetic innervation and other sinuses have trigeminal innervation hence distension can be interpreted as pain / pressure before any rise in ICP.

The work demonstrated in chapter 6 implies that both the retinal and cerebral venous systems distend with hypoxia. Our studies are the first to demonstrate:

1) Cerebral venous distension with hypoxia.
2) Greater headache burden in subjects with relative venous outflow insufficiency.
3) and to correlate intracerebral venous distension with retinal venous distension.

This represents a fundamental change in our understanding of the pathogenesis of high altitude headache. From a clinical perspective, it

demonstrates that the currently static Monro-Kellie doctrine needs to be revised to a dynamic model.

Another study that was carried out as part of the Caudwell Xtreme Everest project was led by Dan Martin. This investigated sublingual microcirculatory changes using sidestream dark-field (SDF) imaging (figure 10-4). He demonstrated both on Cho Oyu (8201m) and on Everest (8848m) (Martin, Ince et al. 2009; Martin, Goedhart et al. 2010) that the microcirculatory flow index reduced in small and medium sized blood vessels. Figures 10-5 and 10-6 show typical images at 75 and 4,900m respectively. It is not yet clear why the microcirculation slows to such a great extent. It may be a rise in haematocrit increases blood viscosity. It has also been suggested that the appearances are very similar to those seen in heart failure resembling a venous stagnation. Although the circulation studied using SDF is extracerebral, it may be that a similar phenomenon is occurring intracerebrally.

Fig 10-4 Dan Martin using the SDF camera to assess his sublingual microcirculation.

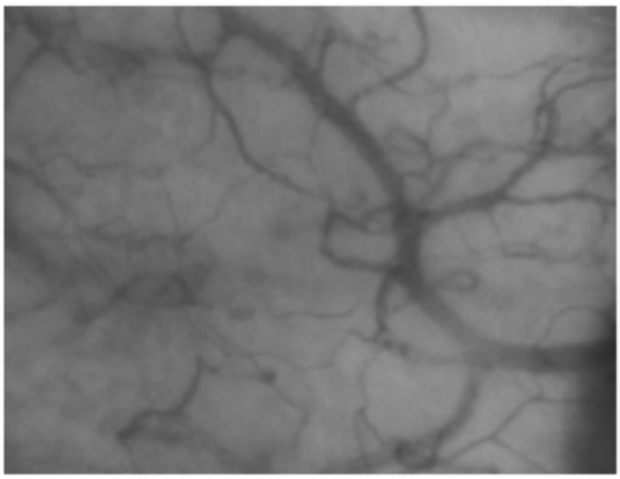


Figure 10-5a Typical still image captured from video footage of sublingual microcirculatory blood flow at sea level (75m).

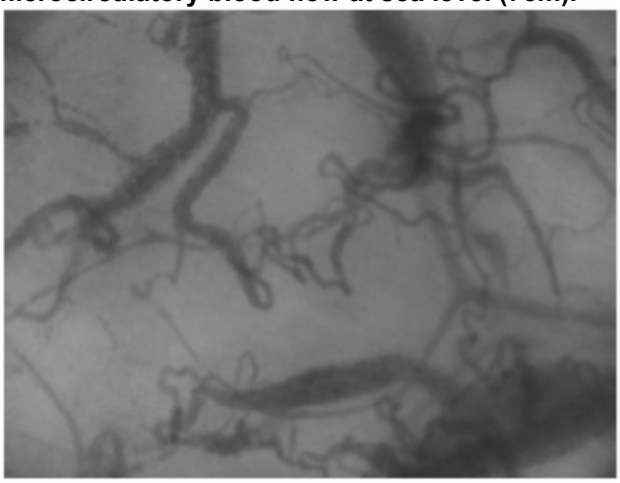


Figure 10-5b Typical still image captured from video footage of sublingual microcirculatory blood flow at altitude (4,900 m).

Since starting my work, a number of other studies have failed to demonstrate any link between ICP / oedema formation and high altitude headache.

Mairer et al have recently demonstrated (again) that simulated hypoxia (FiO_2 = 11%) causes very mild oedema which is increased with hypoxic exercise. Despite this, oedema formation does not correlate with AMS scoring /headache (Mairer, Gobel et al. 2012).

To date studies using different modalities to assess intracranial pressure are failing to show a correlation with headache. Most recently, optic nerve sheath

diameter, probably the best non-invasive ICP monitoring technique currently available, appears not to correlate with high altitude headache (Lawley, Oliver et al. 2012).

10.4.1 Clinical Implications of Venous Findings

10.4.1.1 Idiopathic Intracranial Hypertension (IIH)

Idiopathic Intracranial Hypertension (IIH) is a condition characterised by headache, nausea and vomiting. Both IIH and high altitude headache are commonly and successfully treated with acetazolamide (Bono, Messina et al. 2008). I have drawn extensive comparisons between IIH and high altitude headache and space adaptation syndrome in our hypothesis paper (Wilson, Imray et al. 2011). Bilateral transverse sinus stenosis is found in 90% of IIH sufferers (Pickard, Czosnyka et al. 2008) and is successfully treated with endoluminal stenting (Higgins, Cousins et al. 2003). More recently, Sander (Sander, Poppert et al. 2011) has reported that using Duplex ultrasound with contrast, the "Time to peak" (the time for contrast to go from the antecubital fossa, through the common carotid then peak in the internal jugular) is prolonged in patients with IIH. This itself implies venous congestion.

It may well be that the venous system is involved in more "idiopathic headaches" and hence it should be considered when no other cause can be found. Hypoxia may be a tool to "induce" headaches for study in those susceptible.

10.4.1.2 Trauma

Hypoxia is a common sequelae of trauma. This can occur, for example, with the loss of airway following head injury. In addition to the obvious ischemic effect this has on neurons, Goodman *et al* have demonstrated that inflammatory cytokines (interleukin-6, keratinocyte-derived chemokine, macropharge inflammatory protein-1α and neuron specific enolase) all increased with hypoxic exposure to simulate a flight transfer after head injury in mice (Goodman, Makley et al. 2011). Hence hypoxia following brain injury could contribute to a further secondary injury.

Our study would also imply that hypoxia has a direct cerebrovascular effect resulting in venous hypertension. There is increasing evidence for this clinically as well. Swelling brain compresses venous sinuses which result in greater venous pressures and a cycle of worsening cerebral swelling. This Starling resistor effect may account more for the upstroke in pressure with increasing volume than the "tight fit" closed box model itself. Intervening early to reduce this pressure *on the venous system* may be more important than the intervention to improve cerebral perfusion pressure (Wilson, Wise et al. 2012).

10.4.2 Microgravity

My interest in this field started in 1994 trying to assess intracranial pressure non-invasively in astronauts. At the time it was thought that the rise in intracranial pressure might account for the space adaptation syndrome or space motions sickness that was occurring in over 70% of astronauts in the first few hours of space flight (Torikoshi, Wilson et al. 1995). NASA have not yet flown any of the non-invasive ICP monitoring tools that have been developed, partly because of cuts in budget and partly because none of the techniques developed are yet truly reliable enough to be used for space deployment.

Over the last 2 years however, there has been a dramatic increase in interest in intracranial pressure and the venous system in space. Many astronauts have reported problems with reduced visual acuity, increasing with duration of space flight. This has obvious implications with any planned long duration mission to Mars (minimum duration 630 days).

In post flight questioning of nearly 300 astronauts, 23% reported subjective visual deterioration following short-duration flight (< 2 weeks) while 47% reported deterioration with long-duration (~6 month) flights (Mader, Gibson et al. 2011). Specifically in the 37 crewmembers who have completed longer term International Space Station (ISS) missions, 21 have been evaluated for visual loss, of which 16 have signs and symptoms (of varying severity) (Personal Communication Yael, Barr, Visual Impairment / Intracranial Pressure Project Scientist). A recent evidence report from NASA

(http://humanresearchroadmap.nasa.gov/Evidence/reports/VIIP.pdf)(Alexander, Gibson et al. 2012) goes through many of these cases in great detail and concludes, as I suggested in my hypothesis paper (Wilson, Imray et al. 2011), that the underlying pathology is likely to be venous hypertension.

Jugular venous distension is a consistent and well-described finding upon arrival in microgravity (Herault, Fomina et al. 2000; Arbeille, Fomina et al. 2001). Tom Weiner has coined the term “Space Obstructive Syndrome” (Wiener 2012) and believes that the level of obstruction is the internal jugular vein, principally under the sternocleidomastoid muscle.

10.4.3 Hypoxia in Critical Care

One of the principal tenants of the Caudwell Xtreme Everest project was to learn more of the effects of hypoxia to translate this knowledge into the critical care environment (Grocott, Montgomery et al. 2007). There are many non-neuroscience studies that have results directly relevant to critical care (Grocott, Martin et al. 2009; Martin, Levett et al. 2009; Edwards, Murray et al. 2010; Holloway, Montgomery et al. 2011; Levett, Fernandez et al. 2011; Levett, Radford et al. 2012). My principal findings, relevant to critical care, are that arterial vessel diameter increases with hypoxia (hence TCD may be unreliable) and that the venous system can become engorged when hypoxic. The latter suggests that minimising venous engorgement by preventing hypoxia may be essential in treating venous hypertension and raised intracranial pressure.

10.4.4 Psychological changes

I performed extensive psychological testing as part of my studies with the aim of correlating this with other physiological variables. Bjursten *et al* compared neurocognitive function and S100B (a marker of brain injury) release (Bjursten, Ederoth et al. 2010). They demonstrated a correlation between increased S100B and Lake Louise score and some neurocognitive tests. However, their study sample size was only 7 people, hence little can actually be concluded. Further analysis of our neuropsychological study may reveal correlations with physiology.

10.5 Study Limitations and Further Studies

The studies described within this thesis have a number of limitations. The ascent rate was such that the principal aim was to study the effects of hypoxia and adaptation rather than acute hypoxia (and headache / resulting clinical problems). Also, by performing the investigations at 24 and 48 hours after arrival at each altitude, large acute changes (such as those expected with pupillometry) may have diminished with acclimatisation. Certain tools such as headache burden assessment would be simplified in a future study and raw measurements (e.g. that make up brain oxygenation calculation) would be recorded rather than just the resulting figure.

I have looked at cerebral blood flow independently from cerebral venous drainage. A further study will be to demonstrate that subjects vary in the increase in cerebral blood flow that is required to maintain cerebral oxygenation for a given reduction in FiO_2. Some subjects in my studies have been able to maintain peripheral saturations to a greater extent than others. They have a lower headache burden (chapter 4), presumably because even if they have slight restrictions in outflow, they do not mount such an increase in CBF. Conversely subjects who are unable to maintain SaO_2 probably increase CBF to a greater degree, unmasking any outflow restriction. I am currently undertaking this study with Warwick University in Coventry.

10.6 Summary

This PhD thesis has investigated arterial cerebral blood flow, brain oxygenation and venous outflow in hypoxia. It has used both high altitude (hypobaric) hypoxic and sea-level (normobaric) hypoxia to demonstrate the following:

- Cerebral Arteries:
    - Distend / dilate in hypoxia (demonstrated both with ultrasound and MRI) – which results in a dramatic increase in cerebral blood flow. This has negated the long held assumption applied to the use of transcranial Doppler at altitude, that the vessel diameter remained constant.
- Brain Oxygenation:
    - Brain oxygenation decreases at altitude, that in acute jumps, the left cerebral hemisphere appears to be relatively protected from hypoxia and, since brain oxygen falls to a greater extent than peripheral arterial, an element of cerebral venous engorgement occurs.
- Venous System:
    - The retinal veins distend at altitude and this correlates to headache severity
    - Cerebral veins distend in hypoxia.
    - Transverse sinus anatomy correlates with headache at high altitude and with the degree of venous distension in normobaric hypoxia.

Hence, this PhD has demonstrated that venous congestion may be the underlying mechanism of high altitude headache and subsequent oedema formation.

10.7 Final Conclusion

The Monro Kellie Doctorine is a simple static concept. However, with nearly a litre of blood entering and leaving an individual's cranium each minute, the system is in fact very dynamic. Small changes in this very important dynamic component of intracranial physiology could account for the development of high

altitude headache in the same way that it does in idiopathic intracranial hypertension. A greater understanding of this concept is important in understanding intracranial pressure changes at altitude, in headache syndromes and trauma, and as is now being discovered, in microgravity.

11 References

(1995). "Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group." N Engl J Med **333**(24): 1581-1587.

(2003). "ATS/ACCP Statement on cardiopulmonary exercise testing." Am J Respir Crit Care Med **167**(2): 211-277.

(2004). "The International Classification of Headache Disorders: 2nd edition." Cephalalgia **24 Suppl 1**: 9-160.

Abbott, A. H., D. J. Netherway, et al. (2000). "CT-determined intracranial volume for a normal population." The Journal of craniofacial surgery **11**(3): 211-223.

Ainslie, P. N., K. Burgess, et al. (2007). "Alterations in cerebral dynamics at high altitude following partial acclimatization in humans: wakefulness and sleep." J Appl Physiol **102**(2): 658-664.

Ainslie, P. N., S. Ogoh, et al. (2008). "Differential effects of acute hypoxia and high altitude on cerebral blood flow velocity and dynamic cerebral autoregulation: alterations with hyperoxia." J Appl Physiol **104**(2): 490-498.

Ainslie, P. N. and M. J. Poulin (2004). "Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide." J Appl Physiol **97**(1): 149-159.

Alexander, D. J., O. D. Gibson, et al. (2012). Evidence report: Risk of Space-Induced Intracranial Hypertension and Vision Alterations. Lyndon B Johnson Space Center, Houston, Texas, National Aeronautics and Space Administration.

Ando, S., Y. Yamada, et al. (2010). "Reaction time to peripheral visual stimuli during exercise under hypoxia." J Appl Physiol **108**(5): 1210-1216.

Anooshiravani, M., L. Dumont, et al. (1999). "Brain magnetic resonance imaging (MRI) and neurological changes after a single high altitude climb." Med Sci Sports Exerc **31**(7): 969-972.

Appenzeller, O., V. E. Claydon, et al. (2006). "Cerebral vasodilatation to exogenous NO is a measure of fitness for life at altitude." Stroke **37**(7): 1754-1758.

Appenzeller, O., C. Passino, et al. (2004). "Cerebral vasoreactivity in Andeans and headache at sea level." J Neurol Sci **219**(1-2): 101-106.

Arbeille, P., G. Fomina, et al. (2001). "Adaptation of the left heart, cerebral and femoral arteries, and jugular and femoral veins during short- and long-term head-down tilt and spaceflights." European journal of applied physiology **86**(2): 157-168.

Arjamaa, O. and M. Nikinmaa (2006). "Oxygen-dependent diseases in the retina: role of hypoxia-inducible factors." Exp Eye Res **83**(3): 473-483.

Arzy, S., M. Idel, et al. (2005). "Why revelations have occurred on mountains? Linking mystical experiences and cognitive neuroscience." Med Hypotheses **65**(5): 841-845.

Bailey, D. M. (2003). "Radical dioxygen: from gas to (unpaired!) electrons." Adv Exp Med Biol **543**: 201-221.

Bailey, D. M. (2004). "Ascorbate, blood-brain barrier function and acute mountain sickness: a radical hypothesis." Wilderness Environ Med **15**(3): 231-233.

Bailey, D. M. and B. Davies (2001). "Acute mountain sickness; prophylactic benefits of antioxidant vitamin supplementation at high altitude." High Alt Med Biol **2**(1): 21-29.

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

Bailey, D. M., R. Roukens, et al. (2006). "Free radical-mediated damage to barrier function is not associated with altered brain morphology in high-altitude headache." J Cereb Blood Flow Metab **26**(1): 99-111.

Bartsch, P., D. M. Bailey, et al. (2004). "Acute mountain sickness: controversies and advances." High Alt Med Biol **5**(2): 110-124.

Bartsch, P., M. Maggiorini, et al. (1991). "Prevention of high-altitude pulmonary edema by nifedipine." N Engl J Med **325**(18): 1284-1289.

Bartsch, P., M. Maggiorini, et al. (1991). "Enhanced exercise-induced rise of aldosterone and vasopressin preceding mountain sickness." J Appl Physiol **71**(1): 136-143.

Bartsch, P. and M. Schneider (2001). "Pharmacological prevention of acute mountain sickness. Same ascent rates must be used to assess effectiveness of different doses of acetazolamide." Bmj **322**(7277): 48-49.

Basnyat, B., J. H. Gertsch, et al. (2006). "Acetazolamide 125 mg BD is not significantly different from 375 mg BD in the prevention of acute mountain sickness: the prophylactic acetazolamide dosage comparison for efficacy (PACE) trial." High Alt Med Biol **7**(1): 17-27.

Basnyat, B., J. H. Gertsch, et al. (2003). "Efficacy of low-dose acetazolamide (125 mg BID) for the prophylaxis of acute mountain sickness: a prospective, double-blind, randomized, placebo-controlled trial." High Alt Med Biol **4**(1): 45-52.

Basnyat, B. and D. R. Murdoch (2003). "High-altitude illness." Lancet **361**(9373): 1967-1974.

Basnyat, B., T. Wu, et al. (2004). "Neurological conditions at altitude that fall outside the usual definition of altitude sickness." High Alt Med Biol **5**(2): 171-179.

Basu, M., R. C. Sawhney, et al. (2002). "Glucocorticoids as prophylaxis against acute mountain sickness." Clin Endocrinol (Oxf) **57**(6): 761-767.

Baumgartner, R. W., P. Bartsch, et al. (1994). "Enhanced cerebral blood flow in acute mountain sickness." Aviat Space Environ Med **65**(8): 726-729.

Baumgartner, R. W., A. M. Siegel, et al. (2007). "Going high with preexisting neurological conditions." High Alt Med Biol **8**(2): 108-116.

Baumgartner, R. W., I. Spyridopoulos, et al. (1999). "Acute mountain sickness is not related to cerebral blood flow: a decompression chamber study." J Appl Physiol **86**(5): 1578-1582.

Beall, C. M. (2007). "Two routes to functional adaptation: Tibetan and Andean high-altitude natives." Proc Natl Acad Sci U S A **104 Suppl 1**: 8655-8660.

Beall, C. M., D. Laskowski, et al. (2001). "Pulmonary nitric oxide in mountain dwellers." Nature **414**(6862): 411-412.

Beckmann, N. (2000). "High resolution magnetic resonance angiography non-invasively reveals mouse strain differences in the cerebrovascular anatomy in vivo." Magn Reson Med **44**(2): 252-258.

Beidleman, B. A., S. R. Muza, et al. (2007). "Validation of a shortened electronic version of the environmental symptoms questionnaire." High Alt Med Biol **8**(3): 192-199.

Bellner, J., B. Romner, et al. (2004). "Transcranial Doppler sonography pulsatility index (PI) reflects intracranial pressure (ICP)." Surg Neurol **62**(1): 45-51; discussion 51.

Berry, D. T., J. W. McConnell, et al. (1989). "Isocapnic hypoxemia and neuropsychological functioning." J Clin Exp Neuropsychol **11**(2): 241-251.

Bert, P. (1878). La Pression Barometrique. Paris, Columbus Ohio College Book Company.

Bert, P. (1943). Barometric Pressure; Researches in Experimental Physiology, (1878). Hitchcock MA and HitchcockFA, Columbus: College Book**:** 171-192, 704-707, 963-983.

Besselmann, M., M. Liu, et al. (2001). "MR angiographic investigation of transient focal cerebral ischemia in rat." NMR Biomed **14**(5): 289-296.

Bhambhani, Y., R. Malik, et al. (2007). "Cerebral oxygenation declines at exercise intensities above the respiratory compensation threshold." Respir Physiol Neurobiol **156**(2): 196-202.

Bianchi, M. T. and J. R. Sims (2008). "Restricted diffusion in the splenium of the corpus callosum after cardiac arrest." Open Neuroimag J **2**: 1-4.

Bjursten, H., P. Ederoth, et al. (2010). "S100B profiles and cognitive function at high altitude." High Alt Med Biol **11**(1): 31-38.

Bogduk, N. (1995). "Anatomy and physiology of headache." Biomed Pharmacother **49**(10): 435-445.

Bolmont, B., C. Bouquet, et al. (2001). "Relationships of personality traits with performance in reaction time, psychomotor ability, and mental efficiency during a 31-day simulated climb of Mount Everest in a hypobaric chamber'." Percept Mot Skills **92**(3 Pt 2): 1022-1030.

Bolmont, B., F. Thullier, et al. (2000). "Relationships between mood states and performances in reaction time, psychomotor ability, and mental efficiency during a 31-day gradual decompression in a hypobaric chamber from sea level to 8848 m equivalent altitude." Physiol Behav **71**(5): 469-476.

Bono, F., D. Messina, et al. (2008). "Bilateral transverse sinus stenosis and idiopathic intracranial hypertension without papilledema in chronic tension-type headache." Journal of neurology **255**(6): 807-812.

Bor-Seng-Shu, E., R. Hirsch, et al. (2006). "Cerebral hemodynamic changes gauged by transcranial Doppler ultrasonography in patients with posttraumatic brain swelling treated by surgical decompression." J Neurosurg **104**(1): 93-100.

Bosch, M. M., D. Barthelmes, et al. (2008). "High incidence of optic disc swelling at very high altitudes." Arch Ophthalmol **126**(5): 644-650.

Bosch, M. M., T. M. Merz, et al. (2009). "New insights into ocular blood flow at very high altitudes." J Appl Physiol **106**(2): 454-460.

Boulos, P., C. Kouroukis, et al. (1999). "Superior sagittal sinus thrombosis occurring at high altitude associated with protein C deficiency." Acta Haematol **102**(2): 104-106.

Bouquet, C. A., B. Gardette, et al. (1999). "Psychomotor skills learning under chronic hypoxia." Neuroreport **10**(14): 3093-3099.

Boushel, R., H. Langberg, et al. (2000). "Regional blood flow during exercise in humans measured by near-infrared spectroscopy and indocyanine green." J Appl Physiol **89**(5): 1868-1878.

Bradwell, A. R., A. D. Wright, et al. (1992). "Acetazolamide and high altitude diseases." Int J Sports Med **13 Suppl 1**: S63-64.

Brealey, D., M. Brand, et al. (2002). "Association between mitochondrial dysfunction and severity and outcome of septic shock." Lancet **360**(9328): 219-223.

Brinchmann-Hansen, O., K. Myhre, et al. (1989). "Retinal vessel responses to exercise and hypoxia before and after high altitude acclimatisation." Eye (Lond) **3 (Pt 6)**: 768-776.

Brugniaux, J. V., A. N. Hodges, et al. (2007). "Cerebrovascular responses to altitude." Respir Physiol Neurobiol **158**(2-3): 212-223.

Brzecka, A. (2005). "Brain preconditioning and obstructive sleep apnea syndrome." Acta Neurobiol Exp (Wars) **65**(2): 213-220.

Burgess, K. R., P. Johnson, et al. (2004). "Acute mountain sickness is associated with sleep desaturation at high altitude." Respirology **9**(4): 485-492.

Butler, P. J. (2010). "High fliers: the physiology of bar-headed geese." Comp Biochem Physiol A Mol Integr Physiol **156**(3): 325-329.

Cardoso, E. R. and J. A. Kupchak (1992). "Evaluation of intracranial pressure gradients by means of transcranial Doppler sonography." Acta Neurochir Suppl (Wien) **55**: 1-5.

Casey, D. P., C. H. Imray, et al. (2011). "Comments on point: counterpoint: high altitude is/is not for the birds!" Journal of applied physiology **111**(5): 1520-1524.

Castelló-Roca, A. (1993). Hombre, Montaña y Medicina [Man, Mountains and Medicine]. Barcelona, Editor Service.

Chan, C. W., H. Hoar, et al. (2005). "Effect of sildenafil and acclimatization on cerebral oxygenation at altitude." Clin Sci (Lond) **109**(3): 319-324.

Chan, P. H., J. W. Schmidley, et al. (1984). "Brain injury, edema, and vascular permeability changes induced by oxygen-derived free radicals." Neurology **34**(3): 315-320.

Chen, Y. Y., J. F. Lirng, et al. (2004). "Primary cough headache is associated with posterior fossa crowdedness: a morphometric MRI study." Cephalalgia : an international journal of headache **24**(9): 694-699.

Choy, M., V. Ganesan, et al. (2006). "The chronic vascular and haemodynamic response after permanent bilateral common carotid occlusion in newborn and adult rats." J Cereb Blood Flow Metab **26**(8): 1066-1075.

Clarke, C. (2006). "Acute mountain sickness: medical problems associated with acute and subacute exposure to hypobaric hypoxia." Postgrad Med J **82**(973): 748-753.

Cohen, J. (1992). "A power primer." Psychol Bull **112**(1): 155-159.

Colino, F. L. and G. Binsted (2012). Theory and Practice of MRA-Guided Transcranial Doppler Sonography. Applied Aspects of Ultrasonography in Humans. P. N. Ainslie. Rijeka, Croatia, InTech**:** 41-55.

Curran-Everett, D. C., J. Iwamoto, et al. (1991). "Intracranial pressures and O2 extraction in conscious sheep during 72 h of hypoxia." Am J Physiol **261**(1 Pt 2): H103-109.

Czosnyka, M., H. K. Richards, et al. (1996). "Relationship between transcranial Doppler-determined pulsatility index and cerebrovascular resistance: an experimental study." J Neurosurg **84**(1): 79-84.

Dallimore, J. and E. C. Rowbotham (2009). "Incidence of acute mountain sickness in adolescents." Wilderness Environ Med **20**(3): 221-224.

Dehnert, C., J. Weymann, et al. (2002). "No association between high-altitude tolerance and the ACE I/D gene polymorphism." Med Sci Sports Exerc **34**(12): 1928-1933.

Dekaban, A. S. (1977). "Tables of cranial and orbital measurements, cranial volume, and derived indexes in males and females from 7 days to 20 years of age." Annals of neurology **2**(6): 485-491.

Dickinson, J., D. Heath, et al. (1983). "Altitude-related deaths in seven trekkers in the Himalayas." Thorax **38**(9): 646-656.

Dietz, T. E. (2008). "An Altitude Tutorial." from http://www.issmed.org.

Dorward, D. A., A. A. Thompson, et al. (2007). "Change in plasma vascular endothelial growth factor during onset and recovery from acute mountain sickness." Respir Med **101**(3): 587-594.

Droma, Y., M. Hanaoka, et al. (2008). "Adaptation to high altitude in Sherpas: association with the insertion/deletion polymorphism in the Angiotensin-converting enzyme gene." Wilderness Environ Med **19**(1): 22-29.

Du Boulay, G. H. and L. Symon (1971). "The anaesthetist's effect upon the cerebral arteries." Proc R Soc Med **64**(1): 77-80.

Du, J. Y., X. Y. Li, et al. (1999). "[Effects of acute mild and moderate hypoxia on human short memory]." Space Med Med Eng (Beijing) **12**(4): 270-273.

Dubowitz, D. J., E. A. Dyer, et al. (2009). "Early brain swelling in acute hypoxia." J Appl Physiol **107**(1): 244-252.

Dyer, E. A., S. R. Hopkins, et al. (2008). "Regional cerebral blood flow during acute hypoxia in individuals susceptible to acute mountain sickness." Respir Physiol Neurobiol **160**(3): 267-276.

Edwards, L. M., A. J. Murray, et al. (2010). "The effect of high-altitude on human skeletal muscle energetics: P-MRS results from the Caudwell Xtreme Everest expedition." PloS one **5**(5): e10681.

Ekkekakis, P. (2009). "Illuminating the black box: investigating prefrontal cortical hemodynamics during exercise with near-infrared spectroscopy." J Sport Exerc Psychol **31**(4): 505-553.

Erzurum, S. C., S. Ghosh, et al. (2007). "Higher blood flow and circulating NO products offset high-altitude hypoxia among Tibetans." Proc Natl Acad Sci U S A **104**(45): 17593-17598.

Fagenholz, P. J., J. A. Gutman, et al. (2007). "Evidence for increased intracranial pressure in high altitude pulmonary edema." High Alt Med Biol **8**(4): 331-336.

Fagenholz, P. J., J. A. Gutman, et al. (2009). "Optic nerve sheath diameter correlates with the presence and severity of acute mountain sickness: evidence for increased intracranial pressure." J Appl Physiol **106**(4): 1207-1211.

Fagenholz, P. J., A. F. Murray, et al. (2007). "New-onset anxiety disorders at high altitude." Wilderness Environ Med **18**(4): 312-316.

Faraci, F. M. and D. D. Heistad (1998). "Regulation of the cerebral circulation: role of endothelium and potassium channels." Physiol Rev **78**(1): 53-97.

Farb, R. I., I. Vanek, et al. (2003). "Idiopathic intracranial hypertension: the prevalence and morphology of sinovenous stenosis." Neurology **60**(9): 1418-1424.

Feddersen, B., H. Ausserer, et al. (2007). "Right temporal cerebral dysfunction heralds symptoms of acute mountain sickness." J Neurol **254**(3): 359-363.

Firth, P. G., H. Zheng, et al. (2008). "Mortality on Mount Everest, 1921-2006: descriptive study." Bmj **337**: a2654.

Fischer, R., C. Vollmar, et al. (2004). "No evidence of cerebral oedema in severe acute mountain sickness." Cephalalgia **24**(1): 66-71.

Fishman, R. A. (1975). "Brain edema." N Engl J Med **293**(14): 706-711.

Fog, M. (1938). "The Relationship between the Blood Pressure and the Tonic Regulation of the Pial Arteries." Journal of neurology and psychiatry **1**(3): 187-197.

Forster, P. J. (1985). "Effect of different ascent profiles on performance at 4,200 m elevation." Aviat Space Environ Med **56**(8): 758-764.

Fowler, B., D. D. Elcombe, et al. (1987). "The threshold for hypoxia effects on perceptual-motor performance." Hum Factors **29**(1): 61-66.

Garrido, E., A. Castello, et al. (1993). "Cortical atrophy and other brain magnetic resonance imaging (MRI) changes after extremely high-altitude climbs without oxygen." Int J Sports Med **14**(4): 232-234.

Garrido, E., C. Javierre, et al. (2000). "Hallucinatory experiences at high altitude." Neuropsychiatry Neuropsychol Behav Neurol **13**(2): 148.

Garrido, E., R. Segura, et al. (1995). "New evidence from magnetic resonance imaging of brain changes after climbs at extreme altitude." Eur J Appl Physiol Occup Physiol **70**(6): 477-481.

Germon, T. J., N. M. Kane, et al. (1994). "Near-infrared spectroscopy in adults: effects of extracranial ischaemia and intracranial hypoxia on estimation of cerebral oxygenation." Br J Anaesth **73**(4): 503-506.

Gertsch, J. H., B. Basnyat, et al. (2004). "Randomised, double blind, placebo controlled comparison of ginkgo biloba and acetazolamide for prevention of acute mountain sickness among Himalayan trekkers: the prevention of high altitude illness trial (PHAIT)." Bmj **328**(7443): 797.

Giller, C. A. (2003). "The Emperor has no clothes: velocity, flow, and the use of TCD." J Neuroimaging **13**(2): 97-98.

Giller, C. A., G. Bowman, et al. (1993). "Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy." Neurosurgery **32**(5): 737-741; discussion 741-732.

Giller, C. A., A. M. Giller, et al. (2000). "Evaluation of the cerebral hemodynamic response to rhythmic handgrip." J Appl Physiol **88**(6): 2205-2213.

Glaisher, J. (1862). "Notes of effects experienced during recent balloon ascents." Lancet **2**: 559-560.

Goh, D. and R. A. Minns (1995). "Intracranial pressure and cerebral arterial flow velocity indices in childhood hydrocephalus: current review." Childs Nerv Syst **11**(7): 392-396.

Golalipour, M. J., M. Jahanshaei, et al. (2005). "Estimation of Cranial Capacity in 17-20 year olds in South East Caspian Sea Border (North of Iran)." Int J Morphol **23**(4): 301-304.

Gonzalez, N. R., W. J. Boscardin, et al. (2007). "Vasospasm probability index: a combination of transcranial doppler velocities, cerebral blood flow, and clinical risk factors to predict cerebral vasospasm after aneurysmal subarachnoid hemorrhage." J Neurosurg **107**(6): 1101-1112.

Goodman, M. D., A. T. Makley, et al. (2011). "Hypobaric hypoxia exacerbates the neuroinflammatory response to traumatic brain injury." J Surg Res **165**(1): 30-37.

Gore, C. J., S. A. Clark, et al. (2007). "Nonhematological mechanisms of improved sea-level performance after hypoxic exposure." Med Sci Sports Exerc **39**(9): 1600-1609.

Grissom, C. K., R. C. Roach, et al. (1992). "Acetazolamide in the treatment of acute mountain sickness: clinical efficacy and effect on gas exchange." Ann Intern Med **116**(6): 461-465.

Grocott, M. and H. Montgomery (2008). "Genetophysiology: using genetic strategies to explore hypoxic adaptation." High Alt Med Biol **9**(2): 123-129.

Grocott, M., H. Montgomery, et al. (2007). "High-altitude physiology and pathophysiology: implications and relevance for intensive care medicine." Crit Care **11**(1): 203.

Grocott, M., A. Richardson, et al. (2007). "Caudwell Xtreme Everest: a field study of human adaptation to hypoxia." Crit Care **11**(4): 151.

Grocott, M. P., D. S. Martin, et al. (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, et al. (2010). "Caudwell xtreme Everest expedition." High Alt Med Biol **11**(2): 133-137.

Haack, D. C. and E. C. Meihoff (1971). "A method for estimation of cranial capacity from cephalometric roentgenograms." Am J Phys Anthropol **34**(3): 447-452.

Hackett, P. (2001). High altitude and common medical conditions. An Exploration of Human Adaptation. H. T.F. and S. R.B. New York, Marcel Dekker**:** 839-885.

Hackett, P. H. (1999). "The cerebral etiology of high-altitude cerebral edema and acute mountain sickness." Wilderness Environ Med **10**(2): 97-109.

Hackett, P. H., D. Rennie, et al. (1976). "The incidence, importance, and prophylaxis of acute mountain sickness." Lancet **2**(7996): 1149-1155.

Hackett, P. H. and R. C. Roach (2001). "High-altitude illness." N Engl J Med **345**(2): 107-114.

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, et al. (1988). "Dexamethasone for prevention and treatment of acute mountain sickness." Aviat Space Environ Med **59**(10): 950-954.

Hackett, P. H., P. R. Yarnell, et al. (1998). "High-altitude cerebral edema evaluated with magnetic resonance imaging: clinical correlation and pathophysiology." Jama **280**(22): 1920-1925.

Hadolt, I. and G. Litscher (2003). "Noninvasive assessment of cerebral oxygenation during high altitude trekking in the Nepal Himalayas (2850-5600 m)." Neurol Res **25**(2): 183-188.

Hanlo, P. W., R. H. Gooskens, et al. (1995). "Value of transcranial Doppler indices in predicting raised ICP in infantile hydrocephalus. A study with review of the literature." Childs Nerv Syst **11**(10): 595-603.

Hansen, J. M., D. Pedersen, et al. (2007). "Magnetic resonance angiography shows dilatation of the middle cerebral artery after infusion of glyceryl trinitrate in healthy volunteers." Cephalalgia **27**(2): 118-127.

Harada, K., T. Hayashi, et al. (1993). "[Transcranial Doppler sonography in acute intracranial hypertension model--usefulness of pulsatility index]." No To Shinkei **45**(9): 851-856.

Hartig, G. and P. Hackett (1992). Cerebral spinal fluid pressure and cerebral blood velocity in acute mountain sickness. Hypoxia and Mountain Medicine. J. Sutton, G. Coates and C. Houston. Oxford, Pergamon Press**:** 260-265.

Harvey, T. C., M. E. Raichle, et al. (1988). "Effect of carbon dioxide in acute mountain sickness: a rediscovery." Lancet **2**(8612): 639-641.

Heine, M., A. W. Subudhi, et al. (2009). "Effect of ventilation on cerebral oxygenation during exercise: insights from canonical correlation." Respir Physiol Neurobiol **166**(2): 125-128.

Henson, L. C., C. Calalang, et al. (1998). "Accuracy of a cerebral oximeter in healthy volunteers under conditions of isocapnic hypoxia." Anesthesiology **88**(1): 58-65.

Herault, S., G. Fomina, et al. (2000). "Cardiac, arterial and venous adaptation to weightlessness during 6-month MIR spaceflights with and without thigh cuffs (bracelets)." European journal of applied physiology **81**(5): 384-390.

Higgins, J. N., C. Cousins, et al. (2003). "Idiopathic intracranial hypertension: 12 cases treated by venous sinus stenting." Journal of neurology, neurosurgery, and psychiatry **74**(12): 1662-1666.

Hiraoka, M., M. Firbank, et al. (1993). "A Monte Carlo investigation of optical pathlength in inhomogeneous tissue and its application to near-infrared spectroscopy." Phys Med Biol **38**(12): 1859-1876.

Hiura, M., T. Mizuno, et al. "Cerebral oxygenation in the frontal lobe cortex during incremental exercise tests: the regional changes influenced by volitional exhaustion." Adv Exp Med Biol **662**: 257-263.

Hochachka, P. W., C. M. Clark, et al. (1994). "The brain at high altitude: hypometabolism as a defense against chronic hypoxia?" J Cereb Blood Flow Metab **14**(4): 671-679.

Hochachka, P. W. and C. Monge (2000). "Evolution of human hypoxia tolerance physiology." Adv Exp Med Biol **475**: 25-43.

Holloway, C. J., H. E. Montgomery, et al. (2011). "Cardiac response to hypobaric hypoxia: persistent changes in cardiac mass, function, and energy metabolism after a trek to Mt. Everest Base Camp." FASEB journal : official publication of the Federation of American Societies for Experimental Biology **25**(2): 792-796.

Homburg, A. M., M. Jakobsen, et al. (1993). "Transcranial Doppler recordings in raised intracranial pressure." Acta Neurol Scand **87**(6): 488-493.

Hornbein, T. F., B. D. Townes, et al. (1989). "The cost to the central nervous system of climbing to extremely high altitude." N Engl J Med **321**(25): 1714-1719.

Houston, C. S. (1989). "Incidence of acute mountain sickness at intermediate altitudes." Jama **261**(24): 3551-3552.

Houston, C. S. and J. Dickinson (1975). "Cerebral form of high-altitude illness." Lancet **2**(7938): 758-761.

Hu, Y., J. Liu, et al. (2013). "Recent agents targeting HIF-1alpha for cancer therapy." Journal of cellular biochemistry **114**(3): 498-509.

Huang, S. Y., L. G. Moore, et al. (1987). "Internal carotid and vertebral arterial flow velocity in men at high altitude." J Appl Physiol **63**(1): 395-400.

Iacopino, D. G., C. Zaccone, et al. (1995). "Intraoperative monitoring of cerebral blood flow during ventricular shunting in hydrocephalic pediatric patients." Childs Nerv Syst **11**(8): 483-486.

Imray, C. H., N. J. Barnett, et al. (1998). "Near-infrared spectroscopy in the assessment of cerebral oxygenation at high altitude." Wilderness Environ Med **9**(4): 198-203.

Imray, C. H., S. Brearey, et al. (2000). "Cerebral oxygenation at high altitude and the response to carbon dioxide, hyperventilation and oxygen. The Birmingham Medical Research Expeditionary Society." Clin Sci (Lond) **98**(2): 159-164.

Imray, C. H., T. Clarke, et al. (2001). "Carbon dioxide contributes to the beneficial effect of pressurization in a portable hyperbaric chamber at high altitude." Clin Sci (Lond) **100**(2): 151-157.

Imray, C. H., S. D. Myers, et al. (2005). "Effect of exercise on cerebral perfusion in humans at high altitude." J Appl Physiol **99**(2): 699-706.

Imray, C. H., K. T. Pattinson, et al. (2008). "Intrapulmonary and Intracardiac Shunting With Exercise at Altitude." Wilderness Environ Med **19**(3): 199-204.

Imray, C. H., S. Walsh, et al. (2003). "Effects of breathing air containing 3% carbon dioxide, 35% oxygen or a mixture of 3% carbon dioxide/35% oxygen on cerebral and peripheral oxygenation at 150 m and 3459 m." Clin Sci (Lond) **104**(3): 203-210.

Jafarian, S., F. Gorouhi, et al. (2008). "High-altitude sleep disturbance: results of the Groningen Sleep Quality Questionnaire survey." Sleep Med **9**(4): 446-449.

James, P. B. (1988). "Hyperbaric oxygenation, carbon monoxide, and cerebral edema." Br Med J (Clin Res Ed) **296**(6620): 500-501.

Jansen, G. F., D. A. Kagenaar, et al. (2002). "Basilar artery blood flow velocity and the ventilatory response to acute hypoxia in mountaineers." Respir Physiol Neurobiol **133**(1-2): 65-74.

Jansen, G. F., A. Krins, et al. (1999). "Cerebral vasomotor reactivity at high altitude in humans." J Appl Physiol **86**(2): 681-686.

Jansen, G. F., A. Krins, et al. (2000). "Cerebral autoregulation in subjects adapted and not adapted to high altitude." Stroke **31**(10): 2314-2318.

Jausovec, N. and K. Jausovec (2010). "Resting brain activity: differences between genders." Neuropsychologia **48**(13): 3918-3925.

Jensen, J. B., B. Sperling, et al. (1996). "Augmented hypoxic cerebral vasodilation in men during 5 days at 3,810 m altitude." J Appl Physiol **80**(4): 1214-1218.

Jensen, J. B., A. D. Wright, et al. (1990). "Cerebral blood flow in acute mountain sickness." J Appl Physiol **69**(2): 430-433.

Jobsis, F. F. (1977). "Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters." Science **198**(4323): 1264-1267.

Johnson, B. G., J. Simmons, et al. (2005). "Ataxia at altitude measured on a wobble board." Wilderness Environ Med **16**(1): 42-46.

Johnson, T. S., P. B. Rock, et al. (1984). "Prevention of acute mountain sickness by dexamethasone." N Engl J Med **310**(11): 683-686.

Kallenberg, K., D. M. Bailey, et al. (2007). "Magnetic resonance imaging evidence of cytotoxic cerebral edema in acute mountain sickness." J Cereb Blood Flow Metab **27**(5): 1064-1071.

Kallenberg, K., C. Dehnert, et al. (2008). "Microhemorrhages in nonfatal high-altitude cerebral edema." J Cereb Blood Flow Metab **28**(9): 1635-1642.

Kameyama, M., M. Fukuda, et al. (2004). "Sex and age dependencies of cerebral blood volume changes during cognitive activation: a multichannel near-infrared spectroscopy study." Neuroimage **22**(4): 1715-1721.

Kasamo, K., Y. Okuhata, et al. (1993). "Chronological changes of MRI findings on striatal damage after acute cyanide intoxication: pathogenesis of the damage and its selectivity, and prevention for neurological sequelae: a case report." Eur Arch Psychiatry Clin Neurosci **243**(2): 71-74.

Kellie, G. (1824). "An account of the appearances observed in the dissection of two of the three individuals presumed to have perished in the storm of the 3rd, and whose bodie were discovered in the vicinity of Leith on the morning of the 4th November 1821 with some reflections on the pathology of the brain. ." The Transactions of the Medico-Chirurgical Society of Edinburgh **1**: 84-169.

Kilic, T. and A. Akakin (2008). "Anatomy of cerebral veins and sinuses." Front Neurol Neurosci **23**: 4-15.

Kim, B. S. D., H.; Marks, M. (2004). "Diagnosis and Managements of Cerebral Veous and Sinus Thrombosis." Seminars in Cerebrovascular Diseases and Stroke **4**: 205-216.

Klatzo, I. (1967). "Presidental address. Neuropathological aspects of brain edema." J Neuropathol Exp Neurol **26**(1): 1-14.

Kobrick, J. L. and B. Appleton (1971). "Effects of extended hypoxia on visual performance and retinal vascular state." J Appl Physiol **31**(3): 357-362.

Koehle, M. S., P. Wang, et al. (2006). "No association between variants in the ACE and angiotensin II receptor 1 genes and acute mountain sickness in Nepalese pilgrims to the Janai Purnima Festival at 4380 m." High Alt Med Biol **7**(4): 281-289.

Koyama, S., T. Kobayashi, et al. (1988). "The increased sympathoadrenal activity in patients with high altitude pulmonary edema is centrally mediated." Jpn J Med **27**(1): 10-16.

Kramer, A. F., J. T. Coyne, et al. (1993). "Cognitive function at high altitude." Hum Factors **35**(2): 329-344.

Krasney, J. A. (1994). "A neurogenic basis for acute altitude illness." Med Sci Sports Exerc **26**(2): 195-208.

Krasney, J. A., D. C. Curran-Everett, et al. (1990). High Altitude Cerebral Edema: an animal model. Hypoxia: the Adaptations. J. Sutton, G. Coates and J. Remmers. Ontario, Decker**:** 200-205.

Lang, G. E. and G. B. Kuba (1997). "High-altitude retinopathy." Am J Ophthalmol **123**(3): 418-420.

Lawley, J. S., S. J. Oliver, et al. (2012). "Optic nerve sheath diameter is not related to high altitude headache: a randomized controlled trial." High altitude medicine & biology **13**(3): 193-199.

Levett, D. Z., B. O. Fernandez, et al. (2011). "The role of nitrogen oxides in human adaptation to hypoxia." Scientific reports **1**: 109.

Levett, D. Z., D. S. Martin, et al. (2010). "Design and conduct of Caudwell Xtreme Everest: an observational cohort study of variation in human adaptation to progressive environmental hypoxia." BMC Med Res Methodol **10**: 98.

Levett, D. Z., E. J. Radford, et al. (2012). "Acclimatization of skeletal muscle mitochondria to high-altitude hypoxia during an ascent of Everest." FASEB journal : official publication of the Federation of American Societies for Experimental Biology **26**(4): 1431-1441.

Lewis, M. S., P. Maruff, et al. (2007). "The influence of different error estimates in the detection of postoperative cognitive dysfunction using reliable change indices with correction for practice effects." Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists **22**(2): 249-257.

Luks, A. M. (2008). "Which medications are safe and effective for improving sleep at high altitude?" High Alt Med Biol **9**(3): 195-198.

Lundqvist, C., J. S. Benth, et al. (2009). "A vertical VAS is a valid instrument for monitoring headache pain intensity." Cephalalgia **29**(10): 1034-1041.

Lysakowski, C., E. Von Elm, et al. (2004). "Effect of magnesium, high altitude and acute mountain sickness on blood flow velocity in the middle cerebral artery." Clin Sci (Lond) **106**(3): 279-285.

Mader, T. H., C. R. Gibson, et al. (2011). "Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight." Ophthalmology **118**(10): 2058-2069.

Mader, T. H. and G. Tabin (2003). "Going to high altitude with preexisting ocular conditions." High Alt Med Biol **4**(4): 419-430.

Maggiorini, M., H. P. Brunner-La Rocca, et al. (2006). "Both tadalafil and dexamethasone may reduce the incidence of high-altitude pulmonary edema: a randomized trial." Ann Intern Med **145**(7): 497-506.

Maher, J. T., A. Cymerman, et al. (1975). "Acute mountain sickness: increased severity in eucapnic hypoxia." Aviat Space Environ Med **46**(6): 826-829.

Mairer, K., M. Gobel, et al. (2012). "MRI Evidence: Acute Mountain Sickness Is Not Associated with Cerebral Edema Formation during Simulated High Altitude." PloS one **7**(11): e50334.

Manjunath, K. (2002). "Estimation of Cranial Volume-an overview of Methodologies." J Anat Soc India **1**: 85-91.

Martin, D. S., P. Goedhart, et al. (2010). "Changes in sublingual microcirculatory flow index and vessel density on ascent to altitude." Experimental physiology **95**(8): 880-891.

Martin, D. S., C. Ince, et al. (2009). "Abnormal blood flow in the sublingual microcirculation at high altitude." European journal of applied physiology **106**(3): 473-478.

Martin, D. S., D. Z. Levett, et al. (2009). "Changes in skeletal muscle oxygenation during exercise measured by near-infrared spectroscopy on ascent to altitude." Crit Care **13 Suppl 5**: S7.

Martin, D. S., D. Z. Levett, et al. (2009). "Changes in skeletal muscle oxygenation during exercise measured by near-infrared spectroscopy on ascent to altitude." Critical care **13 Suppl 5**: S7.

Martinoli, C. and L. E. Derchi (1997). "Gain setting in power Doppler US." Radiology **202**(1): 284-285.

Matsuzawa, Y., Kobayashi, T., Fujimoto, K. et al (1992). Cerebral edema in acute mountain sickness. High Altitude Medicine. R. J. Ueda G, Sekiguchi M. Matsumoto, Japan, Sjinshu University Press**:** 300-304.

McArdle, W. D., F. I. Katch, et al. (2006). Exercise Physiology, Lippincott, Williams and Wilkins**:** 357.

Mills, E. M., P. G. Gunasekar, et al. (1999). "Differential susceptibility of brain areas to cyanide involves different modes of cell death." Toxicol Appl Pharmacol **156**(1): 6-16.

Miserocchi, G., A. Passi, et al. (2001). "Pulmonary interstitial pressure and tissue matrix structure in acute hypoxia." Am J Physiol Lung Cell Mol Physiol **280**(5): L881-887.

Moller, K., O. B. Paulson, et al. (2002). "Unchanged cerebral blood flow and oxidative metabolism after acclimatization to high altitude." J Cereb Blood Flow Metab **22**(1): 118-126.

Monro, A. (1823). Observations on the structure and function of the nervous system. Edinburgh, Creech & Johnson.

Moreno, J. A., E. Mesalles, et al. (2000). "Evaluating the outcome of severe head injury with transcranial Doppler ultrasonography." Neurosurg Focus **8**(1): e8.

Morocz, I. A., G. P. Zientara, et al. (2001). "Volumetric quantification of brain swelling after hypobaric hypoxia exposure." Exp Neurol **168**(1): 96-104.

Mosso, A. (1898). Life of Man on the High Alps. London, Fischer Unwin.

Muller, M., K. Schwerdtfeger, et al. (2000). "Assessment of middle cerebral artery diameter after aneurysmal subarachnoid hemorrhage by transcranial color-coded duplex sonography." Eur J Ultrasound **11**(1): 15-19.

Mullner-Eidenbock, A., G. Rainer, et al. (2000). "High-altitude retinopathy and retinal vascular dysregulation." Eye (Lond) **14 Pt 5**: 724-729.

Murkin, J. M., S. J. Adams, et al. (2007). "Monitoring brain oxygen saturation during coronary bypass surgery: a randomized, prospective study." Anesth Analg **104**(1): 51-58.

Murkin, J. M. and M. Arango (2009). "Near-infrared spectroscopy as an index of brain and tissue oxygenation." Br J Anaesth **103 Suppl 1**: i3-13.

Na, S. J., J. M. Hong, et al. (2004). "A case of reversible postpartum cytotoxic edema in preeclampsia." J Neurol Sci **221**(1-2): 83-87.

Nadvi, S. S., M. D. Du Trevou, et al. (1994). "The use of transcranial Doppler ultrasonography as a method of assessing intracranial pressure in hydrocephalic children." Br J Neurosurg **8**(5): 573-577.

Nair, V., A. K. Mohapatro, et al. (2008). "A case of hereditary protein S deficiency presenting with cerebral sinus venous thrombosis and deep vein thrombosis at high altitude." Acta Haematol **119**(3): 158-161.

Nelson, M. (1982). "Psychological testing at high altitudes." Aviat Space Environ Med **53**(2): 122-126.

Nelson, M. T. and J. M. Quayle (1995). "Physiological roles and properties of potassium channels in arterial smooth muscle." The American journal of physiology **268**(4 Pt 1): C799-822.

Nelson, T. O., J. Dunlosky, et al. (1990). "Cognition and metacognition at extreme altitudes on Mount Everest." J Exp Psychol Gen **119**(4): 367-374.

Nicholas, R., P. D. O'Meara, et al. (1992). "Is syncope related to moderate altitude exposure?" Jama **268**(7): 904-906.

Norcliffe, L. J., M. Rivera-Ch, et al. (2005). "Cerebrovascular responses to hypoxia and hypocapnia in high-altitude dwellers." J Physiol **566**(Pt 1): 287-294.

Norelle, A., A. Q. Fischer, et al. (1989). "Transcranial Doppler: a noninvasive method to monitor hydrocephalus." J Child Neurol **4 Suppl**: S87-90.

Nuttall, G. A., D. J. Cook, et al. (1996). "The relationship between cerebral blood flow and transcranial Doppler blood flow velocity during hypothermic cardiopulmonary bypass in adults." Anesth Analg **82**(6): 1146-1151.

Oelz, O., M. Maggiorini, et al. (1989). "Nifedipine for high altitude pulmonary oedema." Lancet **2**(8674): 1241-1244.

Ogoh, S., K. Sato, et al. (2012). "Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries." Experimental physiology.

Ogunshola, O. O. and A. Al-Ahmad (2012). "HIF-1 at the blood-brain barrier: a mediator of permeability?" High altitude medicine & biology **13**(3): 153-161.

Ohmae, E., Y. Ouchi, et al. (2006). "Cerebral hemodynamics evaluation by near-infrared time-resolved spectroscopy: correlation with simultaneous positron emission tomography measurements." Neuroimage **29**(3): 697-705.

Ong, S. G. and D. J. Hausenloy (2012). "Hypoxia-inducible factor as a therapeutic target for cardioprotection." Pharmacology & therapeutics **136**(1): 69-81.

Otis, S. M., M. E. Rossman, et al. (1989). "Relationship of cerebral blood flow regulation to acute mountain sickness." J Ultrasound Med **8**(3): 143-148.

Owen-Reece, H., M. Smith, et al. (1999). "Near infrared spectroscopy." Br J Anaesth **82**(3): 418-426.

Owler, B. K., G. Parker, et al. (2005). "Cranial venous outflow obstruction and pseudotumor Cerebri syndrome." Adv Tech Stand Neurosurg **30**: 107-174.

Pai, S. B., R. G. Varma, et al. (2005). "Microsurgical anatomy of the middle cerebral artery." Neurol India **53**(2): 186-190.

Palma, J., C. Macedonia, et al. (2006). "Cerebrovascular dynamics and vascular endothelial growth factor in acute mountain sickness." Wilderness Environ Med **17**(1): 1-7.

Paola, M. D., M. Bozzali, et al. (2008). "Reduced oxygen due to high-altitude exposure relates to atrophy in motor-function brain areas." Eur J Neurol **15**(10): 1050-1057.

Patel, S., D. R. Woods, et al. (2003). "Angiotensin-converting enzyme genotype and the ventilatory response to exertional hypoxia." Eur Respir J **22**(5): 755-760.

Pelamatti, G., M. Pascotto, et al. (2003). "Verbal free recall in high altitude: proper names vs common names." Cortex **39**(1): 97-103.

Peltonen, J. E., J. M. Kowalchuk, et al. (2007). "Cerebral and muscle tissue oxygenation in acute hypoxic ventilatory response test." Respir Physiol Neurobiol **155**(1): 71-81.

Pendon, J. D. and J. A. King (2003). "Brief altitude exposure in New Zealand white rabbits (Oryctolagus cuniculus) does not produce elevated intracranial pressure." Wilderness Environ Med **14**(3): 183-188.

Perrey, S. (2008). "Non-invasive NIR spectroscopy of human brain function during exercise." Methods **45**(4): 289-299.

Pickard, J. D., Z. Czosnyka, et al. (2008). "Coupling of sagittal sinus pressure and cerebrospinal fluid pressure in idiopathic intracranial hypertension--a preliminary report." Acta neurochirurgica. Supplement **102**: 283-285.

Plutarch (1912). Alexander and Caesar. London, Heinemann.

Pollard, A. J., S. Niermeyer, et al. (2001). "Children at high altitude: an international consensus statement by an ad hoc committee of the International Society for Mountain Medicine, March 12, 2001." High Alt Med Biol **2**(3): 389-403.

Poulin, M. J., P. J. Liang, et al. (1998). "Fast and slow components of cerebral blood flow response to step decreases in end-tidal PCO2 in humans." Journal of applied physiology **85**(2): 388-397.

Poulin, M. J. and P. A. Robbins (1996). "Indexes of flow and cross-sectional area of the middle cerebral artery using doppler ultrasound during hypoxia and hypercapnia in humans." Stroke **27**(12): 2244-2250.

Poulin, M. J.Fatemian, et al. (2002). "Changes in cerebral blood flow during and after 48 h of both isocapnic and poikilocapnic hypoxia in humans." Experimental Phyiology **87**(5): 633-642.

Prior, S. J., J. M. Hagberg, et al. (2003). "Sequence variation in hypoxia-inducible factor 1alpha (HIF1A): association with maximal oxygen consumption." Physiol Genomics **15**(1): 20-26.

Querfurth, H. W., S. W. Arms, et al. (2004). "Prediction of intracranial pressure from noninvasive transocular venous and arterial hemodynamic measurements: a pilot study." Neurocritical care **1**(2): 183-194.

Querfurth, H. W., P. Lieberman, et al. (2010). "Ophthalmodynamometry for ICP prediction and pilot test on Mt. Everest." BMC neurology **10**: 106.

Quinn, M. W. and I. K. Pople (1992). "Middle cerebral artery pulsatility in children with blocked cerebrospinal fluid shunts." J Neurol Neurosurg Psychiatry **55**(4): 325-327.

Rainov, N. G., J. B. Weise, et al. (2000). "Transcranial Doppler sonography in adult hydrocephalic patients." Neurosurg Rev **23**(1): 34-38.

Rajput, C., E. Arif, et al. (2006). "Predominance of interaction among wild-type alleles of CYP11B2 in Himalayan natives associates with high-altitude adaptation." Biochem Biophys Res Commun **348**(2): 735-740.

Ravenhill, T. H. (1913). "Some experiences of mountain sickness in the Andes." Journal of Tropical Medicine and Hygiene **16**: 313-320.

Reese, T., D. Bochelen, et al. (1999). "Magnetic resonance angiography of the rat cerebrovascular system without the use of contrast agents." NMR Biomed **12**(4): 189-196.

Reeves, J. T., L. G. Moore, et al. (1985). "Headache at high altitude is not related to internal carotid arterial blood velocity." J Appl Physiol **59**(3): 909-915.

Regard, M., O. Oelz, et al. (1989). "Persistent cognitive impairment in climbers after repeated exposure to extreme altitude." Neurology **39**(2 Pt 1): 210-213.

Reite, M., D. Jackson, et al. (1975). "Sleep physiology at high altitude." Electroencephalogr Clin Neurophysiol **38**(5): 463-471.

Roach, R. C., P. Bartsch, et al. (1993). Lake Louise AMS Scoring Consensus Committee. The Lake Louise acute mountain sickness scoring system. Hypoxia and Molecular Medicine. S. JR, H. CS and C. G. Burlington, Vt, CHarles S Houston**:** 272-274.

Roach, R. C. and P. H. Hackett (2001). "Frontiers of hypoxia research: acute mountain sickness." J Exp Biol **204**(Pt 18): 3161-3170.

Rodway, G. W. (2007). "Limb paralysis and visual changes during Glaisher and Coxwell's 1862 balloon ascent to over 8800 m." High Alt Med Biol **8**(3): 256-259.

Rohr, A., J. Bindeballe, et al. (2012). "The entire dural sinus tree is compressed in patients with idiopathic intracranial hypertension: a longitudinal, volumetric magnetic resonance imaging study." Neuroradiology **54**(1): 25-33.

Rohwer, N., C. Zasada, et al. (2012). "The growing complexity of HIF-1alpha's role in tumorigenesis: DNA repair and beyond." Oncogene.

Roncin, J. P., F. Schwartz, et al. (1996). "EGb 761 in control of acute mountain sickness and vascular reactivity to cold exposure." Aviat Space Environ Med **67**(5): 445-452.

Rooks, C. R., N. J. Thom, et al. (2010). "Effects of incremental exercise on cerebral oxygenation measured by near-infrared spectroscopy: a systematic review." Prog Neurobiol **92**(2): 134-150.

Ross, R. T. (1985). "The random nature of cerebral mountain sickness." Lancet **1**(8435): 990-991.

Rostrup, E., I. Law, et al. (2002). "Cerebral hemodynamics measured with simultaneous PET and near-infrared spectroscopy in humans." Brain Res **954**(2): 183-193.

Rowell, L. B. (1993). Control of regional blood flow during dynamic exercise. Human Cardiovascular Control. L. B. Rowell. New York, Oxford University Press**:** 204-254.

Rupert, J. L. and M. S. Koehle (2006). "Evidence for a genetic basis for altitude-related illness." High Alt Med Biol **7**(2): 150-167.

Rupp, T., M. Jubeau, et al. (2012). "The effect of hypoxemia and exercise on acute mountain sickness symptoms." Journal of applied physiology.

Rupp, T. and S. Perrey (2009). "Effect of severe hypoxia on prefrontal cortex and muscle oxygenation responses at rest and during exhaustive exercise." Adv Exp Med Biol **645**: 329-334.

Sahin, B., N. Acer, et al. (2007). "Comparison of four methods for the estimation of intracranial volume: a gold standard study." Clin Anat **20**(7): 766-773.

Saito, S., F. Nishihara, et al. (1999). "Exercise-induced cerebral deoxygenation among untrained trekkers at moderate altitudes." Arch Environ Health **54**(4): 271-276.

Sampson, J. B., A. Cymerman, et al. (1983). "Procedures for the measurement of acute mountain sickness." Aviat Space Environ Med **54**(12 Pt 1): 1063-1073.

Sanchez-Elsner, T., J. R. Ramirez, et al. (2004). "A cross-talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SP1 and Smads." J Mol Biol **336**(1): 9-24.

Sander, K., H. Poppert, et al. (2011). "Dynamics of intracranial venous flow patterns in patients with idiopathic intracranial hypertension." European neurology **66**(6): 334-338.

Sanders, D. B., T. Kelley, et al. (2000). "The role of nitric oxide synthase/nitric oxide in vascular smooth muscle control." Perfusion **15**(2): 97-104.

Schoch, H. J., S. Fischer, et al. (2002). "Hypoxia-induced vascular endothelial growth factor expression causes vascular leakage in the brain." Brain **125**(Pt 11): 2549-2557.

Schoonman, G. G., P. S. Sandor, et al. (2008). "Hypoxia-induced acute mountain sickness is associated with intracellular cerebral edema: a 3 T magnetic resonance imaging study." J Cereb Blood Flow Metab **28**(1): 198-206.

Schrader, H., L. J. Stovner, et al. (2001). "Prophylactic treatment of migraine with angiotensin converting enzyme inhibitor (lisinopril): randomised, placebo controlled, crossover study." BMJ **322**(7277): 19-22.

Schreiber, S. J., S. Gottschalk, et al. (2000). "Assessment of blood flow velocity and diameter of the middle cerebral artery during the acetazolamide provocation test by use of transcranial Doppler sonography and MR imaging." AJNR Am J Neuroradiol **21**(7): 1207-1211.

Schwarz, G., G. Litscher, et al. (1996). "Cerebral oximetry in dead subjects." J Neurosurg Anesthesiol **8**(3): 189-193.

Scott, C. J., R. H. Kardon, et al. (2010). "Diagnosis and grading of papilledema in patients with raised intracranial pressure using optical coherence tomography vs clinical expert assessment using a clinical staging scale." Archives of ophthalmology **128**(6): 705-711.

Serrador, J. M., P. A. Picot, et al. (2000). "MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis." Stroke **31**(7): 1672-1678.

Serrano-Duenas, M. (2000). "[Acute mountain sickness: the clinical characteristics of a cohort of 615 patients]." Med Clin (Barc) **115**(12): 441-445.

Severinghaus, J. W. (1995). "Hypothesis: angiogenesis cytokines in high altitude cerebral oedema." Acta Anaesthesiol Scand Suppl **107**: 177-178.

Severinghaus, J. W., H. Chiodi, et al. (1966). "Cerebral blood flow in man at high altitude. Role of cerebrospinal fluid pH in normalization of flow in chronic hypocapnia." Circ Res **19**(2): 274-282.

Shibuya, K., J. Tanaka, et al. (2004). "Cerebral oxygenation during intermittent supramaximal exercise." Respir Physiol Neurobiol **140**(2): 165-172.

Silber, E., P. Sonnenberg, et al. (2003). "Clinical features of headache at altitude: a prospective study." Neurology **60**(7): 1167-1171.

Singh, I., P. K. Khanna, et al. (1969). "Acute mountain sickness." N Engl J Med **280**(4): 175-184.

Smith, K. J. and F. Billaut (2010). "Influence of cerebral and muscle oxygenation on repeated-sprint ability." Eur J Appl Physiol **109**(5): 989-999.

Soehle, M., D. A. Chatfield, et al. (2007). "Predictive value of initial clinical status, intracranial pressure and transcranial Doppler pulsatility after subarachnoid haemorrhage." Acta Neurochir (Wien) **149**(6): 575-583.

Song, S. Y., T. Asaji, et al. (1986). "Cerebral thrombosis at altitude: its pathogenesis and the problems of prevention and treatment." Aviat Space Environ Med **57**(1): 71-76.

Southard, A., S. Niermeyer, et al. (2007). "Language used in Lake Louise Scoring System underestimates symptoms of acute mountain sickness in 4- to 11-year-old children." High Alt Med Biol **8**(2): 124-130.

Stobdan, T., J. Karar, et al. (2008). "High altitude adaptation: genetic perspectives." High Alt Med Biol **9**(2): 140-147.

Subudhi, A. W., A. C. Dimmen, et al. (2007). "Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise." J Appl Physiol **103**(1): 177-183.

Subudhi, A. W., M. C. Lorenz, et al. (2008). "Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance." Am J Physiol Heart Circ Physiol **294**(1): H164-171.

Subudhi, A. W., B. R. Miramon, et al. (2009). "Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia." J Appl Physiol **106**(4): 1153-1158.

Subudhi, A. W., J. T. Olin, et al. (2011). "Does cerebral oxygen delivery limit incremental exercise performance?" J Appl Physiol.

Sutherland, A. I., D. S. Morris, et al. (2008). "Optic nerve sheath diameter, intracranial pressure and acute mountain sickness on Mount Everest: a longitudinal cohort study." Br J Sports Med **42**(3): 183-188.

Suzuki, K., T. Kizaki, et al. (2003). "Genetic variation in hypoxia-inducible factor 1alpha and its possible association with high altitude adaptation in Sherpas." Med Hypotheses **61**(3): 385-389.

Takenouchi, T., L. A. Heier, et al. (2010). "Restricted diffusion in the corpus callosum in hypoxic-ischemic encephalopathy." Pediatr Neurol **43**(3): 190-196.

Tan, H., H. Feng, et al. (2001). "Outcome prediction in severe traumatic brain injury with transcranial Doppler ultrasonography." Chin J Traumatol **4**(3): 156-160.

Tarasow, E., A. Abdulwahed Saleh Ali, et al. (2007). "Measurements of the middle cerebral artery in digital subtraction angiography and MR angiography." Med Sci Monit **13 Suppl 1**: 65-72.

Ter Minassian, A., L. Beydon, et al. (2001). "Doppler study of middle cerebral artery blood flow velocity and cerebral autoregulation during a simulated ascent of Mount Everest." Wilderness Environ Med **12**(3): 175-183.

Theis, M. K., B. Honigman, et al. (1993). "Acute mountain sickness in children at 2835 meters." Am J Dis Child **147**(2): 143-145.

Tissot van Patot, M. C., G. Leadbetter, et al. (2005). "Greater free plasma VEGF and lower soluble VEGF receptor-1 in acute mountain sickness." J Appl Physiol **98**(5): 1626-1629.

Torikoshi, S., M. H. Wilson, et al. (1995). "Ultrasound measurement of transcranial distance during head-down tilt." J Gravit Physiol **2**(1): P145-146.

Tsai, F. Y., A. M. Wang, et al. (1995). "MR staging of acute dural sinus thrombosis: correlation with venous pressure measurements and implications for treatment and prognosis." AJNR Am J Neuroradiol **16**(5): 1021-1029.

Vajda, Z., A. Buki, et al. (1999). "Transcranial Doppler-determined pulsatility index in the evaluation of endoscopic third ventriculostomy (preliminary data)." Acta Neurochir (Wien) **141**(3): 247-250.

Valdueza, J. M., J. O. Balzer, et al. (1997). "Changes in blood flow velocity and diameter of the middle cerebral artery during hyperventilation: assessment with MR and transcranial Doppler sonography." AJNR Am J Neuroradiol **18**(10): 1929-1934.

Van Osta, A., J. J. Moraine, et al. (2005). "Effects of high altitude exposure on cerebral hemodynamics in normal subjects." Stroke **36**(3): 557-560.

Vardy, J. and K. Judge (2006). "Acute mountain sickness and ascent rates in trekkers above 2500 m in the Nepali Himalaya." Aviat Space Environ Med **77**(7): 742-744.

Varnell, R. M., G. K. Stimac, et al. (1987). "CT diagnosis of toxic brain injury in cyanide poisoning: considerations for forensic medicine." AJNR Am J Neuroradiol **8**(6): 1063-1066.

Virues-Ortega, J., G. Buela-Casal, et al. (2004). "Neuropsychological functioning associated with high-altitude exposure." Neuropsychol Rev **14**(4): 197-224.

Voulgaris, S. G., M. Partheni, et al. (2005). "Early cerebral monitoring using the transcranial Doppler pulsatility index in patients with severe brain trauma." Med Sci Monit **11**(2): CR49-52.

Vuyk, J., J. Van Den Bos, et al. (2006). "Acetazolamide improves cerebral oxygenation during exercise at high altitude." High Alt Med Biol **7**(4): 290-301.

Ward, M., J. S. Milledge, et al. (2000). High Altitude Physiology. London, Arnold Publishers.

Watzman, H. M., C. D. Kurth, et al. (2000). "Arterial and venous contributions to near-infrared cerebral oximetry." Anesthesiology **93**(4): 947-953.

Wesensten, N. J., J. Crowley, et al. (1993). "Effects of simulated high altitude exposure on long-latency event-related brain potentials and performance." Aviat Space Environ Med **64**(1): 30-36.

West, J. B. (1984). "Human physiology at extreme altitudes on Mount Everest." Science **223**(4638): 784-788.

West, J. B. (1996). "T.H. Ravenhill and his contributions to mountain sickness." J Appl Physiol **80**(3): 715-724.

West, J. B. (2004). "Paralysis and blindness during a balloon ascent to high altitude." High Alt Med Biol **5**(4): 453-456.

West, J. B., R. B. Schoene, et al. (2007). High Altitude Medicine and Physiology. London, Hodder Arnold.

Weyland, A., H. Stephan, et al. (1994). "Flow velocity measurements as an index of cerebral blood flow. Validity of transcranial Doppler sonographic monitoring during cardiac surgery." Anesthesiology **81**(6): 1401-1410.

Wiedman, M. and G. C. Tabin (1999). "High-altitude retinopathy and altitude illness." Ophthalmology **106**(10): 1924-1926; discussion 1927.

Wiener, T. C. (2012). "Space obstructive syndrome: intracranial hypertension, intraocular pressure, and papilledema in space." Aviation, space, and environmental medicine **83**(1): 64-66.

Williams, A. G., M. P. Rayson, et al. (2000). "The ACE gene and muscle performance." Nature **403**(6770): 614.

Willie, C. K., D. B. Macleod, et al. (2012). "Regional brain blood flow in man during acute changes in arterial blood gases." The Journal of physiology **590**(Pt 14): 3261-3275.

Willmann, G., M. D. Fischer, et al. (2011). "Quantification of optic disc edema during exposure to high altitude shows no correlation to acute mountain sickness." PloS one **6**(11): e27022.

Wilson, M. and H. Montgomery (2007). "Impact of genetic factors on outcome from brain injury." Br J Anaesth **99**(1): 43-48.

Wilson, M. E., M. Imray, C. Wright, A (2008). "Changes in Pupil Dynamics at high altitude - an observational study using a handheld pupillometer." High Altitude Medicine and Biology **In Press**.

Wilson, M. H., I. Davagnanam, et al. (2013). "Cerebral venous system and anatomical predisposition to high-altitude headache." Annals of neurology **73**(3): 381-389.

Wilson, M. H., M. E. Edsell, et al. (2011). "Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia--an ultrasound and MRI study." J Cereb Blood Flow Metab **31**(10): 2019-2029.

Wilson, M. H., C. H. Imray, et al. (2011). "The headache of high altitude and microgravity--similarities with clinical syndromes of cerebral venous hypertension." High Alt Med Biol **12**(4): 379-386.

Wilson, M. H., C. H. Imray, et al. (2011). "The Headache of High Altitude and Microgravity?Similarities with Clinical Syndromes of Cerebral Venous Hypertension." High Alt Med Biol.

Wilson, M. H., D. Z. Levett, et al. (2011). "Stroke at high altitude diagnosed in the field using portable ultrasound." Wilderness Environ Med **22**(1): 54-57.

Wilson, M. H. and J. Milledge (2008). "Direct measurement of intracranial pressure at high altitude and correlation of ventricular size with acute mountain sickness: Brian Cummins' results from the 1985 Kishtwar expedition." Neurosurgery **63**(5): 970-974; discussion 974-975.

Wilson, M. H., S. Newman, et al. (2009). "The cerebral effects of ascent to high altitudes." Lancet Neurology **8**: 175-191.

Wilson, M. H., D. Wise, et al. (2012). "Emergency burr holes: "How to do it"." Scandinavian journal of trauma, resuscitation and emergency medicine **20**: 24.

Winn, H. R., R. Rubio, et al. (1981). "Brain adenosine concentration during hypoxia in rats." Am J Physiol **241**(2): H235-242.

Wolff, C. B. (2000). "Cerebral blood flow and oxygen delivery at high altitude." High Alt Med Biol **1**(1): 33-38.

Wolff, C. B., N. Richardson, et al. (2007). "Near infra-red spectroscopy and arterial oxygen extraction at altitude." Adv Exp Med Biol **599**: 183-189.

Wolff, H. and W. Lennox (1930). "Cerebral circulation XII. The effect on pial vessels of variations in the oxygen and carbon dioxide content of blood." Arch Neurol Psychiatry **23**: 1097-1120.

Wollman, H., T. C. Smith, et al. (1968). "Effects of extremes of respiratory and metabolic alkalosis on cerebral blood flow in man." J Appl Physiol **24**(1): 60-65.

Woods, D. R., S. Allen, et al. (2008). "High altitude arrhythmias." Cardiology **111**(4): 239-246.

Woods, D. R. and H. E. Montgomery (2001). "Angiotensin-converting enzyme and genetics at high altitude." High Alt Med Biol **2**(2): 201-210.

Woods, D. R., A. J. Pollard, et al. (2002). "Insertion/deletion polymorphism of the angiotensin I-converting enzyme gene and arterial oxygen saturation at high altitude." Am J Respir Crit Care Med **166**(3): 362-366.

Wright, A. D., C. H. Imray, et al. (1995). "Intracranial pressure at high altitude and acute mountain sickness." Clin Sci (Lond) **89**(2): 201-204.

Wu, T., S. Ding, et al. (2006). "Ataxia: an early indicator in high altitude cerebral edema." High Alt Med Biol **7**(4): 275-280.

Wu, T. Y., S. Q. Ding, et al. (2007). "Who should not go high: chronic disease and work at altitude during construction of the Qinghai-Tibet railroad." High Alt Med Biol **8**(2): 88-107.

Wu, X., X. Li, et al. (1998). "Effects of acute moderate hypoxia on human performance of arithmetic." Space Med Med Eng (Beijing) **11**(6): 391-395.

Yamakawa, M., L. X. Liu, et al. (2003). "Hypoxia-inducible factor-1 mediates activation of cultured vascular endothelial cells by inducing multiple angiogenic factors." Circ Res **93**(7): 664-673.

Yang, S. P., G. W. Bergo, et al. (1994). "Cerebral pressure-flow and metabolic responses to sustained hypoxia: effect of CO2." J Appl Physiol **76**(1): 303-313.

Yang, Y. B., B. Sun, et al. (1993). "Effects of acute hypoxia on intracranial dynamics in unanesthetized goats." J Appl Physiol **74**(5): 2067-2071.

Yaron, M., S. Niermeyer, et al. (2003). "Physiologic response to moderate altitude exposure among infants and young children." High Alt Med Biol **4**(1): 53-59.

Yaron, M., N. Waldman, et al. (1998). "The diagnosis of acute mountain sickness in preverbal children." Arch Pediatr Adolesc Med **152**(7): 683-687.

Zhang, R., J. H. Zuckerman, et al. (2002). "Autonomic neural control of dynamic cerebral autoregulation in humans." Circulation **106**(14): 1814-1820.

Zhou, H., G. M. Saidel, et al. (2008). "Cerebral blood flow adaptation to chronic hypoxia." Adv Exp Med Biol **614**: 371-377.

Zhou, X. D. (1984). "Transient ischemic attack in young people
at high altitude." Qinghai Med. J. **14**(2): 44-45.

Zrinzo, L. U., M. Crocker, et al. (2006). "Commercial flight and patients with intracranial mass lesions: a caveat. Report of two cases." J Neurosurg **105**(4): 627-630.

Zubieta-Calleja, G. R., Jr., G. Zubieta-Castillo, Sr., et al. (1994). "Acute high altitude hypoventilation that resulted in hyperventilation following hyperoxic tests." from http://altitudeclinic.com/hyperv.html.

Zuj, K. A., D. K. Greaves, et al. (2007). "WISE-2005: reduced cerebral blood flow velocity with nitroglycerin--comparison with common carotid artery blood flow." J Gravit Physiol **14**(1): P65-66.

12 Appendix

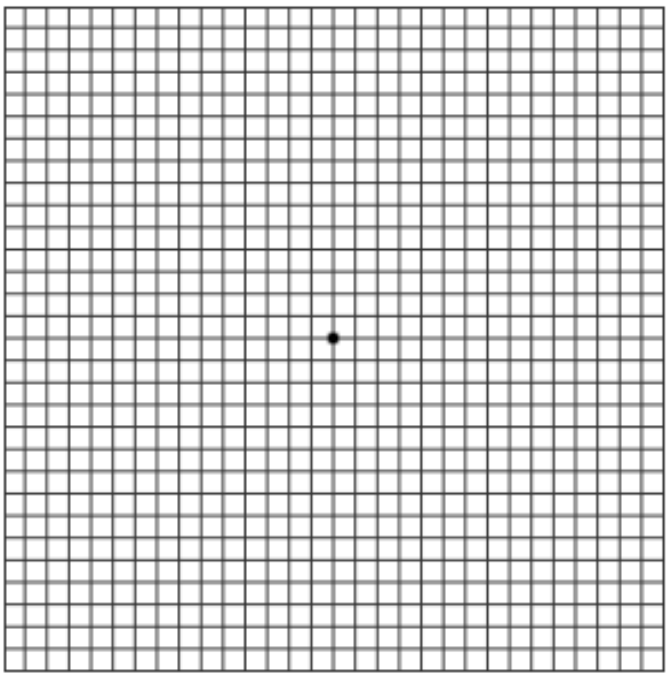
Chapter 2: Methodology Appendix

2A: Amsler Chart:

AMDF American Macular Degeneration Foundation
Saving sight through research and education

American Macular
Degeneration Foundation
PO Box 515
Northampton MA 01061-0515
1.888.MACULAR
1.888.622.8527

AMSLER'S CHART TO TEST YOUR SIGHT

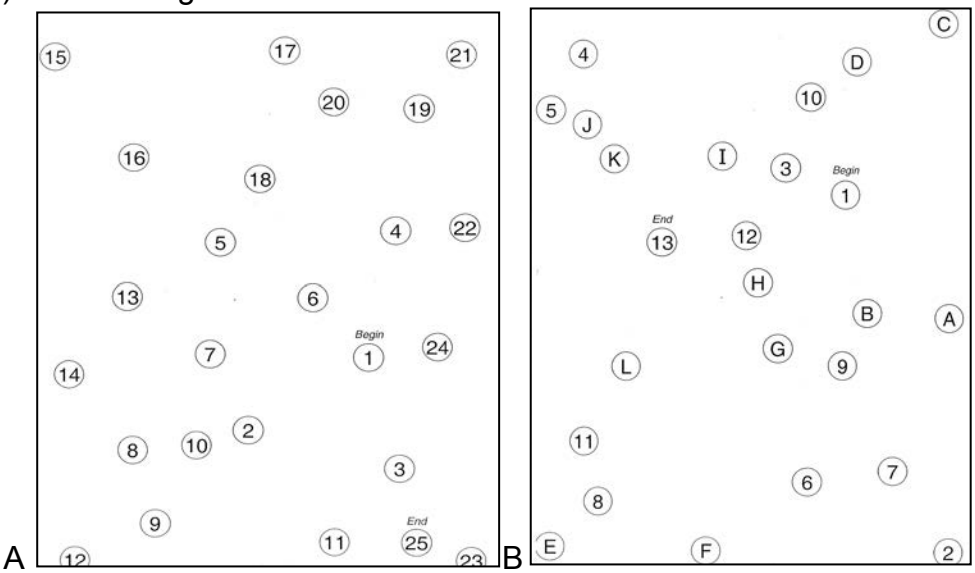


Instructions For Use

1. Print this page.
2. Tape this page at eye level and where light is consistent and without glare.
3. Put on your reading glasses and cover one eye.
4. Fix your gaze on the center black dot.
5. Keeping your gaze fixed, try to see if any lines are distorted or missing.
6. Mark the defect on the chart.
7. TEST EACH EYE SEPARATELY.
8. If the distortion is new to you or has worsened, arrange to see your ophthalmologist at once.
9. *Always* keep the Amsler's Chart the *same distance* from your eyes each time you test.
10. Print new copies of Amsler's Chart as needed.

2B: Neuropsychology:

i) Trail Making:



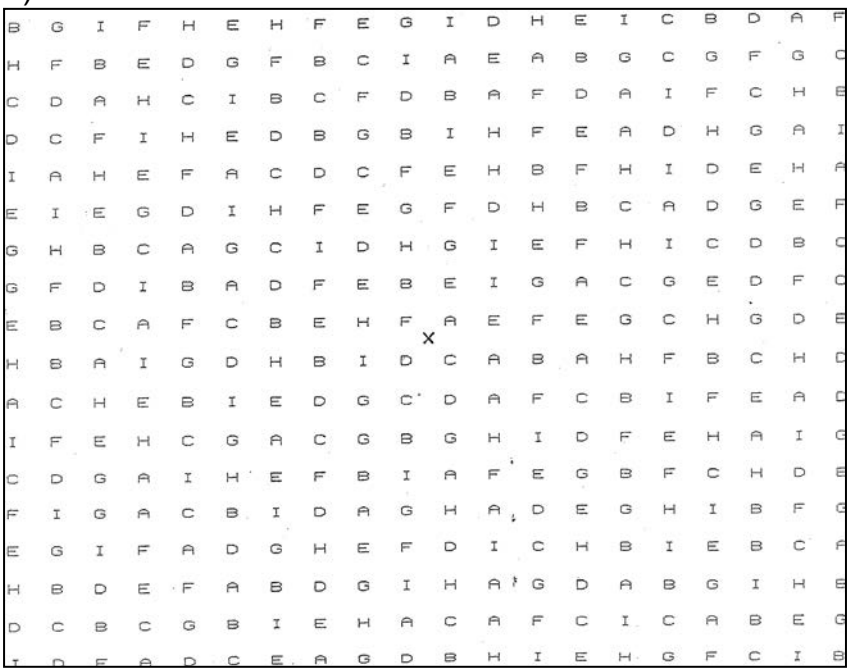
ii) Stroop Color Word Test (SCWT):

Form C Stimulus Sheet

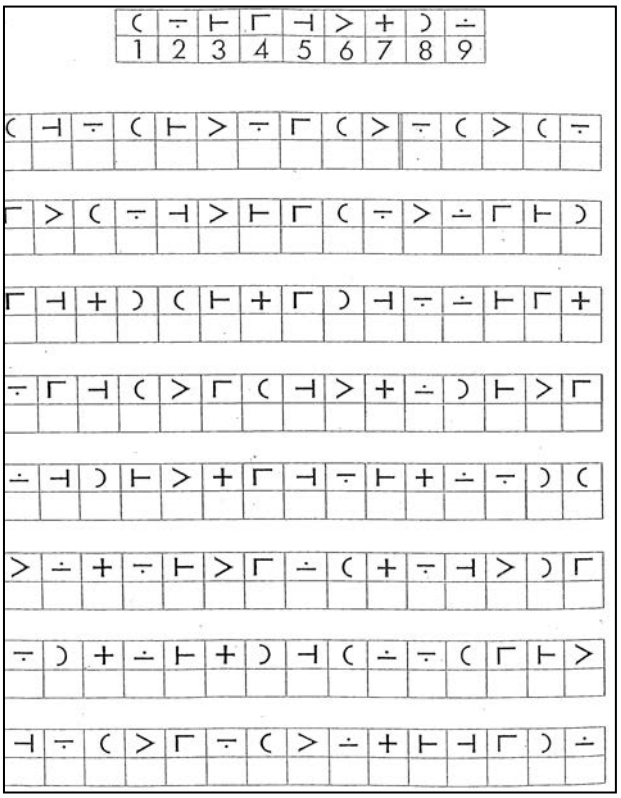
BLUE	RED	TAN	RED
GREEN	GREEN	RED	TAN
TAN	TAN	TAN	RED
RED	BLUE	BLUE	TAN
GREEN	GREEN	TAN	BLUE
BLUE	BLUE	RED	GREEN
GREEN	TAN	GREEN	RED
BLUE	GREEN	RED	BLUE
RED	TAN	BLUE	RED
BLUE	BLUE	TAN	TAN
TAN	GREEN	RED	GREEN
RED	BLUE	GREEN	TAN
TAN	GREEN	RED	BLUE
GREEN	RED	TAN	RED
BLUE	BLUE	BLUE	BLUE
TAN	GREEN	TAN	RED
GREEN	TAN	GREEN	GREEN
RED	RED	TAN	RED
TAN	TAN	BLUE	BLUE
RED	GREEN	TAN	TAN
TAN	TAN	BLUE	BLUE
RED	RED	GREEN	GREEN
GREEN	BLUE	RED	BLUE
RED	RED	GREEN	RED
TAN	GREEN	TAN	BLUE
BLUE	RED	RED	TAN
GREEN	TAN	GREEN	BLUE
TAN	BLUE	BLUE	GREEN

PAR Psychological Assessment Resources, Inc. · 16204 N. Florida Avenue · Lutz, FL 33549 · 1.800.331.8378 · www.parinc.com
Copyright © 1989 by Psychological Assessment Resources, Inc. All rights reserved. 9 8 7 6 5 4 3 2 1 Reorder #RO-1483 Printed in the U.S.A.

iii) Letter Cancellation:



iv) Digit Symbol Modalities test:



v) Rey Auditory Verbal Learning test (RAVLT):

DRUM	DRUM	DRUM	DRUM	DRUM	DESK	DRUM
CURTAIN	CURTAIN	CURTAIN	CURTAIN	CURTAIN	RANGER	CURTAIN
BELL	BELL	BELL	BELL	BELL	BIRD	BELL
COFFEE	COFFEE	COFFEE	COFFEE	COFFEE	SHOVEL	COFFEE
SCHOOL	SCHOOL	SCHOOL	SCHOOL	SCHOOL	STOVE	SCHOOL
PARENT	PARENT	PARENT	PARENT	PARENT	MOUNTAIN	PARENT
MOON	MOON	MOON	MOON	MOON	GLASSES	MOON
GARDEN	GARDEN	GARDEN	GARDEN	GARDEN	TOWEL	GARDEN
HAT	HAT	HAT	HAT	HAT	CLOUD	HAT
FARMER	FARMER	FARMER	FARMER	FARMER	BOAT	FARMER
NOSE	NOSE	NOSE	NOSE	NOSE	LAMB	NOSE
TURKEY	TURKEY	TURKEY	TURKEY	TURKEY	GUN	TURKEY
COLOUR	COLOUR	COLOUR	COLOUR	COLOUR	PENCIL	COLOUR
HOUSE	HOUSE	HOUSE	HOUSE	HOUSE	CHURCH	HOUSE
RIVER	RIVER	RIVER	RIVER	RIVER	FISH	RIVER

vi) Block Design

Chapter 4:Brain Oxygenation Appendix:

Table 4A

		London Resting	Namche Resting	EBC Resting
London Resting rSO2	Pearson Sig. (2-tailed) N	.078 .317 165	-.040 .611 162	.012 .890 139
London Unloaded rSO2	Pearson Sig. (2-tailed) N	.084 .285 165	-.040 .617 162	.024 .783 139
London AT rSO2	Pearson Sig. (2-tailed) N	.044 .578 165	-.038 .629 162	.069 .419 139
London VO2Max rSO2	Pearson Sig. (2-tailed) N	.047 .549 165	-.049 .534 162	.050 .557 139
London Lowest rSO2	Pearson Sig. (2-tailed) N	.050 .521 165	-.044 .577 162	.046 .594 139
Namche Resting rSO2	Pearson Sig. (2-tailed) N	-.040 .603 168	-.007 .929 167	.009 .916 141
Namche Unloaded rSo2	Pearson Sig. (2-tailed) N	-.038 .621 168	-.017 .829 167	.003 .971 141
Namche AT rSO2	Pearson Sig. (2-tailed) N	-.020 .798 168	.081 .300 167	.055 .515 141
Namche VO2Max rSO2	Pearson Sig. (2-tailed) N	-.034 .664 168	.088 .256 167	.116 .170 141
Namche Lowest rSO2	Pearson Sig. (2-tailed) N	-.061 .435 167	.090 .249 166	.127 .135 140
EBC Resting rSO2	Pearson Sig. (2-tailed) N	.040 .637 141	-.014 .874 139	.204 .016 140
EBC Unloaded rSO2	Pearson Sig. (2-tailed) N	.059 .485 144	.014 .871 142	.263 .002 143
EBC AT rSO2	Pearson Sig. (2-tailed) N	.024 .774 141	.008 .924 139	.248 .003 140
EBC VO2Max rSO2	Pearson Sig. (2-tailed) N	.029 .730 141	-.012 .891 139	.202 .017 140
EBC Lowest rSO2	Pearson Sig. (2-tailed) N	.022 .791 142	.023 .790 140	.235 .005 141

Table 4A: Correlations between resting SaO2 and resting rSO2 at each altitude. Only at Everest Base camp does a significant correlation between resting SaO2 and rSO2 occur.

Table 4B

		%age change in rSO2 at VO2Max 75m	Time to VO2Max at 75m	%age Change in rSO2 at VO2Max Namche	Time to VO2Max at 3500m	%age change in rSO2 at VO2Max EBC	Time to VO2 Max at 5300m
%age change in rSO2 at VO2Max 75m	Pearson Correlation	1	.054	.486**	.103	.435**	.193*
	Sig. (2-tailed)		.493	.000	.191	.000	.024
	N	165	165	161	163	136	137
Time to VO2Max at 75m	Pearson Correlation	.054	1	-.263**	-.012	-.193*	-.189*
	Sig. (2-tailed)	.493		.001	.877	.022	.024
	N	165	171	167	169	141	142
%age Change in rSO2 at VO2Max Namche	Pearson Correlation	.486**	-.263**	1	.178*	.604**	.102
	Sig. (2-tailed)	.000	.001		.022	.000	.235
	N	161	167	167	167	137	138
Time to VO2Max at 3500m	Pearson Correlation	.103	-.012	.178*	1	.012	.228**
	Sig. (2-tailed)	.191	.877	.022		.891	.007
	N	163	169	167	169	139	140
%age change in rSO2 at VO2Max EBC	Pearson Correlation	.435**	-.193*	.604**	.012	1	-.140
	Sig. (2-tailed)	.000	.022	.000	.891		.098
	N	136	141	137	139	141	141
Time to VO2 Max at 5300m	Pearson Correlation	.193*	-.189*	.102	.228**	-.140	1
	Sig. (2-tailed)	.024	.024	.235	.007	.098	
	N	137	142	138	140	141	142

Table 4B: Correlation between Time to achieve VO_2Max and Percentage desaturation of rSO_2

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4C

		Headache score at 150m	Headache present at 150m (Y/N)	Headache score at 3500m	Headache present at 3500m (Y/N)	Headache score at 5300m	Headache present at 5300m (Y/N)
Resting rSO2 at 75m	Pearson Corr	-.155*	-.078	-.087	-.045	.075	.062
	Sig. (2-tailed)	.047	.321	.271	.566	.380	.466
Unloaded rSO2 at 75m	Pearson Corr	-.180*	-.096	-.089	-.043	.056	.040
	Sig. (2-tailed)	.020	.219	.256	.588	.513	.638
AT rSO2 at 75m	Pearson Corr	-.196*	-.124	-.099	-.046	.066	.057
	Sig. (2-tailed)	.011	.114	.208	.563	.440	.506
VO2Max rSO2 at 75m	Pearson Corr	-.129	-.090	-.110	-.037	.081	.043
	Sig. (2-tailed)	.098	.251	.162	.641	.340	.618
Lowest rSO2 at 75m	Pearson Corr	-.132	-.091	-.109	-.043	.028	.004
	Sig. (2-tailed)	.090	.243	.168	.585	.747	.966
rSO2 %age Change Unloaded at 75m	Pearson Corr	-.146	-.106	-.002	.018	-.076	-.088
	Sig. (2-tailed)	.061	.176	.982	.822	.370	.302
rSO2 %age Change AT at 75m	Pearson Corr	-.089	-.109	-.042	-.007	-.031	-.018
	Sig. (2-tailed)	.255	.162	.597	.928	.718	.833
rSO2 %age Change VO2Max at 75m	Pearson Corr	-.003	-.038	-.094	-.020	.036	-.009
	Sig. (2-tailed)	.965	.631	.233	.795	.675	.920
rSO2 %age Change lowest at 75m	Pearson Corr	-.002	-.035	-.091	-.028	-.040	-.062
	Sig. (2-tailed)	.975	.659	.249	.719	.638	.464
Resting rSO2 at 3500m	Pearson Corr	-.181*	-.127	-.060	-.034	.164	.109
	Sig. (2-tailed)	.019	.102	.443	.662	.052	.196
Unloaded rSO2 at 3500m	Pearson Corr	-.197*	-.138	-.066	-.047	.197*	.135
	Sig. (2-tailed)	.011	.075	.398	.549	.019	.109
AT rSO2 at 3500m	Pearson Corr	-.189*	-.139	-.058	-.045	.169*	.147
	Sig. (2-tailed)	.014	.073	.455	.566	.044	.080
VO2Max rSO2 at 3500m	Pearson Corr	-.166*	-.130	.017	.055	.069	.056
	Sig. (2-tailed)	.032	.094	.823	.481	.412	.505

Lowest rSO2 at 3500m	Pearson Corr	-.156*	-.123	.015	.050	.091	.080
	Sig. (2-tailed)	.044	.115	.844	.524	.283	.344
rSO2 %age Change Unloaded at 3500m	Pearson Corr	-.144	-.088	-.040	-.061	.160	.128
	Sig. (2-tailed)	.063	.257	.609	.433	.057	.128
rSO2 %age Change ATat 3500m	Pearson Corr	-.085	-.069	-.042	-.062	.067	.112
	Sig. (2-tailed)	.276	.376	.587	.427	.430	.185
rSO2 %age Change VO2Max at 3500m	Pearson Corr	-.085	-.076	.055	.090	-.039	-.014
	Sig. (2-tailed)	.275	.329	.482	.250	.646	.870
rSO2 %age Change Lowest at 3500m	Pearson Corr	-.082	-.073	.051	.079	-.006	.021
	Sig. (2-tailed)	.293	.347	.515	.307	.941	.804
Resting rSO2 at 5300m	Pearson Corr	-.052	-.071	.000	-.068	.020	.003
	Sig. (2-tailed)	.542	.405	.999	.424	.811	.973
Unloaded rSO2 at 5300m	Pearson Corr	-.306**	-.253**	.003	-.058	.058	.042
	Sig. (2-tailed)	.000	.002	.968	.489	.489	.615
AT rSO2 at 5300m	Pearson Corr	-.077	-.098	-.024	-.100	.010	.005
	Sig. (2-tailed)	.365	.250	.783	.240	.907	.956
VO2Max rSO2 at 5300m	Pearson Corr	-.091	-.116	-.010	-.075	.072	.073
	Sig. (2-tailed)	.284	.172	.910	.376	.394	.388
Lowest rSO2 at 5300m	Pearson Corr	-.252**	-.220**	.008	-.072	.101	.102
	Sig. (2-tailed)	.002	.009	.922	.395	.232	.225
rSO2%age change Unloaded at 5300m	Pearson Corr	-.122	-.139	-.040	-.050	.053	.053
	Sig. (2-tailed)	.149	.100	.640	.554	.530	.533
rSO2%age change AT at 5300m	Pearson Corr	-.117	-.118	-.060	-.101	-.033	-.008
	Sig. (2-tailed)	.167	.161	.481	.232	.700	.921
rSO2%age change VO2Max at 5300m	Pearson Corr	-.083	-.099	-.011	-.033	.065	.083
	Sig. (2-tailed)	.328	.244	.898	.697	.444	.325
rSO2%age change Lowest at 5300m	Pearson Corr	-.080	-.089	-.002	-.053	.094	.113
	Sig. (2-tailed)	.350	.298	.986	.538	.267	.184

Table 4C: Correlations between headache as recorded at the time of testing and rSO_2

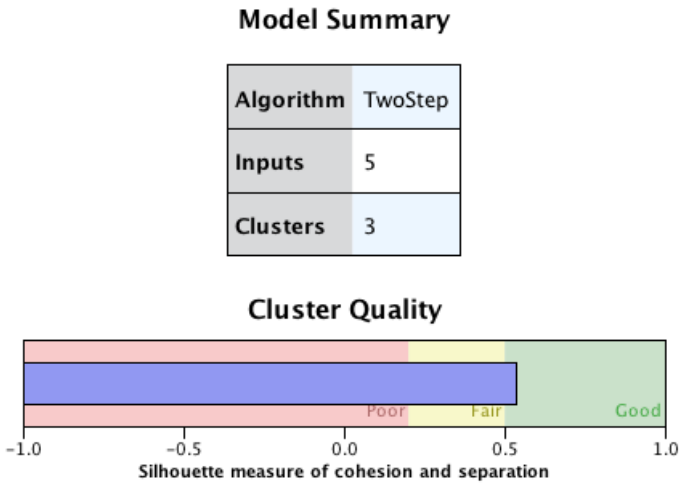
*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

This shows no correlation between rSO_2 values and headache score reported at the time of exercise testing.

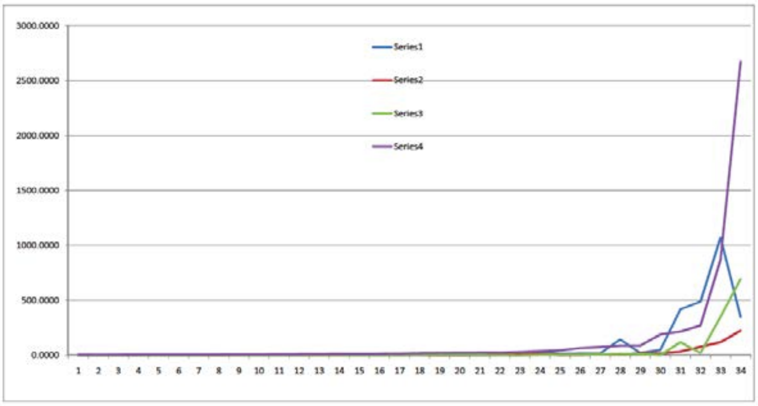
Figures 4A:

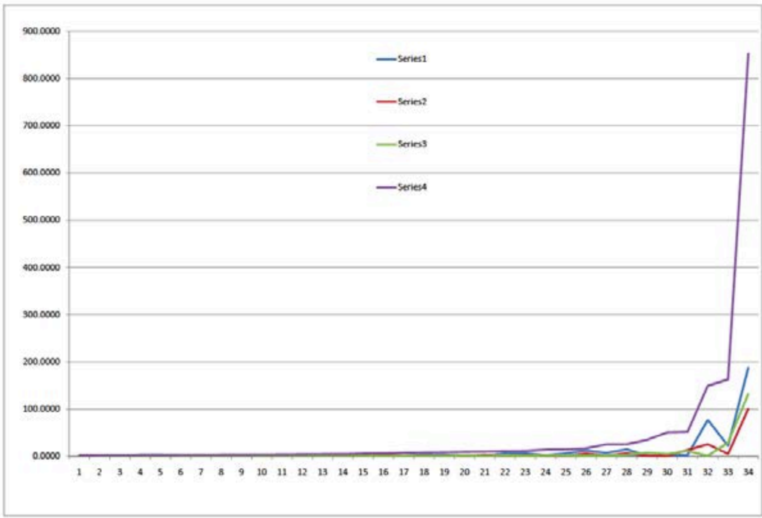
Initial Cluster Analysis.

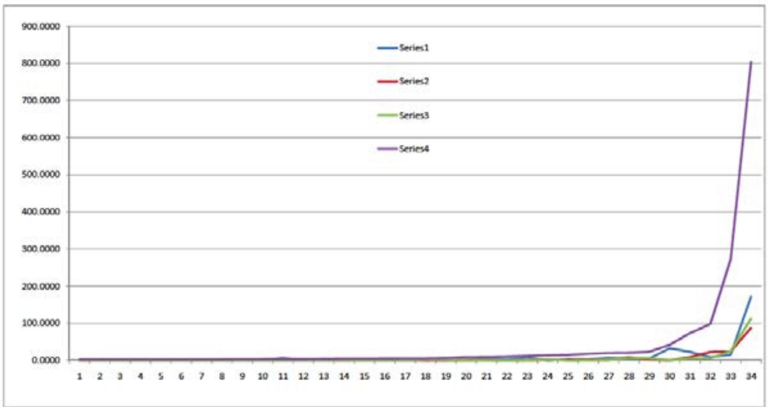
This simply split data into right, medium and low rSO2 values, but starting rSO2 values (which this was mostly being based on) have little meaning between individuals. Cerebral oximtery is usually used as a trend monitor hence a cluster analysis grouping subjects by their pattern of rSO2 changes is more appropriate. Below is the initial cluster.



Allgodendrums to create 2 clusters (Figure 4A - i to iv).







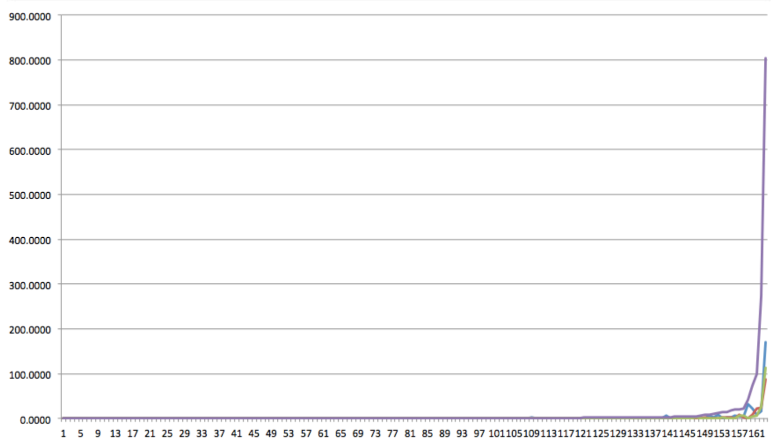


Table 4D: Descriptives

		n	Mean	Std Dev	Std Error	95% CI		Min	Max
						Lower	Upper		
Mean Resting rSO2	1	48	76.09	3.90	.56	74.96	77.22	67.67	86.67
	2	78	68.91	2.96	.34	68.24	69.57	62.50	78.00
	3	39	60.23	5.00	.80	58.60	61.85	37.67	67.3
	Total	165	68.94	6.88	.54	67.89	70.00	37.67	86.67
Mean Unloaded rSO2	1	48	76.07	3.67	.53	75.00	77.13	68.67	87.83
	2	78	69.53	2.96	.34	68.86	70.19	62.83	82.00
	3	39	60.19	5.03	.80	58.56	61.82	36.00	66.00
	Total	165	69.22	6.85	.53	68.17	70.28	36.00	87.83
Mean AT rSO2	1	48	78.87	3.73	.54	77.79	79.96	73.17	92.00
	2	78	71.29	2.87	.32	70.64	71.93	64.00	77.83
	3	39	62.18	4.71	.76	60.65	63.71	40.00	70.67
	Total	165	71.34	7.04	.55	70.26	72.42	40.00	92.00
Mean VO2Max rSO2	1	48	77.94	4.26	.61	76.70	79.17	70.33	89.83
	2	78	66.24	4.821	.55	65.15	67.32	53.16	76.50
	3	39	56.76	5.70	.91	54.92	58.61	40.00	66.67
	Total	165	67.40	9.15	.71	66.00	68.81	40.00	89.83
Mean Lowest rSO2	1	48	75.34	5.20	.75	73.83	76.85	67.67	89.83
	2	78	63.58	4.79	.54	62.50	64.66	52.00	73.33
	3	39	54.66	5.59	.89	52.85	56.47	40.00	65.16
	Total	165	64.89	9.13	.71	63.49	66.30	40.00	89.83

Table 4E:

Clusters actually used:

Cluster 1: A09 B02 B06 B07 B08 B10 C01 C02 C07 D03 D04 D06 D07 E01 E02 E05 E06 E07 E16 F01 F03 F07 F08 F09 G04 G06 G08 H01 H05 I02 I03 I05 I10 J03 J04 K09 L02 L06 L08 M06 M07 A01 A02 B12 B13 B14 C09 C10 C11 C13 C15 D11 D14 D15 E12 E13 F10 G11 G13 H04 I01 I09 I15 J11 J13 K12 K13 K14 L10 L12 L13 L15 L16 M01 M02 M11 M15 M16

Cluster 2: A04 A05 A06 A07 A12 A13 B01 B04 B05 C04 C05 C06 C08 D01 E08 F04 G02 G05 G07 G12 H06 H07 H08 H15 I04 I06 I07 I16 J01 J02 J05 J06 J08 K02 K03 K04 K05 K06 L01 L04 L05 L07 M03 M05 M08 A10 A11 A14 A15 A16 B09 B11 B15 B16 C16 D12 D16 E04 E09 E10 E11 E14 E15 G09 G10 G14 G16 H03 H10 H11 H12 H13 I08 I12 I13 I14 J09 J10 J14 J15 J16 K16 L09 L11 L14 M09 M12

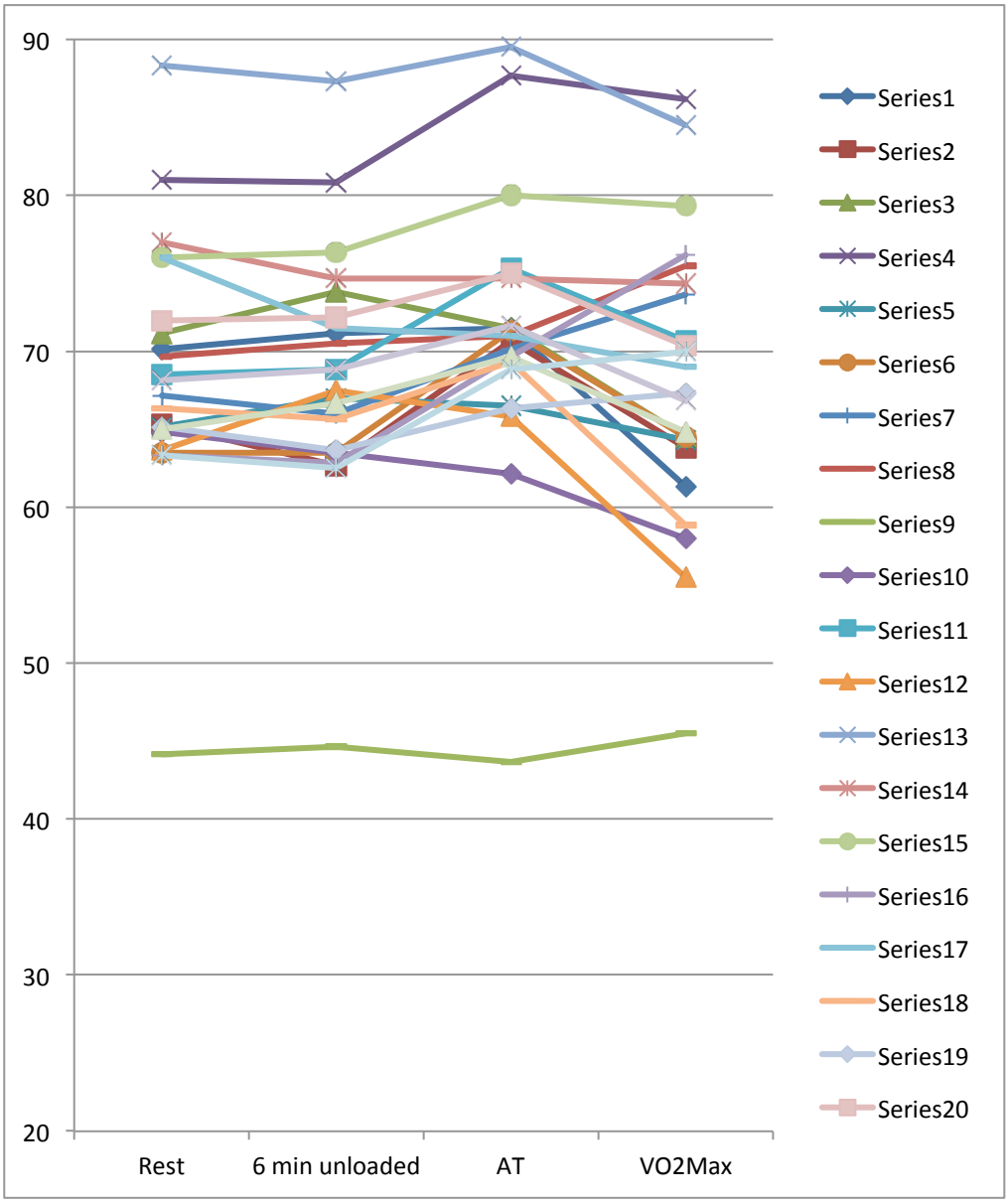
Table 4F:

Table demonstrating mean percentage changes in rSO_2 values between the two clusters.

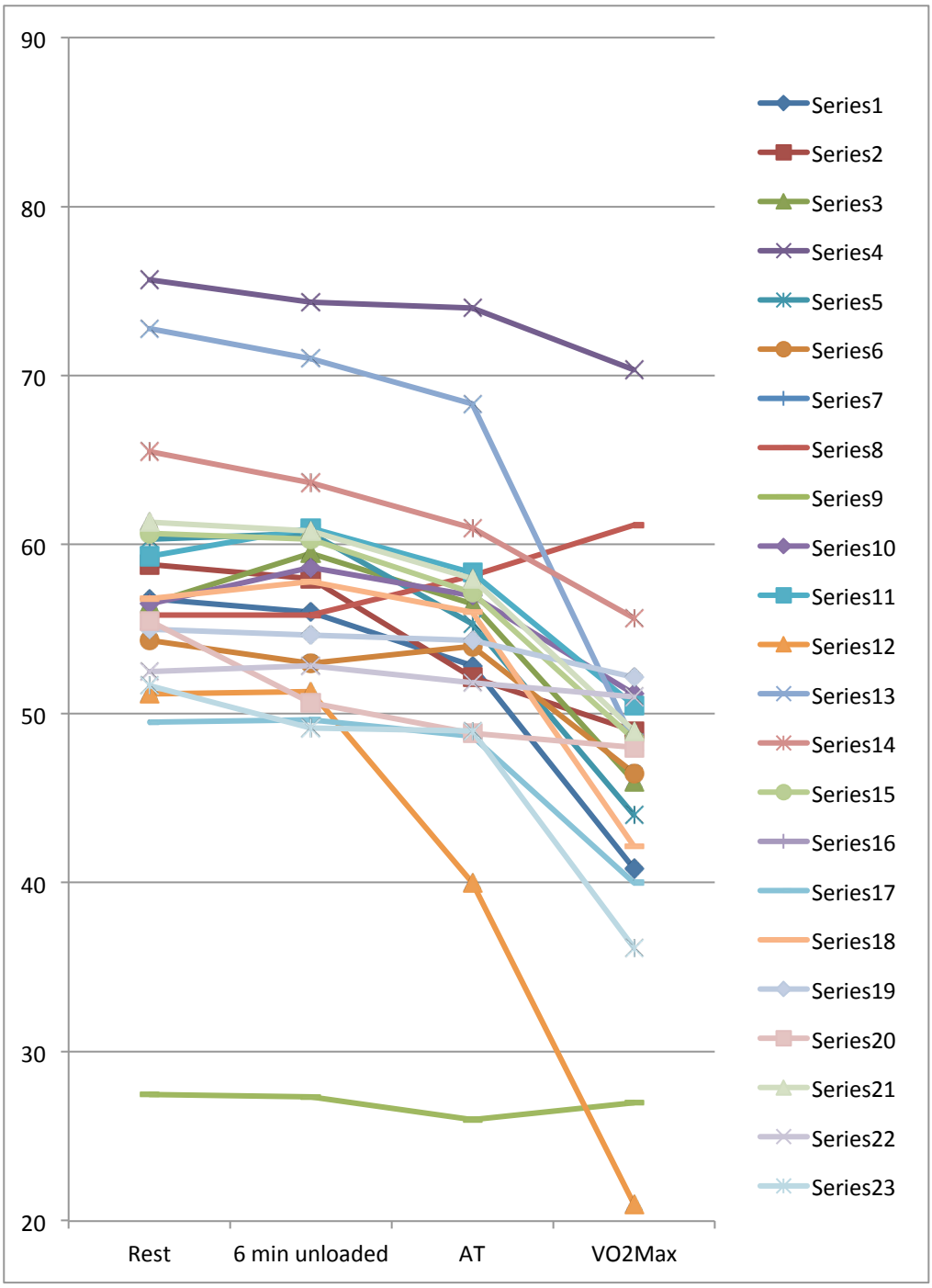
	Mean			Std. Deviation			Std. Error			95% CI for Mean						Minimum			Maximum			
										Lower Bound			Upper Bound									
	1	2	Total	1	2	Total	1	2	Total	1	2	Total	1	2	Total	1	2	Total	1	2	Total	effect size
London Unloaded	0.44	0.43	0.43	2.43	2.30	2.36	0.26	0.25	0.18	-0.11	-0.06	0.07	0.99	0.92	0.80	-7.88	-4.42	-7.88	5.13	6.47	6.47	0.00
London AT	1.33	5.57	3.57	2.96	3.59	3.92	0.34	0.38	0.31	0.67	4.80	2.96	2.00	6.33	4.17	-5.46	-3.93	-5.46	11.29	13.80	13.80	-1.08
London VO2Max	-9.48	4.22	-2.26	6.04	4.52	8.65	0.68	0.49	0.67	-10.84	3.25	-3.59	-8.12	5.18	-0.93	-25.07	-3.24	-25.07	-1.59	16.26	16.26	-1.58
London Lowest rSO2	-12.53	0.00	-5.92	6.24	5.80	8.68	0.71	0.62	0.68	-13.93	-1.24	-7.25	-11.12	1.24	-4.59	-28.37	-15.95	-28.37	-2.49	16.26	16.26	-1.44
Namche Unloaded	-1.19	-0.63	-0.90	3.25	2.58	2.93	0.37	0.28	0.23	-1.92	-1.19	-1.35	-0.46	-0.07	-0.45	-11.97	-10.68	-11.97	6.33	6.32	6.33	-0.19
Namche AT	-8.03	-5.56	-6.76	5.75	4.18	5.14	0.65	0.46	0.41	-9.33	-6.48	-7.56	-6.74	-4.65	-5.96	-26.89	-20.13	-26.89	3.81	2.59	3.81	-0.48
Namche VO2Max	-19.01	-11.53	-15.15	11.21	8.93	10.74	1.27	0.98	0.85	-21.54	-13.48	-16.82	-16.48	-9.58	-13.48	-50.70	-39.82	-50.70	17.06	5.26	17.06	-0.70
Namche Lowest rSO2	-22.41	-14.71	-18.44	11.55	9.24	11.08	1.31	1.01	0.87	-25.01	-16.73	-20.16	-19.80	-12.69	-16.72	-53.78	-41.16	-53.78	16.72	5.26	16.72	-0.69
EBC Unloaded	-3.28	-2.01	-2.63	3.95	3.03	3.55	0.49	0.36	0.30	-4.26	-2.74	-3.23	-2.31	-1.29	-2.03	-15.55	-8.35	-15.55	4.48	6.83	6.83	-0.36
EBC AT	-9.90	-8.20	-9.02	5.46	4.85	5.20	0.67	0.58	0.44	-11.24	-9.35	-9.90	-8.56	-7.05	-8.14	-21.49	-20.54	-21.49	0.34	3.22	3.22	-0.33
EBC VO2Max	-18.23	-13.39	-15.74	9.53	9.13	9.60	1.17	1.09	0.82	-20.57	-15.57	-17.37	-15.88	-11.21	-14.11	-43.15	-42.57	-43.15	-1.03	6.58	6.58	-0.50
EBC Lowest	-21.15	-16.52	-18.78	10.24	8.50	9.64	1.26	1.02	0.83	-23.66	-18.57	-20.42	-18.63	-14.48	-17.14	-52.38	-44.80	-52.38	-6.31	-2.21	-2.21	-0.48

Figure 4B:

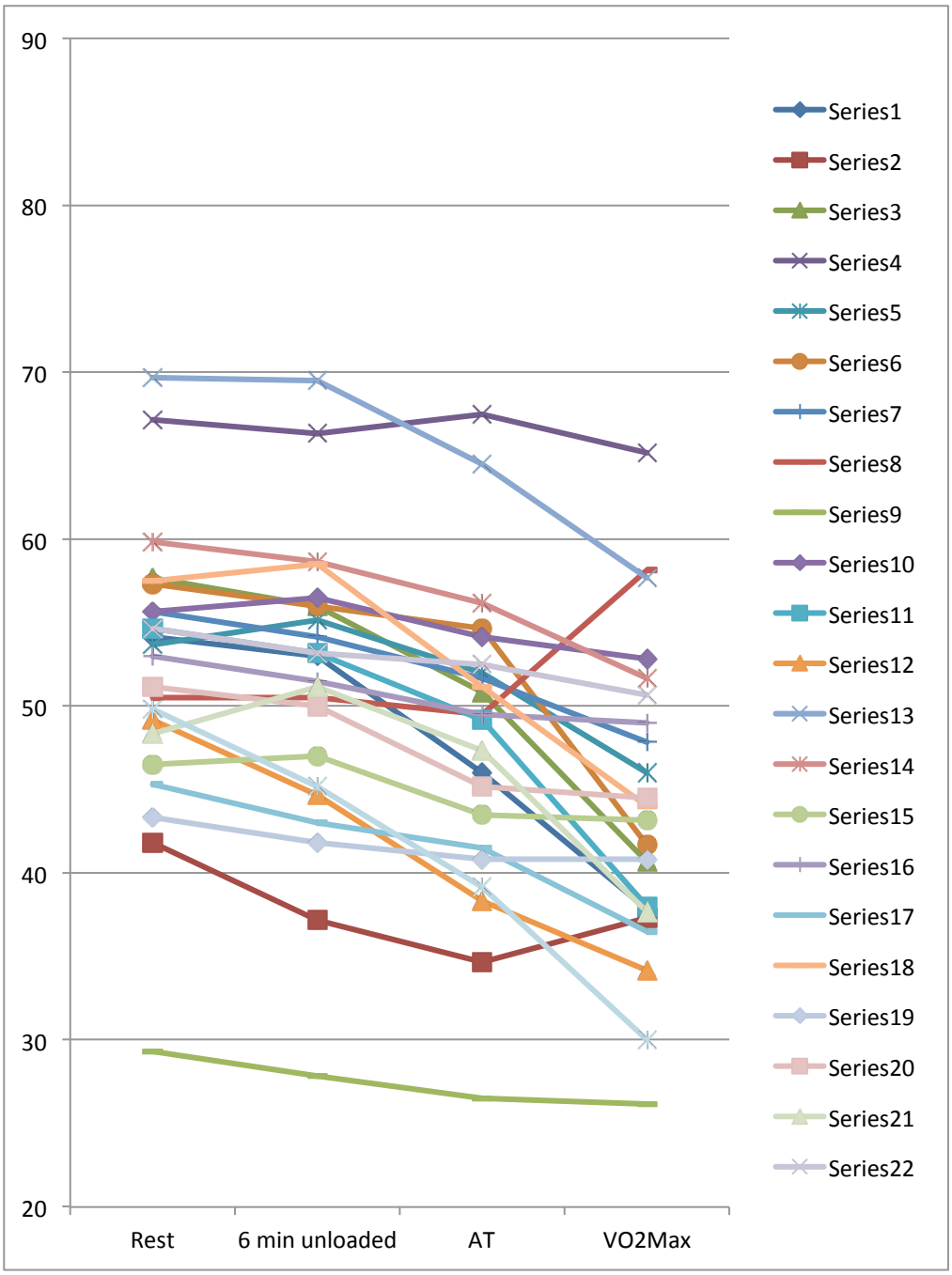
Figure A demonstrates the raw rSO_2 readings at each time point during exercise at a) 75m, b) 3500m and c) 5300m. From this it can be seen that there is wide spread of rSO_2 values, but subjects that start with high rSO_2 values, tend to remain high compared to others when at altitude.



i)rSO_2s for the Core team - Sea-level (75m)



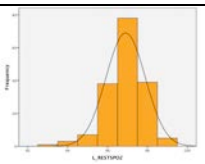
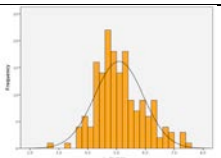
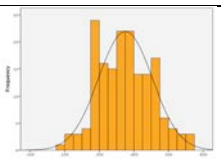
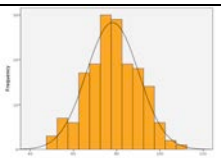
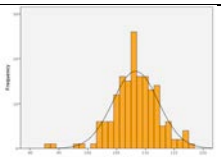
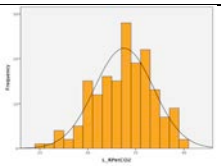
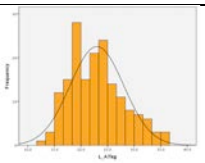
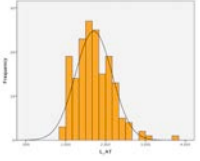
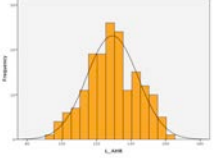
ii) rSO_2s for the Core team - Namche (3500m)

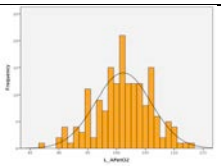
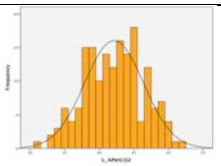
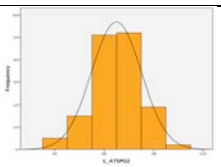
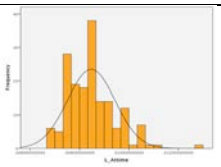
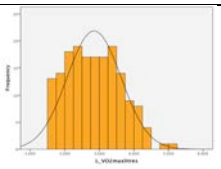
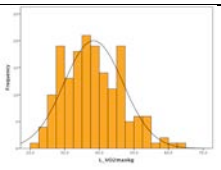
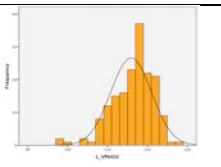
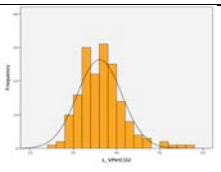
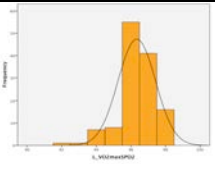
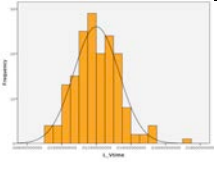


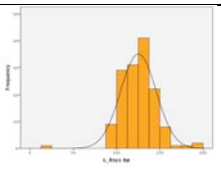
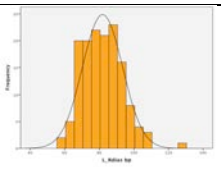
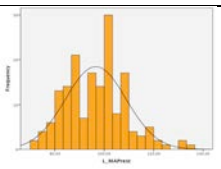
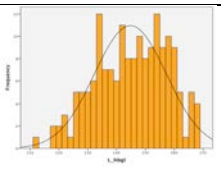
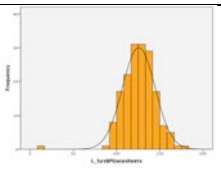
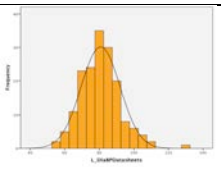
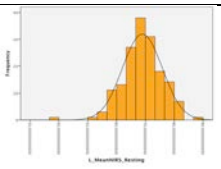
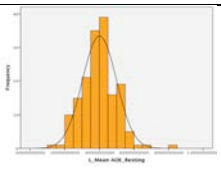
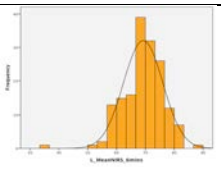
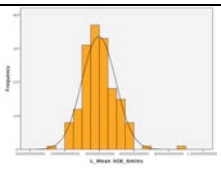
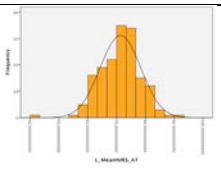
iii) rSO_2s for the Core team - EBC (5300m)

Chapter 5: Headache and Anthropomorphic Data Appendix:

Table 5A:
Basic Data for Physiological Variables in London (150m):

	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles 25th and 75th		Histogram
Resting SaO2/%	171	96.9	0.99	93.0	99.00	96.00	98.00	
Resting VO2/Kg	17	5.08	0.85	2.70	7.40	4.50	5.60	
Resting VO2	171	0.38	0.08	0.20	0.56	0.31	0.43	
Resting Heart Rate	171	78.1	12.1	50.0	111.0	70.00	87.00	
Resting EtO2/mmHg	171	108.30	3.98	93.0	118.0	106.0	111.0	
Resting EtCO2/mmHg	171	33.78	3.06	25.0	40.00	32.00	36.00	
AT / Kg	171	23.0	5.05	13.2	36.50	19.10	26.30	
AT	171	1.70	0.46	0.92	3.71	1.39	2.04	
AT Heart Rate	171	129.25	14.78	93.0	163.0	119.0	141.0	

AT EtO2 / mmHg	171.	101.15	4.87	87.00	113.00	98.00	105.00	
AT EtCO2 / mmHg	171	42.13	4.26	31.00	52.00	39.00	45.00	
AT SaO2 / %	144.	96.49	1.01	94.00	99.00	96.00	97.00	
At Time / hrs	171	0.0885	0.0009	0.007	0.012	0.078	0.009	
VO2Max / litres	171	2.85	0.78	1.51	5.16	2.17	3.44	
VO2Max / Kg	171	38.33	8.53	22.2	63.80	31.40	44.90	
VO2Max heartRatee	171	170.41						
VO2Max EtO2/mmHg	171	115.90	5.14	98.0	127.0	113.0	119.0	
VO2Max EtCO2/mmHg	171	36.06	5.18	25.0	56.00	33.00	39.00	
VO2Max SaO2/%	129	96.3	1.09	92.0	98.00	96.00	97.00	
VO2Max time / hrs	171	0.012	0.0013	0.009	0.017	0.012	0.013	

Resting Sys BP/mmHg	145	125.18	20.66	16.0	195.0	111.0	136.0	
Resting Dia BP/ mmHg	145	81.86	11.62	58.0	128.0	73.00	89.00	
Resting MAP/mmHg	171	96.58	12.36	70.00	134.67	87.00	104.33	
Hb conc.	171	144.78	12.46	112.00	168.00	135.00	155.00	
Sys BP on sheets	171	125.66	18.95	12.0	180.0	114.0	138.0	
Dia BP on sheets	171	80.74	11.32	55.0	128.0	73.00	87.00	
Resting rSO2 %	165	68.94	6.88	37.67	86.67	65.08	73.08	
Resting AOE %	165	0.40	0.10	0.15	0.84	0.34	0.45	
rSO2 at 6mins / %	165	69.22	6.85	36.00	88.00	65.17	74.00	
AOE at 6 mins/ %	165	0.40	0.10	0.13	0.87	0.33	0.46	
rSO2 at AT / %	165	71.34	7.04	40.00	92.00	66.33	75.83	

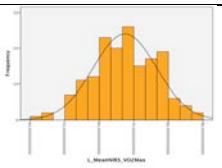
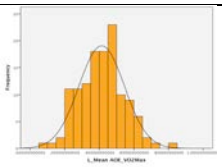
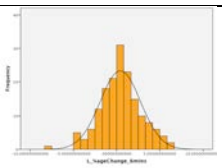
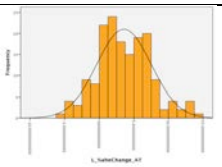
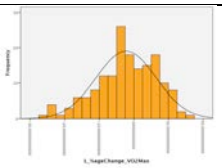
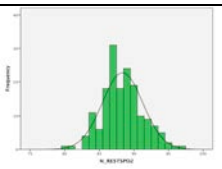
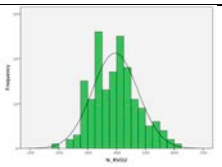
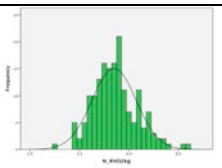
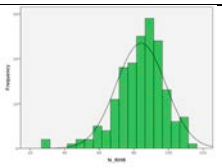
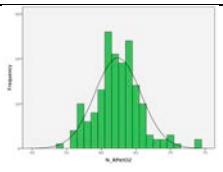
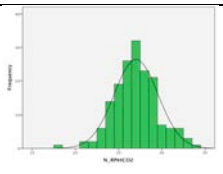
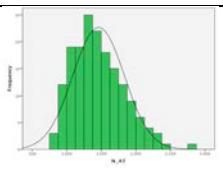
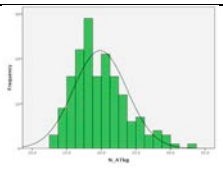
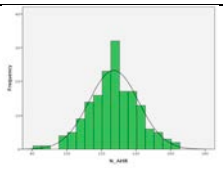
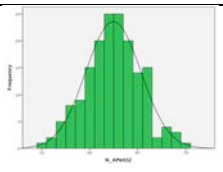
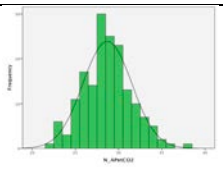
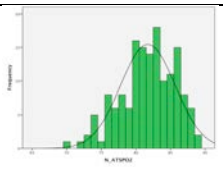
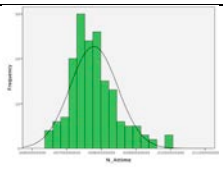
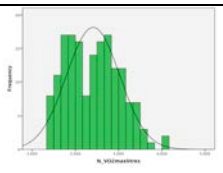
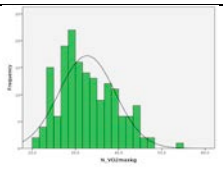
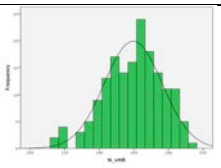
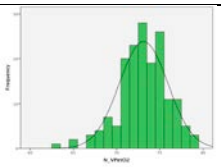
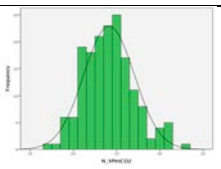
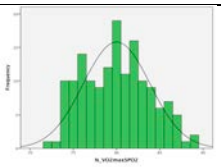
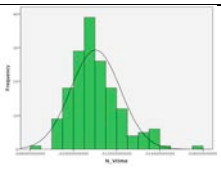
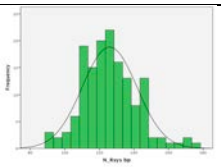
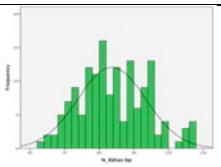
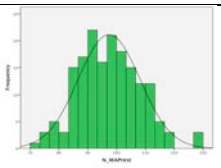
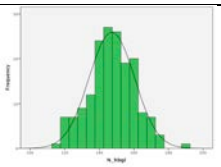
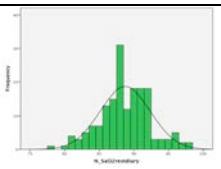
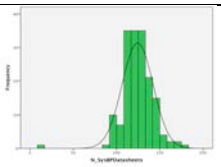
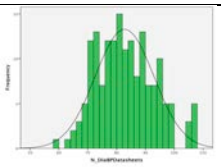
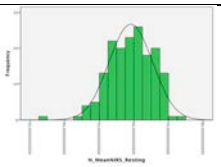
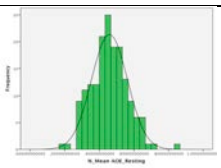
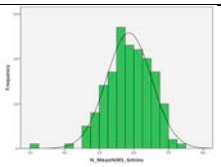
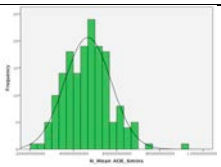
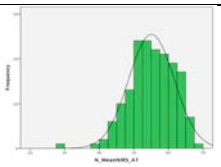
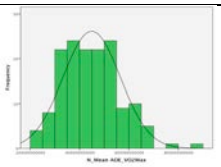
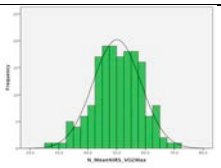
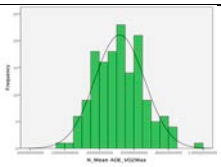
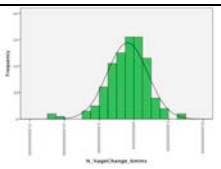
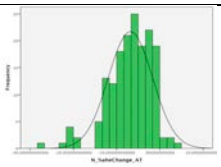
rSO2 at VO2Max / %	165	67.40	9.15	40.00	89.83	61.17	74.67	
AOE at VO2Max / %	126	0.41	0.13	0.09	0.82	0.32	0.50	
rSO2%change6mins	165	0.43	2.36	-7.88	6.47	-1.01	1.76	
rSO2%changeAT	165	3.57	3.92	-5.46	13.80	0.83	6.29	
rSO2%chagneVO2Max	165	-2.26	8.65	-25.07	16.26	-7.69	4.40	

Table 5B:
Basic Data for Physiological Variables in Namche(5300m):

	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles 25th and 75th		Histogram
Resting SaO2/%	168	88.36	2.953	80	97	87	90	
Resting VO2/Kg	169	0.39	0.08	0.19	0.61	0.34	0.45	
Resting VO2	169	5.398	0.8996	3	8.4	4.8	5.95	
Resting Heart Rate	169	84.12	14.375	29	115	76	93.5	

Resting EtO2/mmHg	169	62.38	3.351	54	74	60.5	64	
Resting EtCO2/mmHg	169	27.02	2.559	18	34	25	29	
AT	169	1.46021	0.371932	0.818	2.77	1.171	1.701	
AT / Kg	169	19.828	3.87	13.3	33.5	17	22	
AT Heart Rate	168	127.21	14.429	83	162	118	136	
AT EtO2 / mmHg	169	62.46	2.862	55	70	61	64	
AT EtCO2 / mmHg	169	28.7	2.828	22	38	27	30	
AT SaO2 / %	153	81.73	3.952	70	89	79.5	85	
AT Time / hrs	169	0.007784078	0.000687408	0.006481481	0.009953704	0.007407407	0.008101852	
VO2Max / litres	168	2.41	0.62	1.34	4.16	1.89	2.88	
VO2Max / Kg	168	32.713	6.4998	20.5	54.2	27.85	37.7	

VO2Max HeartRate	167	159.63	16.729	114	193	148	172	
VO2Max EtO2/mmHg	168	73.13	2.816	63	79	71.25	75	
VO2Max EtCO2/mmHg	168	24.13	2.929	17	33	22	26	
VO2Max SaO2/%	153	80.01	3.862	72	89	77	83	
VO2Max time / hrs	169	0.011024956	0.000115096	0.000846064 8	0.015740741	0.010190972	0.011574074	
Resting Sys BP/mmHg	149	125.93	15.801	91	175	114	136	
Resting Dia BP/ mmHg	149	83.61	9.892	63	106	77	91	
Resting MAP/mmHg	171	97.4	10.758	73	128	89.67	104.33	
Hb conc	171	147.23	13.196	115	188	139	156	
NResting SaO2 diary	171	88.74	3.668	78	98	87	91	
Sys BP on sheets	169	124.29	17.934	12	175	114	135.5	

Dia BP on sheets	169	82.75	10.163	59	107	74.5	89.5	
Resting rSO2 %	168	59.11	6.28	34.33	74.50	54.67	63.63	
Resting AOE %	167	0.46	0.10	0.20	0.84	0.39	0.52	
SO2 at 6mins / %	168	58.57	6.501	31	73	54.21	63.5	
AOE at 6 mins/ %	167	0.47	0.11	0.23	0.90	0.39	0.54	
rSO2 at AT / %	168	55.07	6.59	30	69	50.21	60.13	
AOE at AT	152	0.45	0.12	0.21	0.89	0.37	0.52	
rSO2 at VO2Max	168	50.11	8.2956	25.5	70.3	44.708	56.5	
AOE at VO2Max	152	0.52	0.14	0.19	0.96	0.41	0.62	
rSO2%change6mins	168	-0.94	2.91	-11.97	6.33	-2.68	1.03	
rSO2%changeAT	167	-6.80	5.12	-26.89	3.81	-9.59	-3.19	

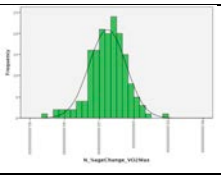
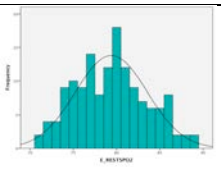
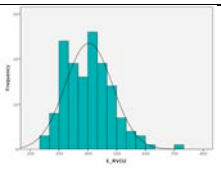
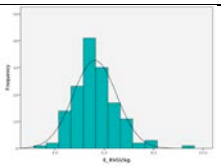
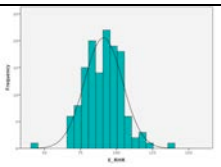
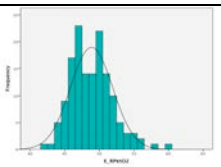
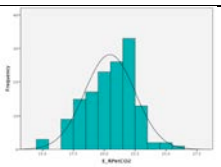
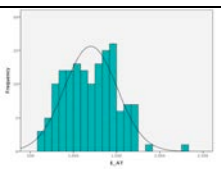
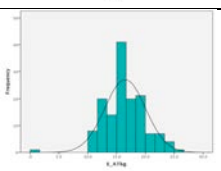
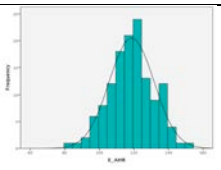
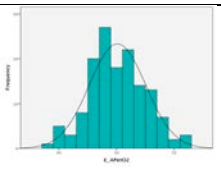
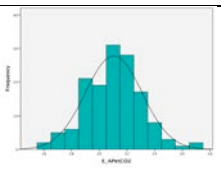
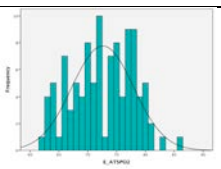
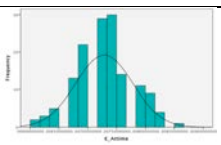
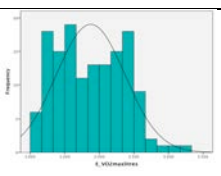
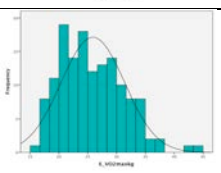
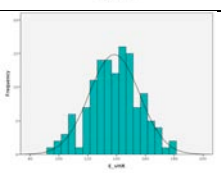
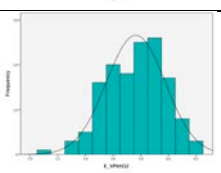
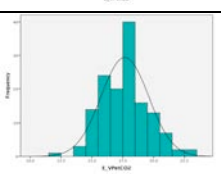
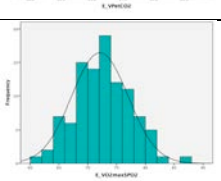
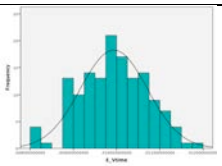
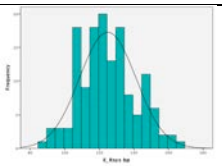
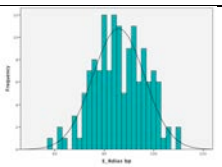
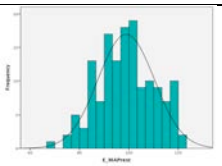
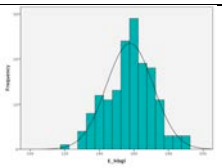
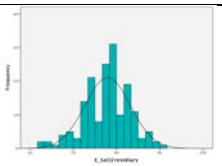
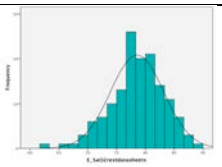
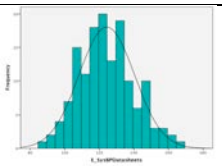
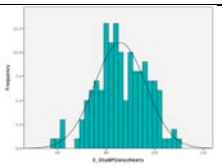
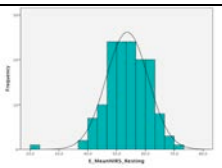
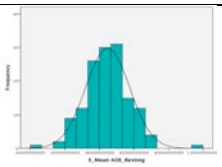
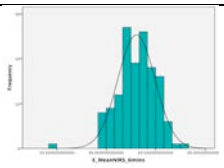
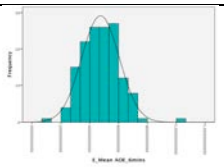
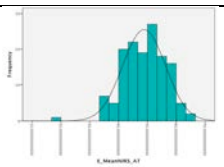
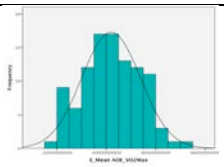
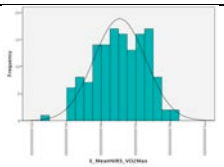
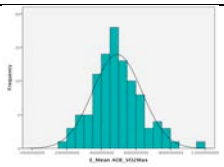
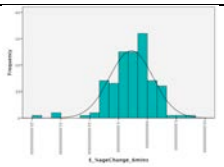
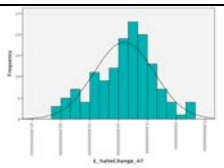
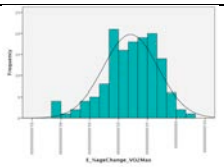
rSO2%chaneVO2Max	167	-15.22	10.64	-50.70	17.06	-21.58	-7.63	

Table 5C:
Basic Data for Physiological Variables at Everest Base Camp (5300m):

	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles 25th and 75th		Histogram
Resting SaO2/%	144	79.4	4.166	71	89	76	82	
Resting VO2	145	0.40381	0.08204	0.248	0.719	0.3355	0.461	
Resting VO2/Kg	145	5.621	0.8878	3.4	9.6	5.1	6.1	
Resting Heart Rate	136	90.91	13.214	43	140	82	99.75	
Resting EtO2/mmHg	145	48.88	3.053	42	60	47	51	
Resting EtCO2/mmHg	144	20.46	2.041	15	26	19	22	
AT	143	1.20117	0.3045	0.601	2.289	0.95	1.426	
AT / Kg	144	16.485	3.5598	0	25.9	14.425	18.7	

AT Heart Rate	133	118.68	12.966	82	152	110	128	
AT EtO2 / mmHg	143	50.08	2.45	44	56	48	52	
AT EtCO2 / mmHg	143	21.04	2.055	16	27	20	22	
AT SaO2 / %	104	72.6	5.347	62	86	68.25	77	
AT Time / hrs	143	0.007285 92	0.000 0495 108	0.00 6018 519	0.0085 64815	0.0069 44444	0.0076 38889	
VO2Max / litres	142	1.87 502	0.49 5845	1.00 5	3.298	1.4377 5	2.2815	
VO2Max / Kg	142	25.87	5.519	16	44	21.18	29.83	
VO2Max HeartRate	130	138.42	17.495	94	180	127	149.25	
VO2Max EtO2/mmHg	142	57.63	2.132	51	62	56	59	
VO2Max EtCO2/mmHg	142	17.67	1.927	12	23	16	19	
VO2Max SaO2/%	101	72.1	4.902	61	86	68	75.5	

VO2Max time / hrs	142	0.009936669	0.000776123	0.008090278	0.011759259	0.009291088	0.010512153	
Resting Sys BP/mmHg	141	124.86	16.28	87	167	113.5	134.5	
Resting Dia BP/ mmHg	141	85.82	10.45	58	109	78.5	93	
Resting MAP/mm Hg	145	98.87	11.369	68	123	91.34	107.33	
Hb conc.	159	157.67	13.524	120	191	149	167	
SaO2rest diary/%	167	77.92	5.314	62	91	74	81	
SaO2rest datasheets	145	78.555	4.645	62	89	76.25	81.65	
Sys BP on sheets	145	124.3	16.04	87	167	112.5	134	
Dia BP on sheets	145	85.77	10.517	58	109	79	93.5	
Resting rSO2 %	144	53.677	7.3291	22	71.7	49.042	59.125	
Resting AOE %	143	0.45	0.13	0.06	0.97	0.38	0.52	

rSO2 at 6mins / %	144	52.23	7.57	17.83	70.17	47.17	58.33	
AOE at 6 mins/ %	143	0.47	0.13	0.09	1.05	0.39	0.55	
rSO2 at AT / %	142	48.85	7.41	18.83	65.67	43.33	54.42	
AOE at VO2Max	103	0.42	0.12	0.16	0.73	0.34	0.51	
E_MeanNIRS_VO2Max	141	45.33	7.46	22.83	61.33	39.83	51.50	
AOE at VO2Max	100	0.49	0.14	0.17	0.96	0.40	0.57	
rSO2%change6mins	144	-2.80	3.77	-18.94	6.83	-4.94	-0.56	
rSO2%changeAT	142	-9.03	5.20	-21.49	3.22	-12.02	-5.82	
rSO2%changeVO2Max	141	-15.83	9.49	-43.15	6.58	-21.59	-8.91	

Chapter 7: Retinal Imaging:

Table 7A: Grades of papilloedema:
Modified Frisén Scale

Table 1. Modified Frisén Scale

Papilledema Grade
0 (Normal Optic Disc)
Prominence of the retinal nerve fiber layer at the nasal, superior, and inferior poles in inverse proportion to disc diameter
Radial nerve fiber layer striations, without tortuosity
1 (Minimal Degree of Edema)
C-shaped halo that is subtle and grayish with a temporal gap; obscures underlying retinal details[a]
Disruption of normal radial nerve fiber layer arrangement striations
Temporal disc margin normal
2 (Low Degree of Edema)
Circumferential halo[a]
Elevation (nasal border)
No major vessel obscuration
3 (Moderate Degree of Edema)
Obscuration of ≥1 segment of major blood vessels leaving disc[a]
Circumferential halo
Elevation (all borders)
Halo (irregular outer fringe with finger-like extensions)
4 (Marked Degree of Edema)
Total obscuration on the disc of a segment of a major blood vessel on the disc[a]
Elevation (whole nerve head, including the cup)
Border obscuration (complete)
Halo (complete)
Grade 5 (Severe Degree of Edema)
Obscuration of all vessels on the disc and leaving the disc[a]

[a] Key features (major findings) for each grade.

Diagnosis and Grading of Papilledema in Patients With Raised Intracranial Pressure Using Optical Coherence Tomography vs Clinical Expert Assessment Using a Clinical Staging Scale
Arch Ophthalmol. 2010;128(6):705-711.
doi:10.1001/archophthalmol.2010.94

Chapter 8: Venous MRI Study:

Figure 8A Calculation of CCS:

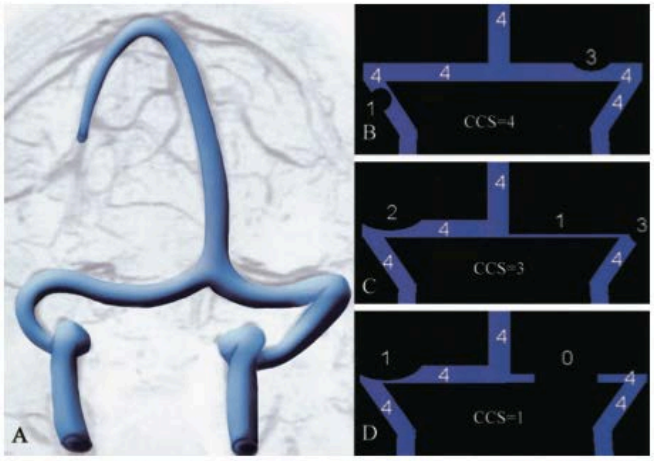


Figure 2. Schematic diagram of the system used for grading the patency of the transverse and sigmoid sinus. The grade for each right and left transverse-sigmoid conduit was determined separately and defined by the highest degree of stenosis encountered from the torcula to the distal sigmoid sinus and given a corresponding number from 0 to 4. 0 = discontinuity (gap) or aplastic segment; 1 = hypoplasia or severe stenosis within a segment of the conduit estimated as less than 25% of the cross sectional diameter of the lumen of the distal superior sagittal sinus; 2 = moderately stenosed segment of the conduit (25–50%), 3 = mildly narrowed segment (50–75%); and 4 = no significant narrowing seen (75–100%). The sum of the right and left provided the combined conduit score (CCS) and generally ranged from 2–8. (A) A normal situation in which each right and left conduit would score a 4 with a resultant CCS of 8, and (B, C, and D) schematic examples of a CCS of 4, 3, and 1.

From Farb RI, Vanek I, Scott JN et al. Idiopathic intracranial hypertension: the prevalence and morphology of sinovenous stenosis. Neurology 2003; 60 (9):141801424

13 Papers Published from this Thesis