

Does Acute Hypoxia and High Altitude Exposure Adversely Affect Cardiovascular Performance?

This dissertation is submitted for the degree of Doctor of Philosophy
(PhD) by Publication with Bournemouth University

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Abstract

Introduction

The cardiovascular adaptations to high altitude (HA) exposure and its relationship to acute mountain sickness (AMS) are incompletely understood.

Aims

This thesis addresses four main hypotheses 1. HA adversely affects biventricular cardiac function leading to an increase in estimated filling pressures which is influenced by the mode of hypoxia. 2. HA exposure leads to myocardial injury that is linked to the development of AMS. 3. HA exposure is associated with a reduction in arterial compliance and an increase in central blood pressure (BP). 4. HA exposure reduces heart rate (HR) variability (HRV) that is linked to AMS an increased risk of cardiac arrhythmias.

Methods

This consisted of eight independent studies conducted at terrestrial and 'simulated' HA (hypobaric hypoxia [HH] and normobaric hypoxia [NH]) Cardiac function and arterial compliance were examined using portable transthoracic echocardiography and pulse contour analysis respectively. Myocardial injury was measured in venous blood by cardiac troponin T (cTnT) quantification. Cardiac inter-beat interval data for HRV analysis was acquired using single lead ECGs and novel finger and patch sensor technologies. Cardiac rhythm was investigated using a novel implantable cardiac monitor.

Results

HA exposure was associated with a non-pathological increase in cTnT, and mild diastolic changes without adversely affecting systolic function or ventricular filling pressures. Resting cardiovascular responses were similar with HH, NH and HA, though notable differences emerged with exercise. Resting central BP, HR and BP-augmentation increased at terrestrial HA. HRV fell (eg reduced time-domain measures, increased LF/HF ratios and less chaos) at HA and was consistently different between men and women. Significant HA (>3500m) was associated with the development of tachyarrhythmia (atrial fibrillation and supraventricular tachycardia) and asymptomatic nocturnal bradycardias and pauses (>3.0 seconds). There were no independent predictors of AMS and its severity.

Conclusion

HA-related hypoxia induces early sympathetic activation leading to an increase in resting HR and central BP and may be proarrhythmic. Parasympathetic activation with acclimatisation can trigger nocturnal pauses at higher altitudes. HA exposure does not adversely affect cardiac function.

Declaration

I declare that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. All of this work was undertaken whilst I was employed as by Poole Hospital NHS trust as a consultant in Cardiology and General internal Medicine.

I declare that the content of the published manuscript are identical to that published with the exception of minor changes to the formatting style which has been undertaken in order to maintain a consistent presentation style and referring throughout the document.

I am aware of and understand the University's policy on plagiarism and I certify that this thesis is my own work, except where indicated by referencing, and the work presented in this thesis has not been submitted in support of another degree or qualification from this or any other university or institute of learning.

Collaborations and Acknowledgements

All of the studies contained in my thesis submission were the result of collaborative research projects. I collaborated with the Defence Medical Services for all of the included studies in this thesis as all of the research volunteers relating to this thesis were serving military servicemen at the time the studies were conducted. My principle collaborators within the Defence Medical Services were Colonel David Woods (Professor of Military Medicine) and Surgeon Commander Dr Adrian Mellor (Royal Navy).

All of the studies conducted using normobaric hypoxia and the Dhaulagiri research involved additional collaborations with Leeds Beckett University and Professor John O'Hara.

I am grateful to the Centre of Aviation Medicine, Henlow, for allowing the use of their hypobaric hypoxic chamber for several of the studies. I would also like to thank the Surgeon General who support for this research has been essential. I wish to acknowledge the contributions of Medtronic, Sonosite, Lumira, the cardiac division of General Electric (GE) and Daily Care Biomedical, Uscom and ithlete. They provided intellectual and financial support whether in the form of a project grant or simply a discount on the costs of their products. I would like to thank the staff at Poole Hospital and my wife for all of their support over this intense seven-year research journey.

I would like to acknowledge the help of Professor Peter Thomas in the Department of Medical Statistics at Bournemouth University for his assistance with SPSS and the conduct of split level ANOVAs. I wish to sincerely thank my Supervisor Professor Alison McConnell for her direction and tutelage during. Finally, I would like to thank the research subjects who agreed to take part in the studies.

List of Key abbreviations

(Less generic abbreviations are qualified within each publication chapter)

AMS	Acute mountain sickness
AP	Augmentation pressure
BNP	Brain Natriuretic peptide
CRP	C-reactive protein
(2,3) DPG	2,3-diphosphoglycerate (2,3-DPG)
ECG	Electrocardiogram
GPS	Global positioning system
HA	High altitude
HACE	High altitude cerebral oedema
HAPE	High altitude pulmonary oedema
HF	High frequency
HRV	Heart rate variability
Hs	High sensitivity
ICM	Implantable cardiac monitor
IF	Impact Factor
IVCT	Isovolumic contraction time
IVRT	isovolumic relaxation time
kPa	kilopascals
LF	low frequency
LLS	Lake Louise scores
LVET	Left ventricular ejection time

MoDREC	Ministry of Defence Research and Ethics Committee
HH	Hypobaric hypoxia
NH	Normobaric hypoxia
NN	Normobaric normoxia
OEII	Operation Everest II
PASP	Pulmonary artery systolic pressure
PCA	Pulse contour analysis
PCO ₂	Partial pressure of oxygen
PO ₂	Partial pressure of oxygen
PP	Pulse pressure
PWV	Pulse wave velocity
SpO ₂	Oxygen saturation
cTn	Cardiac Troponin

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Chapter 1

Introduction

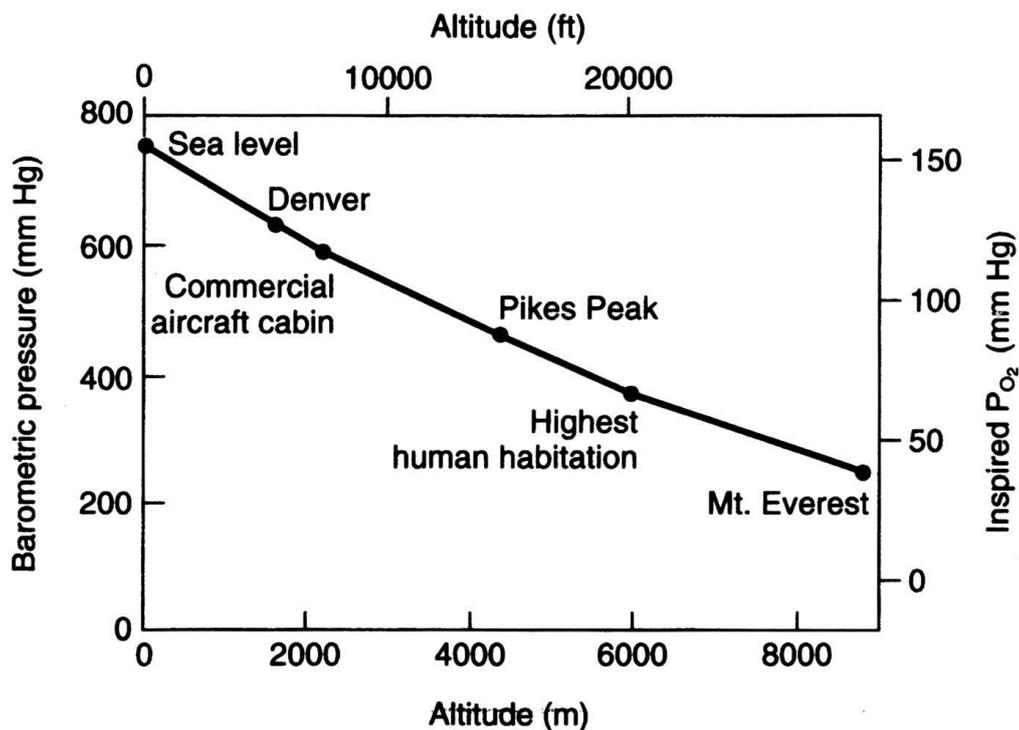
Access to high altitude (HA) has never been easier. Continued improvement in travel access and infrastructure has led to increasing demand to experience this exciting environment. Consequently, there has been a marked rise in the number of people travelling to HA each year. In fact, approximately, 140 million people worldwide live permanently at high altitudes and at least another 40 million people are estimated to travel to HA each year [1]. The HA environment differs markedly from one location to the next (eg the Alps *versus* the Himalayas) and depends on the season (winter *versus* summer or monsoon *versus* dry season), altitude, climate and terrain. With the recent improvements in transport one could, for example, have breakfast in Bournemouth on Saturday morning and potentially be skiing at >2500m in the Alps by the late afternoon.

Beyond the enticing beauty of the scenery, and the unique sporting and recreational opportunities of HA, lie a number of dangers that if not respected, can lead to potentially serious health consequences. The adverse weather (intense cold, high winds, heavy rain or snow), the physical challenges (climbing or long treks and high gradients) and physical risks (eg avalanches and landslides) are some of the obvious dangers, yet they can often be unpredictable. The mental challenges are even more unpredictable and are highly dependent on the HA environment, previous HA experience and an individual's background mental health and resilience [2-5].

One of the greatest risks that all individuals travelling to HA and above 2000m must face is the effects of the falling partial pressure of oxygen (PaO_2). Gases including oxygen, unlike liquids can be compressed and expand and hence whilst the percentage of oxygen in the air remains constant at any altitude within the earth's atmosphere (at 20.9%), with rising altitude

the partial pressure of oxygen (PO_2) falls leading to less available oxygen in the inspired air (Figure 1).

Figure 1 Simple graphical illustration to demonstrate explain the fall in barometric and partial pressure of Oxygen (PO_2) at high altitude (source google images from <http://www.priory.com/anaes/altitude.htm>)



For example, whilst the PO_2 at sea level is approximately 159 mmHg (21 kPa), on the peak of Mount Everest PO_2 it falls to only about 53 mmHg (7kPa) [6, 7]. This leads to hypobaric (reduced pressure) hypoxia (HH) in humans. At HA whilst there is still the same percentage of oxygen (21%; and nitrogen ~79%) in the air as observed at sea level its concentration in air dramatically falls leading to less available oxygen molecules to take part in gas exchange. Consequently, the air is 'thinner' at HA as the weight of gas is less as there are fewer molecules present. This fall in the atmospheric pressure at higher altitude decreases the

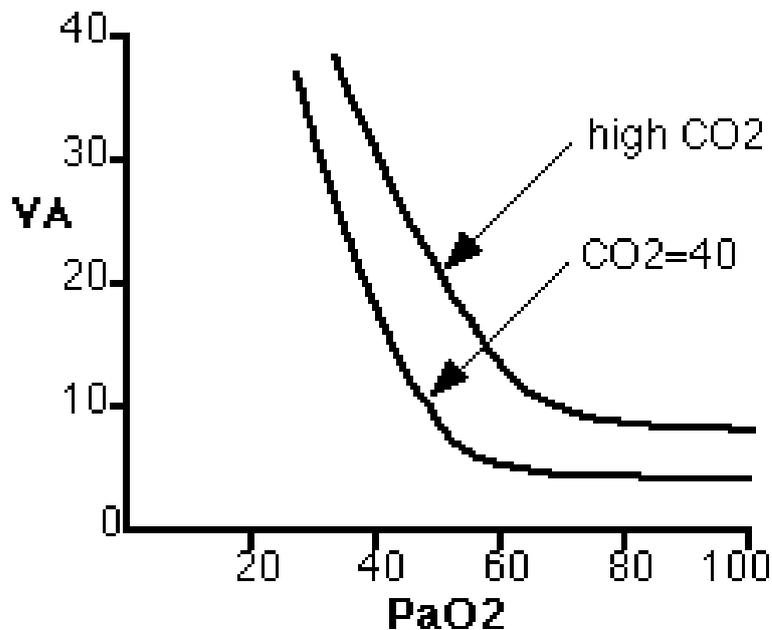
partial pressure of inspired oxygen and hence the driving pressure (or diffusion gradient) for gas exchange in the lungs [6, 7].

Acute Physiological Responses to Hypoxia

Acute Hypoxia leads to a number of well-established compensatory physiological responses which include hyperventilation and increase in resting heart rate [6]. Whilst an increased level of carbon dioxide is the main stimulus to ventilation at sea level at altitude hypoxia only increases ventilation when the inspired partial pressure of oxygen is less than about 100mmHg (13.3 kPa) which equates to an altitude of approximately 3000 m. Owing to the shape of the oxygen dissociation curve at this inspired oxygen pressure the alveolar oxygen pressure (PaO₂) is about 60mmHg (8 kPa), and with further increases in hypoxia ventilation rises exponentially (figure 2) [8].

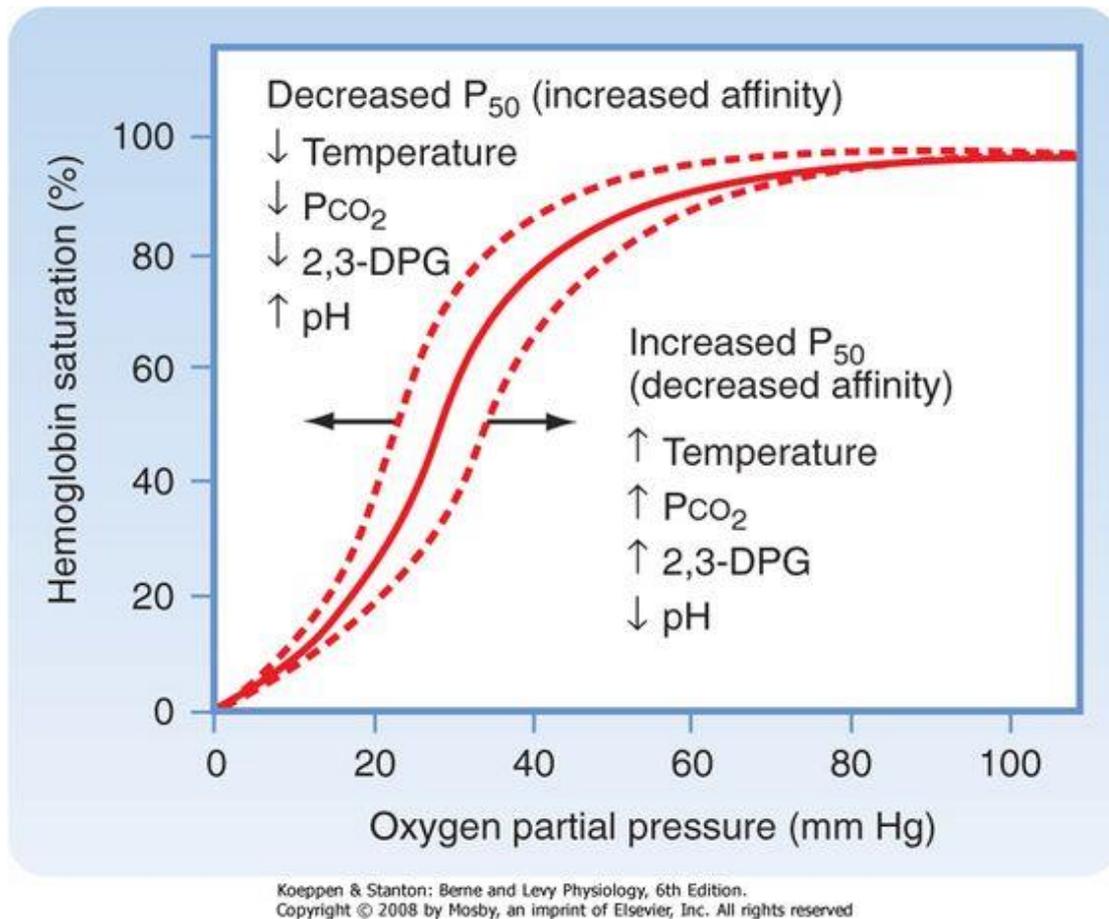
Figure 2 Changes in alveolar minute ventilation (VA) in response to the alveolar partial pressure of Oxygen (PaO₂)

http://www.anaesthesia.med.usyd.edu.au/resources/lectures/ventilation_clt/ventilation.html



This hypoxic hyperventilatory response is one of the earliest homeostatic responses to maintain tissue oxygenation. It is mediated by the carotid body, and its response varies widely between individuals [9]. This hyperventilation leads to a respiratory alkalosis as the PaCO₂ falls in response to the hyperventilation. This leads to a shift in the oxyhaemoglobin dissociation curve to left (the so-called Bohr effect), which acts to enhance the oxygen saturation of blood leaving the lungs (figure 3) [9, 10]. However, as the driving pressure for oxygen from the air to the blood is lower (due to the lower partial pressure of oxygen) coupled with the more rapid transit time of blood across the pulmonary capillary (due to a rise in resting heart rate) there is less time for equilibration of oxygen with the blood [11]. This leads to a diffusion limitation of oxygen across the alveolar–capillary membrane and, thus, more accentuated hypoxemia and a reduction in maximal oxygen consumption and maximal exercise capacity on cardiopulmonary exercise testing [12, 13].

Figure 3 The effects of high altitude on the oxyhaemoglobin dissociation curve



Hypoxaemia within the pulmonary artery circulation leads to a widespread activation of pulmonary artery smooth muscle and pulmonary artery vasoconstriction [14]. This is primarily driven by hypoxic activation of smooth muscle cells within the smaller intrapulmonary arteries in response to the generation of reactive oxygen species within their mitochondria [15]. These changes are thought to improve the delivery of blood to better-oxygenated lung segments in order to optimise ventilation/perfusion matching and systemic oxygen delivery [16]. This response is triggered by changes in the pulmonary artery smooth muscle mitochondrial reactive oxygen species and redox couples in pulmonary artery smooth muscle cells leading to a rise in pulmonary artery systolic pressure (PASP) [16]. Hypobaric hypoxia also stimulates diuresis and natriuresis in the kidneys and a fall in circulating plasma volume (hence relative rise in haemoglobin due to haemoconcentration) which acts to

improve oxygen uptake and delivery [17]. Circulating erythropoietin levels increase which leads to a gradual rise in haemoglobin over many weeks to improve oxygen content and delivery [18]. Gastrointestinal upset is very common with HA exposure. The expansion of gases in the stomach and intestines leads to increased flatulence and even diarrhoea [19]. This factor along with nausea and alterations in circulating gut hormones (eg acylated ghrelin) leads to a reduction in appetite [20, 21]. Insomnia is common and sleep quality shares a strong association and reciprocal relationship with anxiety at altitude [22, 23]. Both the quality and duration of sleep are known to be affected and sleep disordered breathing is not uncommon and is caused by the fall in PaCO₂ which leads to periods to apnoea [24-26]. Cerebral blood flow increases in an attempt to preserve the oxygenation of the brain [15].

High Altitude Related Illness

High altitude-related symptoms are one of the greatest impediments to completing any significant (≥ 2000 m) HA venture. These typically present in the form of one of several recognised syndromes with the most common being that of acute mountain sickness (AMS) [15, 27]. AMS has been historically defined as syndrome of nonspecific symptoms which include headache and one or more of fatigue, altered appetite, dizziness/light headedness and insomnia in the presence of recent altitude gain [28, 29]. Each of these five symptoms are rated on a scale of severity from 0 (not present) to 3 (severe). A total score of ≥ 3 , in the presence of a headache, has been traditionally considered diagnostic for AMS [28, 29]. The definition of AMS has been very recently revised (and subsequent to the publications contained within this PhD Submission) [30]. The reason for this update to the definition related to issues around the inclusion of sleep quality into the definition of AMS. It has been well reported that whilst sleep disturbance is obviously very common at HA it is discordant from other symptoms of AMS [31, 32]. Hence, the latest AMS Consensus Guideline Committee has decided to remove question on sleep from the LLS questions. Consequently

the new consensus definition of AMS now only incorporates four symptoms (insomnia removed) [30].

AMS usually develops >6 hours after a gain in altitude and should not be confused with confounding symptoms related to travel (eg fatigue and dehydration) or responses to acute hypoxia (above). Whilst AMS is usually a relatively benign and self-limiting syndrome its identification and effective management is crucial. It can rapidly worsen and progress to the development of HA cerebral oedema (HACE) [15]. HACE usually appears between 24 and 72 hours after a gain in altitude and is characterised by change in mental status and/or ataxia. It usually (though not always) occurs in persons with AMS or high-altitude pulmonary oedema (HAPE) [15, 30]. HACE is a medical emergency and can rapidly to progress to coma and death if not detected and adequately treated (intravenous steroids, high flow oxygen and descent). HAPE is generally thought to be a non-cardiogenic form of pulmonary oedema which is caused by a heterogeneous and heightened pulmonary artery vasoconstrictor response to alveolar hypoxia [33]. With HAPE there is regional hyper-perfusion of capillaries in areas of lower arterial vasoconstriction leading to increased blood flow into these 'more compliant' (less vasoconstricted) vessels which leads to endothelial disruption and increased capillary leakage of a protein-rich fluid into the alveolar interstitial space and pulmonary 'oedema' [34]. It can lead to right heart dilatation and evidence of myocardial injury, such as a rise in cardiac troponin [35]. High Altitude Pulmonary Oedema can be fatal if not recognised and treated promptly [17, 27]. It affects between 0.1 and 7% of persons travelling to altitudes above 2500m [17, 27]. The pathophysiology of AMS is highly complex and poorly understood. Putative mechanisms include alterations in vascular permeability within the blood brain barrier, attenuation of effective diuresis and natriuresis in response to hypobaric hypoxia [36] with associated alterations in heart rate variability (HRV) and autonomic balance [15].

The reported prevalence of AMS is highly variable and depends on multiple factors. These include the population studied, the amount and intensity of exercise undertaken, the mode of HA ascent (passive eg aeroplane or cable car versus active by climbing or trekking), the acclimatisation protocol as well as the actual altitude achieved [22, 37, 38]. The development of AMS is known to be markedly influenced by individual susceptibility which may have a genetic input, though the genetic hypothesis remains controversial [39]. Furthermore, whilst a consensus definition for AMS does exist other definitions have been suggested and reported [27]. These factors help to explain the marked variation in its reported frequency of HA-related illness across differing published studies and datasets. In general AMS is known to affect at least 1 in 4 persons who ascend to an altitude of $\geq 2000\text{m}$ and $>50\%$ of those travelling to $\geq 5000\text{m}$ [40, 41]. Given the huge number of persons trekking to these altitudes each year the potential burden of HA-related illness and its potential outcomes are enormous.

High Altitude research is of vital importance given both the huge clinical burden of AMS and potentially profound effects of the HA environment on human physiology. Despite intense research into this field a reliable sea level marker that can be used to reliably predict AMS development at HA has still not been identified. The discovery of a simple and reproducible sea level marker of future AMS risk and in severity would be of enormous clinical value and remains one of the holy grails of HA research. There is, however, another perhaps equally persuasive, and translational reason to examine the effects of acute hypoxia and HA on human beings. Tissue hypoxia is one of the central physiological insults of critical illness [42]. This can occur for a host of reasons due to abnormalities of oxygen uptake, extraction and delivery which can all lead to the common insult of tissue hypoxia. Hence HA-related research has enormous translational potential to improve our understanding of the human responses to critical illness [43, 44].

Cardiovascular Adaptations to HA

The cardiovascular responses to HA have been well described [8, 14, 45]. Hypobaric hypoxia at HA leads to an increase in resting sympathetic activity and a consequent rise in resting heart rate and variable changes in brachial blood pressure [17, 45, 46]. The effects of HA-related hypoxia on arterial compliance and central blood pressure are less well understood. Despite the rise in resting heart rate the maximal heart rate is reduced [47]. The reduction in maximal heart rate has been shown to be strongly related to the degree of hypoxia with a roughly linear dose-response between the reduction in arterial oxygen saturation and the reduction in maximal heart rate [45]. The mechanism for this effect is thought to be caused by counter-regulatory stimulation of the parasympathetic nervous system and increased vagal tone [47]. The reduction in circulating plasma volume (due to increased micturition and relative dehydration) coupled with the rise in pulmonary vascular resistance and PASP leads to blunting of the usual increase in stroke volume rise with exercise noted at sea level [48]. This, along with the obvious fall in arterial oxygen content, limits both maximal cardiac output and oxygen consumption ($\text{VO}_2 \text{ max}$) [49]. Despite the blunting of stroke volume, left ventricular ejection fraction, which is measure of radial left ventricular systolic function (and the fraction of blood leaving the heart in a single beat), appears to remain preserved in spite of a fall in left ventricular end diastolic volumes [50].

Until recently the effects of HA exposure on long axis systolic function, diastolic function (cardiac relaxation) and left ventricular filling had been less well described. The effects of HA on complex right ventricular and regional cardiac function is another area that needs greater clarity. The relationship between potential changes in cardiac physiology and function and the development of HA-related illness, and the influence of exercise, are other areas where there are limited data.

There have been several observational reports that have suggested that HA exposure increases the risk of acute and major adverse cardiovascular events. Sudden cardiac death has been reported to be one of the leading causes of non-traumatic deaths in adults at HA particularly during leisure time [51-55]. There are several potential mechanisms that might explain this finding which include reporting bias and the compounding effects of physical exhaustion and dehydration during hypoxia in 'at risk' individuals with 'silent' pre-existing cardiovascular disease [56]. Another plausible explanation is that the HA environment may be pro-arrhythmic [57]. High altitude-related hypoxia leads to sympathetic activation which is a recognised arrhythmogenic stimulus [46]. Unfortunately, there is paucity of previous research conducted during exercise at natural genuine HA, with the majority performed at rest during 'simulated' high altitude using hypoxic chambers or breathing a low oxygen gas mixture.

The ability to better examine the autonomic balance by the quantification of changes in Heart rate variability (HRV) has created exciting prospects for further research at HA. Heart rate variability refers to the changes in the cardiac inter beat interval and hence heart rate which is subject to continuous autonomic control. Sympathetic activation is well known to lead to an increase in heart rate and a reduction in the HRV and the converse following opposing parasympathetic innervation. The measurement of HRV has emerged as non-invasive, albeit indirect, measure of autonomic balance. Its main current clinic applications are in the monitoring of mental resilience, physical fitness, training and injury prevention due to over training [58-61].

The Challenges of High Altitude Research

There are a number of considerable challenges to the conduct research at HA. Firstly, the financial costs tend to be far higher than that for an equivalent study at or near sea level due to the considerable expense of transporting research equipment and personnel to HA. The

hypoxia, obvious cold and potentially limited access to hard structures, sanitation and power add to the logistical challenges and cost. In general there tends to be a reciprocal relationship between altitude and facilities. Hence, the highest altitudes tend to be the most remote with the least available facilities, yet they are the very areas of greatest research interest. The physical risks and dangers of HA research can be considerable leading to high travel and insurance costs. Consequently, the sample size for HA studies tend to be far smaller than for equivalent normoxic sea level studies. The size of the challenge depends on multiple factors, including the type of research study, the season and the actual HA location/environment.

In an attempt to overcome many of the constraints of natural HA, research is often conducted under simulated HA conditions. Simulated high altitude aims to reproduce the degree of hypoxia of genuine/natural HA, but at sea level [62]. This is performed either by breathing a low oxygen gas concentration (eg FiO_2 of 14% rather than 21%) using a tight-fitting face mask or by the use of a normobaric or hypobaric hypoxic chambers. A normobaric hypoxic (NH) chamber (figure 4) also reduces the concentration of inspired oxygen within the room. Conversely, a hypobaric hypoxic (HH) chamber (figure 5 and 6) reduces the oxygen percentage constant whilst reducing its partial pressure [63]. They tend to be more expensive than normobaric chambers and more challenging for research owing to their typically smaller size, high background noise and their restrictions on chamber entry/exit.

Figure 4 Illustration of the inside of the normobaric hypoxic chamber used in several of my studies at Leeds Beckett University (personal photograph)

Figure 5 Example of three Hypobaric Hypoxic Chambers at the centre of Aviation Medicine (Personal photographs taken during one of the studies)

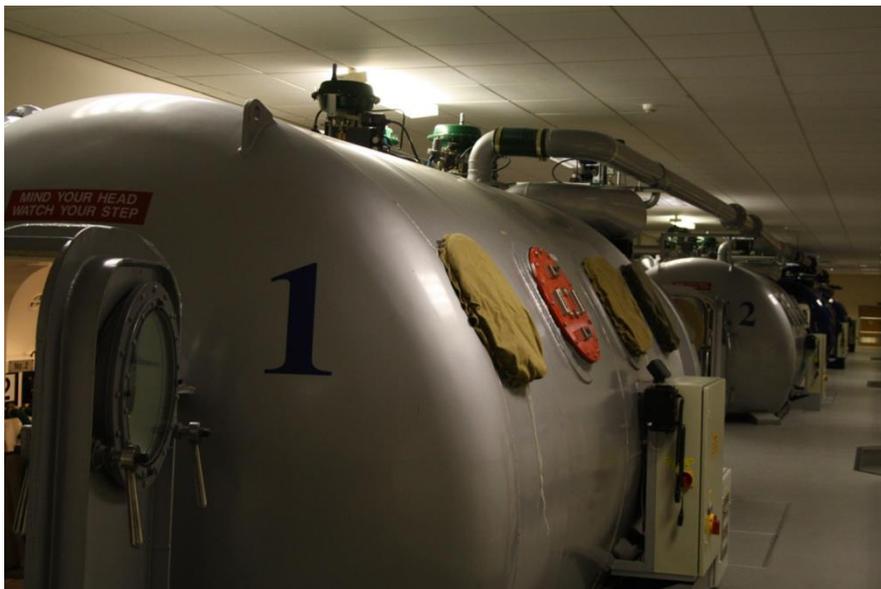


Figure 6 Illustration of the interior of one of the hypobaric hypoxic chambers at the Centre for Aviation Medicine (personal photographs taken during four-way study Chapter 5)



One area of ongoing debate has been whether hypoxic chambers are effective surrogates for natural terrestrial HA. Whilst they can replicate the degree of hypoxia they cannot replicate the hardships of natural HA. These additional challenges of natural HA include the extreme cold, high winds, limited access to varied food choice, insomnia, anxiety, intense exercise and associated muscle aches and physical risks. Conversely, they also cannot reproduce the many positives of genuine HA which include the freedom, the beautiful scenery and the greater sense of accomplishment. There is also considerable debate on the question as to whether HH chambers offer superior HA simulation to that of NH. Comparative studies have tended to focus on one modality *versus* another (eg NH *versus* HH). There has been very little research into the comparative effects of both HH and NH to natural HA. Furthermore, chamber studies tend to be conducted over minutes to hours rather than the days or weeks as would be the case with natural HA exposure.

Another fundamental reason, beyond those cited above, for the dominance of simulated HA research has been the issue of research equipment portability. The difficulties of transporting traditional mains powered stand-alone equipment to terrestrial HA can be considerable. The operating temperatures at genuine HA can damage equipment that was designed to be used at

higher temperatures at sea level. The cold can lead to the rapid depletion of battery life. One way to lessen this challenge is to use more accessible natural HA locations where there is good road links or even cable car access. However, this generally limits the altitude to generally less than 3500m as above this facilities and transport links tend to be far less.

Recent improvements in equipment technology, portability and battery performance have been considerable. This has led to the creation of new opportunities for HA research. For example, in the field of cardiac research (which is the focus of my PhD) we have witnessed the advent of portable and even ultraportable devices that have capabilities exceeding even that of older devices designed for laboratory use. This has generated new prospects for HA research that was previous untenable. These opportunities have been exercised across all of the studies relating to this PhD submission and discussed further in subsequent sections.

Aims and Scope of my PhD Research

This thesis is focussed on the central theme of the cardiovascular responses to acute hypoxia and HA in healthy adults. In order to deliver my thesis aims (stated below) and the science under the challenging research environments used have required the use of portable and often novel cardiovascular equipment and bioassays.

The four main research hypotheses examined in this thesis are as follows:

1. High altitude (HA) adversely affects cardiac diastolic function and increases estimated filling pressures. These changes are influenced by the mode of hypoxia and the hypoxic environment.
2. Exposure to HA leads to an abnormal rise in circulating biomarkers of myocardial injury that are linked to the development of acute mountain sickness.
3. Acute HA exposure reduces arterial compliance and increases central blood pressure.

4. Exposure to HA reduces heart rate variability (HRV) and increases risk of cardiac arrhythmias. The reduction in HRV at HA is influenced by ventilation and biological sex and is linked to the development of acute mountain sickness.

Chapter 2

Research Methods, Literature Review and Supporting Synthesis

This 'PhD by Publication' thesis submission consists of eight published original research studies that were conducted over a period of seven years from 2011 to 2017. The study environments varied considerably across these seven studies and are described below.

Personal Contribution to the Research and Publications

The eight submitted publications for consideration for this PhD all relate to original prospective observational studies conducted on healthy adult British Military Servicemen. Two publications relate to one study. Each study had its own separate research proposal. All of these studies had full ethics approval granted by the Ministry of Defence Research and Ethics Committee (MoDREC) prior to commencement. All ethics submissions required a pre ethics committee meeting attendance (usually Porton Down, Wiltshire) followed by attendance in person at MoDREC at Whitehall, London. I was either first author or co-authored all of the ethics submissions and played a principal role in the preparation and conduct for all of these eight studies relating to this PhD submission. I was personally in attendance 'on the ground' at all of the research locations relating to this thesis with the exception of the Bernese Alps study. I personally sourced all of the key cardiac research equipment via a variety of funding streams which will be discussed below.

I played a principal role in data entry and undertook all of the main data analysis relating to these publications. I was first author on all of the manuscripts comprising this PhD submission. I was supported in all of the studies by my co-authors who assisted with the editing and proof reading of the final versions of all manuscripts. The individual contributions made by my colleagues are cited at the end of each publication. The detailed

methods, findings and limitations of each study are discussed in depth under the individual publications.

Research Methods, Publications and the Research Environment

Publication 1

The 1st publication in this thesis is entitled ‘The effects of acute hypobaric hypoxia on arterial stiffness and endothelial function and its relationship to changes in pulmonary artery pressure and left ventricular diastolic function’ [64]. This study was conducted over five days at the end of 2011. This study aimed to address two of my research hypotheses: high altitude (HA) affects cardiac diastolic function and estimated filling pressures and acute HA exposure reduces arterial compliance and increases central blood pressure. This study was conducted over five consecutive days at the Centre for Aviation Medicine in Henlow, UK. Ten healthy adults were studied at baseline under normobaric normoxia (NN, sea level breathing room air) and at 45, 90 and 150 minutes during 180 minute exposure to hypobaric hypoxia (HH; equivalent to 4800m). Arterial compliance (stiffness index) was measured using a validated device known as the Pulse TracePCA2 (figure 7). The PCA2 estimates large artery stiffness (stiffness index) using the principle of pulse waveform analysis of the digital artery waveform measured at a fingertip via an infra-red finger sensor (photo-plethysmography). This validated device also measures vascular tone and potentially endothelial function using its vascular tone measure known as the reflective index (methods described in greater detail later) [65-67]. A good pulse volume is crucial for this method to be able to detect and analyse the arterial waveform. This led to several challenges at HA under the cold environment where peripheral vasoconstriction led to a reduction in signal strength and the need to warm the fingers.

Figure 7 Graphical Illustration of the Pulse Trace PCA2 Arterial waveform Device
(image source: <https://www.mistrymedical.com/item/9924/carefusion-pulse-trace--36-pt2000-stk->)



Assessments of cardiac function, estimated PASP and vascular resistance were performed using GE Vivid Q transthoracic cardiac ultrasound device (echocardiography) as shown below, at baseline and after 150 minutes of hypobaric hypoxia (figure 8).

Figure 8 Example of a GE Vivid Q portable transthoracic echocardiogram machine

Image source: (google images)

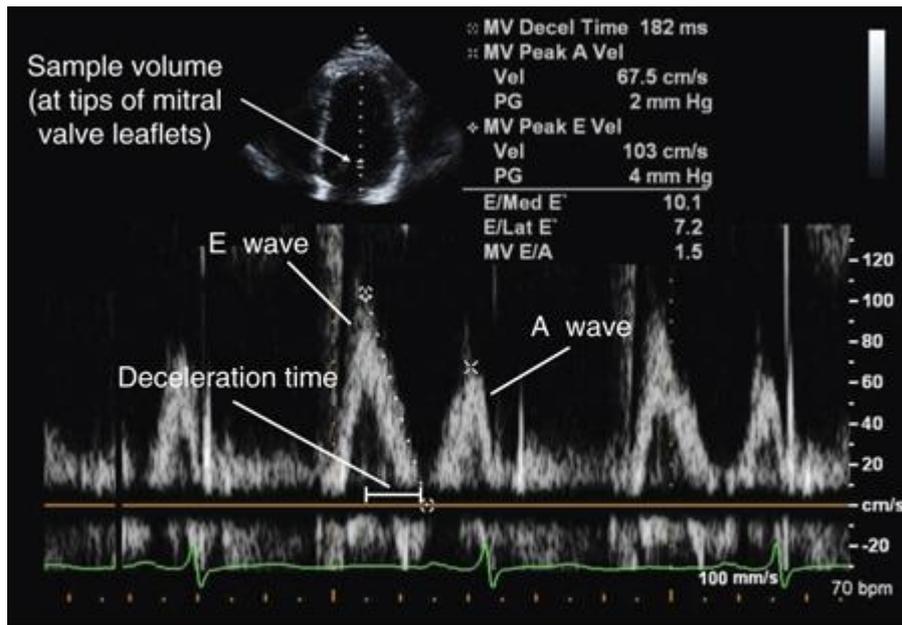


Additional measures undertaken in this study included brachial blood pressure and peripheral oxygen saturations (SpO₂). The echocardiogram and pulse waveform equipment were provided to me as a free research equipment loan from GE Healthcare and CareFusion respectively, following my successful research bid. I attended all of the hypobaric chamber experiments in person.

Diastolic function was assessed using a combination of recognised methods. The first was by examination of the left ventricular inflow by a process known as pulse wave Doppler by sampling the change in blood velocity as it cross the mitral valve using echocardiography as

shown [68] (figure 9). This identifies two discrete waves known as the E wave (marker of early mitral inflow and left ventricular relaxation) and an A wave (later mitral flow and left ventricular filling caused by left atrial contraction) (Figure 9).

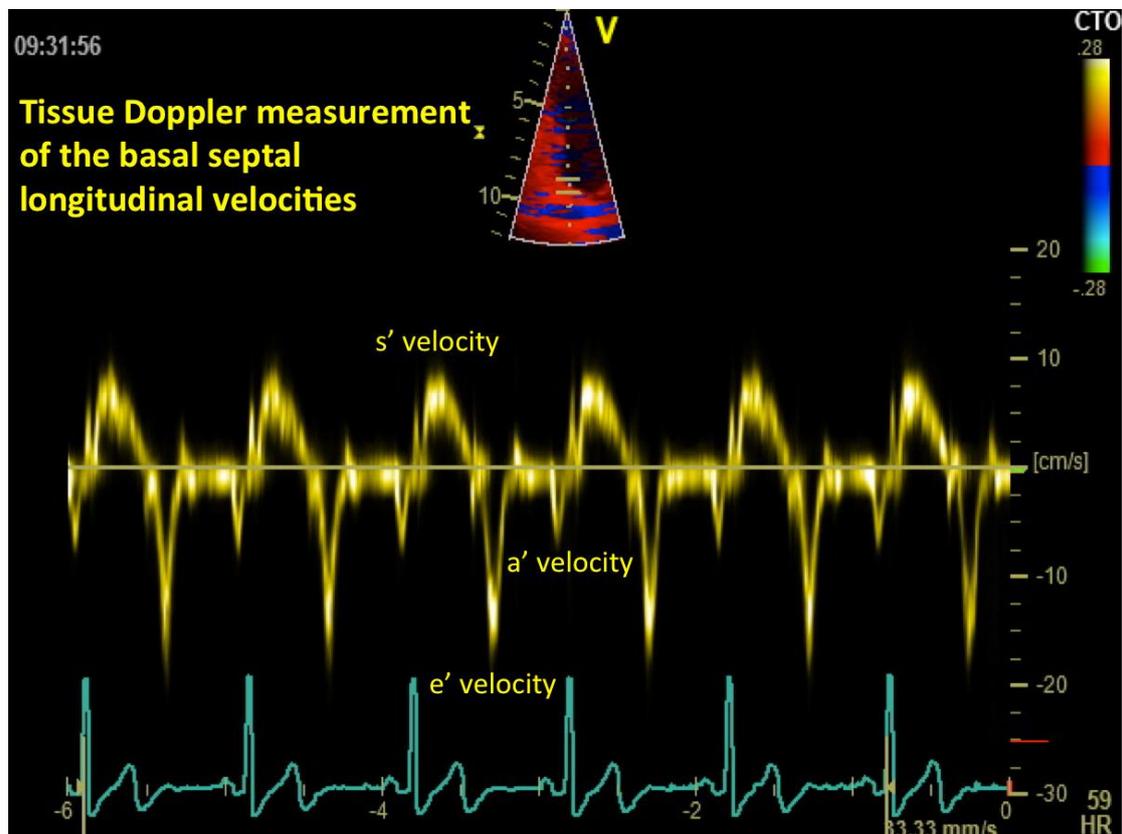
Figure 9 Illustration of the measurement of mitral pulsed wave Doppler to assess diastolic function and estimated left ventricular filling (google images)



Source: Pahlm O, Wagner GS: *Multimodal Cardiovascular Imaging: Principles and Clinical Applications*: www.accessmedicine.com
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Tissue Doppler imaging was used to measure the velocity of the mitral annulus motion to obtain an equivalent but lower early E' and later A' wave deflections related to left ventricular relaxation and atrial contraction respectively (figure 10). Long axis systolic (contractile phase) function of the myocardium can also be quantified by the velocity of the identified S' wave as shown on the image below (figure 10). The data from these measurements were used to build up a comprehensive assessment of diastolic function and estimated left ventricular filling (more detailed methods in main publication).

Figure 10 Graphical Illustration of the typical tissue doppler imaging with sampling from the basal septum (source: <https://isctr.org/chapter-i-7/>)



The findings of this study were published as an original paper in High Altitude Medicine and Biology (Impact factor [IF]: 1.98). To the best of my knowledge was the first study to investigate effects of acute hypoxia on simultaneous assessment of large artery stiffness and endothelial function and its inter-relationship to left ventricular diastolic function and PASP.

Hypobaric hypoxia (HH) led to a significant increase in the estimated PASP and pulmonary artery vascular resistance and a reduction in both systolic blood pressure and the systemic vascular resistance (*versus* baseline) without clinically significant changes in large artery stiffness or left ventricular filling. There was a strong inverse correlation between changes in the arterial reflective index (a marker of medium sized artery tone and endothelial activation)

and in the pulmonary artery vascular resistance. This finding would suggest that haemodynamic responses in the pulmonary artery and systemic arterial circulation to acute hypobaric hypoxia are intricately linked. My results strongly support the concept that acute hypoxia leads to differential haemodynamic effects along the arterial tree with a greater influence on the muscular smaller peripheral versus central elastic arteries (eg aorta) with variable effects on brachial blood pressure [69]. Unfortunately, owing to study design I am unable to infer whether this association equates to causation or a mechanistic relationship whereby the vasomotor changes in pulmonary artery lead to reciprocal changes in the systemic circulation and *vice versa*.

Publication 2

The 2nd publication is entitled ‘the effects of exercise at high altitude on high-sensitivity cardiac troponin release and associated biventricular cardiac function [70]. This study was conducted over a three week period in the Himalayas in March and April 2012. This study addressed the first two hypothesis of my thesis:

1. High altitude (HA) adversely affects cardiac diastolic function and increases estimated filling pressures. These changes are influenced by the mode of hypoxia and the hypoxic environment.
2. Exposure to HA leads to an abnormal rise in circulating biomarkers of myocardial injury that are linked to the development of acute mountain sickness.

Cardiac function, high-sensitivity cardiac troponin T (hs-cTnT, circulating marker of myocardial injury measured) and AMS scores were measured at rest at 1,300m (Kathmandu, Nepal). Cardiac hs-cTnT levels were obtained by peripheral venesection followed by centrifugation for later batch analysis. Cardiac function and PASP were quantified using transthoracic echocardiography (figure 11) and AMS scores were obtained using validated

questionnaires. These three measures were repeated, following exercise and again, at rest 12 h later during incremental terrestrial HA treks to 3,440m, 4,270 m and at 5,150 m (following the summit of Kala Patthar at 5,643 m) on 19 healthy adults.

The echocardiogram machine used was a Sonosite M Turbo (figure 11) which was supplied free of charge for the duration of this study from the manufacturer Sonosite Inc. The cost and conduct of the Cardiac troponin assays were covered following my receipt of an assay grant from the Department of Biochemistry at Poole Hospital NHS Trust. I assisted with data collection and undertook all of the echocardiograms over the duration of this study.

Figure 11 Sonosite M Turbo cardiac ultrasound (echocardiogram) machine
(<https://www.google.co.uk/search?q=Sonosite+M+turbo+Ultrasound+Machine&rl>)



This was the first study to investigate the relationship between changes in cardiac function and biventricular filling and markers of myocardial injury during incremental HA. The manuscript was published in *Clinical Research in Cardiology* (IF 4.5) [45].

The key findings of this study were that exercise at HA led to a significant, but only minor overall increase, in hs-cTnT which was most notable at the highest altitudes in the absence of any notable change in cardiac biventricular filling. The independent predictors of the rise in hs-cTnT were increasing cardiac output (supporting its association to exercise), PASP and decreasing SpO₂ (and hence in proportion to the degree of hypoxia). Cardiac TnT was not an independent predictor of AMS. Hence exercise at HA led to an increase in cardiac troponin levels but not to pathological levels and this rise was not related to any major deleterious effects of cardiac function. The mechanism for the increase in cTnT appears to be the combined effects of exercise and hypoxia. The translational finding of this research are that the rise in cardiac troponin following exercise at HA is transient unlike the sustained and higher levels observed over several days with a myocardial infarction ('heart attack') [71]. Furthermore, there were no clear deleterious effects of exercise on cardiac function at very high altitude. It is unknown whether these findings apply to older subjects with clinical or subclinical cardiac disease as we included a cohort of healthy and generally young adults.

Publication 3

My 3rd publication is entitled 'A Four-Way Comparison of Cardiac Function with normobaric normoxia, normobaric Hypoxia, Hypobaric Hypoxia and Genuine High Altitude' [72]. This study was specifically designed to investigate the influence hypoxia on resting and post exercise changes in cardiac function. The specific hypothesis was that HA would adversely affect cardiac diastolic function and lead to an increase in estimated filling pressures.

This was an extremely challenging collaborative research study conducted over four differing and consecutive (with a washout period in between) research environments in 2013. Transthoracic echocardiography (GE Vivid I Machine – personal purchase), physiological measures and AMS scores were undertaken at rest and at 15 and 120 minutes following two

hours of exercise at sea level normobaric normoxia (NN; Leeds Beckett University), at natural HA at 3,375m (Margherita Hut, Italian Alps, figure 12), normobaric hypoxia (NH; hypoxic chamber at Leeds Beckett University) and with hypobaric hypoxia (HH; Centre of Aviation Medicine, Henlow, UK) to simulate the equivalent hypoxic stimulus to genuine HA.

Figure 12 Image of the view from the Margherita Research Hut where the high altitude component of this study was conducted (personal photo).



The original cohort consisted of 14 participants. However, the sample size varied by study environment with 14 volunteers completing the experiment at genuine HA, 11 with NN and 12 and 6 with NH and HH, respectively.

The original paper relating to this manuscript was published in PLOS One (IF 2.77) [72]. This was the first observational study to compare the effects of exercise on detailed cardiac function and physiological responses under three differing hypoxic environments versus sea level normoxia. The key finding in this study was that the resting cardiac responses to hypoxia were similar under the three hypoxic conditions. However, significant differences emerged following exercise in SpO₂, right ventricular systolic pressure and function. Compared with NH, HH and GHA led to lower oxygen saturations (hence hypoxia), higher heart rates and a greater negative change in the right ventricular Tei (marker of global cardiac function) and increase in the right ventricular systolic pressure [73]. These data emphasises the importance of the type and mode of hypoxic environment as well as the influence of exercise on cardiac function. Data obtained from one environment cannot necessarily be assumed to translate to another environment and simulated hypoxia is not a reliable substitute for genuine HA exposure. Unfortunately, there was a much smaller sample size for the HH protocol (which was the third hypoxic environment to be tested), which was an important confounder.

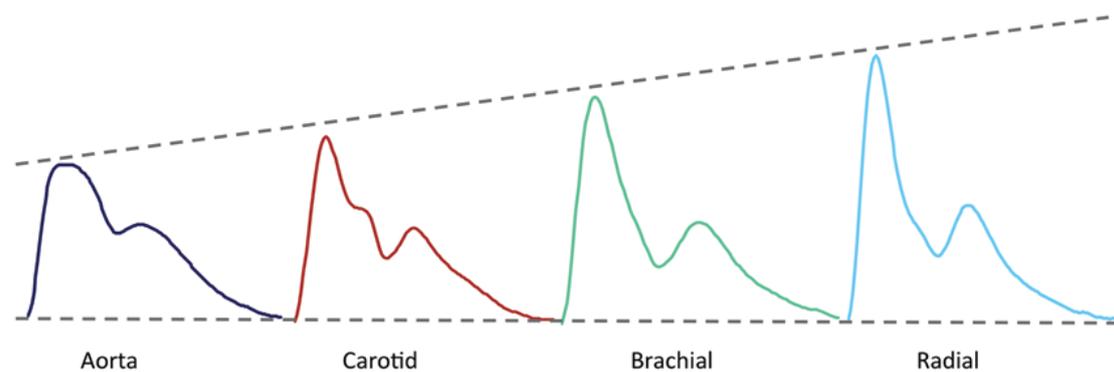
Publication 4

My 4th publication is entitled ‘the effect of high altitude on central blood pressure and arterial stiffness’. This study was conducted in 2016. This study is linked to my third hypothesis which is that high altitude exposure leads to a reduction in arterial compliance and an increase in central blood pressure.

The measurement of brachial blood pressure has been most widely reported using brachial readings. However, relying solely on brachial blood pressure has a number of limitations. Brachial blood pressure reflects the hydrostatic pressure of arterial blood in the upper arm and tends to be higher than the actual central blood pressure [74-76]. The reason for this

phenomenon is due to systolic blood pressure amplification from the central to peripheral arterial tree. This pressure *amplification* is caused by the relative increase in arterial stiffness and vascular tone as the arterial pressure wave travels from the highly elastic central arteries to the stiffer and more muscular peripheral arteries [77]. This causes the upper portion of the arterial wave to become narrower with a more prominent systolic peak and the systolic blood pressure increases (figure 13). Hence the brachial blood pressure does not provide a reliable insight into central blood pressure.

Figure 13 Peripheral amplification of systolic blood pressure image source:
<https://www.researchgate.net/publication/313259486> **I Luso-**
Brazilian Positioning on Central Arterial Pressure/figures?lo=1



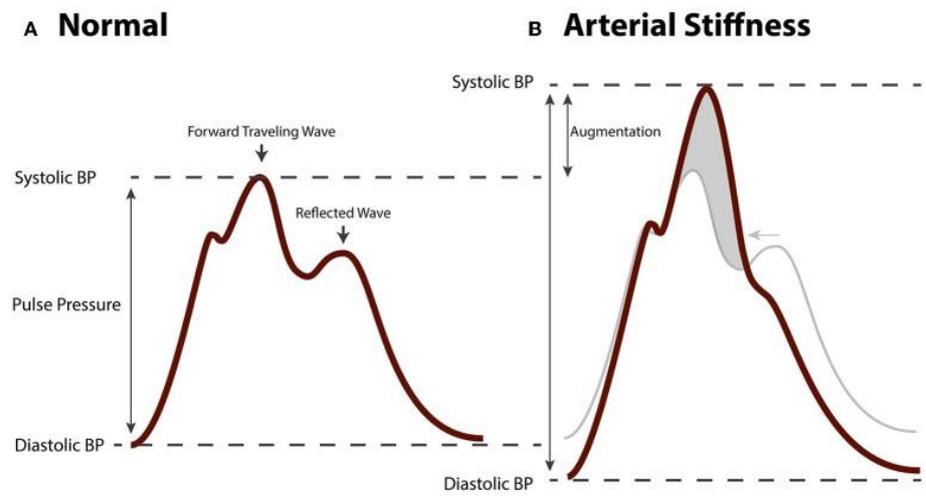
Central blood pressure is a recognised cardiovascular risk factor. It reflects the direct hydrostatic pressure of blood as it leaves the heart and its effects on the organs in close proximity (heart via increased afterload, aorta, brain and kidney). This is thought to explain why central blood pressure has been shown to be a better independent predictor of future cardiovascular risk and adverse cardiovascular endpoints including cardiovascular death and stroke [78, 79].

The differences in the central and brachial blood pressure can vary considerably between individuals and with increasing age and cardiac risk factors. Consequently, accurate quantification of central blood pressure is desirable. Its measurement has traditionally required the use of invasive arterial catheterisation. Technical advances have led to the recent development and availability of several relatively portable devices that can provide accurate (albeit indirect) measures of central blood pressure and large artery compliance/stiffness [80].

Changes in the travelling arterial waveform are caused by alterations in the reflections of the initially forward travelling arterial wave following its ejection from the heart during left ventricular systole (figure 14). The forward travelling arterial wave is followed by a single summated backward-travelling reflected wave (figure 14) which is generated at sites of impedance mismatch such as where the arterial tree tapers or bifurcates [77, 81].

Figure 14 Demonstration of varying arterial augmentation with increasing arterial stiffness with augmentation of the reflected wave lead to an increase in arterial blood pressure

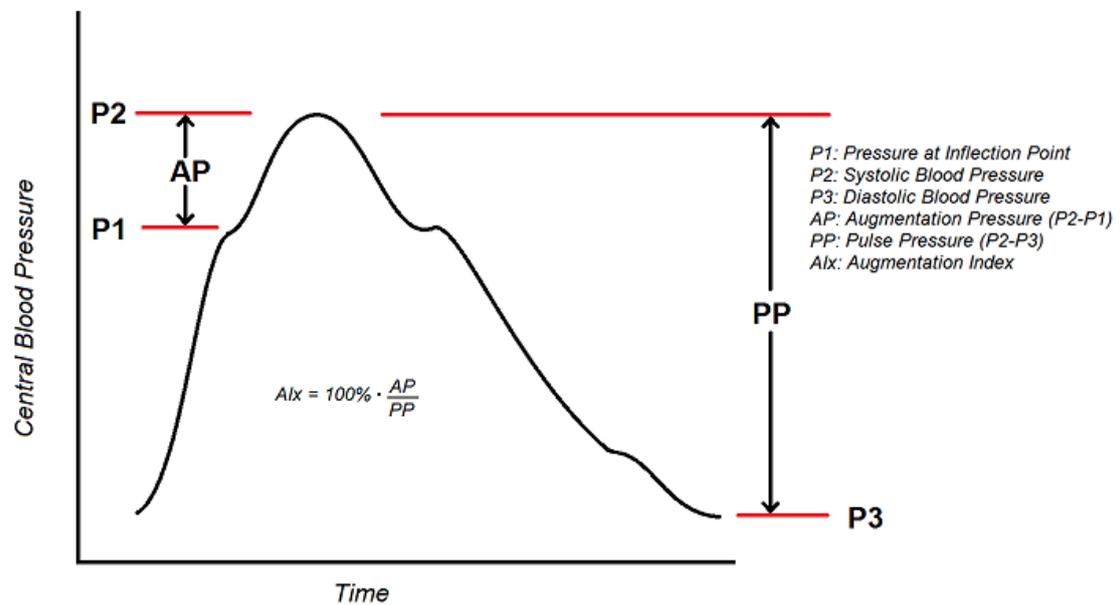
https://www.google.com/search?q=14+Demonstration+of+varying+arterial+augmentation+with+increasing+arterial+stiffness&rlz=1C1CAFB_enGB663GB671&source=lnms&tbn=isch&sa=X&ved=0ahUKEwjPqd2K3NngAhXuVhUIHeTfAkIQ_AUIDigB&biw=1920&bih=933#imgsrc=evSXoVKVcS7P7M:



It is thought that this reflected wave can act to augment and increase the systolic blood pressure in the central arteries. The augmentation index is used to quantify the extent of the augmented pressure relative to the central pulse pressure as shown (figure 14). It is calculated as the percentage ratio of the augmentation pressure (%) relative to the central pulse pressure as shown below (figure 15).

Figure 15 Calculation of the central augmentation index

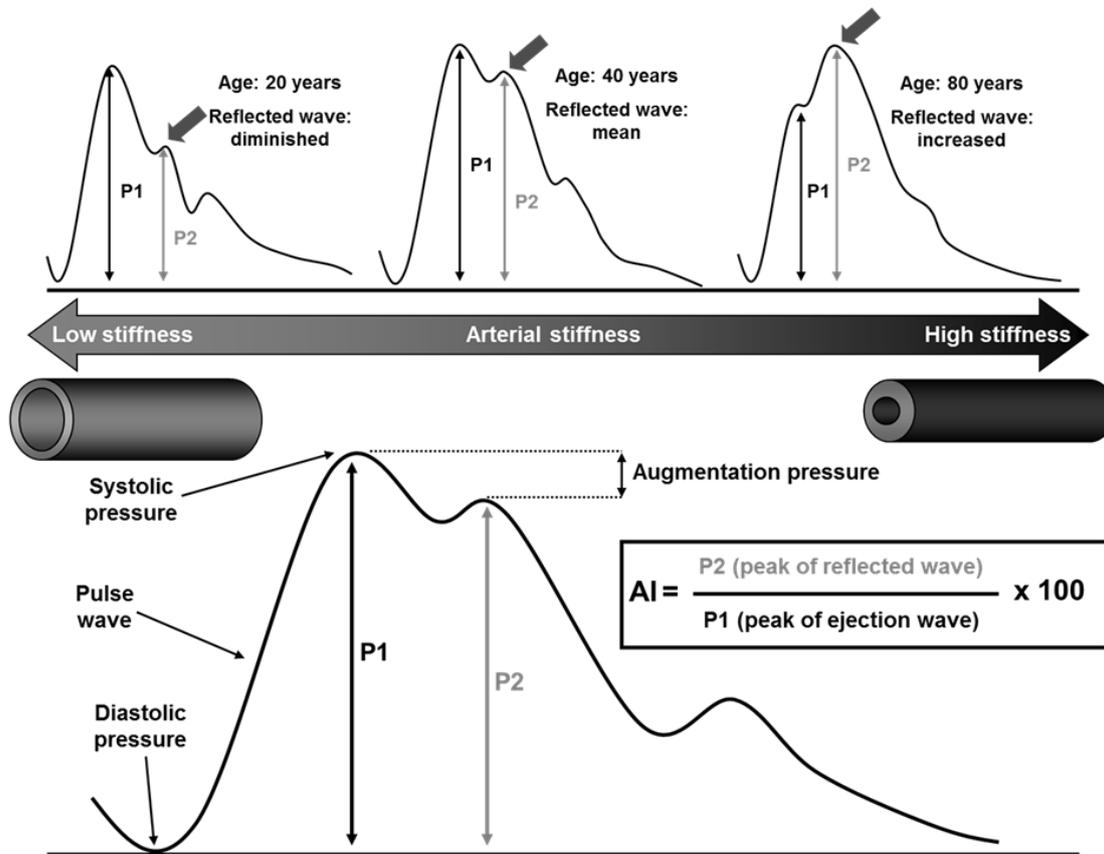
<https://commons.wikimedia.org/wiki/File:Augmentation-index.PNG>



However, the augmentation index can also be calculated from the peripheral (brachial or radial) arterial waveform by the % ratio of the reflected (P2) to the incident (forward travelling P1) wave as shown (figure16).

Figure 16 Calculation of the peripheral (Brachial or radial) augmentation Index

http://file.scirp.org/Html/3-1910314_45642.htm



In general a higher augmentation index can be caused by an increase in arterial stiffness or peripheral vascular tone leading to earlier arterial wave reflection. With an increase in augmented pressure (and augmentation index), the absolute aortic systolic pressure increases (figure 14).

Pulse wave velocity (PWV) is considered by to be the gold standard functional measure of large artery stiffness [82, 83]. It refers to the velocity at which an arterial pressure wave moves along a blood vessel. Its measurement requires the quantification of the desired path length to be examined and a means of calculating the time it takes for the arterial pressure wave to move along the chosen path length (ΔL) as shown (figure 17). This is typically conducted by using two pressure catheters placed at each end of the path length.

Figure 17 Demonstration of the concept of Pulse wave velocity (PWV) and its calculation from the path length (ΔL) and time (Δt) for the arterial waveform to travel from the aorta to the proximal femoral artery

https://www.google.com/search?q=Demonstration+of+the+Concept+of+Pulse+wave+velocity&rlz=1C1CAFB_enGB663GB671&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjnMTh3NngAhWLSRUIHbqZAuMQ_AUIDigB&biw=1920&bih=933#imgrc=4idsyupw5c6AUM

Pulse wave velocity (PWV) and augmentation index have evolved as two of the most commonly cited methods to indirectly determine arterial stiffness. The widely used 'arterial stiffness index' is another frequently quoted measure of arterial stiffness [84, 85]. However, it is a non-specific term and this index can be measured in a number of ways.

In my 4th publication brachial the arterial augmentation index, peripheral and central blood pressure were measured at sea level (UK) using the Uscom BP⁺™ (Pulsecor.com, Sydney NSW 2000 Australia) (figure 18). This device non-invasively measures blood pressure at the brachial artery using a sphygmomanometer. Examination of the low-frequency supra-systolic arterial waveforms waveform at the occluded brachial artery enables the calculation of peripheral augmentation index [86]. The arterial waveform in conjunction with the brachial blood pressure is used to provide a validated measure of central systolic blood pressure [77, 80, 87]. The advantage of the BP⁺ over many other recent central blood pressure reading devices is in its ultra-portability and simplicity of use which is highly operator independent. It can provide a highly accurate and repeatable measurement of central blood pressure within 40 seconds, using a simple oscillometric blood pressure device.

Figure 18 Illustration of the BP⁺ device used to measure central blood pressure and arterial compliance using the augmentation index:
<https://www.uscom.com.au/products/>



Lin et al compared 94 central systolic pressures, estimated using this device to simultaneously measure central aortic pressures obtained at the time of coronary angiography in 37 individuals [87]. He found a very strong correlation in the measurements ($r \geq 0.95$; $p < 0.0001$) with a mean difference (\pm standard deviation) in central systolic pressure of 2.7 ± 3.90 mmHg with a coefficient of variation of 3%. In a further validation study, of 47 healthy adults, the BP⁺ central blood pressure measurements strongly correlated and was in agreement with that of a SphygmoCor device, which is considered the gold standard non-invasive device for arterial stiffness assessment [88].

In my 4th publication, brachial and central blood pressures and the peripheral arterial augmentation index were measured at rest and seated at sea level, 3619m, 4600m and 5140 m in the Mount Dhaulagiri circuit in the Himalayas on 90 healthy adults. I went to the

Himalayas in support of this project but received collaborative help with data capture. The results of this study supported my hypothesis as it was found that the arterial augmentation index (marker of arterial compliance) and central blood pressure both significantly increased at HA. This rise largely reflected subject-related factors (eg age, sex and smoking history) rather than SpO₂, actual altitude or AMS scores. The manuscript was published in the Journal of Human Hypertension (IF 2.433) [89]. Given the time frame of less than three weeks coupled by the information gained from my earlier acute hypobaric hypoxia experiment (publication 1) it can be hypothesised that the increase in central systolic blood pressure and peripheral augmentation index reflect earlier and increased arterial waveform reflection by the peripheral muscular arteries, rather than any material changes in the vascular wall or endothelium. Interestingly the greatest increase in central systolic blood pressure and the augmentation index were at 3619 and 4600m, which thereafter marginally fell suggesting the impact of HA acclimatisation on blood pressure homeostasis. These results emphasise the importance of a gradual ascent profile, which may be of particular importance for those with a history of sustained hypertension.

Publication 5

My 5th publication study is entitled 'the Effect of Sex on Heart Rate Variability at High Altitude' was also conducted in 2016 [90]. Most of the population within this study had also taken part in the Dhaulagiri Blood pressure/augmentation index study above. The measurement of the cardiac inter beat interval for heart rate variability (HRV) analysis was recorded using a novel, validated, and dedicated ultraportable battery operated HRV device known as the CheckMyHeart Plus (Daily Care BioMedical Inc) (figure 19) [91]. I supported the research on the ground during baseline measures and was physically present during the study conduct in the Himalayas.

Figure 19 Illustration of the CheckMyHeart heart rate variability device in which the cardiac inter beat interval is obtained via two electrodes which are attached to the front of the anterior chest wall (personal photograph)



This study is linked to my fourth hypothesis that ‘terrestrial HA exposure reduces HRV and increases risk of cardiac arrhythmias. The changes in HRV at HA are influenced by ventilation and biological sex and are linked to the development of acute mountain sickness.’ Sixty two volunteers were studied during rest, whilst seated, at sea level (SL), 3619m, 4600m, and 5140m.

This was the first study to examine the comparative changes in HRV among men versus women at natural HA. The main results from this study were that HA significantly affected several measures of HRV (discussed later). We also observed a significant main effect for sex

on heart rate and time domain measures of HRV at HA, with men having consistently higher scores and greater variability which is a novel finding. Men also had greater low frequency and total power (on frequency domain analyses) than women. These sex-related differences were maintained with increasing HA with no evidence of a statistical interaction between sex and altitude for any of the HRV indices measured. HRV did not predict either AMS or its severity. This result would suggest that there are clear differences in HRV between men and women and these differences persist. The study was published in *Medicine & Science in Sports and Exercise* (IF 4.291) [90].

Publication 6

The 6th study is entitled a ‘comparison of spontaneous versus paced breathing on heart rate variability at high altitude’ [92]. This prospective observational study was conducted in 2017 on 30 healthy male volunteers over nine days at altitudes ranging from 800-4107m in the Bernese Alps in Switzerland in 2017. Cardiac inter beat intervals were recorded using a simple digital finger sensor attached to a mobile phone (methods discussed in greater detail later). A patented time domain measure of HRV was provided by the *ithlete* App within the phone following a 55 second recording (figure 20) [93, 94].

Figure 20 Image of the ithlete sensor linked to an iPhone to record the cardiac inter beat intervals and the ithlete heart rate variability score

https://www.google.com/search?q=ithlete&rlz=1C1CAFB_enGB663GB671&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiS9tr53tngAhVcVRUIHeKOBt8Q_AUIECgD&biw=1920&bih=933#imgrc=kdrwHlJ5mr6oAM:



This study was linked to my fourth hypothesis that ‘exposure to HA reduces HRV and increases risk of cardiac arrhythmias. The changes in HRV at HA are influenced by ventilation and are linked to the development of AMS.’ One of the specific research questions that I was keen to explore was whether paced breathing would attenuate altitude-related changes in HRV at HA and its potential relationship to AMS. The purchase of the devices was made possible via a research discount from the supplier HRV Fit Ltd. Individual researchers, under my direction, undertook the ‘on the round research in the Alps’ which involved the recording of the HRV data.

The main message of this paper is that whilst HRV scores obtained with paced breathing strongly correlated with that with spontaneous breathing at moderate HA, the values during paced breathing were consistently higher and the measurement variability was lower with paced breathing. This relationship remained consistent across multiple altitudes but appeared

to be affected by the presence of AMS. Given the notable effect of ventilation and breathing rate on HRV, this should have translated into greater discordance in HRV scores between the two breathing modes at higher altitudes. However this was not observed. Although a significant main effect for altitude and breathing modality was observed (higher HRV score with paced breathing), there was no altitude-x-breathing interaction on HRV scores at HA. This supports the hypothesis that paced breathing does negate the HA related changes in the athlete HRV score. However, given that the HRV scores were consistently higher with paced breathing, the two breathing methods cannot be used interchangeably and there is a risk that paced breathing could mitigate genuine changes in HRV with AMS. There is a need for a larger study at higher altitude and burden of AMS cases. This manuscript is due for publication in The Journal of Clinical and Diagnostic Research (IF 0.65).

Publication 7

My 7th study was a parallel study from a subset of the participants from the above Bernese Alps study in 2017. It was entitled ‘High Altitude Affects Nocturnal Non-linear Heart Rate Variability: PATCH-HA Study [95]. This study was very novel as it was the first study to investigate HRV at natural HA using a patch cardiac Monitor and one of very few studies to examine nocturnal HRV. It was also the first study to examine non-linear measures of nocturnal HRV. This study aimed to further examine my hypothesis that HA affects HRV, by investigating the changes in nocturnal HRV and its relationship to AMS and sleep quality. A prototype patch monitor, which is still under development, was used for this study (figure 21). This monitor was unique in that it was far smaller than any other available portable ECG monitor on the market at the time and it could record ‘the wearer’s body position (ie supine one back or side or upright). Moreover it could record a high resolution single lead ECG and the recordings were able to be sent via Bluetooth signal to a linked mobile phone for later cloud storage and offline analysis of HRV using dedicated HRV software. This capability along with its comfort allowed it to be worn during normal sleep (without the distraction and

interference of traditional ambulatory monitors). In this study a one hour continuous period of cardiac inter-beat recording was obtained during nocturnal sleep (at 0200 hours). Sixteen volunteers were studied at baseline (800 m, first night) and over eight consecutive nights, at a sleeping altitude of up to 3600 m.

Figure 21 Example of the Lumira Cardiac Patch Monitor which is applied to skin over the left anterior chest wall (personal photograph)



I won a research grant (Lumira Dx) to cover the technical support and the supply of the patch monitors and the linked iPhones. The data collection was undertaken by the same researchers used for my other HRV study which investigated the influence of sex on HRV at natural HA (publication 6).

The key findings of this study are that non-linear HRV is more sensitive to the effects of HA than time and frequency domain indices. High altitude exposure leads to a compensatory decrease in nocturnal HRV and complexity, which is influenced by the perceived intensity of

exercise in the previous 12 hours. Again changes in HRV failed to predict AMS development. This study highlighted the influence of exercise on subsequent HRV measurement at HA and has challenged the results of several studies that have cited the utility of HRV measures to predict AMS. This study was published in *Frontiers in Physiology* (IF 4.13) [95].

Publication 8

My 8th and final study related to this thesis submission is entitled ‘Assessment of Cardiac Arrhythmias at Extreme High Altitude Using an Implantable Cardiac Monitor: REVEAL HA Study (REVEAL High Altitude)’. This specific hypothesis of this study was that increasing HA exposure is pro arrhythmic and the burden of cardiac arrhythmias is higher at greater altitudes.

One of the greatest limitations of traditional ambulatory ECG monitors is the need for ECG cables which link the surface electrodes to the recording device. Even with excellent skin preparation these devices are highly prone to movement artefacts (eg during sleep, exercise or by rucksack straps) (figure 22). Furthermore, they are expensive and highly vulnerable to water ingress and cold damage at HA, which is not their designed environment of use.

Figure 22 Example of a conventional ambulatory ECG monitor (Lifecard by Spacelabs™)

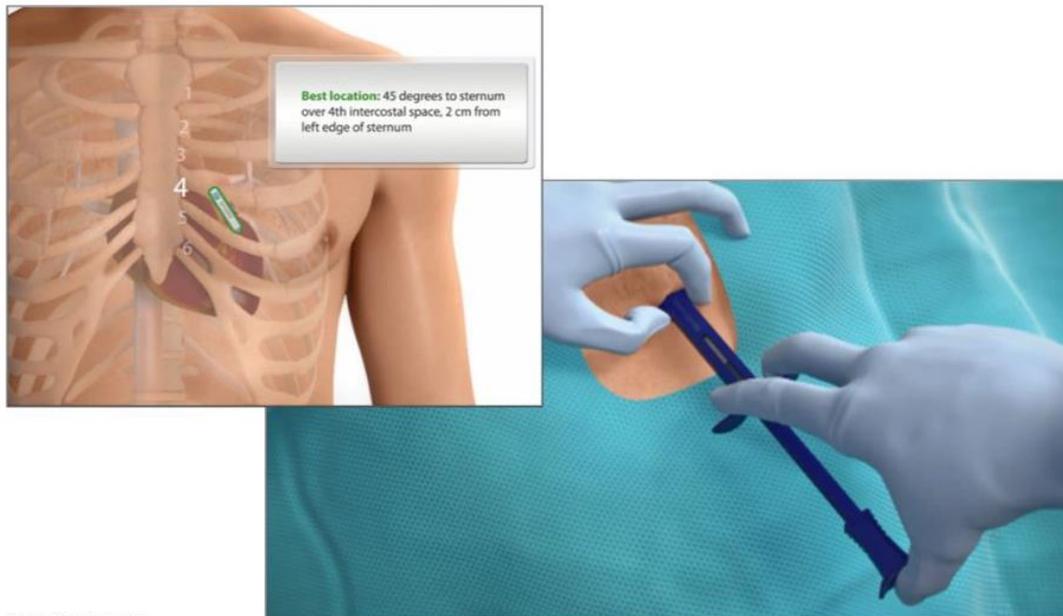


Two major and very recent developments in ambulatory recording are already transforming the monitoring of patients for the presence of important cardiac arrhythmias. The first device is known as an implantable cardiac monitor (ICM). These devices are implanted pre-pectorally over the left anterior chest wall (under the skin and fascia) which means they are far less affected by movement artefact and electrical interference than traditional ambulatory ECGs. Owing to their smaller size, internal fitting (under the human skin and fascia) and avoidance of ECG cables they are also less intrusive than a traditional ambulatory ECG monitors. However, they are considerably more expensive and require a small operation for their insertion. They are ideal devices in circumstances where a prolonged period of cardiac monitoring (>one week) is required as they can record single lead ECG data for up to an incredible three years.

The hypothesis of my 8th study was that exposure to HA would lead to a reduction in HRV and an increased risk of cardiac arrhythmias. This study was unique in that it was the first to investigate the risk of arrhythmia development at HA using a continuously recording ICM. Sixteen healthy adults underwent a detailed clinical assessment to determine their health (clinical history, normal echocardiogram and ECG) at sea level (Poole Hospital NHS Foundation Trust) prior to inclusion. All volunteers underwent the operative insertion of an implantable cardiac monitor (Reveal LinQ, Medtronic) at sea level prior to HA exposure (at Poole Hospital). I was first operator in 14 out of 16 ICM implants (figure 23). The Reveal LINQ has superior ECG signal quality, data storage and arrhythmia detection and battery life to the original Reveal device. Furthermore, its stored ECG data can be uploaded wirelessly to secure cloud storage for remote analysis. I won a research grant from Medtronic that covered the cost of the implantable cardiac monitors, the supply of two dedicated devices to allow the upload of the data stored on the cardiac monitors at HA as well as full technical support.

Figure 23 Graphical illustration of the Reveal LINQ implantable cardiac tor

a. Its method of insertion a (Source Googleimages.co.uk/Medtronic)



Source: Medtronic, Inc.

b. its size in comparison to the previous Reveal device (personal photo)



This study was conducted in 2016 again at Dhaulagiri but on a completely different cohort and at different altitudes to the central blood pressure and HRV Dhaulagiri studies cited above. I went to the Himalayas to support this project. This device allowed for the continuous recording of the subjects' cardiac rhythm which commenced ≥ 7 weeks prior to prior to HA exposure and over a time period of up to two months during an attempted ascent of Mount Dhaulagiri (8,167 m). A maximal altitude of 7550m was achieved by three subjects. The cardiac monitors remained implanted in the volunteers for a least two months post HA exposure. The results of this study confirmed my hypothesis that HA exposure does increase the tendency to cardiac arrhythmia development. Two sustained (>30 seconds) pathological tachyarrhythmias (atrial fibrillation in one subject and supraventricular tachycardia in another) were detected only at HA above 3800m. Multiple cardiac pauses (of >2.5 seconds) were observed in >50% of the subjects at HA only. The frequency and duration of the cardiac pauses increased at higher altitudes. This study has potentially important translational implications for persons with a history of brady- or tachy-arrhythmias who wish to go to HA above >4000m. This manuscript was originally submitted as full paper but changed to a shorter Scientific Letter on the Journal's request and was published in 'Circulation' (IF 19.31) [96].

The deeper relevance of these publications to the background literature and the state of knowledge are explored further below.

Background Literature Review, Research Context and Further Synthesis

This literature review reflects the synthesis and brief overview of some of the most relevant published research that was available at the time of writing the manuscripts related to my publications within this thesis. Entrez-PubMed and Google Scholar was used for all the

literature searches. The vast majority of cited references in this PhD submission relate to peer-reviewed publications that are cited on PubMed.

Hypothesis 1 High altitude (HA) adversely affects cardiac diastolic function and increases estimated filling pressures. These changes are influenced by the mode of hypoxia and the hypoxic environment (publications 1, 2 and 3).

There is a strong rationale to explore the effects of hypoxia on diastolic function in healthy human beings at HA. Across a broad range of clinical diseases that are associated with hypoxia, such as sleep apnoea, pulmonary oedema and chronic obstructive airways disease there is evidence of impairment in left ventricular relaxation and diastolic function. These cardiac changes have been principally observed using transthoracic cardiac ultrasound (known as echocardiography). The mechanism for these changes are multifactorial and are thought to include altered left ventricular filling secondary to the association increase in pulmonary vascular resistance and rise in PASP, pericardial constraint with the increased right ventricular pressure as well as abnormal interventricular septal motion [97]. These observations have led to the obvious question as to whether healthy persons exposed to the physiological hypoxia at HA develop significant diastolic dysfunction which is linked to HAPE susceptibility.

Operation Everest II (OEII) Study was one of the first studies to examine the cardiac effects of HA exposure. At the time it was probably the most extensive and wide ranging ‘high altitude’ study to date [98]. This landmark study sought to examine a wide array of human biological responses during progressive simulated HA exposure in a hypobaric hypoxia (HH) chamber. Eight young adult and healthy (aged 21-31 years) male volunteers were exposed to progressive HH during a ‘simulated’ 40-day ascent of Mount Everest. Impressively the investigators managed to place both systemic arterial and pulmonary arterial catheters

(allowing blood sampling) at rest prior and during maximal exercise at sea level at the start of OE II and at P_B 347 mmHg (~20,000 ft, ~6100 m); P_B 282 mmHg (~25,000 ft, ~7600 m) and the summit equivalent: P_B 253 mmHg (~29,000 ft, ~8848 m).

Six out of the eight participants managed to successfully reach the 'summit'. The key cardiac findings were that maximal cardiac output fell in response to increasing HA, yet despite this ventricular systolic function at all altitudes was similar to that at sea level, or even slightly enhanced [99]. Interestingly, they also found that left ventricular filling pressures actually fell with worsening hypoxia. Right ventricular function (inferred from the physiological data and not directly examined) remained preserved at HA despite the substantial increase in increase in pulmonary vascular resistance and PASP (33 ± 1 mmHg at sea level to 54 ± 2 mmHg at P_B 282 Torr). Despite the increase in PASP right ventricular pressure remained normal and there was no clinical evidence of right heart failure or increase in right atrial and pulmonary capillary wedge (indirect measure of left atrial filling pressure) pressures. Furthermore, whilst acute 100% O_2 breathing did lower cardiac output and the PASP it did not lower the pulmonary vascular resistance [100].

One of the obvious limitations of the Everest II study was that it utilised simulated rather than natural terrestrial HA. Another important limitation was that the investigators could only indirectly assess cardiac function using the data obtained from filling pressures and calculations of cardiac output and stroke volume. They did not have access to echocardiography (cardiac ultrasound) which is now the gold standard measure of 'real time' cardiac function. The Everest II investigators were also unable to measure regional cardiac function or fully quantify diastolic function. As the left ventricular filling pressures were normal in OEII it can be reasonably assumed that there was not severe diastolic function. However, in the absence of transthoracic echocardiography the presence of less severe grades

of diastolic function, in which atrial and ventricular filling pressures are typically usually normal, could not have been excluded.

Using echocardiography, global and regional cardiac systolic function and complex measures of diastolic function can now be quantified non-invasively in a matter of minutes. In the last 20 years we have witnessed the advent of increasingly portable yet robust echocardiogram machines that have even better capabilities than their large, laboratory-based predecessors. These advances have been exploited in three of my publications within this thesis. All three of the portable echocardiogram machines used in the studies linked to this thesis (Sonosite M Turbo 2, GE Vivid I and Q) are no bigger than a large laptop.

In one of the seminal HA studies Alleman et al investigated 41 healthy adults (30 men and 11 women; mean age 41 ± 12 years) at low altitude (550 m) and following a rapid ascent to HA at 4,559 m [101]. Cardiac function was examined using transthoracic echocardiography. HA exposure led to an at least two-fold increase in the PASP which was associated with change in left ventricular diastolic function that was directly correlated with the severity of pulmonary hypertension. They observed a decrease in the mitral E/A ratio consistent with grade I diastolic dysfunction. The authors hypothesised that these changes actually reflected a significant increase in mitral A velocity and augmented atrial contraction. The authors coined these changes as reflecting ‘compensated diastolic (dys)function’ to explain their findings [101].

In a subsequent study by Kjaergaard et al two years later 17 healthy adults underwent transthoracic echocardiography, including tissue Doppler imaging of the septal mitral annulus and basal segments before and after an 18-h overnight stay in a HA simulation tent with a FiO₂ of 12% (equivalent to 4,000 m above sea level) [102]. They measured myocardial velocities using tissue Doppler imaging and also measured the left ventricular Tei Index. The

Tei index is a measure of global left ventricular performance (known as Myocardial Performance Index) that has been validated [73]. It is calculated using pulsed wave Doppler echocardiography measured at the left ventricular outflow tract using the left ventricular ejection time (LVET) and isovolumic contraction (IVCT) and isovolumic relaxation times (IVRT) (Tei et al. 1997). The Tei index is calculated as $(IVCT + IVRT)/LVET$. Again it was shown that hypoxia led to a significant increase in the E/A ratio owing to an increase in mitral A velocity but they also noted that there was a decrease (-26%) in the peak early myocardial velocity (E') despite retained systolic function. Paradoxically the Tei index significantly increased. An increase in Tei function has been generally reported to represent a fall in global cardiac function however this rise was not at the level reported in patients with heart failure, which was the validation patient cohort for this measure, but nevertheless was an intriguing finding and difficult to reconcile given the other findings suggesting enhanced cardiac function.

The concept of potentially deleterious effects of HA exposure on cardiac function failed to diminish following the studies above and if anything gathered greater momentum as the complexity of cardiac functional measures increased. Bernheim et al did not observe any significant changes in diastolic function or estimated left ventricular filling pressures among 39 subjects (including 29 known to be HAPE susceptible) either at rest or during submaximal exercise at low (490 m) or high altitude (4,559 m). Moreover, changes in right ventricular pressure gradients did not correlate to any changes in left ventricular measures of diastolic function or left ventricular filling. Zhou et al examined 96 healthy young male adults (aged 18-35 years; mean 21.8 ± 3.6 years) old following rapid ascent from 1500m to an altitude of 3700m, where they spent 50 days involved in heavy labour (up to 10 hours per day) [103]. Echo parameters were measured on the 50th day after completing their heavy labour duty at HA and on the 2nd and 15th day after a 48-hour by bus return to lower (1500m) altitude. They compared their findings to a control group of similar aged adult men who were from the same

unit but who had not been exposed to HA. They found both the mean PASP and left ventricular Tei index increased. However, contrary to previous work they found that short-term exposure to HA led to a reduction in left ventricular ejection fraction and fractional shortening and that these changes positively correlated with altitude. These negative inotropic effects of HA on cardiac function, were partly inferred as baseline low altitude measures were not undertaken [103]. The authors also observed that compared with day 50 on the 15th day post HA exposure (hence at 1500m), the ejection fraction, systolic shortening and pulmonary levels returned to the same level as those of 98 similar aged male control subjects who were not exposed to HA. The reduction in Tei index following return to near sea level (1500m) was far slower. It is worth noting that the exercise burden in this study was considerable and may be an important confounding factor that was not addressed in the manuscript.

In a more recent study conducted at a similar time to my thesis submission work echocardiography was performed to a defined protocol on 14 healthy adults over three altitudes: in Montreal (30 m) Canada and at Namche Bazaar (3450 m), and Chukkung (4730 m) in the Himalayas [104]. The investigators used cardiac ultrasound to identify the presence of lung comets (acoustic reflections noted on ultrasound related to the presence of increased lung water) [105, 106]. This novel technique entails the identification of lung artefacts related to the presence of extravascular lung water, which is increased in the presence of clinical and subclinical pulmonary oedema. As expected, PASP significantly increased at HA. They measured the right ventricular Tei performance index and a cardiac functional modality which at the time was still in its infancy and known as systolic strain, which is measure of actual myocardial deformation. The investigators noted that the right ventricular Tei index increased significantly (0.32 ± 0.08 at 30 m vs. 0.41 ± 0.10 at 4730 m; $P = 0.046$) and that there was a trend toward deteriorating right ventricular free wall longitudinal strain between 30 and 4730m (-25.9 [5.3%] vs. -21.9 [6.4%]; $p=0.092$). Again, it is worth emphasising that whilst

there an increase these values were not in the typical heart failure range. Contrary to Alleman and Kjaergaard they did not detect any significant changes in diastolic function. Interestingly, whilst there were no cases of clinical HAPE they identified lung comets, in all but one case at 4730 m, suggesting the presence of pulmonary interstitial fluid, [104]. In another acute hypoxic chamber study Hanoaka sought to investigate the relative changes in the myocardial performance Tei Index in a participants with known susceptibility to HAPE (n=11) *versus* those who were more resistant (n=9) [107]. They found that normobaric hypoxia led to enhanced left ventricular myocardial performance yet impaired right ventricular performance in the HAPE susceptible individuals. These differences were not observed in the resistant cases.

Whilst hypoxia is the common physiological stimulus in all of the studies cited above the mode of hypoxia and duration of exposure markedly differ between the studies. Some of the studies have used normobaric or hypobaric hypoxia whereas others have used a genuine natural terrestrial HA challenge. Yet, it is not known if and to what extent the mode of hypoxia influences the cardiac response. Prior to my collaborative work there had been, to my knowledge, only two studies that have attempted to examine the comparative cardiac responses to differing hypoxic environments [108, 109]. Beidleman et al noted that cycling time trial performance was more impaired with HH than NH at the same ambient PO_2 (equivalent to 4,300 m, despite similar cardiorespiratory responses (heart rate, mean arterial pressure and cardiac output) [109]. Miyagawa noted similar changes in heart rate and stroke volume (hence cardiac output) with exercise with normobaric and hypobaric hypoxia. In neither of these studies were echocardiographic assessments of biventricular performance and/or right ventricular systolic pressure measured. A four way comparison of the cardiac adaptations to normobaric hypoxia (NH), hypobaric hypoxia (HH) and genuine HA and its comparison to during similar exercise with normobaric normoxia (normal sea level breathing) had not been performed. This is examined for the first time in one of my thesis studies.

In summary, previous research has clearly shown that acute hypoxia and HA leads to an increase in PASP. The effects of HA on diastolic function are mild and depend on the population studied and may be influenced by the hypoxic environment and its degree. Measures of global and right ventricular function have yielded very mixed results as have the data examining the inter relationships between right and left ventricular function. Acute hypoxia does not appear to lead to an increase in left ventricular filling pressure. There is very limited data on right ventricular filling particularly with terrestrial HA. The majority of the published studies have mainly examined either a single altitude or level of hypoxia and the influence of exercise needs much greater clarification. Little work has been done on the relationship between potential changes in cardiac function to the development of HA related illness and AMS. A comparison of normoxia to the three main modalities of hypoxic challenge had never been previously conducted.

All of these unresolved issues are addressed, at least partially, in the series of independent yet linked research studies within this thesis (studies 1, 2 and 3). My data has confirmed that whilst acute hypoxia and HA exposure leads to minor alterations in diastolic indices it does not lead to an increase in ventricular filling pressures or severe diastolic dysfunction. Whilst the Tei index does increase with HA this does not reflect adverse effects of cardiac filling or global cardiac dysfunction and likely relates to the shortening of the ejection time with increased heart rate. Acute HH and NH elicit similar hypoxic effects on resting cardiac responses to that of genuine HA exposure. However, differences emerge with exercise with a greater increase in RVSP, right ventricular Tei Index and oxygen desaturation. This must be borne in mind when interpreting the results of acute hypoxia and natural cardiac studies.

Hypothesis 2 High Altitude exposure leads to an abnormal rise in circulating biomarkers of myocardial injury that are linked to the development of acute mountain sickness.

There are two cardiac biomarkers that have dominated the clinical and research literature over the least 10 years. The most established and widely used cardiac biomarker is the measurement of cardiac troponins. Cardiac troponins are regulatory proteins that are integral to muscular contraction. Cardiac troponin I and T are more exclusively found in skeletal muscle and hence their release into the systemic circulation is a sensitive and relatively specific marker of myocardial injury [110]. Cardiac troponins are the gold standard circulating biomarker for the diagnosis of myocardial injury, necrosis (cell death) infarction [111].

Brain natriuretic peptide (BNP) is probably the second most widely used and studied cardiac specific biomarker. It is a peptide that is secreted by cardiomyocytes within the left and to a lesser extent (less muscle) right ventricle in response to increased ventricular volumes and pressure overload [112]. It is secreted in two isoforms from a prohormone known as proBNP: NT-proBNP and the biologically active acid polypeptide BNP-32. Both forms can be measured clinically, though NT-proBNP tends to be more typically measured in routine clinic practice given its stability in whole blood of up to 72 hours [112]. Where circulating BNP is examined it needs to be either measured in whole blood within four hours or centrifuged from whole blood within one hour (ideally) and its serum component stored at $\leq -20^{\circ}\text{C}$ for later batched analysis. Along with the use of transthoracic echocardiography the measurement of BNP and NT-proBNP are now the gold standard marker for the diagnosis of heart failure [113].

Given the utility of Cardiac troponins and BNP to identify cardiomyocyte injury and stretch respectively they could have valuable translational uses for the examination of the

controversial issue of whether hypoxia and HA exposure leads to myocardial dysfunction and injury. This is particularly pertinent as it is well known that changes in these biomarkers, particularly troponins can precede any notable impairment in cardiac function, and hence before any significant notable changes observed by echocardiography [114].

In the first study to examine the effects of HA on BNP in healthy humans Tosher investigated 10 healthy non-HAPE-susceptible lowlanders during acute exposure to 5200 m in Bolivia. He found that whilst the estimated PASP, measured using echocardiography, significantly increased as expected at HA, he failed to observe a rise in NT-pro BNP [115].

In another recent study from our research group [37] - not included in this PhD submission) the above findings were challenged. We (Boos C on authorship) found that HA led to a significant rise in both NT-proBNP and BNP which were closely correlated and that BNP levels were significantly higher in those who developed severe AMS *versus* those who did not [37]. Furthermore, BNP levels correlated with total body water supporting an association between the rise in BNP and failure to clear water with AMS. This finding is interesting as one of the fundamental functions of BNP is to physiologically promote diuresis to counteract the volume overload of heart failure. Increased diuresis is fundamental to HA acclimatisation and altered diuresis is thought to be one of the principal mechanisms for the development of AMS which is implicated by our study results [40]. Indeed, animal studies have shown that BNP is released from isolated perfused ventricles in response to local hypoxia [116]. Hence the examination of BNP levels in persons with AMS could be important. Fedderson et al examined 14 (10 men and four women) healthy adult mountaineers who ascended to an altitude of 5050m from 100m over nine days [117]. He found that BNP (measured using a point of care assay on whole blood) values did not significantly increase from baseline to HA, despite evidence of increased diuresis. Furthermore, a relationship between BNP and AMS development was not observed; in fact, BNP levels were similar in the six subjects who

developed AMS *versus* the eight who did not. Interestingly, the authors did note a significant elevation in BNP in a single subject who developed features consistent with HAPE. They concluded that BNP was not the cause of HA-related diuresis [117].

In another study by our group (again not in this thesis, Boos C on authorship) Mellor et al examined 48 healthy adults post-trekking and at rest at three altitudes: 3833 m, 4450 m, and 5129 m. NT-proBNP, hs-cTnT and hsCRP (C reactive protein; marker of inflammation) measured using immunoassays, and PASP, measured using echocardiography [118]. We found that NT-proBNP, hs-cTnT, hsCRP all increased at HA and that the increase in NT-proBNP and hs-cTnT were linked to higher PASP (≥ 40 mm Hg) and the natriuretic peptides to AMS development and the cTnT more closely to exercise. Independent predictors were not examined.

I recently described the case of a highly experienced healthy mountaineer who presented with HAPE at 3800m and a significant simultaneous increase in BNP (111 ng/L; normal < 5), hs-cTnT (43.7 ng/l) and PASP (Boos, Holdsworth et al. 2013). Despite these biomarker increases, particularly in relation to BNP, the estimated left ventricular filling pressures remained normal [35]. In another study Gao et al observed significantly higher levels of NT-proBNP (pg/ml) at HA ($> 3,000$ m) in 21 individuals diagnosed with HAPE *versus* those without HAPE [119]. They also noted that treatment for HAPE led to a drop in the BNP levels to values observed in the control group strengthening the potential utility of BNP as a marker of HAPE and its severity.

The published data on the effects of HA on cardiac troponin levels have also been inconsistent. In one of the seminal studies Davila-Roman studied 14 participants who were competing in a 163-km HA ultra-mountain marathon (elevation 2,350 to 4,300 m)

[120]. Transthoracic echocardiography and cTnT levels were measured before the race, immediately post and then again 18-24 hours later. The lower limit of detection for the cTnI used was 1.5 ng/ml and the upper reference limit was 3.1 ng/ml. Despite evidence of right ventricular impairment (reduced fractional area change with right ventricular dilatation) on measures of cardiac function on echocardiography immediately post exercise in 9 out of the 14 subjects studied cTnI levels were undetectable in all but one subject and this rise was very small (5 ng/ml) [120]. Interestingly, the subject with small rise in cTnI had marked right ventricular dysfunction and the highest PASP at 65 mm Hg, which represented 35 mmHg increase from baseline. At 18-24 hours post-race the subject with the elevated cTnI had detectable but normal (1.8 ng/ml) cTnI levels and all echocardiograms had returned to normal.

Shave et al (n=8) did not observe any significant differences in either systolic or diastolic function following a 50 mile cycle ride under NH and a marginal rise in cTnT was only observed in one athlete [121]. Conversely, Ortega et al observed a significant increase in cTnI immediately after a mountain bike challenge (distance 95 km, cumulative altitude difference 2340 m) conducted on eleven amateur male cyclists [122] and Banfi et al noted a small but significant rise in both cTnT and BNP among 15 mountain marathoners following a race [123].

These publications have identified inconsistencies in the available data on the effects of acute hypoxia and HA exposure on these cardio specific circulating biomarkers. As with the data on cardiac function, the findings have been heavily influenced by the individual, the HA environment and the burden of exercise. These studies have predominantly examined the impact of a single high intensity exercise in non-acclimatised subjects. Furthermore, again it is difficult to fully appreciate the significance of the positive HA studies when a sea level or lower altitude exercise control study on the same population was never conducted. Several of

these studies predate the availability of modern high sensitivity cardiac troponin assays. These assays are able to detect the same biomarker but at much lower concentrations than were previously possible with a high degree of accuracy. High-sensitivity assays are thus able to detect changes in troponin concentrations even within the normal limits in healthy populations. By being able to accurately detect lower concentrations of cardiac Troponin in the blood they are able to identify earlier and smaller changes in troponin which has huge research and clinical applications. For example, the high-sensitivity Roche Elecsys troponin T (hsTnT) assay, used in my local hospital (Poole Hospital NHS) has an upper limit of normal (99thcentile) of 14 ng/L, which removes the need to wait several hours after the onset of chest pain symptoms to reliably confirm or exclude a diagnosis of myocardial infarction.

In this thesis I have presented a study that investigated the effects of increasing altitude on the levels of ultra-high sensitivity cTnT levels (publication 2). The factors influencing the potential rise in cTnT and their relationship to changes in cardiac function (using portable echocardiography) and HA related symptoms are examined for the first time. This research has shown that the rise in cTnT tends to be mild and not in the pathological range. The increase relates to the rise in cardiac output (hence linked to exercise), PASP and degree of hypoxia and not to any changes in cardiac function or filling. Hence, whilst hypoxia and exercise at HA does influence circulating cardiac troponin levels this influence in a non HAPE population is minor, transient (not sustained) and does not reflect the presence of significant myocardial injury.

Hypothesis 3 Acute High altitude reduces arterial compliance and increases central blood pressure.

The vascular response to acute hypoxia is highly complex and involves both local nitric oxide mediated vasodilatation [124] and sympathetically mediated arterial vasoconstriction [125], that act to balance vascular tone. In healthy adults the net effect is a rise in resting heart rate with variable changes on brachial blood pressure depending on the severity and duration of hypoxia [52, 69, 124-126]. Sustained exposure (>24 hours) to terrestrial HA tends to increase systolic and diastolic blood pressure which is mostly evident at night [46, 127-129].

There are a number of rational reasons to explain the potential vasopressor effects of acute hypoxia and HA. As mentioned above acute hypoxia leads to increased sympathetic activation. Another potentially important mechanism relates to the effects of hypoxia on arterial endothelial function. The endothelium is also a principle regulator of arterial stiffness and influences both peripheral and central arterial pressure (Wilkinson et al., 2002; Boos et al., 2007). Acute hypoxia has been shown to cause endothelial activation and even dysfunction within the systemic and pulmonary artery circulation in vulnerable healthy adults [130, 131]. It has been shown that reduced nitric oxide availability and an impairment of vascular endothelial function in the systemic circulation may be a key mechanism for the rise in PASP among HAPE susceptible individuals [132-134].

The measurement of brachial blood pressure is one of the obviously and most practical ways of assessing the potential vasopressor effects of HA. A further and potentially more useful clinical marker of cardiovascular risk would be the measurement of central blood pressure (within the ascending aorta) as it more closely reflects the pressure afterload on the central vital organs (heart, brain and kidneys) [76]. One of the critical obstacles to the measurement of central blood pressure at HA relates to the difficulties of measuring central blood pressure

itself. As mentioned earlier this has traditionally required the use of an arterial sensor catheter delivered into the ascending aorta via peripheral arterial access. This would be very challenging to do at terrestrial HA and particularly above 4000m. Fortunately, there are now several available devices that can provide a non-invasive measure of central blood pressure. This is typically acquired using combined information from the arterial waveform (at the brachial or radial artery) and the knowledge of the brachial blood pressure typically using a generalised transfer function [77]. These indirect peripheral measures of central blood pressure have been consistently shown, with a number of available devices, to strongly correlate with that obtained using gold standard catheter measures [77]. However, the level of agreement (and bias) does appear to be highly dependent on the type of device and its method of central blood pressure estimation [135, 136].

Widespread sympathetic activation and arterial endothelial dysfunction are common to the aetiology of both HAPE and AMS and are implicating factors in the development of systemic hypertension [137]. It would be plausible to hypothesise that HA exposure would lead to an increase central blood pressure and a fall in arterial compliance and that these changes might be linked to the development of HA related illness. This concept has not been proven or demonstrated. The availability of ultraportable devices that are capable of measuring both central blood pressure and arterial compliance has paved new research avenues at HA. The ability to almost simultaneously examine arterial compliance and central blood pressure as well as cardiac performance using echocardiography has provided novel research opportunities at HA that would previously have been impossible.

A number of investigators have examined the effects of acute hypoxia on arterial compliance. The use of simulated hypoxia and very short duration of hypoxic exposure (<1 hour) dominated the earlier research in this field [69, 124, 126]. These studies have shown that acute NH leads as an increase in resting heart rate and a significant reduction in the arterial

augmentation index and systemic vascular resistance with variable effects on arterial blood pressure.

There have been very few terrestrial HA studies in which the effects of genuine HA on arterial stiffness or central blood pressure have been studied and the available data has been conflicting. In the first terrestrial HA study Rhodes et al examined the effects of HA on brachial blood pressure, arterial stiffness (using a measurement known as the stiffness index) and vascular tone (by measurement of the reflective index) using a non-invasive finger photoplethysmography [138]. Seventeen adults (three with a history of hypertension) were studied at sea level, 3,450m and 4,770m over an 11 day ascent at HA. They observed that HA exposure was associated with an increase in brachial blood pressure without affecting the arterial stiffness Index. However, they did note temporal changes in a surrogate measure of endothelial function (and vascular tone known as the reflective index). They observed a non-significant fall in the reflective index (hence lower vascular tone) during first day at 3,450m from $74.4 \pm 7.9\%$ to $70.5 \pm 13.8\%$ ($p > 0.05$) and significantly so by 4,770m ($69.9 \pm 12.0\%$; $p < 0.02$) which then reverted to baseline with acclimatisation. The changes in the stiffness index and reflective index did not relate to changes in blood pressure or the presence of AMS. They did not examine central blood pressure or associated changes in cardiac function. There were also clear limitations in their method of large arterial stiffness assessment which related to difficulties in obtaining good pulse waveform traces at the finger at increasing HA.

In another recent HA study Parati et al (2013) investigated 42 adults (21 males, age 36.8 ± 8.9 years) who were randomised to double blinded treatment with either acetazolamide 250 mg b.i.d. or placebo [128]. They examined brachial blood pressure, pulse wave velocity (PWV) and arterial augmentation index using a high-fidelity PulsePen device. The subjects were studied at baseline sea level and following two days of treatment and after 6h and on 3rd day after exposure to high altitude at (Capanna Regina Margherita, Monte Rosa, 4559

m). HA (*versus* sea level) led to a significant increase in brachial diastolic ($p < 0.005$) and mean blood pressure in the placebo group, which was prevented by the use of oral acetazolamide. There was a non-significant rise in both brachial and central blood pressure which was also prevented by acetazolamide. There was no significant change in carotid femoral PWV. Conversely, HA led to a significant increase in the augmentation index (normalized for a theoretical heart rate of 75/minute) in the placebo but not acetazolamide group. Given that acetazolamide is a recognised first line drug for the prevention and treatment of AMS (mechanism includes reducing alkalosis to improve ventilation [15]) this data further supports this and endorses a potentially additional role for acetazolamide to reduce the haemodynamic effects of hypobaric hypoxia.

My fourth publication in this thesis submission represents the first study to explore the effects of incremental terrestrial HA on both central blood pressure and the arterial augmentation index. The potential factors influencing changes in these parameters at HA, including the degree of hypoxia and their relationship to HA related symptoms were explored. My data showed that HA led to an increase in central blood pressure arterial augmentation index which were strongly correlated (as expected), confirming the data reliability. There was no link between changes in either measure to the development of AMS. This data suggests that the increase in the arterial augmentation index following hypoxia is not explained by a true increase in arterial stiffness which takes many months and generally years to develop. The increased in augmentation index with HA more likely relates to haemodynamic changes in the arterial wall which lead to earlier arterial wave reflections (which act to increase the augmented pressure and augmentation index). These findings have implications for persons with known and particularly poorly controlled hypertension, who plan to travel to HA. These persons need to be aware of the potential for significant further increases in their blood pressure at HA. A further HA study with the inclusion of hypertensive subjects would be insightful as we don't currently know whether the increase in

the blood pressure would be even greater and pose a greater risk to these individuals.

Hypothesis 4 High Altitude exposure leads to a reduction in heart rate variability and an increased risk of cardiac arrhythmias. The changes in HRV at HA are influenced by ventilation and biological sex and are linked to the development of acute mountain sickness

There are a number of possible reasons to explain why acute hypoxia and the HA environment might affect heart rhythm and potentially increase the risk of cardiac arrhythmia development. It has been widely reported that sudden cardiac death is the leading causes of non-traumatic deaths in adults at HA, particularly during leisure time activity [51, 54, 55]. Given that most sudden cardiac deaths are caused by sustained haemodynamically significant cardiac arrhythmias it would be reasonable to hypothesise that hypoxia and HA could be pro-arrhythmic [56, 139]. Furthermore, several of the factors that are known to be provocative in the development of cardiac arrhythmias are synonymous with significant HA exposure and include sympathetic activation, heavy exercise, dehydration, mental stress and sleep deprivation [4, 5].

This hypothetical HA-arrhythmia link has prompted medical researchers to obtain more robust data from detailed ECG monitoring at HA. Delivering this research has been very challenging at terrestrial HA. Consequently, there has been a surprising paucity of real world data during terrestrial HA exposure. The majority of published studies have been performed during passive ascent, simulated hypoxia (HH and NH) and using repeated 12 lead ECGs [140-143]. There is far less data following continuous ambulatory ECG monitoring [144-146].

In a seminal ECG at extreme terrestrial HA study, Hori et al examined nine adults during an ascent of up to 7800m (mean 5710 m) [142]. They acquired 7.5 second ECG print outs, every

60 minutes, over a period of 16-24 hours at HA. They noted that the nocturnal corrected QT interval (QTc) was also significantly prolonged in spite of shortened RR interval. This was challenged in a very large recent simulated HA study whereby the resting 12 lead ECGs of 13 healthy adults were compared at sea level with that at 3000-3600 m [147]. The authors analysed a series of 10 ECG complexes at seven time periods over the 30 minutes. They found that there was no discernible difference in P wave, PR, QRS, and QT interval. However, there was an overall decrease in the T wave amplitude. Coustlet et al investigated 456 adults during normoxia and following 20 minutes exercise under simulated HA using NH (equivalent to 4800m) [141]. They found that whilst the amplitude of all ECG wave deflections had reduced, there were no significant abnormalities and no single ECG change was predictive of AMS development. They did not measure the QT interval.

Kujanik et al studied 20 healthy elderly men (50-64 years) during passive ascent to HA in a cable cabin at 898 m, 1764m, and 2632m [146]. They observed a significant increase in both supraventricular and ventricular premature beats but no evidence of sustained arrhythmias. In a recent study (Boos C et al), not included in thesis submission) of 10 healthy adults during active exercise with ascent and decent at 2610-5140m ventricular premature beats (extrasystoles/ectopy) were observed in all subjects [144]. Supraventricular premature beats were observed in less than half of the cohort. Whilst a trend to higher SVE and VE burden on ascent *versus* descent was observed this did not reach statistical significance. This research raised the suspicion that HA could be pro-arrhythmic. ECG artefacts were relatively high (6.0±14.5%) and most prominent during the most intense exercise which is the period of greatest interest.

There has been only one previous study has an ICM been used to capture ECGs at HA to identify its potential pro-arrhythmic risk. Woods et al studied nine participants at sea level (>2 weeks) and for approximately three weeks at HA (1400 to 5200m) using a first generation

ICM (MedtronicTM, Model 9525) [148]. They observed that development of likely fast atrial flutter (150/minute) lasting 8.5 minutes in one subject (11.1%) immediately after a period of severe exertion at 4500 m. Unfortunately, being a first generation device it did not have auto-detection settings and hence all ECG uploads needed to be triggered at the time of symptoms, undermining its capabilities. Consequently, there was a considerable risk of missing important asymptomatic episodes, particularly during sleep. The Reveal LINQ ICM device has transformed the landscape for cardiac monitoring and its capabilities were examined in my eighth publication [96]. This device is seven times smaller than the device used by Woods et al and can be implanted by an injectable technique with the added capability of being able to remotely monitor its recordings via a conventional wired telephone signal.

In my 8th publication within this thesis I examined the hypothesis that HA exposure increases risk of cardiac arrhythmias by implanting Reveal Linq devices in 16 healthy adults travelling to extreme HA [96]. My findings supported my study hypothesis as more than half of the studied subjects developed significant cardiac arrhythmias at HA. This was manifested as the development of significant brady-arrhythmias and pauses (>2.5 seconds) in the majority, but with the additional observation of two sustained (duration >30 seconds) pathological tachyarrhythmias (atrial fibrillation of >5 hours in one subject and a supraventricular tachycardia of 31 seconds in another) at HA. Interestingly, all of the arrhythmias were detected at >3500m and there were no arrhythmias during ≥ 4 months of recording, including activity at activity at sea level and lower altitudes. This was the first definite study to confirm this. All of the bradyarrhythmias were nocturnal and asymptomatic and the severity and number of pauses increased with altitude gain and likely represent the effects of increased nocturnal vagal tone during possible sleep disordered breathing. These findings have important clinical implication for cardiac patients with a history of brady and tachyarrhythmias that need to be advised that there is a theoretically increased risk perpetuation above 3500m.

The second major technical advance in portable cardiac monitoring has been the very recent development of the ECG patch Monitor. They have the ability to non-invasively combine the features of the present-day Holter and event/loop recorders with real-time data transmission and analysis capabilities [149]. They are attached to the skin via an adhesive carrier with embedded wet gel electrodes. The electrodes within the patch are closely spaced to facilitate the placement of the adhesive patch on the body. This is a rapidly expanding field and the capabilities of these devices are already extending well beyond continuous ECG monitoring to the potential measurement of respiratory rate, skin temperature, physical activity, step counts. There is even the potential to measure capillary SpO₂ recording and record location and track distance and altitude using GPS. Their translational impact on HA research is enormous. A patch with detailed cardiac rhythm monitoring has not been tested at HA.

My 7th publication, in this thesis, represents the use of a novel ‘prototype’ cardiac patch monitor to measure changes in heart rate and its variability at HA [95]. This allowed, for the first time, the ability to non-invasively record the cardiac inter beat interval comfortably whilst sleep at HA. This Lumira cardiac patch monitor has the added advantage of being able to record the position of subjects at the time of recording (upright, on side lying or on back). This enhanced capability is of fundamental importance in the measurement of HRV, given the influence of position of its results.

The third crucial advance in portable cardiac monitoring technology, related to my HA research, has been the advent of other wearable technologies for the measurement of the cardiac inter beat intervals and hence heart rate. This has most notably been with the use of heart rate sensing chest straps linked to smart watches or smart phones and more recently via heart rate sensors embedded within the wrist straps of smart watches or within simple finger

sensors. At present these devices do not have the capabilities for complex heart rhythm detection and function mainly as heart rate monitors to facilitate athletic training. Another form of ‘wearable technology’ is the use of a simple finger sensor linked to smart phone to detect heart rate which was utilised in my 7th publication at natural HA [150].

One common application of all of these devices is their ability to detect the cardiac inter beat interval which is essential for the quantification heart rate variability (HRV). Research has shown that acute hypoxia and HA exposure can lead to significant alterations in HRV [52, 150-160]. The general interpretation of these changes and supported by hormonal data (eg measurement of urine and circulating catecholamines) is that acute hypoxia induces an initial decrease in parasympathetic and increase in sympathetic tone, which is partially reversed with acclimatisation. This interpretation is probably over simplistic but nevertheless, does emphasise the importance of autonomic control in effective HA acclimatisation. Indeed, alterations in autonomic balance have been cited as one of the pathophysiological factors in the development in HA-related illness and HACE [15, 27].

The results of research into the relationship between changes in HRV and HA-related illnesses are inconclusive [15, 161-165]. There are number of factors that might help to explain this. Firstly there was a wide range of differing HRV measures used in these studies. Secondly, there was major heterogeneity in the populations (ages and men *versus* women) studied as well as the HA profile (ascent, height, passive *versus* active ascent). Finally, even the duration of cardiac recordings varied considerably between studies (minutes to hours).

The array of HRV parameters that are currently available is staggering. Whilst many of the simple time and frequency domain measures are highly correlated a number of highly complex and novel measures of HRV measures have evolved. These include detrend

fluctuation analysis, correlations dimension and entropy [166, 167]. The complexity of HRV measures and their interpretation can be very challenging, even for those actively involved in their use (myself included).

In the last 10 years we have witnessed the development of multiple highly portable methods to examine HRV. The capabilities of these devices are highly variable and whilst the majority can provide HRV outputs within seconds of full data capture there is usually the option of later offline further data analysis using a variety of HRV analysis software. Sensors can range from a simple finger probe (figure 12), wrist or chest strap sensor linked to a smartphone (eg ithlete™) or smartwatch to more advanced yet dedicated ECG derived portable HRV devices (eg CheckMyHeart Device™) [93, 94]. These modern portable devices have transformed the research opportunities as traditional HRV devices were both largely non portable and prohibitively expensive.

One of the key potential confounders in HRV analysis relates to the influence of breathing on HRV. It is generally recognised that controlled breathing during the measurement of HRV reduces the variability in the results variance. However, it is still uncertain whether this is better done using the encouragement of slow and relaxed breathing or using timed breathing prompts. This issue becomes even more contentious at HA where hypoxia driven hyperventilation is part of the physiological adaptation [8]. It could be strongly argued that the imposition of a slower breathing pattern could potentially negate the important interaction between physiological breathing on autonomic output. My study of the influence of spontaneous *versus* timed breathing at HA was the first to compare HRV under these conditions.

Another area of uncertainty relates to the influence of biological sex on HRV and whether any observed differences or similarities noted at sea level are maintained at HA. Differences in several measures of HRV and indirect measures of autonomic tone have been reported between men and women [168-171]. Women tend to have lower blood pressure yet relatively higher heart rates than men. There is some evidence to suggest that women are more vulnerable to AMS development and are more likely to suffer with a greater severity [172-174]. This raises the question of whether differences in autonomic regulation and relative HRV between men *versus* women help to explain this finding; a question that was addressed for the first time in publication 5 of this thesis. Whilst there are important biological differences between men and women relative changes in their HRV may add further insight into their differences in HA acclimatisation and susceptibility to HA related illness. A prospective study examining potential sex related changes in HRV at HA has not been previously conducted. In this thesis I have described what is to my knowledge the first study to examine the influence of biological sex on changes in HRV and its links to AMS. In this study I found that, whilst HRV measures differed between men and women, these differences were maintained at HA. This suggests that the effects of HA on HRV remain consistent in both men and women.

Another potential clinical application of HRV is its utility to identify individuals at high risk of developing adverse cardiac events including significant cardiac arrhythmia and even sudden cardiac death. Reduced HRV has been strongly linked to an increased the risk of SCD among a number of different clinical patient groups [52, 175-177]. Its utility for risk prediction in healthy persons has been less well established. However, there is data emerging. For example in the Copenhagen Holter Study of 678 health adults (aged 55-75), it was shown that nocturnal HRV was a strong and independent predictor of the development of stroke [178]. The authors go on state that whilst the mechanisms for this finding was uncertain they hypothesised from their data that reduced parasympathetic activity may have increased the

risk of stroke by increasing the risk of cardiac arrhythmias. Animal data has also supported the link between reduced HRV and arrhythmic risk in healthy hearts. For example it has been shown in a single animal study of 43 healthy dogs that reduced vagal activity using HRV was predictive of higher fatal cardiac arrhythmic risk during their first myocardial ischemic episode. It is still not known whether alterations in HRV at HA are predictive of arrhythmic risk. I was unable to answer this question. I did not find a link between HRV measures and AMS. The patch monitor did not have sufficient rhythm detection capabilities to fully explore this relationship and the Reveal LINQ whilst superb as a method of detecting cardiac arrhythmias its platform did not allow for the detailed inter beat transparency to allow HRV quantification. The integration of the two capabilities would be a worthy future study.

Concluding synthesis

This thesis addresses four hypotheses and presents eight original research studies that have examined the cardiovascular responses to acute hypoxia and natural high altitude exposure in healthy adults.

My first and second studies have led me to partly reject my hypothesis that acute hypoxia and HA exposure adversely affects cardiac diastolic function and increases estimated filling pressures. My data has convincingly shown that minor alterations in diastolic function do occur in response to hypoxia that are sustained, however advanced diastolic function and increase in estimated left ventricular filling pressured do not occur. The changes in diastolic function are influenced by the hypoxic environment (hypobaric versus normobaric hypoxia versus natural HA). Based on the findings of my second study my has led me to reject my second hypothesis HA leads to an abnormal rise in circulating biomarkers of myocardial injury that are linked to the development of acute mountain sickness. Whilst HA exposure did lead to a significant increase in cardiac troponin T the effect size and increase was marginal

and was not associated with the development of AMS or to changes in cardiac function. The troponin rises tended to be affected by exercise and its associated increase in cardiac output and the rise in pulmonary artery systolic pressure with increasing hypoxia.

My first and sixth studies support my third hypothesis that acute HA exposure reduces arterial compliance and increases central blood pressure. However, these effects plateau with HA acclimatisation and relate to haemodynamic effects of hypoxia on the arterial wall and arterial wave reflections rather than any sustained increased in large artery stiffness.

My 5-8th Studies relate to my hypothesis that exposure to HA exposure reduces heart rate variability (HRV) and increases risk of cardiac arrhythmias. The changes in HRV at HA are influenced by ventilation and biological sex and are linked to the development of acute mountain sickness. My data has supports the first part of my hypothesis as HA exposure reduces time domain measures of HRV which then increases with acclimatisation. My data (5th study) suggests that that there are consistent differences in HRV between men and women which become more significant at HA. However, changes in HRV (5th and 6th studies) were not predictive of AMS or its severity.

Finally my eight and final publication supported my hypothesis that HA increases the risk of developing cardiac arrhythmias. My data demonstrated that this is true for both bradyarrhythmias and pauses which increased in number and severity with increasing HA and probably reflected increased vagal tone. HA increase the risk of narrow complex tachyarrhythmias which was manifested as a prolonged episode of nocturnal atrial fibrillation (which was likely vagally-mediated) and one episode of exercise induced supraventricular tachycardia which was likely sympathetically triggered.

The results in this thesis leads me to conclude that acute HA-related hypoxia induces early sympathetic activation followed by delayed parasympathetic activation with acclimatisation. These factors might explain the increase in heart rate, central blood pressure and proarrhythmic risk and the increasing burden of pauses at higher altitudes.

Chapter 3

Publication 1

The effects of acute hypobaric hypoxia on arterial stiffness and endothelial function and its relationship to changes in pulmonary artery pressure and left ventricular diastolic function

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Publication

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The effects of acute hypobaric hypoxia on arterial stiffness and endothelial function and its relationship to changes in pulmonary artery pressure and left ventricular diastolic function

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Running title: Relationship of arterial stiffness and endothelial function to pulmonary artery pressure and diastolic function

Abstract

Background and Aims: This study investigated, for the first time, the effects of simulated high altitude, following acute hypobaric hypoxia (HH), on simultaneous assessment of large artery stiffness and endothelial function and its inter-relationship to left ventricular (LV) diastolic function, pulmonary artery systolic pressure (PASP) and estimated PA vascular resistance (PVR).

Methods: Ten healthy subjects were studied at baseline pre and following acute HH to 4800m for a total of 180 minutes. Assessments of LV diastolic function, mitral inflow, estimated LV filling pressure (E/e'), PVR and PASP were undertaken using transthoracic echocardiography. Simultaneous assessments of arterial stiffness index (SI), systemic vascular resistance (SVR), vascular tone and endothelial function (reflective index [RI]) were performed using pulse contour analysis of the digital arterial waveform.

Results: Acute hypoxia led to a fall in SpO_2 (98.1 ± 0.7 vs. $71.8 \pm 7.1\%$; $p=0.0002$), SVR (1589.1 ± 191.2 vs. 1187.8 ± 248.7 ; $p=0.004$) and RI (50.8 ± 10.3 vs. $33.0 \pm 6.5\%$; $p=0.0008$) with an increase in PASP (24.3 ± 2.2 to 35.0 ± 5.3 mmHg; $p=0.0001$) and estimated PVR (116.40 ± 19.0 vs. 144.6 ± 21.5 ; $p<0.001$). There was no rise in either SI ($p=0.13$), mitral early annular early e' filling velocity or E/e' . There was a significant inverse correlation between SpO_2 and PASP ($r=-0.77$; $p<0.0001$), PVR ($r=-0.57$; $p=0.008$) and between the fall in SpO_2 and change (Δ) in RI (baseline vs. 150 minutes, $r=-0.52$; $p<0.001$). There was a modest inverse correlation between Δ RI (lower Δ RI=worsening endothelial function) and Δ PASP ($r=-0.55$; $p=0.10$) and a strong inverse correlation between Δ RI and Δ PVR ($r=-0.89$; $p=0.0007$).

Conclusions: Acute hypobaric hypoxia does not significantly alter large artery stiffness or cause overt LV diastolic dysfunction. However the degree of hypoxia influences both the systemic endothelial and pulmonary vascular responses. This noted association is intriguing and requires further investigation.

Introduction

Pulmonary arterial (PA) vasoconstriction is one of the key physiological responses to high altitude (HA) exposure and may be a contributory factor to the associated limitation in exercise capacity [49, 179]. The systemic vascular response to hypoxia depends on the balance between local endothelial derived vasodilatation [124] and sympathetically mediated vasoconstriction [125]. Despite hypoxia driven PA vasoconstriction and increase in PA systolic pressure (PASP) there is, in contrast, net systemic vasodilatation and a variable clinical blood pressure response to hypoxia [69, 131].

Endothelial activation and even dysfunction has been observed in both the systemic and PA circulation following hypoxia in healthy controls [130, 131] and among individuals prone to HA pulmonary oedema (HAPE) [133, 134]. The endothelium is also a principle regulator of arterial stiffness [85, 180]. There have been very few studies that have investigated the effects of hypoxia on arterial stiffness and its relationship to endothelial function under hypoxic conditions and the findings have been inconsistent [66, 69, 138]. This has clinical implications as changes in arterial stiffness influence diastolic function and left ventricular (LV) filling [52, 82, 181, 182]. Variable changes in LV diastolic function (including no change in some studies) have been reported [102, 183-185] and in a single study the degree of LV diastolic dysfunction was linked to the rise in PASP [101]. However, the influence of arterial stiffness on LV filling and diastolic function has not reported following acute hypoxia in humans.

In this study, we sought to investigate, for the first time, the effects of simulated HA, following acute hypobaric hypoxia, on simultaneous assessment of arterial stiffness and endothelial function and its inter-relationship to LV diastolic function and PASP. We hypothesized that the increase in PASP would lead to reciprocal changes in endothelial function, arterial stiffness and LV diastolic function.

Methods

This was a prospective interventional study that included ten healthy British military servicemen aged 18-35 years. Confirmation of health status was undertaken via a detailed history and clinical examination by qualified general practitioner and further secondary assessment by an aviation-specialized occupational physician. All subjects were also required to be in sinus rhythm and have a normal baseline echocardiogram study. All subjects avoided any caffeine or stimulants as well as smoking for 12 hours prior to the first baseline measurements. The study was approved by the Ministry of Defence Research and Medical Ethics Committee.

Simulated altitude

Simulated HA was undertaken using a hypobaric chamber. Barometric pressure in the hypobaric chamber was reduced at a rate of 1219 m (4,000ft) per minute until a final simulated altitude equivalent to 4800m (15748ft). All volunteers continued to breathe ambient air throughout the exposure for a total duration of 3 hours at 4800m. The temperature in the chamber was maintained at 22-24°C. The target altitude was held with constant cross-ventilation of the chamber such that the concentration of oxygen in the chamber remained at 20.9% throughout the study. After a total time of 180 minutes at 4800m, the hypobaric chamber was recompressed to ground level at 1219 m per minute. All investigators within the chamber breathed enriched oxygen via a face mask for the duration of the study. HA related symptoms were assessed using the Lake Louise Scoring System (LLS) [29].

Physiological measurements

Simultaneous resting recordings of oxygen saturations (SpO₂) and heart rate were performed using a Nellcor NP-20 pulse oximeter (Covidian, MA, USA). Blood pressure was measured

using an automated blood pressure cuff with the subject sat upright for >10 minutes at rest (Omron MX2; Omron®, Ca, USA)

Assessments of arterial stiffness and vascular tone

Assessments of large artery stiffness, using the stiffness index (SI), and vascular tone, by reflectivity index (RI) were undertaken using pulse contour analysis of the digital volume pulse (DVP) measured from the index finger of the non-dominant (Pulse Trace PCA2; CareFusion™, Basingstoke, Hants, UK [67, 85]). The DVP consists of two traveling pulse waves: the first represents an early systolic peak and relates to pressure waves transmitted along a direct path from the ventricle to the finger, where it generates a consequent measured change in blood volume and the second peak or point of inflection occurs a short time later and reflects pressure waves that have been reflected from along the aorta and larger arteries from major impedance sites in the lower body (figure 1). The reflectivity index (RI) is a measure of vascular tone in the small medium sized muscular arteries, in which the higher the RI, the higher the greater the vascular tone (figure 1) [67]. Quantification of the changes in arterial RI (Δ RI) is a validated method of assessing endothelial function, whereby a lower Δ RI equates to worsening endothelial function as a less reactive vascular system [180, 186]. The arterial SI is relates to the subjects height divided by the time difference between inflection points (the *peak-to-peak time*) (figure 1). As arterial stiffness increases (higher SI) reflected waves will appear closer to the forward wave due to the increased pulse wave velocity [180, 186]. All pulse wave assessments were undertaken with the subjects fully relaxed and seated having rested for at least ten minutes in a temperature controlled environment (22-24°C). Pulse waveform analyses were performed 30 minutes prior to and at 45, 90 and 150 minutes after exposure to simulated HA.

Echocardiographic assessment

All echocardiographic assessments were undertaken using a portable Vivid Q echocardiogram machine (GE Healthcare™, Amersham, Bucks, UK) with a 1.5-3.6 MHz S4 transducer. An initial baseline echocardiogram was performed at 30 minutes prior to and at 150 minutes of simulated HA exposure at the same time as the first and final arterial wave form analysis. PASP was calculated as the sum of the right atrial pressure (assessed by inferior vena caval size and collapse with inspiration) and the right ventricular systolic pressure (assessed using continuous wave Doppler of the tricuspid valve). Cardiac output was assessed by quantification of the LV outflow tract (LVOT) dimension, the LVOT velocity time integral and simultaneous heart rate. The aortic systolic flow velocity integral (SVI), using pulse wave doppler profile of aortic blood flow from the apical five chamber view and the cross sectional area (CSA) of the LVOT was used to calculate the cardiac output ($SV=SVI \times LVOT \text{ CSA}$). The systemic vascular resistance (SVR) was calculated as the mean arterial pressure $\times 80$ divided by the cardiac output (Johnson et al. 2001). The PA vascular resistance was estimated using the following equation $PVR = 80 \times ([10 \times TRV/VTI \text{ RVOT}] + 0.16)$ (dynes / sec / cm^{-5}) where TRV was the maximal tricuspid regurgitation velocity and VTI RVOT was the velocity time integral of the right ventricular (RV) outflow tract velocity measured using pulsed wave doppler at the level of the pulmonary valve in the parasternal short axis view [187, 188]. The pulsed wave sample volume of the conventional doppler was placed at the tips of the mitral valve leaflets. The obtained variables included peak early transmitral flow velocity (E , cm/s), deceleration time (ms) of early diastolic transmitral filling and peak flow velocity (A , cm/s) of late diastolic transmitral filling, the isovolumetric relaxation time (in ms) and the E/A ratio [68]. Tissue doppler imaging (TDI) was used to calculate the early diastolic filling (e') velocity of the basal lateral and basal septal mitral valve annulus which were measured separately (Paulus et al., 2007). TDI was also used to quantify the respective left ventricular isovolumic contraction (IVCT) and isovolumic relaxation times (IVRT). Estimation of LV

filling pressure was undertaken from the ratio of mitral valve E (early filling) velocity divided by average e' [68]).

Statistical analysis and power calculations

Data were analysed using GraphPad InStat version 3.05 and with all graphical figures presented using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com). The Kolmogorov-Smirnov test was undertaken to assess normality of all continuous data. Paired continuous data comparisons were undertaken using the paired t test for normally distributed data and the Wilcoxon matched pairs test for non-parametric data respectively. Time-dependent comparisons of ≥ 3 groups was performed with Repeated measures ANOVA for normally distributed data, with the Tukey post-test for all significant results. Repeated measures of non-parametric continuous data were performed using the Friedman test with post-test for all significant results. Correlation was assessed using Pearson and Spearman correlation coefficients for normal and non-parametric data respectively. A two tailed P value <0.05 was considered statistically significant for all comparisons.

Sample size calculations were performed using GraphPad StatMate version 2.00 for Windows (GraphPad Software). In a previous study of 8 subjects Thomson et al demonstrated a 10% fall in augmentation index following acute hypoxia (Thomson et al., 2006). In another previous study, also of 8 subjects, Blitzer et al demonstrated a significant increase in PA vascular resistance and fall in systemic vascular resistance (SVR) following acute hypoxia [124]. Hence, based on this previous data coupled with our own previously published healthy population data [85] it was calculated that a sample size of 10 subjects would have a $>80\%$ power to detect a $>8\%$ change in arterial stiffness index as well as any significant changes in pulmonary artery vascular resistance and SVR with a two-tailed significance level of 0.05.

Results

The average age of included subjects were 29.7 ± 3.0 years with 70% being male. They weighed 77.9 ± 17.5 kg, were 177.9 ± 17.5 cm tall with an average body mass index and abdominal waist circumference of 25.7 ± 3.4 kg/m² and 84.2 ± 7.6 cm respectively. Acute hypobaric hypoxia lead to an increase in heart rate from 61.0 ± 6.0 to 80.1 ± 11.8 beats/minute ($p=0.0001$) and reduction in oxygen saturations from a baseline level of 98.1 ± 0.7 to $71.8 \pm 7.1\%$ ($p=0.0002$). Compared with baseline levels there was a significant increase in LLS scores (0 vs. 3.4; range 0-10; $p=0.02$).

There was no significant rise in arterial SI ($p=0.13$; table 1). However, acute hypoxia led to a significant reduction in both arterial RI ($p=0.0008$) and the SVR (0.0004) with an associated fall in both systolic blood pressure ($p=0.006$) and mean arterial blood pressure ($p=0.032$) (table 1 and figure2). There was no association between arterial SI and estimated LV filling pressure (E/e' ; $p=0.83$). However, SI correlated with mean arterial blood pressure ($r=0.39$; $p=0.01$). There was a positive correlation between RI and SVR ($r=0.46$; $p=0.043$), systolic blood pressure ($r=0.46$; $p=0.003$), mean arterial blood pressure ($r=0.33$; $p=0.035$) and pulse pressure ($r=0.50$; $p=0.001$) but not diastolic blood pressure (NS).

Acute hypoxia increased (baseline vs. 150 minutes) PASP (24.3 ± 2.2 vs. 35.0 ± 5.3 mmHg; $p=0.0001$) and estimated PVR (116.4 ± 10.0 vs. 144.6 ± 21.4 ; $p<0.0001$). Estimated LV filling pressure (E/e') remained unchanged ($p=0.78$) (table 1). There was no change in mitral early inflow (E) velocity ($p=0.21$) or average early e' velocity ($p=0.13$) with hypoxia (table 2). There was no correlation between arterial SI, RI or PASP and LV filling pressure (E/e'). There was a significant inverse correlation between SpO_2 and PASP ($r=-0.77$; $p<0.0001$), PVR ($r=-0.57$; $p=0.008$) (figure 3) and between the degree of fall in SpO_2 and change (Δ) in RI ($r=-0.52$; $p<0.001$). There was a modest inverse correlation between Δ RI and Δ PAP ($r=-0.55$; $p=0.10$) and a strong inverse correlation between Δ RI and Δ PVR ($r=-0.89$; $p=0.0007$) (figure 4).

Discussion

This is the first study to assess the effects of acute hypoxia on simultaneous assessment of large artery stiffness and endothelial function and its inter-relationship to LV diastolic function and PASP. Our results have shown that acute hypobaric hypoxia led to systemic vasodilatation of the smaller and intermediate sized muscular arteries, demonstrated by a fall in SVR, blood pressure and RI, without a change in large artery stiffness (SI). The only observed significant changes in diastolic indices were an increase in mitral valve inflow A velocity and an associated reduction in the E/A ratio without any changes in tissue doppler derived indices of left ventricular filling or in the estimated LV filling pressure (E/e').

The moderate inverse correlation between Δ RI (marker of endothelial derived vasodilatation and function) and Δ PASP and the strong correlation between PA vascular resistance are novel findings in a healthy human acute HH study. Blitzer et al had previously shown that acute hypoxia (n=8; to oxygen saturations = 84%) led to reciprocal changes in endothelial function (using arterial haemodynamic changes) and PASP, however the authors did not directly examine their correlation [124]. Berger et al demonstrated an inverse correlation between endothelial function (flow mediated vasodilatation) and PASP among HAPE susceptible individuals (n=9) but not among healthy controls (n=9), following acute hypoxic exposure (to SpO₂ 75%) [133]. In their study arterial stiffness, SVR and LV diastolic function were not assessed. Whilst the noted fall in SpO₂ was accompanied by an increase in PASP their direct correlation was not reported (Berger et al. 2005). In the current study we observed a significant inverse correlation between arterial SpO₂ and both PASP and PVR further validating the central role of hypoxia and its severity in influencing PA vascular responses. More recently, Jayet et al demonstrated a strong reciprocal relationship between flow-mediated dilation and PASP in the offspring of mothers with preeclampsia and living at high altitude [189].

In our study changes in PASP and PVR led to a reciprocal impact on systemic vascular tone and endothelial function without overtly affecting arterial stiffness or diastolic function. Our data showed that the subjects who exhibited the lowest fall in RI, and thus a more blunted vasodilatory response to hypoxia, suggestive of worsening endothelial function, developed the greatest increase in PASP and estimated PVR resistance. Furthermore, this process was influenced by the severity of hypoxia and reducing SpO₂ and may relate to reduced availability of endothelial-derived nitric oxide with worsening hypoxia. It has been previously shown that endothelium-derived nitric oxide contributes to systemic vasodilation and serves as a regulatory mechanism to attenuate pulmonary vasoconstriction during acute hypoxia in healthy humans. [124]. The results of the current study may have further clinical applications given the increasingly recognised relationship between worsening vascular endothelial function and susceptibility to HAPE [133, 134]. However, it must be emphasized that our study did not include known HAPE susceptible subjects.

There have been an increasing number of studies that have shown that even short-term exposure to hypoxia is associated with new abnormalities in diastolic function that were not present prior to the hypoxic stimulus [102, 183-185]. However, these changes were generally very mild and noted mainly on pulsed wave mitral inflow only with a reduction in E velocity and an increase in A velocity and were not associated with an increase in estimated LV pressure or overt diastolic dysfunction. This led to the led to the concept of compensated diastolic (dys)function in which minor alterations in mitral inflow as compensated for by enhanced atrial contraction [101, 190]. This finding has been supported by the results of our present study. The mechanism for these minor changes are unknown and might relate to direct effects of hypoxia, upstream effects of pulmonary arterial vasoconstriction and possibly to changes in arterial stiffness. Increasing large artery stiffness increase afterload pressure on the LV and to hypertrophy which subsequently affects cardiac relaxation [181, 182].

The few studies that have assessed the relationship between hypoxia and large artery stiffness have reported inconsistent results. Thompson et al (n=8) noted a significant fall in arterial stiffness, using augmentation index ($-10.1 \pm 1.1\%$) and SVR without changes in blood pressure during 60 minutes of isocapnoeic normobaric hypoxia [126]. In their study the duration of hypoxia was much shorter (one hour), and the average peripheral SpO₂ during hypoxia was much higher than in our study (82.6% vs. 71.8%). In another more recent study, Vedam et al (n=12) noted that acute normobaric hypoxia (20 minutes; to an SpO₂ of 80%) initially increased augmentation index and large artery stiffness with an associated rise in mean arterial pressure [69]. However, during hypoxic recovery there was actually a fall in augmentation index to a lower than baseline value. There were no observed changes in the heart rate adjusted time to pulse wave reflection suggesting that the hypoxia most likely led to changes in the muscular small arteries rather than centrally in the aorta [69]. This is supported by our current data. In a very recently published field study (n=17) to 4770m, Rhodes et al, failed to demonstrate a consistent change in arterial SI with high altitude exposure, but did observe an initial significant fall in RI, as in our study. However, as with all of the above studies assessment of diastolic function and PAP were not performed [138].

Our data appears to suggest that hypoxia leads to differential haemodynamic effects along the arterial tree. This would partly explain the variable changes in reported blood pressure (increase, no change or even decrease) following acute hypoxia documented in previous studies [14, 49, 52, 124, 126, 131]. However, the indices of blood pressure in acute hypoxia studies have been variably reported (eg only, MAP, or only systolic blood pressure and generally not pulse pressure). Blitzer et al demonstrated a small decrease in MAP following acute hypoxic challenge in healthy controls [124]. A similar finding was observed by Johnson et al who noted a fall in MAP and an associated reduction in SVR [131]. In our study hypoxia led to a significant reduction in systolic blood pressure, mean arterial, pulse pressure and RI, reflecting the degree of systemic vasodilatation, yet increased pulmonary

vasoconstriction, with an increase in cardiac output. These physiological changes would allow for greater blood supply to systemic organs in order to meet the metabolic demands of hypoxic tissues [124]. It must be appreciated the mode (eg isopcapnoeic vs poililokapnoeic) and duration (minutes *versus* hours) of hypoxia and the sympathetic and ventilatory reponses will all influence the haemodynamic responses and in part explains the variation in the reported data [14].

This study has several limitations which should be acknowledged. The sample size of the current study was relatively small. Nevertheless, our intervention sample size was larger than many of the previously reported acute hypoxia studies [52, 124, 126] and our study was adequately powered for its principle aims. Moreover, the duration (3 hours) and severity of hypoxia was much greater than the two previously previous simulated HA arterial stiffness studies [69, 126] with the added advantage that we used hypobaric hypoxia, compared with normobaric hypoxia. The PVR and MAP readings are estimated values derived from validation studies using echocardiography and cardiac catheterization [187]. The inclusion of a control group of patients, studied in the chamber over similar time duration, but without actual hypoxic exposure, would have been preferable. However, SI and left LV filling did not significantly change with hypoxia further validating our findings. A larger more detailed study of diastolic function with acute hypoxia is needed.

Conclusions

Acute hypobaric hypoxia does not significantly alter large artery stiffness or LV diastolic function. However, the degree of hypoxia influences both the systemic endothelial and pulmonary vascular responses. This noted association is intriguing and data from further studies investigating simultaneous pulmonary and systemic vascular responses would be helpful.

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Disclosures

The authors have no conflicts of interest or financial ties to disclose.

Table 1 Effects of hypobaric hypoxia on arterial stiffness, symptom scores, and other cardiovascular indices

Variable	Baseline	45 minutes	90 minutes	150 minutes	P Value
Heart rate (minute ⁻¹)	61.0 ±6.0	71.8±7.0	78.8±12.4 ††	80.1±11.8 †††	0.0001
Systolic blood Pressure, mmHg	129.2±10.5	119.0±14.1	115.6±15.6 ††	115.4±14.4 †††	0.006
Diastolic blood pressure, mmHg	74.8 ±7.8	73.6±5.4	72.1±7.1	7.2±9.9	0.58
Mean systemic arterial blood pressure, mmHg	92.9±6.9	88.7±7.4	86.6±8.2 ††	86.5±10.4 †††	0.032
Pulse pressure, mmHg	55.4±11.5	45.4±12.0	43.5±14.7	43.4±11.3 †††	0.04
Arterial stiffness index (m/s)	6.2±0.6	6.6±0.9	6.7±0.9	6.2±0.7	0.13
Arterial reflective index , %	50.8±10.3	42.9±8.1	39.7±12.0 ††	33.0±6.5 †††	0.0008
Oxygen saturations, %	98.1±0.7	70.5 ±6.1 †	72.5±7.7 ††	71.8±7.3 †††	0.0002

†Significant difference (p <0.05) between baseline and 45 minutes; ††Significant difference baseline and 90 minutes; ††† Significant difference baseline and 150 minutes

Table 2 Arterial stiffness, vascular tone and diastolic function responses to hypobaric hypoxia

Variable	Baseline	150 minutes	P Value
Cardiac output, l/minute	4.8 ± 0.8	6.0 ± 1.3	0.0007
Stroke volume (ml)	74.7 ± 14.6	74.5 ± 16.7	0.73
Pulmonary vascular resistance dynes / sec / cm ⁻⁵	116.40 ± 19.0	144.6 ± 21.5	<0.0001
Systemic vascular resistance dynes / sec / cm ⁻⁵	1589.1 ± 191.2	1187.8 ± 248.7	0.0004
Peak Pulmonary artery systolic pressure (mmHg)	24.3±2.2	35.0 ± 5.3	0.0001
Left ventricular Isovolaemic relaxation time (ms)	40.8 ± 6.0	38.3 ± 6.9	0.31
Left ventricular isovolumic contraction times (ms)	61.7 ±	58.3 ± 6.2	0.38
Mitral E velocity, m/s	0.95 ± 0.1	0.89 ± 0.09	0.21
Mitral A velocity, m/s	0.56 ± 0.1	0.67 ± 0.1	0.02
Mitral E/A ratio	1.76 ± 0.4	1.37 ± 0.26	0.01
Mitral E deceleration time, ms	148.6 ± 20.4	152.3 ± 9.3	0.57
Average mitral e' m/s	0.17 ± 0.02	0.16 ± 0.01	0.12
Estimated left ventricular filling pressure (E/e')	5.6 ± 0.7	5.5 ± 0.6	0.78

Figure 1 Illustration of the methods used to calculate the arterial reflective index (RI) and stiffness index (SI)

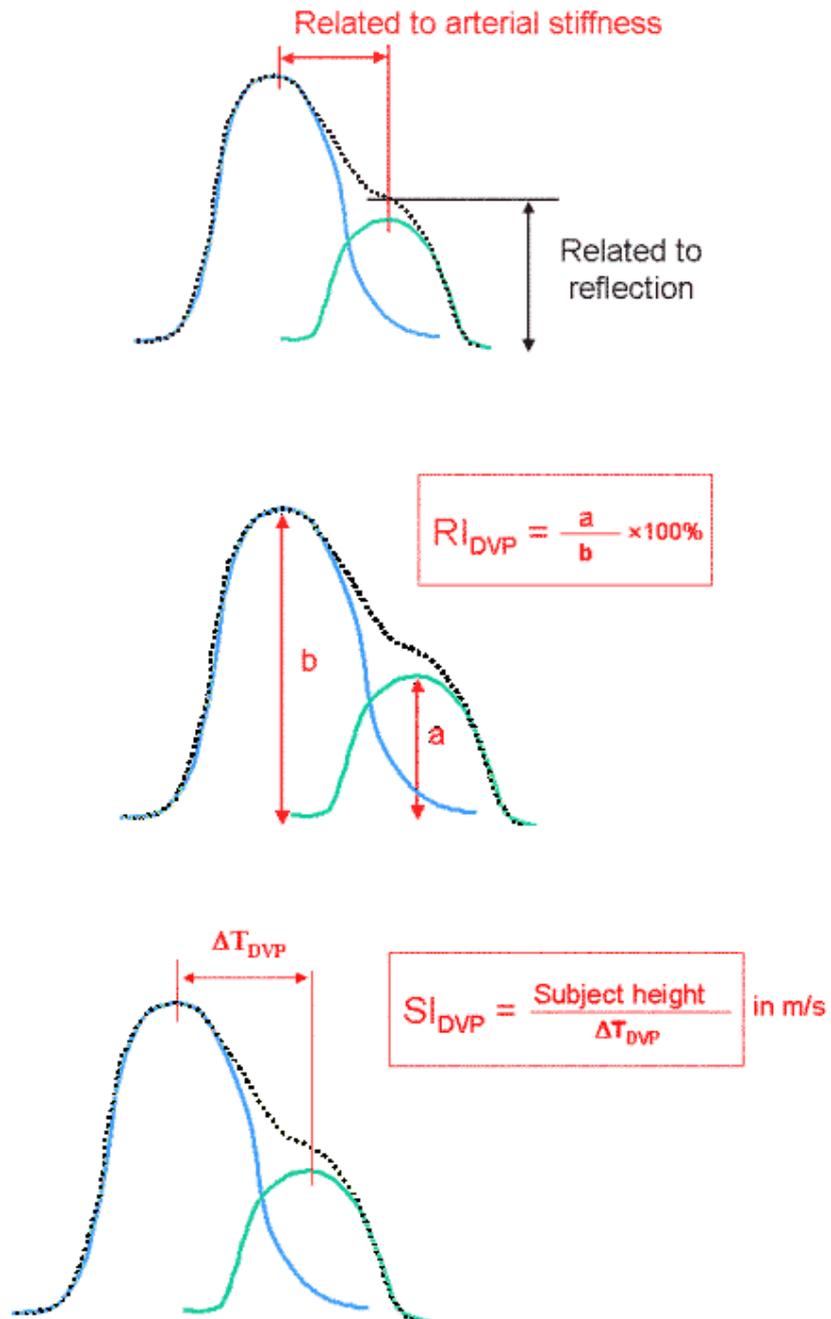


Figure 2 Changes in arterial reflective index (RI) following exposure to acute hypobaric hypoxia

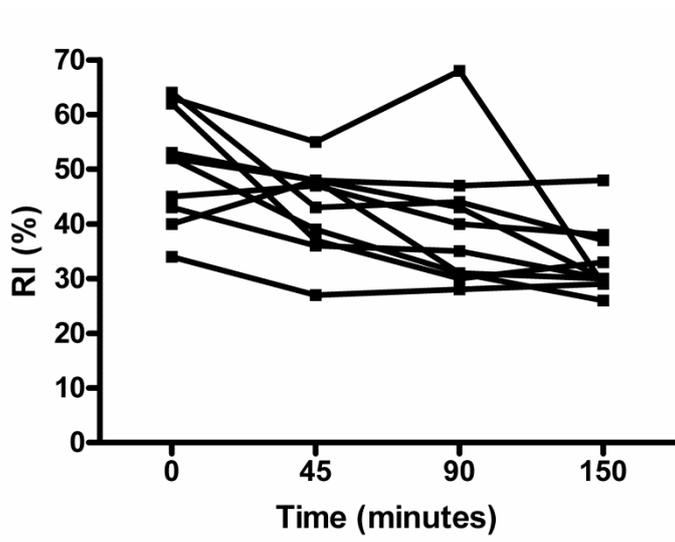


Figure 3 Relationship (correlation) between oxygen saturations and pulmonary vascular resistance index following acute hypobaric hypoxia

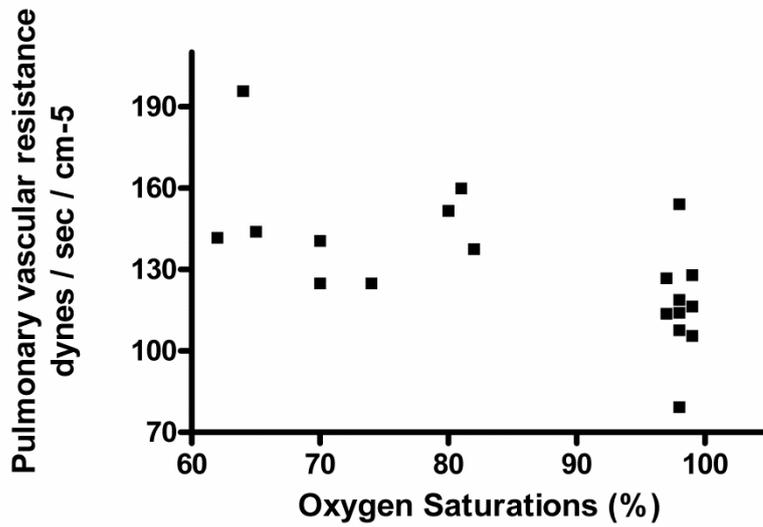
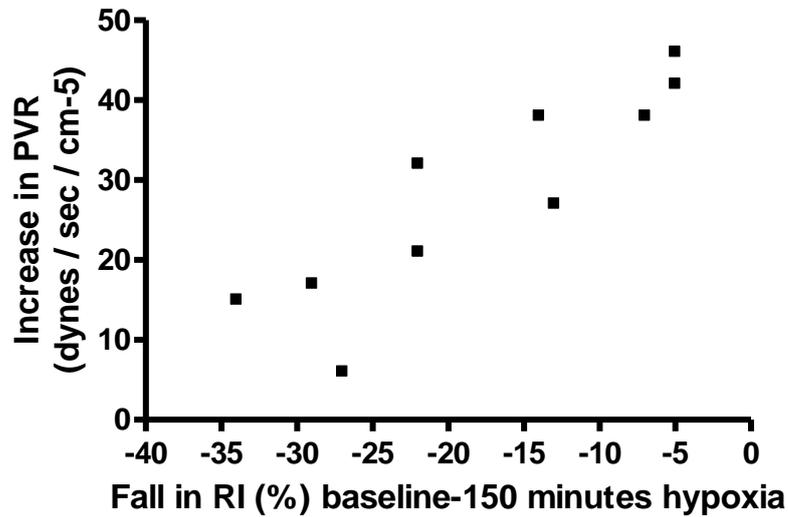


Figure 4 Relationship (correlation) between reducing fall in RI and pulmonary vascular resistance index



Chapter 4

Publication 2

The Effects of Exercise at High Altitude on High-Sensitivity Cardiac Troponin Release and Associated Biventricular Cardiac Function

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The Effects of Exercise at High Altitude on High-Sensitivity Cardiac Troponin Release and Associated Biventricular Cardiac Function

Running title: High altitude, cardiac troponin and cardiac function

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Abstract

Background: It has been consistently shown that heavy exercise leads to cardiac troponin (cTn) release and variable changes in post exercise cardiac function. This relationship has not been explored at increasing or significant high altitude (HA). This study assessed the effects of exercise at progressively increasing HA on high-sensitivity (hs)-cTnT levels and their relationship to biventricular cardiac function and severity of acute mountain sickness (AMS).

Methods: Transthoracic echocardiograms, hs-cTnT levels and AMS scores were measured at rest at 1300m then repeated post exercise and 12 hours later after progressive trekking to 3440 m, 4270m and at 5150m (after trekking to 5643m) on 19 healthy subjects (age $35.4\pm$ years; 52.6% males).

Results: There was a detectable increase ($>5\text{ng/l}$) in post-exercise hs-cTnT with exercise at HA which became significant at 5150m (5.84% at 3440m, 5.2% at 4270m and 56.3% at 5150m; $p=0.0005$). Compared with baseline, HA to 5150m led to a significant rise in post-exercise Lake Louise AMS scores ($p<0.001$) pulmonary artery systolic pressure ([PASP] 23.7 ± 3.8 vs 37.9 ± 11.7 mmHg; $p<0.001$), cardiac output (5.2 ± 1.2 vs 7.5 ± 1.3 l/minute; $p<0.001$) and a fall in SpO_2 ($96.1\pm$ vs $77.4\pm 12.0\%$; $p<0.001$). There was no change in stroke volume ($p=0.10$) or estimated filling pressures (E/E') of the left ($p=0.50$) and right ventricles ($p=0.4$). On multivariate analysis increasing cardiac output ($p=0.02$) and PASP ($p=0.04$) and decreasing SpO_2 ($p=0.01$) were the only independent predictors of increasing cTnT levels (overall $R^2=0.23$; $p<0.0001$).

Conclusions: Moderate intensity exercise at significant HA influences the post-exercise increase in hs-cTnT without overt deleterious effects on cardiac function.

Introduction

Cardiac troponin (cTn) I and T are highly specific markers of myocardial cell injury and damage with increasing levels linked to worsening prognosis among patients with myocardial infarction [110, 191]. Multiple studies have shown that heavy exercise is associated with detectable elevations in cTn levels [114, 192, 193]. This has raised concern as to whether heavy exercise may be deleterious and could lead to subclinical myocardial damage [194]. However, the increase in cTn associated with exercise, is much lower and less sustained compared with the levels observed with ischaemic myocardial injury and has been largely studied in healthy subjects [110, 114]. There is marked inter-individual variation in this exercise-related cTn release and the factors influencing their release are still not fully understood [110, 114, 192]. They include the mode of exercise (eg running > bike) [192], its intensity and duration [195], the timing of sample collection [196, 197] as well as subject-related factors such as age [198, 199] and sex [110], body mass and basic underlying fitness [114, 199, 200].

A number of investigators have coupled cTn testing with non-invasive assessment of cardiac function/injury, following exercise, but a consistent link between the two has not been shown [52, 101, 114, 199, 201, 202]. However, these studies have focused on a single exercise stimulus (eg one race) and were predominantly conducted at sea level. Exercise at high altitude (HA) places a number of additional physiological challenges over the equivalent exercise stimulus at sea level [14]. The consequent hypoxia and increase in pulmonary artery systolic pressure (PASP) reduces maximal exercise capacity, oxygen consumption and associated cardiac output [14, 49]. This has raised the question as to whether exercise at HA leads to a greater or pathological increase in cTn and associated myocardial dysfunction.

The few previous studies to have investigated the effects of exercise on cTn release at HA assessed relatively low altitudes (2500-4300m), predominantly males (>95%), a single exercise

stimulus (eg one race) and utilised 4th or older-generation cTn assays, which lack the sensitivity of hs-cTnT [120-123, 203]. Only two of these studies assessed the associated changes in cardiac function with neither using advances in tissue Doppler imaging (TDI) to more accurately define diastolic and regional systolic function [120, 121]. They did not assess the influence of HA-related symptoms, such as acute mountain sickness scores (AMS) or the independent predictors of cTn release. Our group have recently shown that brain natriuretic peptide levels (BNP) are related to the severity of AMS however, the relationship of cTn to AMS severity is unknown [40]. Consequently, this study aimed to investigate, for the first time, the effects of exercise at progressively increasing and significant HA on hs-cTnT release and its relationship to AMS scores and changes in left and right ventricular systolic and diastolic function.

Methods

Study population

This was a prospective observational study of 19 healthy British military servicemen aged 18-50 years, who had agreed to participate in Exercise Khumbu Ramble. This was trek from Lukla (2840m) to Kala Patthar (KP, 5643 m) in Nepal following assessment of baseline variables at Kathmandu (1400m/1300 m). Participants were allowed to take any medication that they needed to complete their trek. Confirmation of baseline health status was undertaken via a history, clinical examination, bloods, electrocardiogram and transthoracic echocardiogram. All subjects were also required to be in sinus rhythm and have a normal baseline echocardiogram study. The study was approved by the Ministry of Defence Research and Medical Ethics Committee and was conducted according to the standards of the declaration of Helsinki.

High Altitude Ascent profile

After 48 hours acclimatization at 1300m, the subjects flew to Lukla (2840 m) by light aircraft and trekked on to Phakding (2610m) on the same day (day 1) (figure 1). Thereafter a moderate ascent

profile was undertaken which included Namche Bazar (3440 m) on day 2 an acclimatization ascent to Khumjung (3780 m) on day 4, Deboche on day 5 (3710 m), Pheriche (4270 m) on day 6, Lobuche (4910 m) on day 8, Gorak Shep (5150 m) on day 9 and Kala Patthar (5643 m) on day 10 (returning to Gorak Shep).

Blood sampling

Baseline venous blood samples and physiological measurements were taken at 1300m. Thereafter post exercise blood samples and physiological measurements were taken within five minutes of exercise completion after trekking to Namche Bazar (3440 m; altitude gain 830m and trek distance 10.25 km; average trek time 7 hours), Pheriche (4270 m; altitude gain 570m; trek distance 9.0 km; average trek time 6 hours) and Gorap Shep (5150m after return trek to 5643m [Kala Patthar]; 493m of altitude gain; 3.8km; average trek time 3 hours). The samples were repeated at the same altitude after approximately 12 hours rest. Samples for hs-cTnT were analysed on a serum sample that had been immediately centrifuged, separated and frozen at -20 °C. On return to the UK all samples were assayed together using an electro-chemiluminescence immunoassay (ECLIA) on a Cobas e601 immunoassay analyser (Roche Diagnostics, Burgess Hill, UK on non-affected lots - Lot Number 163704) [24]. This assay has a range from 3-10000 ng/L with a lower limit of the blank of 3ng/L and a lower limit of detection (LLD) of 5ng/L. Values below the LLD were reported as 5 ng/L for statistical analysis. The upper reference limit (99th percentile) is 14ng/L. The coefficient of variation at a mean hs-cTnT level of 13.5ng/l is 5.2%.

Physiological measurements

Resting recordings of oxygen saturations (SpO₂) were performed using a Nellcor N-20P pulse oximeter (Nellcor Puritan Bennett, Coventry, UK). Baseline heart rate and blood pressure were measured using an automated blood pressure cuff with the subject sat upright for >10 minutes at

rest M6 (Omron Healthcare, Milton Keynes, UK). Fat free lean mass was calculated using Bodystat® 1500 body composition analyser (Douglas, Isle of Man, British Isles).

AMS scores

HA related symptoms were assessed using the Lake Louise Scoring System twice daily (LLS) {Hackett, 1992}. The LLS scores were recorded on arrival to a new altitude and the following morning. The LLS score allocates a score of 0–3 (symptom not present to severe) for symptoms of AMS (headache, gastrointestinal symptoms, fatigue/weakness, dizzy/light-headedness, difficulty sleeping). A total score of ≥ 3 in the presence of a headache is consistent with AMS and ≥ 6 with severe AMS [28, 40].

Echocardiographic assessment

All echocardiograms were performed using a Sonosite M-Turbo ultrasound machine (Sonosite Inc, Bothell, WA, USA) with a 1.5-3.6 MHz transducer. Pulsed-wave and two dimensional colour images were acquired in the parasternal short axis and apical four-chamber view during a short end-expiration pause. PASP was estimated from the maximum velocity of the trans-tricuspid gradient using continuous wave Doppler imaging added to a fixed value of 5 mmHg (equating to the average right atrial pressure) as previously validated [49, 204]. The pulsed-wave sample volume of the conventional Doppler was placed at the tips of the mitral and tricuspid valve leaflets in order to measure the peak early transvalvular flow velocity (E), and the peak flow velocity (A) of late diastolic filling and the E/A ratios [68]. Pulsed-wave TDI volume samples were recorded at the septal and lateral mitral annulus and over the right ventricular free wall [204]. Samples were taken in duplicate and average values were used for all TDI and pulsed-wave measurements. Estimation of Left and right filling pressure was undertaken from the ratio (E/E') of the mitral and tricuspid valve E velocity divided by TDI-derived early annular filling E' velocity at the averaged (septal and lateral lateral) mitral annulus and the annulus of the

right ventricular free wall respectively [68, 205, 206]. Stroke volume and cardiac output were calculated using the aortic systolic flow velocity integral, using pulsed-wave profile of aortic blood flow from the apical five chamber view and the cross sectional area of the Left ventricular outflow tract [64].

Statistical Methods and power calculations

Data were analysed using GraphPad InStat version 3.05 and with all graphical figures presented using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com). The Kolmogorov-Smirnov test was undertaken to assess normality of all continuous data. Paired continuous data comparisons were undertaken using the paired t test for normally distributed data and the Wilcoxon matched pairs test for non parametric data respectively. Time-dependent comparisons of ≥ 3 groups was performed using a One-way ANOVA (due to variable sample size, due to subject drop out) for normally distributed data, with the Tukey post-test for all significant results. Non-parametric continuous data analyses were performed using the Kruskal-Wallis Test with the Dunn post-test for all significant results. Correlation was assessed using Pearson and Spearman correlation coefficients for normal and non-parametric data respectively. Multivariate analysis was performed to determine the influence of several key collected data factors (including age, sex, blood pressure, resting heart rate and Lake Louise Scores) on the continuous dependent variable of cTnT levels. A cTnT level below the LLD of <5.0 ng/l was quantified as 5.0 ng/L for the purposes of analysis. A two tailed P value <0.05 was considered statistically significant for all comparisons

Sample size calculations were performed using GraphPad StatMate version 2.00 for Windows (GraphPad Software). Banfi et al also noted a significant increase in cTn-I among 4/15 persons running a mountain marathon (peak altitude ≤ 2500 m from race course) (Banfi, Lippi et al. 2010) and Davila-Roman et al [120] noted a detectable increase in cTnI in 13 out of 14 participants

undertaking strenuous exercise at high altitude (max 4300m). Hence, based on this previously published work showing cTn increases in >50% of subjects and our own dataset on healthy subjects at rest, it was calculated that a sample size of ≥ 15 subjects, at multiple altitudes would have >95% power to detect a significant increase in hs-cTnT post exercise at HA compared with baseline using a two-sided alpha of 0.05. Furthermore ≥ 15 subject completions across seven sampling time points (1300m rest, rest and exercise at 3440m, 4270m and 5150m) would allow for ≥ 105 dependent hs-cTnT variables for multivariate analysis.

Results

The average age (\pm SD) of included subjects were 35.4 (\pm) 8.3 years with a near equal balance of males (n=10, 52.6%) and females (n=9, 48.4%) (table 1). Complete data was available on 19 subjects at rest at 1300m, 17 subjects at 3440m, 19 subjects at 4270m and 16 subjects at 5100m following exercise to 5643m. This was related to symptoms of severe AMS.

Changes in hs-cTnT

There was a detectable increase in hs-cTnT in 1/17 (5.8%) immediately post exercise at 3440m with all levels returning to normal 12 hours later with one further isolated rise, which was not noted on the post exercise sample. At 4270m the corresponding figures were 1/19 (5.2%) post exercise with no new increases noted after 14 hours rest. At 5150m, following exercise to 5643m, detectable post exercise increase in hs-cTnT were noted in 9/16 subjects (56.3%) with all returning to the LLD of ≤ 5 ng/L with no new observed rises on the resting sample. Only one subject had a hs-cTnT level above the 99th centile post exercise (28.03 ng/l) which was detected on the highest post exercise sample (at 5150m after exercise to 5643m) but had returned to the LLD of 5.0 ng/l on the post exercise resting sample. There was a significant overall increase in hs-cTnT with increasing altitude (table 2, figure 2) with the difference only being detectable at post exercise at 5150m on post-hoc analysis (Chi-squared test for trend $p=0.0005$). On paired

testing of exercise compared with rest at subsequent altitudes there was no significant paired increase in hs-cTnT at 3440 m ($p=0.53$) or 4270m ($p=0.33$), however there was a significant increase at 5150m following exercise to 5643m compared with rest ($p=0.008$). There was no difference in hs-cTnT rise amongst those with and without severe AMS (figure 3) at any altitude ($p=0.20$).

Changes in cardiac function

Increasing HA led to a progressive increase in PASP which was significant after the first altitude challenge ($p<0.0001$) (table 3). Cardiac output rose from a baseline value of $5.2\pm l/\text{minute}$ to a peak elevation of $7.5\pm l/\text{minute}$ post exercise at 5150m (table 3) without a significant increase in stroke volume. There was a small but significant increase in post exercise Mitral valve A velocity and an overall decrease in the E/A ratio and a decrease in mitral E deceleration time (Table 3). Apart from a significant increase in mitral septal S' velocity there were no changes in other indices of systolic function and no change in either the estimated left ventricular (E/E' ; $p=0.5$) or right ventricular (E/E' ; $p=0.43$) filling pressures (table3).

Factors influencing hs- cTnT elevation

There was a significant inverse correlation between hs-cTnT levels and SpO_2 levels ($r=-0.27$; -0.09 to -0.43 ; $p=0.003$). Hs-cTnT levels correlated with heart rate ($r=0.30$; $0.11-0.46$; $p=0.001$), cardiac output ($r=0.29$; $0.10-0.45$; $P=0.002$), and PASP ($R=0.33$; $0.16-0.49$; $p=0.0003$). However, there were no relationship between hs-cTnT and age ($p=0.53$), sex ($p=0.30$), systolic blood pressure ($p=0.56$), diastolic blood pressure ($r=0.12$) height ($p=0.68$), LLS ($p=0.40$), smoking status ($p=0.69$) total daily total body weight ($p=0.87$), lean body mass ($p=0.97$), body mass index ($p=0.83$), stroke volume ($p=0.87$), mitral lateral E' velocity ($p=0.78$), mitral septal E' velocity ($p=0.79$) mitral E velocity ($p=0.81$), mitral A velocity ($p=0.13$), Mitral E deceleration

time (p=0.35), mitral E/A ratio (p=0.39), Mitral E/E' (p=0.28), RV E' (p=0.87), RV S' (p=0.12), RV E/E' (p=0.73), mitral lateral S' (p=0.73), septal S' (p=0.72) or other echo indices.

We included the 4 significant univariate predictors of increasing hs-cTnT as well as age and sex into a multivariate regression analysis with hs-cTnT as the dependent variable. Increasing cardiac output (p=0.02) and PASP (p=0.04) and decreasing SpO₂ (p=0.01) were the only independent predictors of increasing hs-cTnT levels (overall R²=0.23; p<0.0001).

Discussion

This is the first paper to examine the effects of exercise at progressively increasing HA exposure on hs-cTnT levels and associated cardiac function. It is also the first to assess the influence the effects of AMS and the degree of hypoxia on the elevation in hs-cTnT. Whilst exercise led to minor elevations in hs-cTnT levels, this rise only became significant after exercise to 5643m. Despite this there was no evidence of deleterious changes in either systolic or diastolic function. The only independent predictors of hs-cTnT levels were increasing cardiac output and PASP and reducing SpO₂ levels.

There have been five previous studies on healthy subjects that have investigated the effects of exercise on cTn levels [120-123, 203]. These studies have all used a single exercise stimulus (eg one race or distance), represented predominantly males (>95%) and tested essentially single HA challenge at mild/ moderate HA (≤4300m). Hence, it was not possible to distinguish increases in cTn related to sex or that of heavy exercise from the incremental effects of HA and worsening hypoxia. They observed detectable increases in post-race cTn levels in 7-100% of subjects, however pathological elevations, consistent with a diagnosis on myocardial infarction were not found [120-123, 203]. All of these studies utilised older generation cTn assays and in only two of these studies were assessment of cardiac function performed [120, 121] In the first of these

studies Davila-Roman et al, studied 14 runners (mean age 43 years), who completed a 163km ultra-marathon HA mountain race at elevations ranging from 2350 to 4300m [120]. Exercise led to significant decrease in the left ventricular end-systolic and end-diastolic areas and volumes with no change in the ejection fraction. However, five subjects developed marked right ventricular dilation and global right ventricular hypokinesia, which was corroborated by significant increases in the right ventricular end-systolic areas and end-diastolic areas, and a significant decrease in the right ventricular fractional area change which was associated with greatest increases in PASP [120]. The second study was a simulated altitude study in which comparative changes in post exercise (50km stationary bike ride) cardiac function and cTnT levels were measured following normobaric then hypobaric hypoxia to the same altitude [121]. As in our study, exercise at HA, led to an increase in cardiac output without an increase in stroke volume. No significant differences were observed in either systolic or diastolic function across but quantification of pulmonary artery pressure was not performed.

Our study is the first HA exercise study to use a high sensitivity TnT assay. Newer generation hs-cTn assays have several advantages over previous generation cTn assays used in the previously published HA exercise studies [110]. They are able to detect cTn in a larger number of healthy persons and can more precisely define what is 'normal' (within the 99th percentile) and hence allow for earlier detection of pathological myocardial injury/damage [110, 114, 207]. The pattern of hs-cTnT release observed in our study differs substantially from that observed with acute myocardial infarction where the cTn levels are much higher and usually peak by 12-14 hours later [110, 114, 190, 191, 208]. Yet in our study, despite the additional physiological stress of HA, hs-cTnT levels had largely returned to normal by 12 hours post exercise and followed a similar pattern to the majority of published post exercise cTn data [114, 191-194]. The mechanisms for the exercise-induced cTn rise are still not completely clear. Potential mechanisms include increased membrane permeability and cytosolic release, genuine myocardial necrosis in certain cases or stimulation of integrins by myocardial stretch [114].

In a recently published study, using a similar cohort, our group have recently shown that both BNP and NT-proBNP, markers of ventricular stretch, increased after exercise and also at rest and that BNP levels were significantly higher in those with severe AMS at 5150m [37]. In our current study, hs-cTnT levels bore no relationship to the LLS mountain sickness scores and there was no difference in the degree of hs-cTnT rise amongst those with and without severe AMS (Figure 2). It is interesting that the rise in hs-cTnT only became significant after exercise at the highest altitude (5150 m after trekking to 5643m). Given that the resting levels were normal would suggest that the rise may relate to the confounding effects of the HA on the exercise stimulus. This is partly supported by the results of the multivariate analysis that has shown that increasing cardiac output (suggestive of increased exercise response), reducing SpO₂ levels and increasing PASP (associated with worsening hypoxia and pulmonary vasoconstriction with increasing HA) were independent predictors of the hs-cTnT rise. Whilst the overall model was highly significant the model fit was weak ($R^2=23.3\%$). Hence, the increase in hs-cTnT has been far from fully explained. The observed increases in hs-cTnT, whilst significant post-exercise at the highest altitude, were very mild and lower than that reported in several sea level exercise studies [114]. The reason for this may relate to the fact that whilst the treks were physically challenging, subjects underwent gradual acclimatisation process, and did not race, completing the treks in their own time [14]. Three subjects did not complete the trek to the highest altitude and there were no cases of HA pulmonary oedema (HAPE), both of which may have influenced the results.

Increasing PASP in response to worsening hypoxia (ie reducing SpO₂ levels) is one of the hallmarks of increasing HA exposure with marked inter-individuals variability in responses [14, 208]. It would be reasonable to expect that increasing altitude and worsening hypoxia would have either direct or indirect effects on cardiac function akin to that noted with pulmonary hypertension in acute pulmonary embolism [190]. However, data from the current study and previously published work by our group and others have shown that acute hypoxia and HA are extremely well tolerated [45, 64, 101]. Despite significant increases in PASP, we did not observe

evidence of significant LV or RV systolic or diastolic function. There is early data to suggest that the RV E/E' may be a useful surrogate of RV end diastolic pressure but this is still controversial [205, 206]. Nevertheless, there were no observed increased in the estimated RV end diastolic pressure with increasing HA in this study. There have actually been very few HA studies that have investigated LV diastolic function using tissue Doppler imaging [45, 64, 101] and, to the authors knowledge, this study is the first to assess these changes with progressive HA making it unique.

This study has several limitations that should be acknowledged. Each exercise stage was variable in terms of altitude gain, difficulty and exercise duration, but reflected a real-life established trek to Everest Base Camp. We did not collect individual data on exercise time, medication use, heart rate (during exercise) or levels of perceived exertion, owing to the existing challenges of this type of extreme study. Consequently, it is difficult to disentangle these confounding factors upon the effects of increasing HA. However, the results of the multivariate analysis suggest that the worsening hypoxia and increasing PASP are important contributors. Finally, we are aware that this investigated recreational hard trekking rather than a race or maximal exercise (eg ultra marathon) used in the previous HA cTn studies, which makes comparisons difficult. The exercise stimulus used in our current study is, however, 'more real-life' and representative of the hundreds of thousands of persons undergoing recreational walking to HA each year.

Conclusion

We conclude that moderate intensity exercise at significant HA influences the post-exercise increase in hs-cTnT without overt deleterious effects on cardiac function.

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Author Contributions CJB wrote the manuscript, analysed the data and performed the echocardiograms; CJB, MS, AM, CS and DRW aided with data collection and study conduct; AM and DRW assisted with study design; JB and AH undertook the hs-cTnT analyses; All authors contributed to the writing of the manuscript and approved it.

Competing interests The authors have no conflicts of interest or financial ties to disclose. The Changes in BNP have been published on this cohort of patients before however all hs-cTnT and echocardiographic data are unpublished.

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Table 1 Baseline demographics

Variable	Baseline
Age, years	35.4 ± 8.3
Range	29-51
Males (%)	10 (52.6%)
Height, m	174.79 ± 7.6
Ethnicity, %	
- Caucasian	18 (94.7%)
- Afro Caribbean	1 (5.3%)
Weight, kg	77.0 ± 14.4
Lean mass, kg	62.4 ± 11.4
BMI, kg/m ²	25.0 ± 2.9
Smoking history, %	
- current	2 (10.5%)
- non smoker	17 (89.5%)
Systolic blood pressure, mmHg	130.8 ± 15.4
Diastolic blood pressure, mmHg	78.4 ± 10.3
Haemoglobin, g/dL	14.0 ± 1.5

Table 2 Effects of altitude on Physiological variables and Acute Lake Louise acute mountain sickness scores (LLS)

Variable		1300m		3440m		4270m		5150m		P value
		Rest	Post Exercise							
Heart rate, minute ⁻¹		66.8±9.0	103.0±15.7	80.3±12.1	90.1±14.8	75.1±12.9	96.7±7	78.0±8.1	<0.0001abce	
Systolic blood pressure, mmHg		130.8±15.4	128.6±18.8	123.5±16.4	133.9±10.0	130.5±18.2	127.2±14.5	141.2±17.5	0.08	
Diastolic blood pressure, mmHg		78.4±10.3	80.0±10.9	77.9±9.9	82.6±6.5	79.9±9.4	81.1±10.2	84.8±10.0	0.45	
SpO ₂ , %		96.1±1.9	87.9±4.0	91.5±4.4	85.8±4.5	86.5±4.8	77.4±12.0	75.3±7.8	<0.0001acfg	
Hs-cTnT levels, ng/L		5.0±0.0	5.2±0.9	5.1±0.9	5.0±0.3	5.0±0.0	7.9±5.7	5.0.2±0.0	<0.0001e	
Lake Louise Score		0.1±0.30	2.2±2.6	4.1±4.0	2.2±3.3	2.0±3.2	3.0±3.3	3.3±3.4	<0.0001abcdef	

Post hoc test compared with baseline; a 3440m post exercise; b 3440m rest; c 4270 post exercise, d 4270 rest; e 5150 post exercise and f 5150 at rest; g 3440Ex versus 4270

Table 3 Changes in echocardiogram indices with increasing altitude

Variable	1300m		3440m		4270m		5150m		P value
	At rest	Post exercise	Rest	Post exercise	Rest	Post exercise	Rest		
Cardiac output l/minute	5.2±1.2	6.8±1.5	5.9±1.5	6.8±1.1	5.4±1.1	7.5±1.3	6.0±1.2	<0.0001ace	
Stroke volume, ml	75.6±10.4	67.6±17.3	73.6±17.3	77.6±10.7	73.8±14.5	77.3±14.2	77.1±12.3	0.10	
PASP, mmHg	23.7±3.8	36.2±8.1	31.2±7.9	38.4±9.5	34.6±7.9	37.9±11.7	35.0±8.6	<0.0001acef	
Mitral E velocity, cm/s	93.6±11.8	91.2±26.0	85.0±18.4	97.2±24.4	87.5±16.4	100.3±24.8	92.7±23.1	0.26	
Mitral A velocity, cm/s	61.5±13.0	76.6±17.2	64.3±12.6	76.2±20.2	64.1±15.4	75.8±14.8	69.8±9.3	0.005ac	
Mitral E/A	1.6±0.4	1.2±0.4	1.4±0.3	1.3±0.3	1.4±0.4	1.4±0.3	1.3±0.3	0.04a	
Mitral deceleration time, ms	180.1±3.3	152.6±2.4	185.0±4.3	176.3±2.3	188.9±3.5	165.6±4.1	188.8±4.0	0.02	
Lateral E' velocity, cm/s	18.7±3.3	16.4±3.5	17.3±4.3	16.4±3.8	16.7±3.3	17.8±3.8	15.8±3.5	0.30	
Septal E' velocity, cm/s	14.0±2.5	13.0±3.6	12.7±2.8	13.0±2.0	13.0±2.1	14.3±2.5	13.8±2.0	0.44	
LV E/E'	5.9±1.1	6.1±1.4	5.9±1.0	6.7±1.6	5.9±0.9	6.3±1.0	6.3±1.0	0.50	
Lateral S' velocity, cm/s	12.3±2.5	13.5±2.9	12.1±2.6	11.9±2.6	12.1±2.1	12.7±2.3	11.5±2.0	0.30	
Septal S' velocity, cm/s	9.7±1.6	10.8±1.8	9.5±1.4	9.7±1.2	8.9±0.8	10.1±1.7	9.6±1.3	0.004g	
RV S' velocity, cm/s	14.6±2.8	15.4±1.9	13.6±2.5	14.7±2.4	13.5±2.3	14.4±2.9	13.7±3.0	0.30	
RV E velocity, cm/s	64.1±19.7	69.8±13.1	61.8±16.0	60.0±12.6	57.7±14.1	64.4±12.7	59.6±8.9	0.12	
RV E' velocity, cm/s	14.9±2.7	14.0±4.3	14.2±3.3	12.9±3.7	13.6±3.9	15.0±4.3	13.6±3.9	0.64	
RV E/E'	4.5±1.5	5.5±1.7	4.6±1.7	4.9±1.4	4.6±1.8	4.6±1.1	4.6±1.1	0.43	

LV, left ventricular; RV, right ventricular; Post hoc test compared with baseline; a 3440m post exercise; b 3440m rest; c4270 post exercise, d 4270 rest; e 5150 post exercise and f 5150 at rest; g 3440Ex versus 4270 rest;

Table 4 Results of multivariate Analysis of independent factors influencing cTnT levels with increasing high altitude

Variables	Coefficient	95% Confidence Interval for coefficient	P Value
Age	-0.001	-0.003 to 0.001	0.40
SpO ₂	-0.002	-0.004 to -0.0005	0.01
Heart rate	-0.0008	-0.002 to 0.0005	0.22
Male sex	-0.040	-0.09 to 0.0003	0.06
Cardiac output	0.19	0.003 to 0.03	0.02
PASP	0.002	0.0001 to 0.0.004	0.04

SpO₂, oxygen saturations; PASP, pulmonary artery systolic pressure

Figure 1 Ascent profile with study altitudes and time points highlighted (*)

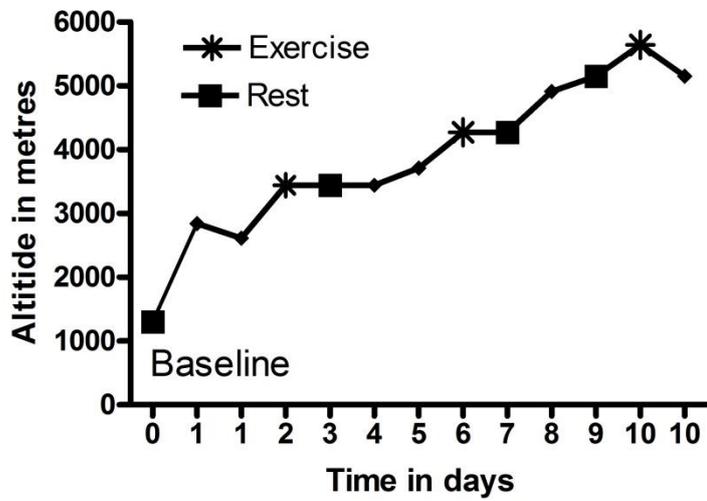


Figure 2 Changes in hs-cTnT levels (ng/l) with increasing altitude post exercise (ex) and at rest (res)

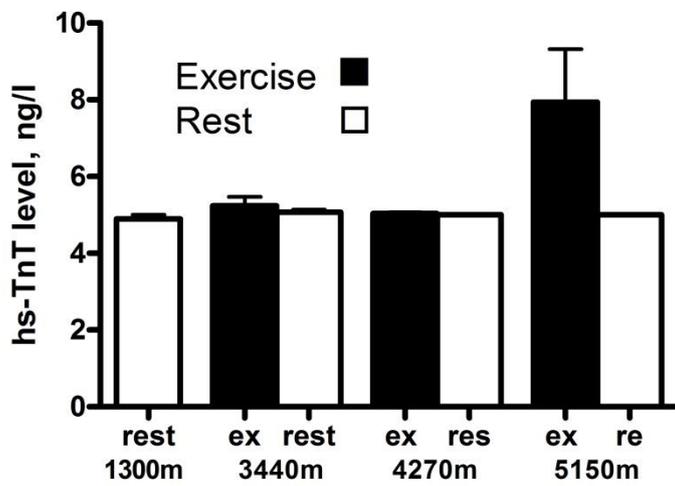
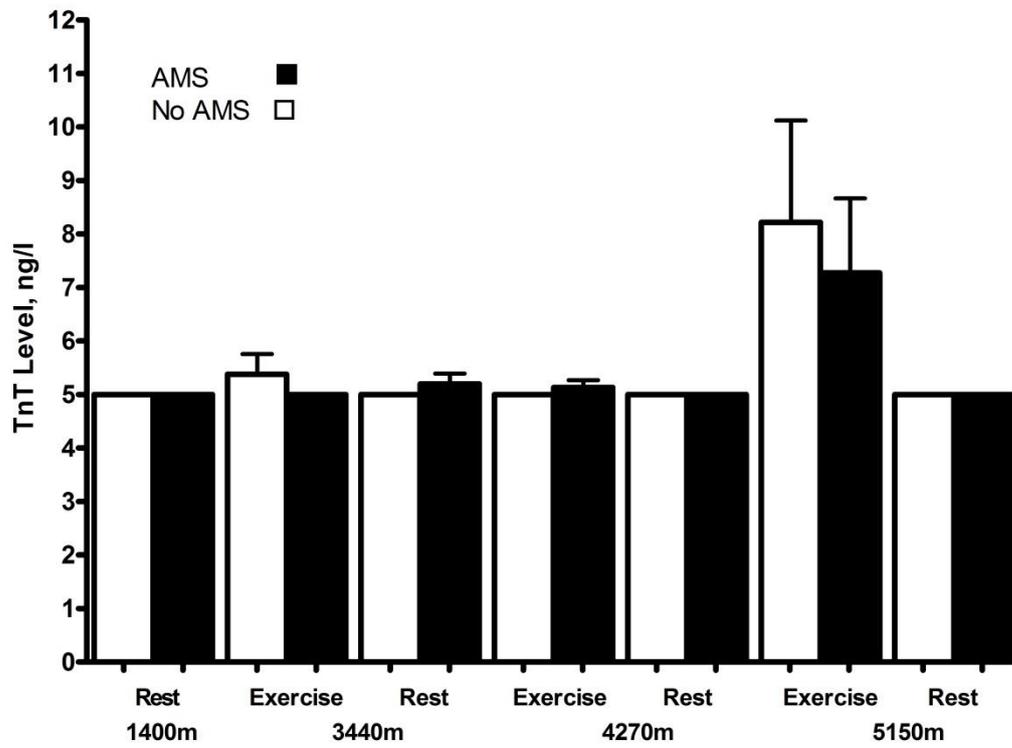


Figure 3 Hs-cTnT levels (ng/l) with increasing altitude and relationship to presence or absence of acute mountain sickness (AMS)



Chapter 5

Publication 3

A Four-Way Comparison of Cardiac Function with Normobaric Normoxia, Normobaric Hypoxia, Hypobaric Hypoxia and Genuine High Altitude

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A four-way comparison of cardiac function with normobaric normoxia, normobaric hypoxia, hypobaric hypoxia and genuine high altitude

Running title: four-way comparison of cardiac function at high altitude

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Abstract

Background

There has been considerable debate as to whether different modalities of simulated hypoxia induce similar cardiac responses.

Materials and Methods

This was a prospective observational study of 14 healthy subjects aged 22-35 years. Echocardiography was performed at rest and at 15 and 120 minutes following two hours exercise under normobaric normoxia (NN) and under similar PiO_2 following genuine high altitude (GHA) at 3375m, normobaric hypoxia (NH) and hypobaric hypoxia (HH) to simulate the equivalent hypoxic stimulus to GHA.

Results

All 14 subjects completed the experiment at GHA, 11 at NN, 12 under NH, and 6 under HH. The four groups were similar in age, sex and baseline demographics. At baseline rest right ventricular (RV) systolic pressure (RVSP, $p=0.0002$), pulmonary vascular resistance ($p=0.0002$) and acute mountain sickness (AMS) scores were higher and the SpO_2 lower ($p<0.0001$) among all three hypoxic groups (GHA, NH and HH) compared with NN. At both 15 minutes and 120 minutes post exercise, AMS scores, Cardiac output, septal S', lateral S', tricuspid S' and A' velocities and RVSP were higher and SpO_2 lower with all forms of hypoxia compared with NN. On post-test analysis, among the three hypoxia groups, SpO_2 was lower at baseline and 15 minutes post exercise with GHA ($89.3\pm 3.4\%$ and $89.3\pm 2.2\%$) and HH (89.0 ± 3.1 and 89.8 ± 5.0) compared with NH (92.9 ± 1.7 and $93.6\pm 2.5\%$). The RV Myocardial Performance (Tei) Index and RVSP were significantly higher with HH than NH at 15 and 120 minutes post exercise respectively and tricuspid A' was higher with GHA compared with NH at 15 minutes post exercise.

Conclusions

GHA, NH and HH produce similar cardiac adaptations over short duration rest despite lower SpO₂ levels with GHA and HH compared with NH. Notable differences emerge following exercise in SpO₂, RVSP and RV cardiac function.

Introduction

Hypoxic exposure has a number of important clinical applications. These include pre-acclimatization training for athletes, the investigation of high altitude (HA) illnesses such as acute mountain sickness (AMS) and clinical diseases complicated by tissue hypoxia [17, 209]. In order to improve the understanding of the clinical effects of genuine HA, hypoxia has been experimentally reproduced typically using normobaric hypoxia (NH) or hypobaric hypoxia (HH). NH lowers the partial pressure of inspired oxygen (PiO_2) by reducing the fraction of inspired oxygen (FIO_2) through addition of exogenous nitrogen (N_2) without altering the barometric pressure whereas HH lowers the PiO_2 by reduction of barometric pressure [17].

There has been considerable and ongoing debate in the medical literature as to whether these differing methods of hypoxic challenge are meaningfully different or clinically important and most importantly whether they are effective surrogate for real life HA [209-215]. A large number of the comparative studies have been in animal models and the human studies have been predominantly two-way comparisons of NH with HH exposure and have not included either a genuine 'real-world' terrestrial HA (GHA) or a normobaric normoxia control (NN) group, akin to normal sea level, limiting the clinical impact of their findings [209]. It has become increasingly appreciated that the physiological responses to GHA at a given altitude are influenced by variations in the ambient barometric pressure due to the differences in latitude, time of year and prevailing weather conditions which may be an important factor in comparative studies. Hence, it is crucial that the ambient pressure for a given field altitude is documented in to allow more reliable comparison both between GHA experiments and with HH chamber studies [127, 216]. Most of the previously published studies to compare differing hypoxia modalities have been undermined by their relatively short periods of hypoxic exposure (<30 minutes), the use of a separate but matched populations for the differing hypoxic challenge groups and the use of only brief wash out periods between each exposure increasing the risk of acclimatization bias [17]. Furthermore, the important stimulus of exercise which is a crucial factor in the majority of HA ventures in real life has been frequently overlooked.

Acute hypoxia leads to a number of recognized cardiopulmonary responses, which notably includes pulmonary vasoconstriction and an associated increase in pulmonary vascular resistance [15, 35, 64, 70, 217]. Whether HA and the associated hypoxia leads to deleterious effects on cardiac function remains still remains a controversial issue [209, 215]. Published studies have consistently shown that acute hypoxia leads to an increase in resting cardiac output and preservation of long axis systolic and radial systolic function [35, 64, 209, 218]. However, more concerning, there is a blunted stroke volume response and variable effects on left ventricular diastolic filling and right ventricular systolic function have been observed [35, 217, 218]. Right ventricular (RV) diastolic function has been barely explored [64, 209]. Evidence to suggest the potential deleterious effects of HA on cardiac performance includes the observed increase in brain natriuretic peptide (BNP) levels at HA compared with sea level and their link to AMS and its severity [37, 64, 118]. It has also been shown that sustained hypoxia can lead to a decline in cardiac energetics, which is linked to adverse changes in left ventricular diastolic function despite preservation of systolic function [219].

There have been only two studies to date that have tried to compare potential changes in cardiac function during exercise following differing modes of hypoxic challenge and in both echocardiographic assessments of biventricular performance and/or right ventricular systolic pressure were not assessed [108, 109]. A four-way comparison of NN, genuine HA (GHA), NH and HH on cardiac function has never been performed. Furthermore, none of the cross comparison studies to date utilized recent advances in echocardiography allowing much detailed assessment of biventricular systolic and diastolic function. Consequently, in this study we sought to investigate, for the first time, the effects of acute and sustained hypoxia on cardiac function at rest and following exercise under NN, NH and HH and GHA.

Materials and Methods

Study population

This was a prospective observational study of 14 healthy British military servicemen aged 22-35 years. In addition to completing a detailed health questionnaire all subjects were required to have a normal baseline ECG and echocardiogram to confirm suitability for inclusion. Baseline health status was undertaken via a history, clinical examination, bloods, electrocardiogram and transthoracic echocardiogram.

Study protocol

All participants completed a standard maximal incremental cycle test to volitional exhaustion at sea level (absolute altitude ~113m) under normobaric normoxia (NN) to determine maximal oxygen uptake and maximal workload (W_{max} [watts]) [220]. This was followed by a maximal incremental test to volitional exhaustion >24 hours later under NH (an FiO_2 equivalent to 3375m/11078ft (PiO_2 ~95 mmHg) in order to establish and ensure equivalent workloads for the hypoxic experimental trials [220].

Participants were then required to complete physiological assessments prior to and during exercise and rest under four different conditions. They were then assessed at GHA at (3375m/11078ft, 'real' altitude, barometric pressure 506.4 ± 1.7 mmHg), followed in order with assessments at NN, NH (TISS, Alton, UK and Sporting Edge, Sherfield on Loddon, UK) and HH (Centre for Aviation Medicine, RAF Henlow, Henlow, UK) ensuring a minimum washout period of >7 days between each experimental condition. This sequence ensured the PiO_2 experienced breathing ambient air during GHA ($PiO_2 = 96.3 \pm 0.4$ mmHg) could be replicated for each individual during subsequent NH and HH exposures.

For NH the FiO_2 ($13.9 \pm 0.2\%$) was manipulated to equate to each individual's PiO_2 established at terrestrial GHA using the following equation, which considers fluctuations in sea level barometric pressure [3,8]:

$$FiO_2 = PiO_2 \text{ (mmHg)} / \text{local PB (mmHg)} - PH_2O \text{ (47mmHg)}$$

For the measurements in the HH chamber participants underwent a decompression period (0.33 mmHg s⁻¹ equivalent to 5 m.s⁻¹ (ascent rate) ~10 minutes) to the target altitude, recreating each participants PiO₂, and on completion of the exposure a recompression period (0.33 mmHg s⁻¹) to the ambient pressure. The chamber was continuously flushed with medical quality gas to maintain the inspired fractions of O₂ and CO₂ at 20.9% and 0.03%, respectively, with nitrogen balance. During the HH exposure participants were continuously monitored by a chief medical officer, who was present in the chamber breathing through an O₂ diluter demand mask. They were in constant contact with the chamber operators and additional medical staff.

The GHA challenge involved rapid ascent by cable car to 3375m after the subjects were driven in a minibus from sea level to 1400m. The NN and NH experiments were undertaken within a NH chamber at Leeds Beckett University and the HH chamber at the Royal Air Force Centre of Aviation Medicine, Henlow.

Each experiment was performed following a 12-hour overnight fast. All subjects underwent 30 minutes of altitude acclimatization followed by a complete 120 minutes of cycling exercise (a progressive intensity warm-up for 15 minutes, followed by 105 minutes at 55% W_{max} based on the NH maximal exercise test). All exercising testing occurred on a bicycle affixed to a bicycle trainer (Compu Trainer Pro Lab, Racer Mate, USA). The cycle ergometer was calibrated following the manufacturer's instructions. The load generator ensured the relative workloads between conditions for each participant were accurately maintained, taking into consideration an individual's natural torque fluctuations with each pedal stroke. The manufacturer's reports an accuracy of 2.5% and repeatability of 1%. Each experimental trial involved the ingestion of a carbohydrate solution (glucose-fructose) so that the dietary intake of each participant was standardised across all four studies. Physiological measurement of

AMS scores, haemodynamics and cardiac function were assessed at rest, at 15 minutes into the rested acclimatization process in each study condition and then again rested 15 minutes and 120 minutes post two hours of cycling exercise in the hypoxic environment. A consistent temperature range of 18-23°C was maintained for all four study conditions.

Ethics

The study was approved by the Ministry of Defence Research and Medical Ethics Committee and was conducted according to the standards of the declaration of Helsinki and all subjects underwent written informed consent.

Physiological measurements

Resting recordings of oxygen saturations (SpO₂) were performed using a Nellcor N-20P pulse oximeter (Nellcor Puritan Bennett, Coventry, UK) following a 15 second continuous recording using the index finger of the right hand with the most consistent reading being used. Blood pressure were measured using an automated blood pressure cuff with the subject sat upright for >10 minutes at rest M6 (Omron Healthcare, Milton Keynes, UK) and heart rate was measured from a single lead ECG at the time of the echocardiogram. The ambient temperature was recorded for each experimental condition (NN.NH, HH and GHA) using a PCE-THB 40 Barometer (PCE Instruments UK Ltd).

Acute mountain sickness (AMS) scores

HA related symptoms were assessed using the Lake Louise Scoring System (LLS) [28]. The LLS score allocates a score of 0–3 (symptom not present to severe) for symptoms of AMS (headache, gastrointestinal symptoms, fatigue/weakness, dizzy/light-headedness, difficulty sleeping). A total score of ≥ 3 in the presence of a headache is consistent with AMS and ≥ 6 with severe AMS [28, 37].

Echocardiographic assessment

All echocardiographic assessments were undertaken using a portable Vivid I echocardiogram machine (GE Healthcare™, Amersham, Bucks, UK) with a 1.5-3.6 MHz S4 transducer. Pulsed-wave and two dimensional colour images were acquired in the parasternal short axis and apical four-chamber view during a short end-expiration pause with the subject lying in the left lateral position. RVSP was estimated from the maximum velocity of the trans-tricuspid gradient using continuous wave Doppler imaging [49, 219]. The pulsed-wave sample volume of the conventional Doppler was placed at the tips of the mitral and tricuspid valve leaflets in order to measure the peak early transvalvular flow velocity (E), and the peak flow velocity (A) of late diastolic filling and the E/A ratios [68]. Pulsed-wave TDI volume samples were recorded at the septal and lateral mitral annulus and over the RV free wall to assess early and late diastolic filling due to left ventricular relaxation (E') and atrial contraction (A') and long axis systolic function (S') [35, 64, 68, 70]. The pulmonary artery vascular resistance (PVR) was calculated using the following equation $PVR = 80 \times TRV/VTI_{RVOT}$ where TRV was the maximal tricuspid regurgitation velocity and velocity time integral of the RV outflow tract velocity measured using pulsed wave doppler at the level of the pulmonary valve in the parasternal short axis view as previously described [64].

Pulsed-wave TDI was used to quantify the respective left and right ventricular isovolumic contraction (ICT) and isovolumic relaxation times (IRT) and the isovolumic contractile velocities (ICV) [3]. Right and left ventricular myocardial performance (Tei) indices (IRT+ICT/ejection time) were performed using TDI [64, 73]. Tricuspid annular plane systolic excursion (TAPSE) was recorded using M-Mode as previously described [221]. The isovolumic contractile velocity was measured at the tricuspid and mitral annulus using PWTDI [49, 68]. Stroke volume and cardiac output were calculated using the aortic systolic flow velocity integral, using pulsed-wave profile of aortic blood flow from the apical five chamber view and the cross sectional area of the Left ventricular outflow tract [35, 70].

Statistical Methods and power calculations

Data were analysed using SPSS[®] statistics version 22. The Kolmogorov-Smirnov test and inspection of the data was undertaken to assess normality of all continuous data. All data are presented as mean \pm standard deviations. Between group comparisons of categorical data for three or more groups were compared using the Fisher's Exact Test. Continuous data across the four experimental altitude groups (NN, HA, NH and HH) were assessed using Ordinary ANOVA with Bonferroni post-test for parametric data and with Kruskal-Wallis and Dunn Post-test for non-parametric data when the P value was <0.05 . Time dependent changes (rest, 15 and 120 minutes post exercise) of continuous data within each group were assessed using Repeated measures ANOVA with Bonferroni post-test for parametric data and using Friedman Test with Dunn Post-test for non- parametric data. Correlation was assessed using Spearman Rank correlation with the 95% confidence interval of R. Further exploratory analyses of the three hypoxia groups only were undertaken using a Two-Way split level 3x3 Repeated Measures ANOVA. The within-subjects main effect of time (before and 15 and 120 min after exercise) and the between-subject main effects mode of hypoxia (GHA, NH and HH) with Bonferroni post-tests and their interactions (and effect size, Eta [n^2]) were assessed. A two tailed P value <0.05 was considered statistically significant for all comparisons.

Sample size calculations were based on previous studies. In 11 out of the 13 prior comparative experimental hypoxia studies the sample size has been between 7-12 subjects [209]. In another very recent comparative study of six subjects Beidleman et al observed that cycling time trial performance was impaired to a greater degree in HH *versus* NH at the same ambient PO₂ equivalent to 4,300 m despite similar cardiorespiratory responses [109]. Hence, based on this previously published work it was calculated that a sample size of ≥ 12 subjects would be sufficient to detect a significant difference in cardiac performance and allow for a minimum group sample size of 6 subjects in the event of any drop outs given the intense and prolonged nature of these four group comparisons.

Results

Fourteen subjects completed the genuine HA phase, 11 the sea level study, 12 with NH and 6 under HH. All subjects completed the exercise task in each group. Non-completion was mainly due to inter-current illness and in the case of HH failure to clear their ears or voluntary withdrawal. There were no significant differences in any of the baseline demographics across the four groups with similar ages, sex, height, and body weight and body mass indices (Table 1).

Physiological and haemodynamic indices

The mean peak oxygen consumption (VO_2) at baseline with NN was 46.3 ± 5.7 ml/kg/minute. There was no difference in the mean peak VO_2 among the subjects who were subsequently included in the GHA, NH and HH groups respectively (46.7 ± 5.7 , 46.2 ± 5.2 and 47.2 ± 4.7 ml/kg/minute; $P=0.98$). As expected peak VO_2 was significantly lower under NH *versus* NN ($38.9 \pm$ vs 46.3 ± 5.7 ml/kg/minute; $P=0.007$).

The ambient temperature was marginally but significantly higher with HH *versus* NH and HH (table 1). There was a significant reduction in SpO_2 across all three hypoxia environments (GHA, NH and HH) compared with NN ($P<0.001$), which was sustained at all three time points (rest, 15 minutes and 120 minutes post exercise) (Table 2). On Post-test analysis SpO_2 was lower at baseline and at 15 minutes post exercise with GHA ($89.3 \pm 3.4\%$ and $89.3 \pm 2.2\%$) and HH (89.0 ± 3.1 and 89.8 ± 5.0) compared with NH (92.9 ± 1.7 and $93.6 \pm 2.5\%$) (Table 2). AMS scores were higher at all time points in the three hypoxic conditions *versus* NN with no between group differences among the hypoxia groups. Absolute resting heart rates were higher across all three hypoxic group time points compared with NN (Table 2). At 120 minutes post exercise, resting heart rates were significantly greater with GHA than NH.

Echo parameters of left ventricular function

There were no differences in any of the echo parameters of left ventricular systolic or diastolic function at rest across the four groups. However, at 15 minutes post exercise Cardiac output

($p=0.01$), septal S' ($p=0.02$) and lateral S' ($p=0.03$) and septal A' ($p=0.003$) velocities were higher with all forms of hypoxia compared with NN with no differences between the hypoxic groups (Table 3). At 120 minutes post exercise cardiac output ($p=0.006$), septal S' ($p=0.04$), mitral A ($p=0.009$) and lateral S' ($p=0.02$) velocities were higher with acute hypoxia *versus* NN, with no intergroup differences among the three hypoxia groups (table 3). Exercise led to an increase in the post exercise left ventricular myocardial performance (Tei) index across all four groups, which was significant at 15 minutes post exercise in the NN, NH and HH groups and only in the HH at 120 minutes post exercise compared with baseline rest (Table 3). Compared with baseline, stroke volume fell at 15 minutes post exercise under all four experimental conditions before increasing again in all except the HH group where the fall in stroke volume was sustained.

Echo parameters of right ventricular function

Resting RVSP ($p=0.0002$), PVR ($p=0.0002$) and tricuspid A velocities ($p=0.01$) were higher with all forms of hypoxia compared with NN with no between group differences among the three hypoxia groups (Table 4). Compared with NN at 15 minutes post exercise RVSP ($p=0.04$), Tricuspid S' ($p=0.009$) and A' ($p=0.0001$) velocities and the right ventricular Tei Index (0.001) were greater with hypoxia. The tricuspid A' velocity was significantly higher with GHA and the Tei index and RVSP with HH on post-test analysis. At 120 minutes post exercise the RVSP ($p=0.0006$), Tricuspid A ($p=0.006$), tricuspid S' ($p=0.03$) and A' ($p=0.0007$) velocities and the RV Tei index ($p=0.004$) were higher with hypoxia than NN (Figure2). The rise in tricuspid A' velocity, RV Tei index and RVSP were greatest among the GHA and HH groups respectively on post-test analysis (Table 4). There was a trend for higher PVR with HH than with either NH or GHA at all three sampling time points ($p<0.05$ for trend). There was a significant correlation between pulmonary vascular resistance and the RV Tei index ($r=0.54$; 95% CI 0.09-0.81).

Two-Way Repeated Measures ANOVA comparing the three hypoxia groups

The main effects of hypoxia and time and the potential interactions (mode of hypoxia x time) and post tests are shown in table 5 for the three hypoxia groups with NN as a reference. There was a significant main-effect for mode of hypoxia on SpO₂, Tricuspid A, A' and ICV as well as the RV Tei index (table 5). There was a significant main effect for time on heart rate, mitral E and A, septal E' and A', lateral S', E', A' and ICV, left ventricular Tei index and stroke volume, tricuspid E, A' and ICV and RV Tei Index. Within group comparisons of the three hypoxic groups revealed no significant interactions between exercise and time for the majority of echo parameters. However there was a significant mode of hypoxia x time interaction effect for the lateral S' (F [2, 56]=3.99; p=0.006; $n^2=0.22$), tricuspid S' (F [2, 58]=3.12; p=0.02; $n^2=0.16$) and the right ventricular Tei index (F [2,58]=4.1; p=0.006; $n^2=0.23$) (figures 1-3, Table 5). The marginal means were consistently higher at 15 minutes post exercise with GHA and HH than NH (figure1-3).

Discussion

This is the first study to assess the comparative changes in physiological and cardiac responses to exercise under four differing altitude conditions of NN, GHA, NH and HH. The key findings were that whilst all three hypoxic environments (GHA, NH and HH) led to similar cardiac adaptations at rest notable differences emerged following exercise. Compared with NH, the RVSP and RV Tei indices were higher with HH and the tricuspid A' was higher with GHA. The degree of hypoxemia was greater with GHA and HH than with NH at both rest and at 15 minutes post exercise. There were no significant interactions between experimental altitude and time with the exception of the RV performance (Tei index) and the RV (tricuspid) and lateral S' velocities.

There has been considerable debate in the literature as to whether differing modalities of hypoxia challenge are synonymous [52, 209-214, 222]. This is an important issue that remains unresolved and has enormous implications for HA research where inferences are often made from sea level chamber or hypoxia studies about potential responses at genuine HA. There have been only two studies, to date, that have assessed the effects of differing hypoxic environments on cardiac

function. Miyagawa et al investigated seven young men who cycled for 40 minutes at 50% peak aerobic power in NN, NH and HH equivalent to 3200m in an artificial climate chamber [108]. Hence, this was a smaller sample size but similar altitude to our current study. Cardiac output and stroke volume were the only specific cardiac functional assessments performed (beyond heart rate) and this was undertaken using pulse dye densitometry using Indocyanine Green [108]. However, both the reliability and reproducibility of this method has been challenged and its comparison to more invasive methods of cardiac output determination has yielded conflicting results [223, 224]. In their study, Miyagawa et al did not find any significant effects of experimental condition (trial) on cardiac output or stroke volume but did note there was a significant interactive effect of [trial \times time] both cardiac output and stroke volume, during exercise, (suggesting that their responses to exercise were significantly different between the experimental conditions [108]. This related to the finding of a marked increase in cardiac output and stroke volume in both hypoxic groups *versus* the NN rather than any observed differences between the two hypoxic groups. Hence, as well as looking at a four-way comparison of the NN, GHA, NH and HH we undertook an additional exploratory analyses of the effects of hypoxia duration (time) and mode and their potential interactions across the three hypoxia groups. In another previous study two separate groups of six subjects were compared following approximately two hours of resting exposure to NH and HH at the equivalent of 4400m [109]. The only cardiac functional assessment performed was cardiac output, which was measured non-invasively using finger pulse waveform analysis [109]. No between group differences in cardiac output were observed however cycling time trial performance was worse with HH than NH.

In our study we assessed both right and left ventricular systolic and diastolic performance as well as markers of pulmonary artery haemodynamics (RVSP and the PVR) and global biventricular function using the Tei Index which adds significant novelty. Moreover, we ensured that the same exercise work and duration was maintained between the groups to reduce the confounding factor of differing exercise burden on any observed results.

One of the most pertinent findings of our study is the observation that it is not only the hypoxic environment but also exercise in this environment, which influences the cardiac and pulmonary vascular responses. Hence, 'resting' comparisons do not adequately reflect the reality of exposure to HA where there is usually an exercise component. This fact is supported in this study by the interaction between experimental conditions and exercise time for the Tei index and lateral left ventricular and right ventricular (tricuspid) S' velocities (figure1-3). Whilst no significant between hypoxic group differences were observed over the short duration at rest, several differences emerged following exercise. For example, the RV Tei index and S' velocities and the lateral left ventricular S' velocities were consistently higher at 15 minutes post exercise with GHA and HH than NH. The higher values of RVSP, right ventricular Tei index and PVR and lower SpO₂ post exercise with HH compared with NH are particularly notable in this study. In a very recent systemic review of crossover trials of HH *versus* NH, Coppel et al (2015) noted that peripheral SpO₂ levels were higher with NH in two out of three short studies involving a hypoxic duration of <30 minutes with no notable differences in studies of ≥ 8 hours [209]. The authors also noted that arterial blood saturations (SaO₂) were lower with HH in all three of the previously published short-term hypoxia-duration studies [209]. Our data is consistent with this limited published literature as we observed significantly lower SpO₂ with HH than with NH. The suggested potential mechanisms to explain these observed differences, include lower minute ventilation, greater intravascular bubble formation and ventilation/perfusion mismatch, increased alveolar dead space as well as differences in alveolar fluid permeability and chemosensitivity with HH *versus* NH [209, 225].

Another factor, which must be considered, is the effect of ambient temperature on SpO₂ readings with finger pulse oximetry. It has been shown that a significant reduction in ambient temperature leads to peripheral vasoconstriction and can lead to a small (≤1.4%) increase in SpO₂ which is thought to be explained by temperature-dependent arteriovenous shunts in the periphery [226, 227]. Variations in core temperature can also affect the SaO₂ (and hence SpO₂) by rightward shift of the HbO₂ dissociation curve [228]. In our current study, we tried maintain a similar exercise intensity and ambient temperature across all four experimental conditions. However, the ambient

temperature was marginally, albeit significantly higher with HH (+2-3°C) versus NH and GHA and unfortunately, we did not record core temperature. Nevertheless, previously published studies have not identified any significant differences in core temperature and thermoregulation between NH and HH [209]. In either case, we would not expect this small variation in ambient temperature across the differing experimental conditions to lead to meaningful differences in core temperature and SaO₂. Nevertheless, the small temperature differences between experimental conditions are still a limitation that should be acknowledged. The fact that SpO₂ was lower and PVR and RVSP were higher with GHA and HH than for NH strengthens our findings, given the well-established reciprocal relationship between SpO₂ and RVSP/PVR, due to hypoxia driven pulmonary arterial vasoconstriction [14, 28, 64].

The observation of a greater increase in the RV Tei index with HH *versus* NH is an interesting and novel finding. The myocardial (Tei) index is a marker of global myocardial performance, which includes both systolic and diastolic functional parameters in its assessment and is independent of heart rate and ventricular geometry [73]. It may be a more sensitive marker of myocardial function than many traditional indices of cardiac function with increasing and higher values (>0.40-0.45) indicating worsening cardiac performance [73, 221]. The RV Tei index has been shown to positively correlate with both mean pulmonary artery pressure and PVR in patients with pulmonary artery hypertension and is highly susceptible to the effects of treatment [103, 229]. The baseline (0.29-0.32) and post exercise increase in the RV Tei Index (up to 0.41) in our study is consistent with the published literature [103, 229, 230]. Huez et al previously noted that the RV Tei Index increased by approximately 50% (versus up to 33% in our study) following acute but more prolonged exposure to a higher altitude of 3750 among 15 healthy Caucasian adults [231]. More recently Page et al noted that the RV Tei Index increased significantly at genuine field HA (0.32±0.08 at 30 m to 0.43±0.15 at 3450m and 0.41±0.10 at 4730 m; P = 0.046) and was associated with subclinical pulmonary oedema in 13 out of the 14 subjects [104]. This increase in the Tei Index was nearly identical to that in our study that is also in keeping with that noted among patients with treatment responsive pulmonary hypertension [68, 73]. We noted an interaction between exercise and HA environment on the RV Tei (figure3). It has been previously demonstrated that heavy exercise under hypoxia leads to increased

capillary disruption and fluid leak, which may be one of the mechanisms in the development of high altitude pulmonary oedema [232]. We observed that the PVR positively correlated with the RV Tei index and interestingly the HH group had both the highest Tei index and increase in PVR and PASP strengthening our findings. Our data suggests that increased PVR may have a negative effect on RV function, perhaps by increasing RV afterload due to increased PASP. The RV Tei Index appears to be particularly sensitive to even short-term changes in the hypoxic environment supporting previous work [103, 229].

The interaction between time and experimental conditions on the left and right ventricular S' velocity appeared to relate to a more sustained increase in long axis function with GHA in relation to NN and NH where the changes were less marked (figure1 and 2). We also observed marked differences in the right ventricular systolic (S' and ICV), diastolic (E and A') and global function (Tei) depending on the hypoxic environment.

This study has a number of limitations that need acknowledgement. The sample size in the HH was smaller than among the other three groups, which could lead to selection bias and reduced power to detect a difference that was not appreciated. However, the demographics on the smaller HH group were similar to the other three groups and this sample size is at least as large as several previous comparative studies. Despite allowing for a reasonable 'washout' period between studies, it is uncertain whether the order of the studies could have influenced symptom scores and cardiac performance over time due to changes in physical fitness and experience of HA exposure. Furthermore, the effects of hyobarbia was not independently assessed in this study through a hypobaric normoxia condition, as this was not feasible, and as such, the findings of this study should be reviewed in the context in which they have been presented.

In conclusion, HH, NH and HH produce similar cardiac adaptations at rest. However, notable differences emerge following exercise in the degree of hypoxemia, RVSP, RV systolic, diastolic and global function. This was most marked with HH and GH *versus* NH where the post exercise RV Tei and S' velocity respectively were greater. The type of hypoxic environment and exercise

performed in this environment significantly influence the cardiac response. Observed changes in cardiac function with NH are not necessarily predictive of similar changes with genuine HA or HH and vice versa.

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Competing interests

The authors have no conflicts of interest or financial ties to disclose.

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Table 1 Baseline demographics

Variable	NN	GHA	NH	HH	P value
Number	11	14	12	6	
Age, years	26.4 ± 4.0	25.9 ± 3.8	26.1 ± 4.1	26.3 ± 3.8	0.96
Range	22-35	21-35	21-35	22-33	
Males (%)	7 (64%)	8 (57%)	8 (67%)	4 (67%)	0.99
Height, m	175.4 ± 9.7	174.4 ± 9.6	175.5 ± 10.0	178.3 ± 9.7	0.88
Weight, kg	72.5 ± 8.7	71.5 ± 9.9	72.9 ± 10.1	72.8 ± 11.4	0.99
BMI, kg/m ²	23.5 ± 1.9	23.4 ± 1.90	23.6 ± 2.0	22.8 ± 1.8	0.90
Current smokers n,%	6 (54.5%)	7 (50%)	5 (35.7%)	2 (33.3%)	0.89
Blood haemoglobin g/dL	14.5 ± 1.6	14.5 ± 1.7	14.7 ± 1.5	15.2 ± 1.4	0.80
Temperature, °C	20.8±1.7	19.1±1.4	19.3±0.5	22.1±2.0	0.008a,b

NN, normobaric hypoxia; GHA, genuine high altitude; NH, normobaric hypoxia; HH, hypobaric hypoxia

Post-test differences: a, NH vs HH; b, GHA vs HH;

Table 2 Changes in acute mountain sickness (AMS) and haemodynamic variables

Variable	NN	GHA	NH	HH	P Value
Lake Louise Scores					
-Rest	0	1 ± 1.0	1.4 ± 2.3	0.7 ± 1.2	0.02 ^{ab}
-15 minutes post exercise	0	2.7 ± 3.0	2.6 ± 2.5	2.7 ± 3.4	0.004 ^{ab}
-2 hours post exercise	0	2.3 ± 2.2	2.1 ± 2.1	2.7 ± 3.0	0.004 ^{ab}
Heart Rate , minute ⁻¹					
-Rest	60.1 ± 9.9	72.1 ± 9.0	65.7 ± 10.6	65.7 ± 7.5	0.02 ^a
-15 minutes post exercise	79.9 ± 11.1*	89.7 ± 8.6*	86.6 ± 9.9*	88.7 ± 8.7*	0.09
-2 hours post exercise	67.5 ± 9.4**	89.4 ± 10.0**	76.6 ± 15.3**	80.2 ± 11.6**	0.0005 ^{a,d}
Oxygen Saturations, %					
-Rest	98.6 ± 1.4	89.3 ± 3.4	92.9 ± 1.7	89.0 ± 3.1	<0.001 ^{abcdf}
-15 minutes post exercise	98.4 ± 1.4	89.3 ± 2.2	93.6 ± 2.5	89.8 ± 5.0	<0.01 ^{abcdf}
-2 hours post exercise	98.9 ± 1.5	91.6 ± 3.1**	92.8 ± 4.3	91.8 ± 5.2	<0.01 ^{abc}

NN, normobaric normoxia; GHA, genuine high altitude; NH, normobaric hypoxia; HH, hypobaric hypoxia; LLS, Lake Louise Scores t; AMS-C, Acute Mountain Sickness Scores

Post-test differences: a, NN vs GHA; b, NN vs NH; c, NN vs HH; d, GHA vs NH; e, GHA vs HH; f, NH vs HH

Table 3 Changes in Echo derived markers of Left ventricular function

Variable	NN	GHA	NH	HH	P Value
Stroke volume, ml					
-Rest	71.5 ± 11.9	74.1 ± 11.7	73.6 ± 13.6	74.7 ± 8.4	0.85
-15 minutes post exercise	60.6 ± 8.2 *	62.7 ± 8.5*	66.5 ± 11.5 *	69.8 ± 8.5	0.16
-2 hours post exercise	66.1 ± 10.2	66.6 ± 9.3**	70.9 ± 12.6	69.7 ± 9.5	0.58
Cardiac output, L/minute					
-Rest	4.3 ± 0.8	5.3 ± 1.1	4.9 ± 1.3	4.9 ± 1.0	0.24
-15 minutes post exercise	4.8 ± 0.8	5.6 ± 1.0	5.9 ± 1.1*	6.2 ± 0.9*	0.01 ^{b,c}
-2 hours post exercise	4.4 ± 0.5	5.9 ± 1.1	5.4 ± 1.2	5.6 ± 0.9	0.006 ^a
-Mitral E velocity, cm/s					
-Rest	88.6 ± 9.6	90.1 ± 12.5	92.5 ± 18.2	95.2 ± 11.7	0.78
-15 minutes post exercise	72.0 ± 16.2*	81.8 ± 13.2	77.4 ± 15.2*	84.5 ± 18.5	0.19
-2 hours post exercise	76.5 ± 15.0	85.4 ± 14.0	79.8 ± 13.3**	76.7 ± 17.6 **	0.48
Mitral A velocity, cm/s					
-Rest	48.5 ± 14.4	51.8 ± 9.2	55.2 ± 11.0	58.2 ± 11.4	0.39
-15 minutes post exercise	56.7 ± 9.5	63.3 ± 17.3	68.5 ± 12.1*	64.3 ± 11.0	0.23
-2 hours post exercise	46.6 ± 12.4	59.9 ± 14.8	67.8 ± 16.7 **	62.0 ± 10.1	0.009 ^b
Mitral E/A ratio					
-Rest	1.71 ± 0.27	1.79 ± 0.42	1.71 ± 0.37	1.67 ± 0.26	0.87
-15 minutes post exercise	1.08 ± 0.25*	1.39 ± 0.45*	1.15 ± 0.26*	1.35 ± 0.36	0.13
-2 hours post exercise	1.25 ± 0.42**	1.53 ± 0.55	1.26 ± 0.42 **	1.27±0.24*	0.32
Septal S' velocity, cm/s					
-Rest	8.9 ± 1.4	10.2 ± 1.4	9.8 ± 1.6	9.0 ± 0.9	0.33
-15 minutes post exercise	8.3 ± 1.2	10.1± 1.7	9.4 ± 1.2	9.8 ± 0.8	0.02 ^a
-2 hours post exercise	8.9 ± 1.4	10.9 ± 1.7	9.8 ± 1.2	9.7 ± 1.2	0.04 ^a
Septal E' velocity, cm/s					
-Rest	13.0 ± 1.6	12.9 ± 1.9	13.3 ± 3.3	13.2 ± 1.5	0.97
-15 minutes post exercise	10.8 ± 1.5*	12.9 ± 1.9	12.7 ± 3.0	12.7 ± 1.5	0.11
-2 hours post exercise	11.6 ± 1.6**	12.7 ± 2.5	12.0 ± 2.0	11.0 ± 1.8**	0.36
Septal A' velocity, cm/s					
-Rest	7.9 ± 2.3	8.6 ± 2.2	9.6 ± 2.2	8.3 ± 2.0	0.09
-15 minutes post exercise	8.2 ± 1.2	11.2 ± 3.4*	11.1 ± 2.2*	10.8 ± 2.3*	0.003 ^{abc}
-2 hours post exercise	8.5 ± 1.8	10.7 ± 3.1**	10.9 ± 2.6	10.0 ± 1.1	0.11
Septal ICV, cm/s					
-Rest	6.5 ± 1.8	7.1 ± 1.6	7.0 ± 2.1	6.8 ± 1.3	0.80
-15 minutes post exercise	7.1 ± 2.1	8.4 ± 2.9	7.2 ± 2.2	6.8 ± 2.4	0.55
-2 hours post exercise	7.2 ± 1.9	8.8 ± 3.2	7.3 ± 2.1	7.7 ± 2.2	0.46
Lateral S' velocity, cm/s					
-Rest	11.1 ± 2.2	12.1 ± 2.5	12.6 ± 2.4	10.7 ± 1.7	0.06
-15 minutes post exercise	10.9 ± 1.4	13.8 ± 2.8	12.1 ± 2.4	13.5 ± 2.2*	0.03 ^a

-2 hours post exercise	12.1 ± 2.3	15.1 ± 2.9**	12.9 ± 2.3	12.8 ± 1.6**	0.02 ^a
Lateral E' velocity cm/s					
-Rest	18.3 ± 3.1	18.6 ± 2.1	20.8 ± 2.8	17.8 ± 2.8	0.90
-15 minutes post exercise	16.5 ± 2.8	17.9 ± 3.2	17.2 ± 2.5*	15.5 ± 1.6	0.52
-2 hours post exercise	17.4 ± 2.7	18.0 ± 3.8	18.1 ± 3.2**	16.0 ± 2.3	0.57
Lateral A' velocity cm/s					
-Rest	7.9 ± 2.5	9.4 ± 2.8	9.0 ± 2.6	7.7 ± 2.2	0.30
-15 minutes post exercise	8.4 ± 2.3	10.6 ± 2.4	10.1 ± 2.5	10.5 ± 2.2	0.13
-2 hours post exercise	8.9 ± 2.2	10.7 ± 3.5	9.9 ± 3.2	9.8 ± 2.9	0.54
Lateral ICV, cm/s					
-Rest	8.1 ± 2.0	9.1 ± 2.8	7.6 ± 2.0	7.7 ± 1.6	0.34
-15 minutes post exercise	8.6 ± 1.8	9.6 ± 3.0	9.2 ± 2.5	10.7 ± 3.1*	0.52
-2 hours post exercise	9.2 ± 1.8	8.9 ± 3.6	9.5 ± 2.5**	8.8 ± 1.8	0.94
Left ventricular Tei Index					
-Rest	0.28 ± 0.04	0.30 ± 0.03	0.29 ± 0.04	0.29 ± 0.03	0.59
-15 minutes post exercise	0.33 ± 0.04*	0.32 ± 0.04	0.33 ± 0.03*	0.37 ± 0.06*	0.15
-2 hours post exercise	0.32 ± 0.04	0.33 ± 0.03	0.33 ± 0.03	0.36 ± 0.02**	0.13

NN, normobaric normoxia; GHA, genuine high altitude; NH, normobaric hypoxia; HH, hypobaric hypoxia ; ICV, isovolumic contractile velocity; Between group post-test differences: a, NN vs GHA; b, NN vs NH; c, NN vs HH; Within groups repeated measures post-test difference * rest vs 15 minutes post exercise; ** rest vs 2 hours post exercise

Table 4 Changes in Echo derived markers of right ventricular function

	Sea Level	GHA	NH	HH	P Value
Right ventricular systolic pressure, mmHg					
-Rest	21.8 ± 4.0	31.8 ± 3.7	31.5 ± 5.8	31.7 ± 4.0	0.0002 ^{abc}
-15 minutes post exercise	27.6 ± 5.2*	31.9 ± 6.1	31.4 ± 5.7	35.2 ± 5.7	0.04 ^c
-2 hours post exercise	28.2 ± 4.0**	32.0 ± 6.4	32.0 ± 5.6	36.1 ± 8.1	0.0006 ^{cf}
Pulmonary vascular resistance dynes/cm ⁵					
-Rest	86.5 ± 13.8	107.5 ± 12.2	106.1 ± 15.9	118.7 ± 15.1	0.0002 ^{a,b,c}
-15 minutes post exercise	111.5 ± 11.3*	116.8 ± 20.1	118.8 ± 19.3*	127.3 ± 13.8	0.11
-2 hours post exercise	106.7 ± 12.8**	114.5 ± 22.3	117.2 ± 15.6	129.1 ± 12.2	0.04 ^c
TAPSE, cm					
-Rest	2.6 ± 0.4	2.2 ± 0.3	2.4 ± 0.3	2.6 ± 0.3	0.05
-15 minutes post exercise	2.3 ± 0.4*	2.3 ± 0.3	2.4 ± 0.3	2.5 ± 0.3	0.63
-2 hours post exercise	2.3 ± 0.3**	2.5 ± 0.3**	2.4 ± 0.3	2.6 ± 0.3	0.27
Tricuspid E velocity cm/s					
-Rest	56.2 ± 8.3	63.4 ± 18.1	62.2 ± 12.9	66.2 ± 11.5	0.43
-15 minutes post exercise	50.4 ± 9.1	58.2 ± 12.5	50.8 ± 9.6*	57.7 ± 9.0	0.16
-2 hours post exercise	53.9 ± 10.2	58.5 ± 14.0	58.4 ± 10.5	54.3 ± 10.6 **	0.67
Tricuspid A velocity cm/s					
-Rest	33.7 ± 5.2	37.6 ± 1.0	45.9 ± 9.6	43.3 ± 10.0	0.01 ^b
-15 minutes post exercise	39.9 ± 9.0*	45.8 ± 12.3*	51.5 ± 13.1	45.2 ± 13.7	0.16
-2 hours post exercise	35.4 ± 8.4	40.4 ± 7.4	51.7 ± 16.0	40.2 ± 7.1	0.006 ^b
Right ventricular S' velocity, cm/s					
-Rest	14.7 ± 1.3	15.5 ± 1.1	15.4 ± 1.6	14.5 ± 2.8	0.45
-15 minutes post exercise	13.2 ± 1.7	15.4 ± 1.5	13.4 ± 1.8*	15.2 ± 1.9	0.009 ^a
-2 hours post exercise	13.9 ± 1.9	16.4 ± 2.0	14.3 ± 2.3	14.8 ± 2.9	0.03 ^a
Right ventricular E' velocity, cm/s					
-Rest	14.2 ± 2.9	16.4 ± 3.5	14.8 ± 2.8	15.7 ± 2.9	0.30
-15 minutes post exercise	13.3 ± 3.8	16.6 ± 3.8	15.3 ± 4.7	15.3 ± 4.5	0.23
-2 hours post exercise	13.7 ± 3.0	16.4 ± 2.7	15.4 ± 3.3	15.2 ± 5.5	0.23
Right ventricular A' velocity, cm/s					
-Rest	11.1 ± 1.9	11.5 ± 2.1	10.2 ± 1.9	12.2 ± 1.5	0.16
-15 minutes post exercise	10.9 ± 2.5	17.5 ± 3.1*	13.2 ± 4.1*	14.8 ± 1.5	0.0001 ^{ad}
-2 hours post exercise	12.5 ± 2.4	18.1 ± 3.7**	14.9 ± 3.3*	14.8 ± 1.3**	0.0007 ^a
Right ventricular ICV velocity, cm/s					
-Rest	9.5 ± 2.7	10.5 ± 2.3	8.9 ± 1.9	9.1 ± 1.6	0.43
-15 minutes post exercise	10.8 ± 2.7	12.5 ± 3.1	10.2 ± 2.7	11.7 ± 2.1*	0.14
-2 hours post exercise	11.3 ± 3.1	12.2 ± 3.3	10.6 ± 1.9	9.7 ± 1.8	0.24
Right ventricular Tei Index					
-Rest	0.29 ± 0.05	0.32 ± 0.04	0.29 ± 0.03	0.30 ± 0.06	0.29
-15 minutes post exercise	0.33 ± 0.04	0.37 ± 0.05*	0.33 ± 0.03	0.41 ± 0.05*	0.001 ^{cf}
-2 hours post exercise	0.32 ± 0.06	0.31 ± 0.05	0.34 ± 0.07**	0.39 ± 0.05**	0.04 ^e

NN, normobaric normoxia; GHA, genuine high altitude; NH, normobaric hypoxia; HH, hypobaric hypoxia; TAPSE, trans

annular plane systolic excursion; ICV, isovolumic contractile velocity; Between group post-test differences: a, NN vs GHA; b, NN vs NH; c, NN vs HH; d, GHA vs NH; e, GHA vs HH; f, NH vs HH. Within groups repeated measures post-test difference * rest *versus* 15 minutes post exercise; ** rest *versus* 2 hours post exercise;

Table 5 Results of Two-Way Repeated Measures ANOVA comparing the Main Effects of Duration (time) and mode of hypoxia across the three hypoxia groups

	Mode of hypoxia		Time		Interaction	
	F	P value	F	P value	F	P Value
SpO ₂	6.55	0.004 ^{ac}	2.4	0.10	1.2	0.34
Heart Rate	2.32	0.12	75.47	<0.001 ^{abc}	2.18	0.08
Mitral E velocity	0.17	0.84	11.97	<0.001 ^{ac}	1.2	0.32
Mitral A velocity	1.20	0.33	5.9	0.005 ^{ac}	0.35	0.85
Mitral E/A ratio	1.93	0.16	10.79	<0.0001	0.43	0.79
Septal S' velocity	2.38	0.11	0.99	0.38	0.72	0.58
Septal E' velocity	0.20	0.82	3.37	0.04 ^b	0.77	0.55
Septal A' velocity	0.29	0.75	12.32	<0.001 ^{ab}	0.36	0.83
Septal ICV	1.0	0.40	2.70	0.08	1.0	0.42
Lateral S' velocity	1.40	0.27	10.28	<0.001 ^{ab}	3.99	0.006
Lateral E' velocity	2.20	0.13	7.56	0.001 ^{ab}	1.12	0.36
Lateral A' velocity	0.40	0.68	6.97	0.002 ^{ab}	0.58	0.68
Lateral ICV	0.12	0.89	6.99	0.002 ^a	2.14	0.09
LV Tei Index	2.32	0.12	6.48	0.003 ^a	1.54	0.10
LV stroke volume	0.39	0.68	10.37	0.004 ^{ab}	0.93	0.45
Cardiac output	0.23	0.80	13.8	<0.001 ^{ab}	2.52	0.06
RVSP	0.70	0.51	1.47	0.24	0.88	0.48
Pulmonary vascular resistance	2.10	0.14	5.85	0.005 ^{ab}	0.27	0.76
Tricuspid E velocity	0.55	0.58	5.15	0.009 ^a	0.88	0.48
Tricuspid A velocity	3.90	0.03 ^a	1.9	0.16	0.58	0.68
TAPSE	1.21	0.31	1.1	0.31	2.4	0.06
Tricuspid S' velocity	2.60	0.09	1.3	0.30	3.12	0.02
Tricuspid E' velocity	0.66	0.53	0.01	0.99	0.10	0.98
Tricuspid A' velocity	8.20	0.002 ^a	22.3	<0.001 ^{ab}	1.59	0.19
Tricuspid ICV	3.40	0.048 ^a	6.7	0.002 ^a	0.78	0.54
Right ventricular Tei Index	5.0	0.01 ^c	16.10	<0.001 ^{ab}	4.10	0.006

TAPSE, trans annular plane systolic excursion; RVSP, right ventricular systolic pressure; ICV isovolumic contractile velocity; Results of post hoc tests – time: a baseline *versus* 15 minutes post exercise, b baseline *versus* 2h post exercise, c 15 minutes *versus* 2 hours post exercise, mode of hypoxia: a GHA vs NH, b GHA *versus* HH, NH *versus* HH

Figure 1 Changes in the left ventricular lateral S' velocities (marginal means) with differing experimental conditions and duration of hypoxia (time 1= baseline rest, time 2= 15 minutes post exercise and time 3 =120 minutes post exercise). * demonstrates between group differences on post test

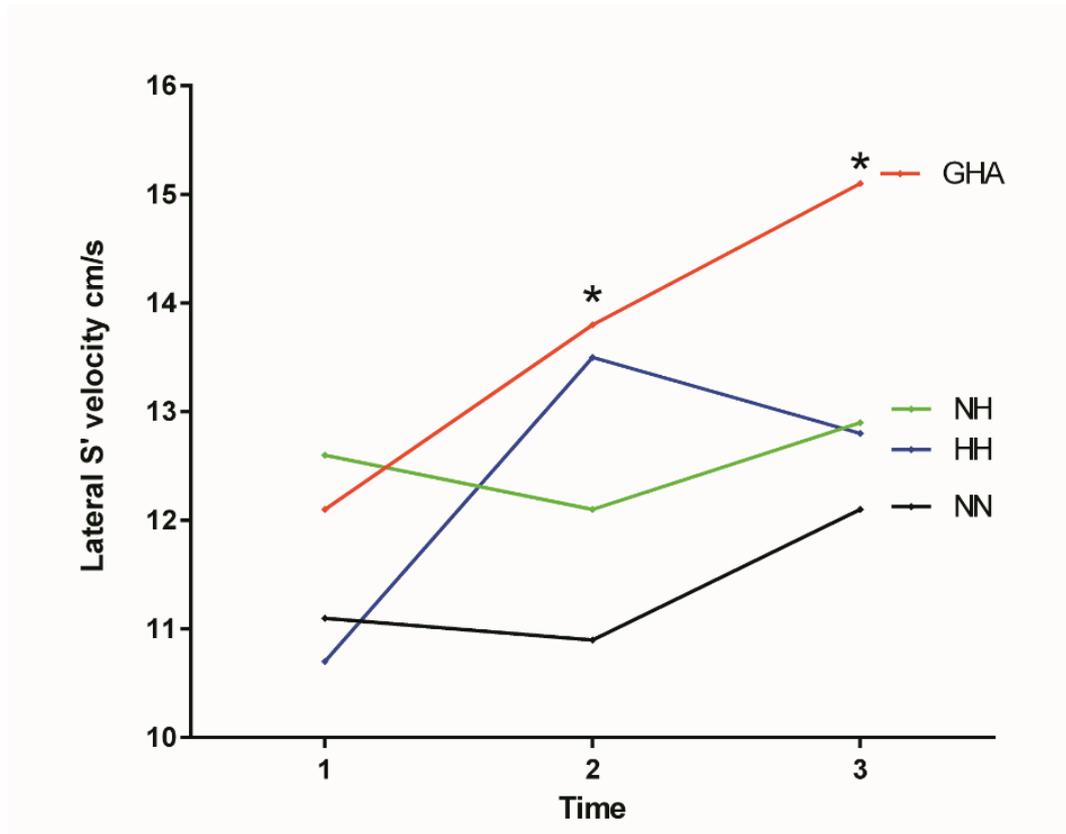


Figure 2 Changes in the right ventricular S' velocities (marginal means) with differing experimental conditions and duration of hypoxia (time 1= baseline rest, time 2= 15 minutes post exercise and time 3 =120 minutes post exercise). * demonstrates between group differences on post test

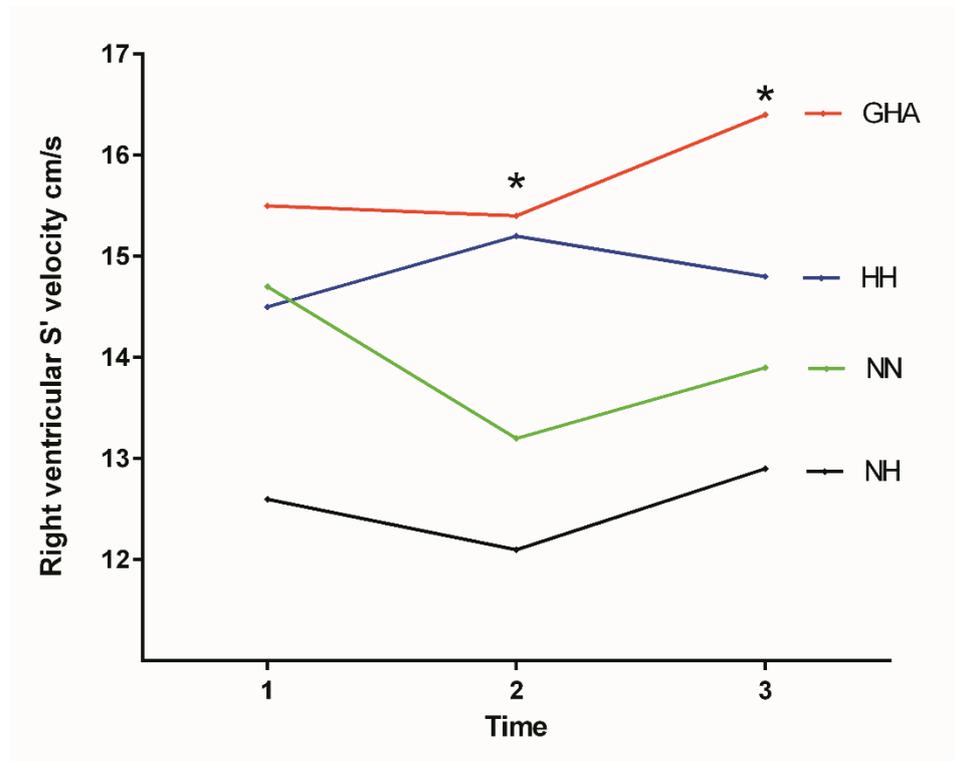
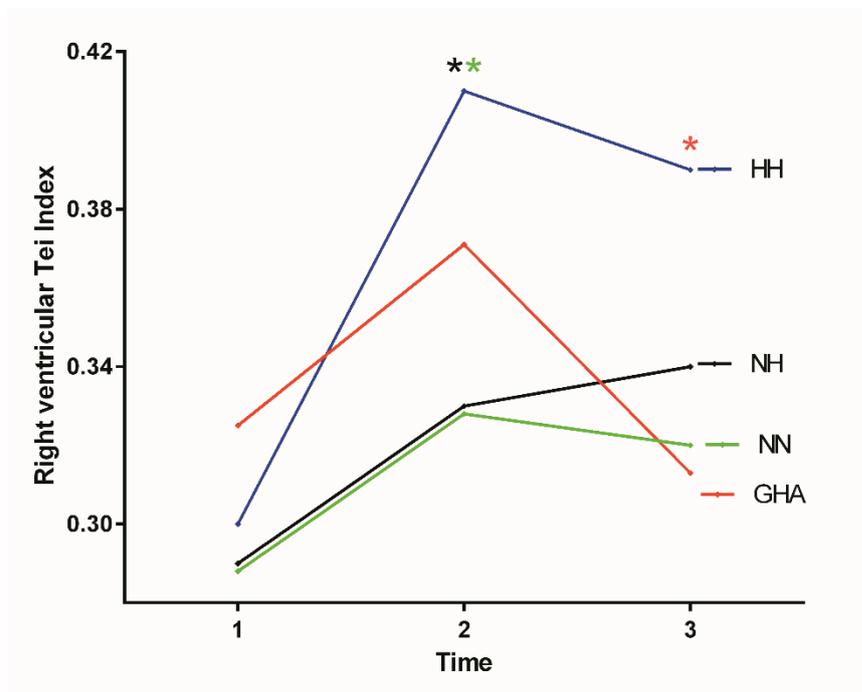


Figure 3 Changes in the right ventricular Tei Index (marginal means) with differing experimental conditions and duration of hypoxia (time 1= baseline rest, time 2= 15 minutes post exercise and time 3 =120 minutes post exercise) * demonstrates between group differences on post test



Chapter 6

Publication 4

The effect of high altitude on central blood pressure and arterial stiffness

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The effect of High Altitude on Central blood pressure and arterial stiffness

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Abstract

Central arterial systolic blood pressure (SBP) and arterial stiffness are known to be better predictors of adverse cardiovascular outcomes than brachial SBP. The effect of progressive high altitude (HA) on these parameters has not been examined.

Ninety healthy adults were included. Central BP and the augmentation index (AI) were measured at the level of the brachial artery (Uscom BP+ device) at <200m and at 3619m, 4600m and 5140m. The average age of the subjects (70% men) were 32.2 ± 8.7 years. Compared with central arterial pressures, brachial SBP ($+8.1 \pm 6.4$ mmHg; $p < 0.0001$) and pulse pressure ($+10.9 \pm 6.6$ mmHg; $p < 0.0001$) were significantly higher and brachial DBP was lower (-2.8 ± 1.6 mmHg; $P < 0.0001$). Compared <200m, HA led to a significant increase in brachial and central SBP. Central SBP correlated with AI ($r = 0.50$; 95% CI: 0.41 to 0.58; $p < 0.0001$) and age ($r = 0.32$; 21 to 0.41; $p < 0.001$). AI positively correlated with age ($r = 0.39$; $p < 0.001$) and inversely with subject height ($r = -0.22$; $p < 0.0001$) weight ($r = -0.19$; $p = 0.006$) and heart rate ($r = -0.49$; $p < 0.0001$). There was no relationship between acute mountain sickness scores (LLS) and AI or central BP. The independent predictors of central SBP were male sex (coefficient, $t = 4.7$; $P < 0.0001$), age ($t = 3.6$; $p = 0.004$) and AI ($t = 7.5$; $p < 0.0001$; overall $r^2 = 0.40$; $p < 0.0001$). Subject height ($t = 2.4$; $p = 0.02$), age ($t = 7.4$; $p < 0.0001$) and heart rate ($t = 11.4$; $P < 0.0001$) were the only independent predictors of AI (overall $r^2 = 0.43$; $p < 0.0001$). Central BP and AI significantly increase at HA. This rise was influenced by subject-related factors and heart rate but not independently by altitude, LLS or SpO₂.

Introduction

Cardiovascular death is a leading cause of non-traumatic deaths in adults at high altitude (HA) [51]. Despite this fact, there has been limited research into cardiovascular risk assessment at HA [51]. HA exposure leads to an increase in resting heart rate, compared with that at sea level, yet paradoxically, maximal heart rate is reduced [17]. The stroke volume rise noted with exercise at sea level is blunted at HA [17, 70]. Consequently, whilst resting cardiac output is higher at HA, *versus* sea level, at peak exercise it is comparatively lower [14, 17, 70]. These factors along with the notable reduction in arterial oxygen content act to limit peak exercise capacity and oxygen consumption [14, 17]. Other reported cardiovascular responses include an increase in resting brachial artery systolic blood pressure (SBP) and 24-hour arterial blood pressure (BP), which along with the increase in resting heart rate could be potential implicating factors in the increased cardiovascular risk [127, 128, 138, 233].

The effects of HA on central arterial haemodynamics, such as central arterial BP and large artery stiffness, are far less well understood and have been barely reported. Central arterial BP and large artery stiffness are known to be more powerful predictors of adverse cardiovascular outcomes, including stroke and cardiovascular death than brachial artery BP as they more closely reflect the haemodynamic loading of vital central organs such as the heart, brain and kidneys [76, 77]. Brachial artery BP does not reliably reflect central BP due to the effects of peripheral amplification which is highly variable between individuals [76, 77].

Unfortunately, the accurate non-invasive assessment of central BP and large artery stiffness has been traditionally very difficult. It had required the need for either arterial catheterisation or less portable and expensive non-invasive equipment limiting its research utility at HA, explaining the paucity of published research at genuine terrestrial HA [14, 233].

In the only study to investigate the influence of terrestrial HA on both large arterial stiffness and central BP Parati et al. observed a significant increase in both central SBP and the arterial

augmentation index (AI, marker of arterial stiffness) in untreated subjects travelling to HA.⁷ However, the altitude gain was very rapid (4559m within 28 hours of ascent) and only a single altitude was studied. Nevertheless, their findings are potentially important given the huge numbers exposed to HA worldwide [17, 51].

The Uscom BP⁺ is a novel device which is able to estimate central blood pressure using a simple oscillometric BP cuff on the upper arm [86]. It has shown excellent agreement with catheter based assessments of central BP and gold standard measures of arterial stiffness [87, 234]. It utilises pulse wave analysis to assess the AI which reflects the enhancement (augmentation) of central aortic systolic pressure by reflected arterial pulse waves. It has the advantage over several competing devices. It is highly portable and only requires the use of an upper arm cuff therefore avoiding the need to assess either the radial or digital pulse where the signal to noise ratio may be less favourable.

In this study we sought to utilise this available technology to investigate, for the first time the effects of a step-wise increasing terrestrial HA on both central BP and AI during a trek to >5000m.

Methods

Study design and participants

Ninety healthy British Military servicemen aged >18years were included. Inclusion was entirely voluntary and represented a large subset of military servicemen who had been selected to take part in a quadrennial military adventure training exercise to HA. Significant mountaineering experience was not essential but those with very limited experience were encouraged to attend a winter skills course (<1200m) within 3 months of departure. The subjects were assessed at near sea level (<200m) and during progressive ascent in the Dhaulagiri region in the Himalayas in March/April 2016. Health status was confirmed following a detailed baseline questionnaire. All subjects were assessed to be medically fit for a high altitude venture by their general practitioner.

To be considered fit they were all required to have passed their annual military basic fitness test which includes a 1.5 mile timed run. Key exclusion criteria included a history of hypertension and/ or atrial fibrillation. All participants were low altitude dwellers and none had prior exposure to >1400m terrestrial altitude in the four weeks prior to this study. The subjects were studied consecutively in groups of 8-10 individuals with a two day stagger between successive groups. HA related symptoms were assessed using the Lake Louise Scoring System (LLS) [28, 29].

High Altitude Ascent and descent profile

The subjects flew from the UK to Kathmandu (1400m day 1-3) where they underwent a short period of local acclimatisation at 1400m. From there they travelled by a staged road move to Darbang (1030m). From there they commenced trekking on foot with loads of up to 12kg over the ensuing 11 days (to day 14) to an altitude of 5140m (with an overpass of 5360m) before commencing their descent (day 15) on foot to Marpha (2719m) and then by road back to Kathmandu (figure 1). Research assessments were performed at sea level and at static research camps at 3619m (day 9), 4600m (day 12) and 5140m (day 14) during ascent.

Physiological assessments and central blood pressure measurement

Oxygen saturations (SpO₂) were measured using a Nonin Onyx (Nonin Medical Inc, Plymouth, Minnesota, USA) pulse oximeter. Blood pressure and arterial stiffness assessments were obtained at the same time using an Uscom BP⁺ device (Uscom, Sydney, NSW, Australia) as previously reported [86-88, 234]. The upper arm cuff was attached to the dominant arm of seated subjects. All subjects were rested for at least five minutes prior to BP assessment and they were not permitted to drink caffeine or smoke for at least three hours and alcohol for ≥ 10 hours prior to BP measurements [83]. The subjects were advised not to speak during the recordings. The BP⁺ device measures both central and peripheral BP (mmHg) using supra systolic oscillometry. Following an initial inflation-deflation the cuff is re-inflated to approximately ≥ 30 mm Hg above the recorded suprasystolic pressure for 10 seconds, during which suprasystolic BP and pulse wave assessments are recorded via the arm cuff. All recordings were stored on a mini SD card

within the device and later exported for data analysis. Only readings with a signal-to-noise ratio of ≥ 6 were included and tests with a ratio of < 6 were repeated.

The BP⁺ calculates a number of additional haemodynamic indices that were of interest to this study, including the AI. Its quoted AI is the arterial augmentation pressure (difference between the second and first systolic peaks of the central pressure waveform) expressed as a percentage of the pulse pressure and it is an *indirect* measure of large arterial stiffness. Further parameters that we were specifically interested in for this study were the time to systolic wave Reflection (TR) and the suprasystolic pulse pressure variation (ssPPV). The reflected Wave Transit Time is an indirect measure of pulse wave velocity and large arterial stiffness. The ssPPV is a novel measure of fluid responsiveness and is heavily influenced by respiratory variation and left ventricular stroke volume, both of which can be affected at HA [235-237]. The BP⁺ calculates the ssPPV as the difference between maximum and minimum pulse pressures divided by the average pulse pressure over the 10 second rhythm strip.

Ethics

Participation was entirely voluntary and all participants underwent detailed written informed consent. The study was approved by the Ministry of Defence Research and Medical Ethics Committee (MODREC) and was conducted according to the standards of the declaration of Helsinki.

Statistical analysis

Data were analysed using GraphPad InStat version 3.05 and with all graphical figures presented using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). Sample size calculations were performed using a proprietary determined sample- size calculator using (GraphPad StatMate version 2.00 for Windows). The Kolmogorov-Smirnov test was

undertaken to assess normality of all continuous data and all continuous data are presented as mean \pm standard deviations and median \pm interquartile range for parametric and non-parametric data respectively. Comparison of unpaired data was performed using an unpaired T test or the Mann-Whitney Test for parametric and non-parametric data respectively and with a paired t test and Wilcoxon matched pairs test for equivalent paired data. Continuous data from ≥ 3 groups were compared using a one-way Analysis of Variance (ANOVA) with either Tukey post-hoc tests or a Kruskal-Wallis test with Dunn post-test for parametric and non-parametric data respectively. Correlations were performed using Pearson and Spearman rank correlation ($\pm 95\%$ confidence interval, CI) for parametric and non-parametric data respectively. A two tailed P value < 0.05 was considered statistically significant for all comparisons. All univariate predictors of central arterial systolic blood pressure were entered into a multiple linear regression analysis model in order to identify its independent predictors. A two tailed P value < 0.05 was considered statistically significant for all comparisons.

Sample size calculations

Parati et al studied 44 subjects who travelled from sea level to 4559m within 29 hours [128]. From this group there were 22 subjects who were randomised not to receive prophylactic medication to prevent acute mountain sickness. In this group they observed a non-significant increase in central systolic blood pressure from 103.7 ± 10.7 to 108.8 ± 8.0 mmHg from sea level to that after 48h at HA. The AI significantly increased at HA *versus* sea level. Based on this data and the average standard deviation of their central BP readings, we calculated that a sample size of at least 60 subjects would have $> 80\%$ power to detect a ≥ 5 mmHg change in central SBP and a $\geq 7\%$ change in AI at HA at a significance level (alpha) of 0.05 (two-tailed).

Results

Ninety subjects were included. The average age of the subjects were 32.2 ± 8.7 years with 70% being male. Heart rate and LLS increased and SpO_2 fell at HA compared with sea level (table 1). The average 1.5 mile run time for included subjects was 9.9 ± 1.2 minutes.

Overall brachial arterial SBP (+8.4 [5.0 to 12.0] mmHg; $p < 0.0001$) and pulse pressure (+11 [7.0 to 15.0] mmHg; $p < 0.0001$) were significantly greater than that observed centrally. Conversely the brachial artery DBP was lower (-2.6 [-3.4 to -2.0] mmHg; $P < 0.0001$) than the equivalent central readings.

Compared with baseline sea level values there was a significant increase in both brachial and central SBP and in brachial but not central arterial pulse pressure at HA (table 2). The highest increase in both brachial and central SBP was between sea level and 4619m (+7.0 [-5.0 to 16.0] and +7.0 [-4.5 to 18.0] mmHg respectively) (table 2; figure 2).

The AI and ssPPV both increased at HA whereas the reflected wave transit time and systolic ejection period decreased *versus* sea level (table 2; figure 3). Adjusting the AI to an average heart rate of 75 per minute (AI@75) did not alter the findings.

There were significant correlations between central SBP and both AI ($r = 0.50$; 0.41 to 0.58; $p < 0.0001$) and age ($r = 0.32$; 0.21 to 0.41; $p < 0.001$). Other independent, albeit weak predictors, of central SBP were SpO₂ ($r = -0.14$; -0.25 to -0.05; $p = 0.02$), heart rate ($r = -0.16$; -0.27 to -0.05; $p = 0.003$) male sex ($r = 0.15$; 0.04 to 0.26; $p = 0.004$) ethnicity ($r = 0.15$; 0.04 to 0.25; $p = 0.007$) smoking status ($r = 0.18$; -0.28 to -0.07; $p = 0.001$) and altitude ($r = 0.10$; $p = 0.05$). AI positively correlated with age ($r = 0.39$; $p < 0.001$) and inversely with subject height ($r = -0.22$; $p < 0.0001$) weight ($r = -0.19$; $p = 0.006$), and heart rate ($r = -0.49$; $p < 0.0001$). There was no relationship between LLS and either AI or central BP.

Multivariate analysis was performed to assess the independent predictors of central systolic BP. Only the univariate predictors were included in the model. The independent predictors of central SBP were male sex (coefficient, $t = 4.7$; $P < 0.0001$), age ($t = 3.6$; $p = 0.004$) and AI ($t = 7.5$; $p < 0.0001$; overall $r^2 = 0.40$; $p < 0.0001$). If AI was removed from the model (overall $r^2 = 0.29$; $p < 0.0001$) then

the independent predictors of central systolic BP were age, heart rate and smoking history. Subject height (coefficient 2.4; $p=0.02$), age (7.4; $p<0.0001$) and heart rate (11.4; $P<0.0001$) were the only independent predictors of AI (overall $r^2=0.43$; $p<0.0001$). The order of the trekking groups did not influence the findings when included in the multivariate analysis.

Discussion

To the author's knowledge, this is the first study to assess the effects of stepwise increasing terrestrial HA on arterial stiffness and central BP over a conventional and progressive HA trek. We found that HA exposure led to a significant increase in central SBP and AI. Neither altitude nor the SpO_2 were independent predictors of AI and central SBP. Heart rate was a significant determinant of both AI and central BP.

HA exposure leads to a wide range of complex effects on both the pulmonary and systolic circulation which have been well described [14, 17, 70, 72]. Hypobaric hypoxia leads to widespread sympathetic activation leading to an increase in resting heart rate [64, 238, 239]. The reported effects on BP are variable and are highly dependent on the degree of hypoxia and speed and duration of exposure. Furthermore, the type of hypoxic environment may be a major confounder [209]. Several previously published studies have used simulated hypoxia (using either a normobaric or hypobaric chamber) in an attempt to replicate the degree of hypoxia observed at genuine HA [64, 70, 72, 209]. Whilst they are very useful surrogates for HA exposure, simulated hypoxia does not fully reproduce the environmental and geographical effects genuine terrestrial HA such as the cold or the exercise burden. The reported literature has tended to focus on the effects of HA on brachial artery BP and largely following a relatively short periods (≤ 6 hours) of simulated hypoxia [72, 209]. Available data at terrestrial HA has shown that HA exposure typically leads to an increase in both resting systolic and 24 hour blood pressure which may be more pronounced in those with background hypertension [127]. The effects of HA on central BP and arterial stiffness have been barely examined at HA, yet they are well recognised to be better predictors of cardiovascular risk than brachial BP [76, 77]. Given the

vast numbers of patients with known hypertension and cardiovascular disease who undergo recreational HA exposure annually the ability to better define cardiovascular risk in these individuals would be important. This has added importance given that cardiovascular death is a leading cause of non-traumatic death at HA [51]. An improved understanding of the effects of HA on central BP and other non-invasive measures of cardiovascular risk such as arterial stiffness might allow for tailored medical therapy at HA to reduce the cardiovascular risk to individuals. We observed a significant increase in brachial but not central pulse pressure suggesting differences in BP behaviour in the peripheral *versus* the central circulation. Indeed whilst the brachial SBP was higher than that observed centrally the increase in central SBP was greater and was significant across all three altitudes studied (table 2).

There has only been one previous study to investigate the effects of HA on measures of both arterial stiffness and central BP at terrestrial altitude. Parati et al studied 44 subjects who were randomised to placebo or to oral acetazolamide prior to and during HA exposure [128]. Following sea level assessment the subjects ascended to 4559m within 28 hours by road to 1130m, then cable car to 3647m before completing the rest of the ascent on foot. Measurements at HA were obtained within 4-6 hours of arrival at 4559m and again after two days at this altitude. They observed a non-significant increase in both central and peripheral SBP but an even greater and significant increase in DBP. AI@75 significantly increased from Sea level to HA. However, whereas the SBP continued to increase from 4-6 hours to two days at HA there was no further increase in the AI@75 beyond the early increase. In our study we noted a similar sized increase in both brachial and central SBP to that in this previous study and the significance in our current study likely relate to our much larger sample size. Our data would seem to suggest that the increase in heart rate is a significant independent predictor of the increase in AI at HA which was not directly related to either the SpO₂ or altitude. The observed increase in heart rate, AI, brachial and central SBP would strongly suggest that these increases relate to sustained sympathetic activation at HA as has been well described rather than a genuine increase in large artery stiffness [238].

In one of the only previously published studies to assess the effects of HA on arterial stiffness and brachial BP during a conventional trek Rhodes et al studied 17 subjects over an ascent from 80m to 4770m over 11 days [138]. They found that HA led to a transient increase in large artery stiffness index (using finger photoplethysmography) noted at day four at 3450 m before returning to baseline levels. A significant rise in both systolic and diastolic BP were observed at 3450m and the increase was sustained throughout the HA exposure [138]. Interestingly, they observed that the increase in BP was not related to changes in arterial stiffness nor was there a link between the increase in arterial tone and the presence of AMS. We did not identify a relationship between LLS, SpO₂ and either AI, which is an indirect measure of large artery stiffness and central systolic BP at HA.

Consistent with previous research we found that the AI related to the subjects age and inversely correlated with height and heart rate [180, 240]. This is explained by the fact that the time of the reflected wave is related to the dimensions of the body and heart rate. In shorter individuals, a reduced return time for reflected waves leads to an increase in central pressure augmentation [240]. As a result of the noted influence of heart rate on AI it has been suggested that AI should be adjusted for the effects of heart rate and this has traditionally been to an average of 75 per minute (AI@75) [241]. Adjusting the AI@75 to account for heart rate did not alter our findings. It has also been more recently suggested that adjusting for heart rate on multivariate analysis of AI is more appropriate and this has been additionally done in our analysis [65]. Our data has shown that heart rate was the independent variable with the greatest impact on AI. Indeed augmentation of central BP is influenced by heart rate and therefore the duration of systole and shifting the reflected arterial wave to diastole and reducing the time to wave reflection as has been observed in our study [241]. Therefore it is reasonable to assume that the increase in AI at HA is largely related to the associated increase in heart rate leading to a rise in arterial augmentation and central BP rather than actual changes in large artery stiffness over only 14 days HA exposure.

In this study we were also interested in the effects of HA on the ssPPV. This is a measure of the variation in the pulse pressure averaged over the 10 second arterial waveform recording using the BP⁺ device. The beat to beat variation in pulse pressure is known to be influenced by a number of factors including left ventricular preload, stroke volume and ventilation, which are all known to be affected at HA [72]. Clinically, probably the most widespread use of ssPPV has been to assess fluid responsiveness in mechanically ventilated patients intra-operatively and on intensive care [236, 237]. During inspiration negative intrathoracic pressure leads to an increase in venous return and ultimately an increase in ventricular filling. Its effect on left ventricular stroke volume is influenced by hydration and intravascular filling, which is dependent on the relative position on the Frank-Starling curve [235]. HA-related hypoxia has been shown to affect both right and left ventricular stroke volume with variable effects on ventricular filling [64, 70, 72]. The mechanisms to explain these changes are complex and include the known hypoxia mediated pulmonary vasoconstriction leading to an increase in pulmonary artery systolic pressure and right ventricular afterload [14]. HA acclimatisation is known to lead to relative dehydration and hypoxia-mediated hyperventilation all of which may affect biventricular stroke volume. Whilst the ssPPV cannot be used in isolation serial measurements can be used to assess filling and fluid responsiveness. In our study the ssPPV was very susceptible to the effects of HA exposure but was not related to LLS. HA led to a marked increase in the ssPPV, despite no significant increase in the central arterial pulse pressure.

This study has a number of limitations that require acknowledgement. The subjects were studied in groups two days apart. This was done to accommodate the large sample size of the study and ensure excellent reproducibility of the measures and ensure that subject BP measurements were conducted robustly at each individual research station by trained researchers. The environmental factors, such as temperature and barometric pressure would not have been identical for the study groups at the time of their data collection which could have potentially influenced the findings. However, we did not observe any significant influence of the trekking group order of study on either AI or central systolic blood pressure. Unfortunately, we did not measure hormonal markers of sympathetic activation, such as circulating catecholamines, to better investigate the mechanism

for the increase in SBP and AI, however, we did note that the increases did not relate to the degree of hypoxia (SpO₂) or LLS.

In conclusion in this study we found that HA exposure led to an increase in brachial and central SBP and a rise in AI compared with near sea level baseline levels. The increase in central SBP and AI was not related to the degree of hypoxia and SpO₂ at HA nor to LLS. The observed changes likely relate to increased sympathetic activation rather than any genuine change in large artery stiffness.

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Conflict of Interest

The authors have no conflict of interest to declare.

What is known about the topic?

- HA exposure leads to an increase in heart rate and there is evidence from a single study of rapid largely cable car ascent to 4559m that it leads to an increase in central SBP and arterial AI.

What this study adds?

- This is the first study to examine the effects of stepwise increasing terrestrial HA on arterial stiffness and central BP over a conventional and progressive HA trek to >5000m.
- We have discovered that the HA exposure led to a significant increase in central SBP and AI.

- Neither altitude nor the SpO₂ were independent predictors of AI and central SBP.
- The increase in AI related to the increase in heart rate at HA and did not reflect a genuine change in large artery stiffness.

Table 1: Baseline Demographics

Demographic	Result
Age, years (range)	32.2±8.7 (18-56)
Males n, %	63 (70%)
Height, cm	173.5±9.1
Weight, kg	73.4±12.3
Body mass index kg/m ²	24.38±2.7
Ethnicity, %	
- Caucasian	78 (86.7%)
- Nepalese	11 (12.2%)
- South Asian	1 (1.1%)
Smoking status (N, %)	
- Current	9 (10%)
- Ex	11 (12.2%)
- Never	70 (77.8%)

Table 2 Effect of high altitude on measured vascular parameters including central blood pressure and augmentation index

Parameter	Sea level	3619m	4600m	5140m	P value
Heart rate/ minute	65.2±12.8	69.6±11.8	77.3±15.3	78.2±13.6	<0.0001*†‡
Oxygen Saturations, %	97.7±1.4†	91.9±3.4	82.8±6.3	80.4±5.3	<0.0001*†‡
Lake Louise Scores	0.23 (0.64)	1.1 (1.9)	1.4 (1.6)	1.3 (1.4)	<0.0001*†‡
Brachial artery systolic BP, mmHg	132.8±14.0	136.9±13.4	138.8±13.3	138.6±13.9	0.04†‡
Brachial artery diastolic BP, mmHg	81.8±11.7	84.7±9.4	83.7±9.8	83.9±9.7	0.28
Mean brachial arterial BP, mmHg	99.3±12.9	102.0±9.9	102.1±9.9	102.2±9.8	0.23
Brachial artery pulse pressure, mmHg	51.6±11.3	52.1±9.7	55.5±10.9	54.7±11.3	0.02†
Central systolic BP, mmHg	124.7±14.8	130.1±14.2	131.4±15.4	129.4±14.3	0.02*†‡
Central diastolic BP, mmHg	84.0±11.6	87.5±9.6	86.8±9.6	87.3±9.5	0.09
Central artery pulse pressure, mmHg	40.7±9.5	42.6±9.6	44.6±13.4	42.1±9.9	0.26
Augmentation index, %	55.3±34.9	71.1±34.1	61.8±36.7	56.6±32.7	0.001†
Reflected wave transit time, s	0.16±0.02	0.16±0.02	0.14±0.02	0.14±0.01	<0.0001*†‡
Systolic ejection period, s	0.30±0.03	0.31±0.02	0.29±0.03	0.28±0.02	<0.0001†‡
Supra Systolic pulse pressure variation	0.23±0.13	0.28±0.15	0.37±0.20	0.34±0.19	<0.0001*†‡

BP, blood pressure; results of post hoc tests vs baseline sea level, *3880m, † 4400m, ‡ 5140m

Figure 1 Ascent Profile the altitude and timing of data collection

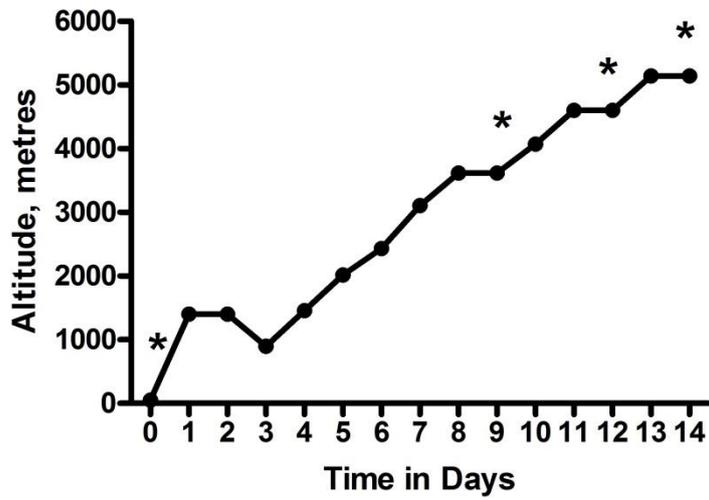


Figure 2 Changes in systolic blood pressure with HA exposure. Symbol* denotes significant difference vs baseline level

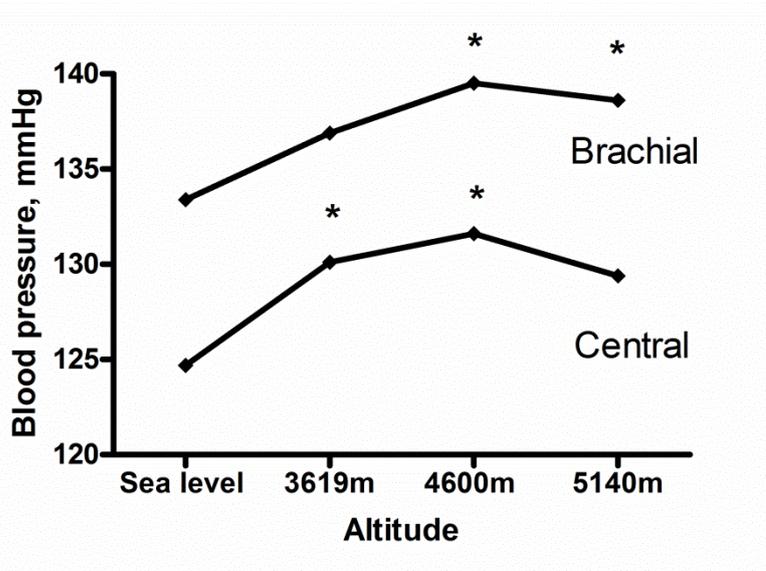
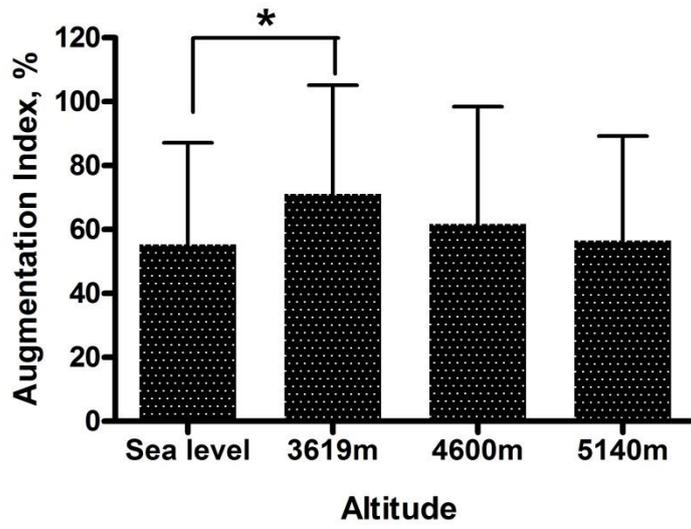


Figure 3 Change in Augmentation Index with high altitude



Chapter 7

Publication 5

The Effect of Sex on Heart Rate Variability at High Altitude

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The effect of Sex on Heart Rate Variability at High Altitude

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Abstract

Introduction

There is evidence to suggest that high altitude (HA) exposure leads to a fall in heart rate variability (HRV) that is linked to the development of acute mountain sickness (AMS). The effects of sex on changes in HRV at HA and its relationship to AMS are unknown.

Methods

HRV (5-minute single lead ECG) was measured in 63 healthy adults (41 men and 22 women) aged 18-56 years at sea level (SL) and during a HA trek at 3619m, 4600m and 5140m respectively. The main effects of altitude (SL, 3619, 4600 and 5140m) and sex (men vs women) and their potential interaction were assessed using a Factorial Repeated Measures ANOVA. Logistic regression analyses were performed to assess the ability of HRV to predict AMS.

Results

Men and women were of similar age (31.2 ± 9.3 vs 31.7 ± 7.5 years), ethnicity, body and mass index. There was main effect for altitude on heart rate, SDNN (standard deviation [SD] of normal-to-normal [NN] intervals), RMSSD (Root mean square of successive differences), NN50 (number of pairs of successive NNs differing by >50 ms), pNN50 (NN50 / total number of NNs), very low frequency (VLF), low frequency (LF), high frequency (HF) and total power (TP). The most consistent effect on post hoc analysis was reduction in these HRV measures between 3619 and 5140m at HA. Heart rate was significantly lower and SDNN, RMSSD, LF, HF and TP were higher in men compared with women at HA. There was no interaction between sex and altitude for any of the HRV indices measured. HRV was not predictive of AMS development.

Conclusions

Increasing HA leads to a reduction in HRV. Significant differences between men and women emerge at HA. HRV was not predictive of AMS.

Introduction

The assessment of heart rate variability (HRV) has rapidly evolved from what was predominantly a research tool to an increasingly appreciated clinical modality [58]. Its most widespread translational use at present is in the assessment of psychological stress, physical fitness and the prevention of overtraining [58, 167]. The improved portability and reduced cost of HRV-measurement equipment have also played a significant role in this regard. HRV assessment relies on the detailed assessment of the variations in the time-intervals between consecutive heart beats which are subject to continuous autonomic control [60, 167]. From these data, the changes in the beat-beat intervals over time (time-domain analyses) can be more robustly quantified from as little as 1-5 minutes of recording [58, 164, 170]. The beat-to-beat data can be further examined by frequency domain analysis whereby the generated sinusoidal waveforms of these intervals over time, are placed into various frequency components, allowing for a more in depth analysis of autonomic balance [60, 167].

An area of recent interest has been in the effects of high altitude (HA) on HRV [91, 150, 151, 216]. HA exposure challenges several physiological systems that are heavily reliant on continuous autonomic control and are likely to influence [151, 152, 187, 216, 242]. Acute hypoxia and HA leads to marked sympathetic activation yet paradoxically there is also evidence of increased competing parasympathetic activity which contributes to the reduction in maximal heart rate in proportion to the altitude gained [137, 151, 152, 216]. Hypobaric hypoxia, cold, exercise, stress and fatigue, which are synonymous with HA exposure, are all known individually to influence HRV [151, 187, 243].

There are data to suggest that acute hypoxia and HA exposure leads to a decline in HRV [150, 151] with conflicting data on its potential link to HA-related symptoms and acute mountain sickness (AMS) [162-164]. Published studies on HRV at HA have been derived from relatively small cohorts, with very little data on the effects of genuine terrestrial, rather than simulated HA [91, 162, 163]. Moreover, there has been an under-representation of women, in current datasets,

despite their obvious physical and potentially important physiological differences compared to men. Resting heart rate tends to be higher in women than men, yet their stroke volumes and cardiac outputs are lower and these differences are sustained and even enhanced with hypoxia [168, 244]. Resting minute ventilation, which affects HRV, is relatively greater in women under both normoxia and hypoxia (23). Time-domain measures of HRV are typically higher in healthy men (<50 years) compared with age-matched women (Boos, Mellor et al. 2016, Koenig and Thayer [153]. However, the power spectral density (PSD) of HRV in females is usually characterized by less total power (TP), greater or similar high-frequency (HF) and lower low-frequency (LF) power and LF/HF ratios [60, 168, 170].

There are some data to suggest that women may be also more vulnerable to both AMS development and worsening symptom severity compared with men [172-174]. Given the possible sexual dimorphism in HRV and AMS incidence/severity coupled with the reported links between HRV and AMS an investigation of comparative HRV in men and women and its relationship to AMS development is warranted.

In this study, we aimed to investigate, for the first time, the influence of sex on time and frequency-domain measures of HRV with increasing terrestrial HA and its potential link to AMS development.

Methods

Study design and participants

Sixty-three healthy British Military servicemen aged >18years were included. They were all assessed at near SL (<200m) and again at three further altitudes during progressive HA ascent in the Dhaulagiri region of the Himalayas in March/April 2016. Health status was confirmed following a detailed baseline questionnaire. For inclusion, all subjects needed to be low altitude dwellers and were required to be deemed medically fit for HA exposure by their medical practitioners. All participants were required to have successfully completed their mandatory

military Personal Fitness Assessment 1.5 mile run in accordance with published standards (adjusted to age and sex) prior to inclusion. This run was undertaken in sports clothing on a flat surface. Subjects with a history of cardiac arrhythmias were excluded. The subjects were studied consecutively in groups of 8-14 at sea level and at HA with a two-day stagger between successive groups. All trekking groups followed an identical ascent and exercise recovery profile with similar morning wake times. Sea level (SL) baseline assessments were performed in the UK approximately six weeks prior to each departure.

High Altitude Ascent and descent profile

The subjects flew from the UK to Kathmandu (1400m) where they underwent two days of local acclimatisation (Days 1-3). Thereafter, they travelled by road over two days to 1030m (Dabang). From there they commenced trekking on foot over the ensuing 11 days to an altitude of 5140m (with an overpass of 5360m) before commencing their descent on foot to Marpha (2719m) and then by road back to Kathmandu. Research assessments were performed at SL and at static research camps at 3619m, 4600m and 5140m during HA ascent.

Physiological Assessments and Heart Rate Variability

Oxygen saturations (SpO₂) were measured using a Nonin Onyx (Nonin Medical Inc, Plymouth, Minnesota) pulse oximeter with sampling over approximately 15 seconds. HA-related symptoms were recorded using the Lake Louise Scoring (LLS) system. AMS was defined as a LLS of ≥ 3 in the presence of headache [28, 30]. HRV assessments were undertaken using dedicated battery-operated portable HRV devices which records a single lead ECG at a sampling rate of 250/second (CheckMyHeart Plus™ Daily Care Biomedical, Taiwan) as previously described. The first of the two surface ECG electrodes were placed at the right sternal edge at one finger breadth below the suprasternal notch and the second over the left 5th intercostal space at the mid clavicular line (i.e. cardiac apex). Measurements were taken on fully rested subjects over a five-minute period in the early morning post-micturition and prior to breakfast or caffeine [91]. All subjects were studied seated in a warm building at sea level and wearing warm clothing and in a

tent at HA and were advised not to talk during HRV assessment. All stored recordings were exported via USB hook up for offline data analysis (CheckMyHeart Plus™ R30 V4, Daily Care Biomedical, Taiwan).

The R waves of the stored ECG were used as the fiducial point to determine the beat to beat interval with full ECG disclosure. Non-normal-to-normal-(NN) intervals and ectopic beats were identified using customised software non-linear algorithms and were highlighted by colour coding within the HRV software to ease their identification. All ECG data was inspected in six second windows for further identification and manual editing of potential non NN intervals if necessary. All confirmed non NN intervals due to ectopy were excluded. The average five-minute heart rate, and the SDNN, RMSSD, NN50 and pNN50 time domain measures, as previously described, were recorded [60, 167]. The SDNN refers to the standard deviation of the NN intervals from the acquired ECG. The RMSSD (Root mean square of successive differences) is the square root of the mean of the squares of the successive differences between adjacent NN intervals. The NN50 describes the number of pairs of successive NNs that differ by >50 ms and the pNN50 refers to the proportion of NN50 divided by total number of NN intervals. Frequency-domain analysis was performed using the non-detrend method of fast Fourier transformation (FFT) with full graphical display of the power spectral data. Key frequency band data collected were the HF power (0.15-0.40 Hz), LF power (LF; 0.04-0.15 Hz), very low frequency power (VLF; 0.01-0.04 Hz), TP and the LF: HF ratio as previously defined [152, 245]. Normalized HRV values of LF (LFnu) and HF (HFnu) were calculated as a percentage of the total spectral power minus the VLF respectively [60].

Ethics

All participation was voluntary and all subjects underwent detailed written informed consent. This study was approved by the Ministry of Defence Research and Medical Ethics Committee (MODREC) and was conducted according to the standards of the Declaration of Helsinki.

Statistical analysis

Data were analysed using GraphPad InStat version 3.05 and SPSS[®] statistics version 22 with all graphical figures presented using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). Sample size calculations were performed using a proprietary determined sample size calculator using (GraphPad StatMate version 2.00 for Windows). Data inspection and the Kolmogorov-Smirnov test was undertaken to assess normality of all continuous data, which were presented as mean \pm standard deviations and as the standard error of the mean (SEM) for figures. Categorical variables were compared using a Chi-Squared test. Comparison of unpaired data was performed using an independent t test and a Mann-Whitney test for parametric and non-parametric data respectively. Correlations were performed using Pearson and Spearman rank correlation (\pm 95% confidence interval, CI) for parametric and non-parametric data respectively. A Factorial Repeated Measures ANOVA with Bonferroni correction (to minimise type I error) was performed to assess the main effect of sex (men vs women) over the four altitude time points (SL, 3619m, 4600m and 5140) and any interaction of altitude x sex on HRV scores. Binary logistic regression analyses (enter) were undertaken to assess potential continuous HRV and other univariate predictors of AMS development (yes or no) and its coefficient (B). We also undertook an additional exploratory analysis of the categorical HRV measures of RMSSD <30ms, LF:HF >1.3 and LFnu <20% that have been previously reported to be associated with AMS [162, 163]. Non-parametric data was log (Ln) transformed and normality confirmed for the ANOVA and logistic regression analyses. A two tailed P value <0.05 was considered statistically significant for all comparisons.

Sample size calculations

In a recent pilot study in Dhaulagiri, which included 12 subjects, we observed a non-significant but 11% (-7.9 ms) fall in the RMSSD time domain measure of HRV from baseline to 3600m, using identical HRV (CheckMyHeart Plus[™]) devices [91]. Hence, by studying an even greater altitude of >5000m we calculated that a sample size of >18 subjects in each group (men vs

women) would have >80% power to detect a significant change in the RMSSD at HA at a significance level (alpha) of 0.05 (two-tailed). In another recent study Saleem et al documented that the SDNN was significantly higher in 27 healthy men versus 18 healthy women (24). We estimated that a sample size of at least 18 women and >30 men studied across four differing altitudes would have sufficient power to detect a significant sex difference in HRV including SDNN.

Results

HRV data were available on 62 subjects at SL and at 3619 m and on 58 subjects at 4600m and 5140m respectively. The men (31.2 ± 9.3 years) and women (31.7 ± 7.5 years) were well matched for age, ethnicity, smoking history and body mass index (table 1). As expected, the men were on average taller and heavier with higher systolic blood pressures at baseline, with faster completion times for their mandatory annual 1.5 mile running fitness test ($P < 0.0001$) (table 1).

HA exposure led to a significant fall in SpO_2 and an increase LLS among both men and women, compared to baseline with no effect for subject sex (table 2). Heart rate (five-minute average) increased at HA in both sexes, with women having consistently higher rates than men both at SL and at HA (table 2 and 4).

There was a significant main effect for altitude on all time-domain measures of HRV. On post-hoc analysis this represented a significant reduction in time-domain measures of HRV most consistently between 3619m to 5140m (table 2 and 4; figure 1). There was a significant main effect for altitude on LF, HF and TP. This difference was again most marked on post-hoc analyses between 3619m and 5140m where significant reductions in LF, HF and TP were observed (table 3 and 4; Figures 2).

Time domain measures of HRV were non-significantly higher in men at SL and significant differences emerged at HA, where all measures were notably higher in men (table 2 and 4; figure

1). There was also a main effect for sex among the frequency domain measures of TP, LF and HF power which were all significantly higher in men at HA (table 3 and 4, figure 2). There were no interactions between sex (men vs women) x altitude (SL, 3619m, 4600m and 5140m) on any measures of HRV (table 4) or heart rate.

SpO₂ inversely correlated with LLS ($r=-0.38$; 95% CI -0.50 to -0.24; $p<0.0001$) and positively with RMSSD ($r=0.16$; $p=0.02$), SDNN ($r=0.18$; 0.05 to 0.30; $p=0.007$), VLF ($r=0.17$; 0.04 to 0.30; $p=0.01$), LF ($r=0.16$; 0.03 to 0.29; $p=0.2$) and TP ($r=0.17$; 0.03 to 0.29; $P=0.02$).

The prevalence of AMS increased at HA from 15.2% at 3619m to 27.3% at 4600m and 32.5% at 5140m ($p=0.004$). Reducing SpO₂ (B -0.13; $P<0.0001$) and increasing altitude (B 0.80; $P<0.0001$) and mean heart rate (B 0.03; $P=0.04$) were the only univariate predictors of AMS. None of the continuous measured HRV parameters or the categorical variable of subject sex (men vs women) were predictive of AMS. RMSSD <30ms, LF:HF >1.3 and LFnu <20% were not predictive of or associated with AMS.

Discussion

This is the largest study to assess the effects of HA on HRV, and to the author's knowledge the first study to investigate the influence of sex on HRV at terrestrial HA. In this study HRV was influenced by HA. Minor sex-related differences in HRV that were observed at SL were sustained at genuine terrestrial HA. A link between HRV and symptoms of AMS were not found.

We observed a significant fall in resting SpO₂ and an increase in LLS with increasing HA. There was also a significant main effect for altitude on heart rate (which increased) and all the evaluated time-domain measures of HRV. The most consistent change was between 3619 and 5140m, and hence at higher altitude, where there was a significant fall in SDNN, RMSSD, NN50, PNN50, LF and HF power. These findings are in keeping with published data that has shown a fall in time-domain measures of HRV at HA [162-164]. These changes are in part explained by a number of

factors linked to the HA environment. These include reducing sleep quality, extremes of cold and heat, physical exhaustion and increasing anxiety which are all known to adversely affect and reduce time domain measures of HRV [137, 163, 171].

We also observed a significant main effect for sex on heart rate and time domain measures of HRV at HA, with men having consistently higher scores and greater variability. This is a novel finding. Published data have shown a consistent trend to higher time domain measures of HRV in young adult men *versus* women at SL [153, 168, 246]. This is the first comparative study at HA. The trend to higher time domain HRV measures at SL became significant at HA. There was no interaction of altitude on sex on the time domain HRV parameters. This finding can be partly explained by the sex differences in heart rate which was consistently lower in the men. Heart rate is well known inversely correlate with all main time domain measures of HRV [60].

We also observed a significant effect of altitude (SL, 3619m, 4600m and 5140m) on TP, VLF, LF and HF power. The most consistent finding, on post-hoc analyses was a reduction in these parameters at the highest altitude of 5140m vs sea level and 3619m. HA exposure was also associated with a significant main effect of sex with greater TP, LF and HF power among the men. Results from a very recently published meta-analysis of comparative HRV measures among men and women at SL, that included more than 60,000 participants, demonstrated that when compared to that seen in women, PSD in men is generally characterised by lower HF power and greater LF, TP and LF/HF ratios [168]. This is thought to reflect their higher resting sympatho-vagal tone (hence greater LF and LF/HF ratios) compared with women. Our LF data supports this previous data. However, contrary to the published data we found that HF power was actually higher with variable effects on LF/HF power among the men. There are several potential factors that might explain these results. It is known that LF, HF power and their relative ratios (LF/HF) can be markedly influenced by a number of factors which include age, respiratory rate, recording length and heart rate [60, 167, 168, 187, 245, 246]. Whilst the ages were similar between the men and the women the greater heart rates in women would have led to the analysis of a higher number of beat-to-beat intervals, despite an identical recording period, which could be an

important confounder. Secondly, whilst increasing heart rate and minute ventilation are HA are thought to relate to enhanced sympathetic activation there is also evidence of elevated parasympathetic neural activity [72, 152, 187, 216, 242]. This increase in competing vagal activation at HA is thought to contribute to the reduction in maximal heart rate at HA [216]. LF power and the LF/HF ratio have been traditionally thought to represent sympathetic activation and net sympatho-vagal balance respectively with RMSSD and HF power reflecting parasympathetic nerve activity [60]. However, there is evolving evidence to show that these arbitrary assumptions about the discrete autonomic effects these HRV measures, may be overly simplistic [247].

Our identified sex-related dissimilarities in the time and frequency domain HRV measures at HA could also relate to differences in fitness levels. Indeed, the men in our study had higher time domain measures of HRV and lower 1.5 mile run times. Our findings could also relate to dissimilarities in acclimatisation in men vs women. Acclimatisation encompasses the cumulative effects of multiple factors such as hydration, ventilation and enuresis that are known to influence autonomic balance and HRV [248]. HRV, and in particular frequency domain analysis can be significantly affected by breathing pattern and ventilation, which are markedly affected at HA where hypoxia driven hyperventilation predominates [168, 173, 249]. In our study paced breathing during HRV assessments were not performed, but the participants were encouraged to relax and breathe normally. The majority of published studies on HRV at HA have utilised spontaneous non-paced breathing, hence we were keen to utilise a comparative methodology [153, 172, 216, 248]. Our participants were assessed at far higher altitudes and under greater hypoxia than most of the previous HA HRV studies to date, hence the potential challenge to paced breathing was likely to have been greater. We anticipated that at 4600 and 5140m controlled breathing under significant hypoxia and a high ventilatory drive might paradoxically increase subject anxiety and perceived breathlessness. By enforcing a similar paced breathing protocol in both men and women we risked neutralising genuine sex-related differences in HRV related to well-reported dissimilarities in ventilation between men and women at HA [137, 247]. Unfortunately, we did not measure comparative respiratory rate and ventilation among the men

vs women. This is an obvious limitation as sex-related differences in their spontaneous breathing it could have provided further insight into the observed differences in HRV identified.

We did not observe a link between AMS and HRV in this current study. There is limited evidence linking changes in HRV to AMS, raising the prospect of using HRV as a non-invasive predictor of AMS development [162, 163]. In a previous study Karinen et al investigated 36 different healthy climbers ascending from 2400 m to 6300 m altitudes during five differing expeditions and noted that a lower RMSSD and HF at 2400m was a marker of AMS at 3000 to 4300m [163]. However, contrary to our study, the speed of ascent varied between their five studied groups. Furthermore, they measured HRV over two rather than five minutes. In another study, of similar size (n=32), Hang et al noted that a HF% <20% (nu) or LF/HF ratio >1.3 at lower altitudes was predictive of AMS at 3400m [162](9). These HRV parameters failed to be either associated or predict AMS in our study. Willie et al in a prolonged normobaric hypoxia study and our group in another recent study (athlete RMSSD-derived HRV score), failed to identify a clear link between AMS and HRV supporting our data [91, 164].

The potential reasons for the contradictory findings in HRV to predict AMS may relate to differences in study design, HA environment, ascent/hypoxic profile, HRV recording time as well as the actual HRV parameters measures. Even the definitions of AMS that were used differed between these studies. For example, Karinen et al defined AMS as a LLS of ≥ 3 in their study whereas in the study by Willie they a LLS ≥ 4 was used to define AMS [163, 164]. In our study we used the Lake Louise Consensus definition (1992) for AMS, which refers to LLS score of ≥ 3 in the presence of headache [28, 72]. It is well known that AMS is a highly complex and heterogeneous condition. Its causative mechanisms include changes in cerebral arterial blood flow and increased vascular permeability within the blood brain barrier, both of which may be influenced by local autonomic control [15]. Whilst HRV reflects overall cardiac autonomic control it is relatively non-specific and is not indicative of local autonomic balance [247].

This study has several additional limitations that should be mentioned. The subjects were studied in consecutive groups of 8-14 two days apart and not all together in one batch. This was because of the large sample size for this type of remote field study and the need to undertake at HRV in the early morning pre-breakfast and caffeine. We measured five minute HRV which may be more vulnerable to short-term sex and situational bias than that obtained from longer recordings [60]. However, five minute HRV measurement is well-validated and endorsed by the current HRV Task Force Guidelines and is more potentially applicable to clinical practice than that of longer recordings [60]. We included a larger proportion of men than women and cannot exclude the possibility of sample bias, despite their similarities in age, ethnicity, smoking history and body mass index.

In conclusion our findings indicate that increasing HA was associated with a reduction in HRV which was most notable at 4600m and above. There were significant sex related differences in HRV between men and women which were sustained at HA. There was no interaction between sex and altitude on any of the HRV parameters measured. These sex-related differences may reflect dissimilarities in their autonomic balance and acclimatisation to HA. HRV was not predictive of AMS.

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Disclosures

Professor Boos has received speaker's fee and consultation fees from Pfizer, Bristol Myers Squibb, Astra Zeneca and Boehringer-Ingelheim. Professor Mellor has received speaker fees from Medtronic. The other authors report no conflicts.

Table 1: Baseline Demographics

Demographic	Overall	Men	Women	P value*
Number (%)	63, 100%	41 (65%)	22 (35%)	
Age, years (range)	31.41 ± 8.7 (18-56)	31.2 ± 9.3	31.7 ± 7.5	0.83
Height, cm	173.4 ± 9.7	177.1±9.3	166.5±5.8	<0.0001
weight	72.5 ± 13.0	77.4±12.1	63.2 ± 9.3	<0.0001
Body mass index, kg/m ²	24.0 ± 2.8	24.2 ± 3.0	23.5± 2.6	0.36
Systolic blood pressure	132.1 ± 15.6	136.9 ± 15.1	123.7 ± 12.7	<0.0001
Diastolic blood pressure	80.5 ± 14.6	80.8 ± 12.4	78.7 ± 10.7	0.53
Basic fitness time (minutes) (1.5 mile run)	9.9 ± 1.3	9.5 ± 1.1	10.8 ± 1.2	<0.0001
Ethnicity, %				
- Caucasian	56 (88.9%)	35 (85.4%)	21 (95.5%)	
- Non Caucasian	7 (11.1%)	6 (14.6%)	1 (5.5%)	0.40
Smoking status (%)				
- Never	51 (81.0%)	33 (80.4%)	18 (81.8%)	
- Current	4 (6.3%)	4 (9.8%)	0 (0%)	
- Ex	8 (12.7%)	4 (9.8%)	4 (18.2%)	0.23

*P value refers to results of comparison of men vs women only

Table 2 Changes in SpO2 and time domain measures of Heart rate Variability at sea level to increasing high altitude

	Sea level	3619m	4600m	5140m	Post-test Paired differences for effects of altitude
SpO2 (%)					
-Men	97.7 ± 1.3	93.1 ± 3.2	83.5 ± 6.0	81.3 ± 5.3	abcef
-Women	97.9 ± 1.3	90.9 ± 4.5	80.4 ± 8.5	78.6 ± 5.4	
Lake Louise Scores					
-Men	0.4 ± 0.6	1.0 ± 2.1	1.9 ± 2.2	1.5 ± 1.5	abc
-Women	0.5 ± 0.9	1.5 ± 2.4	2.3 ± 1.2	1.3 ± 1.4	
Mean heart rate/minute					
-Men	57.6 ± 9.0	63.6 ± 12.0	72.7 ± 15.2	74.5 ± 15.7	abcde
-Women	60.8 ± 9.7	69.5 ± 9.4	79.5 ± 12.8	81.1 ± 11.8	
SDNN (ms)					
-Men	78.1 ± 25.2	94.8 ± 55.9	85.0 ± 451.7	72.1 ± 49.2	abcde
-Women	76.0 ± 28.3	74.6 ± 29.1	52.5 ± 20.8	51.4 ± 28.0	
RMSDD (ms)					
-Men	67.0 ± 31.6	93.2 ± 72.9	76.2 ± 56.4	62.2 ± 54.6	cde
-Women	59.6 ± 32.2	60.8 ± 30.2	37.3 ± 19.5	41.5 ± 32.6	
NN50					
-Men	104.8 ± 38.6	113.0 ± 76.0	105.5 ± 75.2	81.9 ± 64.0	ce
-Women	96.3 ± 54.6	104.8 ± 59.1	54.4 ± 51.4	60.0 ± 65.3	
pNN50 (%)					
-Men	37.8 ± 19.8	39.6 ± 27.8	34.1 ± 25.6	26.5 ± 22.2	cde
-Women	32.8 ± 19.8	32.2 ± 18.8	14.7 ± 14.3	16.4 ± 18.8	

Results of post hoc paired differences with time (altitude) for both men and women: a, sea level vs 3619m; b sea level vs 4600m; c sea level vs 5140m; d, 3619m vs 4600m; e, 3619 vs 5140m; f 4600m vs 5140m

Table 3 Changes Frequency domain measures of Heart rate Variability at sea level to increasing high altitude

	Sea level	3619m	4600m	5140m	Post-test Paired differences for effects of altitude
Total power (ms ²)					
-Men	7.87 ± 0.7	7.95 ± 1.4	7.86 ± 1.6	7.34 ± 1.5	cef
-Women	7.75 ± 0.7	7.73 ± 0.8	7.05 ± 0.7	7.0 ± 1.0	
VLF (ms ²)					
-Men	6.06 ± 0.7	6.11 ± 1.2	6.00 ± 1.3	5.62 ± 1.3	ce
-Women	6.16 ± 0.8	5.92 ± 0.9	5.44 ± 0.7	5.28 ± 1.1	
LnLF (ms ²)					
-Men	6.80 ± 0.8	6.61 ± 1.4	6.62 ± 1.7	6.19 ± 1.4	ce
-Women	6.44 ± 0.7	6.67 ± 1.0	5.89 ± 0.9	5.76 ± 1.0	
LnHF (ms ²)					
-Men	6.55 ± 0.8	6.87 ± 1.7	6.50 ± 2.1	6.09 ± 1.8	e
-Women	6.23 ± 1.0	6.36 ± 0.9	5.47 ± 1.0	5.43 ± 1.3	
LF%, nu					
-Men	55.4 ± 15.9	44.7 ± 18.0	52.7 ± 17.9	52.0 ± 18.8	NS
-Women	44.5 ± 18.8	56.8 ± 15.6	58.8 ± 16.4	56.8 ± 20.6	
HF%, nu					
-Men	44.6 ± 15.9	55.4 ± 18.0	47.3 ± 17.6	48.0 ± 18.8	NS
-Women	45.7 ± 18.4	43.2 ± 15.6	41.2 ± 16.4	43.2 ± 20.6	
LF / HF					
-Men	1.7 ± 1.3	1.0 ± 0.80	1.6 ± 1.5	1.6 ± 1.5	NS
-Women	1.4 ± 1.1	1.6 ± 1.0	2.1 ± 1.8	2.2 ± 2.2	

NS, Non-significant; Results of post hoc paired differences with time for men and women combined: a, sea level vs 3619m; b sea level vs 4600m; c sea level vs 5140m; d, 3619m vs 4600m; e, 3619 vs 5140m; f 4600m vs 5140m

Table 4 Results of Two-way Repeated Measures ANOVA comparing the main effects of altitude (SL, 3619m, 4600m and 5140m) and sex (men vs women) on measures of heart rate variability

	Time (altitude)		Sex		Interaction	
	F	P value	F	P value	F	P Value
SpO ₂	165.20	<0.001 ^{abcef}	0.49	0.48	0.21	0.89
Lake Louise Scores	4.30	0.008 ^{abc}	0.37	0.55	0.63	0.60
Mean heart rate	47.3	<0.001 ^{abcde}	4.10	0.04	0.43	0.73
SDNN	40.6	<0.001 ^{abcde}	4.00	0.04	1.14	0.26
RMSDD	8.10	<0.001 ^{cde}	4.40	0.04	0.94	0.43
NN50	8.20	<0.001 ^{ce}	3.20	0.08	0.82	0.47
PNN50	10.7	<0.001 ^{cde}	3.50	0.06	0.94	0.41
SD1 / SD2	1.89	0.13	4.90	0.03	0.34	0.80
Total power	8.40	<0.001 ^{cef}	4.20	0.04	1.87	0.14
VLF	7.10	<0.001 ^{ce}	2.00	0.17	1.76	0.16
LF	7.10	<0.001 ^{ce}	3.80	0.048	1.70	0.17
HF	5.80	0.001 ^e	5.80	0.02	1.26	0.29
LFnu	0.39	0.76	3.10	0.09	1.70	0.17
HFnu	0.39	0.76	3.10	0.09	1.70	0.17
LF / HF	0.50	0.70	1.60	0.20	3.50	0.07

SpO₂, oxygen saturations; Results of post hoc paired differences with time (altitude) a, sea level vs 3619m; b sea level vs 4600m; c sea level vs 5140m; d, 3619m vs 4600m; e, 3619 vs 5140m; f 4600m vs 5140m

Figure 1 Comparative Changes in the RMSSD (mean \pm SEM) among men and women at sea level and increasing high altitude. Post-test differences on repeated measures ANOVA: * *versus* sea level, ‡ 3619 vs 5140m, †3619 vs 4600m.

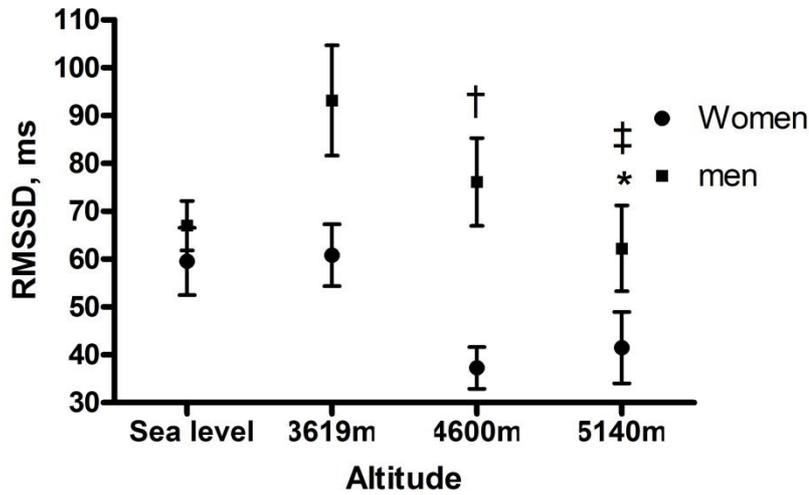
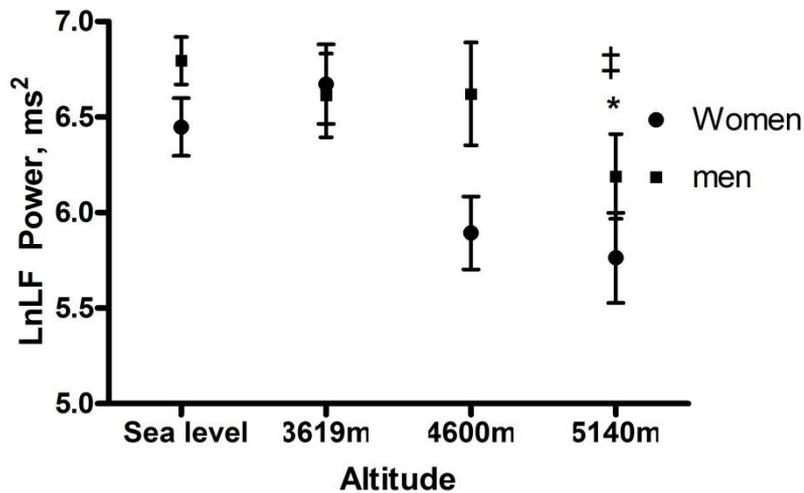


Figure 2 Comparative Changes in Low frequency (LnLF) Power (mean \pm SEM) among men and women at sea level and increasing high altitude. Post-test differences on repeated measures ANOVA: * *versus* sea level, ‡ 3619 vs 5140m.



Chapter 8

Publication 6

Comparison of Spontaneous Versus Paced breathing on Heart rate Variability at High Altitude

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Comparison of Spontaneous *Versus* Paced breathing on Heart rate Variability at High Altitude

Running title: heart rate variability at high altitude

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Key words Perceived effort, acute mountain sickness, autonomic, parasympathetic

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Abstract

Introduction

There is conflicting data at sea-level to suggest that paced (PB) *versus* spontaneous breathing (SB) during short-term heart rate variability (HRV) measurement improve data reliability.

Aims

This study sought to examine the effects of SB *versus* PB on HRV, at high altitude (HA).

Material and Methods

This was a prospective observational study on thirty healthy adult men who were investigated over nine days at altitudes of 800-4107m. Cardiac inter-beat interval data were measured over 55 seconds, twice daily, using an athlete finger sensor linked to a mobile phone to generate an HRV score. Agreements in the paired (SB vs PB) HRV scores were examined using paired t tests, correlation coefficients and F-Testing. A factorial Repeated Measures ANOVA was used to examine the main effect of altitude and breathing method on the paired differences in HRV Scores

Results

HA led to a significant reduction in SpO₂ and increase in acute mountain sickness (AMS) Scores. HRV scores (511 paired scores) were consistently higher with PB *versus* SB (mean difference +6.0; 96.1% within 95% agreement limit), though the variance was lower (F = 1.2; P=0.04) and the scores strongly correlated (r=0.78; p<0.0001). HRV scores were lower with AMS (*versus* without AMS), but this difference was only significant with SB (68.1±12.1 vs 74.3±11.4 vs; p=0.03) but not PB (76.3±11.8 vs 80.3±10.4 vs; p=0.13). There was a significant main-effect for altitude (F=5.3; p<0.0001) and breathing (F=262.1; p<0.0001) on HRV scores but no altitude-x-breathing interaction (F=1.2; p=0.30).

Conclusion

Athlete HRV scores obtained with PB and SB strongly correlate at moderate HA but are consistently higher and the variance lower with PB. Whilst the actual per se does not affect this difference, the presence of AMS may be an important confounder.

Introduction

Heart rate variability (HRV) refers to the temporal changes in the beat-to-beat intervals in the heart, which is subject to continuous autonomic nervous system (ANS) and competing sympathetic *versus* parasympathetic control. HRV assessment has rapidly progressed in recent years from being predominantly a research-based tool to its translational use across several mainstream clinical and sporting applications [59, 61, 250]. These include the prevention of overtraining in relation to physical performance and in the management of mental stress [58, 59, 61, 251, 252]. The miniaturisation and increased portability of HRV equipment with the availability smartphone-based platforms has significantly helped in this regard.

There is now a plethora of available HRV measures of varying complexity, however it is some of the short-term (≤ 5 minutes) measures that have provided the greatest use in day-to-day practice [58, 250-252]. One of the most utilized HRV parameters is the RMSSD (root mean square of successive differences), which can be obtained from ultrashort beat-to-beat recordings of less than two minutes [59, 60, 93, 253]. It is thought to be a non-invasive surrogate for parasympathetic activity and vagal tone [60, 250].

One of the areas of novel clinical interest in HRV assessment has been in the field of high altitude (HA) medicine. HA related hypoxia leads to a compensatory rise in respiratory rate and tidal volume, known as the hypoxic ventilatory response, which acts to preserve tissue oxygenation [10, 254]. This physiological response may be of considerable practical importance in relation to HRV measurement at HA, given the recognized influence of respiratory rate and tidal volume (ie minute ventilation) on HRV and its measurement [255].

An area of ongoing debate is the issue of whether controlled/paced breathing during HRV measurement generates more reliable HRV data than with relaxed and regular spontaneous breathing [166, 256, 257][14-18]. The comparative effects of spontaneous *versus* paced breathing on HRV have never been examined at HA. Establishing their potential influence on HRV at HA is a crucial methodological consideration. It plausible to hypothesize that by enforcing a paced breathing pattern genuine changes in HRV appreciated with the physiological hyperventilation of HA, could be obscured [258]. Furthermore, the ventilatory influence of HA-related illnesses such as HA pulmonary oedema (HAPE), and acute mountain sickness (AMS) on HRV could also be potentially mitigated by the use of paced breathing during HRV measurement. This issue assumes even greater importance given several recent publications suggesting a potential link between changes in HRV and AMS development [162, 165, 187, 252]. The published HRV at HA studies to date have mainly utilized spontaneous breathing during HRV measurement and the comparative effects of spontaneous *versus* paced breathing has not been examined [90, 91, 150, 162, 163, 165].

In this study we aimed to assess, for the first time, the effect of spontaneous versus paced breathing on HRV at HA as well as the influence of AMS on their level of agreement.

Materials and Methods

This was a prospective observational study conducted over nine days on 30 healthy British military servicemen trekking in the Bernese Alps in Switzerland in June 2017. Baseline data was collected at 800m (basecamp; days 0-1). On day 2 the participants moved by road to 1200m then on foot to Blumlisalputte (2840m) over 4 hours carrying a weight of 15kg. There they spent three days (days 2-5) with training serials on a nearby glacier before returning to basecamp (day 5). At basecamp the subjects were split into three groups. Team 1 (n=9) remained at Basecamp (800m) until day 9 (pm). Team 2 (n=12) went to Mönchsjoch

Hut (3658m) by train and then light trek over the last one hour. From there they climbed to the 4107m over days 5-8 before returning to basecamp (day 8) where they stayed till the end of the data collection (day 9). Team 3 (n=9) travelled by road followed by a 4 hour trek to 2543m. From there they climbed to 3583m on days 5-8, before returning to basecamp (days 8-9). Measurement of HA-symptoms, HRV and heart rate were measured twice daily. Saboul et al observed significant differences in short term daily measures of HRV including RMSSD and heart rate between spontaneous and controlled breathing in 10 healthy subjects [255]. Based on this data and an expected correlation between pairs of >0.70 we calculated that at sample size of >20 subjects measured twice daily over at least 7 days (>280 paired samples) would have $>90\%$ power to detect a mean difference in HRV score of ≥ 1.5 .

Physiological and Physical assessments

HA-related symptoms were recorded using the Lake Louise Scoring (LLS) system. AMS was defined as LLS of ≥ 3 in the presence of headache and a recent altitude gain [28, 38]. The Borg Rating of Perceived exertion was recorded (Borg) at the end of each day. This is a 15 point numerical scale numbered from 6 – 20, with values of 6 representing the resting state and 20, exhaustive exercise (Borg 1970) that has been used at HA previously [28]. The highest Rating of perceived exertion (RPE) during the day was recorded to reflect the overall effort [259].

Assessment of Heart Rate Variability

This was performed twice daily post micturition and prior to breakfast/dinner or caffeine. Conditions were kept consistent with previous studies of HRV at HA with all subjects being seated in a covered environment, wearing warm clothing for at least five minutes before the HRV recordings were obtained [90, 91]. HRV variability was obtained using a finger sensor

attached to mobile phone (Apple™ iPhone 6s) installed with the ithlete HRV app (HRV Fit Ltd. Southampton, UK) as previously described and validated [91, 94, 150, 187, 260]. Two consecutive 55 second HRV recordings were obtained separated by a one minute wash out rest period. This validated time period is set by the device and cannot be altered. The first HRV reading was undertaken with the subjects breathing spontaneously, after 5 minutes of relaxation. The second HRV measurement was recorded during paced breathing at frequency of 7.5 breaths per minute. This guided breathing protocol within the ithlete mobile phone app is delivered via visual onscreen prompts to guide the speed and duration of both inspiration and expiration [91, 94, 150, 260].

The ithlete™ HRV score modifies the acquired RMSSD (root mean squared of successive differences) by taking the natural log transformation and multiplying by twenty ($\ln\text{RMSSD} \times 20$). This provides a more interpretable figure for the user on a ~100 point scale [94, 260]. In a previous study of 12 healthy subjects studied over seven altitudes from the 1400-3600m the coefficient of variation for paired HRV readings using paced breathing was 5.5% [91].

Statistical Analysis

Sample size calculations were performed using a proprietary determined sample-size calculator (GraphPad StatMate version 2.00 for Windows). Data was analysed using GraphPad InStat version 3.05 and with all graphical figures presented using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com). Data inspection and the Kolmogorov-Smirnov test was undertaken to assess normality of all continuous data. Results are presented as mean \pm standard deviation (SD). Paired comparisons of parametric and non-parametric continuous data were assessed using a paired t test and a Wilcoxon matched pairs test and their correlation using Pearson and Spearman correlation coefficients \pm 95% confidence interval (CI) respectively. Only correlations with

an $R > 0.20$ were reported. An F-Test was performed to assess whether any potential differences in standard deviations in HRV scores between spontaneous *versus* paced breathing were significant.

The accuracy of agreements in HRV Scores between that obtained from spontaneous versus paced breathing were assessed using Bland-Altman plots [91, 261] in which the difference between the two values were compared with the average values from the comparative two readings. The bias was defined as the mean \pm standard deviation [SD] of the difference between the readings. Reasonable agreement was defined as $<5\%$ of readings being within $1.96SD$ (95% CI) from the mean.

A factorial Repeated Measures ANOVA was undertaken to assess the main effects of altitude and breathing (spontaneous vs paced) on HRV scores and any potential interactions of altitude and breathing method on the paired differences in HRV Scores. A p value of <0.05 was considered significant for all comparisons.

Results

The average age of the 30 included participants was 33.3 ± 7.7 years with an age range of 22-54 years. They were all Caucasian and non-smokers with a mean body mass index of $26.0 \pm 2.3 \text{ kg/m}^2$. There was a significant reduction in SpO_2 and an increased in LLS and heart rate at higher altitudes of $\geq 2543\text{m}$ (Table 1). There was an inverse correlation between SpO_2 and LLS ($r = -0.25$; -0.33 to -0.16 ; $p < 0.0001$). The prevalence of AMS was 13.3% at 2840m, 22.2% at 2543-3658m and 41.7% at 3583-4107m. The cases AMS were generally mild ($>90\%$ LLS 3-5).

There were a total of 511 paired HRV scores obtained. Overall, HRV scores obtained from spontaneous *versus* paced breathing strongly correlated (511 pairs; $r=0.79$: 0.75-0.82; $P<0.0001$) (Figure1). This significant correlation was consistent at both lower ($<2543\text{m}$) altitudes and higher altitudes ($\geq 2543\text{m}$). There was significant inverse correlation between heart rate and the HRV score ($r=-0.68$; -0.73 to -0.63; $P<0.0001$). Borg RPE scores positively correlated with resting heart rate ($r=0.26$; 0.13 to 0.38; $P<0.0001$) and inversely with HRV Score ($r=-0.30$; -0.42 to -0.17; $P<0.0001$) measured at the same time.

The HRV scores were consistently higher with paced *versus* spontaneous breathing (73.3 ± 11.4 vs 80.1 ± 10.4 ; mean difference +6.1; $P<0.0001$) (Table 1, figure 2.). The standard deviation around HRV scores was marginally, yet significantly higher, for spontaneous *versus* paced breathing ($F = 1.2$; $P=0.04$). HRV scores were lower in those with vs without AMS for both breathing methods. However, this difference was only significant for spontaneous (74.3 ± 11.4 vs 68.1 ± 12.1 ; $p=0.03$) but not paced breathing (80.3 ± 10.4 vs 76.3 ± 11.8 ; $p=0.13$).

On Bland Altman analysis identified a strong level of agreement between HRV scores (511 pairs: 96.1% within the 95% limit of agreement) obtained with spontaneous *versus* paced breathing but with a consistent bias to higher scores with paced breathing (mean difference [bias] +6.00; 95% CI -8.0 to 20.1) (figure 3). This bias further increased when only those ($n=15$) with AMS were examined (93.8% within 95% limit of agreement; bias + 8.0: 95% CI -5.5 to +22.0).

There was a significant main effect for altitude ($F=5.3$; $p<0.0001$) and breathing ($F=262.1$; $p<0.0001$) on the paired HRV scores. However there was no altitude-x-breathing interaction ($F=1.2$; $P=0.30$). For the dependent variable of heart rate there was a main effect for altitude

($F=7.0$; $p<0.0001$) and breathing ($F=10.7$; $P=0.001$) but again no altitude-x-breathing interaction ($F=0.60$; $P=0.70$).

Discussion

This is the first study to assess the comparative effects of paced *versus* spontaneous breathing on short term measures of HRV at sea level and HA. We found that there was a strong agreement between the two methods. HRV scores were consistently higher, though less variable with PB. These differences increased with AMS. Whilst there was a significant main effect for altitude and method of breathing on HRV scores there was no overall interaction between altitude and breathing method on HRV scores. The RPE inversely correlated with HRV, with higher perceived exertion being linked to lower HRV.

The influence of ventilation on heart rate and HRV has been well investigated at sea level [52, 166, 169, 255-257]. The concept behind using paced breathing during HRV assessment is that by standardizing the breathing pattern (respiratory rate, tidal volumes, inspiratory and expiratory time) the inter-sampling variability between HRV measurements should be reduced and validity improved. Published comparative data on differences in HRV measurements between spontaneous *versus* paced breathing have yielded inconsistent results. This variability may, in part relate to relative differences in environmental conditions, the duration and frequency of paced breathing used and the HRV parameters examined [255, 256, 262]. These disparities probably explain why a consensus guideline on paced breathing during HRV measurement has not yet been established.

The effects of breathing method during HRV measurement at HA had not previously investigated, despite an increasing number of published studies in this environment, which was the impetus for this study. Increasing minute ventilation is one of the most consistent

physiological effects of HA exposure and acclimatization [10, 254]. Hypoxic stimulation of arterial chemoreceptors leads to compensatory hyperventilation in order to limit the fall in alveolar PO₂ and the degree of arterial hypoxaemia [10]. Despite these factors we still observed a strong correlation in the athlete HRV scores ($r=0.79$) between spontaneous and paced breathing over several days at variable altitudes. This degree of correlation is remarkably similar to the comparative published sea level data (correlation coefficients of ≥ 0.70) [255].

The paced breathing rate generated by the athlete app was fixed throughout our study, whereas it was not controlled during spontaneous breathing. Respiratory rate and minute ventilation (respiratory rate x tidal volume) are known to rise in response to the worsening hypoxia at increasing HA [10, 254]. We anticipated that this would lead to greater relative differences in the minute ventilation between spontaneous and paced breathing at higher altitudes during HRV measurement: as whilst the spontaneous respiratory rate would be expected to increase the paced rate, would obviously remain unchanged at 7.5 breaths per minute. Given the recognized influence of respiratory rates on HRV, this should have translated into greater discordance in HRV scores between the two breathing modes at higher altitudes, yet this phenomenon was not observed. Although we noted a significant main effect for altitude and breathing modality (higher HRV score with paced breathing), there was no altitude-x-breathing interaction on HRV scores at HA. This would suggest that paced breathing does not appear to negate the HA related changes in the athlete HRV score and RMSSD. However, given that the higher HRV scores were with paced breathing, the two methods cannot be used interchangeably.

HRV scores tended to be lower with AMS (*versus* without) and inversely correlated with increasing RPE, which is an expected finding and consistent with previous research [52, 91, 95, 162, 163]. Nevertheless, it is interesting that whilst this difference was observed for both

spontaneous and paced breathing this difference was only statistically significant for spontaneous breathing. This might be a chance effect or related to bias due to the smaller sample size for the AMS group. A further exploration of comparative HRV measurements at higher altitudes and with a greater AMS burden and severity is clearly warranted. This is needed to better determine whether paced breathing could act to mitigate some genuine changes in HRV with very HA and significant AMS, by the implementation a short period of forced and unnatural breathing at HA.

We only assessed one measure of HRV obtained over an ultrashort recording time using the ithlete validated HRV protocol [91, 167]. This device was specifically selected as it is battery operated (via the mobile phone charge), portable, user friendly and affordable which is ideal for HA research and maximizes its translational potential [94, 263]. Its HRV score is derived from the RMSSD which is strongly correlated with other time domain measures of HRV [7,23]. The ithlete HRV score is thought to reflect parasympathetic control and is thus highly responsive to acute changes in vagal tone and may be useful as a marker of HA acclimatization [10, 94, 150]. It has been shown that HA acclimatization is associated with changes in parasympathetic output and the withdrawal of cardiac vagal modulation has been implicated in AMS development [90, 264].

The order of the breathing in the protocol was fixed throughout this study with spontaneous breathing always preceding paced breathing. This sequence was chosen for two important reasons. Firstly this order is consistent with several other published comparative studies [166, 169, 257, 258]. Secondly, this sequence is logistically easier and makes physiological sense as the first 55 second HRV measurement during spontaneous breathing was conducted immediately after five minutes of spontaneous breathing. We felt that reversing this order could lead to negative bias. A parallel study of age matched groups randomized to

spontaneous then fixed breathing and vice versa would have been ideal but would have required a larger sample size.

Limitations

This study has a number of limitations that should be acknowledged. The altitudes studied were modest, the incidence of AMS was relatively low and the majority of AMS were very mild (predominantly LLS 3-4), which may have limited the impact of our finding. We only measured one measure of short term HRV using the RMSSD derived athlete HRV score obtained over only 55 seconds. Hence we cannot be certain of applicability of these findings to other HRV measures obtained over a longer recording period (>1 minute). We did not measure the comparative respiratory rates and tidal volumes of the two breathing strategies, hence we do not know for certain what their true differences were.

In conclusion we found that the athlete HRV score measured using spontaneous breathing was strongly correlated with that for paced breathing at moderate HA. However the score was consistently and significantly higher and the variance lower with paced breathing. Whilst the relative differences in HRV with spontaneous and paced breathing were not affected by altitude, the presence of AMS may be an important confounder. Further data at higher altitude and with a greater of AMS would be useful to further explore this finding.

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Compliance with Ethical Standards:

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Conflict of Interest: All authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

1 **Table 1 The effects of High altitude on HRV, Physiological and Mountain sickness scores.**

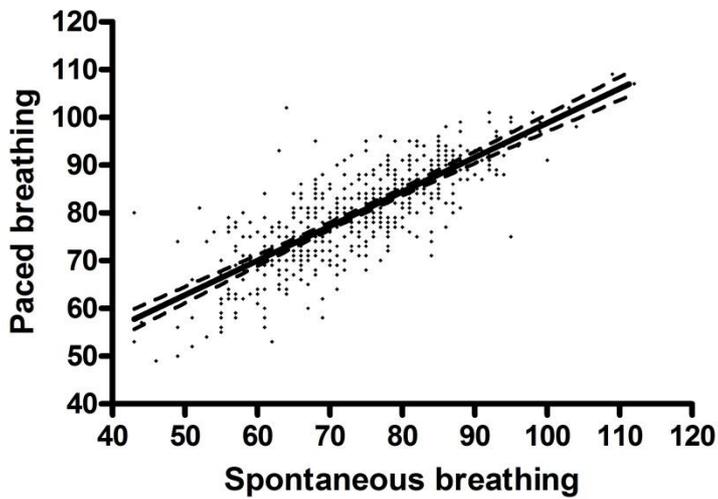
Altitude	800m	2840m	800m	2543 - 3658m	3583- 4107m	800m	P Value
HRV Score							
-Spontaneous breathing	75.2±10.8	74.1±12.2	71.3±10.6†	75.5±11.4	72.2±10.9	78.8±9.9†	0.0006
-Paced breathing	80.9±10.1*	81.1±10.6*	76.5±10.2†*	81.0±10.3*	78.8±10.3*	84.1±8.9†*	<0.0001
Heart rate							
-Spontaneous breathing	65.4±8.5	70.1±11.3†	72.9±10.6†	69.3±8.6†	70.6±8.4†	65.1±10.1	<0.0001
-Paced breathing	65.3±8.8	69.3±10.7†	71.8±10.2†	68.6±8.7†	69.1±8.2†	64.7±8.8	<0.0001
SpO ₂	95.9±2.6	91.9±3.0†	96.0±1.8	89.8±3.3†	89.9±4.3†	96.6±2.2	<0.0001
Lake Louise Score	0.21±0.6	1.0±4.3†	0.22±0.7	1.2±1.5†	1.3±1.4†	0.30±0.60	<0.0001

†Significance vs baseline on Post-test; *Significant paired differences between spontaneous vs paced breathing

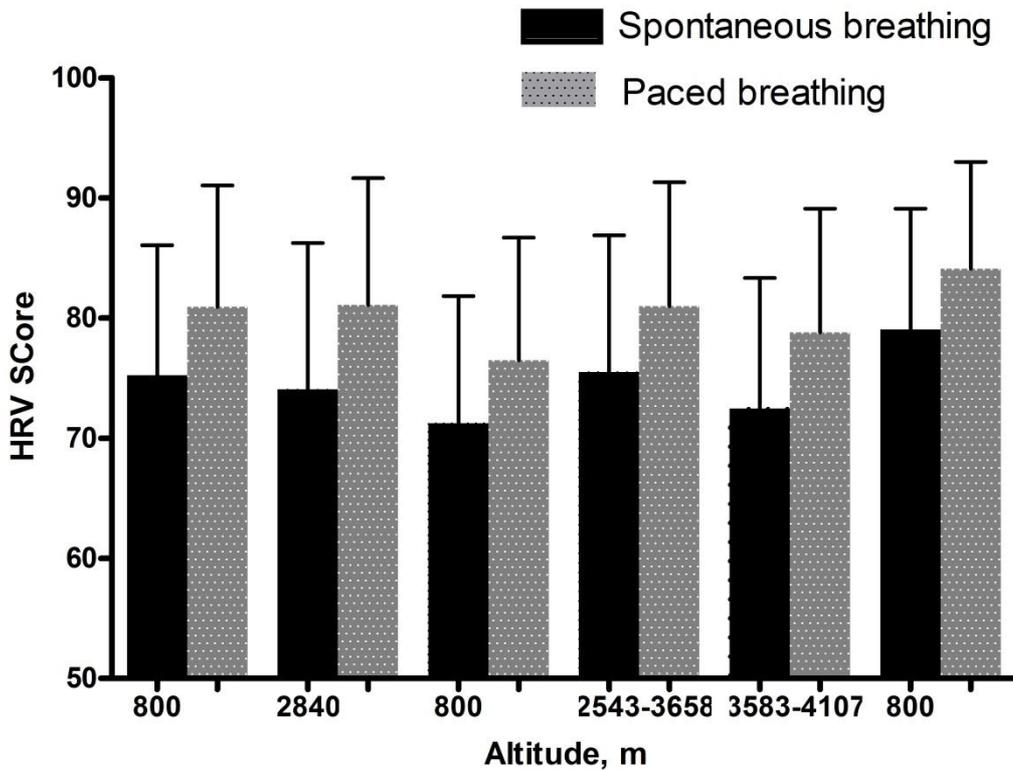
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4 Figure 1 Linear regression (95% CI) of comparative HRV scores obtained with
 5 spontaneous *versus* paced breathing.

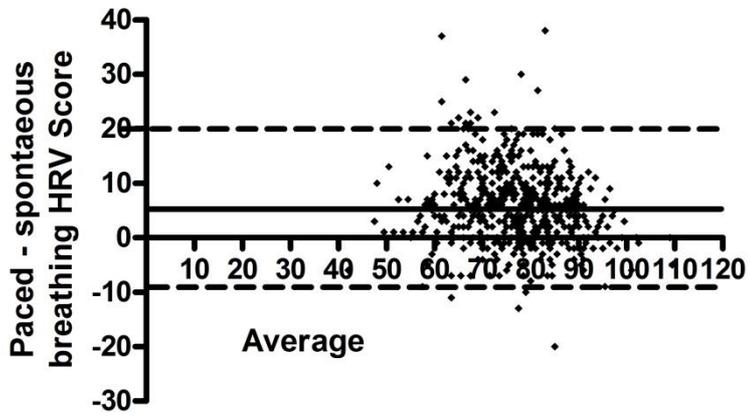


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 7 Figure 2 Comparison of relative HRV scores (mean± standard deviation) with each
 8 altitude for spontaneous *versus* paced breathing



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10 Figure 3 Comparison of HRV scores obtained by spontaneous *versus* paced breathing over
11 all time points: differences (Y axis) are compared with the average HRV score



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Chapter 9

Publication 7

High Altitude Affects Nocturnal Non-linear Heart Rate Variability:

PATCH-HA Study

Authors

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34 **High altitude affects Nocturnal Non-Linear Heart rate Variability: PATCH-HA Study**

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50 **Key words** heart rate variability, high altitude, cardiac patch, acute mountain sickness, non-

51 linear, rating of perceived exertion

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56

57

Abstract

58 **Background**

59 High altitude (HA) exposure can lead to changes in resting heart rate variability (HRV), which
60 may be linked to acute mountain sickness (AMS) development. Compared with traditional HRV
61 measures, non-linear HRV appears to offer incremental and prognostic data, yet its utility and
62 relationship to AMS have been barely examined at HA. This study sought to examine this
63 relationship at terrestrial HA.

64

65 **Methods**

66 Sixteen healthy British military servicemen were studied at baseline (800m, first night) and over
67 eight consecutive nights, at a sleeping altitude of up to 3600m. A disposable cardiac patch
68 monitor was used, to record the nocturnal cardiac inter-beat interval data, over one hour (0200-
69 0300 hours), for offline HRV assessment. Non-linear HRV measures included Sample entropy
70 (SampEn), the short (α_1 , 4-12 beats) and long-term (α_2 , 13-64 beats) detrend fluctuation
71 analysis slope and the correlation dimension (D2). The maximal rating of perceived exertion
72 (RPE), during daily exercise, was assessed using the Borg 6-20 RPE scale.

73 **Results**

74 All subjects completed the HA exposure. The average age of included subjects was 31.4 ± 8.1
75 years. HA led to a significant fall in SpO_2 and increase in heart rate, LLS and RPE. There were
76 no significant changes in the ECG-derived respiratory rate or in any of the time domain
77 measures of HRV during sleep. The only notable changes in frequency domain measures of
78 HRV were an increase in LF and fall in HFnu power at the highest altitude. Conversely,
79 SampEn, SD1/SD2 and D2 all fell, whereas α_1 and α_2 increased ($p < 0.05$). RPE inversely
80 correlated with SD1/SD2 ($r = -0.31$; $p = 0.002$), SampEn ($r = -0.22$; $p = 0.03$), HFnu ($r = -0.27$;
81 $p = 0.007$) and positively correlated with LF ($r = 0.24$; $p = 0.02$), LF/HF ($r = 0.24$; $p = 0.02$), α_1

82 ($r=0.32$; 0.11 to 0.49; $p=0.002$) and α_2 ($r=0.21$; $p=0.04$). AMS occurred in 7/16 subjects (43.8%)
83 and was very mild in 85.7% of cases. HRV failed to predict AMS.

84

85 **Conclusions**

86 Non-linear HRV is more sensitive to the effects of HA than time and frequency domain indices.
87 HA leads to a compensatory decrease in nocturnal heart rate variability and complexity, which is
88 influenced by the RPE measured at the end of the previous day. HRV failed to predict AMS
89 development.

90 **Introduction**

91 High altitude (HA) exposure leads to a number of well recognized physiological responses under
92 hypobaric hypoxia [10]. These include increases in resting minute ventilation and pulmonary
93 artery systolic pressure [10]. Resting cardiac output increases which is principally driven by a
94 rise in resting heart with little change in stroke volume [72].

95

96 The influence of HA on the changes in cardiac inter-beat intervals (IBI), known as heart rate
97 variability (HRV), has been an area of significant recent research interest [90, 91, 150, 162,
98 163]. This attention relates, in part, to the fact many of the factors that are known to affect HRV
99 (eg fatigue, stress, insomnia, hypoxia and cold) are predominant at HA [10, 251, 252]. The
100 improved portability and reduced cost of HRV recording equipment has helped to create new
101 research opportunities at HA, that were previously untenable. Cardiac patch monitoring
102 represents a significant advance in this regard. Patch monitors can non-invasively and accurately
103 measure the cardiac IBIs, whilst negating the need for intrusive chest straps or electrocardiogram
104 cables that are prone to interference and detachment. Despite these advantages, their utility to
105 assess HRV at HA has not been examined.

106

107 There is evidence to suggest that HA exposure is associated with significant changes in HRV
108 compared with sea-level/low altitude [90, 91, 150, 162, 163]. However, the majority of the
109 published data relate to short-term HRV recordings (1-5 minutes) obtained conducted in hypoxic
110 chambers during 'simulated' rather than genuine terrestrial HA [265-268]. The hypoxic period
111 examined has been generally brief (minutes to <8 hours) with a tendency to assess at a single
112 HA [165, 269] leading to an under appreciation of the influence of acclimatization on HRV.
113 Furthermore, despite the known influence of sleep on autonomic function, there has been a
114 distinct lack of research into nocturnal HRV at HA [270, 271].

115

116 Despite these research limitations there is some, albeit limited data, supporting a potential link
117 between changes in HRV at HA and acute mountain sickness (AMS) development [90, 162, 163,
118 165]. However, there are marked inconsistencies in the published results. This may relate to the
119 heterogeneity, in the methods used to assess HRV, the HA environment (terrestrial *versus*
120 simulated; severity and duration of hypoxia), exercise intensity and in the populations studied
121 [38, 91].

122

123 There is an increasing appreciation that traditional time and frequency domain measures of HRV
124 that have dominated the literature, provide an incomplete representation of the complexity of IBI
125 variability and autonomic balance [167]. Consequently, a number of non-linear measures of
126 HRV have emerged that provide incremental and prognostic data [167]. To date these
127 parameters have barely been examined at terrestrial HA [272].

128

129 In this study we aimed to assess, for the first time, the use of a cardiac patch monitor to assess
130 both linear and non-linear measures of HRV at terrestrial HA.

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132

133 **Materials and Methods**

134 *Subjects*

135 Sixteen healthy British military servicemen, undergoing military training in the Bernese Alps,
136 were studied (the ascent profile is shown in figure 1). All participants arrived by road to their
137 800m basecamp, where they spent their first night. Thereafter, their 2nd and 3rd nights were at
138 2840m (accessed by road to 1200m then on foot over 4 hours carrying a weight of 15kg). During
139 their days at 2840m they underwent training serials on a nearby glacier. Their 4th night was spent
140 back at the 800m basecamp. Following this they were split into two equal groups of eight
141 participants with one group of eight spending their 5th and 6th nights in huts at 3658m and the
142 other at 2543m. Those based at 3658m underwent a daytime HA acclimatization climb to
143 4100m, whereas those at 2543m climbed to 3583m. Both groups descended to their huts to
144 sleep. All subjects spent their 7th and 8th nights back at basecamp (800m) where they stayed till
145 the end of the data collection (day 9). The subjects slept in sleeping bags in tents at 800m and in
146 beds with sleeping bags in huts at the higher altitudes.

147

148 *Physiological and Physical assessments*

149 Assessment of SpO₂ and HA related symptoms were measured, during seated rest, in the early
150 morning at each altitude. HA-related symptoms were recorded using the Lake Louise Scoring
151 (LLS) system [29]. This allocates a symptom score ranging from 0 (none) to 3 (severe) to the
152 following five symptoms: difficulty sleeping, gastrointestinal symptoms, fatigue/weakness,
153 dizziness/lightheadedness and headache. A LLS of ≥ 3 in the presence of headache and a recent
154 altitude gain was used to define AMS as previously described [29, 91]. Mild AMS was defined
155 as the presence of AMS and a LLS of 3-4 and severe when the LLS was ≥ 5 [29, 91]. The rating
156 of perceived exertion during the day (sessional RPE) was assessed at the end of each day, using
157 a 6-20 scale as previously described [38, 259, 273].

158

159 *Assessment of Heart Rate Variability*

160 Continuous cardiac IBIs for each subject were recorded using a lightweight (6 grams) disposable
161 120 x 42 x5 mm adhesive patch monitor (Healthstrip, LumiraDx; [274] that was provided for
162 this study free of charge. It was made available for investigational use in this study and is
163 currently awaiting a CE mark. The Healthstrip contains two internal hydrogel electrodes (Figure
164 2). The patches were placed following simple skin preparation (shaving and an alcohol wipe).
165 The subjects wore their patches continuously throughout the study period and the patches were
166 replaced as necessary. The patches were placed over the 2nd to the 4th intercostal space at angle
167 of 45 degrees (towards the cardiac apex). The data was stored on the patch monitor prior to daily
168 data Bluetooth transfer to an iPhone 6s smartphone. This coded cardiac data were then securely
169 transferred using Wi-Fi for later HRV analysis.

170

171 HRV was assessed over a one hour nocturnal cardiac recording period from 0200 to 0300 hours
172 during sleep each of the eight consecutive nights. The Healthstrip also records physical activity
173 and body position (upright, supine on back, lying on side) by a movement sensor, which
174 supports the confirmation of genuine sleep, which was documented as an activity
175 percentage/minute. A 36 second full disclosure single lead ECG (Sampling frequency 250Hz)
176 was additionally recorded over the middle 36 seconds (0230 hours) of each one hour.

177

178 HRV analysis of the exported nocturnal one hour IBI data from the Healthstrip were performed
179 on desktop computer using dedicated HRV software (Kubios® Premium ver. 3.0.2;
180 <http://www.kubios.com/>) as previously described [275]. Prior to HRV computation all IBI data
181 were visually inspected for correctness and then underwent automatic artefact correction. The
182 default sample length was set to 3600s (one hour) and over the number of IBIs generated in this
183 time frame.

184

185 Time and frequency domain measures of HRV were calculated according to the HRV Task
186 Force Guidelines [60]. The following established time domain measures of HRV were assessed:
187 SDNN (standard deviation of the NN intervals), RMSSD (root mean square of successive
188 differences), pNN50% (the number of NN intervals that differ by >50ms divided by the total
189 number of NN intervals). Prior to calculation of the spectral HRV parameters, the Kubios default
190 smoothness priors detrending was employed (Lambda, λ value=500) as previously described
191 [275, 276]. The IBIs were transformed to evenly sampled time series with 4-Hz interpolation
192 resampling rate. The detrended and interpolated IBIs were used for the frequency-domain HRV
193 analysis. HRV spectra were calculated by using the fast-Fourier-transform (FFT) with Welch's
194 periodogram method (50% overlap window and 300 s window width) as previously described
195 (Gasior et al., 2016). We reported the LF (low-frequency) (0.04-0.15 Hz) and HF (high-
196 frequency) power (0.15-0.40 Hz) and the LF/HF ratio as previously described [60]. Due to
197 skewed distributions, LF and HF power were transformed by natural *logarithms* (ln). In order
198 obtain greater insight into the relative HF power it was also reported in normalized units HF
199 (HFnu) which was calculated as HF/(LF + HF) (Task Force., 1996). Non-linear HRV assessment
200 were examined as previously described [167] using Poincaré plots and the derived ratio of the
201 standard deviation (SD) of long term (SD2) to short term (SD1) variability known as SD1/SD2
202 ratio, Sample entropy (SampEn), the short (α_1 , 4-12 beats) and long term detrend fluctuation
203 analysis (DFA) slopes (α_2 , 13-64 beats) and the correlation dimension (D2) [167]. The Poincaré
204 plot is a scatterplot in which current IBIs are plotted as a function of previous interval. SD1
205 represents the standard deviation of short-term HRV and SD2 (or major axis) the continuous
206 longer term IBIs [60]. The SD1/SD2 ratio measures the unpredictability of the RR time series
207 [277]. SampEn is a measure of the regularity and fluctuation of a time series with lower values
208 representing less complexity and greater self-similarity in a time series [278]. DFA detects the
209 simple correlations between successive RRIs over differing time scales with α_1 reflecting the
210 slope over shorter fluctuations and α_2 over longer time periods [277]. D2 is an estimation of the
211 number of independent variables necessary to describe a systems behavior with a higher value
212 representing greater complexity [167].

213 Calculation of the respiratory rate was obtained by ECG-derived respiration (EDR) software
214 within the HRV analysis package as previously described, using the 36 second Healthstrip ECG
215 recording [279].

216

217 *Statistical Analysis and sample size calculation*

218 Data were analyzed using GraphPad InStat version 3.05 and with all graphical figures presented
219 using GraphPad Version 3.10 (GraphPad Software, San Diego, CA, USA; www.graphpad.com).
220 Data inspection and the Kolmogorov-Smirnov test was undertaken to assess normality of all
221 continuous data. Results are presented as mean \pm standard deviation (SD) for all data.
222 Comparison of continuous data between altitudes was performed using a one-way ANOVA with
223 Tukey posttest and with Kruskal-Wallis test with Dunn-Post test for parametric and non-
224 parametric data respectively. Correlations of continuous data were assessed using the Pearson
225 and Spearman rank correlation coefficients (r) and 95% confidence interval, for parametric and
226 non-parametric data respectively. A two-sided p value of <0.05 was considered as significant for
227 all analyses.

228

229 In a previous, yet recent study, we had observed a significant 11% (7.9ms) change in the
230 RMSSD-derived HRV score between baseline sea level and 3619m in a cohort of 12 persons
231 [91]. Zhang et al recently observed a significant fall in SampEn in eight healthy male subjects,
232 following short term exposure to simulated HA from sea level to 3600 m [280]. Based on this
233 later data, we calculated that a sample size of at least 14 subjects would have sufficient power to
234 examine for differences in RMSSD and a $\geq 80\%$ to detect a difference in mean SampEn of ≥ 0.18
235 at a significance level (α) of 0.05 (two-tailed) (GraphPad Statmate).

236

237

238 **Ethics**

239 All participation was entirely voluntary and all subjects underwent detailed written informed
240 consent >24hours after being sent a participant information sheet for the study. This study was
241 approved by the Ministry of Defence Research and Medical Ethics Committee (MODREC) and
242 was conducted according to the standards of the Declaration of Helsinki.

243

244 **Results**

245 The average age of included subjects was 31.4 ± 8.1 years. They had mean height of 179.8 ± 5.0
246 cm, weight of 84.6 ± 11.0 kg and body mass index of 26.1 ± 2.7 kg/m². All of the participants were
247 non-smokers and were not on any regular medication.

248

249 Compared with baseline, ascent to HA ≥ 2543 m was associated with a significant fall in SpO₂,
250 higher RPE scores, and an increase in average heart rate, LLS and in the average sleep score
251 component of the LLS (table 1).

252

253 The mean number of patches used per subject over the study was $1.94 \pm .25$ (range 1-2), with all,
254 but one subject, requiring two patches. The recorded cardiac IBI data were good quality with an
255 artefact rate of <3% at all altitudes studied, but was significantly higher at higher altitude at
256 ≥ 2543 m *versus* baseline 800m (table 1). There were no significant overall changes in any of the
257 time domain measures of HRV (table 2). The only notable change in frequency domain
258 measures of HRV was an increase in LF power and fall in HFnu at the highest altitude (table 2).
259 There were significant changes in all of the non-linear measures of HRV at HA: SampEn,
260 SD1/SD2 and D2 fell, whereas $\alpha 1$ and $\alpha 2$ increased ($P < 0.05$) (table 3).

261

262 RPE inversely correlated with HFnu, SD1/SD2 and SampEn and positively correlated with LF,
263 LF/HF, α_1 and α_2 , but not the other HRV indices (table 4). Among the non-linear indices only
264 SD1/SD2 inversely correlated with α_1 ($r=-0.87$; -0.91 to -0.81: $p<0.0001$), α_2 ($r=0.28$; - 0.44 to -
265 0.10: $p=0.003$). SampEn inversely correlated with α_2 ($r=-0.27$; -0.44 to 0.01: $p=0.003$) and D2
266 inversely with α_2 ($r=-0.35$; - 0.51 to -0.17: $p=0.0001$).

267

268 Seven out of the sixteen subjects (43.8%) suffered with AMS. These were all self-limiting and in
269 all but one were mild episodes (LLS score 3-4). HRV scores failed to predict AMS.

270

271 **Discussion**

272 This is the first study to assess the utility of a cardiac patch monitor to assess non-linear
273 measures of HRV at HA. We found that non-linear HRV was more sensitive to the effects of HA
274 than traditional time and frequency domain HRV measurements. HA led to a significant fall in
275 SD1/SD2, D2 and SampEn and an increase in α_1 and α_2 . We observed a significant relationship
276 between nocturnal HRV and RPE measured at the end of the previous day. HRV measures failed
277 to predict the development of AMS.

278

279 We chose to examine the effects of HA on several non-linear HRV parameters, given the paucity
280 of data at HA and their potential advantages over established time and frequency domain HRV
281 parameters. Their advantages include its lower sensitivity to the presence of cardiac ectopy,
282 artifacts and to the recording period, which is of greater relevance at HA [281]. The non-linear
283 HRV parameters examined in this study were the SD1/SD2 ratio obtained from Poincaré plots,
284 SampEn, α_1 and α_2 and D2. We found that SampEn was significantly lower at HA at both 2840
285 and 2543-3658m compared with baseline. Conversely the α_1 and α_2 increased from baseline to
286 2480 and 2543-3658m, with a return to near baseline levels at 800m. D2 and SD1/SD2 values
287 were also significantly lower at HA *versus* 800m. These findings suggest that HA to ≥ 2543 m

288 leads to a compensatory change in autonomic balance with increased regularity (lower HRV)
289 and lower complexity and chaos in the cardiac IBI signal [277].

290

291 Our data is largely consisted with that obtained from several recent acute hypoxia studies. In
292 their study of eight healthy men exposed to acute normobaric hypoxia (equivalent to 3600m) for
293 10 minutes, Zhang et al also reported a fall in SampEn, measured over a one minute recording
294 period [280]. Their observed values of α_1 , which was also studied, were similar to our current
295 study and very close to 1.0, but did not change significantly. In another study of ten healthy
296 men, supine HRV was measured during intermittent periods of acute normobaric hypoxia
297 (simulated HA; FIO₂ down to 9.8%) [282]. Again, a significant fall in SampEn was observed,
298 but on this occasion it was associated with a significant increase in α_1 , which is consistent with
299 our data. Due to the brevity of the HRV recording period in their study, α_2 and other non-linear
300 HRV measures were not examined.

301

302 In this study we assessed HRV during sleep at HA. Our interest in specifically examining this
303 period was stimulated by a number of factors. Firstly, it has been well shown that sleep and its
304 stages are associated with marked variability in autonomic modulation of cardiac activity that is
305 typified higher parasympathetic tone during normal non-Rapid Eye Movement Sleep (REMS)
306 and a shift toward sympathetic predominance during normal REMS [52, 270, 283]. Secondly,
307 ventilation, which can have a significant influence on HRV, is affected by HA [60]. Alterations
308 in breathing patterns and even periodic breathing (PB) are a well-established phenomenon at
309 high altitude [24]. PB represents an abnormal ventilatory pattern in which apneas and hypopneas
310 alternate with periods of hyperventilation [24]. 2016). The worsening hypobaric hypoxia at HA
311 leads to compensatory hyperventilation until a point when the arterial PCO₂ (PaCO₂) falls below
312 the threshold required to stimulate breathing leading to either hypopnea or even apnea, followed
313 by the restoration of hyperventilation as the hypoxia worsens and the paCO₂ resets [24]. This
314 phenomenon is subject to marked individual variability, but is generally observed at >2000m

315 [24]. Unfortunately, we were not able to measure ventilation throughout the one hour recording
316 period, but did quantify the ECG derived respiratory rate during part of the HRV recording
317 period. It is highly likely that there were cases of PB and the observed swings in the IBI raise
318 this suspicion. Nevertheless, wary of the confounding effect of sleep stage and potentially PB on
319 HRV we selected a one-hour HRV recording period in preference to a traditional five-minute
320 recording to minimize this potential bias [60].

321

322 By using an adhesive cardiac patch monitor (and avoiding ECG cables and minimizing
323 movement artifact) we were able to overcome the obvious challenges of accurately, yet non-
324 intrusively measuring HRV during sleep at HA. However, poor sleep was still a significant
325 contributor to the total LLS at each altitude in our study. The sleep score component of the LLS
326 was significantly higher at 2840m compared with 800m, indicating perceptually worse sleep at
327 higher altitude (table 1). Whilst reduced HRV with insomnia is a widely accepted concept, it has
328 not been well supported by empirical evidence [284]. Reduced sleep quality and insomnia are
329 common at HA, but its effect on HRV has not been examined. Unfortunately, we were unable to
330 assess the sleep stages during the HRV recording or the total sleep time prior to HRV recording.
331 However, the subjects generally went to sleep before 2300 hours each night and the activity
332 sensor on the Healthstrip confirmed that subjects were supine and largely inactive during the
333 HRV defined nocturnal HRV recording period.

334 The significant, yet modest, relationship between end of day RPE and nocturnal HRV is a novel
335 finding at HA. Our data support the temporal effects of heavy exercise and exhaustion on HRV
336 [271]. Higher RPE appeared to be associated with lower nocturnal HRV and greater LF/HF
337 dominance.

338

339 This study has a number of additional strengths and limitations that should be acknowledged. The
340 fact that we studied three separate terrestrial altitudes, yet included a far larger sample

341 size than the majority of published acute hypoxic chamber studies are obvious strengths. The
342 wide breadth of HRV parameters and physiological measurement examined, allowed for a
343 comprehensive assessment of HRV at HA. Baseline HRV studies were performed at 800m rather
344 than sea level due to practical issues, which could have reduced the effect size. It was not
345 possible to control the subjects sleeping position (eg prone or on side) and their sleeping
346 conditions varied with altitude which may be potential confounders [285]. The altitude studied
347 was modest and the majority of AMS cases were mild, hence we cannot be certain whether our
348 findings would be reproducible at higher altitudes and with worsening AMS severity. The
349 duration and intensity of exercise varied with altitude, which whilst being a relevant confounder,
350 reflects the reality of a real world terrestrial HA venture. We were only able to absolutely
351 confirm the presence of normal sinus rhythm at the time of the 36 second ECG capture. Whilst
352 this does not fully exclude the possibility of arrhythmias at other time points visualization of the
353 IBI data coupled with the altitude and healthy population studied would strongly suggest against
354 the presence of an undetected significant cardiac arrhythmia [96].

355

356 In conclusion this study demonstrated that moderate terrestrial HA exposure leads to significant
357 changes in resting nocturnal non-linear HRV that is typified by increased regularity and lower
358 complexity and chaos of the cardiac inter-beat signal. These changes are influenced by the
359 intensity of exercise over the previous day. Nocturnal HRV was not predictive of AMS.

360

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363 support and for the subjects who undertook what was a very challenging clinical study to
364 perform.

365

366

367 **Author Contributions**

368 CB, LS, CB and KB performed all the experiments. CB, AM, DW, TQ and MS initiated the
369 project. CB, TQ and LS performed the data analysis. All the authors contributed to data paper
370 writing.

371

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374 funded by LumiraDx.

Table 1 Changes in physiological measurements, Lake Louise Scores and rating of perceived exertion (RPE)

Altitude	800m(1)	2840m	800m(2)	2543-3658m	800m(3)	P Value
SpO ₂ , %	96.8±1.2	92.2±2.4	96.1±2.0	90.0±3.3	95.9±3.0	<0.0001 ^{acef}
Mean Heart rate, minute ⁻¹	53.2±7.3	68.8±14.9	68.9±17.9	65.0±13.4	60.8±9.3	0.001 ^{abc}
ECG-derived Respiratory rate	16.7±3.9	17.3±2.0	16.8±3.6	16.9±3.4	16.9±4.6	0.90
Lake Louise total Scores	0.2±0.6	1.0±1.1	0.5±1.2	1.4±1.5	0.4±0.70	<0.0001 ^{acf}
Lake Louise Sleep Scores	0.0±0.2	0.6±0.8	0.0	0.3±0.7	0.1±0.5	<0.0001 ^{ae}
RPE Score	10.8±2.8	11.40±2.6	10.2±3.5	12.4±2.5	10.4±1.9	0.02 ^{cef}

SpO₂, capillary oxygen saturation; RPE rating of perceived exertion (Borg 14-20 score);

Significant post-test differences vs baseline 800m (1): a 2840m; b 800(2) m; c 2543-3658m; d 800(3) m;

e 800(2)m vs 2840m; f 800(2)m vs 2543-3658m

Table 2 Comparative Time and Frequency Domain heart rate variability parameters at high altitude

Altitude	800m (1)	2840m	800(2)m	2543-3658m	800(3) m	P Value
Artefact %	1.1±1.1	2.4±1.8	1.6±1.0	2.6±1.7	1.1±0.7	0.001 ^{acg}
LnSDNN	4.2±0.4	4.3±0.6	4.2±0.7	4.5±0.40	4.0±0.8	0.09
LnRMSSD	4.4±0.5	4.2±0.8	4.2±0.8	4.40±0.60	4.0±0.9	0.45
pNNI%	39.4±18.9	28.9±23.3	21.8±25.1	37.7±18.4	25.4±22.7	0.35
Triangular Index	15.1±3.70	15.1±8.70	16.6±13.3	19.6±11.2	14.2±9.1	0.09
LnLF	7.6±0.6	7.6±1.0	7.5±1.3	8.4±0.8	7.0±1.5	0.004 ^{eg}
LnHF	7.3±1.0	7.1±1.6	7.2±1.6	7.5±1.2	6.8±1.6	0.59
HFnu,%	40.5±15.1	36.4±17.4	43.3±16.3	31.9±16.0	43.3±15.7	0.04 ^{fg}
LF/HF ratio	1.7±1.0	2.8±2.6	1.7±1.2	2.9±2.0	1.6±01.1	0.09

SDNN, standard deviation of normal-to-normal; RMSSD, root mean square of successive differences; pNNI%, Percentage of successive RRs >50ms; LF. Low frequency; HF high frequency; Significant post-test differences: vs baseline 800m (1), a 2840m, b 800(2) m, c 2543-3658m, d 800(3) m; e 800(2)m vs 2840m; f 800(2)m vs 2543-3658m; g 800(3)m vs 2543-3658m

Table 3 Effect of high altitude on Non-linear measures of Heart rate Variability

Altitude	800m (1)	2840m	800(2) m	2543-3658m	800(3) m	P Value
SD1/SD2	0.6±0.1	0.5±0.2	0.6±0.2	0.5±0.2	0.6±0.2	0.02 ^{cf}
Sample Entropy	1.6±0.2	1.4±0.3	1.6±0.3	1.4±0.2	1.7±0.2	0.0004 ^{ace}
DFA α 1	1.0±0.2	1.2±0.3	1.0±0.3	1.1±0.2	0.9±0.2	0.006 ^{acef}
DFA α 2	0.4±0.10	0.5±0.10	0.4±0.1	0.4±0.1	0.4±0.2	0.03 ^{ac}
D2	3.4±1.4	2.1±1.3	2.3±1.5	2.9±1.3	2.1±1.6	0.03 ^{ad}

SD, standard deviation; DFA detrend fluctuation analysis; D2, Correlation Dimension;

Significant post-test differences vs baseline 800m (1): a 2840m; b 800(2) m; c 2543-3658m; d 800(3) m; e 800(2)m vs 2840m; f 800(2)m vs 2543-3658m

Table 4 Correlation between Rating of Perceived Exertion (RPE) and HRV Measures

HRV parameter	Correlation coefficient	95% Confidence interval	P value
HFnu	-0.27	-0.45 to -0.07	0.007
LF	0.24	0.04 to 0.44	0.02
LF/HF	0.24	0.04 to 0.42	0.02
SD1/SD2	-0.31	-0.49to -0.10	0.002
Sample Entropy	-0.22	-0.40 to 0.02	0.03
$\alpha 1$	0.32	0.11 to 0.49	0.002
$\alpha 2$	0.21	01 to 0.40	0.04

LF. Low frequency; HF high frequency; SD, standard deviation

Legend for Figures

Figure 1 Ascent Profile of the subjects. HRV recordings were taken at 0200-0300 each night

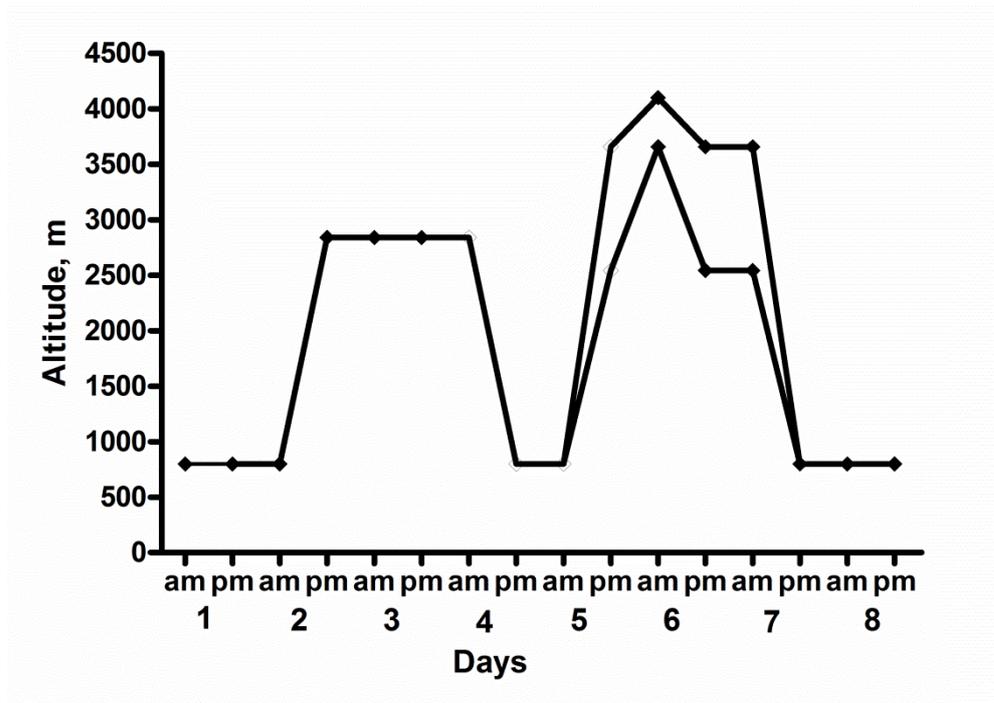


Figure 2 Illustration of the Healthstrip Cardiac Patch Monitor



Chapter 10

Publication 8

Assessment of Cardiac Arrhythmias at Extreme High Altitude Using an Implantable Cardiac Monitor: REVEAL HA Study

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Assessment of Cardiac Arrhythmias at Extreme High Altitude Using an Implantable Cardiac Monitor: REVEAL HA Study

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Key words high altitude, arrhythmias, implantable cardiac monitor

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It has been suggested, though still unproven, that high altitude (HA) exposure is proarrhythmic and could potentially contribute to an increased risk of sudden cardiac death (SCD).^{1,2} However, there are very limited data, particularly above 5000m to substantiate this claim. We hypothesised that extreme HA leads to an increased risk of pathological cardiac tachyarrhythmias, detected using an implantable cardiac monitor (ICM).

Sixteen healthy adult Caucasian male British Military servicemen underwent continuous ECG monitoring, using a Reveal LINQ ICM (Medtronic Ltd), for ≥ 7 weeks before, during and >8 weeks after an attempted summit of Mount Dhaulagiri (8,167 m). They were required to have a normal 12-lead ECG and transthoracic echocardiogram at recruitment and were excluded if they had a history of cardiac arrhythmia. They underwent written informed consent and the study was approved by the Ministry of Defence Research and Medical Ethics Committee. The participants flew from the UK to Kathmandu, Nepal (1400m, days 1-2), then by road (day 3-4) to 2679m. Thereafter, they trekked carrying moderate loads to 3720m (day 5), 4150m (day 7) and 5140m where they remained (days 11-24) for attempts on 6035m and 6800m peaks. One subject aborted at 4100m, due to severe gastrointestinal symptoms. On day 25, five subjects descended and 10 climbers remained at 4800m for an attempted summit of Mount Dhaulagiri over days 26-51(days 26-51). The subjects were monitored wirelessly pre and post-departure (Medtronic MyCareLink™ Monitor) and during trekking using a portable Medtronic Programmer every 2-5 days, depending on environmental conditions.

The subjects were 35.1 ± 6.6 (24-48) years. Fifteen (93.8%) achieved an altitude of ≥ 6035 m, six to 6800m, one to 7100m and three to 7500m. Unfortunately, an attempted summit of Mount Dhaulagiri became impossible, due to adverse weather conditions. SpO₂ significantly fell at increasing HA from $96.4 \pm 1.6\%$ at 1400m to $93.2 \pm 2.8\%$ at 2650m, $88.8 \pm 3.5\%$ at 4100m, $80.6 \pm 5.0\%$ at 5140m and $78.1 \pm 4.5\%$ at 5340 (Ordinary ANOVA $P < 0.0001$). The ICM rhythm-detection settings are shown in table 1 (footnote). Significant rhythm abnormalities were observed in 9 out of 16 subjects (56.3%) at HA and only at ≥ 4100 m. Symptom-related device activation was triggered on 18 occasions in 8/16

subjects at HA and related to extreme breathlessness and palpitations. Subject five developed an episode of nocturnal symptomatic rapid atrial fibrillation (AF) at 4100m, during the initial ascent phase, which occurred immediately after drinking cold water. It lasted for 282 minutes at a mean ventricular rate 133/minute. Subject four, developed an episode of supraventricular tachycardia (SVT) lasting for 30.8 seconds (mean rate 207/ minute). It occurred immediately on attempting to lift a 30kg load, at 5200m and was associated with sudden and transient light-headedness and breathlessness.

Significant pauses (>3 seconds) were identified at HA in 8 out of 15 (53.3%) subjects at HA at $\geq 4800\text{m}$ only, with none detected in any subjects below this altitude (Fisher's Exact Test $p=0.0008$). There were 82 pauses (3.0-7.0s) in total, which were sinus in 80 with evidence of high grade heart block in two cases (mean number 10.3 ± 14.1 ; range 1-41) (table 1). The number of pauses increased with altitude gain from 0 at $<4800\text{m}$ to 4.2 at 4800m and 14.3 at $>6000\text{m}$ (Kruskal-Wallis Test $P<0.0001$) with 19.3 ± 20.6 pauses at 7550m ($n=3$) *versus* 1.9 ± 4.2 among the rest of the subjects ($n=13$; Chi-squared Test $p=0.007$). The number of pauses increased with duration of HA exposure: 6 during first 17 days (tercile, 15-16 subjects), 29 during days 18-34 (10-15 subjects) and 47 (10 subjects) during days 35-51. The pauses typically occurred following cyclical periods of heart rate acceleration then deceleration preceding it.

This is the first study to convincingly demonstrate the pro-arrhythmic risks of significant HA and to the author's knowledge the first to continuously monitor healthy subjects above 6325m at terrestrial HA. In the only previous ICM study at HA nine subjects were studied using first generation Reveal ICM, which lacked auto-detection capabilities and only two subjects were assessed at 6325m.¹ They observed one short-lived episode of atrial flutter at 150/minute (8.5 minutes) immediately after a severe exertion at 4500 m. The episode of nocturnal AF detected in our study would suggest vagally-mediated AF. We would also postulate that the pauses observed in our study were likely physiological and also related to the effects of increased nocturnal vagal tone and sleep-disordered breathing which

are well recognised at HA.^{3,4} We believe the episode of SVT relates to the combination of sympathetic activation, hypoxia and sudden explosive exercise at HA. In addition to the factors outlined above the proarrhythmic effects of HA may be partly explained by other factors including acclimatization, changes in heart rate variability, sleep deprivation, dehydration and anxiety.⁵

In conclusion HA exposure to $\geq 4100\text{m}$ is associated with significant brady and tachy-arrhythmias in healthy adult men supporting a potential proarrhythmic risk. There was no link between HA and sustained ventricular arrhythmias linked to an increased risk of SCD.

Disclosures

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Table 1. ICM detection settings, key cardiac rhythm findings and altitudes achieved

Subject number	Age	Maximal altitude achieved	Significant Findings	Key Abnormalities	No of pause Episodes	Longest duration in seconds
1	30	6035m	No	None	-	-
2	40	6200m	No	None	-	-
3	41	7550m	Yes	Pauses	41	6.0
4	30	6800m	Yes	SVT and pauses	5	5.0
5	32	7550m	Yes	Atrial fibrillation	-	-
6	24	4100m	No	None*	-	-
7	38	6200m	Yes	Pauses	15	4.0
8	25	6800m	No	None	-	-
9	48	6800m	Yes	Pause	1	3.3
10	30	7550m	Yes	Pauses	17	7.0
11	33	6035m	Yes	Pause	1	4.4
12	41	7100m	No	None	-	-
13	38	6800m	No	None	-	-
14	38	6035m	No	None	-	-
15	32	6800m	Yes	Pause	1	3.4
16	41	6200m	Yes	Pause	1	5.0

SVT, supraventricular tachycardia; *descended due to gastrointestinal illness; Tachycardias were defined as a heart rate/minute >230 -age in years for ≥ 16 beats; bradycardia as a heart rate <30 /minute for ≥ 12 beats, pause R-R interval >3 seconds and atrial tachycardia/fibrillation/flutter as episodes fitting morphology detection criteria lasting >10 minutes.

Chapter 11

Conclusions

This thesis presents the conduct and findings of a series of studies using both simulated and terrestrial HA, with a specific focus on elucidating the cardiovascular effects of hypoxia using novel portable equipment. The key findings of this research are as follows.

- Biventricular cardiac systolic function remains preserved with acute hypoxia and HA exposure.
- Whilst minor changes in biventricular diastolic function were observed this likely reflects augmented atrial contraction as the estimated biventricular filling pressures remains unchanged and were not increased.
- Minor elevations in cardiac troponin and natriuretic peptides can occur at HA and are more likely to reflect the influence of exercise at HA and the increase in PASP rather than related to adverse changes in biventricular function.
- Genuine HA, NH and HH produce similar short term cardiac adaptations, at rest. Following exercise the fall in SpO₂ and increase in right ventricular systolic pressure appears to be greater with HH and natural HA compared with NH.
- High Altitude exposure is associated with an increase in brachial and central blood pressure and the peripheral augmentation index. These increases are likely explained by increased wave reflections and peripheral vasoconstriction of the more peripheral muscular arteries in response to hypobaric hypoxia rather than persistent changes in the arterial wall.
- Increasing HA affects HRV and leads to a reduction in time domain measures of HRV, increased low frequency power initially and high frequency power during acclimatization. Changes in non-linear HRV indices are characterized by greater self-similarity and reduced signal chaos and entropy. Changes in HRV at HA were not predictive of AMS development or its severity.

- There are consistent differences in HRV scores between men and women at sea level which. These differences were maintained at HA. There is no evidence for a sex-x-altitude/hypoxia interaction on the changes in HRV at HA.
- Increasing HA leads to an increasing risk of nocturnal bradycardia and significant pauses and HA is pro-arrhythmic.
- My data from this thesis would largely suggest that in healthy adults moderate HA exposure does not pose any over effects on cardiovascular function.
- However HA exposure does lead to an increase in both peripheral and central blood pressure and more extreme HA above 4100m is associated with a pro-arrhythmic risk. This has important implications for persons with poorly controlled hypertension or at risk of cardiac arrhythmias where there the potential risk of exacerbation at HA may be increased.
- Further carefully conducted research amongst 'at-risk populations' including those with known hypertension are needed.

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