**Research plan and experiment protocol**

Renal bicarbonate handling and acute mountain sickness

**Background**

Acute mountain sickness (AMS) develops when people climb too quickly and affects millions of people at mountainous regions around the world. It is characterised by symptoms such as headache, nausea, fatigue/lassitude, vomiting and dizziness, which can lead to more life-threatening illness resulting in death [1, 2]. Despite considerable efforts over the past two centuries, we still do not know why some people are more prone to developing AMS when they ascent to high altitude [3]. A better understanding of its cause will help clinician identify individuals who are at greater risk of developing AMS, thereby preventing this disease.

During ascending to high altitude, increases in breathing causes the blood pH to rise (respiratory alkalosis) [4-6]. In order to restore blood pH towards normal values, the kidneys must work hard to excrete the excess bicarbonate ions via our urine in order to restore blood acid-base balance [5, 6]. However, evidence from early studies which indicate that this process does not completely return blood pH at high altitude [4, 7, 8], and some wondered whether poor kidney function at high altitude might account for the differences in AMS susceptibility between people. In support of this notion, studies have found a link between the severity of AMS and urine acidity [9, 10]. Furthermore, one of the ways by which Diamox (a common treatment for AMS) alleviate AMS is by improving the functions of the kidneys at altitude [11].

One way by which poor kidney functions can lead to AMS is through its effects on brain blood flow. We have found changes in blood pH alters blood flow regulation in the brain at high altitude [12]. Due to its symptoms, it has been speculated that AMS originates from the brain [3], via excessive brain blood flow and a breakdown of the blood-brain-barrier [13, 14]. Therefore, changes in blood pH in individuals with poorer kidney function at high altitude, might lead to improper brain blood flow regulation, resulting in AMS.

**Aim**

The aim of the proposed study is to examine the role of kidney function on brain blood flow and severity of AMS during prolonged high altitude exposure. Using the unique Global Energetic and Environmental Simulation Suite (GEnESiS) at the Centre for Translational Physiology, University of Otago, Wellington, we test the hypothesis that individuals with poor kidney function will have higher AMS scores and poorer brain blood flow regulation during prolonged exposure to altitude.

**Hypothesis**

We will test the hypothesis that reduced bicarbonate excretion by the kidneys are associated with elevated brain perfusion and increased AMS.

**Significance**

Due to advanced transportation, millions of people are at risk of AMS worldwide each year. Further, a significant portion of AMS cases can develop into life-threatening high altitude pulmonary oedema and cerebral oedema. A better understanding of the role of the kidneys is paramount in the ongoing research in the prevention and treatment of AMS.

**Recruitment.** Healthy individuals will be recruited from the Wellington region.

**Inclusion criteria**

* Healthy individuals (age 18-45 years), specifically:
* Free of history of long-term disease
* Not currently taking any medication that may influence measures in this study

**Exclusion criteria**

* Anyone who has been above 2,500 m in the previous 2 months
* Pregnancy
* Respiratory or cardiovascular diseases
* Renal impairment
* Currently taking diuretics, antacids, proton pump inhibitors, or histamine blockers
* Body mass index greater than 30

**Experimental design**

The participants will be required to visit the laboratory three times. Following a familiarisation visit (visit one), the participants will undergo visit a 10h sea-level session (visit two) and a 10h high altitude session (visit three) in a single blinded manner. All experimental sessions will take place in the environmental simulation suite at the Centre for Translational Physiology at the University of Otago, Wellington. Each experimental session will take around ten hours and the participants will be seated in a semi-reclined position at a comfortable ambient temperature.

Participants will be asked to refrain from alcohol and avoid exercise for at least 24 hours prior to study commencement. Further, strict dietary guidelines will be adhered for the morning of the study. All participants will be required to drink at least 500mls of water on the morning of the study.

**Experimental protocol**

Following baseline, pre-exposure assessment, the participants will be exposure to a simulated altitude of 5000 m. Studies have found 5000 m to be the ideal altitude for studying AMS development [15]. Assessments will be conducted every two hours. The participants will be allowed to consume food and drink water *ad libitum*. In addition, body fluid loss via urine will be replenish by drinking the equivalent volume of their last urine output. The altitude exposure sessions will be terminated if:

1. AMS score above, which indicates severe AMS
2. resting heart rate above 140 bpm
3. peripheral O2 saturation below 65%
4. subject intolerance
5. metal abnormalities as assessed by the clinician (i.e., confusion, incoordination of motor skills).

**Measurements**

*AMS scores*

The Lake Louise Questionnaire (LLQ) is used worldwide as a gold-standard method of assessing both the presence and severity of AMS during altitude exposure [16]. The LLQ will be used to measure and monitor the wellbeing of the participants during the experimental sessions. The Environmental Symptom Questionnaire (ESQ) was development to help researchers quantify symptoms a person may experience during exposure to environmental conditions [17]. The ESQ will be used as complementary measures to provided additional information on the participants’ wellbeing.

*Resting metabolism*

Indirect calorimetry is the gold-standard measurement for resting energy expenditure (REE). REE is the amount of energy required to maintain vital functions whilst at rest and makes up to 60-70% of the daily energy requirements. Measuring REE using our indirect calorimetry system (Promethion, Sable Sys., NV, USA) will help us determine the role of metabolism on AMS. Partial pressure of end-tidal CO2 (PETCO2) and O2 (PETO2) will be sampled from a nasal cannula and measured using a gas analyzer (model ML206; ADInstruments, New Zealand). Ventilation will be measured using a heated pneumotach (800L, Hans Rudolph Inc, USA).

*Anthropometry measurements*

Body composition will be assessed using Dual-energy X-ray absorptiometry (Horizon A, Hologic, NSW, Australia). This is a gold-standard measure for measuring changes in the body’s composition of muscle and fat. Height will be measured at baseline, while near-nude body mass will be assessed throughout the exposure period.

*Cognitive functions*

Cognitive function tests provide valuable insight into the brain function of the participant, which is particularly important during environmental exposes where decision making is crucial. Previous studies have found an association between the decline in brain function and AMS development [14, 18]. Standardised cognitive and psychological assessments will be performed using a laptop computer. These tests include simple reaction time-1, simple reaction time-2, code substitution-simultaneous, match to sample and procedural reaction time.

*Blood and urine samples*

Changes in blood acidity, oxygen saturation and kidney function are important measures of an individual’s ability to acclimatise to altitude [15]. Venous blood samples will be taken to assess blood oxygen content and acidity, as well as biomarkers of AMS and kidney function. With the subject in a relaxed recumbent position, venous blood sample (10 mL) will be collected. A small portion (1 mL) of the blood sample will be analysed immediately for blood gas parameters as well as blood creatinine levels for glomerular filtration rate (index of kidney function). The remaining blood sample will be centrifuged and then placed in storage inside a -80°C freezer. An arterialised blood sample will also be taken from the earlobe using a lancet.

The body’s tissue oxygenation will be measured continuously using pulse oximetry. Pulse oximetry is a clinical tool routinely used in hospitals to determine an individual’s blood oxygen saturation. In additional, brain tissue oxygenation will be measured invasively using Near-infrared spectroscopy probes placed on the forehead (OxiplexTS, ISS Inc, USA).

Urine volume and acidity are important markers of kidney function and will be collected and measured during each session.

*Resting blood pressure and cerebral blood flow*

Resting blood pressure is routinely used by clinicians as an index of cardiovascular health. Resting blood pressure will be regularly assessed and monitored using pulsatile pressure-flow waveform analysis (BP+, Uscom, Australia) to determine the effects of altitude training on cardiovascular health. In addition, continuous blood pressure will be measured non-invasively using finger pulse plethysmography (Finometer Midi, Finapres Medical Systems, The Netherlands). A 3-lead ECG will be recorded with surface electrodes on the chest to monitor heart rate.

Duplex Doppler ultrasound is an accurate and effective method for assessing brain blood flow. With the participant resting quietly, ultrasound imagining will be used to measure beat-to-beat carotid and vertebral artery diameters (Terason t3000, Teratech Corporation, USA). Longitudinal section of the left carotid and vertebral artery, 2 cm proximal to the bifurcation will be imaged and recorded for offline analysis using custom edge tracking software.

*Cerebrovascular CO2 reactivity test*

Cerebrovascular CO2 reactivity is the most commonly used technique to assess brain blood flow regulation. Brain blood flow velocity will be measured in the M1 segment of the left or right middle cerebral artery (MCA) using 2-MHz pulsed wave transcranial Doppler ultrasound (ST3 Digital Transcranial Doppler System; Spencer Technologies, USA). The participants will inhale a 5% CO2 gas mixture for 100 sec. At the end of this period, the subjects will be instructed to hyperventilate to lower their PETCO2 (level of CO2 in the body) for 2-3 min.

*Flow-mediated dilatation test*

Flow-mediated dilatation (FMD) is a gold-standard measure of blood vessel function [19]. Since blood vessel dysfunction is known to exacerbate AMS development [20], the participants will undergo FMD tests to assess changes in blood vessel function. This involves an inflatable arm cuff being applied to the left forearm immediately distal to the elbow. While resting in supine position, the cuff will be inflated for 5 minutes. Following this period of inflation, the cuff will be deflated rapidly and brachial blood flow measurements will be taking using the duplex Doppler ultrasound.

All analogue data will be acquired on a computer-based data acquisition and analysis system (PowerLab 16SP, ADInstruments, New Zealand).

**Analysis**

The primary dependent variables of interest are AMS scores, biomarkers of kidney function, CBF measures. The key independent factor is experimental condition (sea-level vs. simulate altitude) and duration of exposure. We will control for gender, as these are potential confounding factors. Differences and interactions between experimental condition and exposure duration will be parsimoniously assessed using generalized linear mixed-effects models.

**Sample size**

Previous studies have reported ~80% of the participants develop AMS following acute exposure to ~5000 m, with 50% experiencing moderate AMS [15]. Assuming simulated altitude exposure to 5000 m can induce similar incidence of AMS, a sample of 30 individuals would provide >80% power to detect an effect size that corresponds to a ~50% of the participants developing moderate AMS, assuming a standard deviation of 3 AMS scores at a two-tailed significance level of 0.05.

**Timeline**

The project will be completed within a three-years period as detailed below**.**

|  |  |  |
| --- | --- | --- |
| **Year one** | **Year two** | **Year three** |
| * Participant recruitment * Data collection | * Complete participant recruitment and Data collection intervention * Data processing and analysis | * Complete data processing and analysis * Publications |

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