CONTROL OF BREATHING AND CARDIO-RESPIRATORY RESPONSE TO HYPOBARIC AND NORMOBARIC HYPOXIA

by

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Abstract

We examined the control of breathing, cardio-respiratory effects and the prevalence of acute mountain sickness (AMS) in humans exposed to hypobaric hypoxia (HH), normobaric hypoxia (NH), and under two control conditions (hypobaric normoxia and normobaric normoxia). Subjects (n = 11) were familiarised with all tests prior to their first exposures. The order of conditions was randomized, each exposure lasted for 6 hours, and consecutive exposures were separated by a one-week washout period. Prior to and following exposures, subjects underwent hyperoxic and hypoxic Duffin rebreathing tests, measuring CO_2 threshold and sensitivity, and a hypoxic ventilatory response test (HVR), measuring sensitivity to O_2. Inside the environmental chamber, minute ventilation (V_E), tidal volume (V_T), frequency of breathing (f_B), blood oxygenation (S_PO_2), heart rate (HR) and blood pressure (BP) were measured at 5min, 30min and hourly until exit. Symptoms of AMS were evaluated hourly using the Lake Louise score (LLS). Both the hyperoxic and hypoxic CO_2 thresholds were lowered after HH and NH during the Duffin rebreathing test. Hypoxic sensitivity in the Duffin rebreathing test was only increased after HH exposure. No changes occurred in the HVR after any of the four exposures. Ventilatory parameters, S_PO_2 and HR were higher in the hypoxic exposures as opposed to the normoxic exposures. No major differences were observed for V_E or any other cardio-respiratory variables between NH than HH. The LLS was greater in AMS-susceptible than in AMS-resistant subjects, but LLS was similar in HH and NH. We conclude that 6 hours of hypoxic exposure is sufficient to lower the peripheral and central

CO_2 threshold, but it is too short in duration to induce differences in cardio-respiratory variables between HH and NH or to create differences in AMS severity.

Preface

A section of the introduction has been published.

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Ethical review for all techniques has been carried out by the University of British Columbia Clinical Research Ethics Board for Experiments Involving Human Subjects (UBC CREB). The study CREB approval certificate is #H10-03151.

Table of Contents

List of Tables

List of Figures

Glossary

2,3-DPG: 2,3-diphosphoglycerate
AAE: Alveolar air equation
Acute exposure: Greater than 5 minutes but less than 1 hour
AHVR: Acute hypoxic ventilatory response
Brief exposure: 5 minutes or less
Chronic exposure: Greater than 1 day
CBF: Cerebral blood flow
f_B: Frequency of breathing
F_IO_2: Fraction of inspired oxygen
F_IN_2: Fraction of inspired nitrogen
HVD: Hypoxic ventilatory decline
Hypercapnia: Increased P_ACO_2 levels
Hypercarbia: Increased P_aCO_2 levels
Hypocapnia: Lowered P_ACO_2 levels
Hypocarbia: Lowered P_aCO_2 levels
Isocapnia: Steady P_ACO_2 levels
mmHg: Millimeter of mercury
Intermediate altitude: Altitudes between 4000 m and 5000 m
Mild altitude: Altitude less than 3000 m
P_ACO_2: Partial pressure of alveolar CO_2
P_aCO_2: Partial pressure of arterial CO_2
P_AO_2: Partial pressure of alveolar oxygen
P_aO_2: Partial pressure of arterial oxygen
P_AN_2: Partial pressure of alveolar nitrogen
P_B: Barometric pressure
P_CCO2: Central PCO_2
PCO_2: Partial pressure of CO_2
$P_{ET}CO_2$: Partial pressure of end-tidal CO_2
$P_{ET}O_2$: Partial pressure of end-tidal oxygen
PH_2O: Partial pressure of water vapour
P_IO_2: Partial pressure of inspired oxygen
PN_2: Partial pressure of nitrogen
PO_2: Partial pressure of oxygen
Poikilocapnia: Freely fluctuating P_ACO_2 levels
RER: Respiratory exchange ratio
RQ: Respiratory quotient
S1: Sensitivity of the first slope in Duffin test
S2: Sensitivity of the second slope in Duffin test
Severe altitude: Altitude greater than 7000m
S_PO_2: Percent blood saturation
Sub-acute exposure: Greater than 1 hour but less than 24 hours
V_A: Alveolar ventilation
VAH: Ventilatory acclimatization to hypoxia
V_B: Basal ventilation
VCO_2: Carbon dioxide production
V_D: Deadspace ventilation
V_E: Minute ventilation
VO_2: Oxygen consumption
VRT: Ventilatory response threshold
V_T: Tidal volume
WOB: Work of breathing

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Dedication

To Jacinthe and Daniel Richard

1.0 Basic knowledge and application

1.1 Oxygen

Hypoxia deprives the body of its most vital need: oxygen. Limiting our discussion to environmental factors, decreased oxygen availability can be induced by reducing barometric pressure (P_B) or by reducing the fraction of inspired oxygen (F_IO_2). Hypobaric hypoxia (HH) results from a decreased atmospheric P_B and is experienced in leisure, work and research purposes. Both altitude ascent and use of hypobaric chambers induce HH. Normobaric hypoxia (NH) is used in research settings, attempts at pre-acclimatization and in athletic training. In NH the F_IO_2 is reduced from 0.2093 to lower fractions without altering the P_B; this decreases the inspired PO_2 (P_IO_2). Lowering of the F_IO_2 is typically accomplished with the addition of exogenous nitrogen (N_2).

The P_IO_2 is the product of P_B (minus water vapour pressure) and F_IO_2. At sea level, P_B is approximately 760 mmHg and dry air contains 0.2093 O_2, resulting in a PO_2 of 159 mmHg. Since air is humidified upon entering the upper airways, we must account for water vapour pressure (P_{H2O}); therefore humidified air has a PO_2 of 149mmHg [760 mmHg – 47 mmHg (water vapour pressure) • 0.2093 = 149 mmHg]. At sea level, the P_IO_2 of 149 mmHg is mixed with the contents of the lung, and decreases to approximately 100 mmHg. The alveolar air equation (AAE), predicts alveolar PO_2 (P_AO_2) as seen in equation 1.

$$\text{Eq1.} \quad P_AO_2 = P_IO_2 - P_ACO_2 \bullet [(F_IO_2 + (1\text{-}F_IO_2/RQ)]$$

where

$$P_ACO_2 = (VCO_2/V_A) \bullet k$$

and

$$RQ = VCO_2/VO_2$$

as well as

$$P_IO_2 = F_IO_2 \bullet (P_B - P_{H_2O})$$

The alveolar PCO_2 (P_ACO_2) is a function of resting CO_2 production (VCO_2) divided by alveolar ventilation (V_A) multiplied by a conversion factor (k). The respiratory quotient (RQ), seen in equation 1, is calculated as the resting VCO_2 divided by the resting oxygen consumption (VCO_2/VO_2) with a value nearing ~ 0.82. The conversion factor k = [(273+*t*)*(760/273)] is broken down as 273℃ representing 0 Kelvin, *t* representing body temperature in℃, and 760 representing standard atmospheric pressure in mmHg [1]. This conversion factor therefore varies with body temperature and barometric pressure. It is worth noting that this equation is accurate as long as the inhaled inert gases (i.e. N_2) are in equilibrium and that barometric pressure has stabilized. This is of importance to brief but severe hypoxic exposures. Thus, consideration of the above in order to avoid potential errors in HH and NH exposure calculations should be undertaken. The AAE can be approximated as follows, in equation 2, for practicality reasons.

Eq2. $P_AO_2 = P_IO_2 - P_ACO_2/RQ$

At sea level, the AAE (149mmHg – 40mmHg/~0.82) yields a P_AO_2 of approximately 100 mmHg. A further decrease in PO_2 may occur when crossing the alveolar-blood barrier. Arterial PO_2 (P_aO_2) is 95-100 mmHg as a consequence of

ventilation-perfusion mismatch. More specifically, some pulmonary capillaries remain closed at rest (approximately two thirds are unavailable), yet their availability can be increased with distension and/or recruitment as occurs during hypoxia or exercise. Within the blood, release of oxygen from haemoglobin is facilitated by increased heat, acidity (decreased pH) and 2,3-diphosphoglycerate (2,3-DPG), while cold, alkalosis (increased pH) and decreased 2,3-DPG enhance oxygen binding. Respiratory alkalosis, as seen at altitude, increases blood pH thus moving the haemoglobin dissociation curve to the left and improving oxygen uptake at the lungs.

1.2 Control of breathing

One of the most visible and researched responses to hypoxia is the increase in minute ventilation (V_E). To better understand this response, review of the underlying mechanisms regulating control of breathing is necessary. Excluding behavioral influences, exercise or disease at sea level, the arterial partial pressure of CO_2 (P_aCO_2) controls the ventilatory response centrally beyond a basal drive to breathe (V_B); while at altitude the effects of hypoxia tend to heighten both the peripheral and central chemosensory responses [2, 3] Resting ventilation is controlled centrally by the medulla and the pons located in the brain stem. Afferent input from mechanoreceptors (e.g. lung stretch receptors) and from the peripheral and central chemoreceptors also assist in the regulation of ventilation [4]. The central chemoreceptors, situated ventrolaterally on the medulla [5], are stimulated when overlying cerebrospinal fluid (CSF) pH falls below its normal value (approximately 7.32). The blood-brain barrier is impermeable to blood H^+

and HCO_3^-, therefore central stimulation ultimately depends on the proton concentration obtained through diffused CO_2 (affecting CSF pH) which stimulates the central chemosensors.

The ventilatory response to CO_2 under hypoxic and hyperoxic conditions can be observed in the laboratory with the administration of either a progressively rising or steady-state CO_2 stimulus. The central chemoreceptors are stimulated by P_aCO_2 upon reaching a certain threshold. The CO_2 threshold refers to the P_aCO_2 point below which there is no increase in V_E for an increase in CO_2. Therefore, increased ventilation only occurs once the P_aCO_2 reaches this threshold referred to as the ventilatory recruitment threshold (or VRT) [6]. If plotted against CO_2, V_E would be constant below the VRT and would progressively increase above it. The linear slope of the increase in ventilation past the VRT is termed the CO_2 sensitivity, referred to as S1, is mediated by an increased tidal volume (V_T) and is often expressed in $l \cdot min^{-1} \cdot mmHg^{-1}$ [6]. In some, a second slope, usually a function of increases in breathing frequency (f_B) is seen; this is termed S2[6]. With sea level residents at altitude (10 days), the ventilatory response to iso-oxic (steady PO_2 tensions) CO_2 rebreathing tests appears to be facilitated by an increase in S1, [7, 8]. In high-altitude natives (Andeans, Himalayans) the response to hypoxic CO_2 rebreathing is generally mediated by a lowered CO_2 VRT [7, 9]. Threshold and sensitivity are affected by genetics, site of residency, and type of exposure (hyperoxic vs. hypoxic, isocapnic vs. poikilocapnic) [8]. The peripheral chemoreceptors, located in the aortic and carotid bodies, primarily respond to decreased arterial PO_2 (P_aO_2) but also to increases in P_aCO_2 [10]. Furthermore acidosis, P_aCO_2 and temperature also seem to have an influence.

Therefore, immediately after exposure to hypoxia, a rapid increase in V_E, termed the acute hypoxic ventilatory response (AHVR) occurs. The peripheral chemoreceptors respond rapidly to a single hypoxic breath due to their location proximal to the newly circulated blood [11]. Mechanistically, the carotid glomus cells are triggered when P_aO_2 falls below 80-85mmHg. Their afferent input to the sinus nerve merges into the glossopharyngeal nerve synapsing into the nucleus tractus solitarius. The slope of the increase in ventilation for a given change in blood oxygen saturation ($l \cdot min^{-1} \cdot \%S_aO_2^{-1}$) is often used to quantify the ventilatory response to hypoxia (HVR) in laboratory tests.

1.3 Measuring the central and peripheral chemoreflex response

The ventilatory response to increased carbon dioxide (hypercapnia) can be measured using several techniques such as the steady-state, Read rebreathe and Duffin rebreathing methods. The steady-state method is achieved via prospective targeting or end-tidal forcing [12, 13]. In both of these methods, a predetermined end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) is selected. During prospective targeting participants breathe from two bags. The first bag contains the computed gas necessary to achieve the pre-determined $P_{ET}CO_2$ with the bag volume being slightly less than the subject's V_T. Once the initial bag is emptied a low resistance valve recruits the second bag that contains previous expirate to provide the rest of the V_T [13, 14]. In end-tidal forcing, sampling of the expired air allows a computer model to determine the gas mixture necessary to achieve the wanted end-tidal tension by addition/removal of gases (CO_2, O_2, N_2, air) to the next inhalation on a breath-by-breath basis. By measuring the steady-state response at

multiple tensions in either hypoxia or hyperoxia, a sensitivity (slope) can be determined and a threshold extrapolated from resting ventilation. Two points are necessary to determine a slope, but the steady-state test tends to be more similar to the Duffin test slope when three points are included [15]. These techniques are costly and require extensive setup. Additionally, slopes may be inaccurate if the selected $P_{ET}CO_2$ tensions are below the VRT or greater than the maximum voluntary ventilation achievable by the subject.

In the Read rebreathe method the participant rebreathes form a bag containing an initial concentration of CO_2 [16]. Through rebreathing, CO_2 slowly rises providing a stimulus to breathe. A VRT is extrapolated using the subject's basal breathing. This test may mask the VRT if the bag CO_2 content is too high or may not be attained if the subject is artificially hyperventilating prior to the start of the test. The method described by Read relies on moderate (150 mmHg) hyperoxia thus masking the response to hypercapnia in the presence of hypoxia.

The modified Read rebreathe method, now called the Duffin method [15], implemented two major changes. Firstly, a 5-min coached hyperventilation, where subjects lower their $P_{ET}CO_2$ to between 19 and 25 mmHg, was added. This facilitates determination of the VRT by allowing P_aCO_2 to gradually rise until ventilation increases from V_B (at the VRT). Note that this hyperventilation induces temporary alkalosis. Two versions of the test were implemented to determine the response of a rising CO_2 stimulus in hypoxic and hyperoxic environments. The hypoxic level ($P_{ET}O_2$ 50 mmHg), allows estimates of the central and peripheral input while the hyperoxic tension ($P_{ET}O_2$ 150

mmHg), according to some [2] silences the peripheral chemosensors and, yields the central response. Caveats to this method include respiratory muscle fatigue prior to test start, short-term-potentiation (STP) and hypocapnia-induced changes in cerebral blood flow.

The AHVR challenges respiratory homeostasis as the acute increase and subsequent gradual rise in ventilation causes hypocarbia and blood alkalosis, which affects the haemoglobin dissociation curve. The term AHVR will be used when examining the ventilatory response over an acute time period (< 5min) while the term HVR refers to hypoxic challenge tests that quantify one's response to a hypoxic stimulus. Use of HVR tests where the $P_{ET}CO_2$ is not maintained constant (termed poikilocapnic HVR tests) tend to blunt the ventilatory response as the hypocarbia-induced ventilatory depression "quiets" the central chemosensory input, lowering the ventilatory stimulus. Therefore, isocapnia (P_aCO_2 levels held constant) is often used in HVR testing to avoid this confounding factor. There is significant inter- and intra-individual variation in the HVR with coefficients of variation reported to range from 19-76% [17]. The ventilatory response to hypoxia can be measured using a variety of techniques such as rebreathing with CO_2 scrubbing, using a Duffin-type test in conjunction with a 20min hypoxic isocapnic and poikilocapnic challenges, steady-state end-tidal forcing, single breath challenges or with a progressively decreasing isocapnic or poikilocapnic challenge. The rebreathing method entails the use of a "hypoxicator" (commercially available under the name Altipower by GO2Altitude). The unit is set up so that a mask is connected to a bag where the participant rebreathes their own expirate progressively rendering it hypoxic

while CO_2 is scrubbed out to prevent hypercapnia. Although simple, this method is not very effective as the decrease in F_IO_2 varies from subject to subject and the scrubbing is not always effective. To measure the ventilatory response to hypoxia Duffin has suggested using the difference in response between hyperoxic and hypoxic rebreathing tests in parallel with two 20-min isocapnic and poikilocapnic hypoxia challenge which allow observation of the hypoxic ventilatory decline in (HVD) [8]. This method although very thorough is quite time consuming and equipment intensive. The steady-state method for hypoxic ventilatory response test is similar to the one used for CO_2 tolerance type tests. Briefly a computer model predicts the amount of gas needed in the next inspired breath to reach a predetermined hypoxic $P_{ET}O_2$; isocapnia can be maintained [14, 18]. Caveats to this technique are similar to the CO_2 setup; the outfit is extensive and one must join a minimum of 2 steady-state values together to determine a slope. Single breath hypoxic challenges predominantly measure the peripheral response to various stimuli. Briefly, after quantifying V_B in the resting subject, hypoxic gas is introduced into the breathing apparatus without warning [11]. Changes in ventilation in the subsequent breaths allow estimation of the response. Benefits include basic equipment set-up (pneumotach, gas analyzers) and time efficiency. Caveats to this method are the absence of HVD data, and possibility for noise interference since very few breaths are used to quantify the response. Finally, in progressive hypoxia challenges, the F_IO_2 is gradually lowered by the addition of N_2 to the inspired gas mixture in such that blood saturation (S_pO_2) reaches the 75% range over 5-8min [17]. The length of the test allows for measure of the acute increase in ventilation but is generally too short for HVD. Isocapnia is

maintained by manual addition of CO_2 into the inspired gas as determined by observation of resting $P_{ET}CO_2$ *a priori*. It must be emphasized that subjects must be completely relaxed to avoid ventilatory input from other sources (anxiety, voluntary hypo-hyperventilation, etc.), which would inflate/mask the measured response.

1.4 Acute mountain sickness

Acute mountain sickness or AMS is a self-limiting illness experienced by some when exposed to hypoxia. This condition usually manifests itself 6-12 hours after arrival at altitude, is potentiated by exercise and is believed to be caused by exposure to hypoxia over a period of several hours; hypobaria appears to play a role [19]. By definition, AMS is suggested if recent ascent to altitude (greater than 2500 m) is followed by the presence of a headache with one or more of the following symptoms: nausea/vomiting, insomnia, general fatigue, and dizziness [20]. The Lake Louise Score (LLS) and the Environmental Symptoms Questionnaire (ESQ) [21, 22] are two self-report questionnaires that are used to confirm the presence of AMS. The LLS, used predominantly in the literature, grants a score of 0-3 for each of five symptoms, for a total score between 0 and 15. Physiologically, when exposing susceptible (AMS+) and resistant (AMS-) subjects to the same level of hypoxia, AMS+ subjects tend to have a higher heart rate, mean blood pressure, f_B, lower body temperature and higher concentrations of circulating catecholamine [23]. The prevalence of AMS varies greatly; some are more apt to develop symptoms while others are resistant. Interestingly, anthropometrics, athletic training and use of alcohol have little to no influence in predicting the occurrence of AMS prior to

ascent [24]. Conflicts exist in the present literature, and the range of prevalence is dependent on multiple factors, making an estimate inappropriate. It is agreed that factors such as rate of ascent, recent prior exposure, altitude reached, preventative drug use, and experience seem to play a major role on AMS occurrence or severity[25].

No consensus has been established regarding the pathophysiology of AMS. Mild AMS has often been associated with hypoventilation, poor gas exchange, retention and relocation of fluids and with a greater sympathetic response [26]. Past hypotheses have included variability in the ratio of cerebrospinal fluid to brain volume; those with a greater ratio have more room for swelling thus are less prone to exhibit certain symptoms[27]. Additionally, previous authors have shown a synergistic effect of hypoxia alongside hypobaria as opposed to hypoxia alone in AMS symptoms and blood variables when comparing HH, NH and hypobaric normoxia (HN) [23]. Furthermore, anecdotal evidence suggests that the use of hyperbaric tent is efficacious in treating AMS in conjunction with supplemental O_2 [28]. Therefore, pressure, alongside hypoxia, seems to have a role in the development of AMS, thus warranting the examination of HN.

Attempts at predicting AMS susceptibility are perplexing. Some have tried correlating HVR test, measured as one's increase in ventilation for a given blood oxygenation ($l \cdot min^{-1} \cdot \%S_aO_2^{-1}$), to AMS incidence without success [29-31] while some have found a correlation [32]. Others have tried correlating S_PO_2 upon arrival to altitude yet no concrete evidence exits [33]. Differences in CO_2 control of breathing in sea level dwellers and high altitude residents have been noticed [8, 9] and a recent study shows that AMS+ subjects (n = 12) have a higher hyperoxic VRT and a lower S1 [34]. This

relationship remains to be thoroughly examined and has yet to be studied pre- and post-hypoxia exposure in relationship to AMS.

1.5 Review of previous studies examining HH and NH

The upcoming section examines the current literature comparing the cardio-respiratory and symptomatic responses to HH and NH. In the 19th century, French scientist Paul Bert was one of the first to artificially induce HH by reducing P_B. He observed detrimental symptoms that were relieved by breathing supplemental O_2, and proposed that lowering PO_2 was harmful to humans [35]. Furthermore, in the early 1900's, Sir Joseph Barcroft demonstrated that decreasing the F_IO_2 alone could also induce hypoxemia in his classic "Glass House" experiment [36]. Since these observations, the scientific community has speculated whether physiological responses to equivalently lowered PO_2 differ between HH and NH. A common axiom among physiologists is that equivalent hypoxia levels (i.e. P_IO_2) induced either by HH or NH delivers equivalent physiological responses, regardless of F_IO_2 or P_B manipulations, making NH a suitable alternative for simulating HH. Identical hypoxic doses may be calculated and delivered using HH or NH; however it is yet to be confirmed if physiological responses are equivalent for the two conditions. Past studies have sought to determine the responses to NH and HH but with a lack of congruency rendering it difficult to compare results. It is therefore necessary to conduct a thorough examination to better understand the current state of knowledge regarding response to HH and NH.

A literature search was conducted in English using PubMed and Google Scholar with the following key terms in combination or alone: *hypobaric hypoxia, normobaric hypoxia, acute mountain sickness* (or *AMS*), *carbon dioxide* (or CO_2), and *simulated altitude*. Papers were collected from peer-reviewed journals and academic reports pertinent to the field. The selected studies had a set P of 0.05, compared HH to NH, selected subjects who were healthy adults (< 50 yrs old), with above average fitness and residents of sea level or very mild altitude for at least six months (< 1600 m). Ten major studies compared HH to an NH equivalent with four considering hypobaria [37-40] and two others combining hypobaria with hypoxia [41, 42]. Of the ten, three of the studies [23, 43, 44] included women; no note was made regarding variations in ventilation due to changes in the menstrual cycle. The selected studies seen in Table 3 in the appendices had sample sizes ranging from six to 43 participants with an overall mean of 15. Seven of the studies reported randomizing the exposure order [37, 39-43, 45, 46] while three did not for logistical or unmentioned reasons [37, 38, 44]. Washout periods between exposures ranged from multiple same day exposures [42-44] to 24 hours [41] to one week [37, 39, 40, 45, 46]. We classified hypoxic dose using the selected altitude as follows: mild (< 3000 m), intermediate (> 3000 m, < 5000 m) and severe (> 5000 m). The examined chamber studies were either classified as brief, acute or sub-acute. The brief exposures lasted ≤ 5 minutes [41, 42, 44] using severe hypoxic exposures (7620 m) while the acute studies (< 1hr) [45, 46] used intermediate levels of hypoxia (4500 m). The sub-acute (> 1hr) chamber exposure studies [37-39] used intermediate levels of hypoxia (~ 4500 m) and ranged from 2.5-10 hour exposures; when available field studies were

included in this section and considered chronic (> 1 day). Table 4 (appendix 1) classifies the ten reports in terms of study quality, separating studies into high, medium and low level of evidence. Criteria used to evaluate study quality included method of hypoxia delivery, gas analysis method, sample size, subject training, anthropometric data availability, study design/methods by means of the Downs-Blacks score [47], blinding, washout and randomizations. Of the ten studies, the authors categorized one study as being of high evidence [41], four of medium evidence [39, 44-46] and five of low evidence [37, 38, 40, 42, 43]. Unless noted otherwise, the described study altitudes in meters are those reported by the authors' as converted from feet or calculated from pressures using the 1976 "standard atmosphere" arithmetic [48] seen in equation 3:

$$\text{Eq3.}\quad P_{B\,Hypo}\ (\text{mmHg}) = 760 \bullet [288.15 / (288.15 - 6.5 \bullet \text{altitude (km)})]^{-5.25588}$$

1.5.1 Respiratory response to hypoxia

The following three paragraphs describe the ventilatory response to brief and acute hypoxia exposures. Aircrews participating in cabin depressurization exercises (i.e. hypobaric hypoxia) are at risk for both decompression sickness and barotrauma from sudden decompression. Researchers have used combined altitude and depleted oxygen (CADO) (3048 m & F_IO_2 of 0.10) as an alternative to HH in order to alleviate this risk. When CADO was compared to equivalent HH (7620 m) for 5 minutes, Singh et al. reported that the rate of desaturation was more rapid in HH than in CADO in 43 subjects [41]. In a similar project, Evetts et al. exposed 11 subjects for 5 minutes to NH alone (F_IO_2 0.069), combined HH to NH (F_IO_2 of 0.103 with 3050 m) and HH alone (7620 m)

[42]. Normobaric hypoxia elicited the greatest V_E and $P_{ET}CO_2$; combined NH and HH elicited the lowest V_E but similar $P_{ET}CO_2$ to HH [42]. The authors proposed that the said differences were a function of unequal gas densities. In 2011 Self et al. sought to determine if NH yielded comparable physiological responses to HH by exposing 43 subjects to both conditions. The examination of alveolar gases in brief (5-minute) but severe hypoxic (7620 m) exposures revealed higher P_AO_2, lower P_ACO_2 and quicker blood desaturation in HH as compared to NH [44].

Two of the key studies comparing NH and HH under acute conditions have been performed by Savourey et al., in 2003 and 2007, studying 18 subjects at a simulated intermediate altitude of 4500 m under NH and HH conditions for 40 minutes [45, 46]. In their 2003 study, V_E was greater in NH than HH; this was not the case in their 2007 study, where V_E was similar in both HH and NH. Ventilatory pattern examination demonstrated a greater V_T in NH compared to HH, while f_B tended to be lower for a given V_E in NH as opposed to HH. Frequency of breathing was significantly higher in HH than NH after 5 minutes, but reached similar values in both conditions at the end of the 40-minute exposure. Similar respiratory pattern findings were observed in their subsequent 2007 study also measuring 18 subjects at the same simulated altitude [46]. When looking at end-tidal gases ($P_{ET}CO_2$, $P_{ET}O_2$) in 2003, Savourey et al., observed similar $P_{ET}CO_2$ in HH and NH. At the same time, $P_{ET}O_2$ was significantly lower in HH for the first 15 minutes of hypoxia, but increased to values similar to NH for the remainder of the 40-minute exposure [45]. However, the results for the end-tidal gases were different for the subsequent 2007 study. Specifically there were no differences

between conditions for $P_{ET}O_2$ while $P_{ET}CO_2$ was lower in HH than NH for the first 5 minutes [46]. In their examination of blood gases in 2003, P_aO_2 and P_aCO_2 were lower in HH, despite having equivalent end-tidal partial pressures to NH, suggesting an increase in deadspace ventilation. Shallow and rapid breathing increases the movement of air in the anatomical deadspace while a slower rate and deeper breaths tend to maximize movement of air to the exchange sites. Physiological deadspace is often calculated as the ratio of alveolar or end tidal CO_2 to arterial CO_2 as seen in the Bohr equation (Eq4) where V_D represents deadspace ventilation [49].

$$\text{Eq4. } V_D/V_T = P_aCO_2 - P_{ET}CO_2/P_aCO_2$$

The two studies of Savourey et al. contradict each other in some findings, yet conclusions can still be drawn. Specifically, compared to NH, individuals in HH demonstrated increased f_B and deadspace ventilation, and more profound alkalosis, hypocarbia and hypoxemia [45]. The authors concluded that lower V_T and V_E along with increased f_B, were a "specific response" to HH [45]. Increased deadspace ventilation in HH, as proposed by Savourey et al., would diminish gas exchange and therefore blood oxygenation which could potentiate AMS. Self et al., Singh et al., and Savourey et al., [41, 44, 45] all demonstrated more rapid blood desaturation (S_PO_2) in HH. If this occurs predominantly in HH, subjects are hypoxemic for longer durations, perhaps an explanation for the greater negative symptoms. Additionally, the greater hypocarbia and correspondingly higher pH reported in both of Savourey et al., studies [45, 46] in the HH

condition could consequently have a lesser influence on the central chemosensors drive to breathe.

The three major chamber studies in the following section examined six subjects for 2.5 hours [38], nine subjects for 9 hours [40], and nine subjects for 10 hours [37] using intermediate altitude for sub-acute durations. One of the key differences noted between the conditions was that of V_E during the hypoxic exposure. For a given PO_2, V_E was the greatest in NH, followed by HH[37, 38]. The differences in respiratory variables among the conditions of NH, HH, and HN are seen in Table 5 (appendices). Over 10 hours at simulated altitude of 4570 m, Loeppky et al. [37] observed a significantly greater V_E in NH than HH in their nine subjects. With respect to oxygenation, $P_{ET}O_2$ and blood oxygen saturation (S_PO_2) were similar in HH and NH throughout the test. Likewise, Loeppky et al. (in 1996) also reported no major difference in S_PO_2 between HH and NH, but showed that the respiratory exchange ratio (RER) was higher in NH than HH [43]. The RER differs from the RQ (which only reflects VCO_2/VO_2 at rest at the tissue level) in that it takes into account whole body fluctuations such as respiratory (hyper- or hypoventilation) and metabolic (exercise, fever, etc.) influences. The RER is an indicator of energy substrate use such that it nears 0.82 at rest, as fats are the main fuel source, whilst during maximal exercise RER ranges from 1.1-1.2, with muscle and liver glycogen as primary fuel sources. Under normal circumstances, CO_2 production is proportional to workload, thus at higher work rates more CO_2 is produced, increasing the RER. Since there was no reason for the subjects to be metabolizing different substrates between the two conditions, another mechanism may have been at work to explain the difference in

RER. Such results could suggest that the work of breathing (and hence overall metabolic rate) is lower in HH than NH exposure [37].

1.5.2 Mechanisms for differences in short duration and sub-acute exposures

In brief (≤5 minutes) hypoxic exposures, hypoxemia and desaturation are more pronounced in HH than NH [42, 44]. During acute (<1 hour) exposures, desaturation is greater in HH along with f_B while $P_{ET}CO_2$ is also initially lower than in NH [46]. Work of breathing (WOB) is a function of gas density, elastic work, airway resistance and V_E. Early work suggested that increasing ventilation raises the oxygen uptake of the respiratory muscles [50]. Nemery et al. addressed this hypothesis using a randomized design by comparing the work of breathing in air versus a less dense helium-oxygen mixture (HeO_2) [51]. In six resting non-blinded subjects, the authors noted a mean RER of 0.9 for air and 0.84 for HeO_2, although these were not reported as statistically different. When Loeppky et al.[43] examined RER during a 30-minute exposure to NH and HH they found a significantly higher RER in NH as compared to HH, hypothesizing that increased flow rates and gas density elevate the WOB in NH. Furthermore, Petit et al. demonstrated reduced WOB with decreased P_B in two subjects exposed to altitudes ranging from sea level to 7500 m leaning towards a lowered WOB [52]. During basal ventilation, air movement through lower sections of the respiratory tree tends to be laminar with resistance being low. Higher flow rates precipitate turbulence, increasing resistance and WOB; although this is a speculation, this may be the case in NH.

One alternative hypothesis to account for the differences in end-tidal gases between HH and NH, relates to the dissimilarities in nitrogen equilibrium. In normobaric hypoxia, the F_IO_2 is reduced by increasing the fraction of inspired N_2 (F_IN_2), whereas in hypobaric hypoxia, the PO_2 and the partial pressure of N_2 (PN_2) both decrease proportionally along with P_B. During NH exposure, N_2 follows the pressure gradient from the chamber gas (high N_2) to the tissues (low N_2) quickly achieving N_2 equilibrium as a result of the smaller gradient. During HH exposure, the ambient PN_2 is initially lower than the body's therefore N_2 initially diffuses from the tissues to the alveoli as the tissue stores are diminished. This gradient is larger in the HH condition and thus takes longer to achieve equilibrium [53]. Until this equilibrium is achieved, the P_AO_2 and P_ACO_2 are lowered as a result of the relatively higher P_AN_2 in HH as compared to NH. This mechanism fits nicely with the findings of Savourey et al., showing significantly lower $P_{ET}O_2$ and $P_{ET}CO_2$ in HH than NH, initially, and then no difference in end-tidal gases over time [45, 46]. Upon decompression, some nitrogen is released from tissues and dissolves in the blood forming venous gas embolism or VGE. To date, supportive evidence remains elusive regarding the role of VGE as a contributing factor to the variations seen in ventilation during NH and HH.

1.6 Chronology of the ventilatory response to hypoxia

1.6.1 Acute hypoxic ventilatory response

In the context of short duration hypoxia, Savourey et al. reported the poikilocapnic HVR as the $\Delta V_E/\Delta S_PO_2$ for each minute during NH and HH. They found no differences between the two modalities in 18 subjects over the 40-minute hypoxic exposure to 4500 m [46]. Isocapnic HVR was not assessed, so the absence of differences may have been the result of the confounding effect of poikilocapnia. Despite the blunted respiratory response of the poikilocapnic HVR test; these tests can be relevant because they are more similar to true altitude than an isocapnic HVR test.

The following field studies have measured the HVR during chronic hypoxia exposures to examine its relationship to AMS. Sato et al. demonstrated a doubling of HVR measured during 5 minutes of isocapnic hypoxia over the course of a 12-day acclimatization to 3810 m in six subjects. Increased peripheral chemosensitivity to hypoxia was proposed to be part of the acclimatization process to HH [54]. However, research linking AMS to HVR is conflicting. Hohenhaus et al. noted a lower isocapnic HVR in subjects who had already suffered AMS, yet based on their findings could not predict AMS risk based on HVR in their study of 30 mountaineers with known AMS susceptibility (AMS+) or resistance (AMS-) [30]. Milledge et al. examined the correlation of HVR to AMS on two occasions [29, 31]. In 1988, 32 subjects underwent HVR tests prior to altitude sojourns to 4500 and 5400 m; again, no correlation was found with AMS incidence [29]. Similar results occurred in 1991 when measurement of HVR

was not related to AMS in 17 subjects visiting 4500 m [31]. Conversely, Moore et al. found a blunted isocapnic HVR, lower V_E at altitude, and greater hypoxemia in known AMS+ subjects while at simulated altitude for 7 hours (4800 m) [32]. Similarly, Bartsch et al. examined both the poikilocapnic and isocapnic HVR (*n = 24*) during ascent to intermediate altitude (4559 m) for three days [33]. A significant increase in poikilocapnic and isocapnic HVR from baseline to day 2 occurred in AMS- but not in AMS+ subjects. A key reason for the inconsistencies in the relationship between HVR and AMS incidence is the variety of methodologies of measuring HVR (HH vs. NH, isocapnic vs. poikilocapnic, 5-minute vs. 20-minute, etc.). A consensus method for measuring HVR has not been established; until a common method of measuring HVR is employed it will remain difficult to compare the studies. Exercise and work upon arrival at altitude seems to exacerbate AMS. Moreover the ventilatory response to hypoxia during exercise and the degree of desaturation during exercise in hypoxia appear to be strong predictors of AMS[55]. Furthermore, factors other than the HVR such as rate of ascent, altitude reached and genetic predisposition [56] also play important roles in determining AMS predisposition.

1.6.2 Hypoxic ventilatory decline

In humans, approximately 5 to 30 minutes following the onset of hypoxia, a decrease in ventilation, termed the hypoxic ventilatory decline (HVD) occurs [57]. The HVD, mediated by decreased V_T, is not caused by blood alkalosis or hypocapnia as it occurs in both poikilocapnic and isocapnic hypoxia [57]. The exact site of action for

HVD is uncertain; however, its occurrence could result from changes in peripheral chemoreceptor sensitivity or a central mechanism [57, 58]. The afferent input of the carotid and aortic baroreceptors and peripheral chemoreceptors both appear to reach the nucleus tractus solitarius [59]. Unloading of the baroreflex using negative pressure seems to potentiate the HVR whilst activating the baroreflex appears to blunt the HVR, pointing towards a common integration centre [59]. Using lower body negative pressure (-37.5 mmHg), Koehle et al. demonstrated an increase in HVR but no effect on HVD [58]. If the integrating mechanism responsible for HVD were centrally located, as for the HVR, negative pressure would also affect HVD. These findings further support a peripheral and not central site of action of HVD. Loeppky et al. demonstrated decreased f_B during a 10-hour HH exposure to 4400 m, but the magnitude of the HVD has not yet been compared between NH and HH exposures [37].

1.6.3 Ventilatory acclimatization to hypoxia: a chronic perspective

The subsequent increase in ventilation following HVD is termed the ventilatory acclimatization to hypoxia (VAH) and occurs within hours to days [57]. Sato et al. showed that the isocapnic HVR increased once at altitude (3810 m) and remained elevated up to 3 days after return to sea level in their six male subjects, indicating an increase in peripheral chemosensitivity over the 6-day sojourn [60]. Changes in HVR during altitude exposure indicate that peripheral chemoreceptors have an important role in the acclimatization to hypoxia. In the same study, the hypercapnic ventilatory response to CO_2 (HCVR) only increased significantly from baseline values on days 5 and 6 at

altitude and days 1 and 2 following return to sea level, suggesting that changes in central chemosensitivity are mediated over a longer time course. The time lag in the increase of HCVR, as seen above, suggests that in sea level residents VAH is initially a function of increased peripheral chemosensitivity. Other changes associated with the VAH include a rise in pH. Blood pH increased within 1 day at altitude and remained elevated until day 3 post-altitude, correlating with the isocapnic HVR [60]. Further evidence comes from animal models. In rats, long-term hypoxia (8 days NH F_IO_2 0.10) stimulates the hypertrophy of the carotid body glomus cells and increases ventilation [61]. Sato et al. observed an increase in S_PO_2 after a few days at altitude in conjunction with the rise in AHVR in their six subjects [54]. The increase in saturation was not linked to increased ventilation, suggesting that other factors such as improved ventilation/perfusion ratio or a decrease in pulmonary oedema could play a role in the VAH [54, 60]. In summary, increased ventilation seems to have protective effects in preventing AMS, which may be independent of saturation. An often underappreciated part of the long-term acclimatization to hypoxia regards central chemosensitivity. Promising work has been done examining the central chemoreceptor's role in control of breathing by means of rebreathing tests [8]. The relationship of CO_2 threshold and sensitivity to AMS remains to be thoroughly investigated.

1.7 Cardiovascular effects of hypoxia

1.7.1 Brief and acute exposures

Brief exposure of 20 subjects to severe hypoxia (7620 m), generated a greater heart rate (HR) in HH after 1 minute, but reached similar levels to NH after 5 minutes [44]. When CADO was compared to equivalent HH (7620 m) for 5 minutes, the increase in HR and the decrease in saturation in 43 subjects occurred more rapidly in HH; however, both parameters reached similar values after one minute of exposure [41]. Both CADO and HH induced extreme blood desaturations of 56.91% ± 11.4% and 58.6% ± 12.9% respectively. Indeed, one must keep in mind that exposure length and methodologies used varied when interpreting these data; however, it appears that HR tends to respond similarly to NH and HH although the HR may be slightly higher (at least initially) in HH. Using intermediate altitude (4500 m) in acute exposures Savourey et al. observed a higher HR in HH as opposed to NH in 2003 but no difference between HH and NH in their 2007 study using a very similar protocol [45, 46]. When measuring the hypoxic cardiac response (HCR), quantified as the increase in heart rate for a given change in oxygen saturation ($\Delta HR/\Delta S_PO_2$), the HCR showed no difference between HH and NH [46].

1.7.2 Sub-acute and chronic exposures

Tucker et al. (1983) reported that a hypoxic exposure of 2.5 hours to 4750 m in NH and HH increased blood pressure and HR similarly [38]. Furthermore, earlier research conducted by Savourey et al. showed no association between AMS-environmental symptoms questionnaire score and hypoxic cardiac response (HCR) in 11 subjects during a 12-day expedition to the Andes (4510 m) [46]. Although data are limited it appears that both HH and NH tend to increase HR similarly in short duration, sub-acute and chronic exposures.

1.8 Differences in subjective symptoms induced by hypoxia

Investigating short-term hypoxia, Self et al. looked at subjective hypoxia symptoms, such as headache, dizziness and visual impairment, assessed during a brief hypoxic bout of 5-minutes at 7620 m [44]. Symptoms were greater in HH at 1 minute, but equivalent to NH at 3 and 4 minutes, respectively. This pattern of differences is likely attributable to the aforementioned more rapid desaturation seen in HH. Symptom severity also increased in both HH and NH from 1 minute to 3 minutes [44]. When comparing CADO to HH using a hypoxia symptom questionnaire, no major differences between the two modalities were observed[41]. In Evetts' study, no differences in severity or onset of symptoms appeared between HH and NH. It is interesting to note that arterial saturation (S_aO_2) was similar in both NH and HH, which might explain the lack of differences [42].

The above findings seem to indicate that short but severe exposures to hypoxia are poorly tolerated under either condition and no clear consistent differences in subjective symptoms exist.

In the three studies comparing sub-acute exposures, under HH and NH conditions, using either the LLS or ESQ, all reported higher AMS symptoms in the HH exposure [38-40]. Early chamber comparisons revealed that the Altitude Illness Symptom questionnaire (a measure of participants' AMS symptoms) scores were higher in HH than NH over 2.5 hours [38]. Similarly, mean LLS were higher in HH (3.7) than NH (2.0) and HN (0.4) over a 9-hour exposure to 4564 m [43]. Similar findings were observed by Loeppky et al., who noted higher LLS in HH compared to NH over a 10-hour exposure in nine subjects [39]. Detecting AMS susceptibility and severity prior to altitude sojourns would prove advantageous; to date a validated test does not exist. The previously mentioned 2007 Savourey et al. study pre-exposed 18 subjects to an acute 40 minute HH and NH challenge (4500 m), prior to an expedition in the Andes (*n = 11*) and in the Himalayas (*n = 7*) [46]. They then developed two algorithms to predict AMS susceptibility in altitude sojourns, equation 5 and 6, to predict maximal and mean AMS symptoms based on data gathered during the HH and NH exposures correlated to AMS experienced during the expeditions.

$$\text{Eq5. } AMSmax = 9.47 + 0.104 \bullet P_{ET}O_2 - C_PO_2$$

$$\text{Eq6. } AMSmean = 3.91 + 0.059 \bullet \Delta f + 0.438 \bullet HCR - 0.315 \bullet C_PO_2$$

Where Δf is frequency of breathing in min^{-1}, HCR in $bpm\%^{-1}$, $P_{ET}O_2$ in hPa, calculated peripheral blood O_2 content (C_PO_2) in ml/dl, AMSmax being the most severe

AMS symptoms and AMS mean being the average symptoms to be expected. Savourey et al. concluded that the use of a 30-minute NH test would be the most practical [46].

Regarding tests to predict AMS susceptibility, it has recently been reported [62, 63] that commercially available NH devices often used to simulate HH or determine AMS+/- susceptibility tend to overestimate the calculated HH dose especially by failing to include the effects of water vapour in their hypoxia calculations thus fail to deliver an equivalent hypoxic dose. It is therefore crucial that the calculated NH hypoxic dose is accurate as possible to the targeted HH when using NH exposures to determine AMS susceptibility, or for athletic training, and research.

2.0 Research questions

The purpose of this research was to investigate the differences observed in control of breathing, cardio-respiratory variables and AMS severity occurring between hypobaric and normobaric hypoxia for an iso-oxic dose (i.e. identical P_IO_2). In order to isolate the role of hypobaria, a hypobaric normoxic (HN) condition was added. These differences will be examined over the course of a six-hour exposure to HH, NH, HN and a sham normobaric normoxia (NN) condition respectively.

Our hypotheses are threefold:

1) Exposure to HH will produce the highest AMS scores; scores will correlate negatively with a lower V_E, S_PO_2, and positively with a higher $f_{B.}$
2) Exposure to HH will produce a lower post-test HVR measure compared to NH. The lower HVR in HH will correlate with the greater AMS symptoms seen in HH.
3) Those with a higher CO_2 threshold and a lower sensitivity to CO_2 as measured prior to the exposure will demonstrate higher LLS.

3.0 Methods

3.1 Inclusion criteria

Selected participants (n = 12) were non-smoking, healthy males 18 to 50 years of age with no recent travel to altitude (> 2500 m), or history of cardio-respiratory disease. Subjects were recruited by means of posters distributed throughout the Simon Fraser University (SFU) and University of British Columbia (UBC) campus, and by verbal communication. Upon subject-initiated communication with the primary contact, a consent form and study description were sent out via email. Interested subjects then scheduled a familiarization session lasting two hours. The familiarization took place at SFU and allowed subjects to experience the environmental chamber. After the chamber demonstration, anthropometric data were collected and basic spirometry testing (FVC, FEV_1) conducted in accordance with the American Thoracic Society standards to exclude those with lung disease. Next, subjects were familiarized with the three control of breathing tests (Duffin hyperoxic and hypoxic rebreathe and HVR test); this procedure helped minimize anxiety in subsequent tests. Subjects provided written consent after receiving written and verbal descriptions of the entailed project. This study was approved by the UBC and SFU Ethics Board and conformed to the standards of the Declaration of Helsinki.

3.2 Environmental chamber

The chamber (Perry Baromedical, Florida) is capable of accommodating seven participants. It is located at SFU (365 m) and is cylindrical in shape measuring 1.9 m by 4.5 m. The chamber is composed of three sections, the main lock (ML) where participants stayed during the exposure, an entry/exit lock (EL) used only for entering and exiting the chamber and a wet pot used for diving type experiments. A vacuum is used to simulate HH, while exogenous nitrogen is artificially added inside to reduce the F_IO_2 for NH. The chamber is equipped with CO_2, O_2, P_B, humidity and temperature sensors (Analox Sub-EIR1 5R, Stokesley, North Yorkshire) and a two-way radio type communication system with the outside operator (AMCOM 11 2820-4003, Gaithersburg, MD). The activities inside the chamber were continuously recorded and displayed on outside screens for safety purposes. During the study, a research assistant was inside the main lock (ML) tending to subjects and undertaking the necessary measures at their appropriate time point. The ML research assistant was at all times breathing from a mask to ensure they remained normoxic in the hypoxic exposures (HH, NH) and for a placebo effect in the other two exposures (HN, NN). Furthermore, the research assistant continuously wore a radio headset allowing communication with the outside operators or the main researcher. The radio was also used to prompt the research assistant about upcoming measurements. Oxygen masks were readily available for each subject inside the chamber if an emergency were to arise. A travel tender was present to accompany the

subjects inside the EL as they "traveled" from ambient pressure and inspired gas to the conditions inside the ML.

3.3 Entry and exit protocol

Subjects changed into the provided scrubs and removed any street clothing and shoes, to avoid contaminating the chamber with hydrocarbons.

A) Initial entry: each subject entered the EL with the travel tender. In the EL subjects breathed a normoxic gas mix. The EL was depressurized at a rate of 450 m per minute (in the case on HH, and HN) or hypoxicated (in the case of NH) to equilibration with the ML. In the normobaric exposures, the EL was slightly depressurized (to 1500 m) then re-pressurized in an attempt to blind participants as to which condition they were going to experience. Once equilibration was achieved between the EL and the ML, the subject removed his mask and entered the ML where data collection began.

B) Subjects exited at 60-minute intervals with the aid of the travel tender who facilitated the travel from the ML to the EL. Participants breathed the ambient EL air upon "descent".

Gas partial pressures and simulated altitudes are presented in Table 4. Our calculated hypoxic doses were equivalent at the alveolus as previously discussed above.

Table 1. Exposure altitude and oxygen availability

	Hypobaric Hypoxia	**Normobaric Hypoxia**	**Hypobaric Normoxia**	**Normobaric Normoxia**
Altitude (m)	4500	0	4500	0
Pressure (mmHg)	427	760	427	760
F_IO_2 (%)	0.198	0.105	0.395	0.2093
PO_2 (mmHg)	85	80	166	160
P_AO_2 (mmHg)	75	75	150	150

3.4 Test schedule

Prior to all testing, participants were asked to refrain from alcohol and heavy exercise for 12 hours. Participants were also asked not modify their caffeine intake which could influence the subjective rating of hypoxia-related headaches. Subjects presented themselves on five separate occasions. The first session served as the familiarization session that has been described above. The subsequent four sessions were nine hours in duration including pre-exposure measures, the chamber exposure and post-exposure measurements. Exposures were conducted in a pseudo-randomized crossover, single-blind fashion allowing a minimum 14-day washout between hypoxic exposures and a 7-day washout between normoxic exposures. Participants entered at 60-minute intervals, and were exposed for 6 hours while taking part in relaxing activities (reading, playing cards, talking) in between tests; light snacks (Nature Valley Sweet and Salty© and fresh bananas), juice boxes and water were provided *ad libitum*. Testing was performed both

outside and inside the chamber. Outside of the chamber participants underwent the control of breathing tests prior to and after the six-hour exposure. This was to observe any chances induced by the hypoxic exposure. The inside chamber testing schedule was broken down into immediate (5min), acute (30min) and sub-acute measures (hourly). The rationale for early measures was to be able to examine the immediate responses to hypoxia such as the poikilocapnic HVR and the HCR, and the following decline (hypoxic ventilatory decline) in these parameters. Following the acute and subacute assessments, the hourly procedures tracked changes in the initial measures for trends.

3.5 Duffin CO_2 rebreathing test

Duffin hyperoxic and hypoxic rebreathe tests were conducted in order to determine the respiratory CO_2 VRT and S1 [6]. The hyperoxic test was conducted first followed by the hypoxic test once ventilation returns to resting values. Lying supine on a massage table, wearing nose clips, subjects breathed through a mouthpiece (9060 series, Hans Rudolph, Shawnee, KS) connected to a filter and heated pneumotach (3813 Athletic series, Hans Rudolph, Shawnee, KS) measuring flow upon which f_B and V_T were determined. The mouthpiece, connected to a three-way valve (ER2870, Hans Rudolph, Shawnee, KS), was initially open to the ambient air. The valve was attached to a 10 l rebreathe bag into which the participant rebreathed their expirate. End-expiratory oxygen levels were maintained to either a hypoxic (50 mmHg) or hyperoxic tension (150 mmHg) by sampling air directly from the mouth and adjusting the bag's O_2 partial pressure accordingly by means of a computer-controlled solenoid valve. Return lines conveyed the

sampled gases back to the rebreathe bag. Prior to the start of the test, participants were coached through a 5-minute hyperventilation, breathing room air, in order to reduce and maintain their $P_{ET}CO_2$ between 19 and 25 mmHg. After the hyperventilation, participants maximally exhaled and the valve was switched from room air to the rebreathe bag where participants were asked to take three large breaths to equilibrate the gas in their lungs with the gas in the rebreathe bag. For hyperoxic tests the bag contained 6.5% CO_2, 26% O_2 and balance N_2. In the hypoxic test the bag contained 6.5% CO_2, 6% O_2 and balance N_2. Participants were then instructed to breathe normally. Test termination occurred once ventilation reached 100 $l \cdot min^{-1}$, if $P_{ET}CO_2$ values reached 60 mmHg or if the subject was experiencing severe discomfort. Analog data were collected using a National Instruments DAQ board (NI USB-6229, National Instruments, Austin, Texas) and specialized software (LabVIEW 10.0, National Instruments, Austin, Texas) displaying real-time ventilatory parameters and end-tidal gases over time. After completion of the test, V_E was graphed against $P_{ET}CO_2$ on a breath-by-breath basis expressed as $l \cdot min^{-1} \cdot mmHg^{-1}$ using customized software (LabVIEW 10.0, National Instruments, Austin, Texas). From these data, CO_2 VRT and S1 were established. The Duffin hyperoxic and hypoxic rebreathe test were correlated to LLS. Previous research has measured the reliability of this test with a within-subject coefficient of variability of 0.18-0.32 for CO_2 sensitivity, and of 0.03-0.038 for CO_2 threshold [64].

The Duffin rebreathing method has been chosen as opposed to the steady-state method and the original Read rebreathe method for two reasons. The steady-state protocol does not include a prior hyperventilation; therefore the measure of baseline

ventilation is absent if the initial CO_2 stimulus is above the CO_2 threshold. Thus the threshold may be hidden if the initial starting PCO_2 is higher especially during hypoxic type tests. Secondly, slope sensitivity may vary. The possibility of slope error in steady-state test occurs if one of the chosen points is below the VRT is included or if the chosen steady-state tension is above the participant's maximum ventilation [15].

3.6 Hypoxic Ventilatory Response test

The hypoxic ventilatory response (HVR) was measured on the familiarization day, prior to and after exposures with procedures based on methods conducted by research groups from our laboratories [17, 65]. Resting supine on a plinth, listening to relaxing music, participants wore nose clips and breathed through a mouthpiece and heated pneumotach connected to a one-way non-rebreathing valve (2700 series, Hans Rudolph, Shawnee, KS). Participants breathed naturally for 2-5 minutes in order to establish baseline $P_{ET}CO_2$ values; the isocapnic CO_2 level used during the test. At the onset of the HVR test, oxygen levels were lowered from an F_IO_2 of 0.2093 to approximately 0.05 over 5 minutes through the addition of 100% N_2 to the inspired air via a custom made 25 l mixing chamber (specifications available upon request). The flow of N_2 was adjusted such that the F_IO_2 was constantly decreasing without prominent plateaus. Isocapnia was maintained by means of CO_2 addition using a manually-controlled gas regulator. End-tidal gas levels were analyzed breath-by-breath using O_2 and CO_2 analyzers (Vacumed Fast Response Edition 17625 and 17630, Ventura, CA) connected to the mouthpiece by sample lines. Data were acquired and converted to digital

signals (PowerLab 16/30 ADinstruments, Colorado Springs, CO) and viewed in real-time using commercially-available software (LabChart, ADinstruments, Colorado Springs, CO). A pulse oximeter (Avant 9600, Nonin Medical Inc., Plymouth, MN) was attached to the index finger and the test terminated once S_PO_2 reached 75%. From the acquired data, V_E was plotted against S_PO_2. A linear slope was fit using computer software (Microsoft Excel 2010, Redmond, WA); the magnitude of this slope was interpreted as the HVR.

3.7 Cardio-respiratory variables

A series of cardio-respiratory variables were measured upon entry at 5 minutes, 30 minutes and hourly until exit. Heart rate was measured by means of the pulse oximeter. Blood pressure was measured using an automated blood pressure cuff taking three readings which were averaged (BPM 200, BpTRU, Coquitlam, BC) and S_PO_2 using right earlobe pulse oximetry (CANL-425SV-A, Med Associates Inc., St. Albans VT). During the exposure, ventilation was measured at specific time points by means of a mouthpiece connected to a one-way valve and pneumotach. End-tidal gases were measured by O_2 and CO_2 sensors (Vacumed Gold Edition 17518 and 17515, Ventura, CA). The hypoxic cardiac response (HCR) and poikilocapnic HVR were subsequently determined by measuring the change in HR and V_E for a fixed hypoxic stimulus during the first 5 minutes of exposure to HH and NH in chamber.

3.8 Subjective hypoxia symptoms

Subjective hypoxia symptoms were measured using the Lake Louise Score (LLS), which has successfully been used in identifying the onset of AMS [21]. This was measured hourly until exit. The question inquiring about sleep quality was omitted as subjects did not stay overnight; therefore scores were graded out of 12 as opposed to the conventional score of 15.

3.9 Statistical methods

The primary outcomes examined in this research experiment were control of breathing and cardio-respiratory variables. Descriptive statistics (means and standard deviations) were calculated for cardio-respiratory parameters, AMS scores, HVR, and rebreathing test measures. A two-way repeated measures ANOVA was undertaken for cardio-respiratory variables, AMS, HVR, and rebreathing tests at their previously mentioned time points, and for each of the four conditions (HH, NH, HN, NN); Bonferroni's test was used to compare pairs of columns (i.e. the different times or environmental conditions). When comparing pre- and post- for control of breathing test within the same condition a one-way ANOVA was used and a Tukey's post-hoc test conducted. A $P < 0.05$ was deemed statistically significant. Pearson`s *r* correlations (two-tailed) were conducted between LLS scores and cardio-respiratory variables, and with pre- and post-test HVR and rebreathing data scores for each exposure. Unpaired t-tests were used to compare AMS+ vs. AMS- subjects when necessary.

3.10 Safety measures

Consultation with the National Fire Protection Association and the SFU Environmental Medicine and Physiology Unit Director deemed safe the enrichment of air with 0.395 O_2 in order to achieve an inspired PO_2 of 150 mmHg in our hypobaric normoxia condition. A physician was present outside the chamber during all hypoxic and hypobaric exposures as was a qualified first aid and CPR/AED provider. Subjects were removed from the chamber upon reaching a LLS > 9, a steady S_PO_2 reading < 70% or subject request.

4.0 Results

A total of $n = 11$ subjects completed the four exposure days; one subject dropped out as he misunderstood the time commitments. We considered our blinding effective; subjects reported a difference between the hypoxic (HH and NH) and normoxic exposures (HN, NN) but couldn't differentiate normobaric from hypobaric exposures as determined by post-experiment interviews. Descriptive statistics for the subjects' resting baseline parameters gathered on the familiarization day are seen in Table 5. Subjects were predominantly fit and most led an active lifestyle.

Table 2. Baseline and anthropometric data.

Subject	Age	Height (cm)	Weight (kg)	BMI	FVC	FEV_1	FEV_1/FVC	Systolic (mmHg)	Diastolic (mmHg)	HR (bpm)	AMS+/-
s005	18	181	74.6	22.7	4.31	4.05	94	125	76	82	+
s006	20	185.5	76	22.1	7.66	6.17	80.5	118	78	69	+
s007	19	172	61.6	20.1	5.08	4.09	80.4	104	69	77	+
s009	20	173	72.5	24.2	6.23	5.22	83.8	107	73	80	+
s004	47	174	63.6	21	6.23	5.22	83.8	103	67	79	-
s012	19	168.5	60	21.1	4.74	3.84	81	119	71	60	-
s013	21	185	77	22.4	5.54	4.41	80.9	120	71	74	-
s014	27	183	74	22.1	5.5	4.55	82.7	125	70	68	-
s016	23	190	90	24.9	8.8	7.22	82	111	58	60	-
s017	32	178	73	23.1	6.34	5.2	82	112	79	54	-
s018	34	183	112	33.5	5.56	4.54	81.7	115	76	100	+
AMS+											
Mean*	22.2	178.8	79.3	24.5	5.8	4.8	83.8	113.8	74.4	81.6	
SD	6.6	6.1	19.1	5.2	1.2	0.89	5.9	8.5	3.5	11.4	
AMS+-											
Mean**	28.2	179.7	72.9	22.4	6.2	5.07	82.1	115	69.3	65.8	
SD	10.3	7.8	10.6	1.4	1.4	1.2	1.09	7.9	6.8	9.5	

*n = 5 **n = 6

4.1 Prevalence of AMS

The LLS was used to quantify AMS severity and then classify subjects as either AMS+ or AMS- . Scores recorded at hour 6 or from the last questionnaire completed inside the chamber were used for analysis. Some subjects were taken out of the chamber prior to completion of the 6-hour period for safety purposes as their LLS were > 9. The LLS was greater in the two hypoxic conditions ($P < 0.05$) as opposed to the normoxic conditions (Figure 1). When separated into AMS+ and AMS- subjects, the AMS+ group had significantly elevated LLS scores ($P < 0.0001$) (Figure 2). Group and AMS+ /AMS- scores can be seen in Table 7 and individual LLS scores per condition can be seen in Table 8 in the appendices. No order effect was seen between HH and NH for the LLS.

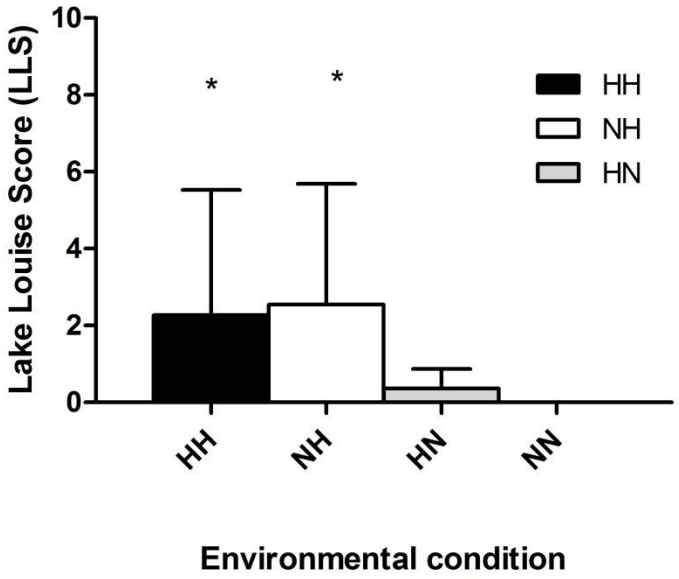

Figure 1. Mean LLS of all subjects at hour 6 across four conditions. The * indicates a significant difference between group means ($P < 0.05$). Bars represent means, and error bars represent standard deviation. For each group, n=11.

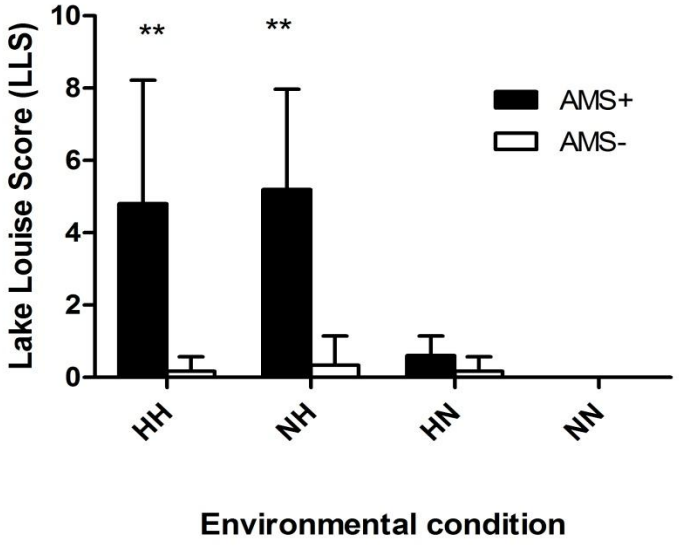

Figure 2. Mean LLS of AMS+ and AMS- at hour 6 across all four conditions. The ** denotes significance ($P < 0.0001$). Black bars represent AMS+ subjects (n = 5) and white bars represent AMS- subjects (n = 6).

4.2 Control of breathing

Hyperoxic and hypoxic Duffin CO_2 rebreathing tests were conducted pre- and post- chamber exposure. Figure 3 represents a typical response to a hyperoxic rebreathing test, whilst Figure 4 represents the typical response to a hypoxic test. Note that in Figure 4, a second slope (S2) is seen; when present this change in slope was mediated by an increase in f_B. Pre-chamber group means and standard deviations for the VRT and S1 for the hyperoxic and hypoxic Duffin tests per conditions can be seen in Table 9 found in the appendices.

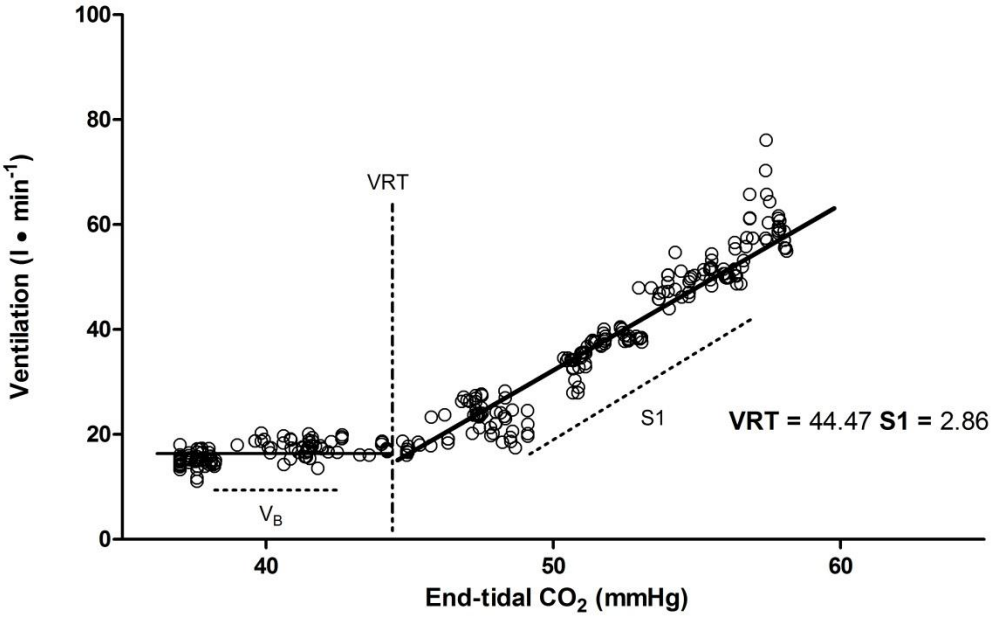

Figure 3. Hyperoxic Duffin rebreathing test. The VRT indicates the point upon which ventilation increases linearly with PETCO2. The S1 indicates the sensitivity of this slope. VRT = Ventilatory response threshold, S1 = Slope sensitivity, VB = Basal ventilation.

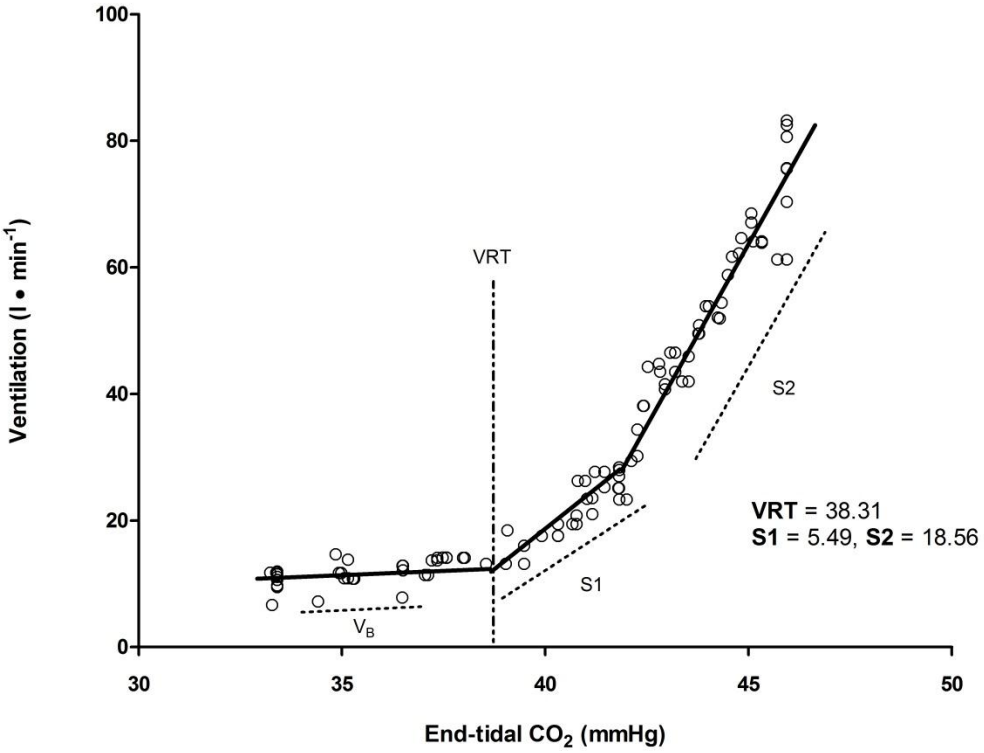

Figure 4. Hypoxic Duffin rebreathing test. The VRT indicates the point upon which ventilation increases linearly with $P_{ET}CO_2$. The S1 indicates the sensitivity of this slope. VRT = Ventilatory response threshold, S1 = Slope sensitivity, V_B = Basal ventilation. S2 = Second slope sensitivity

As expected, the pre-chamber VRT (values are expressed as mean and ±SD) for all of the hyperoxic Duffin tests (45.98, ±1.65 mmHg) was higher than that for the hypoxic Duffin test VRT (41.15 ±2.26 mmHg, $P < 0.0001$). The sensitivity for S1 was lower in the hyperoxic test (3.96 ±2.5 $l \cdot min^{-1} \cdot mmHg^{-1}$) than in the hypoxic test (5.84 ±1.71 $l \cdot min^{-1} \cdot mmHg^{-1}$, $P < 0.01$). The sensitivity for S2, when present, was 3.41 ±1.95 $l \cdot min^{-1} \cdot mmHg^{-1}$ for the hyperoxic test and 5.42 ±3.67 $l \cdot min^{-1} \cdot mmHg^{-1}$ for the hypoxic test. The coefficients of variation for the pre-chamber VRT in the hyperoxic and hypoxic test were 0.036 and 0.056 respectively; this included the familiarization session. The S1 coefficient

of variability was 0.50 for the hyperoxic test and 0.28 for the hypoxic test. When comparing all of the Duffin tests (hyperoxic and hypoxic pre-chamber), S2 were present 38% of the time in AMS+ subjects and 32% in AMS- subjects. The hypoxic Duffin test elicited S2 patterns in 31% of AMS+ and in 29% of AMS- subjects; these differences were non-significant.

4.2.1 Hyperoxic Duffin rebreathing test

The hyperoxic VRT decreased from pre- to post-exposure in HH and NH (Pre: HH 46.30 ±1.88 mmHg, NH 45.17 ±1.92 mmHg, Post: HH 43.25 ±1.8 mmHg, NH 41.01 ±1.47 mmHg, $P < 0.0001$) as seen in Figure 5. Difference between the pre-chamber test and the post-chamber test (VRT pre- minus VRT post-) were seen. The Δ VRT difference was larger in NH than HN and NN (NH: 4.15 ±2.05 mmHg; HN: 1.22 ±2.05 mmHg; NN: 1.02 ±2.34 mmHg $P < 0.01$) but no difference was seen between HH and the other conditions. No differences in the hyperoxic test were observed pre- to post- in all conditions for S1 as seen in Figure 6. When comparing the V_B in hyperoxic Duffin test pre-to post-exposure, only in HH was post-test V_B higher (Pre: 12.07 ±5.29 $l \cdot min^{-1}$, vs. Post: 15.95 ±6.53 $l \cdot min^{-1}$, $P < 0.01$); this was mediated by a greater V_T (Pre: 0.94 ±0.59 l vs. Post: 1.18 ±0.65 l $P < 0.05$). Finally, the post-chamber hyperoxic VRT correlated negatively to LLS (i.e. lower VRT in AMS+) in NH ($r = 0.376$, $P < 0.05$). Group and individual data for hyperoxic test can be seen in the appendices (Table 10 and 11). Figure 14, in the appendices shows a typical trace, incorporating selected channels during a hyperoxic Duffin rebreathing test.

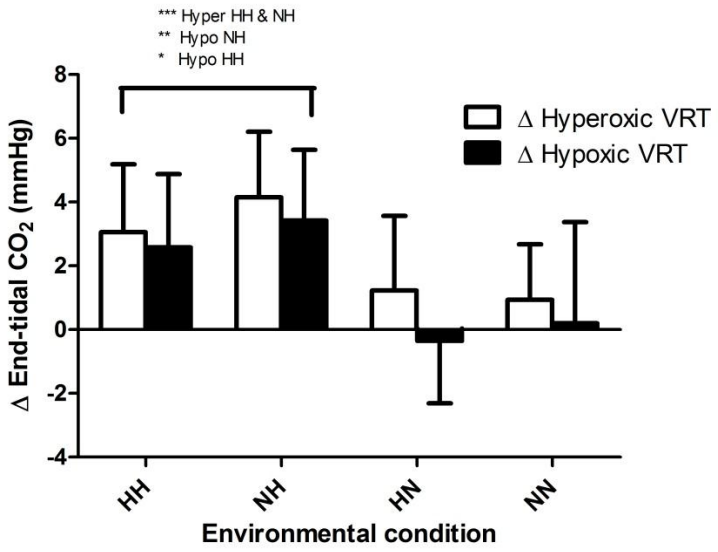

Figure 5. Difference in the hyperoxic (white bars represent the Δ in hyperoxic VRT) and hypoxic VRT (black represent the Δ in hypoxic VRT). The * denotes $P < 0.05$, the ** signifies $P < 0.01$, and the *** represents $P < 0.001$. VRT = Ventilatory response threshold. Hypo and hyper refer to hyperoxic and hypoxic rebreathing tests.

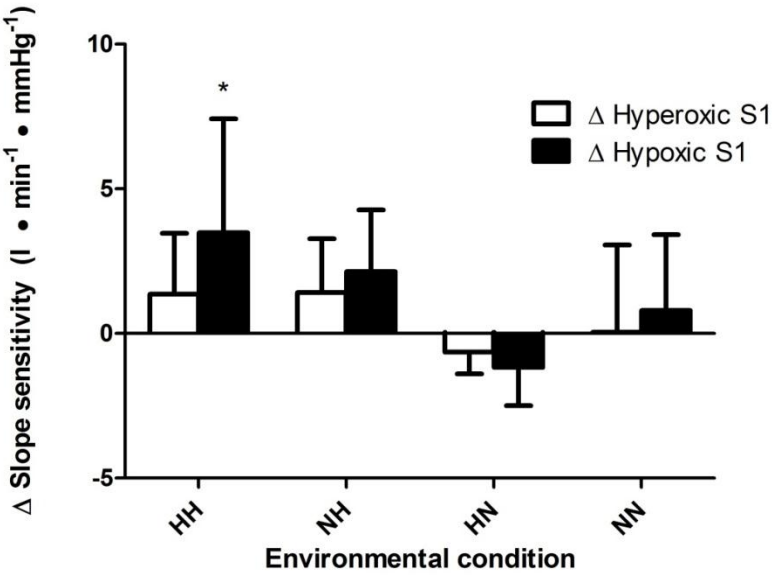

Figure 6. Difference in hyperoxic (white bars represent Δin hyperoxic S1) and hypoxic S1 (black bars represent Δ in hypoxic S1). Note n = 10; s013 was removed as he had an unexpected response. The * denotes that a significance $P < 0.05$ was only observed in theΔ S1 in the HH hypoxic Duffin test. S1 = Slope sensitivity.

4.2.2 Hypoxic Duffin rebreathing test

The hypoxic VRT decreased significantly following the two hypoxic exposures (Pre: HH 41.96 ±2.14 mmHg, NH 40.96 ±2.63 mmHg, Post: HH 39.38 ±2.07 mmHg, NH 37.53 ±2.06 mmHg, $P < 0.01$ and $P < 0.001$). When comparing the difference between the pre-exposure test and the post-exposure test (VRT pre- minus VRT post-chamber) the hypoxic Δ VRT difference was larger in NH than HN and NN (NH 3.421 ±2.2 mmHg, vs. HN 0.98 ±2.17 mmHg, & NN 0.56 ±2.47 mmHg, $P < 0.01$). Similarly, the difference in pre- to post- Δ VRT in HH was larger than in HN and NN (HH 2.57 ±2.30 mmHg, vs. HN 0 .98 ±2.17 mmHg, & NN 0.56 ±2.47 mmHg, $P < 0.05$) (Figure 5). The S1 showed a difference pre- to post- in NH initially (Pre: 6.23 ±2.93 $l \cdot min^{-1} \cdot mmHg^{-1}$, Post: 12.27 ±13.73 $l \cdot min^{-1} \cdot mmHg^{-1}$, $P < 0.05$) however, after removal of a subject with an unusual response (extremely high S1 in the post-chamber hypoxic Duffin test; HH 26.56 $l \cdot min^{-1} \cdot mmHg^{-1}$, post- NH 52.18 $l \cdot min^{-1} \cdot mmHg^{-1}$) this difference was nullified. Removal of this subject (s013) from all conditions in the hypoxic S1 analysis showed a difference pre- to post- in S1 in the HH condition (Pre: 4.09 ±1.87 $l \cdot min^{-1} \cdot mmHg^{-1}$, Post: 7.91 ±4.64 $l \cdot min^{-1} \cdot mmHg^{-1}$, $P < 0.05$). No differences were observed between AMS+ and AMS- subjects with respect to VRT or S1.

Initially, there was no increase in the V_B stage in the hypoxic Duffin test post- HH exposure ($P = 0.11$). Upon re-examination, removal of an outlier (s018) showed a significantly greater post-exposure V_B in HH (Pre: 12.61 ±6.52 $l \cdot min^{-1}$, vs. Post: 19.69 ±10.67 $l \cdot min^{-1}$, $P < 0.01$). Accordingly, both f_B (Pre: 15.35 ±5.52 min^{-1}, vs. Post: 17.7

±5.65 min^{-1}, $P < 0.05$) and V_T (Pre: 0.93 ±0.62 l, vs. Post: 1.32 ±0.93 l, $P < 0.05$) increased post-exposure during the V_B stage of the hypoxic Duffin test after HH. No correlations were observed between LLS and the hypoxic VRT and S1 in HH and NH. Tables 10 and 12, in the appendices, shows group and individual data across all conditions for the post-chamber hypoxic test.

4.2.3 Hypoxic ventilatory response test

Representative HVR and HCR plots are seen in Figures 7 and 8. In both cases the test officially began at the start of desaturation upon inhalation of the hypoxic gas. As the F_IO_2 gradually decreased, V_E and HR increased in a progressive manner. By convention, the x-axis (blood oxygenation) is displayed from left to right, allowing graphing of a positive slope.

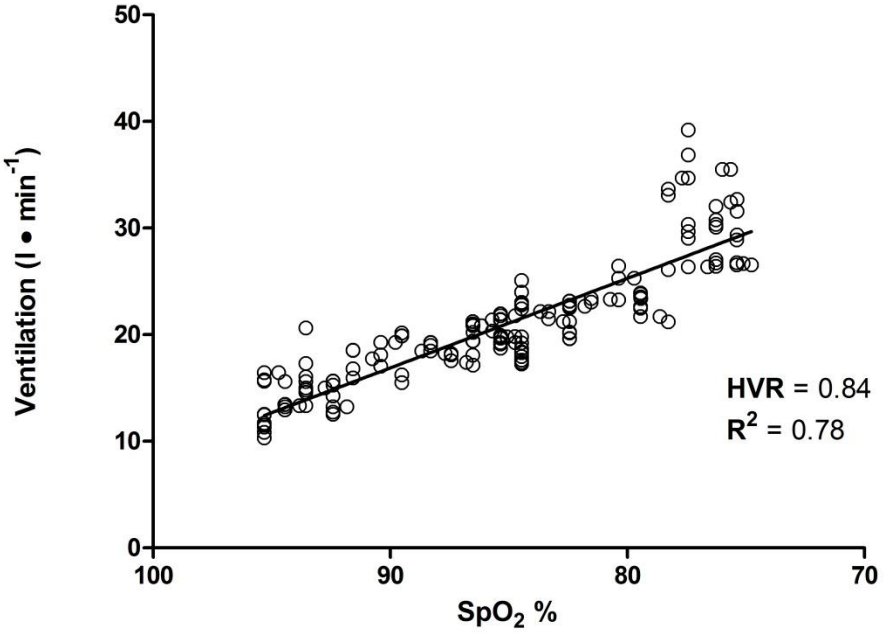

Figure 7. Hypoxic ventilatory response test.

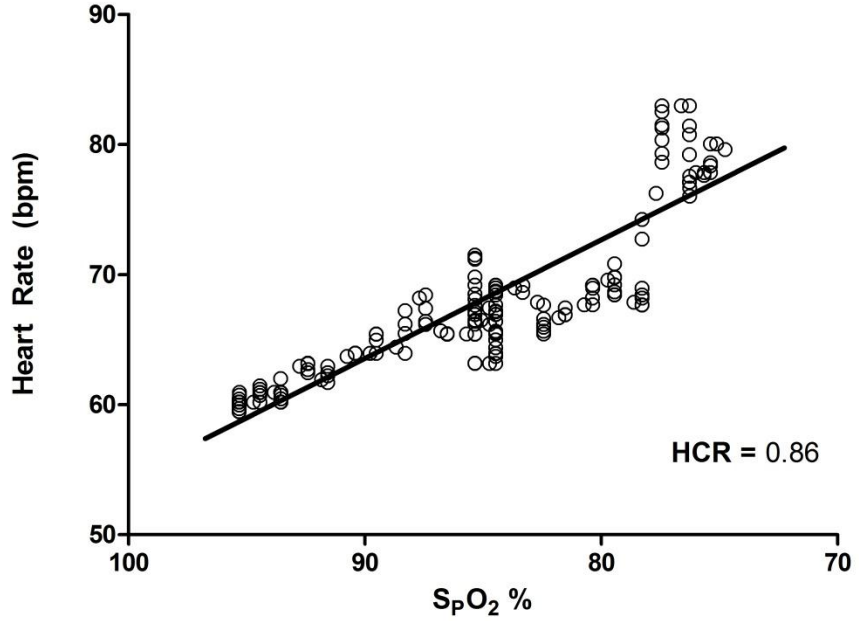

Figure 8. Hypoxic cardiac response test

Table 13 illustrates the HVR and HCR measured pre-exposure while Table 14 demonstrates the HVR and HCR values seen post-exposure. The mean HVR for all pre-exposure measures was 0.55 ±0.16 $l \cdot min \cdot \%S_aO_2^{-1}$ and was 0.51 ±0.24 $l \cdot min \cdot \%S_aO_2^{-1}$ following HH and 0.69 ±0.4 $l \cdot min \cdot \%S_aO_2^{-1}$ following NH exposure; there were no significant differences (P = 0.14). Group and AMS+/AMS- mean pre-and post-chamber HVR and HCR scores can be seen in Tables 15 and 16 (appendices). The HVR and the HCR did not differ significantly when compared pre- post-chamber between or within conditions. Comparison of post-chamber HVR and HCR scores again showed no statistical significance. Both the pre-exposure HVR and HCR were evaluated for their coefficient of variability yielding 0.448 and 0.389 for HVR and HCR respectively (Table 17 appendices). Furthermore no difference was observed between AMS+ and AMS- subjects before (HVR four-condition mean; AMS+ 0.62 ±0.23 $l \cdot min \cdot \%S_aO_2^{-1}$, AMS- 0.49 ±0.05 $l \cdot min \cdot \%S_aO_2^{-1}$. HCR four-condition mean; AMS+ 0.69 ±0.37 $l \cdot min \cdot bpm^{-1}$, AMS- 0.649 ±0.23 $l \cdot min \cdot bpm^{-1}$) or after the chamber exposure using an unpaired t-test (P > 0.05). Figure 15 (appendices) illustrates a sample trace taken during a HVR test; the S_PO_2 and F_IO_2 both progressively decrease while ventilation increases. No significance or correlations whatsoever were found with the poikilocapnic HVR and HCR.

4.3 Cardio-respiratory parameters

Cardio-respiratory parameters were measured at 5min, 30min, and hourly until chamber exit. The following analyses used the 5min and 30min time points and averaged

the values for the last 5 hours; referred to as the 5hr-mean time point. Group mean for all cardio-respiratory parameters can be seen in Table 18 in the appendices. At all time points, V_E showed a strong environmental condition effect ($P < 0.0001$). At 5min V_E during HH and NH was significantly higher than during the two normoxic conditions (HH 18.95 ±6.68 $l \cdot min^{-1}$, NH 18.47 ±4.67 $l \cdot min^{-1}$ vs. HN 14.79 ±3.77 $l \cdot min^{-1}$, NN 14.82 ±3.28 $l \cdot min^{-1}$ $P < 0.05$). At the 5hr-mean time point V_E in NH was higher than HN and NN (NH 17.58 ±3.0 $l \cdot min^{-1}$, vs. HN 14.79 ±2.87 $l \cdot min^{-1}$, $P < 0.05$ & NN 14.39 ±2.6 $l \cdot min^{-1}$, $P < 0.01$). The V_E in HH (16.48 ±3.65 $l \cdot min^{-1}$) was only higher than NN at the 5hr-time point. The above mentioned differences in V_E can be observed in Figure 9. No differences were seen between AMS+ and AMS- subjects, nor were there any correlations observed between V_E and LLS. No difference from the above-mentioned was seen when V_E was normalized for body surface area (BSA).

There were no differences between AMS+ and AMS- subjects for f_B at any time point in or between conditions. A correlation was observed between f_B at 5hr-mean and LLS with AMS+ subjects having a higher rate of breathing in HH ($r = 0.406$, $P < 0.05$) and NH ($r = 0.434$, $P < 0.05$). When normalized for BSA, f_B was greater in HH at 5min than at 5hr-mean (10.36 ±4.4 $b \cdot min^{-1}$, vs. 9.07 ±3.78 $b \cdot min^{-1}$, $P < 0.05$). This difference was absent when reported as absolute values. Figure 10 illustrates f_B in all conditions at all time points; note the large standard deviations.

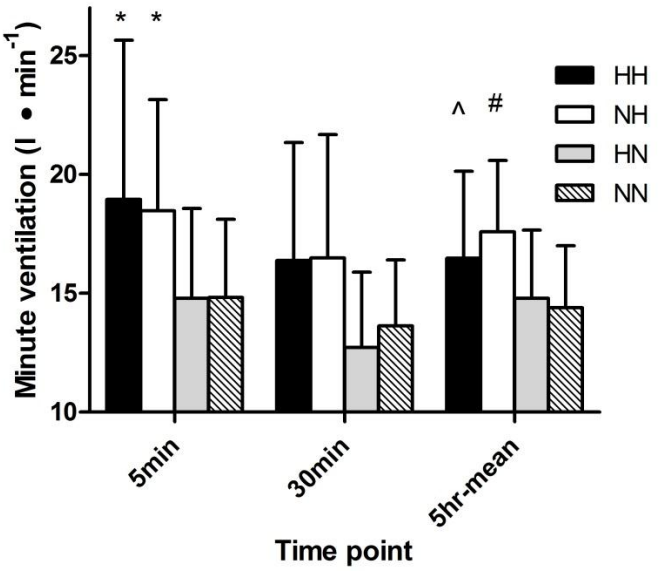

Figure 9. Overall V_E in all conditions at all time points. The * indicates that HH, NH > NN, HN at 5min ($P < 0.05$). The # denotes that NH > HN ($P < 0.01$) and NN ($P < 0.05$) at 5hr-mean The ^ signifies that HH > NN at 5hr-mean ($P < 0.05$).

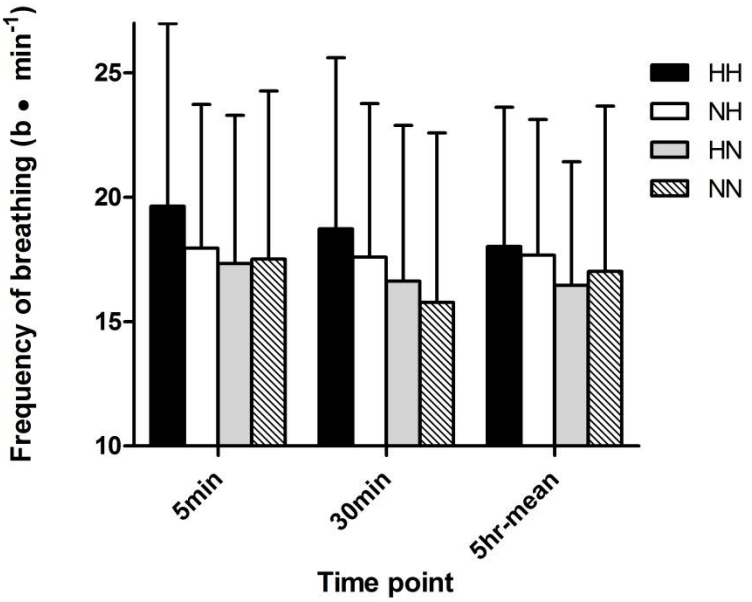

Figure 10. Overall f_B in all conditions at all time points. No significance was observed.

Tidal volume (Figure 11) was higher in NH than HN at 5min (1.88 ±0.52 l, vs. 0.98 ±0.40 l, $P < 0.05$) and 30min (1.12 ±0.59 l, vs. 0.90 ±0.40 l, $P < 0.05$) and greater than NN at 5hr-mean (1.57 ±0.41 l, vs. 0.99 ±0.33 l, $P < 0.05$). A correlation was observed between AMS+ and V_T. The AMS+ subjects had a lower 5hr-mean V_T in both the HH and NH condition (HH; $r = 0.3982$ $P < 0.05$, NH; $r = 0.4824$ $P < 0.05$).

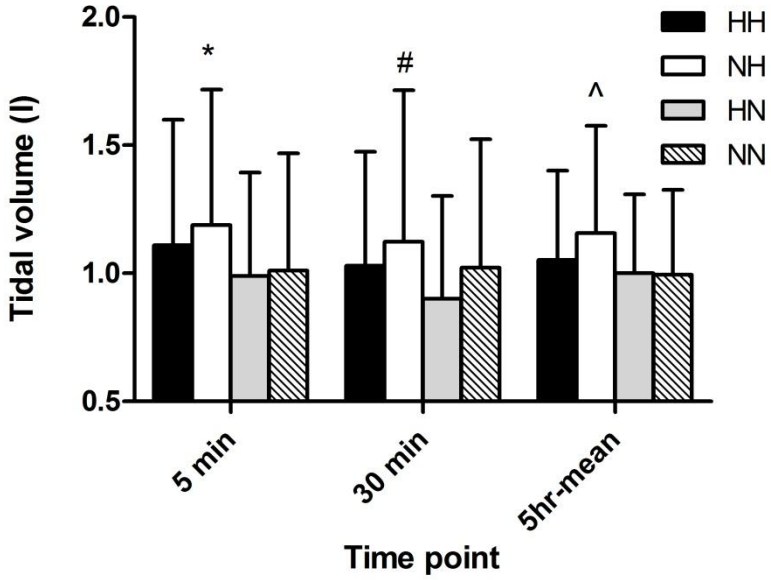

Figure 11. Overall V_T in all conditions at all time points. The * indicates NH > HN at 5min. The # denotes NH > HN at 30min. The ^ signifies NH > NN at 5hr-mean. All $P < 0.05$

Blood oxygen saturation was significantly lower in both hypoxic conditions as opposed to the normoxic conditions at all time points ($P < 0.0001$) as seen in Figure 12. In HH, S_PO_2 was lower at 30min than 5min (86.36 ±5.93 S_PO_2, vs. 80.53 ±8.62 S_pO_2, $P < 0.05$). In NH, S_pO_2 was lower at 30min than 5min and 5hr-mean (85.58 ±5.34 S_pO_2, vs. 80.47 ±6.58 S_pO_2, & 80.61 ±5.26 S_pO_2, $P < 0.01$). No difference was observed between

AMS+ and AMS- subjects for S_PO_2 at any of the time points, nor were there any significant correlations between LLS and S_PO_2.

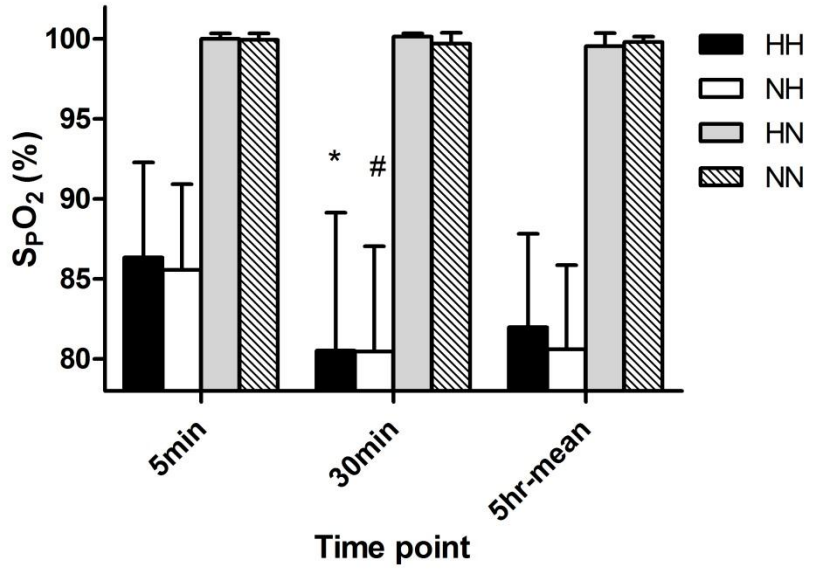

Figure 12. Overall S_PO_2 in all conditions at all time points The * denotes HH 30min < 5min ($P < 0.05$). The # signifies NH 30min < 5min and 5hr-mean ($P < 0.01$).

An environmental condition effect was observed for HR ($P < 0.0001$). Both hypoxic conditions elicited greater HR than the normoxic conditions at all time points (Figure 13). Heart rate differed in all conditions from 5min to 5hr-mean as seen in HH (85.6 ±19.77 bpm, vs. 96.56 ±12.58 bpm, $P < 0.01$) in NH from 5min to 5hr-mean (87.82 ±10.87 bpm, vs. 95.18 ±14.05 bpm, $P < 0.05$) in HN (72.3 ±12.05 bpm, vs. 80.43 ±11.56 bpm, $P < 0.001$) and in NN (71.52 ±13.97 bpm, vs. 76.3 ±10.16 bpm, $P < 0.05$). The HR data in both normoxic conditions (NN and HN) did not differ from control HR data taken out of the chamber during the familiarization. No difference was observed between

AMS+ and AMS- subjects for HR at any time. Individual HR data can be seen in the appendices (Table 19).

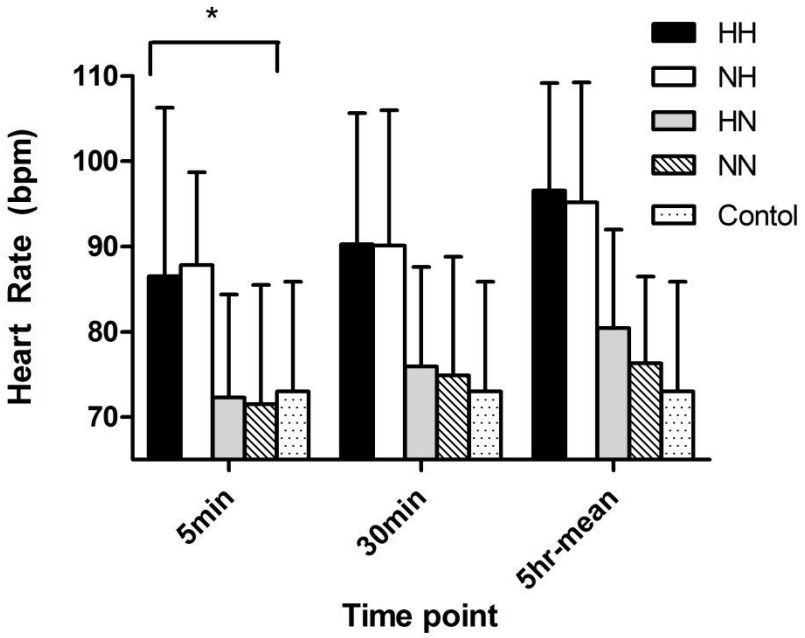

Figure 13. Overall HR in all conditions at all time points. The * denotes that in all conditions HR at 5min < 5hr-mean ($P < 0.01$ HH, $P < 0.05$ NH, $P < 0.001$ HN, $P < 0.05$ NN). Control data was gathered during the familiarization session.

No significance was observed for systolic and diastolic BP between and within condition at all time points. When compared to control values taken outside of the chamber diastolic pressure was higher in HH at 5min (81.45 ±6.78 mmHg, vs. 71.63 ±5.9 mmHg, $P < 0.05$) and in HN at 5min and 30min (81.54 ±6.7 mmHg, & 81.0 ±7.71 mmHg, vs. 71.63 ±5.9 mmHg, $P < 0.05$). There were no differences between control systolic pressure and any of the environmental conditions. Comparisons of AMS+ and AMS- subjects showed no difference, nor were any correlations pertinent.

5.0 Discussion

The primary purpose of this study was to compare and examine the control of breathing and cardio-respiratory responses to equivalent hypobaric hypoxia and normobaric hypoxia exposures. The discussion will be broken into two major sections. Firstly the control of breathing tests (Duffin rebreathing and HVR) conducted outside of the chamber prior to and upon exit will be discussed. Secondly we will elaborate on the cardio-respiratory parameters (V_E, V_T, f_B, S_PO_2, HR and BP) measured inside the chamber. In both of the aforementioned sections, we will discuss the relationship of the control of breathing test and of the measured cardio-respiratory parameters to AMS and their applicability in the prediction of this condition. To date, this is the first crossover design study using four environmental conditions (HH, NH, HN, NN) and equivalent hypoxic doses to systematically examine the control of breathing with a sufficient duration to induce acute mountain sickness in susceptible subjects.

5.1 Control of breathing

We demonstrated repeatability in our control of breathing test similar to that of previously published studies. Our coefficient of variability pre-chamber exposure for the VRT in the hyperoxic and hypoxic test were of 0.036 and 0.056, respectively. The S1 coefficient of variability was 0.50 for the hyperoxic test and 0.28 for the hypoxic test. Similarly Jensen et al. observed a coefficient of variability of 0.03 and 0.038 for the VRT and of 0.185 and 0.327 for S1 [64]. Conversely, Mahamed and Duffin observed a

coefficient of variability of 0.076 and 0.07 for the VRT and of 0.59 and 0.85 for the hyperoxic and hypoxic S1 respectively [66].

Our pre-exposure values for the VRT in hyperoxia (45.98 ±1.65 mmHg) and hypoxia (41.15 ±2.26 mmHg) align with those previously reported. Somogoyi et al. [67] reported pre-hypoxia exposure values of 49.6 ±1.06 mmHg and 46.0 ±1.65 mmHg for hyperoxic and hypoxic tests, and Mahamed et al. [68] reported values of 42.4 ±1 mmHg and 38.7 ±1.1 mmHg, respectively. Our pre-exposure values for the S1 were 3.96 ±2.5 $l \cdot min^{-1} \cdot mmHg^{-1}$ and 5.84 ±1.71 $l \cdot min^{-1} \cdot mmHg^{-1}$ for the hyperoxic and hypoxic tests. In comparison, for hyperoxic and hypoxic sensitivity Somogyi et al. [67] reported pre-hypoxia exposure values of 3.3 ±0.51 $l \cdot min^{-1} \cdot mmHg^{-1}$ and 4.9 ±0.69 $l \cdot min^{-1} \cdot mmHg^{-1}$, Mahamed et al. [68] reported 5.1 ±1.3 $l \cdot min^{-1} \cdot mmHg^{-1}$ and 6.8 ±1.5 $l \cdot min^{-1} \cdot mmHg^{-1}$, and Koehle et al. [65] reported 3.4 ±0.3 $l \cdot min^{-1} \cdot mmHg^{-1}$ and 5.2 ±0.6 $l \cdot min^{-1} \cdot mmHg^{-1}$, each for hyperoxic and hypoxic tests, respectively. Based on these comparisons, the quality of our rebreathing tests is acceptable.

Our findings are broadly consistent with the current literature. Following hypoxic exposure, both the hyperoxic and hypoxic VRT are typically reduced. Three hours of isocapnic hypoxia, as well as consecutive intermittent hypoxia bouts over a week, have been reported to lower the hypoxic VRT measured with the Duffin rebreathing tests [65, 66, 68] but were not sufficient to induce changes in S1. However, in the present study, 6-hours of hypoxia were sufficient to increase the S1 (and V_B) in the hypoxic Duffin rebreathing test but only after the HH exposure (i.e. these variables did not increase post-NH exposure). Ainslie and Burgess observed similar increases in peripheral sensitivities (S1) in 5 subjects after 9 days at a comparable altitude (5000 m; i.e. HH), again using

hypoxic Duffin rebreathing tests [69]. Hence, it appears that shorter hypoxic exposures can decrease the peripheral threshold but that longer exposures are needed to increase sensitivity of the peripheral chemoreflex. In terms of hyperoxic VRT testing, intermittent hypoxia [65] studies and field studies [69, 70] have reported lowering of the hyperoxic VRT as seen by using rebreathing and steady-state tests. Few hypoxia exposure or acclimatization studies have shown increases of the hyperoxic S1 or central sensitivity. Of the few using hyperoxic rebreathing, Fan el al. demonstrated increased S1 after 2-4 days at 5050m [70]. Using steady-state tests, increases in hyperoxic S1 have been noticed by Ainslie et al., who exposed 12 subjects to 0.123 F_IO_2 overnight in NH for 5 days [71], by Fetamian and Robbins, who exposed 10 subjects to 8 hours of poikilocapnic and 8 hours of isocapnic hypoxia on separate days ($P_{ET}O_2$ 55mmHg) [72], and by Koehle et al. [65], who used bouts of intermittent NH. Some of the aforementioned studies used the steady-state method, which tends to underestimate the S1 as opposed to rebreathing type tests (i.e. the response slope is shifted to the left). The leftward shift in steady-state type tests results from the $P_{ET}CO_2$-P_CCO_2 (central CO_2) gradient. Consequently in steady-state tests, $P_{ET}CO_2$ most accurately represents P_aCO_2, while in rebreathing tests, $P_{ET}CO_2$ better approximates P_cCO_2 as the P_aCO_2-P_CCO_2 gradient is minimalized with the gradual rise in P_aCO_2 [15].

In conclusion, it appears that changes in peripheral VRT require a shorter hypoxic stimulus than do changes in the S1. This change in peripheral VRT could be regarded as an early protective mechanism, similarly to the increased HVR often seen after acute hypoxic exposure [60, 65, 73]. Since Somogoyi et al. 2005 did not observe changes in S1, we speculate that changes in the hypoxic S1 require a more severe hypoxic dose (3840 m

vs. 4500 m in this current study and 5000 m in Ainslie et al.); alternatively Somogoyi et al. may have been underpowered and unable to show significance, as their sample size of 6 was approximately half of ours (i.e. n = 11) and that of Ainslie and Burgess (n = 12). In regard to central regulation, a set time or hypoxic dose necessary to induce a re-setting or simulation of the central chemoreflex has yet to be determined. Nonetheless, following the trend of the peripheral chemoreflex, changes in central chemosensitivity seem to need longer and more severe hypoxia than changes in peripheral chemosensitivity.

Compared to our other conditions (NH, HN, NN), V_B after HH was unexpectedly high. A few hypotheses are possible to explain the higher V_B in both the hyperoxic and hypoxic Duffin tests following HH. An increase in sympathetic activation could be present in HH as opposed to other conditions, favouring greater V_B afterwards. The increased DBP in HH would further support this. Alternatively, perhaps a higher incidence of subclinical HAPE occurred in the HH condition; stimulating higher ventilation to offset the impaired gas exchange. If present, subclinical HAPE would have lowered S_PO_2; however being mild, the increased V_B would have offset the decrease in gas exchange, thus normalising S_PO_2 in comparison to NH. This increase in V_B could also account for the lack of differences in V_E, observed in our study, between HH and NH. Examination of lung diffusion capacity would be needed to clarify this speculation. It is interesting that the change in Δ VRT was significant only in the NH condition for the hyperoxic test and that it was significant in both HH and NH for the hypoxic test. If accurate, this would suggest that NH evoked changes in central VRT while HH did not. Previous studies did not make this observation so further research is warranted. One modest correlation was observed between LLS and hyperoxic VRT ($r = 0.376$, $P < 0.05$)

in NH yet no correlation was observed in HH (r = 0.092, P = 0.3646); again more validations are needed. The most conservative explanation for this finding would be the possibility of a type 1 error. We therefore suspect that those with a higher CO_2 threshold and a lower sensitivity to CO_2 as measured prior to the exposure are not predisposed to AMS. Future work investigating CO_2 chemosensitivity and AMS predictability should continue using HH when possible and utilize subjects with known altitude tolerance matched to resistant subjects using a repeated-measures design.

The HVR has frequently been discussed as a possible predictor of altitude tolerance; however, to date, altitude chamber studies have not examined HVR prior to and following hypoxic exposure. The HVR did not change following either HH (P = 0.41) or NH (P = 0.098); however, we expected increased HVR after the hypoxic exposures, as this result was previously reported by authors using field or intermittent hypoxia exposures [60, 65, 73]. These changes were reported after exposure to multiple intermittent hypoxia bouts or after several days at altitude where factors such as VAH (chemosensitivity changes) or long-term plasticity (changes in respiratory motor neuron activity) may come into effect [57]. In the context of our study, perhaps the time period over which the HVR was tested after the chamber exposure (~45 minutes) allowed for a sufficient washout to restore ventilation to baseline, or the exposure to the Duffin rebreathing tests immediately prior to the HVR test, confounded any potential increase in V_E. There was a time gap from chamber exit to start of the chemosensitivity tests (10-13 minutes) to allow the subjects to change, use the restroom, and complete two Duffin tests (30 minutes), both of which included a hyperventilation period. Past reports examining

the time to ventilatory recovery post-hypoxia have observed that 7 minutes of normoxic air breathing did not lower ventilation after 25 minutes of hypoxia (S_aO_2 80%), 15 minutes restores it partially, and one hour restores it fully [74]. Interestingly, 7 minutes of severe hyperoxia (F_IO_2 1.0) fully restored ventilation, while 15 minutes of moderate hyperoxia (F_IO_2 0.3) almost fully restored ventilation to resting levels (within 5% of baseline) [74]. Thus, we estimate that subjects spent approximately 45 minutes breathing normoxic air and were exposed to short hypercapnic, hyperoxic and hypoxic bouts prior to the post-exposure HVR test. Therefore, it is possible that the lack of an increase in the HVR post-chamber exposure could in part be due to a confounding effect from these other perturbations. Another potential cause for the lack of differences in the HVR could be due to HVD, as the subjects had recently been exposed to a $P_{ET}O_2$ of 50 mmHg during the hypoxic Duffin test for approximately 4-6 minutes. As the HVD can last from minutes to an hour [57], this might have interfered with our HVR measures. The variation in our subjects' blood glucose levels might also have influenced the post-chamber HVR. Food intake inside the chamber was *ad libitum* and was not recorded. The peripheral chemosensors are acknowledged to respond to hypoglycemia and hyperglycemia [10] and some have shown increases in the HVR in the presence of hypoglycemia [75]. Therefore, variations in food intake between subjects could be a causative factor in the lack of differences between HVR analyses. Finally the HVR test has significant inter and intra-individual variability. Although our CV was in the middle of the range of those previously reported, it may have been large enough to mask any small changes in HVR post-exposure [17, 76]. Therefore, similar to previous studies [29, 30], we did not find a strong correlation between HVR and AMS susceptibility. In light of the lack of

significant findings, we have demonstrated that HVR is not increased to a greater extent following HH exposure and that AMS susceptibility does not correlate to HVR.

5.2 Cardio-respiratory parameters

The most striking finding was the absence of differences in V_E between HH and NH, which is in disagreement with previous experiments [37, 38, 45]; however, some previously reported differences in ventilatory patterns were observed in the present study [37, 45, 46]. Specifically, in NH, V_T seemed to be greater than in HH; yet this difference was not significant. However, this altered breathing pattern (higher V_T in NH than HH) in NH did not affect LLS or S_PO_2 in hypoxia. Correspondingly, f_B tended to be higher in HH at all time points; yet this difference was not significant. The LLS correlated positively to f_B (greater LLS in those with higher f_B) at 5hr-mean in AMS+ subjects in both the HH and NH condition. Subject discomfort (headache, nausea) potentially favoured this breathing pattern; however, the higher f_B in AMS+ subjects did not yield differences in V_E or S_PO_2 between AMS+ and AMS- subjects. Although we were unable to measure RER, if the work of breathing is greater in NH because of the denser air, perhaps utilizing a greater V_T as opposed to increased f_B is an integrated efficiency mechanism specific to NH. This remains speculative, as larger breaths would increase work against the elasticity of the lung and the chest wall. Analysis of end-tidal gases would allow us to further examine the difference in WOB between NH and HH. Of interest was the chronologic ventilatory response to hypoxia. Both HH and NH caused an abrupt increase in V_E, followed by a depression lasting approximately one hour, followed by a progressive rise.

Accordingly, S_PO_2 followed suit and was lowest at the 30min time point before gradually rising to a new steady-state. The HR response increased with ventilation time points in the hypoxic conditions, which is not surprising. Of interest was the finding that HR increased significantly throughout the exposure in both the HN and the control NN condition. One possible explanation for this elevation in resting HR is the stress of being in the chamber for such a prolonged time. Although the subjects were well familiarized with the chamber prior to the commencement of the study, it was still an unusual environment with restrictive quarters, loud noise and a set testing schedule. Another explanation for the gradual increase in HR could be due to cardiac drift caused by dehydration due to inadequate water consumption inside the chamber. A few subjects reported minimizing their water intake to avoid urinating inside the chamber. Blood pressure did not differ between conditions even though three measures were taken at each time point; we expected greater values in the hypoxic conditions. This absence of differences may warrant more accurate instruments (e.g. a Finapres®) which has been used accurately in the measure of blood pressure in the presence of hypoxia [77, 78].

In summary, we were unable to link our cardio-respiratory parameters to AMS susceptibility. Individual resting cardio-respiratory variables are, according to this study, poor predictors of AMS. We therefore reject our third hypothesis that neither S_PO_2, V_T nor V_E were linked to AMS susceptibility. Analysis of end-tidal gases remains an unexplored subject and would shed light on efficiency of breathing, substrate utilization and dead space ventilation and could further illuminate differences between AMS+ and AMS- subjects as well as HH and NH.

In contrast to the previous literature, the LLS score did not differ significantly between HH and NH as previously reported [38-40]. We demonstrated very similar LLS, with NH having slightly higher scores than HH in both the overall group and the AMS+ group. Since our blood oxygenation where similar to Roach's 1996 experiment and our hypoxic doses similar but slightly lower (P_IO_2 80mmHg vs. 75mmHg), a suitable explanation is not evident. The number of reports comparing HH and NH is limited to a handful, with Loeppky's 1997 and 2005 studies and Roach's 1996 experiment being the most similar to ours in terms of length measurements, sample size and hypoxic dose [37, 39, 40]. The only notable comparison was that our exposure length was slightly shorter than that of Roach et al. and Loeppky et al. (9hr vs. 6hr in this study); therefore, perhaps we would have seen similar results with a longer exposure. However ventilation in Loeppky et al. 1997 study was nearly identical at 6hr and 9hr (10.6 vs. 10.3 $l \cdot min^{-1}$) and no significant difference was reported for V_T or f_B. Since only the two above-mentioned studies resemble our study, it is unclear as to the cause of the divergent findings. As discussed in our review of literature, current research has leaned towards HH generating greater LLS than NH. Our results challenge this precept and propose that HH is not more severe than NH for a given equivalent hypoxic dose. Our demonstration of a slightly higher LLS score in NH supports this assertion, as does the absence of major significant physiological differences between NH and HH. This has also been recently reported by Schommer and Bartsch who stated that 18 hours of NH (F_IO_2 0.12) elicited similar LLS when compared to an overnight stay at 4559 m [79]. Inclusion of a HN exposure allowed us to separate the effects of hypobaria and hypoxia. Lack of pertinent findings in HN (only a minor difference in DBP) and of major differences between HH and NH suggests

that pressure *per se* might have been overlooked. We recognize that HH and NH are different, but this study shows that the differences are not as exaggerated as we have previously thought.

5.3 Critique of methods

All exposures were separated by a one-week washout and all hypoxic exposures (HH and NH) were arranged so that they were two weeks apart. Previous research from our laboratory examining week-long intermittent hypoxia protocols has demonstrated a return to baseline in the HVR after a one-week washout [65]. Additionally, Loeppky et al., whose protocol most resembles ours, has not reported residual effects following a one-week washout after 9 hours hypoxic exposure [37].

Despite our efforts to keep ambient room CO_2 partial pressure equivalent to atmospheric air, the chamber's environmental control unit experienced minor difficulties. We strived to maintain the chamber air PCO_2 within 1-1.5 mmHg throughout the 12 exposure days. Table 6 in the appendices display the chamber internal atmospheric conditions per condition and exposure trial. In a similar study [37], environmental PCO_2 has been reported being maintained below 3.7 mmHg. Regardless, since ambient PCO_2 was similar in all exposure days, we are confident this factor did not influence our measured parameters.

The gold standard in determining blood oxygenation is direct arterial sampling followed by immediate analysis. Since saturation values were needed on a real-time basis for the chemosensitivity test, and four subjects were inside the chamber simultaneously, it

would have been unfeasible to utilize arterial catheterization. Blood oxygenation throughout this study was established using pulse oximetry at rest, at ambient room temperature in healthy young subjects. Anaesthesiology-focused reviews of S_PO_2 propose an error of 5% below saturations of 80% [80] while high-altitude-focused reviews propose that accuracy falls below the 70-75% range [81]. Both of the devices used in this study claimed accuracy of ± 2% up to 70%; saturations rarely drop below such values. Although errors in S_PO_2 readings might have occurred, use of a crossover and within-subject design mitigated these factors.

Compared to other fields, our sample sizes are worthy of critique; this remains a caveat of small but intensive physiological experiments. In order to gain a proper understanding of the physiological differences between HH and NH studies with a larger sample of know AMS+ and AMS- subjects, of duration sufficient to induce AMS and using a crossover design will be necessary. On a broader spectrum, this being a chamber study of 6 hours in duration does not truly encompass the acclimatization process seen during multiday field acclimatization studies. By utilising a chamber model we eliminated confounding factors (cold, exhaustion, poor nutrition); however, caution is warranted when inferring results from laboratory studies to terrestrial altitude.

6.0 Conclusions

In this systematic investigation of the interaction between hypoxia and hypobaria, we found very minimal differences between NH and HH in terms of physiological parameters and AMS incidence and severity. We demonstrated a lowering of the central and peripheral ventilatory response thresholds to CO_2 following a 6-hour exposure to intermediate hypoxia (4500 m equivalent). An increase in peripheral sensitivity following exposure to HH was also observed. It appears that longer exposures (> 6 hours), or more severe hypoxia (< P_B 427 mmHg or F_IO_2 0.105) are needed to reset central sensitivity. No major discrepancies in LLS or in cardio-respiratory variables were observed between HH and NH. As only a handful of studies have compared HH to NH, conclusive discrepancies are elusive and our study further reinforces this. The timely publication of a debate between the presence and absence of differences between HH and NH further supports our views that a consensus between the differences in not evident [82, 83]. We conclude that a 6-hour intermediate hypoxic exposure is too short to allow the development of significant differences in chemosensitivity, AMS or cardio-respiratory variables between HH and NH. Further research will need to compare HH and NH over a longer time course (> 24 hours), in the presence of exertion and using known AMS+ and AMS- subjects.

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Appendix.

Individual and group data for select measures

Table 3. Comparison of hypobaric and normobaric hypoxia studies.

Investigators	**Exposure type**	**Time**	**Altitude (m)**	***n***	**LLS/ESQ* Higher in HH (P<0.05)**	**Ventilation**	
						V_E	**V_T & f_B**
Self et al. 2011	HH,NH	5min	7620	20	No	n/a	n/a
Singh et al. 2011	HH, CADO†	5min	7620	43	No	n/a	n/a
Evetts et al. 2005	HH, NH,HH+NH‡	5min	7620	11	n/a	HH ↑	n/a
Loeppky et al. 1996	HH, NH, HN, NN	30min	4570§	6	n/a	HH↑	NH↑ V_T
Savourey et al. 2007	HH, NH	40min	4500	18	n/a	n/a	HH ↑f_B, NH ↑V_T
Savourey et al. 2003	HH, NH	40min	4500	18	n/a	NH ↑	HH ↑f_B, NH ↑V_T
Tucker et al. 1983	HH, NH	2.5hrs	4570	6	Yes	NH ↑	NH ↑ f_B
Loeppky et al. 2005	HH, NH, HN, NN	10hrs	4540§	9	Yes	n/a	n/a
Loeppky et al. 1997	HH, NH, HN	10hrs	4540§	9	n/a	NH ↑	NH ↑
Roach et al. 1996	HH, NH, HN	9hrs	4564	9	Yes	n/a	n/a

*Lake Louise score, Environmental symptoms score and hypoxia symptoms score. †Combined altitude depleted oxygen, ‡Combination of reduced P_B and F_IO_2, §Author's calculations using equation 3.

Table 4. Comparison of study design and level of evidence in hypobaric and normobaric hypoxia studies

Investigators	Method of hypoxia	Gas analysis	Continuous vs. interrupted	*n*	Subject training	Height/ weight	Downs Black score	Blind	Wash out length	Random -ization	Level of evidence
Self [44]	Chamber	Spectrometry	Interrupted	20	n/a	Yes	19/31	Double	Same day	No	Medium
Singh [41]	Mask checked for leaks	n/a	Continuous	43	Yes	n/a	24/31	Double	24hr	Yes	High
Evetts [42]	Mask	Spectrometry	n/a	11	n/a	n/a	19/31	Single	n/a	Yes	Low
Loeppky [43]	Mask	Spectrometry	Interrupted	6	n/a	Yes	18/31	Single	Same day	Yes	Low
Savourey [45]	Mask	Spectrometry	Continuous	18	Yes	Yes	21/31	Single	1 week	Yes	Medium
Savourey [46]	Mask	Spectrometry	Continuous	18	Yes	Yes	22/31	Single	1 week	Yes	Medium
Tucker [38]	Chamber	Fuel cell	Continuous	6	Yes	n/a	15/31	No	Weeks	No	Low
Loeppky [39]	Chamber	n/a	Continuous	9	Yes	Yes	20/31	Single	1 week	Yes	Medium
Loeppky [37]	Chamber	Spectrometry	Continuous	9	n/a	Yes	22/31	Single	1 week	No	Low
Roach [40]	Chamber	n/a	Continuous	9	Yes	n/a	19/31	single	1 week	Yes	Low

Criteria used for determining level of evidence: Method of hypoxia: chamber air, mask checked for leaks, mask. Gas Analysis: mass spectrometry, fuel cell. Continuous vs. interrupted: live real-time data recording vs. recording of data at specific time intervals. Subject training: were the subjects familiarized with the procedures *a priori* to minimize anxiety and hyperventilation. Height/Weight of subject reported. Downs-Black score assessing study design [47]. Blinding: no blinding, single (only subjects), double (subjects and chamber tenders). Washout: length of time between exposures. Randomization: where order exposure randomized

Table 5. Comparison of cardio-respiratory parameters in NH and HH over brief, acute and sub-acute hypoxic exposures.

	Brief (≤5min)			Acute (<1hr)		Sub-acute (>1hr)		
	Severe (>7000m)			Medium (4000-5000m)		Medium (4000-5000m)		
	HH*	NH †	HH ⁂ NH‡	HH*	NH†	HH*	NH†	HN§
Minute Ventilation	↑ [42]	↑ ↑ [42]	↑ [42]	↑ [45, 46]	↑ ↑ [45]	↑ [37, 38]	↑ ↑ [38]	↔[37]
Frequency of breathing	↑ [42]	↑ [42]	↑ [42]	↑ ↑ [45]	↑ [45]	↑ ↑ [37]	↑ [37]	↔[37]
Tidal Volume	Ø	Ø	Ø	↑ [45, 46]	↑ ↑ [45, 46]	↑ [37]	↑ ↑ [37]	↔[37]
Blood pH (alkalinity)	Ø	Ø	Ø	↑ [45]	↑ [45, 46]	Ø	Ø	Ø
SpO_2 ¶	↓ [42]	↓ [42]	↓ ↓ [42]	↓ ↓ [45]	↓ [45, 46]	↓ [38]	↓ [38]	↔[37, 39]
$P_AO_2/P_{ET}O_2$ **	↓ [42, 44]	↓ ↓ [42]	↓ [42]	↓ ↓ [45, 46]	↓ [45, 46]	↓ [37]	↓ [37]	↔[37]
$P_ACO_2 /P_{ET}CO_2$	↑ [42]	↑ ↑ [42]	↑ [42]	↑ [45, 46]	↑ [45, 46]	↑ [37]	↑ ↑ [37]	↔[37]
P_aCO_2 ††	↑ [44]	↑ ↑ [44]	↑ [42]	↑ [45, 46]	↑ ↑ [45, 46]	Ø	Ø	Ø
Negative hypoxia symptoms	↔[41, 42]	↔[44]	↔[41, 42]	Ø	Ø	↑ ↑ [39, 40]	↑ [38]	↔[39, 40]
HR ‡‡	↑ ↑ [44]	↑ [41]	↑ [42]	↑ ↑ [45]	↑ [45, 46]	↑ ↑ [38]	↑ [38]	Ø
BP §§	↑ [42]	↑ [42]	↑ [42]	Ø	Ø	↑ [38]	↑ [38]	Ø
RER ¶¶	Ø	Ø	Ø	↔[43]	↑ [43]	↑ [37]	↑ ↑ [37]	↑ [37]

↑ higher; ↑ ↑ significantly higher; ↓ lower; ↓ ↓ significantly lower; ↔ equal or no change; Ø no data available *hypobaric hypoxia, †normobaric hypoxia, ‡combination of reduced P_B and F_IO_2, §hypobaric normoxia, ¶ blood oxygen saturation (%), **P_A/P_{ET}: alveolar/end tidal partial pressure, ††P_a: arterial partial pressure, ‡‡heart rate, §§blood pressure, ¶¶ respiratory exchange ratio.

Table 6. Environmental chamber atmospheric gas composition.

		HH	NH	HN	NN
PO_2 (mmHg)	Exp1	84.31	80.48	166.36	155.26
	Exp2	84.46	80.63	166.51	156.76
	Exp3	84.98	80.26	166.4	156.76
	Mean	84.58	80.46	166.51	156.26
	SD	0.35	0.19	0.078	0.87
PCO_2(mmHg)	Exp1	0.87	0.975	1.1	1.13
	Exp2	1.1	1.43	0.9	1.04
	Exp3	1.03	0.89	1	1.01
	Mean	1	1.09	1.02	1.06
	SD	0.12	0.28	0.11	0.06
Temp (°C)	Exp1	23.4	26.6	25.1	23.2
	Exp2	24.4	25.2	24.8	24.7
	Exp3	24.3	25.3	25.8	25.7
	Mean	24.03	25.7	25.23	24.53
	SD	0.55	0.78	0.51	1.27
Hum (%)	Exp1	42.3	50.3	54.7	51.3
	Exp2	41.8	50.3	35.3	44.3
	Exp3	37.8	45.3	45.9	47.6
	Mean	40.63	48.63	45.3	47.73
	SD	2.47	2.88	9.71	3.5

Exp = Exposure, SD = Standard deviation

Table 7. Overall group and AMS+ AMS- LLS across conditions

	HH		NH		HN		NN	
Group mean	2.27 *		2.54*		0.36		0	
Group SD±	3.29		3,14		0.51		0	
	AMS+	**AMS-**	**AMS+**	**AMS-**	**AMS+**	**AMS-**	**AMS+**	**AMS-**
AMS+/- mean	4.80**	0.16*	5.2*	0.33**	0.60	0.1	0	0
AMS+/- SD±	3.42	0.41	2.77	0.82	0.55	0.41	0	0

*Denotes P < 0.05 compared to normoxic conditions, **P < 0.0001 compared to AMS-

Table 8. Last hour individual subject LLS per condition

	s005	s006	s007	s009	s004	s012	s013	s014	s016	s017	s018
HH	4	4	7	9	0	0	0	0	1	0	0
NH	6	2	9	6	0	0	2	0	0	0	3
HN	1	0	0	0	0	0	0	0	1	0	1
NN	0	0	0	0	0	0	0	0	0	0	0

Table 9. Pre-chamber hyperoxic and hypoxic Duffin test parameters

Hyperoxic	**VRT**		**S1**		**S2**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**n of S2***
HH	46.3	1.88	5.16	8.83	1.98	0	1
NH	45.1	1.92	3.41	1.8	1.9	0.46	2
HN	46.4	1.66	3.55	1.51	5.61	5.93	3
NN	46.1	1.83	3.69	2.7	3.33	1.47	4
Hypoxic	**VRT**		**S1**		**S2**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**n of S2***
HH	41.9	2.14	4.51	2.25	4.77	2.46	7
NH	40.9	2.63	6.23	2.93	4.85	2.42	7
HN	40.7	2.55	6.85	3.29	8.75	6.9	4
NN	40.9	2.83	5.78	3.11	4.56	2.93	8

*not all subjects exhibited an S2

Table 10. Post-chamber hyperoxic and hypoxic Duffin test parameters

Hyperoxic	VRT		S1		S2		
	Mean	SD	Mean	SD	Mean	SD	n of S2*
HH	43.2	1.8	4.721	3.2	18.7	0	1
NH	41	1.478	5.447	3.1	7.62	5.4	3
HN	45.2	1.406	2.958	1.6	2.4	0	1
NN	45.1	1.584	3.642	1.4	0	0	0

Hypoxic	VRT		S1		S2		
	Mean	SD	Mean	SD	Mean	SD	n of S2*
HH	39.4	2.1	9.6	7.1	8.5	4.2	9
NH	37.5	2.1	12.3	13.7	10.8	5.4	5
HN	41.6	1.2	5.4	2.8	4.4	2.3	7
NN	40.7	1.8	6.3	2.1	7.5	5.9	6

*not all subjects exhibited an S2

Table 11. Individual hyperoxic post-chamber VRT and SI per condition

													Group n = 11		AMS+ n = 5		AMS- n = 6	
		s005	**s006**	**s007**	**s009**	**s004**	**s012**	**s013**	**s014**	**s016**	**s017**	**s018**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
HH	VRT	42.2	40.1	43.3	42.9	41.1	45.2	44.8	43.1	45.1	45.6	42.2	43.2	1.9	42.1	1.2	44.2	1.7
	S1	2.83	2.2	0.9	2.9	4.9	8.2	11.7	2.7	6.6	6.56	2.4	4.7	3.3	2.3	0.8	6.8	3
	S2								18.7				18.7				18.7	
NH	VRT	40.3	40.9	39.7	38.3	39.3	41.6	40.9	42	42.7	43	42.3	41	1.5	40.3	1.5	41.6	1.3
	S1	3.4	7.9	3.9	4.2	4.4	9.4	11.2	1.8	6.8	5.33	1.5	5.4	2.9	4.2	2.3	6.5	3.4
	S2			2.9					6.33	13.6			7.6	5.4	3		10	5.1
HN	VRT	43.5	45.6	43.4	44.3	48.2	45.9	46.6	45.4	45.1	44	45.2	45.2	1.5	44.4	1	45.9	1.4
	S1	1.2	1.2	3.4	1.6	1.2	2.3	3.7	6.5	1.9	5.12	3.2	3	1.7	2.5	0.8	3.4	2
	S2										2.37		2.4				2.4	
NN	VRT	45.5	45.2	41.7	47.4	45.4	45.1	47.4	45.5	45.4	44.2	43.7	45.1	1.6	44.7	2.1	45.5	1.1
	S1	2.8	3.4	2.4	3.3	2.9	4.3	3.9	1.7	5.87	6.5	2.9	3.6	1.5	3	0.4	4.2	1.8
	S2																	

Table 12. Individual hypoxic post-chamber VRT and SI per condition

													Group n = 11		**AMS+ n = 5**		**AMS- n = 6**	
		s005	**s006**	**s007**	**s009**	**s004**	**s012**	**s013**	**s014**	**s016**	**s017**	**s018**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
HH	VRT	37.4	38.6	41.2	42.5	37.8	39	36.6	41.5	37.1	41.6	39.9	39.4	2.2	39.9	2	38.9	2.2
	S1	3	6.4	4.16	14.3	7.3	6.5	26.6	5.3	7.2	18	6.9	9.6	7.5	7	4.4	11.8	8.6
	S2	2.8	10.4	2.8	11.6	5.2		13.8	9.2	13		8.2	8.6	4.4	7.2	4.2	10.3	3.9
NH	VRT	35.7	35.8	40.5	38.7	35.9	35.9	35.1	38.6	37.5	41.2	37.8	37.5	2.2	37.7	2	37.4	2.3
	S1	4.2	11	6.6	11.4	15.8	5.8	52.2	3.3	9.4	9.7	5.6	12.3	14.3	7.8	3.3	16	18.2
	S2		12.9			8.1	8.2		4.5	20.2			10.8	6.1	12.9		10.3	6.9
HN	VRT	41.2	41.6	39.7	41.1	41.4	42.8	41.8	40.1	43.8	42.6	42.3	41.7	1.2	41.2	1	42.1	1.3
	S1	4	4.9	4.9	2.4	4.4	4.9	13.1	2.8	6.8	5.9	5.7	5.5	3	4.4	1.3	6.3	3.6
	S2	3.3	7.96	4.3		1.6	6.9		4.2	2.4			4.4	2.3	5.2	2.4	3.8	2.4
NN	VRT	38.3	40.5	41.5	44.8	41.1	41.5	40.5	39.9	38.5	39.7	41.7	40.7	1.8	41.4	2.4	40.2	1.1
	S1	4.3	8.4	7	5.4	5.9	5.1	9.8	3.1	6.8	9.1	5	6.3	2.2	6	1.7	6.6	2.5
	S2	4.1	8.9	3.3		3.2				4.8	18.5		7.1	6	5.4	3	8.9	8.4

Table 13. Pre-chamber HVR and HCR scores

HVR	s005	s006	s007	s009	s004	s012	s013	s014	s016	s017	s018
HH	0.66	0.22	0.35	0.79	0.54	0.68	0.36	1.23	0.46	0.69	0.91
NH	0.80	0.17	0.38	0.69	0.17	0.55	0.68	0.38	0.47	0.41	0.55
HN	0.57	0.11	0.2	0.94	0.32	0.47	0.98	0.12	0.42	0.45	0.7
NN	0.90	0.33	1.66	0.52	0.69	0.17	0.25	0.49	0.43	0.46	0.99
Mean	**0.73**	**0.21**	**0.64**	**0.74**	**0.43**	**0.47**	**0.57**	**0.55**	**0.44**	**0.5**	**0.77**
SD	0.14	0.09	0.68	0.18	0.23	0.22	0.33	0.47	0.03	0.13	0.2
HCR											
HH	1.05	0.71	1.49	0.83	0.94	0.89	1.11	1.58	0.63	0.53	0.54
NH	0.85	0.52	0.59	0.85	0.29	0.75	1.16	1.02	0.39	0.39	0.34
HN	0.67	0.5	0.63	0.31	0.08	0.75	0.86	0.76	0.27	0.71	0.62
NN	0.75	0.35	2.63	0.58	0.3	0.5	0.5	0.78	0.49	0.49	0.46
Mean	**0.83**	**0.52**	**1.34**	**0.64**	**0.40**	**0.72**	**0.91**	**1.03**	**0.45**	**0.53**	**0.49**
SD	0.16	0.15	0.96	0.25	0.37	0.16	0.3	0.38	0.15	0.14	0.12

Table 14. Post-chamber HVR and HCR

HCR	s005	s006	s007	s009	s004	s012	s013	s014	s016	s017	s018
HH	0.60	1.54	1.16	0.62	0.11	1.3	1.07	0.29	0.55	1.14	0.52
NH	0.81	0.88	1.44	1.12	0.28	0.9	0.67	1.23	0.76	0.88	0.41
HN	0.61	0.08	0.63	0.26	n/a*	0.59	0.7	0.46	0.75	0.56	0.68
NN	0.78	0.27	0.49	0.4	0.16	0.81	0.99	0.91	0.53	0.65	0.64
HVR											
HH	0.38	0.99	0.6	0.75	0.27	0.41	0.34	0.21	0.58	0.71	0.35
NH	0.85	0.98	1.03	0.88	0.17	0.12	1.48	0.56	0.58	0.67	0.36
HN	0.64	0.0002	0.09	1.23	n/a*	0.13	0.81	0.47	0.26	0.39	1.01
NN	0.53	0.063	0.41	0.66	0.03	0.73	0.76	0.77	0.54	0.52	0.89

* lost file

Table 15. Pre-and post-group HVR and HCR across conditions

	HH		**NH**		**HN**		**NN**	
HVR	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Mean	0.63	0.51	0.48	0.69	0.48	0.46	0.62	0.54
SD	0.29	0.24	0.21	0.4	0.29	0.41	0.42	0.28
HCR								
Mean	0.94	0.81	0.59	0.69	0.5	0.47	0.7	0.6
SD	0.11	0.14	0.13	0.19	0.08	0.08	0.2	0.08

Table 16. Mean HVR and HCR scores in AMS+ and AMS- post-chamber across condition.

	AMS+		**AMS-**	
HVR	**Mean**	**SD**	**Mean**	**SD**
HH	0.64	0.12	0.42	0.08
NH	0.82	0.19	0.59	0.2
HN	0.59	0.11	0.36	0.11
NN	0.56	0.12	0.56	0.12
HCR				
HH	0.89	0.44	0.74	0.49
NH	0.58	0.9	0.78	0.31
HN	0.42	0.33	0.54	0.2
NN	0.52	0.2	0.67	0.29

All $P > 0.05$

Table 17. Coefficient of variability pre-chamber for all condition per subject

	s005	s006	s007	s009	s004	s012	s013	s014	s016	s017	s018	**Mean**
HVR	0.2	0.44	1.05	0.24	0.54	0.46	0.59	0.85	0.06	0.25	0.25	**0.45**
HCR	0.2	0.28	0.72	0.39	0.92	0.22	0.33	0.37	0.34	0.25	0.26	**0.39**

Table 18. Overall cardio-respiratory parameters at all time points in all conditions

		HH		NH		HN		NN	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
V_E	5min	18.9	6.7	18.5	4.7	14.8	3.7	14.8	3.3
	30min	16.4	4.9	16.5	5.2	12.7	3.2	13.6	2.8
	5hr-mean	16.5	3.6	17.6	2.9	14.8	2.9	14.4	2.6
V_T	5 min	1.11	0.5	1.2	0.5	0.98	0.4	1	0.4
	30 min	1.03	0.4	1.1	0.6	0.9	0.4	1.02	0.5
	5hr-mean	1.05	0.3	1.2	0.4	1	0.3	0.99	0.3
f_B	5min	19.6	7.3	17.9	5.8	17.3	5.9	17.5	6.7
	30min	18.7	6.9	17.6	6.2	16.6	6.3	15.8	6.8
	5hr-mean	18	5.6	17.8	5.4	16.5	4.9	17	6.6
S_PO_2	5min	86.4	5.9	85.6	5.3	100	0.3	99.9	0.4
	30min	80.5	8.6	80.5	6.6	100	0.2	99.7	0.7
	5hr-mean	82	5.8	80.6	5.3	99.5	0.8	100	0.3
HR	5min	86.5	19.8	87.8	10.9	72.3	12.1	71.5	13.9
	30min	90.2	15.4	90.1	15.9	75.9	11.7	74.9	13.9
	5hr-mean	96.6	12.6	95.2	14.1	80.4	11.5	76.3	10.1
Systolic	5min	117.9	7.1	113.6	11.9	117.2	9.8	114	7.3
	30min	115.7	7.4	109.1	10.1	113.9	7.5	114.4	9.6
	5hr-mean	112.4	4.41	110.9	9.5	113.9	8.5	112.6	5.6
Diastolic	5min	81.5	8	78.4	6.4	81.5	6.8	79.1	7.7
	30min	80.4	6	76.1	6.7	81	7.7	77.3	3.9
	5hr-mean	79.6	5.4	77.3	7.1	79.2	6	77.1	5.7
LLS at	Group								
hour 6	n = 11	2.3	3.3	2.5	3.1	0.3	0.5	0	0
	AMS+								
	n = 5	4.8	3.4	5.2	2.8	0.6	0.5	0	0
	AMS-								
	n = 6	0.17	0.41	0.3	0.8	0.17	0.4	0	0

Table 19. Individual HR data in all conditions

		s005	s006	s007	s009	s004	s012	s013	s014	s013	s017	s018
HH	**5min**	91	108	87	76	71	96	79	76	76	61	131
	30min	90	91	95	87	90	96	94	80	70	72	128
	5hr-mean	103	90	98	90	90	105	104	89	83	85	127
NH	**5min**	90	82	94	91	92	89	92	89	73	67	107
	30min	99	73	102	93	91	93	89	86	70	70	125
	5hr-mean	99	93	90	86	86	103	105	87	82	85	131
HN	**5min**	73	83	71	61	81	84	73	63	59	54	92
	30min	77	76	83	67	92	80	88	63	66	56	88
	5hr-mean	80	83	89	66	93	81	92	67	70	67	98
NN	**5min**	77	71	70	70	65	63	82	68	62	53	107
	30min	83	72	75	72	69	66	86	68	67	56	109
	5hr-mean	74	74	80	79	72	71	90	67	75	61	98

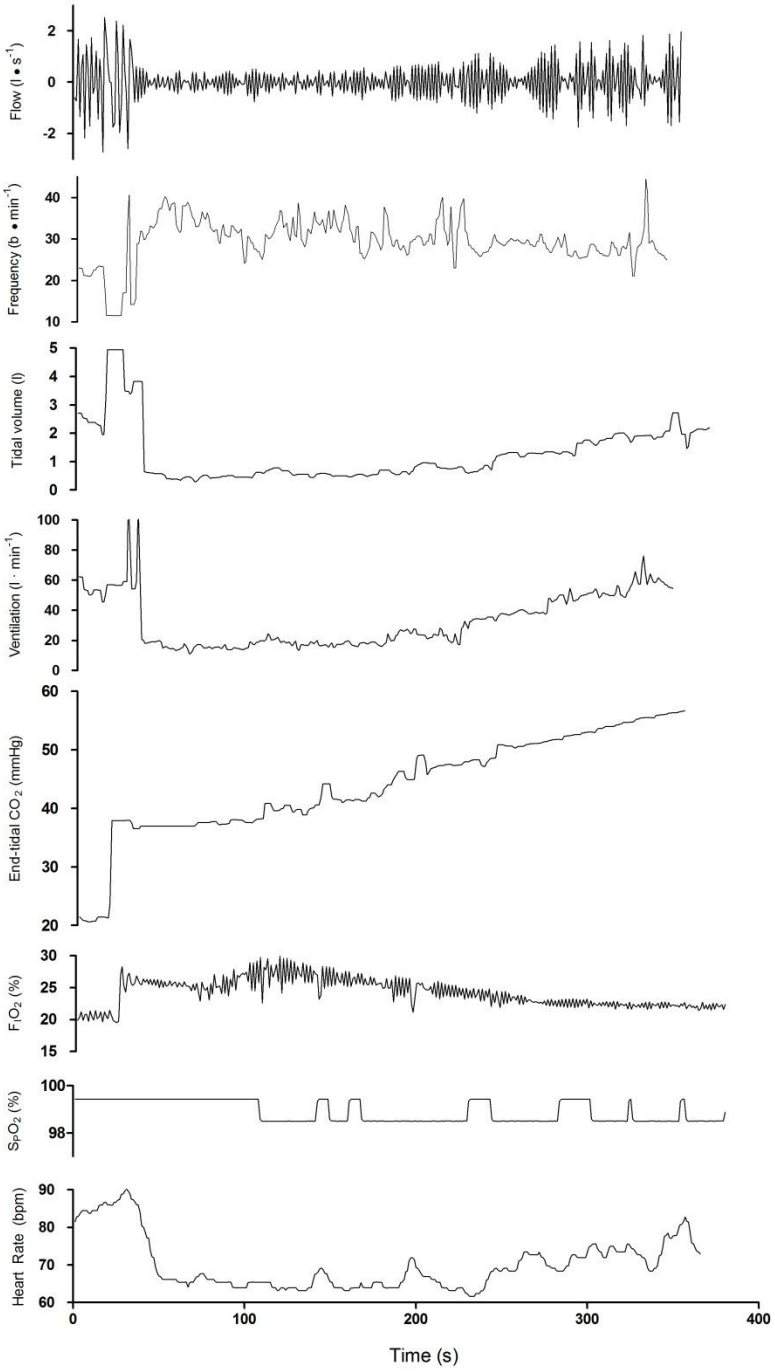

Figure 14. Sample tracing of a Hyperoxic Duffin rebreathing test.

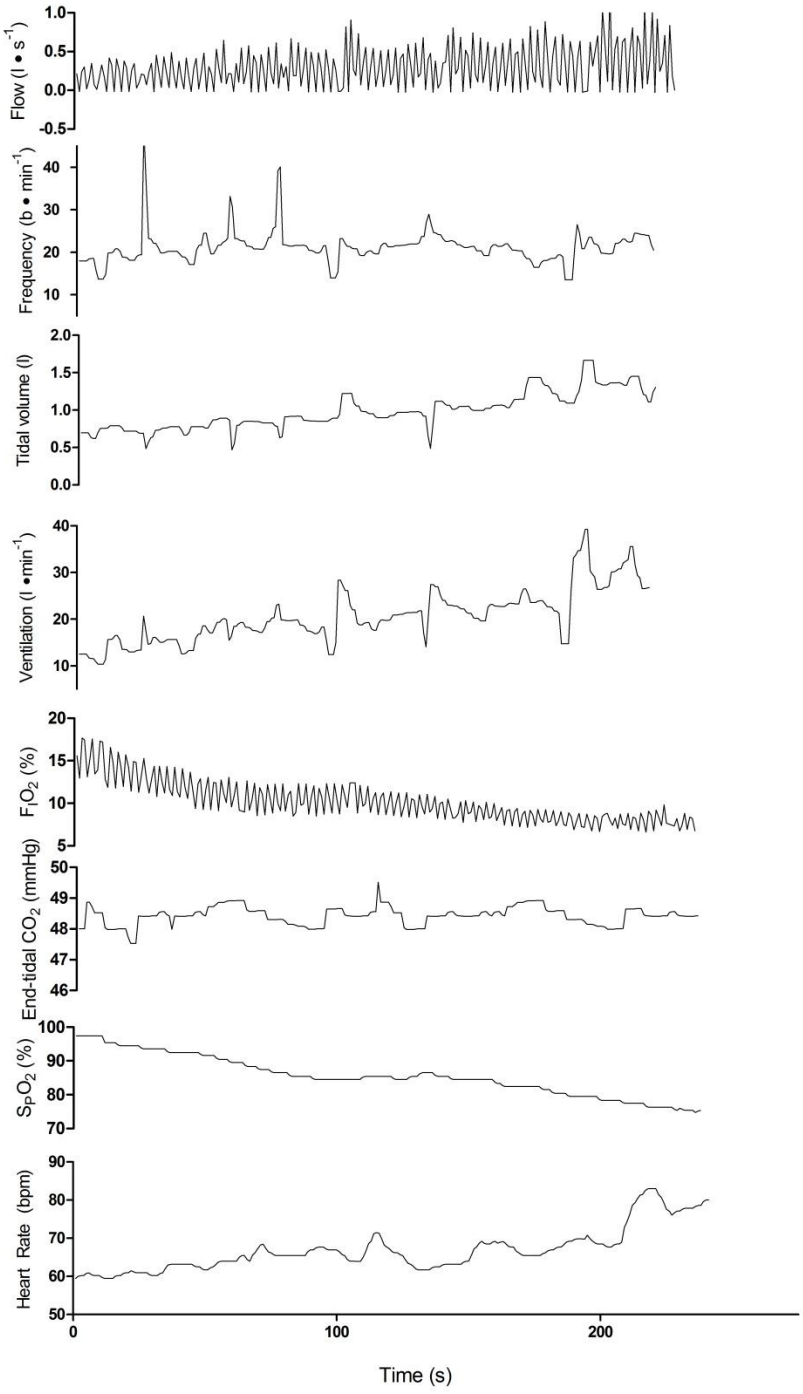

Figure 15. Sample tracing of a HVR test.