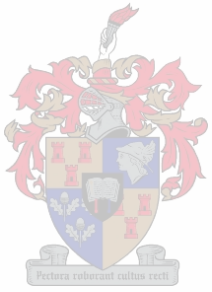
A familial comparison of hypoxic sensitivity in two South-African populations



Jonathan Steed Terblanche

Writers Declaration

I hereby declare that all the work presented in this thesis is my own original work, with the exception of part of Chapter 2, which was used for the publication of Fahlman *et al.*, (2002).

Andreas Fahlman, Sue Jackson, **John Terblanche**, Joseph A. Fisher, Alex Vesely, Hiroshi Sasano, and Kathryn H. Myburgh. (accepted 7 August 2002). **A simple breathing circuit to maintain isocapnia during measurements of the hypoxic ventilatory response**. Respir. Physiol. & Neurobiol.

Thesis Summary (English version)

Chapter 1 presents a general literature review on the acute isocapnic hypoxic ventilatory response (HVR).

The main findings from Chapter 2 indicate that our modified breathing circuit effectively measured the HVR while maintaining isocapnia. The measured ventilatory variables changed significantly with repeated short-term exposure to hypoxia over a 30-minute period, and the within- and between-day variability did not differ significantly. Furthermore, the variability in the HVR response (as measured by the coefficient of variation, (CV)) amounted to approximately 27% between tests in both parameters. Repeated measures are recommended in future determinations of the HVR.

In Chapter 3 the main findings were that hypoxic sensitivity does not differ between Caucasian and Xhosa sea-level populations in South Africa, and that ventilatory components in both normoxia and hypoxia differed between these two populations. Two distinct patterns of breathing were evident: shallow, rapid breathing among Xhosa subjects, and deeper, slower breathing among Caucasians. Moreover, lower arterial oxygen saturation levels during hypoxia among Xhosa subjects suggest that these two patterns of breathing differ in the effectiveness with which they oxygenate the blood.

Inter-individual variation in HVR within each population is of the same high magnitude as that reported in the literature (Beall *et al.*, 1997), further supporting the use of repeated measures in future studies.

As previously reported (Sahn *et al.*, 1977, Reeves *et al.,* 1993), in Chapter 3 I document a significant correlation between HVR and partial pressure of end-tidal CO_2 (PET_{CO_2}). Future studies of HVR should consider PET_{CO_2} as a covariate, despite the fact that my analyses of covariance (ANCOVA) showed no inter-population differences in HVR.

In Chapter 4 I report that regression analysis shows that the HVR of parents is not a predictor of that of their offspring. No significant heritability was evident for any of the additional key variables of hypoxic $\dot{V}_E$, hypoxic SaO_2, and the CV for HVR, but *a priori* analyses showed that I tested too few subjects to be able to demonstrate heritability (or the lack thereof) conclusively by means of regression analyses. Importantly, repeatability estimates within populations (86 %) revealed that despite its high variability, the HVR is highly repeatable, and therefore remains a useful comparative research tool for studies of human adaptation to hypoxia.

Tesis Samevatting (Afrikaans weergawe)

Hoofstuk 1 gee 'n algemene literatuuroorsig van die akute isokapniese hipoksiese ventilatoriese reaksie (HVR).

Die hoofbevindinge uit Hoofstuk 2 dui aan dat ons gemodifiseerde asemhalingsbaan HVR effektief meet terwyl isokapniese toestande gehantaaf word. Die ventilatoriese veranderlikes gemeet, het betekenisvol verskil met herhaalde korttermyn blootstelling aan hipoksie in a 30-minuut periode, en die binne- en tussen-daagse afwykbaarheid het nie betekenisvol verskil nie. Verder het die afwykbaarheid van die HVR reaksie (soos bepaal deur die koëffisiënt van variasie (KV)) ongeveer 27 % beloop tussen toetse van beide parameters. Herhaalde metings word vir toekomstige bepalings van die HVR voorgestel.

In Hoofstuk 3 was die hoofbevindinge dat hipoksiese sensitiwiteit nie verskil tussen Kaukasiese- en Xhosa- seevlak populasies in Suid-Afrika nie, en dat ventilatoriese komponente in beide normoksie en hipoksie verskillend was tussen hierdie twee populasies. Twee definitiewe asemhalingspatrone was duidelik merkbaar: vlak, vinnige asemhaling in Xhosa proefpersone, en dieper, stadiger asemhaling in Kaukasiërs. Verder het laer arteriële suurstof versadigingsvlakke gedurende hipoksie in Xhosa proefpersone daarop gedui dat hierdie twee asemhalingspatrone moontlik verskil in hul effektiwiteit om die bloed met suurstof te verryk.

Inter-individuele variasie in HVR binne elke populasie was van dieselfde groot omvang as wat in die literatuur gerapporteer word (Beall *et al.*, 1997), wat die gebruik van herhaalde metings in toekomstige studies verder ondersteun.

Soos voorheen gerapporteer (Sahn *et al.*, 1977, Reeves *et al.,* 1993), dokumenteer ek in Hoofstuk 3 'n merkbare korrelasie tussen HVR en parsiële druk van eind-tidale CO_2 (PET_{CO_2}). Verdere HVR studies behoort PET_{CO_2} as a kovariant te beskou, ten spyte van die feit dat my analise van kovariansie (ANCOVA) geen inter-populasie verskille in HVR getoon het nie.

In Hoofstuk 4 rapporteer ek dat regressie analise bewys dat die HVR van ouers nie 'n voorspeller van dié van hul kinders is nie. Geen betekenisvolle oorerflikheid was duidelik vir enige van die addisionele sleutelveranderlikes van hipoksiese $\dot{V}_E$, hipoksiese SaO_2, of die KV van HVR nie, maar 'n vorige analise het getoon dat ek te min proefpersone getoets het om oorerflikheid (of die gebrek daaraan) m.b.v. regressie analises te kan demonstreer. Dit is belangrik dat intra-populasie herhaalbaarheidsskattings (86 %) getoon het dat ten spyte van sy hoë afwykbaarheid, die HVR hoogs herhaalbaar is, en daarom 'n nuttige vergelykende navorsingshulpmiddel is vir studies rakende menslike aanpassing by hipoksie.

Acknowledgements

Chapter 1.

I would like to thank Laura Stockwell and Marc Rütschlin for their personal time proof-reading and their useful comments for this chapter.

Chapter 2.

I am particularly grateful to Alan Thomas, Nick Robinson, Shaun Thaysen and the staff of National Hyperbarics (Pty Ltd) for their help with gas mixing, helping us save both money and time. Thanks to Peggy Schlie of Dräger (Medizintechnik GmbH, Germany) for providing the Oxidem 3000 demand valve. The quality and quantity of work on this project are directly attributable to the dedication and professionalism of our mechanical engineers, Henry Bennett, Jos Weerdenburg, and Heinie Foot, and our electrical engineer Willie van Rooyen, who helped us develop the HVR system. We are grateful to all our subjects for their cooperation.

Chapter 3.

First, I would like to thank our subjects for their participation in this project from which the data for Chapters 3 and 4 were generated. I also thank those who were instrumental in helping me meet the families and recruit the subjects, in particular Danie Moolman.

I would again like to thank the staff of National Hyperbarics (Pty Ltd) in particular Alan Thomas, Nick Robinson, and Shaun Thaysen, for their help with mixing gases and providing the air so generously.

Chapter 4.

I am impressed by the level of expertise in his field and remain extremely grateful to Bill Louw (formerly of the Department of Genetics), for his generosity and assistance. He has dedicated much personal time and knowledge on quantitative genetics and data analysis for this chapter. I also thank him for his patience and the excellent recommendation of literature in this field.

General:

I thank Andreas Fahlman for teaching me the methodology of the breathing circuit, statistics, and educating me on the finer appreciation of caffeine, without which we would surely be lost. *Muchas gracias* to Sue Jackson for listening to my ideas and giving me the freedom to explore them. I also thank Sue for her patience and dedication to the project, while playing a crucial role in the facilitation of my wildest dreams. Above all, thank you to both Andreas and Sue for the right balance of constant support and encouragement; the most essential ingredients in a student's education! Thanks also go to Kathryn Myburgh for financial support and insightful comments throughout the last two years. I also extend my thanks to Carine Smith for the speedy translations and technical assistance.

I am extremely grateful to Benjamin Harper for writing the data-capturing upgrades and file conversion programme, and his excellent and personal service on matters of computer maintenance. I would like to thank Carl Kritzinger for writing the Gas Conversion programme, and his infuriating ease in understanding of data management and technical

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I must thank Corlie Moolman for learning the technical jargon in Xhosa, assisting in translating, and for her level of involvement which far exceeded her obligations as my girlfriend. I thank my brother, Benjamin Herr, for insightful comments and unmatched lateral thinking, and my mother, Charlene Herr, for the inspiration and support (not only financial) without which I definitely would not have achieved my goals.

Lastly, I thank Maarten Turkstra, and in particular those mentioned above, who have provided opportunities and contributed to my development as a scientist, student and researcher. Hopefully I am a credit to them and will remain so in the future.

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Chapter 1

Introduction to the Hypoxic Ventilatory Response

Introduction to Hypoxia

An increase in altitude is associated with a decrease in the partial pressure of oxygen (PO_2) which has the same effect as a reduced oxygen concentration at 1 atm. Both result in reduced PO_2, which leads to decreased O_2 diffusion across the alveoli, followed by reduced hemoglobin (Hb) saturation and decreased arterial oxygen saturation (SaO_2). This causes reduced aerobic performance, and an associated cognitive perception of difficulty to breathe (air "hunger"). Humans compensate for decreased inspired PO_2 by a progressive, time-dependent increase in ventilation, termed ventilatory acclimation to hypoxia (VAH). The mechanisms involved differ in their absolute inhibitory or simulatory effects on tidal volume (V_T), respiration frequency (f_R), and the time course of these responses (Powell *et al.*, 1998). The severity of hypoxia dictates the degree of response. However, even with a fixed severity of hypoxia, individuals vary in their degree of response.

To immediately place the literature review in the context of my thesis I present here at the outset the objectives of my thesis. They are to:

1) Determine whether or not oxygen sensitivity as expressed by the ventilatory parameter known as the Hypoxic Ventilatory Response (HVR), differs significantly between two different populations.
2) Assess the heritability of the HVR by means of quantitative genetics.

General Introduction

I will first present an overview defining hypoxia and the physiological mechanisms applicable to the HVR. From there I will supply a background of the literature for my project, including a description of current methods and principles relevant to HVR research. In Chapter 2, I will present the methodology and setup of our system that was used for the testing. Also in Chapter 2 I will present data regarding the variability of HVR. These data were collected by a Postdoctoral Fellow with myself as research assistant. Chapter 3 compares HVR in two populations and Chapter 4 assesses the heritability and repeatability of this parameter. Finally, Chapter 5 is a summary and conclusion.

A definition of HVR

HVR can be defined as the magnitude of the change in ventilation in response to a hypoxic challenge. It is expressed relative to a 1% change in arterial oxygen saturation, with units of $L \bullet min^{-1} \bullet \%^{-1}$, i.e. the difference between an individual's expired minute ventilation ($\Delta \dot{V}_E$) in normoxia and in hypoxia, divided by the corresponding change in that individual's oxygen saturation (ΔSaO_2) (Rebuck & Campbell, 1974). The HVR test is most commonly used to quantify levels of peripheral oxygen sensitivity in human subjects.

A definition of the severity of hypoxia

Experimental hypoxia can be induced in two ways: first, by using a hypobaric chamber to achieve reduced total and partial gas pressures; second, by reducing the percentage of oxygen in air at a fixed atmospheric pressure. Measurements of HVR almost always use the latter technique. Although there are no clearly defined terms for the severity of hypoxia, for the sake of this thesis I will use the following categories of hypoxia: "mild" refers to gas mixtures containing more than 18 % O_2, "moderate" to those with 13 – 18 % O_2, "severe" to those with 12 – 10 % O_2 and "extreme" to gas mixtures containing 9 % O_2 or less. To understand more clearly the relationship between altitude, and PO_2 and % O_2 see Figure 1.1a & 1.1b.

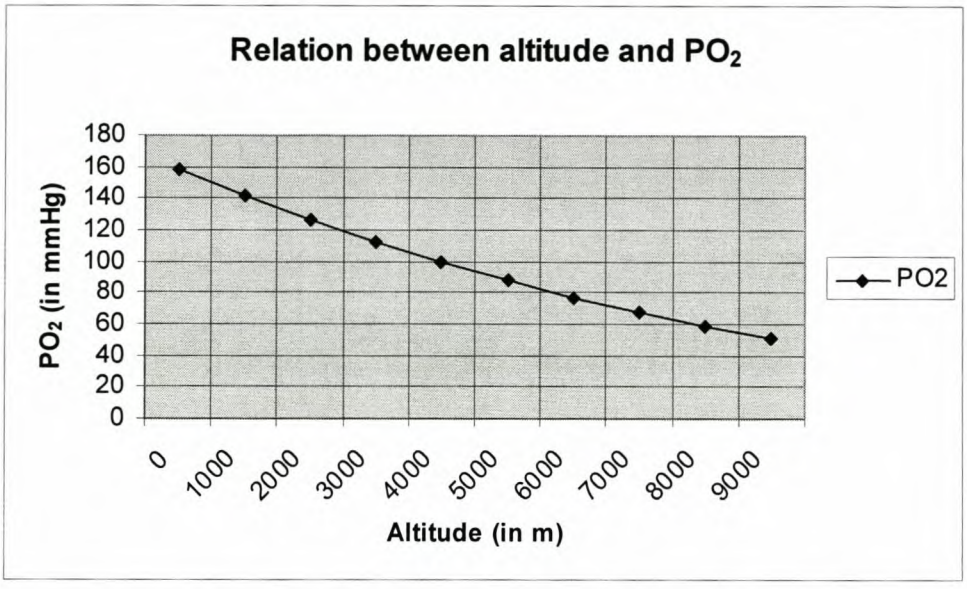

Figure 1.1a. The relationship between altitude and PO_2 expressed as a partial pressure (mmHg).

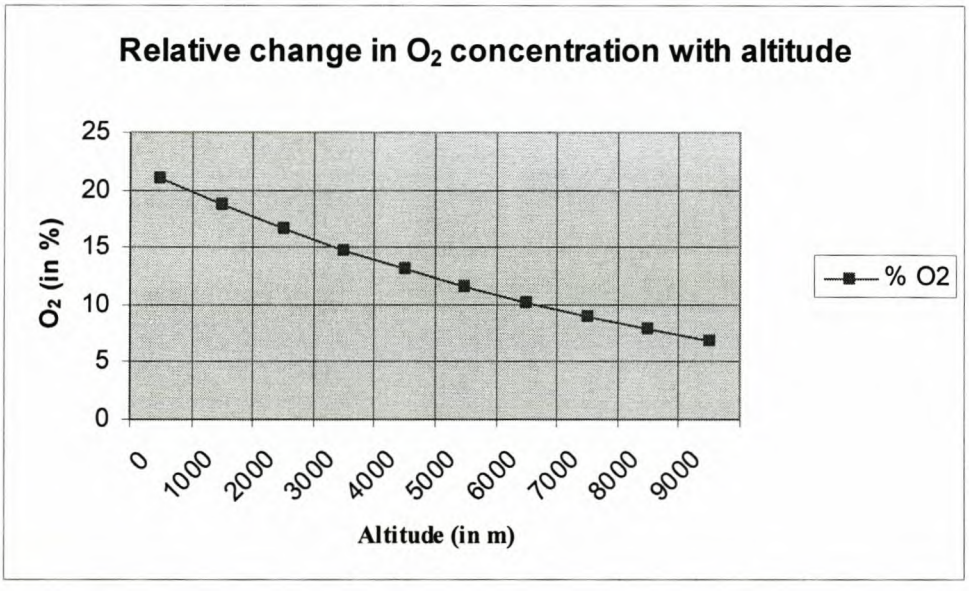

Figure 1.1b. The relationship between altitude and O_2 concentration expressed as a percentage (%) at 1 atm.

ACUTE AND CHRONIC HYPOXIC EXPOSURE

General Mechanisms

The ventilatory response to hypoxia has the following components, discussed below in the order in which they occur (Fig. 1.2):

1) The Acute Hypoxic Ventilatory Response (AHVR)
2) Hypoxic Ventilatory Depression (HVD)
3) Ventilatory Acclimation to Hypoxia or High Altitude (VAH)

1) The Acute Hypoxic Ventilatory Response

Ventilatory responses to hypoxia are influenced by both the severity and pattern of the hypoxic exposure, and are mediated through several physiological mechanisms (Powell *et al.*, 1998). The simplest visible components of hypoxic exposure are increased f_R and V_T, which together contribute to a significant increase in expired $\dot{V}_E$ above resting values (Weil & Zwillich, 1976, Easton *et al.*, 1986).

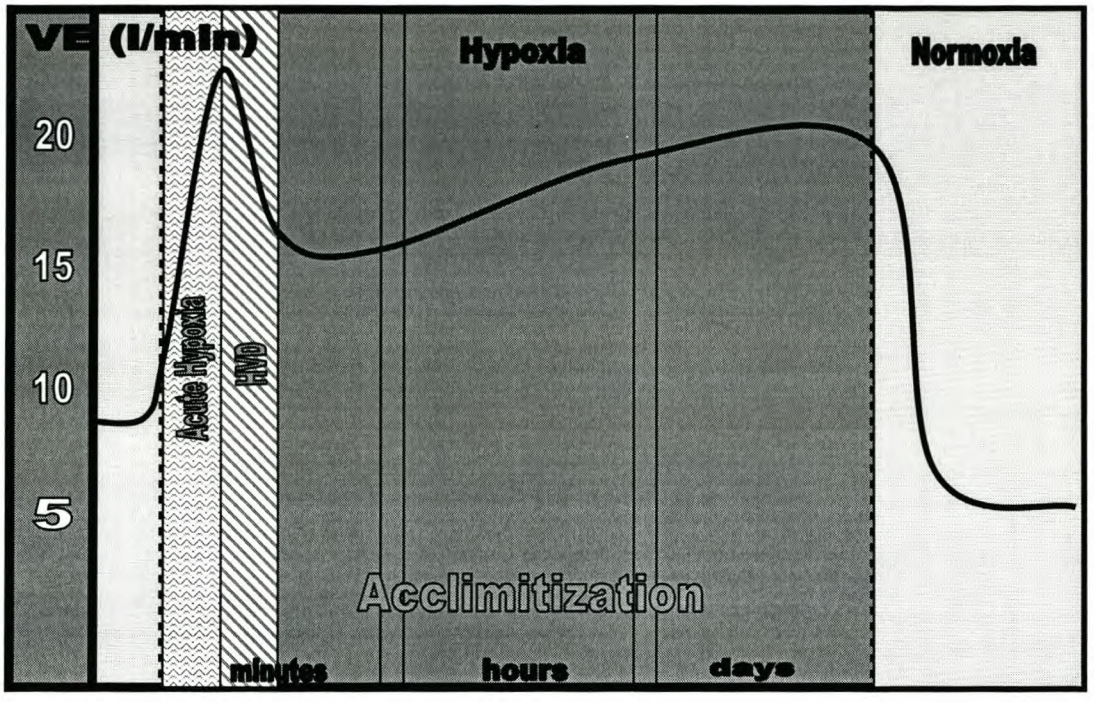

Figure 1.2. Time course of $\dot{V}_E$ during ventilatory acclimation to hypoxia (adapted from High Altitude, pp 141, 2001)

The AHVR is triggered by peripheral chemoreceptors in the carotid body, which are sensitive to a reduction in the PO_2 of the arterial blood (PaO_2), and send signals to the respiratory control center in the medulla oblongata of the central nervous system. This control center responds by increasing ventilatory drive, in an attempt to restore normal oxygen delivery to the tissues. Carotid body-resected humans show no HVR (Honda, 1992).

The two main ventilatory control mechanisms identified in the control of hypoxic ventilatory responses were a) peripheral (i.e. carotid aortic body) chemoreceptors and b) central (i.e. superficial ventral medullary) chemoreceptors (Mitchell *et al.*, 1963).

The general function of the peripheral and central chemoreceptors is to maintain homeostasis in the body by means of feedback control from the central nervous system (CNS) which interacts to restrict cellular hypoxia as well as limit respiratory and metabolic pH shifts.

The main stimuli of the peripheral chemoreceptors are pH (directly) and arterial hypoxemia (O_2), while the central chemoreceptors are primarily stimulated by CO_2 (pH indirectly). The peripheral sensors are stimulated by the effect of reduced CO_2 (as a result of respiratory or metabolic changes) and the consequent effects on H^+-ion concentration. The central chemoreceptors respond to cerebrospinal fluid pH changes that are determined by both the arterial CO_2 concentration and the environmental extracellular fluid (ECF) of cerebrospinal fluid HCO_3^- concentration (Mitchell *et al.,* 1963) Quantifying the relative contributions of the peripheral and central chemoreceptor has been attempted for the purposes of better understanding the ventilatory response to hypoxia. However, an understanding of the relative contributions of the peripheral and central components is complicated by the possibility of increased sensitivity of the carotid bodies with duration of acclimatization (Smith *et al.,* 2001).

The acute response involves an immediate escalation of minute ventilation at the onset of hypoxia. At high altitude or during hypoxic exposure, the arterial hypoxemia stimulating the peripheral drive increases ventilation, which promptly lowers the $PaCO_2$, and raises the pH of cerebrospinal (central ECF). This decreases the central ventilatory drive, which consequently decreases the synaptic output to the respiratory muscles and thus lowers

depth and rate of ventilation. The acute ventilatory response results from the reflex activation of a variety of respiratory muscles, and may terminate after the afferent input caused by hypoxia has returned to normal (Powell *et al.*, 1998). This may occur within one breath of PaO_2 changing at the carotid bodies. There is a decrease in ventilatory activity at the termination of hypoxia. The acute response represents the effects of changes in peripheral chemoreceptor afferent input to glutamatergic (and possibly other) synapses in the nucleus of the solitary tract (Powell *et al.*, 1998). This synaptic input alters during the course of the different phases of the continuous respiratory cycle. While the ventilatory response to acute hypoxia includes changes in both the respiratory timing and amplitude, the pattern of change in fR and VT is highly variable and differs between species (Powell *et al.*, 1998). In unacclimatized low-altitude residents, an exponential relationship exists between PaO_2 and minute ventilation, with a marked increase in ventilation occurring when the PaO_2 drops into the low (60 mmHg) range (Sahn *et al.*, 1977). The greater the ventilatory response to hypoxia, the higher will be the alveolar PO_2 and hence the arterial PO_2 and consequently the arterial oxygen saturation (although other factors may serve to increase the alveolar-arterial oxygen tension difference) (Smith *et al.*, 2001). In light of the above information it is clear that the control of CO_2 is of importance in the accurate measurement of the ventilation response to hypoxia."

Various secondary response mechanisms (such as psychological factors, Kawakami *et al.*, 1982) complicate the primary physiological response, which makes analysis of respiratory behaviour during hypoxia quite complex. Secondary responses may also

contribute to the large inter-individual variability reported in the literature (Khamnei & Robbins, 1990; Liang *et al.,* 1997).

2) Hypoxic Ventilatory Depression

The immediate increase in expired minute ventilation ($\dot{V}_E$ (in $L \cdot min^{-1}$)) in response to acute hypoxic exposure is followed by a decline in this parameter to a higher-than-normal baseline value, a phenomenon known as Hypoxic Ventilatory Depression (HVD) (Easton, *et al.,* 1986). The hypoxic response is also accompanied by a decline in alveolar, hence end-tidal CO_2 (P_ACO_2 and $P_{ET}CO_2$), as a consequence of the increased ventilation volume *(poikilocapnic hypoxia)* (Fig. 1.3). This has two important consequences: first, hypocapnia decreases peripheral chemoreceptor sensitivity and second, it depresses central chemoreceptor activity in the medulla (Easton *et al.*, 1988), resulting in a progressive fall in $\dot{V}_E$ during poikilocapnic hypoxia.

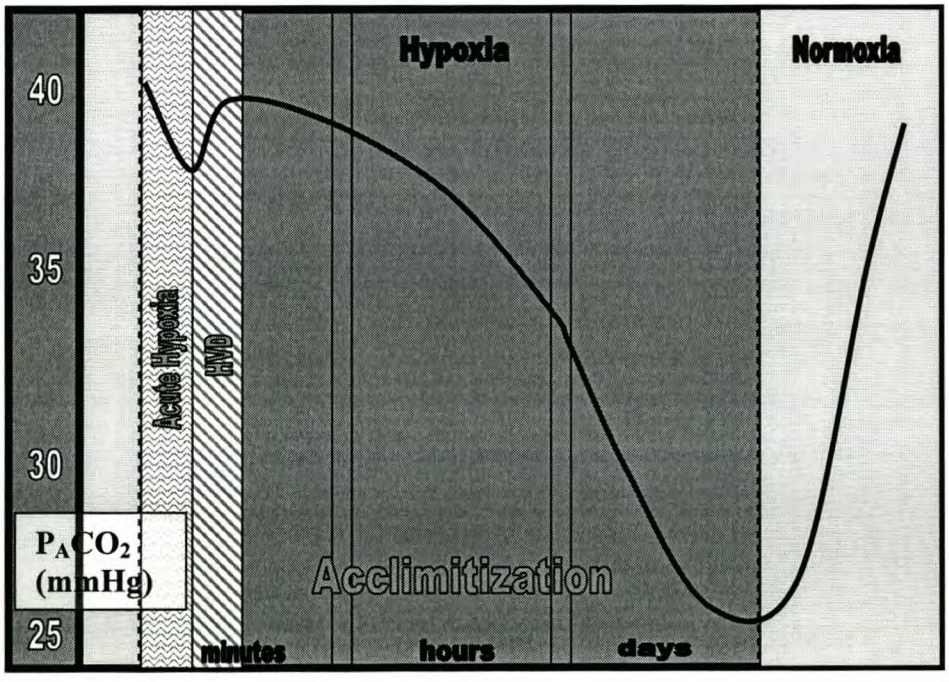

Figure 1.3 Time course of P_ACO_2 during ventilatory acclimation to hypoxia (adapted from High Altitude, pp 141, 2001).

To remove the confounding effect of altered CO_2 levels on ventilation, experimental hypoxic challenges such as the AHVR test are performed while maintaining constant P_ACO_2 levels (*isocapnic hypoxia)*, thereby isolating the ventilatory response of the peripheral chemoreceptors to oxygen alone. During both isocapnic and poikilocapnic hypoxic exposure the ventilatory response is biphasic; with an initial peak followed by HVD. The mechanisms of HVD are not fully elucidated, but more common explanations for it are:

a) The metabolic suppression of the neurons induced by the hypoxic exposure (Central Ventilatory Depression).
b) Inhibitory effects of neuro-effectors such as GABA (Gamma-aminobutyric acid), adenosine, lactic acid, and endogenous opioids, (Smith *et al.*, 2001), and
c) Hypoxaemic destabilization of the respiratory control system (Takahashi & Doi, 1993).

The influence of HVD on acclimation to hypoxia has not been assessed (Lahiri, 2001), neither has the extent of inter-individual variation in HVD nor its interaction with VAH.

3) Ventilatory Acclimatization to Hypoxia

The primary factor characterizing ventilatory adaptation to prolonged hypoxia is the gradual increase in $\dot{V}_E$ (Powell *et al.*, 1998). This acclimatization is a time-dependent

process that results in systemic adaptations which can be measured as physiological responses, e.g. changes in the HVR or haemoglobin concentration.

The process of ventilatory acclimatization has been linked to the carotid body-initiated response (Beidleman *et al.*, 1997, and references therein; Lahiri & Cherniack, 2001).

Genotypic alterations in HVR have been invoked in inter-population comparisons (e.g. Beall *et al.*, 1997), and may have occurred in the following three ways (Hochachka, *et al.,* 1999):

a) changes in physiological systems due to genetic drift;

b) changes in physiological systems due to natural selection at rates proportional to selection pressure; or

c) conservation of physiological systems for long time periods by stabilizing selection.

3.1) Broad mechanisms of adaptation to hypoxia

Humans can adapt to hypoxia, although to a lesser degree than can other mammals such as goats (Powell *et al.*, 1998), by compensating at several levels (Samaja, 1997). A chronological list of the various mechanisms of compensation that contribute to the process of acclimatization follows:

a) Hypoxic stimulation of peripheral chemoreceptors causes an immediate increase in alveolar ventilation and $\dot{V}_E$ (Powell *et al.,* 1998).

b) Initially, respiratory alkalosis resulting from lowered $PaCO_2$ shifts the oxygen-hemoglobin curve to the left, facilitating alveolar oxygen loading in the period before erythropoiesis (c, below) has occurred. Subsequently, increased 2,3-diphosphoglycerate (DPG) production by erythrocytes shifts the oxygen-hemoglobin dissociation curve back to the right, facilitating oxygen unloading into the tissues (Vander *et al.*, 2001).

c) Hypoxia stimulates secretion of the hormone erythropoietin (EPO), which stimulates erythropoiesis in the bone marrow.

d) On a longer time scale, hypoxia increases mitochondrial concentration, muscle myoglobin concentration and capillary density, all of which increase O_2 transfer rates (Kayar & Weiss 1992).

e) An increased loss of sodium and water in the urine is associated with arterial hypoxemia (Hildebrandt *et al.*, 2000). This diminishes the plasma volume, resulting in an increased concentration of the erythrocytes in the blood, and in extreme cases an increased viscosity. This can be deleterious, for example in the case of "blood-doping" (see for example Spivak, 2001) but of more relevance to

altitude-related studies plasma-volume contraction may confound the measurement of hematocrit (Hct), and can sometimes be mistaken for increased RBC synthesis.

f) Combined exercise and hypoxia enhance Exercise-Induced Hypoxaemia (EIH), providing a potent stimulus for the up-regulation in mitochondrial enzymes and for a simultaneous down-regulation in Na^+-K^+-ATPase pump expression (Green *et al.*, 1999).

3.2) Circulatory adaptations to hypoxia

Hypoxia increases the effectiveness of circulatory oxygen delivery by the following mechanisms (Samaja, 1997):

1) Immediate increases in cardiac output, followed by return to near normal levels in a few days, when other systems (see above) have adapted sufficiently.
2) Increase in tissue capillarity.
3) Increase in erythrocyte production of DPG.
4) Release of ATP from erythrocytes is believed to cause local vasodilation (Ellsworth, 2000), hence improved oxygen delivery.

3.3) Functional plasticity of respiratory responses

Physiological flexibility plays a crucial role in organisms' responses to variable environments, a fact that has long been acknowledged by comparative physiologists and more recently by human physiologists in some fields. Some of the compensatory mechanisms described above retain a degree of functional plasticity in the ventilatory

control system. An increase in carotid body sensitivity to hypoxia occurs with acclimation and most likely plays a key role in this natural adjusting process. Phenotypic or genotypic alterations in carotid body sensitivity may contribute to respiratory plasticity (Lahiri & Cherniack, 2001).

FACTORS INFLUENCING THE HVR

1) Gender and Age

The timing of HVR testing in female subjects is important, because ovarian hormones cause increased ventilation (Hannhart *et al.*, 1990). Gender influences on HVR were ignored in early studies, leading to contradictions between these and more recent findings. The phases of the menstrual cycle may influence HVR, (White *et al.,* 1983; Muza *et al.*, 2001), and women may have lower HVR values than men (White *et al.,* 1983), but Muza *et al.* found no differences (2001). Variations in reported HVR values for women may reflect the wide variation in the endogenous ovarian hormone levels that are common during phases of the menstrual cycle (Hannhart *et al.*, 1990), as well as the large degree of inherent intra-individual variability in the HVR (Sahn *et al.*, 1977, Zhang & Robbins, 2000). Metabolism, which differs between genders, also influences the degree of hypoxic sensitivity (Sahn *et al.,* 1977).

While a HVR has been extensively measured in young adults, there is relatively little literature on the ventilatory responses to acute or sustained isocapnic hypoxia in older healthy adults (Smith *et al.*, 2001). Although some studies suggested that HVR among

older men is reduced relative to that of younger men (Kronenberg *et al.,* 1972), a recent and very thorough study that accounted for many of the listed factors in this chapter, showed that the acute isocapnic HVR is maintained with no decline into the eighth decade in healthy, moderately active elderly men (Smith *et al.*, 2000). Such contradictions may result from the use of different techniques to measure HVR. These are summarized below.

2) Inherent Variability of HVR

Large inter- and intra-subject variability in HVR confounds study of this parameter. Original estimates were between 8 % and 64 % for intra-individual variability of the HVR with later values of approximately 26 % (Sahn *et al.,* 1977; Zhang & Robbins, 2000). Early studies showed that the inter-day variability is greater than the intra-day variability (Sahn *et al.*, 1977), but recent research does not support this (Zhang & Robbins 2000; Fahlman *et al.*, 2002). Such variability in HVR may reflect intra-individual fluctuations in sensitivity as the effects of hypoxia on the brain alter the arterial chemoreceptor signal or modify the interpretation of that signal by central respiratory neurons (Lahiri & Cherniack, 2001). For more comprehensive discussion of variability in the HVR, see Chapter 2.

3) The Effect of pH on HVR

Maintenance of isocapnia during HVR testing is important for two reasons: first, central ventilatory drive is affected by $PaCO_2$; second, pH changes directly stimulate peripheral chemoreceptors. Recall that plasma PCO_2 has a profound influence on pH. Reduced

blood pH elicits a right-shift in the O_2-haemoglobin curve, just as increased pH causes a left-shift. Such shifts influence arterial oxygen saturation, a commonly measured component of HVR, but to a lesser degree than the effect of a nett decrease in PaO_2, especially in the <60 mmHg range where the Hb-O_2 relationship is steepest (Wagner *et al.,* 2001).

In addition, if $PaCO_2$ changes during measurement of the HVR, concomitant changes in pH directly influence the central respiratory drive (as discussed earlier). Furthermore, it has been noted that in low-altitude residents an inverse relationship exists between end-tidal CO_2 and HVR (Reeves *et al.,* 1993), such that it may be implied that even sea-level PaO_2 can influence resting ventilation (Smith *et al.,* 2001). Maintenance of isocapnia during HVR testing is therefore of crucial importance. Diurnal variation in CO_2 sensitivity may also influence the HVR (Spengler *et al*., 2000; Stephenson *et al.,* 2000). Care should thus be taken to test all subjects at the same time of day. Inter-individual comparisons show that sea-level HVR is inversely related to resting $P_{ET}CO_2$ (Sahn *et al.*, 1977; Reeves *et al*., 1993).

The effects of pH on the ventilatory system are reviewed by Powell *et al.* (1998). End tidal PCO_2 is highly variable between subjects (Moore *et al.*, 1984; Huang *et al*., 1984). When steady-state conditions are maintained (e.g. diet, exercise, caffeine consumption, altitude of residence, and time of day), within-individual variation of end-tidal PCO_2 is low (e.g. Moore *et al.*, 1984; Regensteiner *et al.*, 1989). In addition, variability of the HVR within an individual may be linked to fluctuations in blood pH (Anderton et al.,

1964; Reeves *et al.*, 1993). pH and temperature-induced shifts in O_2 dissociation at a given arterial PO_2 contribute to variation in chemosensitivity. Measurement of PO_2 is affected by changes in blood pH, PCO_2, and temperature (Vander *et al.*, 2001). Furthermore, the measurement of end-tidal PCO_2 is the most sensitive measure of the effects of menstrual cycle variation of ovarian hormones on resting ventilatory drive in women residing at a constant altitude (see for example Muza *et al.*, 2001).

4) Population Differences

With applications ranging from biochemistry to anthropology and evolutionary genetics, research into physiological variability between populations has far-reaching consequences. Hypoxia tolerance in widely different populations living above 4000 m has been well-studied, particularly among Andean and Himalayan peoples (Beall *et al.*, 1997, Zhuang *et al.*, 1993; more detail will be presented on this in Chapter 3). Hypoxic tolerance in such populations may have a high degree of genetic heritability, implying that variations in response to hypoxia may have a genetic basis (Neubauer, 2001). This implication is supported by the fact that Quechuas do not lose their hypoxia tolerance after short-term adaptation to sea-level (Hochachka *et al.*, 1999).

Physical limits have been compared between Kenyans living at moderate altitude and Scandinavians adapted in the short-term to moderate altitude (Saltin *et al.* 1995a; Saltin *et al.*, 1995b). However, there have been no published studies of hypoxic ventilatory responses of any African population, either from high or low altitude. The only published study of ventilatory sensitivity in Africans investigated Nigerians' responses to

hypercapnia in which Nigerians exhibited lower hypercapnic ventilatory responses compared to young Nigerians (Elegbeleye & Femi-Pearse, 1980). Some measures of lung capacity such as forced vital capacity (FCV) have been explored in Ethiopians (Harrison *et al.,* 1969). Brutsaert's (2001) recommendations for studying adaptation to hypoxia suggest that a comparison of the widely-separated South-African Xhosa and Caucasian populations will enhance our understanding of the effects of genes *vs.* environment on the HVR. These two populations have lived for many generations at the same altitude but last shared a common ancestor approximately 100 000 years ago (Cavalli-Sforza *et al.*, 1994). East Africans are regarded as one of the three main HA populations (Hochachka *et al.*, 1999) yet the only information regarding ventilatory sensitivity in Africans is Elegbeleye & Femi-Pearse's (1980) study of hypercapnic sensitivity in West Africans.

It is unclear to what degree improved tolerance to hypoxia is a result of living at altitude for a single lifespan, or of altitude adaptation over generations. In summary, physiological adaptations to hypoxia in humans are well-documented but their genetic basis and their importance relative to social factors and lifestyle choices still warrant investigation, particularly in Africa (Moore, 2001).

6) Advances in HVR Testing

Many studies do not agree in terms of their findings in this research field, but this may be in part, or even largely attributed to the different techniques and protocols that have been used in these studies. Other differences occur between species (Neubauer, 2001).

The earliest method of measuring HVR involved exposure of subjects to a certain fraction of inspired O_2 and measurement of their expired $\dot{V}_E$ (Cormack *et al.*, 1957). Subsequently, progressive hypoxia was induced using the re-breathing technique (Rebuck & Campbell, 1974), which does not allow for switching between gases. This method was superseded by the computer-controlled dynamic end-tidal forcing technique (Robbins *et al.*, 1982; Howson *et al.,* 1987), which permits rapid changing of gas fractions between normoxia and hypoxia, and employs pre-mixing of gases to obtain subject-specific end-tidal gas levels. However, this technique is expensive and non-portable, and so its use in the field is impractical and non-existent. Furthermore, it may be construed as being non-physiological, because it induces changes in P_AO_2 far more suddenly than would occur under non-laboratory conditions. These changes are achieved when subjects breath several breaths of anoxic or hyperoxic gas mixtures.

The breathing circuit developed by Sommer *et al.*, (1998) controls alveolar CO_2 (see Chapter 2) and was refined in our laboratory by Fahlman *et al.*, (2002) to allow for complete study of all the characteristics of the HVR, while keeping the setup portable and relatively inexpensive. This system induces less rapid changes between gas mixtures than does the dynamic end-tidal forcing technique, and the length of the hypoxic exposure is increased accordingly, but not enough to cause complicating effects such as HVD or VAH (Powell *et al.*, 1998).

HVR measurements only permit quantification of the rate of change, or speed of an individual's response, when the data acquisition system being used has a sampling

frequency that permits breath-by-breath or greater resolution. Mathematical models (Khamnei & Robbins, 1990) and computer simulation techniques (Ursino *et al.*, 2001) have been developed to help assist understanding and clarification of the HVR's complex and inter-related mechanisms, yet even with these advanced methods, ventilatory research retains its difficulties.

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Chapter 2

Variability of the ventilatory response to isocapnic hypoxia.

***Note*:** This chapter has in part been used for two manuscripts: 1) "**A simple breathing circuit to maintain isocapnia during measurements of the hypoxic ventilatory response**" by Andreas Fahlman, Sue Jackson, **John Terblanche**, Joseph A. Fisher, Alex Vesely, Hiroshi Sasano, and Kathryn H. Myburgh (accepted 7 August 2002, Respiration Physiology & Neurobiology); 2) "**Inter- and intra-day variability of the hypoxic ventilatory response**" by Andreas Fahlman, **John Terblanche**, Sue Jackson, Charles McClure, and Kathryn Myburgh in preparation for the Journal of Applied Physiology. I acted as research assistant during these studies.

Abstract

We report the testing of a simple breathing circuit for measurement of the isocapnic hypoxic ventilatory response (HVR) in humans and the assessment of the HVR variability within and between days. The circuit permits rapid switching between two gas mixtures with different partial pressures of oxygen. Subjects (n = 15) breathed repeated cycles of exposure to normoxia (21 % O_2, balance N_2) and hypoxia (8.1 ± 0.1 % O_2, balance N_2). Hypoxia induced significant increases in minute ventilation and its components, breathing frequency and tidal volume ($P < 0.05$). In addition, the system successfully maintained isocapnia in all volunteers. Subjects experienced mild, but significant hypoxic ventilatory depression (HVD) with repeated hypoxic exposures, but HVD was not detected during the first hypoxic interval. There were no systematic changes in any respiratory variables between tests done on the same day, indicating that 60 min between tests was long enough to reverse the extent of HVD seen in this protocol. To assess between and within day variability of the HVR, subjects were tested on a total of three days, either once ($n = 6$) or three times per day ($n = 9$), intervened by a 60 min rest period. Variability of the HVR was computed using only the data from the first normoxic and hypoxic exposure in which no HVD was detected. The variability in HVR within-day and between days did not differ, and amounted to ~ 27% between tests.

Introduction

Chronic exposure to hypoxia elicits a triphasic ventilatory response in humans. Primarily, hypoxia causes increases in expired minute ventilation volume ($\dot{V}_E$, $L \cdot min^{-1}$) which is contributed to by amplified tidal volume (V_T, L) and breathing frequency (f_R, breaths $\cdot$ min^{-1}) above resting levels (Weil & Zwillich, 1976; Easton *et al.*, 1986). This initial acute response is rapid and develops over a few seconds (Powell *et al.*, 1998; Zhang & Robbins, 2000), but has been difficult to measure because of contamination of the data with those from subsequent phases. During stable hypoxic exposure lasting longer than two to five minutes, there is a second phase entailing decreased ventilation, termed hypoxic ventilatory decline or depression (HVD; Easton *et al.*, 1986; Powell *et al.*, 1998). During this second phase, V_T returns towards resting values while f_R remains elevated for the entire hypoxic exposure (Easton *et al.*, 1986). In experiments to determine the hypoxic ventilatory response (HVR), the imposed hypoxic challenge should be long enough for the full response to develop but short enough to prevent the development of HVD (Severinghaus, 1976; Khamnei & Robbins, 1990; Mou *et al.*, 1995). In the third phase, called ventilatory acclimatization to hypoxia (VAH), ventilation rises over orders of hours during hypoxic exposure (Powell *et al.*, 1998).

The most common technique used to measure HVR in humans is the re-breathing of a fixed volume of air in a bag from which the CO_2 is partially removed to maintain the desired end-tidal CO_2 partial pressure ($P_{ET}CO_2$; Rebuck & Campbell, 1974; Beall *et al.*, 1997). This technique permits a single hypoxic exposure, but not the switching between hypoxic and normoxic mixtures that allows repeated measurements.

Repeated measurements are important for assessing the considerable intra- and inter-individual variability in ventilatory parameters (Zhang & Robbins, 2000).
Another method is the end-tidal forcing system (Robbins *et al.*, 1982; Howson *et al.*, 1987), which permits rapid changes of inhaled O_2 and CO_2 concentrations. This system allows for repeated measurements of HVR within the same experiment, and also permits exposure of subjects to rapidly alternating cycles of hypoxia and normoxia for repeated measurements of HVR within the same experiment without eliciting HVD. However, it requires a chamber with a high gas turnover and complex computerized mixing equipment and is therefore costly and non-portable.

Sommer *et al.* (1998) recently developed a technique that controls alveolar CO_2 concentration by providing a fixed flow of gas and a second flow of gas on demand. For the current study, this circuit was modified so that it permits switching between inspired gases with different P_{O_2} concentrations over several cycles of alternating hypoxia and normoxia. The first goal of this chapter is to report tests of this system's ability to keep subjects isocapnic during changes between two gas mixtures of different O_2 concentrations when applying a square wave protocol similar to that described by Zhang and Robbins (2000), albeit without instantaneous attainment of hypoxia.

The second main goal of this chapter is reporting the extent of variability inherent in the HVR. Inter-individual variability in HVR is relatively well-documented (e.g. Hirshman *et al.*, 1975; Kronenberg *et al.,* 1972; Rebuck *et al.*, 1973). Although intra-individual variability of this response between days is less well understood, early studies suggested that this ranged from 7.6 % to 64 %, and repeated tests on different

days on the same individual showed that HVR differs significantly between days in some individuals (Sahn *et al.*, 1977). Zhang and Robbins (2000) estimated that between-day variability in HVR is on average approximately 26%. However, these authors could not estimate within-day variability because tests performed on the same day used different protocols. These authors found no significant differences between the different protocols employed, but other studies have shown that different patterns of hypoxic exposure yield different results for HVR (Mahutte & Rebuck, 1978). It has also been suggested that different methods of analyzing the data may account for the large variability (Sahn *et al.*, 1977). Still, single measurements of HVR have been used to evaluate physiological differences between and within populations where conclusions have been made regarding the genetic differences of the populations (Beall *et al.*, 1997; Hochachka & Monge, 2000).

I report here the results of repeated tests to assess within- and between-day variability of HVR. We aimed to establish whether the variability in HVR differs between tests carried out 60 minutes apart, a time period long enough to reverse the HVD (Easton *et al.*, 1988), and between repeated tests carried out on different days. It would be useful to know whether within-day variability in HVR is similar to or possibly even less than that measured on different days, as time could be saved during testing and subjects need not agree to so many visits. We are not aware of any published studies that have done this. Furthermore, to establish HVR as a measurable and repeatable physiological indicator of altitude adaptation (Beall *et al.*, 1997; Hochachka & Monge, 2000), we require a better understanding of its inherent variability (Sahn *et al.*, 1977; Zhang & Robbins, 2000).

Methods

Subjects & Laboratory conditions

Fifteen healthy subjects (seven male and eight female) of a low-altitude residency (altitude <100m) participated voluntarily. Their mean body mass was 69.4 ± 11.8 kg (± 1 SD), mean height 173.5± 10.3 cm, and age 25.5 ± 5.1 y (individual data are presented in Table 2.2). All experiments were performed at sea-level (<100 m) in a laboratory. As measured by the metabolic system (MetaMax™, Cortex Biophysik GmbH, Leipzig, Germany), mean laboratory ambient temperatures, mean exhaled air temperatures for all subjects and mean atmospheric pressure inside the laboratory were 22.9 ± 1.5 °C, 31.3 ± 0.4 °C, 1005.8 ± 2.8 mBar (or 754.6 ± 2.1 Torr) respectively for all the experimental days. None of the above varied significantly between days (repeated measures ANOVA, $P > 0.3$).

All test procedures were fully explained to each subject, verbally and in written form, before he/she signed a consent form. All subjects understood that they were free to withdraw from the study at any time. Ethical approval for all procedures was granted by the Subcommittee C of the Research Committee of the University of Stellenbosch, which conforms to the internationally accepted ethical guidelines detailed in the Declaration of Helsinki.

Isocapnic breathing circuit

The circuit described by Sommer *et al.* (1998) comprises a one-way valve, the inspiratory port of which is connected to two gas sources. One gas source (fresh gas, FG) is provided at a constant flow rate. During exhalation this constant flow allows a gas reservoir to collect FG and provides it for inhalation with the next breath. The

second gas (termed reserve gas, RG) is provided via a demand regulator only if the ventilatory demand exceeds the flow of FG, causing the gas reservoir to collapse. The RG contains gas comprising the desired fraction (F_{O_2}) or partial pressure of O_2 (P_{O_2}), and 5.5 % CO_2 (F_{CO_2}) (Sommer *et al.*, 1998). The equation given by Sommer *et al.* (1998) describing the effect of various breathing parameters on alveolar ventilation is described by:

$$\dot{V}_A = FGF + (\dot{V}_E - FGF) \bullet (P\bar{v}_{CO_2} - PRG_{CO_2}) \bullet (P\bar{v}_{CO_2})^{-1} \qquad [Eq.2.1]$$

where $\dot{V}_A$ is the alveolar ventilation ($L \bullet min^{-1}$), or the ventilation that contributes to CO_2 exchange, the FGF is the fresh gas flow ($L \bullet min^{-1}$), $\dot{V}_E$ the expired ventilation during hyperpnea ($L \bullet min^{-1}$), and $P\bar{v}_{CO_2}$ and PRG_{CO_2} are the partial pressures of CO_2 of the mixed venous blood and the RG, respectively.

[Eq.2.1] has two theoretical shortcomings. First, the rationale for the use of $P\bar{v}_{CO_2}$ in the reserve gas is not correct. To eliminate the effects of ventilation on arterial P_{CO_2} (Pa_{CO_2}), the reserve gas P_{CO_2} should be equal to that in the alveoli ($P_{A_{CO_2}}$) or arterial blood. Indeed, Sommer *et al.* (1998) found that adjusting the FRG_{CO_2} to 5.5 % (which corresponds approximately to a normal $P_{A_{CO_2}}$) maintained isocapnic hypoxia better than FRG_{CO_2} of 6.5 %, which corresponds more closely to $P\bar{v}_{CO_2}$. In preliminary testing we found the same and, accordingly, have used FRG_{CO_2} = 5.5 %.

Second, [Eq.2.1] does not take into account anatomical dead space. Clearly, in the second term, the ventilation obtained from the reserve gas will not be ($\dot{V}_E$ - FGF) but ($\dot{V}_E$ - $\dot{V}$Dan - FGF), where $\dot{V}$Dan is the minute ventilation of the anatomical dead

space. A modification of the equation described by Sommer *et al.* (1998) taking these factors into account is:

$$\dot{V}_A = FGF + (\dot{V}_E - \dot{V}Dan - FGF) \bullet (P_{A_{CO_2}} - PRG_{CO_2}) \bullet (P_{A_{CO_2}})^{-1} \quad [Eq.2.2]$$

where the $P_{A_{CO_2}}$ is assumed to be equal to the PET_{CO_2}.

To allow switching between normoxic (21 % O_2, balance N_2) and hypoxic (8 % O_2, balance N_2) gas mixtures while maintaining isocapnia, a duplicate circuit of FG and RG gas bottles were added containing hypoxic gas mixtures (Fig. 2.1). Consequently, we had two FG bottles containing different fractions of O_2 (8.3 % and 21 %, balance N_2), and two RG bottles containing 5.5 % CO_2 in addition to these two fractions of O_2 (balance N_2). The circuit worked as follows. The inspired gas was supplied from a 2 L reservoir filled continuously from a compressed gas cylinder containing normoxic (21 % O_2, balance N_2) or hypoxic (8.3 ± 0.1 % O_2, balance N_2, n = 3 bottles used) gas (Fig. 2.1). Flow into the bag was measured by a flow meter (Ohmeda, BOC Healthcare, England) calibrated with air and with an 8 % O_2, 92 % N_2 mixture using a water spirometer. Estimated flows were reproducible within 4 %.

The flow of FG was set to equal each subject's $\dot{V}_A$ [Eq. 2.1], estimated as:

$$\dot{V}_A = 0.70 \bullet \dot{V}_E \quad [Eq. 2.3]$$

where $\dot{V}_E$ was measured during the initial 5 min period before the hypoxic/normoxic exposure, and 0.70 is the estimated fraction of $\dot{V}_E$ contributing to gas exchange (Tortora & Grabowski, 1996). When $\dot{V}_E$ was equal to or less than FG, inspired gas consisted entirely of FG. When $\dot{V}_E$ exceeded that supplied by the FG, the reservoir emptied and a low resistance demand valve (opening pressure -0.5 mBar; Oxidem

3000, Dräger Medizintechnik GmbH, Germany) opened to supply the RG with 5.5 % CO_2 and the same F_{O_2} as the FG. This arrangement maintained the subject's $P_{ET_{CO_2}}$ at his or her normal resting values during both normoxia and hypoxia, despite the increases in ventilation produced by hypoxia. The seal of the facemask was ensured by asking the subject to cover the port (the large-diameter opening in the mask where connection to the switch-apparatus is made) with his or her hand and attempting to inhale and exhale. If air leaked during this procedure, a mouthpiece was substituted for the facemask.

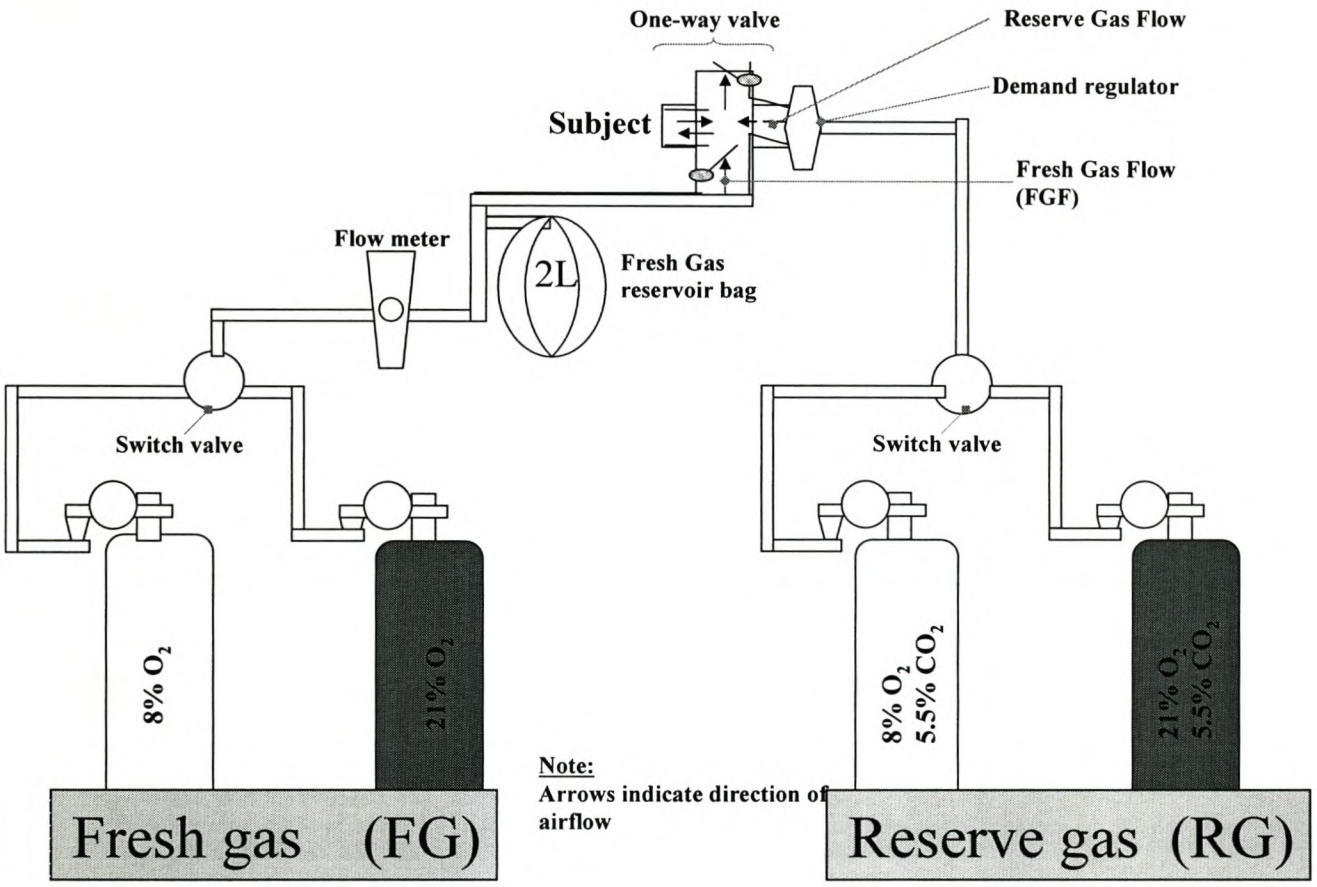


Figure 2.1. Schematic representation of the breathing circuit designed to maintain isocapnia in subjects breathing either a normoxic (21% O_2, balance N_2) or a hypoxic gas mixture (8% O_2, balance N_2).

Gas Exposure Protocol

After the initial resting normoxic phase (Pre), subjects were exposed to 4 square-waves of inspired O_2 that alternated every 120 s between 8.3 (± 0.1) and 21 % O_2. Each test was therefore divided into nine intervals, five exposures to normoxic gas and four to hypoxic gas. The period of each of the four waves was therefore 240 s and longer than the 120 s used by Zhang and Robbins (2000) because their dynamic end-tidal forcing system allows steady $P_{ET}O_2$ values to develop within 5 s (one to two breaths) of switching from normoxia to hypoxia. The $P_{ET}O_2$ of subjects breathing through our circuit only stabilised one minute after switching. If inspired P_{O_2} is not dropped to extremely low values for one or two breaths at the start of the hypoxic exposure, mixing of inhaled air with that in the dead space and the residual volume causes arterial P_{O_2} to lag behind inspired P_{O_2} (Anthonisen & Fleetham, 1987). Our subjects inhaled a gas mixture with a constant F_IO_2 (8.3 %; P_{O_2} = 60 mmHg) and therefore required approximately 60 s to reach steady $P_{ET}O_2$ values, as did subjects in a recent study using a partial re-breathing circuit (Garcia *et al.*, 2001). Thereafter, fifty seconds at a steady, hypoxic $P_{ET}O_2$ is long enough for full development of the acute HVR yet short enough to prevent a significant HVD (Mou *et al.*, 1995; Zhang & Robbins, 2000).

Published studies using various protocols, some of which, like ours, do not instantaneously induce hypoxia, show that ventilatory decline begins two to three minutes after introduction of hypoxia (Severinghaus, 1976; Weil & Zwillich, 1976; Easton *et al.*, 1986; Khamnei & Robbins, 1990; Bascom *et al.*, 1992; Paterson *et al.*, 1993; Powell *et al.*, 1998; Garcia *et al.*, 2001). Although Howard and Robbins (1994) and Mou *et al.* (1995) recommend use of a 50 sec bout of hypoxia, the slower

switching of our circuit meant that this would not have been long enough for our subjects to reach the required $P_{A}O_2$. Powell *et al.* (1998) suggest that 120-180 s of hypoxic exposure may be short enough to prevent HVD. We therefore chose a hypoxic interval of 120 s to induce adequate hypoxia, causing significant desaturation of arterial blood (to a mean value of 82.5 ± 5.5%) while reducing the risk of development of HVD. The total period incorporating one hypoxic and one normoxic interval was thus 240 s. For each subject, we repeated this 240 s hypoxia-normoxia cycle four times.

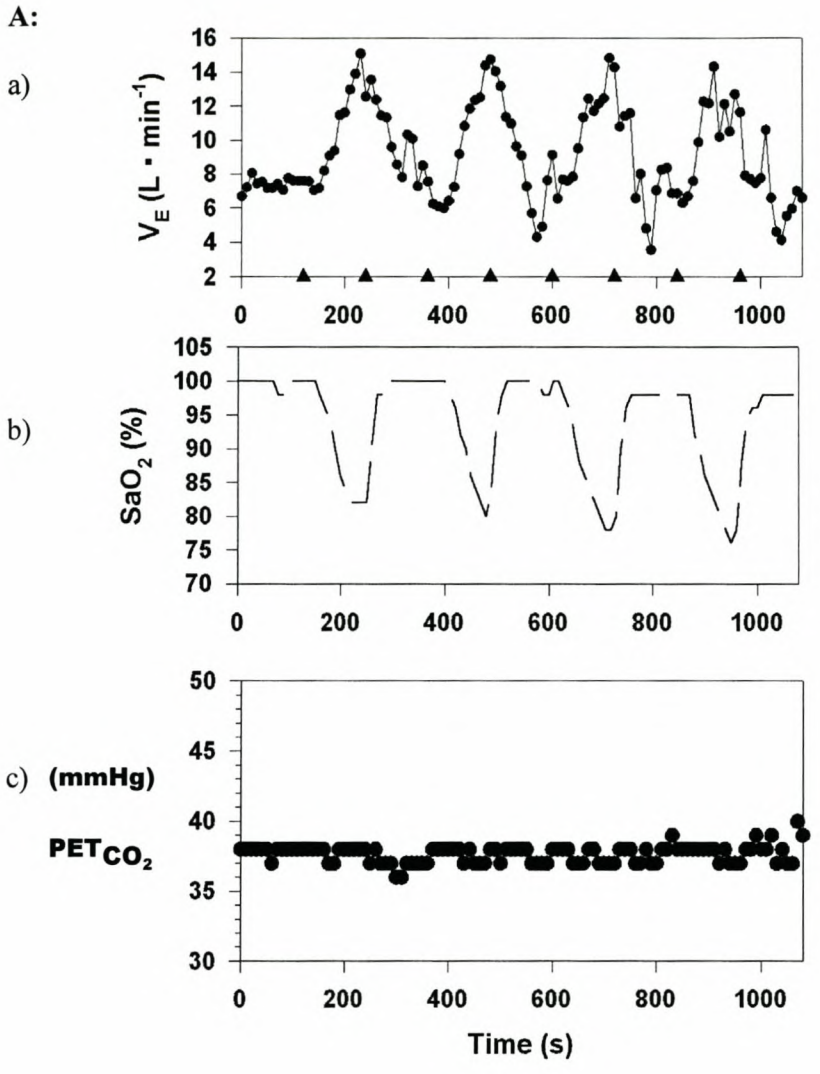
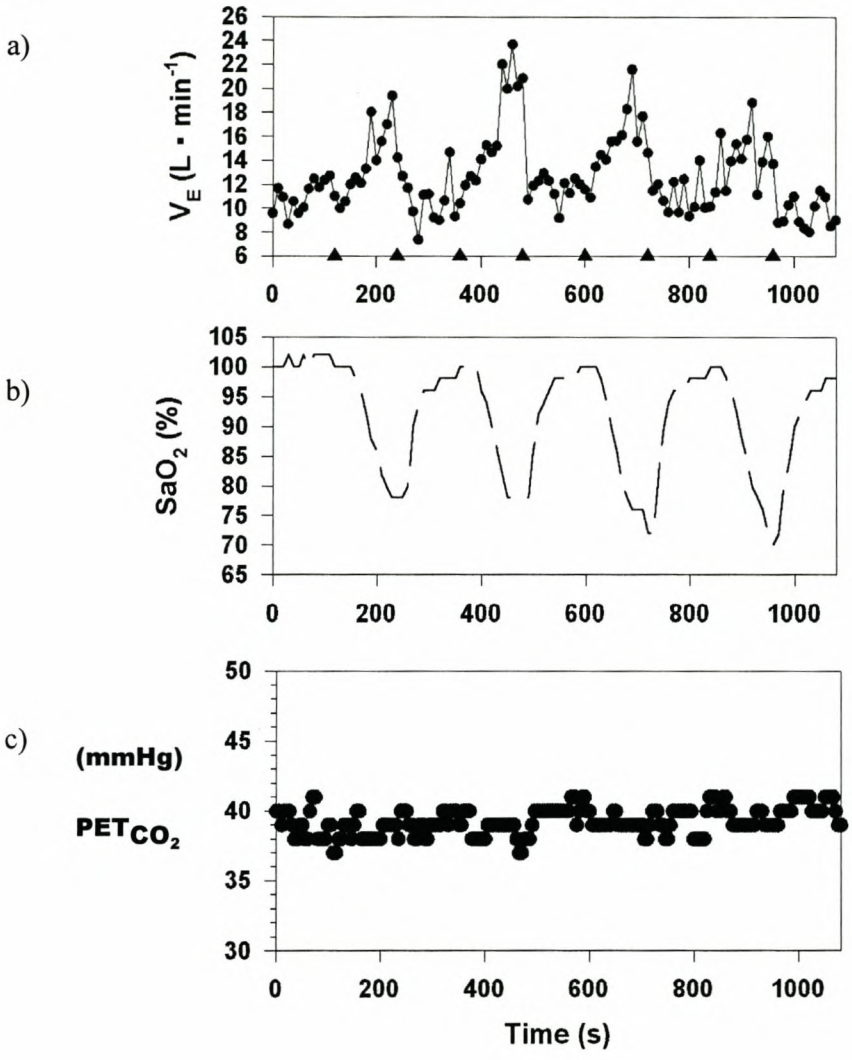


Figure 2.2. Representative responses of minute ventilation (a) $\dot{V}_E$, L • min^{-1}; upper panel), arterial oxygen saturation (b) SaO_2, expressed as a percentage; middle panel), and end-tidal CO_2 (c) $P_{ET}CO_2$, mmHg; lower panel) to repeated 2 min bouts of exposure to air (21 % O_2, balance N_2) and hypoxic gas (8.3% O_2, balance N_2) in a single subject with a 'steady' response (A; subject ID 8, Table 2.2) and illustrated on the next page, a subject with a variable response (B; subject ID 7, Table 2.2).

Triangles on axes represent switch in gas mixture. $\dot{V}_E$ and $P_{ET}CO_2$, are expressed in BTPS.

B:



Protocol for testing variability

The subjects were randomly assigned to two groups depending on the number of visits they were willing to make to the laboratory. The protocol structure, sample and number of tests is represented in Table 2.1. Each member of Group 1 (Gr1) underwent three HVR tests on a single day, each separated by 60 min, and repeated this series of

three tests on three separate days (n = 9; five males and four females, Table 2.1). Each member of Group 2 (Gr2) conducted three identical HVR tests on each of three different days (n = 6; two males and four females). Tests on different days were separated by at least two and at most 36 days. All eight female subjects were studied during the follicular phase of their menstrual cycle. Subjects were asked to refrain from drinking alcohol and caffeine-containing beverages from the evening before the tests. Before the study, each subject completed one or two preliminary tests for familiarization with the breathing circuit and the study protocol. During the first of these preliminary tests the subject's height (cm) and weight (kg) were measured. The number of preliminary tests for each subject was dictated by his or her comfort and ability to relax, demonstrated by stable and consistent resting values for $\dot{V}_E$ and breathing frequency (f_R, breaths • min^{-1}).

Table 2.1. Group number (Gr1 or Gr2), number of subjects in each group (n), number of tests within each day (WD), or between days (BD), and total number of tests for each group for all subjects.

Group	Within Day (WD)	Between Day (BD)	Total
Gr1 (n = 9)	3	3	81
Gr2 (n = 6)	1	3	18

Data recorded

Expired minute volume ($\dot{V}_E$, L • min^{-1} at standard temperature and pressure dry, STPD), tidal volume (V_T, L, STPD), and breathing frequency (f_R, breaths per minute) were sampled by a metabolic system (MetaMax™, Cortex Biophysik GmbH, Leipzig,

Germany) and average values recorded every 10 s. The end-tidal partial pressure of CO_2 (PET_{CO_2} at body temperature, pressure and saturation, BTPS) was sampled by a capnograph placed approximately 5 cm from the face-mask attachment point (Microstream™, Microcap, Oridion Medical Ltd, Jerusalem, Israel) and average values recorded every 5 s and converted to STPD.

Arterial O_2 Saturation (SaO_2, %)

During the tests, subjects' SaO_2 was measured continuously using a pulse oximeter (Nellcor N-395 Pulse Oximeter, Mallinkrodt, Inc., St Louis, MO, USA). Each subject was fitted with a forehead sensor (Nellcor RS10, Mallinkrodt, Inc., St Louis, MO, USA) that measured the SaO_2 (%). To improve the blood flow to the region of the sensor, the subject's forehead was rubbed with an ointment containing capsaicin (0.25 g per 100 g, Sloan Heat Rub, Warner-Lambert, South Africa) (Benoit *et al.*, 1997). The SaO_2 data were captured every 4 s and averaged over a 30 s period corresponding to the $\dot{V}_E$ values' 30 s interval.

Data Assessment and Statistical Analysis

All values are reported as means ± 1 standard deviation (SD), unless otherwise specified. Data from the start of the test up to the last two minutes of the Pre period were discarded.

Means of all variables for the last 30 s of the initial normoxic interval before hypoxic exposure (Pre) were regarded as baseline values for that subject and that test. The

remaining four intervals of normoxia (N1-N4) each alternated with an interval of hypoxia (H1-H4). Mean values of all variables were calculated using only the final 30 s (60 s for $P_{ET}CO_2$) of each 120 s interval.

Initially, the 30 s means for each of the five normoxic (Pre, N1-N4) and four hypoxic intervals (H1-H4) for each subject were compared, using repeated measures ANOVA to test for differences in each of the respiratory variables with interval number for each condition. A Bonferroni multiple comparison test was used to determine any systematic differences in mean values for intervals in normoxia or hypoxia. Repeated measures ANOVA was also used to determine if there were systematic differences in the HVR between the three tests conducted on each day, and between those conducted on different days. Coefficient of variation (CV) was calculated as the SD divided by the mean. For analysis of within-day variability, only subjects from Group 1 who were tested repeatedly on the same day were used (n = 9). For analysis of between-day variability, all subjects from Groups 1 and 2 were used, but only the data from the first test of each day were used (n = 15). The Kolmogorov-Smirnov non-parametric test was used for data with unequal variances and F-tests were used for non-parametric repeated measures data with unequal variances. Departures from normality were corrected by appropriate transformations in the case of unequal variances non-parametric statistics were used. Statistical analyses were performed using the NCSS 2000 statistical package (NCSS statistical software, Kaysville, Utah). Acceptance of significance was set to the $P < 0.05$ level, unless otherwise stated.

Estimation of HVR

The change in $\dot{V}_E$ ($\Delta \dot{V}_E$) in response to hypoxia is linearly related to the change in SaO_2 (Rebuck & Campbell, 1974; Sahn *et al.*, 1977). Consequently, we estimated HVR for each hypoxic interval as $\Delta \dot{V}_E \bullet \Delta SaO_2^{-1}$ ($L \bullet min^{-1} \bullet \%^{-1}$) using the 30 s means for $\dot{V}_E$ and SaO_2 from the normoxic period that preceded it, i.e. Pre *vs.* H1. We called the magnitude of this variable HVR. The initial base line $\dot{V}_E$ was computed as the average $\dot{V}_E$ of the last 30 s of the Pre period. During the hypoxic period, data from the last 30 s of $\dot{V}_E$ and SaO_2 were selected for the estimation of the hypoxic response.

Results

Subjects

In some subjects, $\dot{V}_E$, SaO_2, and PET_{CO_2} were repeatable between each hypoxia/normoxia cycle (Fig. 2.2, panel A), while others showed irregular ventilatory patterns (Fig. 2.2 panel B).

All subjects completed all three days of the study, and in only one case did a test end early (at the last normoxic interval) because the subject felt light-headed. No other subject complained of discomfort, although several reported that their breathing felt difficult during hypoxia. Several subjects also reported a sensation of relief during the normoxic period following certain hypoxic periods. Each subject's $P_{ET}CO_2$ remained stable with minor fluctuations immediately after the switch between gas mixtures (Fig. 2.2).

Rates of oxygen consumption and carbon dioxide production

For all subjects, neither $\dot{V}O_2$ nor $\dot{V}CO_2$ measured during Pre differed between days, nor were there differences between tests on the same day in subjects from Group 1 ($P > 0.3$, repeated measures ANOVA). The mean and standard deviation for each subject are given in Table 2.2.

Table 2.2. Group number (Gr1 or Gr2), subject identification number (ID), gender, weight, height, mean oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) rates for each subject in the variability study. $\overline{X}$ is the grand mean value, ± the SD of the $\overline{X}$.

Group	ID	Gender	Age (years)	Weight (kg)	Height (cm)	$\dot{V}O_2$ ($L \cdot min^{-1}$)	$\dot{V}CO_2$ ($L \cdot min^{-1}$)
Gr1	S1	M	31	81.5	186.5	0.40 ± 0.02	0.33 ± 0.02
Gr1	S2	M	28	78.2	179.0	0.42 ± 0.04	0.36 ± 0.03
Gr1	S3	M	22	86.0	183.5	0.43 ± 0.05	0.38 ± 0.05
Gr1	S4	F	22	65.2	159.5	0.33 ± 0.03	0.29 ± 0.03
Gr1	S5	F	21	52.0	163.5	0.23 ± 0.04	0.21 ± 0.04
Gr1	S6	F	21	60.0	166.0	0.34 ± 0.04	0.31 ± 0.04
Gr1	S7	F	22	66.0	170.5	0.35 ± 0.04	0.28 ± 0.04
Gr1	S8	M	33	67.0	171.5	0.42 ± 0.03	0.34 ± 0.02
Gr1	S9	M	25	81.5	185.5	0.32 ± 0.04	0.26 ± 0.04
Gr2	S10	F	23	46.2	158.5	0.34 ± 0.02	0.30 ± 0.02
Gr2	S11	F	28	76.4	174.0	0.39 ± 0.01	0.35 ± 0.02
Gr2	S12	F	38	65.4	176.0	0.31 ± 0.01	0.28 ± 0.01
Gr2	S13	M	21	82.5	188.5	0.39 ± 0.07	0.35 ± 0.06
Gr2	S14	M	24	73.6	179.5	0.47 ± 0.03	0.40 ± 0.04
Gr2	S15	F	23	60.0	160.0	0.29 ± 0.05	0.24 ± 0.04
$\overline{X}$			25.5	69.4	173.5	0.36	0.31
SD			± 5.1	± 11.8	± 10.3	± 0.06	± 0.05

Table 2.3 a. Comparison of minute ventilation ($\dot{V}_E$, ± 1 SD) and end-tidal PCO_2 (PET_{CO_2}) at rest and during hyperventilation in air (without addition of CO_2 to maintain hypercapnia). Resting $\dot{V}_E$ (n = 13) and PET_{CO_2} (n = 25) were averaged during the two minutes prior to the first hyperventilation; values of hyperventilating $\dot{V}_E$ and PET_{CO_2} were averaged from three measurements during the last 30 s. $\overline{X}$, grand mean for all subjects. All values given as BTPS.

	Rest		Hyperventilation	
Subject ID	$\dot{V}_E$ ($L \cdot min^{-1}$)	PET_{CO_2} (mmHg)	$\dot{V}_E$ ($L \cdot min^{-1}$)	PET_{CO_2} (mmHg)
1	8.7 ± 1.1	36.4 ± 1.1	18.9 ± 0.7	28.6 ± 1.1
3	6.5 ± 1.8	36.6 ± 05	19.7 ± 3.5	29.7 ± 1.6
4	11.5 ± 0.8	38.9 ± 0.8	18.8 ± 1.2	31.8 ± 0.4
5	8.1 ± 1.8	37.7 ± 0.6	16.8 ± 0.8	27.4 ± 1.7
6	9.5 ± 1.6	34.8 ± 0.4	24.7 ± 1.5	25.6 ± 0.4
7	9.1 ± 0.8	39.4 ± 1.0	19.8 ± 3.0	33.1 ± 0.8
8	6.0 ± 0.5	37.5 ± 0.5	12.7 ± 1.0	31.4 ± 0.7
9	8.9 ± 0.6	39.5 ± 0.5	19.6 ± 1.1	32.0 ± 1.6
$\overline{X}$	8.5	37.6	18.9	30.0
SD	± 1.7	± 1.6	± 3.3	± 2.6

Testing the breathing circuit: maintenance of isocapnia

To compare changes in $P_{ET}CO_2$ with and without the addition of CO_2, eight of our 15 subjects returned to repeat the test in 1 atm air without the addition of CO_2 (Table 2.3a).we chose to do these additional experiments in normoxic air to avoid the discomfort of hyperventilation in combination with hypoxia. Subjects breathed normally for 5 min while we measured their resting $\dot{V}_E$ and $P_{ET}CO_2$. Each subject was then instructed to increase his or her $\dot{V}_E$ to a level equivalent to that measured during the hypoxic exposure, for 1 min. $\dot{V}_E$ and $P_{ET}CO_2$ were measured and averaged during the last 30 s of hyperventilation. This procedure was repeated three times, allowing enough time between each measurement for $P_{ET}CO_2$ to return to the subject's resting level. Averages of the three $\dot{V}_E$ and $P_{ET}CO_2$ values from each run were compared with corresponding values for each subject obtained during hypoxia.

Table 2.3 b. Comparison of PET_{CO_2} values (mean ± SD in mmHg; given in BTPS) for Pre Normoxic and Hypoxic intervals. The mean of three tests Pre Normoxic values (n =3) as obtained from averaging the last two minutes of the normoxic period PET_{CO_2} (in mmHg); the Hypoxic means for three tests and each of the 4 cycles within those three tests (n = 12), which uses data from the last 30 seconds of the respective period.

	PET_{CO_2}(in mmHg)			
	Pre Normoxia		**Hypoxia**	
Subject ID	**Mean**	**± SD**	**Mean**	**± SD**
1	34.32	1.37	35.00	1.00
2	34.15	0.47	34.21	0.76
3	33.21	0.61	33.78	0.97
4	32.96	0.96	33.52	0.86
5	32.17	1.92	33.22	2.05
6	38.80	1.44	38.79	1.099
7	30.75	0.73	31.69	0.89
8	34.97	0.63	35.47	0.84
9	33.69	0.47	33.48	0.57
10	36.00	0.63	36.14	0.86
11	34.66	2.44	34.10	1.85
12	34.89	1.60	34.37	0.70
13	32.85	1.84	32.98	1.03
14	35.14	0.79	35.48	0.34
15	33.51	0.76	33.67	0.43

$P_{ET}CO_2$ differed significantly before and after hyperventilation in normoxia ($P < 0.01$, paired two-tailed t-test) when the mean change was –7.6 ± 1.4 mmHg (range -6.1 to -10.3 mmHg), or -20.4 ± 4.3% (Table 2.3a). The mean decrease in $P_{ET}CO_2$ during hyperventilation in hypoxia with the addition of CO_2 was -0.2 ± 0.3 mmHg (range 0.4 to -0.8 mmHg, Table 2.3a), or -0.4 ± 0.8%, All subject's $P_{ET}CO_2$ values for Hypoxia were within our target range of within 1 mmHg of that subject's resting Pre Normoxia $P_{ET}CO_2$ (Table 2.3b). A range of 1 mmHg has been considered acceptable for maintaining isocapnia in recent studies of isocapnic hypoxia (e.g. Banzett *et al.*, 2000). The change in $P_{ET}CO_2$ of -20.4% (Table 2.3a, n = 8) during hyperventilation in air was significantly different from the change of -0.4% during hyperpnea in hypoxia with the addition of CO_2 (Table 2.3a, n = 11, Mann-Whitney, $P < 0.01$).

Table 2.4. Estimated absolute hypoxic ventilatory rate response (HVR, L • min^{-1} • $\%^{-1}$) and pre-hypoxic exposure ventilatory rates ($\dot{V}_E$, L • min^{-1}) calculated as the average $\dot{V}_E$ for all the hypoxic intervals (H1-H4) minus the normoxic baseline value (Pre, N1-N4), per unit SaO_2 (HVR and $\dot{V}_E$). The values are means of all three tests on each of the three successive days, for subjects 1-9 (n = 3), and for all three tests on day one for subjects 10-15 (n=3). $\overline{X}$ represents the mean ± 1 SD of the group (n = 3 tests). The coefficient of variation (CV) for each day is calculated using the mean CV for all subjects for that day and is expressed as a percentage.

Subject ID	**HVR**			$\dot{V}_E$		
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
S1	1.35 ± 0.49	1.38 ± 0.27	1.48 ± 0.11	7.3 ± 0.2	7.5 ± 0.5	7.4 ± 0.4
S2	0.58 ± 0.07	0.64 ± 0.24	0.66 ± 0.35	8.5 ± 1.7	8.8 ± 0.4	8.1 ± 0.4
S3	1.54 ± 0.45	1.58 ± 0.26	1.65 ± 0.12	9.3 ± 2.4	9.4 ± 1.4	9.0 ± 1.3
S4	0.55 ± 0.11	0.53 ± 0.28	0.43 ± 0.03	8.1 ± 0.2	7.7 ± 0.8	8.0 ± 0.5
S5	0.11 ± 0.04	0.06 ± 0.06	0.15 ± 0.07	4.6 ± 1.2	6.0 ± 1.4	4.5 ± 0.5
S6	0.22 ± 0.02	0.24 ± 0.05	0.24 ± 0.04	6.9 ± 0.7	5.7 ± 1.3	6.6 ± 0.1
S7	0.49 ± 0.20	0.93 ± 0.37	0.87 ± 0.21	7.7 ± 0.6	8.4 ± 1.2	9.4 ± 1.0
S8	0.37 ± 0.12	0.39 ± 0.08	0.34 ± 0.05	9.6 ± 0.4	8.6 ± 1.2	9.8 ± 0.2
S9	0.68 ± 0.32	0.53 ± 0.14	0.40 ± 0.08	6.1 ± 0.6	7.1 ± 0.4	6.5 ± 1.6
S10	0.26 ± 0.05			6.9 ± 0.7		
S11	0.64 ± 0.06			8.7 ± 0.6		
S12	0.45 ± 0.06			7.1 ± 0.5		
S13	0.70 ± 0.12			8.6 ± 1.3		
S14	0.24 ± 0.04			10.7 ± 0.8		
S15	0.40 ± 0.14			5.9 ± 1.1		
CV	30	38	22	12	13	9
$\overline{X}$	0.57	0.70	0.69	7.7	7.7	7.7
SD	± 0.40	± 0.51	± 0.54	± 1.5	± 1.3	± 1.7

Table 2.5. Estimated absolute hypoxic ventilatory rate response (HVR, L • min^{-1} • $\%^{-1}$) and pre-hypoxic exposure ventilatory rates ($\dot{V}_E$, L • min^{-1}) calculated as the average $\dot{V}_E$ for the first hypoxic interval (H1) minus the normoxic baseline value (Pre), per unit S_aO_2 (HVR and $\dot{V}_E$), using data for all intervals (Pre, N1-N4, H1-H4). The values are means (n = 3) for subjects (S1-S9) across three days for the first, second and third tests (Tests 1, 2 and 3) conducted on each day. $\overline{X}$ represents the mean ± 1 SD (n = 3 tests). The coefficient of variation (CV) for each test is the mean CV for all subjectS for that test expressed as a percentage.

Subject ID	$\dot{V}_E$			HVR		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
S1	7.5 ± 0.2	7.1 ± 0.1	7.7 ± 0.3	1.30 ± 0.12	1.52 ± 0.29	1.39 ± 0.45
S2	8.3 ± 0.5	7.8 ± 1.0	9.3 ± 0.9	0.70 ± 0.30	0.64 ± 0.29	0.55 ± 0.06
S3	8.0 ± 0.5	8.7 ± 0.6	11.1 ± 0.8	1.44 ± 0.34	1.50 ± 0.18	1.83 ± 0.08
S4	7.8 ± 0.6	7.7 ± 0.4	8.3 ± 0.4	0.43 ± 0.04	0.60 ± 0.21	0.48 ± 0.19
S5	4.3 ± 0.9	5.6 ± 1.4	5.3 ± 1.2	0.11 ± 0.03	0.08 ± 0.05	0.12 ± 0.11
S6	6.5 ± 0.2	5.7 ± 1.3	7.0 ± 0.6	0.24 ± 0.03	0.26 ± 0.03	0.21 ± 0.03
S7	9.1 ± 0.7	8.8 ± 1.4	7.6 ± 0.6	0.78 ± 0.47	0.76 ± 0.22	0.74 ± 0.36
S8	9.2 ± 0.4	8.9 ± 1.5	9.8 ± 0.2	0.29 ± 0.01	0.37 ± 0.03	0.44 ± 0.09
S9	6.3 ± 1.0	6.3 ± 1.1	7.1 ± 1.0	0.54 ± 0.11	0.58 ± 0.40	0.49 ± 0.05
CV	10	14	9	21	33	30
$\overline{X}$	7.4 ± 1.5	7.4 ± 1.3	8.1 ± 1.7	0.65 ± 0.46	0.70 ± 0.50	0.69 ± 0.56

Ventilatory variables

It is well known that extended exposure to hypoxia causes HVD (Howard & Robbins, 1994, Powell *et al.*, 1998). To determine if our protocol caused HVD with repeated exposure to hypoxia and normoxia, seen as a decrease in the ventilatory response with increasing cycle number, we analyzed the mean values separately for the hypoxic and normoxic exposures. For this analysis we used repeated measures ANOVA, with cycle number as a fixed factor and gender as a nested factor, followed by Bonferroni multiple comparisons in the case of significant differences between cycles. We used all the data for each subject and assumed that each test was independent. For clarity, Figures 2.3 to 2.12 show the mean values for all subjects in addition to the mean values with genders separated. Where gender dependence was not significant, the genders were combined for multiple comparison analyses and when computing mean values. When the repeated measures ANOVA analysis showed a significant difference between genders, multiple comparisons were also made within each gender.

Table 2.6. Estimated absolute hypoxic ventilatory response (HVR, $L \cdot min^{-1} \cdot \%^{-1}$) and pre-hypoxic exposure ventilatory rates ($\dot{V}_E$, $L \cdot min^{-1}$) for all subjects (S1-S15) calculated as the average $\dot{V}_E$ for the first hypoxic interval (H1) minus the normoxic baseline value (Pre), per unit SaO_2 (HVR and $\dot{V}_E$). The values are means of all tests for subject (S) 1-15 (n = 9, S1-9; n = 3, S10-15).

Subject ID	$\dot{V}_E$	HVR
S1	7.4 ± 0.3	1.40 ± 1.29
S2	8.5 ± 1.0	0.63 ± 0.22
S3	9.3 ± 1.5	1.59 ± 0.27
S4	7.9 ± 0.5	0.50 ± 0.16
S5	5.1 ± 1.2	0.10 ± 0.07
S6	6.4 ± 0.9	0.24 ± 0.03
S7	8.5 ± 1.1	0.76 ± 0.33
S8	9.3 ± 0.9	0.37 ± 0.08
S9	6.6 ± 1.0	0.54 ± 0.21
S10	6.9 ± 0.7	0.26 ± 0.05
S11	8.7 ± 0.6	0.64 ± 0.06
S12	7.1 ± 0.5	0.45 ± 0.06
S13	8.6 ± 1.3	0.70 ± 0.12
S14	10.7 ± 0.8	0.24 ± 0.04
S15	5.9 ± 1.1	0.40 ± 0.14

Normoxia

Because body size differs between genders, this Chapter focuses on systematic changes in ventilatory variables with increasing cycle number rather than on gender differences within each square wave period. In Chapter 3, size-related gender differences are further explored using ANCOVA with Body Mass Index and/or gender as a covariate.

When comparing the different normoxic periods, Figure 2.3 shows a moderate but statistically insignificant increase in $\dot{V}_E$ from Pre to N1 with males and females combined ($P > 0.1$). $\dot{V}_E$ differed between genders ($P < 0.01$), but not between cycles

(Figure 2.3, $P > 0.05$). The SaO_2 during normoxia decreased after each successive exposure hypoxia (Figure 2.4, $P < 0.05$). F_R increased from a mean Pre value of 14.1 ± breaths • min^{-1} to 15.2 ± breaths • min^{-1} during N3 ($P < 0.05$, multiple comparison), but there were no significant differences across the other intervals (Fig 2.5). V_T among males was approximately ~ 0.12 L larger in males ($P < 0.05$, repeated measures ANOVA), but did not differ from baseline values for either gender (Figure 2.6). The PET_{CO_2} increased between the baseline and the N1-N4 intervals ($P < 0.05$; Figure 2.7).

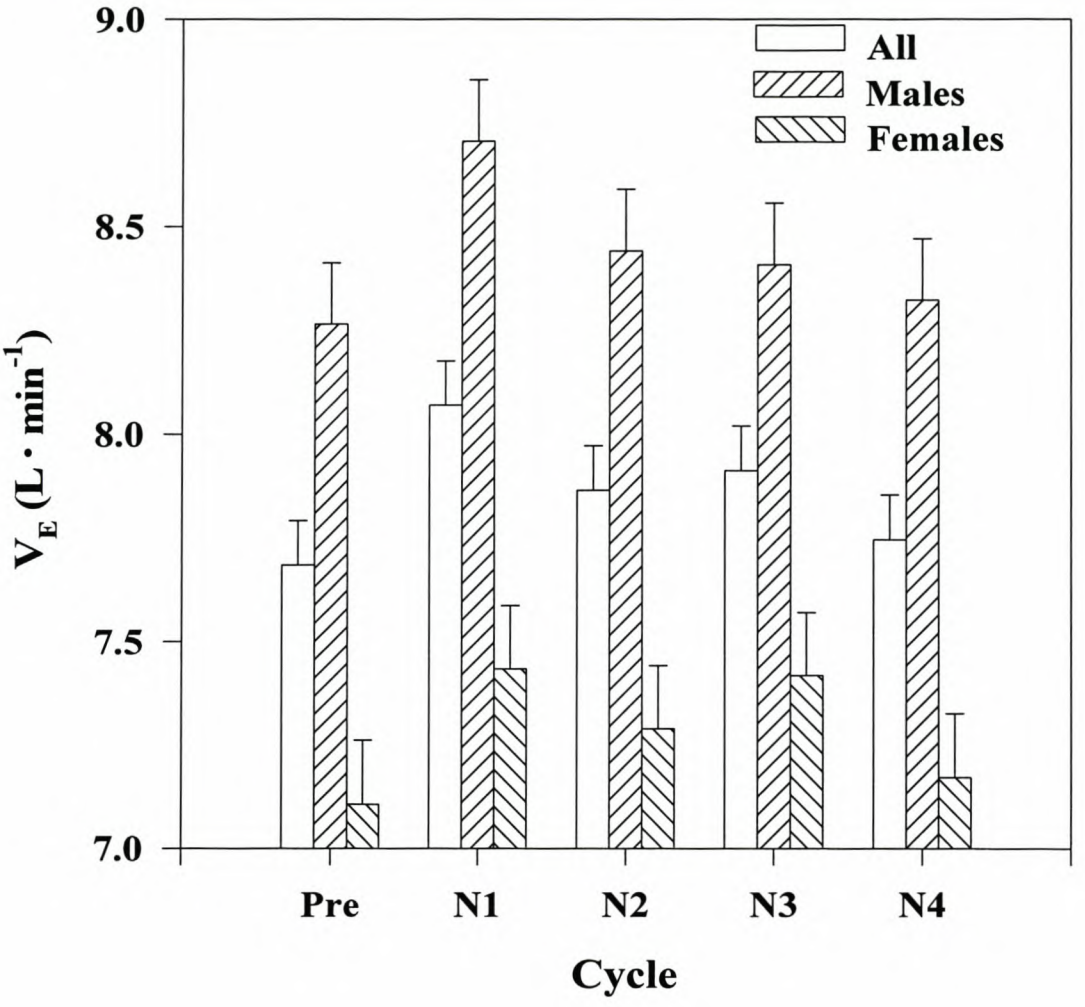


Figure 2.3. Mean minute ventilation ($\dot{V}_E$, L • min^{-1} ; ± SEM for the purposes of multiple comparisons) for the initial baseline resting period (Pre) and each of the four intervals (N1-N4) for all subjects (n = 15) breathing normoxic gas (21 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8). Males were significantly larger than females ($P < 0.01$). Bonferroni multiple comparisons revealed no systematic change in $\dot{V}_E$, with increasing cycle number for all subjects, and for males and females considered separately ($P > 0.05$ repeated measures ANOVA). All values are expressed at STPD.

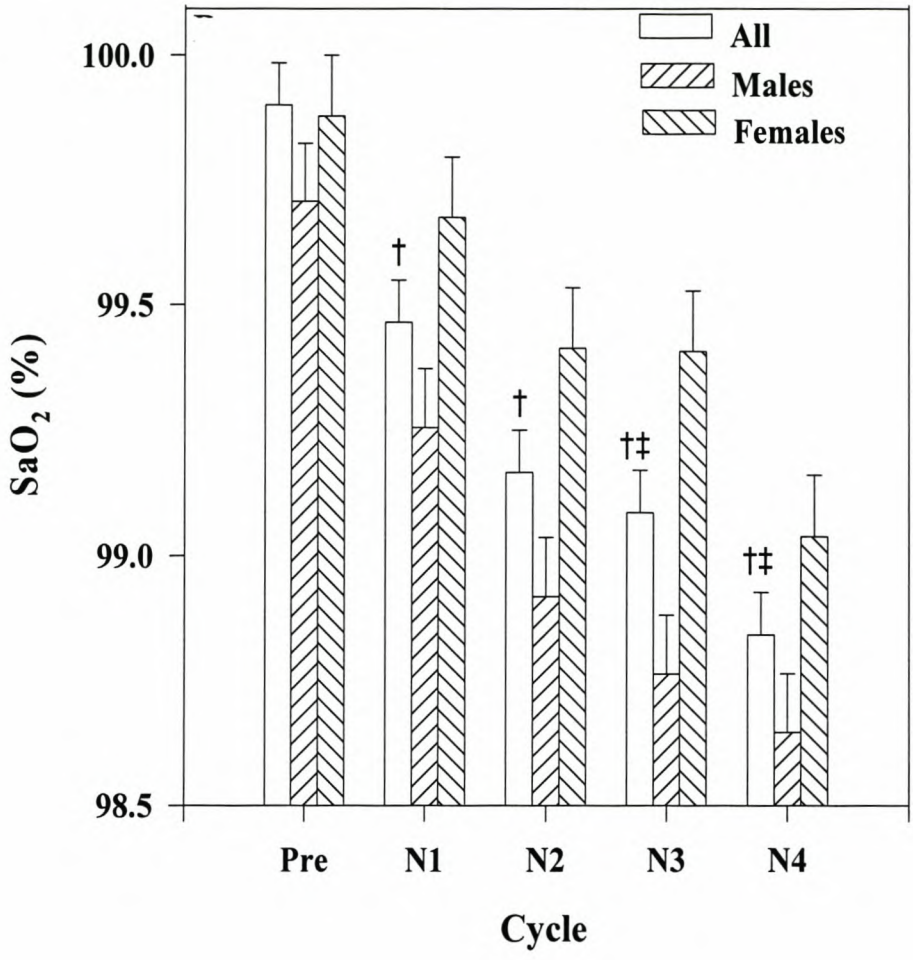


Figure 2.4. Mean arterial O_2 saturation (SaO_2, %; ± SEM) for the initial baseline resting period (Pre) and each of the four intervals (N1-N4) for all subjects (n = 15) breathing normoxic gas (21 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8), but males and females did not differ consistently ($P > 0.05$ repeated measures ANOVA). †Significant difference relative to the initial baseline resting period (Pre), or ‡ to the first normoxic interval ($P < 0.05$, Bonferroni multiple comparison for all subjects pooled).

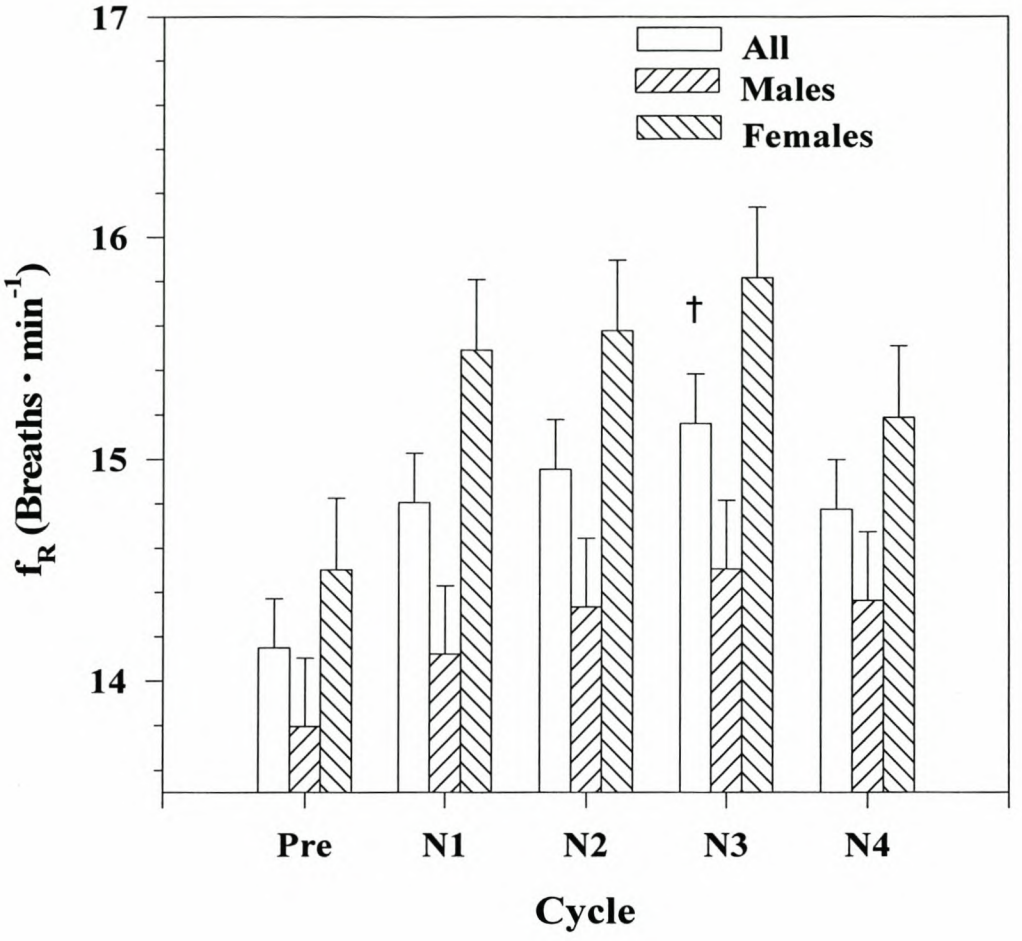


Figure 2.5. Mean breathing frequency (f_R, breaths • min^{-1}; ± SEM) for the initial baseline resting period (Pre) and each of the four intervals (N1-N4) for all subjects (n = 15) breathing normoxic gas (21 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8), but males and females did not differ consistently ($P > 0.05$ repeated measures ANOVA). †Significant difference relative to the initial baseline resting period (Pre; $P < 0.05$, Bonferroni multiple comparison for all subjects pooled).

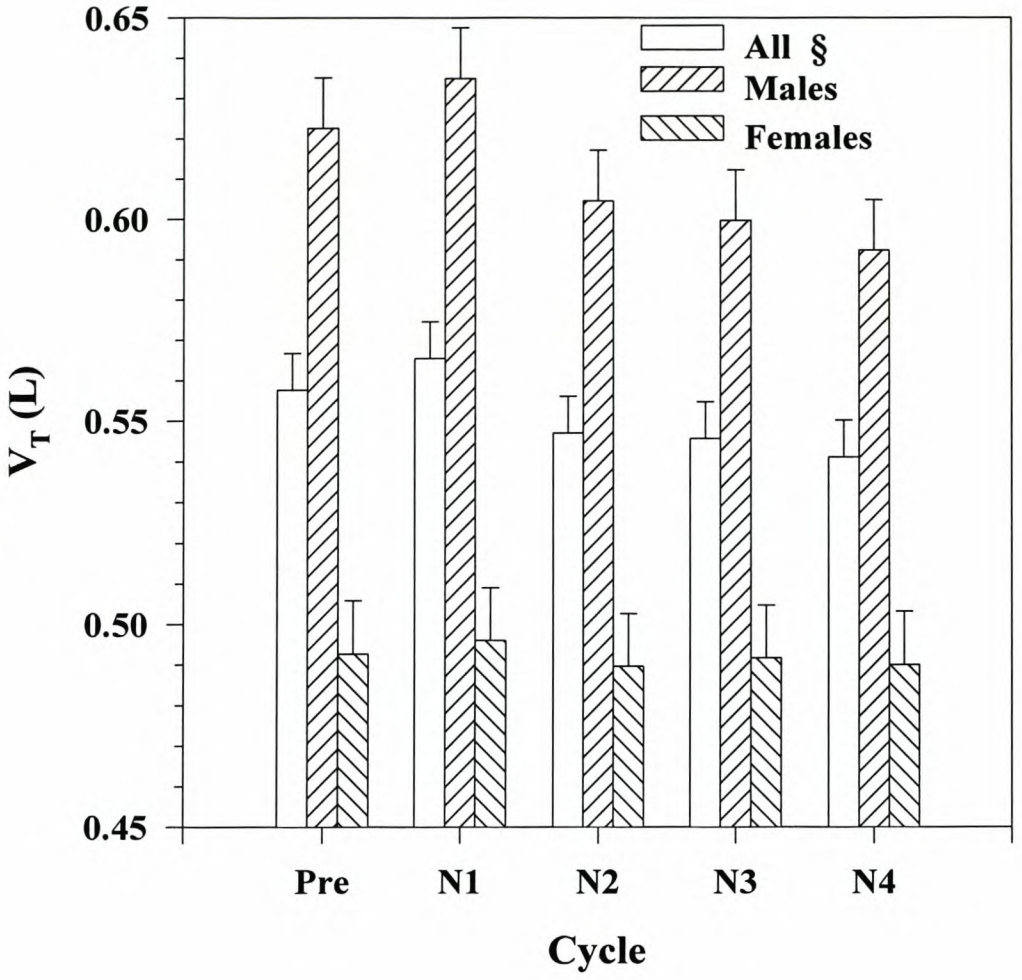


Figure 2.6. Mean tidal volume (V_T, L; ± SEM) for the initial baseline resting period (Pre) and each of the four intervals (N1-N4) for all subjects (n = 15) breathing normoxic gas (21 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8), and values for males were consistently larger than those for females (§, $P > 0.05$ repeated measures ANOVA). All values are expressed at STPD.

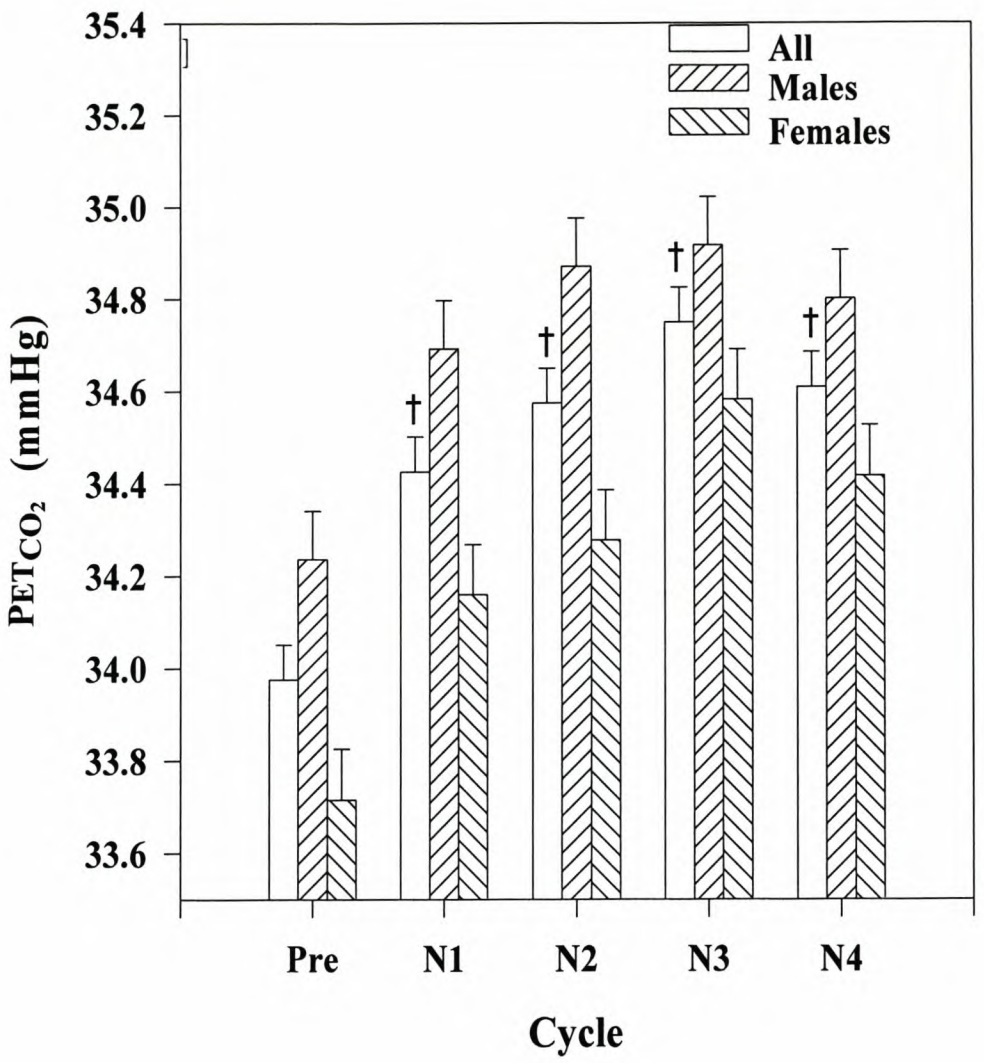


Figure 2.7. Mean arterial end-tidal PCO_2 ($P_{ET}CO_2$, mmHg; ± SEM) for the initial baseline resting period (Pre) and each of the four intervals (N1-N4) for all subjects (n = 15) breathing normoxic gas (21 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8), but males and females did not differ consistently ($P > 0.05$ repeated measures ANOVA). †Significant difference relative to the initial baseline resting period (Pre; $P < 0.05$, Bonferroni multiple comparison for all subjects pooled). All values are expressed at STPD.

Hypoxia

$\dot{V}_E$ was significantly higher among males than females (Fig 2.8, $P < 0.05$), and decreased with increasing interval number in male subjects (Fig 2.8, $P < 0.05$). Males hypoxic SaO_2 values were significantly higher ($P < 0.05$) than females (Fig 2.9). SaO_2 declined steadily with increasing hypoxic interval number, the trend was not statistically significant in males, although SaO_2 changed with interval number in females ($P < 0.05$). F_R was higher during H1 than during H2 and H4 (Fig 2.10, $P < 0.05$). V_T among males was 0.37-0.54 L higher than among females ($P < 0.05$) (Fig. 2.11). V_T among males changed significantly with repeated exposures to hypoxia ($P < 0.05$), whereas PET_{CO_2} was unchanged between intervals ($P > 0.3$)(Fig. 2.12) and males and females did not differ consistently.

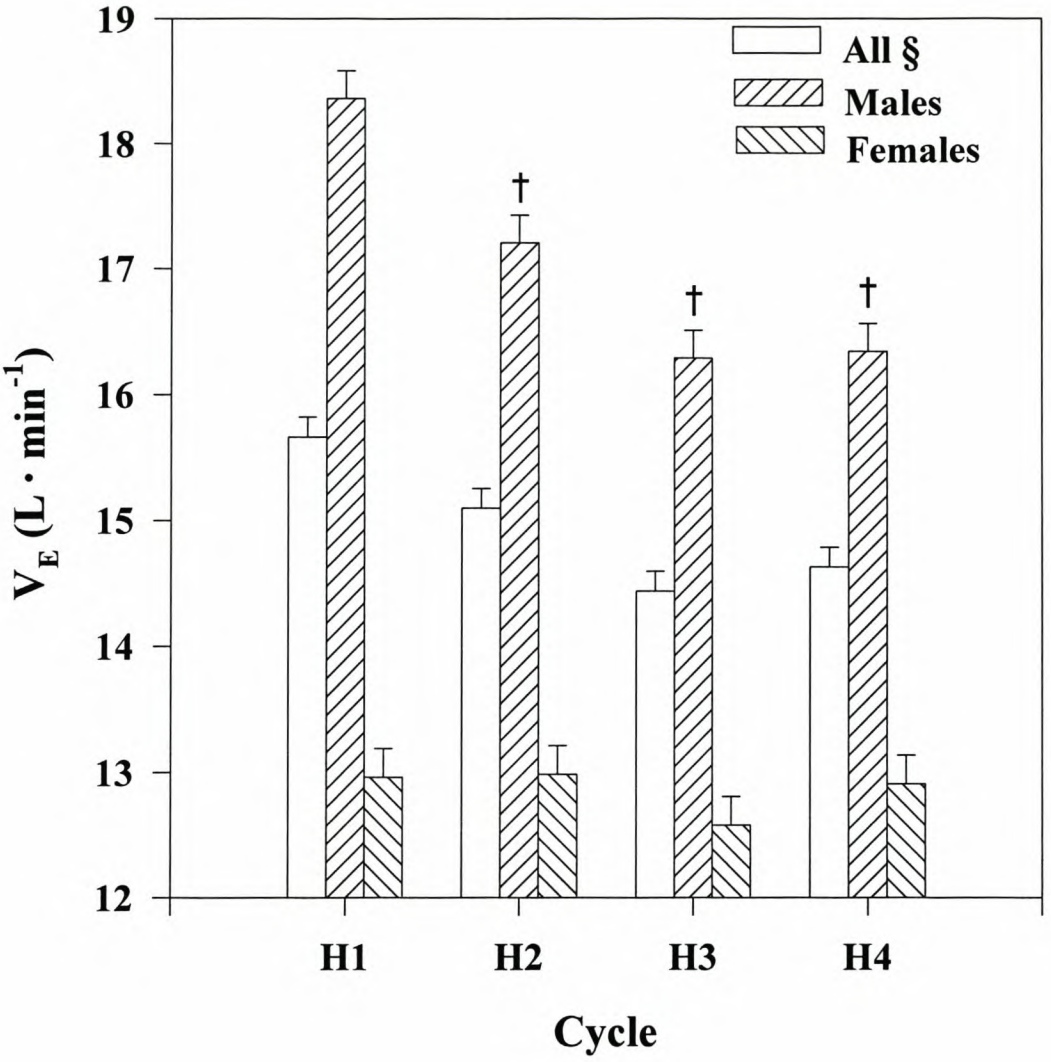


Figure 2.8. Mean minute ventilation ($\dot{V}_E$; L • min^{-1}; ± SEM) for each of the four intervals (H1-H4) for all subjects (n = 15) breathing hypoxic gas (8 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8). Values for males were consistently larger than those for females (§, $P < 0.05$ repeated measures ANOVA). †Significant difference relative to the first hypoxic exposure (H1; $P < 0.05$, Bonferroni multiple comparison for all subjects pooled). All values are expressed at STPD.

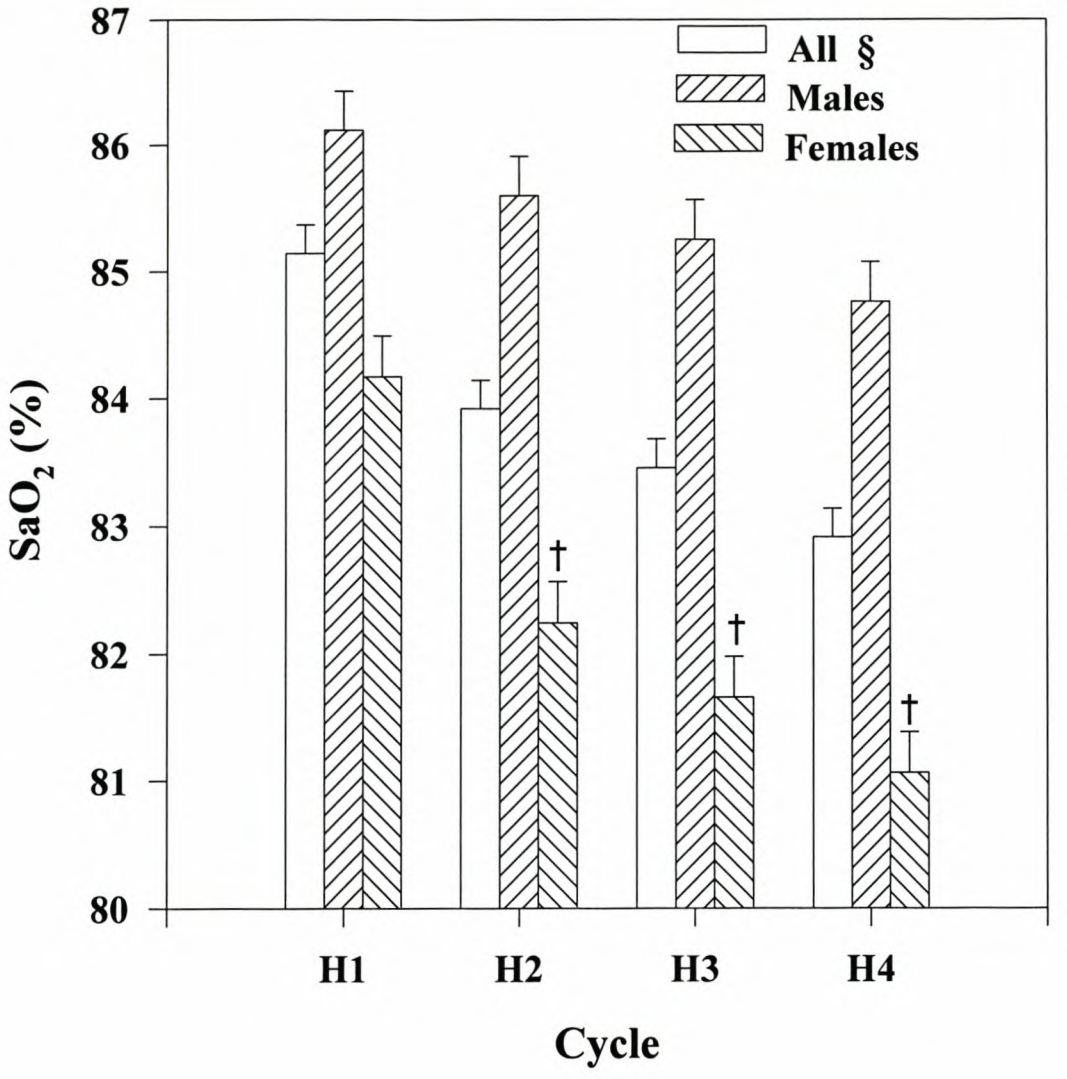


Figure 2.9. Mean arterial O_2 saturation (SaO_2, %; ± SEM) for each of the four intervals (H1-H4) for all subjects (n = 15) breathing hypoxic gas (8 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8). Values for males were consistently higher than those for females (§, $P < 0.05$ repeated measures ANOVA). †Significant difference relative to the first hypoxic exposure (H1; $P < 0.05$, Bonferroni multiple comparison for all subjects pooled).

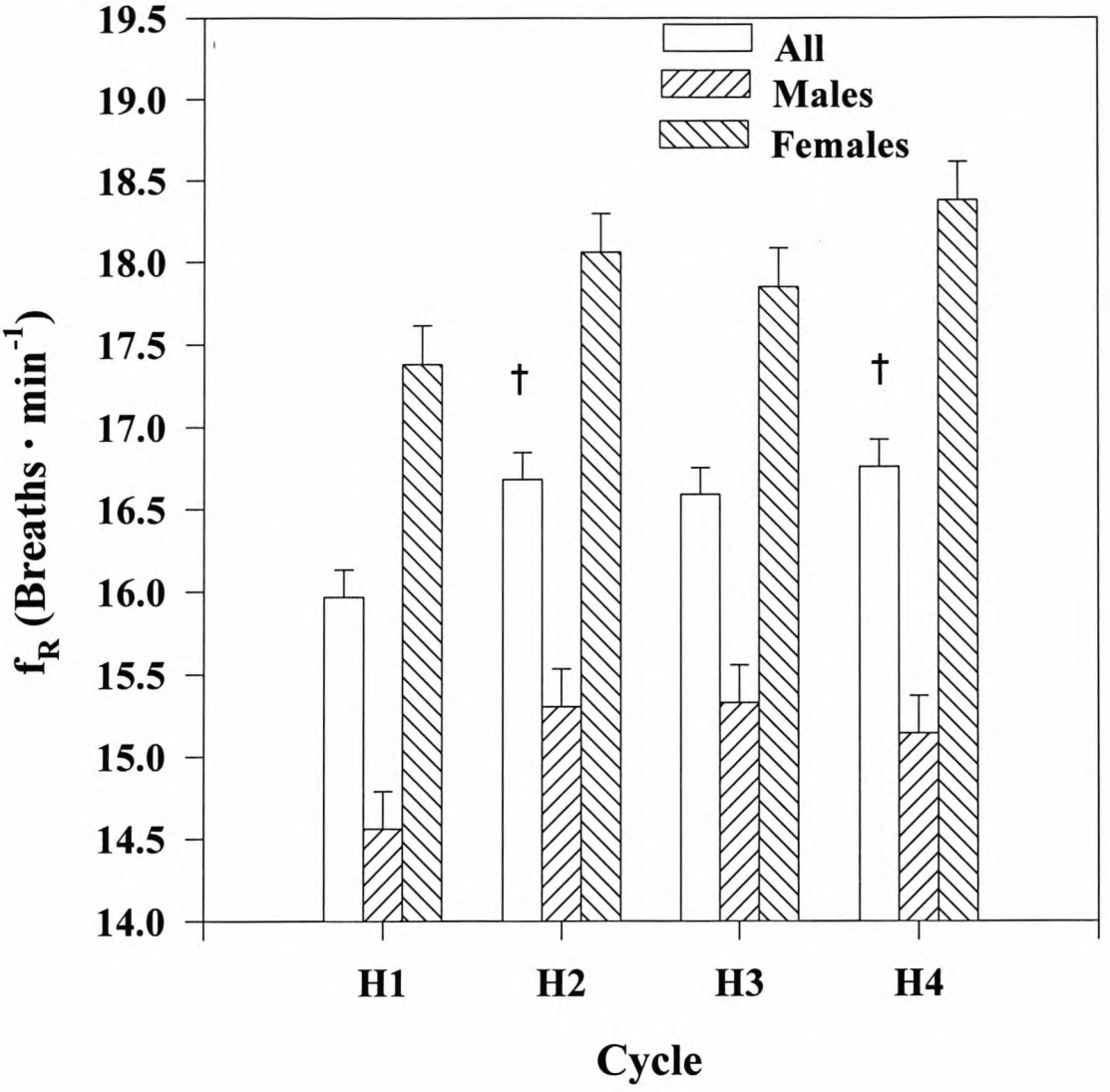


Figure 2.10. Mean breathing frequency (f_R, breaths • min^{-1}; ± SEM) for each of the four intervals (H1-H4) for all subjects (n = 15) breathing hypoxic gas (8 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8), but males and females did not differ consistently ($P > 0.05$ repeated measures ANOVA). †Significant difference relative to the first hypoxic period (H1; $P < 0.05$, Bonferroni multiple comparison for all subjects pooled).

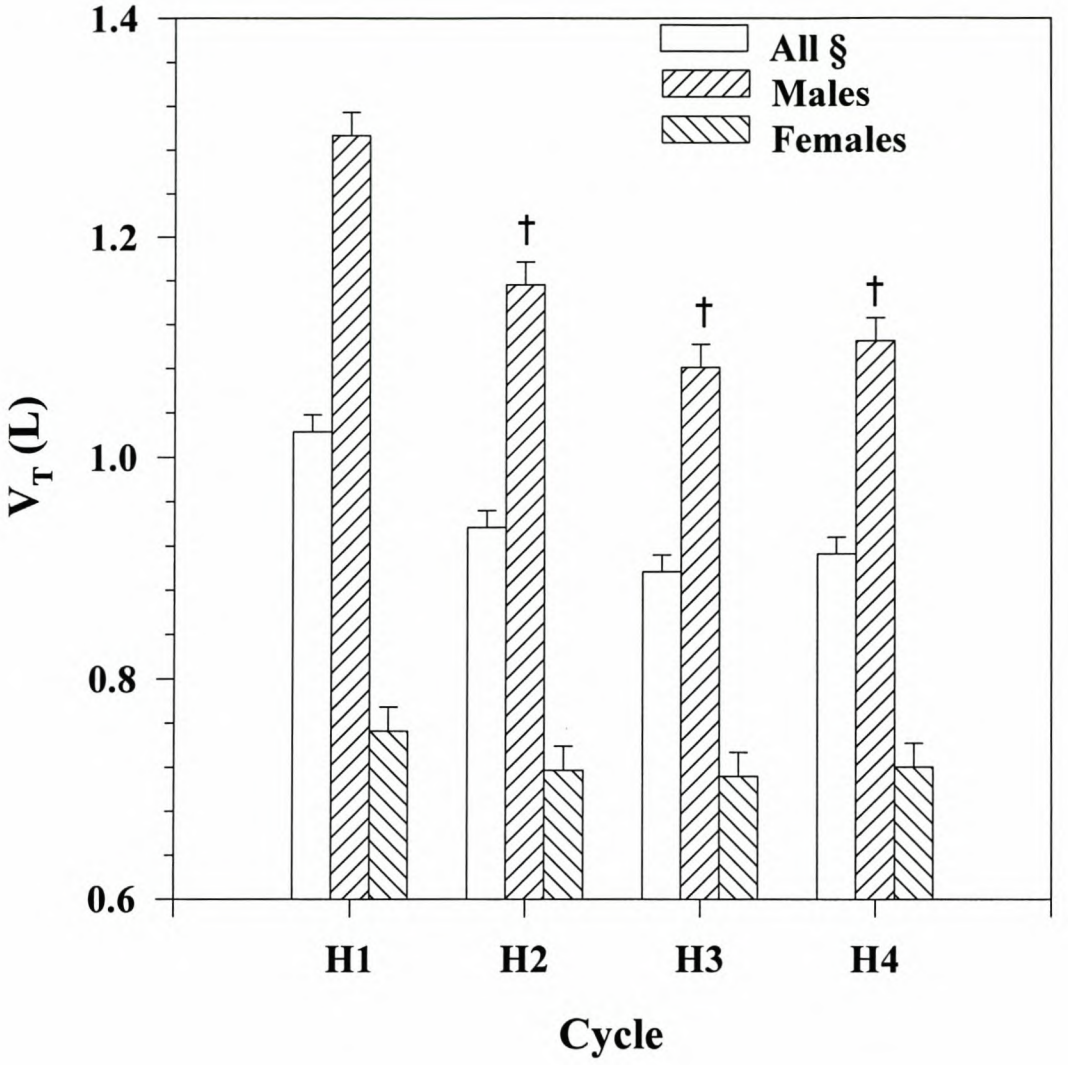


Figure 2.11. Mean tidal volume (V_T, L; ± SEM) for each of the four intervals (H1-H4) for all subjects (n = 15) breathing hypoxic gas (8 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8). Values for males were consistently larger than those for females (§, $P < 0.05$ repeated measures ANOVA). †Significant difference relative to the first hypoxic exposure (H1; $P < 0.05$, Bonferroni multiple comparison for all subjects pooled). All values are expressed at STPD.

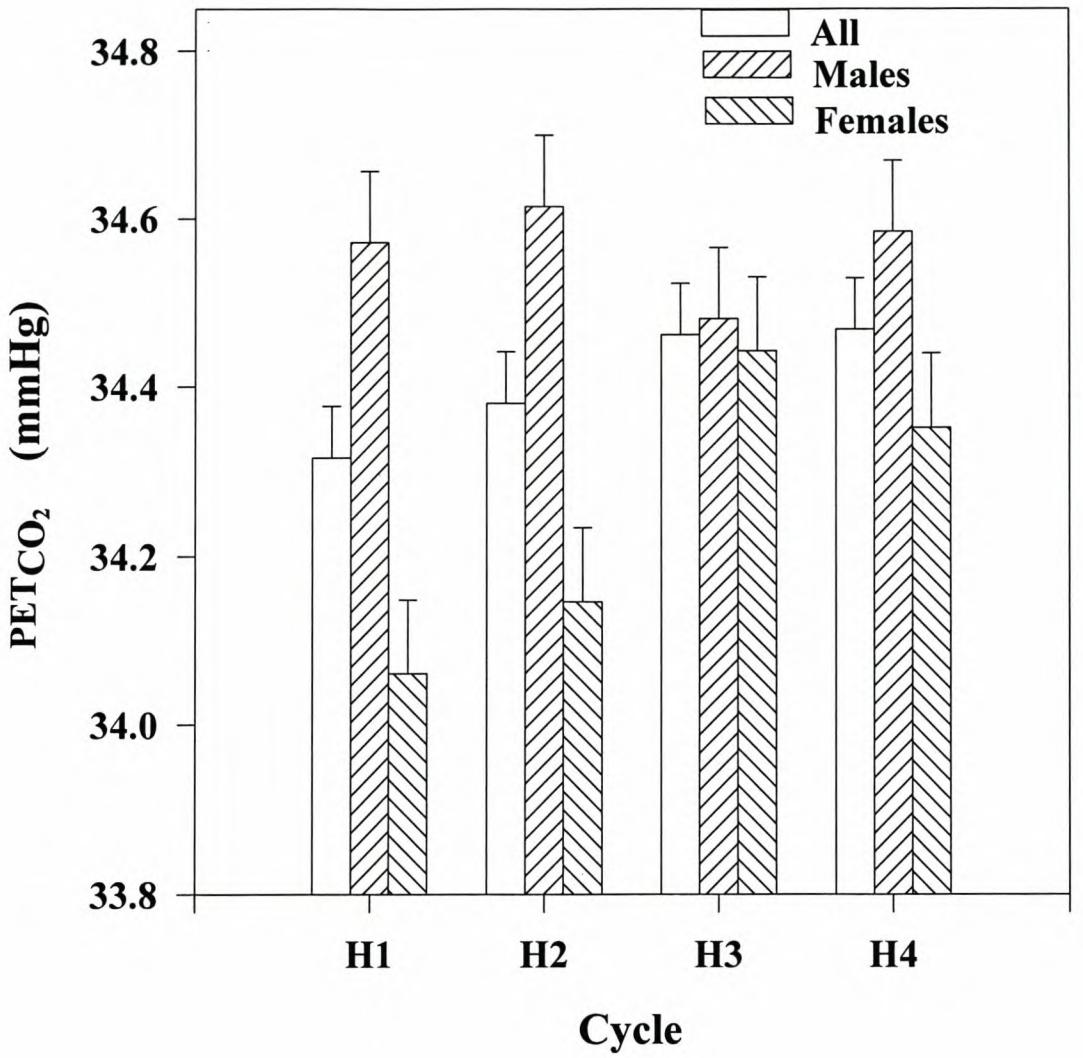


Figure 2.12. Mean end-tidal PCO_2 (PET_{CO_2}, mmHg; ± SEM) for each of the four intervals (H1-H4) for all subjects (n = 15) breathing hypoxic gas (8 % O_2, balance N_2). Overall means for males and females are presented both pooled, and separated by gender (male n = 7, female n = 8) but males and females did not differ consistently ($P > 0.05$ repeated measures ANOVA). Bonferroni multiple comparisons revealed no systematic change in PET_{CO_2} with increasing cycle number for all subjects, and for males and females considered separately ($P > 0.05$ repeated measures ANOVA). All values are expressed at STPD.

HVR: Variability within and between days

The consistent and significant differences between $\dot{V}_E$ with increasing interval number suggested that the subjects experienced HVD during repeated exposures to hypoxia, but the actual HVR values did not change with increasing interval, indicating that HVD (as it is recognized in the literature) was not experienced. The HVR is calculated as the change in $\dot{V}_E$ with each percentage change in SaO_2 ($L \bullet min^{-1} \bullet \%^{-1}$, Vargas *et al.*, 1998). But for repeated tests performed on the same day, HVR was not significantly different from each other ($P > 0.3$, repeated measures ANOVA). Therefore, in Table 2.4, the HVR and $\dot{V}_E$ are averaged over all tests on a given day. Table 2.5 shows the average HVR and $\dot{V}_E$ for each test over the three days. There was no systematic change in the HVR between days ($P > 0.4$, repeated measures ANOVA). The variability (estimated using the coefficient of variation (CV)) for HVR, within a particular day was between 22-38 % and between days it was 21-33 %. For $\dot{V}_E$ the within day CV was between 9-16 % and between days it was 9-15 %.

Changes in the magnitude of HVR versus changes in variability

There was a significant positive correlation between a subjects average HVR and the variability (measured as standard error of mean) between tests (Fig 2.13, $P < 0.05$, $r^2 = 0.55$).

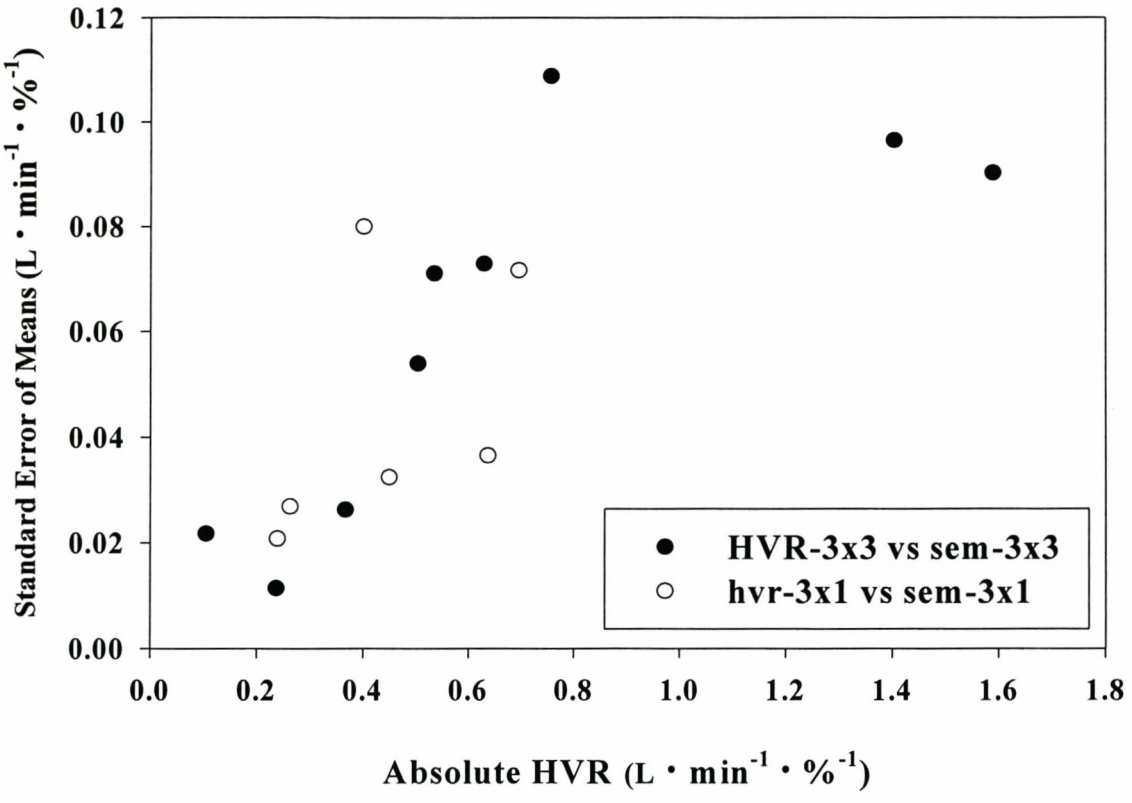


Figure 2.13. Mean HVR for all tests versus standard error of mean (SEM) for all subjects in group 1 (Gr1, n = 9 subjects over 9 tests) and group 2 (Gr2, n = 6 subjects over 3 tests). There was a significant positive correlation ($P < 0.05$, $r^2 = 0.552$).

Discussion

The main findings from this study are:

a) the system effectively measured the HVR while maintaining isocapnia during hypoxia,

b) the measured ventilatory variables changed significantly with repeated short-term cycles of exposure to hypoxia over a 30-min period, and

c) the within- and between-day variability in HVR did not differ significantly, and amounted to approximately 27% between tests.

Breathing circuit

The breathing circuit successfully maintained isocapnia in our subjects, who showed minimal but significant changes in $P_{ET}CO_2$ from their eucapnic resting levels during both hypoxic and normoxic intervals. $P_{ET}CO_2$ did not differ between hypoxic and normoxic intervals, but between normoxic intervals increased slightly by a maximum of 0.7 mmHg from the Pre interval. There are two possible explanations for this. First, subjects may have hypoventilated for a few seconds during the transition from hypoxia to normoxia, as was reported in subjects exposed to five minutes of hypoxia (Georgopoulos *et al.*, 1989; Holtby *et al.*, 1988). Such hypoventilation would result in increased $P_{ET}CO_2$. The $\dot{V}_E$ was computed from the last 30 s of the Pre interval, representing the resting normoxic $\dot{V}_E$ before hypoxia exposure. There was no systematic change in the $\dot{V}_E$ between Pre and N1-N4, which one would expect if subjects hypoventilated in normoxia after exposure to hypoxia. This apparent discrepancy between $\dot{V}_E$ and $P_{ET}CO_2$ may be explained by the fact that the $\dot{V}_E$ was computed as the average during the last 30 s of each normoxic

interval, excluding changes during the first 90 s after the change in inspired gas. Thus, the significantly higher $P_{ET}CO_2$ in normoxia could reflect only a transient hypoventilation in normoxia, hypoventilation that was invisible in the analyses of $\dot{V}_E$ based on a restricted portion of the data. This highlights the importance of using all data within the time window selected when making conclusions about respiratory changes over short intervals, and advises against selecting certain portions for analysis.

Second, the slight increase in $P_{ET}CO_2$ may be a function of the design of our breathing circuit (see Fahlman *et al.*, 2002). For maintenance of isocapnia with this circuit, it is important that the reserve gas have a fractional concentration of CO_2 (FRG_{CO_2}) equal or close to the subject's arterial value, and that the fresh gas flow (FGF) be equal to the subject's alveolar minute ventilation ($\dot{V}_A$) (see Fahlman *et al.*, 2002). Consequently, the slight increases in $P_{ET}CO_2$ between different normoxic intervals that we observed may result from a normoxic FRG_{CO_2} that was slightly higher than most subjects' resting values, or a FGF set slightly lower than the subjects' $\dot{V}_A$. $P_{ET}CO_2$ did not differ between normoxic and hypoxic intervals, or Pre and hypoxic intervals. We sought to maintain isocapnia in association with hypoxia, the primary stimulus under investigation, and since the $P_{ET}CO_2$ was not different between Pre and any of the hypoxic intervals it is unlikely that this slight elevation of $P_{ET}CO_2$ during intervening normoxic episodes influenced our subjects' HVR.

The non-significant decrease in $P_{ET}CO_2$ of -0.2 mmHg in hypoxia relative to normoxia may be due to a slightly lower CO_2 fraction in the RG (FRG_{CO_2}) than in each subject's mixed venous blood, or to an FG flow slightly higher than resting $\dot{V}_A$. Both situations would lead to enhanced elimination of CO_2 from the blood into the lungs and thence the expired air. Maintenance of isocapnic eucapnia requires an FRG_{CO_2} that is not too low and a FG flow equal to $\dot{V}_E$. Moreover, the $\dot{V}_E$'s of several subjects declined to slightly less than their initial normoxic baseline values immediately after a switch from hypoxia to normoxia. This result forced us to either increase the $P_{ET}CO_2$ during the hypoxic interval or to maintain it at a level consistent with initial resting values. We chose to do the latter, to maintain isocapnia at eucapnic levels.

We modified the breathing circuit described by Sommer *et al.* (1998) by adding an additional pair of FG and RG gas cylinders. The original circuit described by Sommer *et al.* (1998) was designed for a different purpose and maintained isocapnia during increases in ventilation using a single gas mixture drawn from one tank of FG and one of RG. Our modification facilitates changes in the inspired gas mixture that permit rapid alteration between experimentally-induced normoxia and hypoxia in isocapnic human subjects.

The breathing circuit described here allowed us to adequately titrate the subjects' $P_{ET}CO_2$ during rapid changes in inspired F_{O_2}. We were acutely aware of the need to avoid HVD during the slightly prolonged hypoxic intervals mandated by the lag in stabilization of SaO_2 that we observed because of our switching system.

Weiskopf and Gabel (1975), Weil and Zwillich (1976) and Easton *et al.* (1986) were among the first to report the development of HVD during more than three minutes of exposure to hypoxia. They reported an initial acute ventilatory increase during which both V_T and f_R rise significantly. Following this, V_T returns almost to the base line value, while f_R remains elevated for the entire hypoxic exposure. The net effect is an initial rapid increase in $\dot{V}_E$ followed after two to five minutes by a decline that nonetheless does not reach the resting value for the duration of the entire hypoxic exposure (Easton *et al.*, 1986). Our choice of hypoxic exposure falls within the period preceding development of HVD described by these authors. Had our prolongation of the hypoxic exposure from 60 to 120 s led to HVD we might have expected systematic changes in $\dot{V}_E$ with progressive cycles of hypoxia, with V_T increasing less while f_R remained elevated. We saw no such changes, suggesting that the hypoxic exposure interval of 120 s we used is short enough to preclude the development of HVD. Moreover, there were no systematic changes in the HVR itself with repeated exposures to hypoxia, further suggesting that our subjects' sensitivity to hypoxia was not altered. However, the slight non-significant decline ($P > 0.05$) in the SaO_2 with repeated exposures to hypoxia and normoxia and the small sample size suggest that this protocol requires further study, and that repeated hypoxic exposures following the initial one be treated with caution.

Across all individuals, $\dot{V}_E$, f_R and V_T in every cycle and every test increased significantly from normoxia to hypoxia. For all subjects, there was a non-significant

increase in mean $P_{ET}CO_2$ of 0.2 mmHg in normoxia relative to hypoxia, with the largest difference in any one subject being +0.8 mmHg. Ventilatory responses to decreased P_{CO_2} (Sahn *et al.*, 1977; Ren & Robbins, 1999; Mahamed & Duffin, 2001) suggest that a decrease in alveolar P_{CO_2} of ~1 mmHg would elicit a change in $\dot{V}_E$ of approximately 3 L • min^{-1}. Comparison of this value with the standard deviation around the increased $\dot{V}_E$ resulting from hypoxia reported here (0.4 – 3.2 L • min^{-1}) shows that the potential increase in $\dot{V}_E$ due to stimulation of the CO_2-sensitive central chemoreceptors is well within the confidence limits of our measurements. Moreover, separate analyses of the mean $P_{ET}CO_2$ values during normoxia and hypoxia for each subject reveal that in no subject did $P_{ET}CO_2$ differ in hypoxia versus normoxia ($P > 0.05$, two-tailed t-test). As mentioned earlier, the main difference was from the Pre to the other normoxic sections of the test. In addition, no subject's $P_{ET}CO_2$ for individual tests exceeded our experimental criteria range of ± 1 mmHg during the hypoxic versus the normoxic periods. Our criteria lie within the accepted range for the maintenance of isocapnic hypoxia (e.g. Howard & Robbins, 1994).

Our second experiment on subjects coached to hyperventilate while breathing air showed that $P_{ET}CO_2$ changed substantially (~ 40 fold) more during hyperventilation on air without the addition of supplementary CO_2, far more than it did during hypoxia when CO_2 was added via the circuit's demand valve. This observation is a clear indication that the circuit satisfactorily compensated for potential changes in $P_{ET}CO_2$.

The HVR values reported here are comparable to those obtained using the end-tidal forcing (Zhang & Robbins, 2000) and the re-breathing (Rebuck & Campbell, 1974; Beall *et al.*, 1997) techniques. The CV between subjects was 70% and is consistent with data in the literature (Zhang & Robbins, 2000, 47%; Rebuck & Campbell, 1974, 72%; and see Chapter 1). We conclude that the HVR can be measured using this circuit, but the inherent variation in HVR should be taken into account and be acknowledged in discussion of results when this parameter is measured in future studies.

Responses of ventilatory variables to repeated hypoxic exposures

The literature suggests that a steady hypoxic $P_{ET}O_2$ over 50 s is long enough for full development of the acute HVR, yet short enough to prevent a significant HVD (Mou *et al.*, 1995), whereas 120 s of steady $P_{ET}O_2$ led to HVD (Howard & Robbins, 1994; Zhang & Robbins, 2000). Alternatively, induction of stepwise hypoxia using seven 50 s periods of increasing or decreasing $P_{ET}O_2$ (Mou *et al.*, 1995) did not lead to significant HVD, suggesting that as long as the exposure to a steady hypoxic $P_{ET}O_2$ does not exceed 50 s, HVD can be avoided. In our subjects the switch from normoxic to hypoxic inspired gas was followed by a transitional period where the subjects' SaO_2 remained stable for ~ 20 s, then dropped continuously for the next 40-50 s before stabilizing for an additional 50-60 s, presumably indicating a steady $P_{ET}O_2$. Therefore, our chosen protocol using intervals of 120 s should adequately prevent the development of HVD during each test. This time to reach steady SaO_2 is similar to that reported by Easton *et al.* (1986), and can be explained by the mixing of inhaled gas with that contained in the dead space and the residual volume, causing alveolar PO_2 to lag behind inspired PO_2 (Anthonisen &

Fleetham, 1987). In contrast, the dynamic end-tidal forcing system (Zhang & Robbins, 2000) allows steady PET_{O_2} values to develop within five seconds (one to two breaths) of switching of the inhaled gas mixture, after which the subjects remain at a steady PET_{O_2} for 60 s. Previously, we argued that alternation of hypoxic and normoxic intervals of 120 s each would prevent significant changes with repeated intervals, because hypoxia was not at steady state for more than 50-60 s (Fahlman *et al.,* 2002). However, in this study both male and female subjects consistently showed systematic decreases in SaO_2 in both hypoxia and normoxia with increasing interval number although this trend was statistically significant only among females. Since the SaO_2 (an indication of the hypoxic challenge) changed with interval number, the $\dot{V}_E$ should have increased with interval number if there was no HVD. However the $\dot{V}_E$ in hypoxia decreased for males but was unchanged for females. In addition, significant differences in f_R and V_T between hypoxic intervals suggest that each exposure to hypoxia affected the subsequent response.

HVD within each interval

The above trends suggest that our subjects experienced HVD during the course of the 4-cycle test. However, it was not clear if HVD also developed during each hypoxic interval. To investigate this possibility, we computed average $\dot{V}_E$'s using data from the period from 80 to 110 s after gas switching (T-10) and 70 to 100 s after switching (T-20), and compared these with values computed with data from the last 30 s (T, 90 to 120 s after switching) of each interval. Across all subjects, mean $\dot{V}_E$ calculated for the T-10 period was not different from the T period, but that for the T-20 period, on the other hand, tended to be lower than that calculated for the T period ($P < 0.09$, one way

ANOVA followed by Bonferroni multiple comparisons). If 120 s of hypoxic exposure was long enough for HVD to develop within each interval, we expected the $\dot{V}_E$ for the T-10 or T-20 periods to be higher than that during the T period. The results suggested that this was not the case, and contrary to supplying evidence for HVD, suggested that when using this circuit the full HVR only develops after 90-120 s. In addition, this is consistent with the literature (Easton *et al.*, 1988).

Comparison with other studies

Recovery from hypoxic exposure lasting 25 min takes 15 – 60 min and is accelerated by inhalation of 100 % O_2 (Easton *et al.*, 1988). Consequently, if variables for each hypoxic interval are not independent of those for previous intervals within the same test due to a carry-over effect, and if the intervening normoxic periods are too short to allow full recovery, one would expect a certain degree of HVD to occur over the course of each test. Across all subjects, we report an initial increase in V_T during the first hypoxic interval, followed by a decline, suggesting that repeated hypoxic exposures caused a certain degree of HVD in our subjects (Easton *et al.*, 1988) and that the decline is not short-term ventilatory depression (Powell *et al.,* 1988). Furthermore, Easton *et al.* (1988) reported that the subjects showing the largest ventilatory response to hypoxia also showed the largest HVD (Easton *et al.,* 1986). Accordingly, we compared ventilation changes between the Pre, H1, and H4 intervals, but we found no indication that this was so in our data ($P > 0.1$, $r^2 = 0.17$, $n = 15$).

The consistent but non-significant decline in SaO_2 with increasing interval number that occurred both in hypoxia and normoxia may have resulted from too short a recovery time between hypoxic exposures, so that subjects' $\dot{V}_E$'s did not regain the original value. An alternative possibility for the decline in SaO_2 values with cycle number may be that the effect of the capsaicin ointment wore off to some degree as interval number increased.

Zhang and Robbins (Fig. 1, 2000) reported data suggesting a similar decrease in $\dot{V}_E$ with increasing interval number, although they did not analyze and point this out. By extrapolating the values on their graph, we estimated that mean resting $\dot{V}_E$ before hypoxia was ~ 20 L • min^{-1} and that during the first hypoxic interval the mean $\dot{V}_E$ increased to ~ 55 L • min^{-1}. After four hypoxic intervals the mean $\dot{V}_E$ had decreased by ~ 5 L • min^{-1} to ~ 50 L • min^{-1}, a decrease of ~ 14 % of the initial increase of 35 L • min^{-1}. Therefore, measuring multiple exposures with a 120 s recovery between hypoxia does not seem to be feasible option for us.

The HVR values that we report are comparable in magnitude to those from other studies (Garcia *et al.*, 2001; Zhang & Robbins, 2000), but the mean value (~ 0.60 L • min^{-1} • $\%^{-1}$) is only half that reported by researchers using the end-tidal forcing technique mainly as a result of the chosen units in BTPS (Zhang & Robbins, 2000), as opposed to ours which are given in STPD. Our method is a combination of rapidly induced hypoxia during the transition period immediately after switching the gas mixture, followed by approximately 50-60 s of steady-state hypoxia. Mahutte and Rebuck (1978) suggested that the HVR estimated by induction of gradual hypoxia might be smaller than that observed during

steady state hypoxia such as that used by Zhang and Robbins (2000). Moreover, the protocol used by Zhang and Robbins (2000) maintains a hypercapnic isocapnia, whereas we chose to maintain isocapnia at eucapnic levels. Their increased level of $P_{ET}CO_2$ (approximately 2 mmHg) for the maintenance of isocapnia can contribute to a 6 L/min difference in $\dot{V}_E$ and this would account for the observable differences in HVR values between our study and that of Zhang and Robbins (2000). Alternatively, hypercapnia elicits increased $\dot{V}_E$ (Sahn *et al.*, 1977) and enhances hyperventilation in hypoxia (Lloyd *et al.*, 1958; Sahn *et al.*, 1977), and may present an additional explanation for the difference between our results and those of Zhang and Robbins (2000).

Inter- and intra-day variability in HVR

Many studies estimating HVR have reported its intrinsically large variability between and within subjects (Hirshman *et al.*, 1975; Kronenberg *et al.*, 1972; Rebuck *et al.,* 1973), but very few have investigated the intra- and inter-day variability within each subject (Sahn *et al.*, 1977; Zhang & Robbins, 2000). Moreover, early studies suggested that the magnitude of this variability may differ between different test protocols (Anderton *et al.,* 1964; Kronenberg *et al.*, 1972), but this was not supported by later studies (Sahn *et al.*, 1977; Zhang & Robbins, 2000). Our study has quantified this inter- and intra-day variability preparatory to use of our breathing circuit as a comparative research tool. As in other studies (Sahn *et al.*, 1977, Zhang & Robbins, 2000), our data showed no systematic difference in HVR within or between days. Unlike Sahn and co-workers (Sahn *et al.*, 1977), we report similar coefficients of variation (CV) both within and between days. However, we only repeated the test three times on a given day and on

three different days separated by 36 days at most, while Sahn *et al.* (1977) repeated their test five times within 2 h, and up to seven times over seven months. This may have influenced the lower mean variation reported by them. The consistent and considerable variation in HVR reported here and by others (Sahn *et al.*, 1977; Zhang & Robbins, 2000) further highlights the importance of conducting repeated tests for comparative purposes. Knowledge of the magnitude of HVR variability will help determine the sensitivity of comparisons and the likelihood that differences between the groups under investigation are masked by that variability.

How might HVR variability influence comparative studies?

To use a physiological parameter in comparative studies, the variability of the parameter should not mask differences between the populations under study. Examples of the use of HVR in comparative studies include exploration of human adaptation to altitude by comparison of HVR between highlanders and lowlanders (Beall, 2000; Beall *et al.*, 1997; Lahiri *et al.*, 1976; Sahn *et al.*, 1977) and within and between highland residents from different parts of the world (Curran *et al.*, 1997; Moore *et al.*, 1998). Within-population studies of HVR in lowlanders (Lahiri *et al.*, 1976) or in highlanders living at different altitudes (Curran *et al.*, 1995), and of the responses of individuals translocated to high altitude (Sato *et al.*, 1992), have explored the phenotypic plasticity of the HVR, as have training studies focusing on the relationship between endurance training and ventilatory responsiveness (Levine *et al.*, 1992). The genotypic component of the HVR has been assessed in familial studies (Collins *et al.*, 1978, Scoggin *et al.*, 1978), and the

development of ventilatory sensitivity through the life of individuals has been studied in rats (Ling *et al.*, 1997) and humans (Lahiri *et al.*, 1976).

All of the studies mentioned above used a single value for each individual subject in their analyses. The high inter-individual variability we report here begs the question: how representative of each individual are such values? The larger an individual's HVR, the greater the variability in his or her ventilatory responsiveness to hypoxia. For each study population, we recommend that repeated measures of HVR on each test subject be incorporated into study designs to permit informed decisions about the best protocol to use, and to increase the power of the comparisons being made. Without such variability analyses, researchers may fail to detect real differences and risk drawing invalid conclusions from their data.

In addition, we conclude that our circuit can be used successfully to measure HVR. Because it is simple, inexpensive to construct and maintain, and portable, the circuit is particularly well-suited for studies requiring large sample sizes or many repeated experiments. Our data suggest that further assessments of variation in HVR are warranted, and field comparisons of HVR between human populations with differing degrees of altitude adaptation. However, we suggest that the protocol be changed to include only one hypoxic interval of 120 s preceded and followed by a normoxic period. Repeated tests can be performed on the same day given at least 60 min between tests.

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Chapter 3

A comparison of oxygen sensitivity in two South-African sea-level populations.

Abstract

Ventilatory sensitivity to hypoxia differs between populations native to high altitude (HA) and those native to low altitude (LA), and even between two HA populations of different heritage. I am not aware of any published studies comparing hypoxic sensitivity between two LA populations of distinctly different heritage. Here, I make such a comparison using measurements of the ventilatory parameter known as the acute isocapnic hypoxic ventilatory response (HVR; $L \cdot min^{-1} \cdot \%^{-1}$) in two LA South African groups represented by twenty families (10 Caucasian (C); 10 Xhosa (X); total n = 63). The HVR was calculated as the change in minute ventilation ($\Delta \dot{V}_E$, $L \cdot min^{-1}$) divided by the change in arterial oxygen saturation (ΔSaO_2, %). Caucasians were taller (C: 1.79 ± 0.09 m; X: 1.59 ± 0.07 m; $P < 0.001$), while their BMI was lower (X: 28.6 ± 8.30 $kg \cdot m^{-2}$; C: 23.0 ± 2.44 $kg \cdot m^{-2}$; $P < 0.001$) than that of Xhosas. ANCOVA with BMI and gender as covariates showed no significant difference between HVR's of the two groups ($F = 1.04$; $P > 0.31$) with mean absolute HVR values of 0.323 ± 0.395 and C: 0.432 ± 0.417 $L \cdot min^{-1} \cdot \%^{-1}$ for Xhosas and Caucasians respectively. Minute ventilation ($\dot{V}_E$) was similar for both groups under normoxic and hypoxic conditions ($P > 0.40$). Estimates of alveolar ventilation confirmed that effective ventilation did not differ between groups. However, the components of $\dot{V}_E$ differed significantly, with C showing larger tidal volumes (V_T; $P < 0.012$) and lower breathing frequencies (f_R, breaths • min^{-1}; $P < 0.010$) than did X for both normoxia and hypoxia. Similar differences in breathing patterns have been reported for different mouse populations, for which HVR may be regulated by as few as two major genetic determinants. Moreover,

hypoxic oxygen saturation (SaO_2) was higher among Caucasians then Xhosas, suggesting that differences existed with respect to oxygenation of the blood.

Introduction

Ventilatory sensitivity to hypoxia differs between populations native to high altitude (HA) and those native to low altitude (LA) (Zhuang *et al.*, 1993; Hochachka, Gunga, *et al.,* 1998 Hochachka *et al.*, 1999), with HA natives exhibiting blunted hypoxic sensitivity. Two HA populations living at similar altitudes in different global regions differ in hypoxic sensitivity (Beall, Strohl *et al.,* 1997), and also in two HA populations with different lineage living at similar elevations and in similar global regions (Zhuang, J. *et al.*, 1993). Although Lahiri *et al.* (1976) did a within population study in lowlanders, I am not aware of published studies comparing hypoxic sensitivity between two LA populations of distinctly different heritage, such as those living in coastal South Africa. Furthermore, I am not aware of any published inter-population differences in hypoxic sensitivity in either LA or HA populations in African (Niermayer *et al.*, 2001), although East Africans are recognised as one of the three main HA populations in the world (Hochacka *et al.*, 1999). This study serves to assess baseline ventilatory responses to hypoxia in a low-altitude African population, and additional comparison with low-altitude southern-African Caucasian peoples.

By using Brutsaert's (2001) optimal study design to assess genetic influence of adaptation to hypoxia, a within-population comparison of East Africans living at high and low altitude would enhance our understanding of the effects of genes and environment on the hypoxic ventilatory response. However, the present exploratory study seeks to establish baseline values for ventilatory sensitivity to hypoxia in African populations living only at sea level, since a field study was beyond the scope of the assignment. This

study selected two low altitude populations both residing in the same town. The two populations last shared an ancestor approximately 100 000 years ago (Cavalli-Sforza *et al.*, 1994). Motivation for the selection of the Xhosa population was the fact that this study site is one of very few LA locations where these two populations reside in the same town. This is the first study of hypoxic sensitivity among Africans. Furthermore, the study assessed feasibility of working in the field of ventilatory chemosensitivity in Africa.

The hypoxic ventilatory response (HVR) is a widely accepted measure of hypoxic sensitivity for inter-population comparisons (Beall, Strohl, *et al.*, 1997; Hochachka & Monge, 2000). Accumulating evidence of the high variability in HVR with repeated testing (Sahn & Zwillich, 1977; Zhang & Robbins, 2000; Fahlman *et al.*, submitted) should influence the design of testing protocols. Such variability does not reduce the usefulness of HVR as a research tool, but without repeated measurements the accuracy of the deductions that can be made from the acquired data is severely limited, and perhaps even false. Studies using single measures with fewer than 6 subjects to support conclusions about ventilatory responses are not uncommon (e.g. Insalaco *et al.*, 1996). Existing knowledge of the high intra-individual variability in the HVR mandates further testing of theories based on such limited data. High variability in HVR within populations (Beall *et al.* 1997; Hochachka *et al.,* 1998; Hochachka *et al.*, 1999; Hochachka & Monge 2000) does not preclude comparisons of HVR between populations, but necessitates large sample sizes.

By measuring HVR, I aimed to determine non-invasively whether two sedentary LA South African populations, both residing at similar altitudes and living under similar environmental conditions, differ in their hypoxic sensitivity. In view of the variability in HVR (Chapter 2), my second aim was to calculate HVR values more representative of each individual by repeated testing and appropriate subsequent treatment of the raw data. These values will be used in the inter-population comparisons that follow.

Methods

Subjects

20 South African families (10 Caucasian, C, and 10 Xhosa, X, total individuals n = 63) who participated voluntarily in the study.

Family Criteria

The families invited to participate in the study had to comply with the following selection criteria.

a) The progeny were offspring of the parent(s) tested.
b) A minimum of two progeny and one parent were tested.
c) The youngest of the progeny was no younger than 15 years of age.
d) The oldest parent was less than 70 years of age.
e) All individuals had non-athletic lifestyles and never participated in national or international sports.
f) All families lived at sea-level in the same town.
g) For Xhosa speaking families, the family for at least two previous generations was of only Xhosa-speaking origin, i.e. they had not interbred with any people from another native South African tribe.

Where necessary, language differences between the investigators and subjects, were overcome using translators. All experimental procedures were fully explained, verbally and in written form, before each subject signed a consent form. Under-age subjects signed a consent form in the presence of their parent. Participants understood that they

were free to withdraw from the study at any time. Ethical approval for all procedures was granted by the Subcommittee C of the Research Committee of the University of Stellenbosch, which conforms to the internationally accepted ethical guidelines detailed in the Declaration of Helsinki.

Questionnaires

Each subject completed a questionnaire encompassing the following characteristics. Subjects were identified as either smokers (S) or non-smokers (N/S) (including ex-smokers). Subjects were classified as having an altitude history (AH; having been born at an altitude of greater than 1000 m above sea level, either with a history of acute hypoxia such as prior involvement in similar tests or frequent high altitude exposures, as may be seen in mountaineers or pilots) or not (N/AH; born at LA, and with no previous altitude exposures). All subjects had lived in Stellenbosch for more than 11 years. Subjects were further identified as having respiratory (e.g. asthma) or haematological (such as anaemia) disorders whether treated or untreated) (D), or having no disorders (N/D). Subjects with chronic respiratory or haematological disorders were excluded. Female subjects were asked when they had last menstruated enabling me to determine whether they were in the follicular (F) or luteal (L) phases. Subjects who were not menstruating (NM) were either post-menopausal, breastfeeding or using injectable contraceptives (e.g. Depo-provera) that prevent menstrual cycles.

Isocapnic breathing circuit

During exposure to hypoxia or air (normoxia), isocapnia at normocapnic partial pressures was maintained during hyperventilation using the non-rebreathing method described in Chapter 2 (see also Fahlman *et al.*, 2002). Additional modifications to the system were completed prior to this study, namely a) the reduction of the dead space to approximately 220 ml, (half the dead space in the original circuit described by Fahlman *et al.*, 2002), and b) the relocation of the demand valve to nearer to the mouthpiece, permitting more rapid changes in the inspired flow of oxygen (FiO_2) and the subsequent SaO_2 response curve, which proved successful (Appendix 1).

Protocol

Before the study, the subjects each completed one or two preliminary experiments involving normoxic and hypoxic exposures identical to the actual experiments for familiarisation with the breathing circuit and the study protocol. The number of preliminary experiments was determined by each subject's comfort and ability to relax, demonstrated by stable and consistent resting values for minute ventilation ($\dot{V}_E$, L • min^{-1}) of at least 5 min, or longer when necessary. Data from preliminary experiments were used to calculate coefficients of variation for the HVR and establish levels of end-tidal CO_2 partial pressure (PET_{CO_2}), otherwise data were discarded. Experiments were conducted on each subject a minimum of three and a maximum of five times including familiarisation tests, separated by a minimum of 60 min. Subjects were asked to refrain from drinking alcohol and caffeine-containing beverages from the evening before the experiments. Subjects were reassured that they could remove the mask and discontinue

the test if they became uncomfortable, in which case the experiment would be re-started. Restarting of the test was not required more than once per individual, and on not more than three occasions in each population group and when this occurred, the subject was allowed 10 – 15 minutes to recover. During each test the HVR ($L \cdot min^{-1} \cdot \%^{-1}$) was measured using the square wave protocol described by Fahlman *et al.*, (2002) with a specific modification (see later).

Each subject was seated in front of the apparatus with his or her face level with the directional valve (see Chapter 2, Fig 2.1), and allowed to rest for 10-15 min, during attachment of oximeter probe (Nellcor RS10, Mallinkrodt, Inc., St Louis, MO, USA) and headphones. Subjects read and/or listened to music on a personal stereo. The subject was then fitted with a facemask (8930 Series, 47.2 mL dead space, Hans Rudolph Inc., Kansas City, MO, USA) and the seal of the mask was checked as described in Chapter 2, (page 47). If the facemask did not seal properly, the subject breathed through a mouthpiece attached to the same circuit. Expired volume ($\dot{V}_E$, $L \cdot min^{-1}$, STPD), tidal volume, (V_T, L, STPD), and f_R (breaths $\cdot$ min^{-1}) was sampled by a metabolic system (MetaMax™, Cortex Biophysik GmbH, Leipzig, Germany) and average values recorded every 10 sec. The end-tidal CO_2 partial pressure ($P_{ET}CO_2$ at body temperature, pressure and saturation, BTPS) was sampled by a capnograph (Microstream™, Microcap, Oridion Medical Ltd, Jerusalem, Israel) and average values recorded every 5 sec. All values are given in STPD unless otherwise stated.

For the first five minutes subjects breathed normoxic air (21 % O_2, balance N_2). Both subject's resting $\dot{V}_E$ and PET_{CO_2} were averaged during the last two minutes of this period. If these two variables were not stable, the initial period was extended until stable values were attained. Inspired gas was then switched instantaneously to 8.2 % (± 0.3 %, n = 3 bottles of compressed gas) followed after 120 sec by 21 % O_2 (± 0.2 %, n = 7 bottles of compressed gas). The full period of this "square wave" was therefore 240 sec (Chapter 2; Fahlman *et al.*, 2002). Although Fahlman *et al.* (2002) used four such waves, only one for this study was used.

I modified the experimental protocol of Chapter 2 (see also Fahlman *et al.*, 2002) by reducing the number of hypoxic exposures from four to one, because it was found that a non-significant decline occurred in the HVR over the period of four square waves, indicating mild hypoxic ventilatory decline (HVD; Chapter 2; Fahlman *et al.*, 2002). Use of only one hypoxic exposure per test eliminated the possibility of acute HVD. The total experimental time was thus nine minutes and comprised the following: an initial resting phase (N_1) lasting 5 minutes or more until stable resting values were reached; followed by two of hypoxia (H), then two minutes of normoxia (N_2), during which the subject was simply monitored to ensure full recovery of all the ventilatory parameters and SaO_2 to resting levels. Tests were performed a minimum of 60 min apart.

Each subject's PET_{CO_2} was maintained at his or her normocapnic levels (± 1 mmHg), ascertained during the final two minutes of N_1. Subjects were separated from the switch controls by a screen, and were therefore blind to gas switching. Care was taken to ensure

similar levels of gas pressure in all cylinders to reduce any obvious noises accompanying the switch between gas cylinders.

Arterial O_2 Saturation (SaO_2, %)

SaO_2 was measured using a pulse oximeter (Nellcor N-395 Pulse Oximeter, Mallinkrodt, Inc., St Louis, MO, USA) with a forehead sensor (Nellcor RS10, Mallinkrodt, Inc., St Louis, MO, USA). The area of application of the sensor was massaged with a mild capsaicin ointment (0.25 g per 100 g, Sloan Heat Rub, Warner-Lambert, South Africa) approximately two minutes before attachment to promote surface blood flow. Analogue signals from the oximeter were relayed to the metabolic system, which recorded SaO_2 every 10 seconds.

Data Processing

Data from the start of the experiment up to the last two minutes of the initial resting period (N_1) were discarded. Resting values of $\dot{V}_E$, SaO_2, V_T and f_R for each subject were calculated as means for the final 120 seconds of N_1 (number of data points, $n = 22 \pm 1$), except in the case of $P_{ET}CO_2$ where the last 60 seconds were used (number of data points, $n = 20 \pm 1$). For all variables during the hypoxic exposure, a 30 second period was used (H; number of data points, $n = 7 \pm 2$).

Calculations

The average of the last 120 seconds of the N_1 period was used in the calculation of the resting normoxic $\dot{V}_E$ for determination of the HVR♦. Normoxic variables were calculated as the last 120 sec for N_1. Hypoxic variables were calculated during the lowest 30 seconds of SaO_2. This was always the last 30 second period before SaO_2 started to rise. The averages of two test values (calculated immediately, but excluding the familiarisation tests) were used, unless the coefficient of variation (CV) of the calculated HVR values between the two experiments was greater than 26% (Zhang & Robbins, 2000), in which case a third experiment was performed and the median of the three test values was used to calculate population or group means for all variables.

Statistical Analysis

All data are reported as means ± 1 standard deviation (SD), unless otherwise specified. Initially, paired comparisons between N_1 and H were made to test for the effects of hypoxia and isocapnia in all subjects. Thereafter, inter-population comparisons were done using chi-square tests for categorical data, or two-tailed Student's t-tests, or when appropriate, Mann-Whitney tests to determine differences in mean values between tests or groupings (Zar, 1996).

Multiple regressions were performed to isolate suitable covariates for analyses of covariance. Gender, and weight and height (combined as BMI) were thus identified as

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♦ Mean values for $\dot{V}_E$ over the last 120 seconds of the N_1 period and for the last 30 seconds of the same period were compared using a paired Student's t-test. In some individuals 120 sec and 30 sec averages differed significantly. To obtain values that were as representative as possible, I chose the longer time period.

covariates for respiratory variables. In all cases of repeated data sampling, repeated measures analysis of variance or covariance was used when justifiable. For each subject, averages (n = 2) or medians of data (n = 3) were used to assess differences between and within the groups (Winer *et al.*, 1991). The HVR and primary components of the HVR, namely $\dot{V}_E$ and SaO_2 (both for N_1 and H) were analysed by means of ANCOVA. In these cases, BMI, but not gender, proved to be a statistically justifiable covariate for inter-population ANCOVA comparisons of the HVR and $\dot{V}_E$, as required by the analyses, but, these factors did not prove to be significant covariates in either hypoxic or normoxic periods for comparisons of SaO_2. For SaO_2 the only justifiable covariant was PET_{CO_2}, and this was duly used for ANCOVA's. PET_{CO_2} was also justifiable as a covariate in ANCOVA comparisons of inter-population HVR.

Further inter-population comparisons were performed on the components of $\dot{V}_E$, namely, V_T and f_R, for both the H and N_1 periods. BMI and gender were significantly justifiable covariants for ANCOVA comparisons of V_T during H and N_1. Neither BMI nor gender was justifiable as a covariant for ANCOVA comparisons of f_R and therefore standard Student's t-tests and ANOVA's were used for the inter-population comparisons of this parameter during H and N_1.

Analyses were performed using NCSS 2000 statistical package (NCSS statistical software, Kaysville, Utah). Acceptance of significance was set to the $P < 0.05$ level, unless otherwise stated. Whole body variables were not divided by body mass or by

metabolic rate, rather I used ANCOVA with BMI and or gender as covariates (Hayes, 2001), as stated previously.

Results

Across all subjects, V_T, f_R, $\dot{V}_E$, and SaO_2 differed significantly between normoxic and hypoxic intervals, but PET_{CO_2} did not differ between N_1, H and N_2 (31.5 ± 0.16, 31.1 ± 0.16 and 31.7 ± 0.16 mmHg, respectively; ANOVA, $P > 0.18$).

1. Inter-Population Comparisons:

Subject characteristics:

Of the 63 subject's significantly more Caucasians smoked than Xhosas (Table 3.1). Although all subjects had resided at sea-level for many years, a significantly larger proportion of the Caucasian group had been born at moderate altitude (from 1000 m to 1600 m). Of the 11 % with diseases reported in Table 3.1, 65 % were asthmatics, using occasional self-medication when required, and had not recently suffered any episodes. The others who fell into this category had disorders such as elevated blood pressure, and all cases were self-medicated.

Table 3.1. Caucasian and Xhosa subjects who were smokers, had historically lived at high altitude (<1000m), or had respiratory or haematological disorders.

Group	Smokers (%)	Altitude History (%)	Diseases (%)
C (n = 33)	61	51	9
X (n = 30)	13*	10*	13

Chi-square test (* $P < 0.05$).

C were significantly taller than X ($P < 0.01$), and BMI's were significantly lower ($P < 0.01$) among C (Table 3.2). All other parameters in Table 3.2 were not different between the two populations. Since height and BMI were different between the two groups, and there were differences between genders within the two groups, these factors were tested for their influence on the respiratory variables using correlations and comparisons of P-values.

Table 3.2. Characteristics of Xhosa (X) and Caucasian (C) subjects.

Group	Age (years)	Height (m)	Weight (kg)	BMI ($kg.m^{-2}$)
C (all) (n = 33)	32.9 ± 15.4	1.79 ± 0.09	73.5 ± 10.9	23.0 ± 2.44
X (all) (n = 30)	27.4 ± 12.3	1.59 ± 0.07†	71.5 ± 16.8	28.6 ± 8.30*
C (♂) (n = 23)	30.5 ± 15.2	1.83 ± 0.06	64.8 ± 4.90	22.7 ±1.83
X (♂) (n = 5)	22.0 ± 5.66	1.69 ± 0.03†	60.5 ± 8.51	21.3 ± 3.51
C (♀) (n = 10)	38.5 ± 15.4	1.69 ± 0.02	77.3 ±10.6	23.1 ± 2.70
X (♀) (n = 25)	28.4 ± 13.1	1.57 ± 0.01†	73.7 ± 17.3	30.1 ± 8.23*

Student's t-test (unpaired, two-tailed) or Mann-Whitney comparison between Xhosa and Caucasian subjects. (†: $P < 0.01$). *: $P < 0.05$).

Respiratory variables:

Equal variance and F-tests confirmed normal distributions of $\dot{V}_E$ values ($P > 0.1$) in normoxia and hypoxia, and for $P_{ET}CO_2$ and $\dot{V}O_2$ ($P > 0.3$) during the N_1 period for both groups.

For the following comparisons of HVR, the mean of two tests was used when the CV was less than or equal to 26 %, and the median of 3 tests was used when the HVR values CV was greater than 26 %. Each subject is therefore represented by one value, ($n = 30$ for X, and $n = 33$ for C). Misrepresentation of a subject's HVR caused by one extreme value is minimized with this system. The mean intra-individual CV for HVR among the Xhosas (173 ± 389 %) was significantly greater than that for the Caucasians (30 ± 117 %; Mann-Whitney, $P < 0.01$).

Beta-coefficients and their P-values for all ANCOVA's exploring the effects of gender and race on respiratory variables are given in Table 3.5.

HVR Differences:

HVR's did not differ between the Xhosa and Caucasian populations (ANOVA, $P > 0.35$; t-test, $P > 0.16$; Table 3.3). ANCOVA with normoxic $P_{ET}CO_2$ as a covariate, found no significant difference between X and C ($P > 0.43$). An *a priori* power analysis, using the obtained difference (0.42 *vs.* 0.34, an $\alpha = 0.05$, and a $\beta = 0.7$) showed that a sample size of greater than 260 would be necessary to detect any significant difference in HVR between X and C. Regression analysis indicated that the BMI accounted for more

variability than did age, weight or height successively, and additional consequent multiple regression analyses of HVR between groups using BMI and gender as co-factors found no difference ($P > 0.34$, $F = 1.04$, $df = 59$).

Table 3.3. Means of N_1 $\dot{V}O_2$, N_1 PET_{CO_2} and HVR in Xhosa (X) and Caucasian (C) subjects.

Group	HVR $^{\Psi}$ ($L \cdot min^{-1} \cdot \%^{-1}$)	$\dot{V}O_2$ § ($L \cdot min^{-1}$)	PET_{CO_2} § (mmHg)
C (all) (n = 33)	0.42 ± 0.33	0.37 ± 0.007	32.0 ± 3.6
X (all) (n = 30)	0.34 ± 0.36	0.36 ± 0.007	31.5 ± 4.2

§ Unpaired two-tailed Student's t-test (NS: Not significantly different, $P > 0.5$).

$^{\Psi}$ ANCOVA (Not significantly different, $P > 0.4$)

Corrected HVR

The large intra-individual variation in HVR prompted me to calculate a "corrected HVR", using $\% \Delta \dot{V}E$ instead of Δ in absolute $\dot{V}E$ ($L \cdot min^{-1}$), divided by ΔSaO_2 (%). Corrected HVR did not differ between the two study populations (two-tailed unpaired t-test, $P > 0.09$), although in this case the Xhosas revealed a trend towards lower corrected HVR values than the Caucasians.

HVR Components:

Table 3.4. Normoxic (N_1) and Hypoxic (H) values for $\dot{V}_E$, VT, f_R, and SaO_2 in all subjects for Caucasians (C) and Xhosa (X).

Group	$\dot{V}_E$ (L•min^{-1})	VT (L)	f_R (breaths•min^{-1})	SaO_2 (%)
N_1 C (n = 33)	8.52 ± 2.38	0.66 ± 0.21	13.8 ± 3.8	99.3 ± 1.6
N_1 X (n = 30)	9.41 ± 3.59	0.50 ± 0.19*	19.6 ± 5.3*	99.6 ± 0.9
H C (n = 33)	15.08 ± 4.29	1.11 ± 0.32	14.3 ± 4.2	81.7 ± 4.7
H X (n = 30)	16.18 ± 4.57	0.75 ± 0.20 †	22.2 ± 5.7 †	78.4 ± 4.7*

* Statistically different ($P < 0.05$). † Statistically different ($P < 0.01$).

Table 3.5. Beta-coefficients and their P-values for all ANCOVA's exploring the effects of gender and race on respiratory variables.

		Dependant Variable			
		Gender		Race	
Independent Variable	**Significant Covariate**	**β-co-efficient**	**P-Value**	**β-co-efficient**	**P-Value**
$\dot{V}_E$ (Hyp)	BMI	0.896	0.496	0.215	0.872
VT (N_1)	BMI; Gender	0.114	0.056	-0.153	0.012
VT (H)	BMI; Gender	0.218	0.006	-0.338	0.000
HVR	PET_{CO_2}	-0.003	0.817	0.109	0.311
SaO_2 (H)	PET_{CO_2}	1.794	0.204	-3.04	0.036

$\dot{V}_E$

$\dot{V}_E$ was similar in both populations in normoxia (N_1) (t-test, $P > 0.20$), and in hypoxia (H) (X: 16.2 ± 4.6 L • min^{-1}; C: 15.1 ± 4.3 L • min^{-1}; t-test, $P > 0.16$; ANCOVA, $P > 0.80$, $df = 59$; see Fig 3.1) and ANCOVA with BMI as covariate revealed no differences ($P > 0.70$, $df = 59$). In both of these cases gender was not a significant covariate, but gender differences in $\dot{V}_E$ (see below) prompted me to perform multiple regression analysis using gender as a co-factor, which also detected no differences between populations ($P > 0.4$). In both groups hypoxic exposure elicited an increase in $\dot{V}_E$ (Fig 3.1).

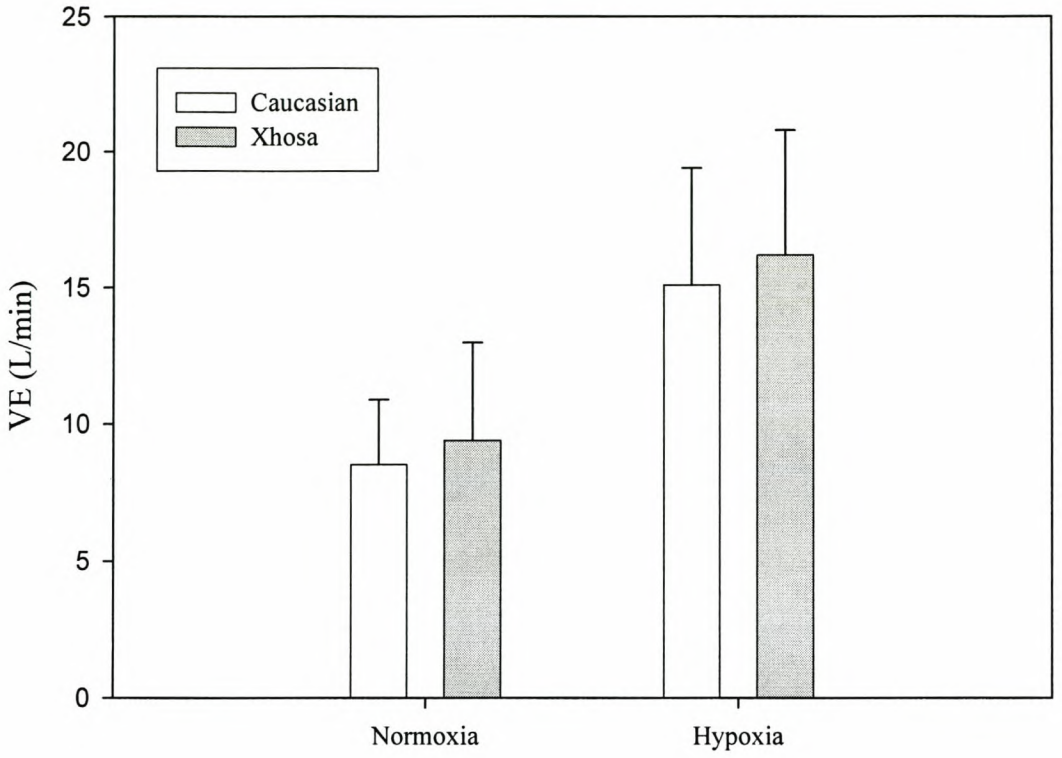

Figure 3.1. Mean $\dot{V}_E$ (L • min^{-1}) in hypoxia and normoxia (ANCOVA, P > 0.7) comparing groups.

f_R

Neither BMI nor gender proved justifiable as covariates for ANCOVA of f_R. Xhosas displayed significantly higher f_R for N_1 (19.6 ± 5.3 breaths • min^{-1}; t-test, P < 0.01; ANOVA, P < 0.01), and H periods (22.2 ± 5.7 breaths • min^{-1}; t-test, P < 0.01; ANOVA, P < 0.01) than did Caucasians (N_1: 13.8 ± 3.8 breaths • min^{-1}; H: 14.3 ± 4.2 breaths • min^{-1}; see Fig 3.2).

VT

ANCOVA with BMI and gender as covariates revealed significantly larger VT values in Caucasians than Xhosas for N_1 (X: 0.499 ± 0.185 L; C: 0.661 ± 0.210 L; $P < 0.01$, $F = 6.66$, $df = 59$) and H (X: 0.750 ± 0.19 L; C: 1.110 ± 0.32 L; $P < 0.01$, $F = 19.44$, $df = 59$) (Fig 3.3).

When BMI was used as a covariate, there was a significant interaction between gender and race (F-Ratio 4.86; $P < 0.05$). Caucasian Males exposed significantly larger VT than Caucasian Females, Xhosa Males, and Xhosa Females.

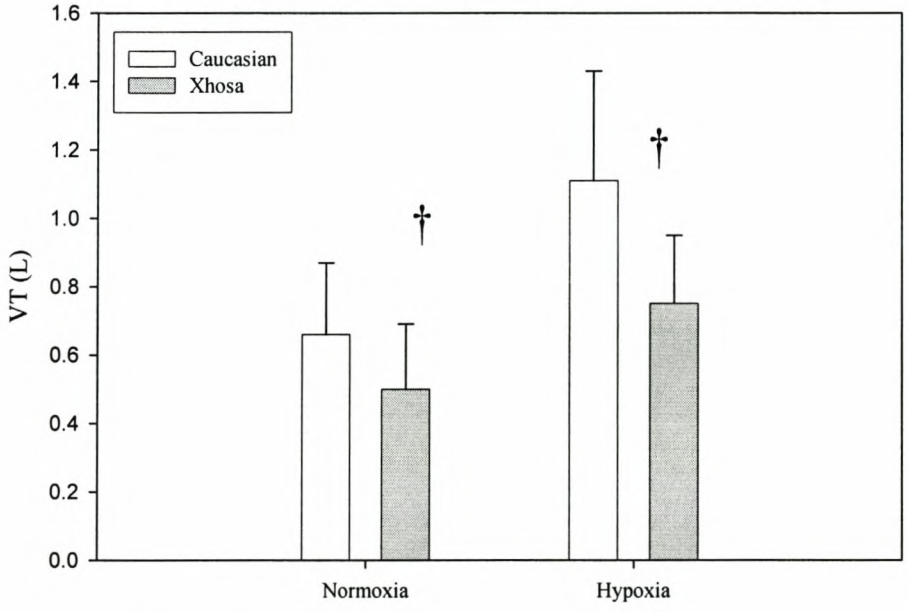

Figure 3.2. Mean fR (breaths • min^{-1}) in H and N_1 for Caucasian and Xhosa groups.
†: $P < 0.01$; two-tailed t-tests and ANOVA.

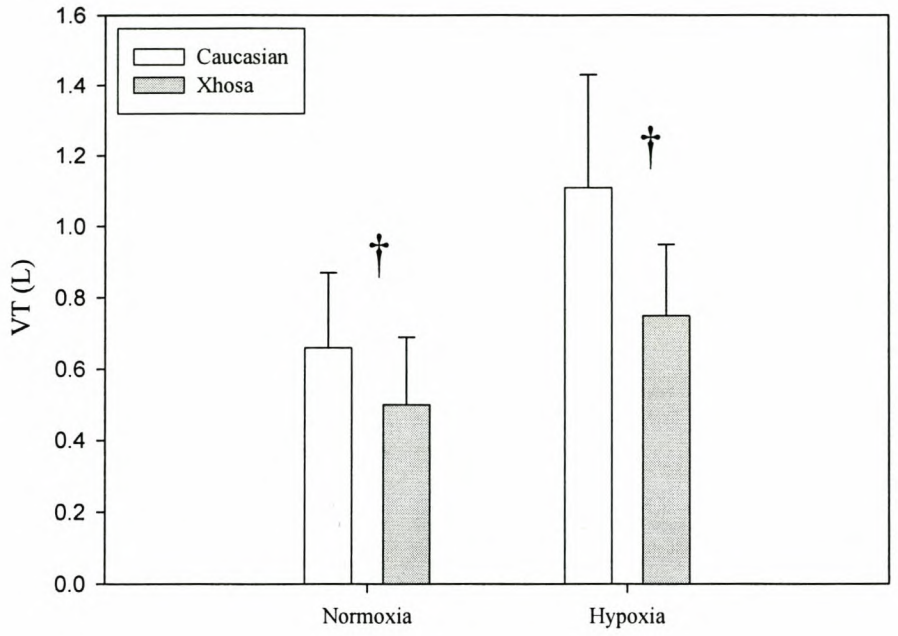

Figure 3.3. Mean VT (L) in normoxia and hypoxia for Caucasian and Xhosa groups.
†: ANCOVA, $P < 0.01$.

SaO_2

Baseline N_1 SaO_2 did not differ between populations (X: 98 ± 1 %; C: 97 ± 2 %; $P > 0.41$), but hypoxic SaO_2 was significantly lower among Xhosas (X: 77 ± 5 %; C: 80 ± 5 %), using either ANCOVA with $P_{ET}CO_2$ as the covariate, ($P < 0.05$, $F = 4.60$, $df = 59$) (Fig 3.4); or an unpaired, two-tailed t-test, ($P < 0.01$). After two minutes of normoxia, the SaO_2 of both populations returned to similar baseline levels of SaO_2 (X: 99 ± 2 %; C: 98 ± 4 %, $P > 0.21$).

Alveolar Ventilation Rates

Using Eq. 2.3 (page 46) alveolar ventilation rates ($\dot{V}_A$; L/min) was determined and compared between X and C. Similar levels of mean $\dot{V}_A$ were obtained for both groups (C: 10.6 ± 3.0 L/min; X: 11.3 ± 3.2 L/min; two-tailed T-test; $P > 0.33$).

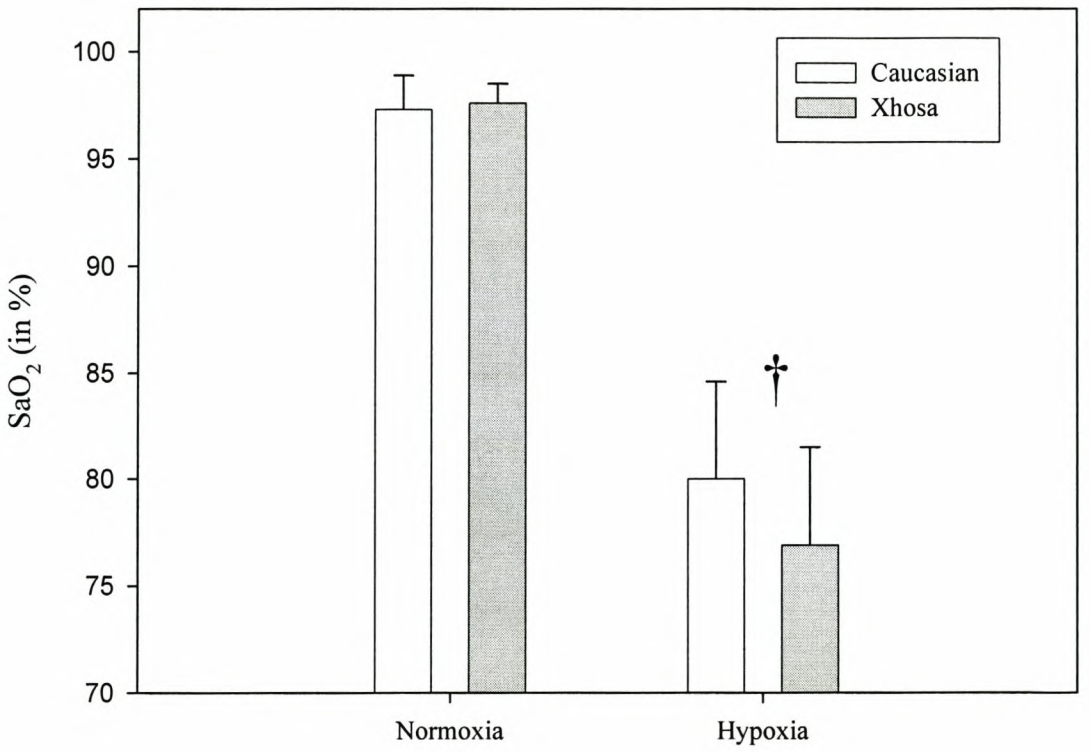

Figure 3.4. Mean SaO_2 (%) in normoxia and hypoxia for Caucasian and Xhosa groups.

†: ANCOVA, $P < 0.05$; unpaired, two-tailed t-test, $P < 0.01$).

2. Inter-Population Gender Comparisons:

Anthropometric data:

Caucasian males (n = 23) were significantly taller than Xhosa males (n = 5) ($P < 0.01$) (Table 3.3 for this and all anthropometrical comparisons), and Caucasian females (n = 10) were significantly taller than Xhosa females (n = 25), ($P < 0.01$). In contrast to the male subjects, where Caucasians were significantly heavier than Xhosas ($P < 0.01$), Caucasian females BMI's were significantly lower than Xhosa females ($P < 0.01$).

HVR Differences:

Caucasian males displayed HVR's of significantly greater magnitude (i.e. far more hypoxic sensitivity) than did Xhosa males (C: -0.46 ± 0.35; X: -0.13 ± 0.10; ANCOVA, $P < 0.05$) in contrast to the lack of difference in HVR between groups when genders are pooled. There were no differences in HVR among females between different phases of their menstrual cycle.

HVR Components:

For brevity, only significant differences will be reported in this section.

Caucasian females had significantly lower $\dot{V}_E$ than Xhosa females during N_1 ($P < 0.05$), and H ($P < 0.01$). Caucasian males had significantly larger $\dot{V}_E$ in H than Xhosa males ($P < 0.05$). Caucasian females had significantly lower f_R than Xhosa females in the N_1 ($P < 0.01$), H ($P < 0.01$), and N_2 period ($P < 0.01$). Caucasian males had significantly lower f_R values in H than Xhosa males ($P < 0.05$). Caucasian males' V_T's were significantly

larger than Xhosa males in H ($P < 0.01$). Caucasian males SaO_2 was significantly greater than Xhosa males in H ($P < 0.01$). There were no significant differences in PET_{CO_2} for any of the above comparisons. A robust regression weighted for number of tests showed a significant correlation between normoxic PET_{CO_2} and HVR ($r^2 = 0.44$, df = 60, $P < 0.001$) for all subjects (Fig. 3.5).

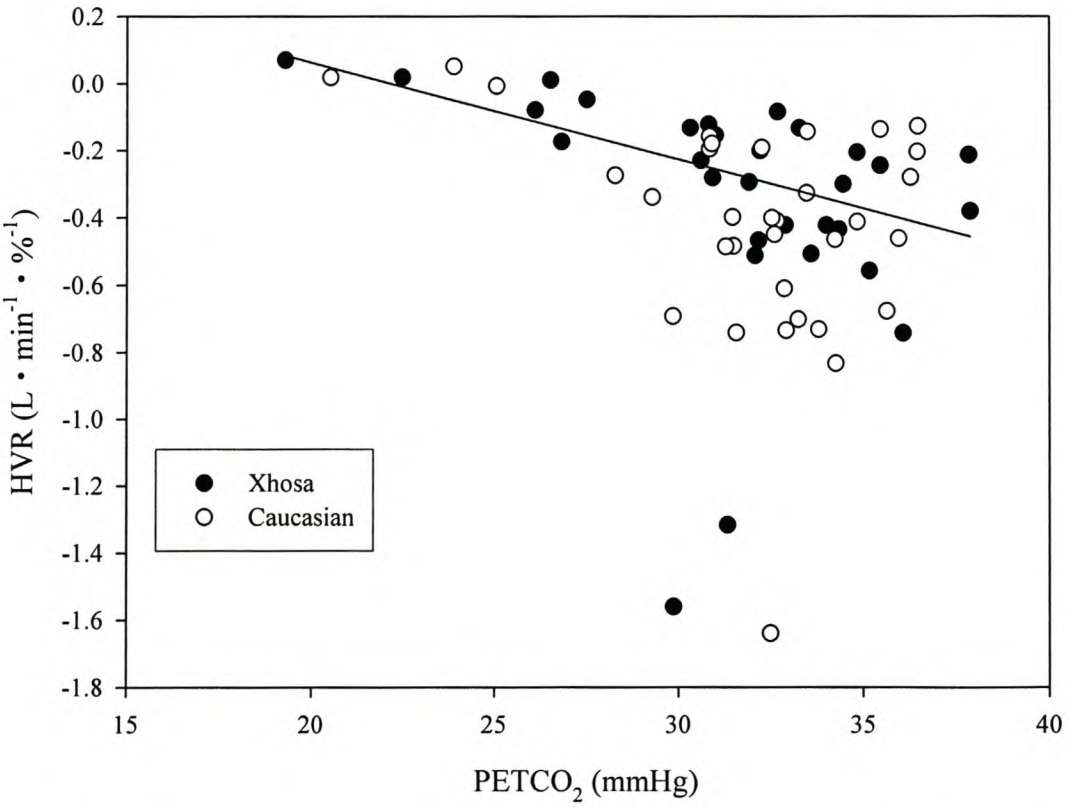

Figure 3.5. PET_{CO_2} (mmHg) and HVR ($L \cdot min^{-1} \cdot \%^{-1}$) are significantly correlated for all subjects (robust regression, $r^2 = 0.44$, $P < 0.001$). Recall that, because HVR carries a negative sign, the negative correlation above means that individuals with greater hypoxic sensitivity have higher resting PET_{CO_2}.

Discussion

The main findings from this study are that *a)* hypoxic sensitivity (HVR) does not differ between the two populations, and *b)* ventilatory components (V_T and f_R) differ between Caucasian and Xhosa subjects during both normoxia and hypoxia. This suggests two distinct patterns of breathing: Xhosas demonstrated shallower, more rapid breathing, and Caucasians exhibited deeper, slower breathing. Despite these differences, we estimated that alveolar ventilation was similar in the two groups. This being so, the lower arterial oxygen saturation levels during hypoxia among the Xhosa subjects suggest less effective oxygenating of the blood, which will be more fully discussed below.

$P_{ET}CO_2$ and SaO_2 results indicate that the breathing circuit successfully induced hypoxia while maintaining isocapnia in all subjects as was previously shown for the same circuit (Fahlman *et al.*, 2002) prior to the reduction of dead space that I introduced.

It is clear from the CV for the HVR that the intra-individual variation within each population studied is as large as that reported in the literature (Beall *et al.*, 1997), supporting the use of repeated measures in future studies as recommended in Chapter 2. While Xhosa subjects exhibited significantly larger intra-individual CV for their HVR than did Caucasians, and it is possible that additional testing may reduce the amount of variability significantly, one should remain cognisant of the fact that under the same experimental conditions these two populations exhibited vastly different levels of variability, which warrant study in future. As this is not the key focus of the study I will

not pursue these findings in depth, and without further investigation it is difficult to comment extensively on this point. However, the likelihood that these differences between the two populations occur as a direct result of innate intra-individual variability does exist.

A priori analysis of power and repeated measures

I obtained the desired repeated measures of the HVR by continuous assessment of the CV between tests within an individual. This reduced the possibility of false conclusions regarding differences in hypoxia tolerance between the two populations. The *a priori* power analyses for the HVR data suggest that this measure of hypoxic sensitivity may require so many subjects that its effectiveness as a research tool is greatly reduced, and it may be necessary to consider alternative methods of assessing hypoxic sensitivity in future. Potential problems arise with ANCOVA if invalid covariates are selected or because of error in the measurement of covariates. In order to assess this possibility analyses of variables were performed on data with gender pooled and separately.

Corrected HVR calculated using the percentage change in $\dot{V}_E$ rather than the absolute change may be useful in correcting for differences in subjects' body sizes but it should be used cautiously as body size is not the only factor affecting HVR. For example, it does not resolve inter-individual $P_{ET}CO_2$ differences. This method of calculating HVR, *if* used in conjunction with statistical analyses such as ANCOVA that take variation in $P_{ET}CO_2$ into account, may prove to be a useful comparative tool.

Baseline comparisons

Comparisons between studies are difficult because HVR is highly variable. Inter-population differences in mean height and BMI underline the need for correction for body size using ANCOVA. Methodologies and numerical conventions differ e.g. Smith *et al.* (2001) standardised $\dot{V}_E$ for height by use of the equation $\Delta \dot{V}_E$ x (mean height)^2 x (subject height)$^{-2}$ (as originally proposed by Burr *et al.* (1985)) while Townsend *et al.* (2002) use no correction of $\dot{V}_E$. Therefore, I propose that general conventions should be developed and utilised to facilitate comparisons between published studies in this field. In light of the current information, new attempts should be made to group the existing population information in a coherent and comparable manner. I recommend that the design of protocols become standardised, while repeatability and methodological issues (such as fitness levels, gender and statistical analyses) are taken into consideration.

The similarity in baseline normoxic (N_1) $\dot{V}O_2$ levels across the two study populations implies similarity in resting metabolic rate. Normality of data distribution and similar normoxic $\dot{V}E$ and PET_{CO_2} means, and standard deviations were confirmed in subjects in both groups prior to hypoxic exposure. Similar PET_{CO_2} in both populations further indicates similar stimuli for ventilatory drive under sea level conditions. Conversations with subjects, and their behaviour before the tests, indicated that subjects in both populations were comfortable with the test environment. Both groups were sampled randomly from within their communities and neither group contained any individuals who had participated previously in tests of ventilatory sensitivity. Furthermore, while the Xhosa population may be of a different social status to the Caucasian, and not living

equally distributed throughout the town, there is daily interaction between the two groups in work environments.

Inter-population HVR comparisons

The two populations presented similar levels of hypoxic sensitivity as expressed by the HVR even after covarying for body size. Our data for these two groups provide us with a comparative means for assessing baseline hypoxic sensitivity in LA African populations without the confounding effect of HA environments. Cross-sectional studies in endurance athletes have demonstrated a diminished HVR compared with mountaineers or sedentary controls (Schoene, 1982; Masuyama *et al.*, 1986). Conversely, short-term altitude acclimatization may increase the HVR (Levine *et al.,* 1992). Natives to HA have a blunted HVR (Severinghaus *et al.*, 1966; Milledge & Lahiri 1967; Zhuang *et al.*, 1993; Hochachka & Monge, 2000). Beall *et al.* (1997) showed a difference of approximately 50 % in HVR between two HA populations from different global regions. Understanding the ~25 % lower HVR, albeit non-significant, in Xhosas compared to the Caucasian subjects is complicated. It is difficult to assess subjects' previous residence at high altitude, which may cause a blunting in HVR (Leon-Velarde *et al.,* 1996) Intermittent hypoxia may be a more potent stimulus for adaptation than is continuous hypoxia (Prabhakar, 2001), along with the developmental plasticity effects which confound the study of chemosensitivity (Gozal & Gozal, 2001; Mitchell *et al.*, 2001; Okubo & Mortola, 1990), these results remain difficult to interpret thoroughly.

The complete absence of published information on hypoxic tolerance in African populations limits my ability to compare my data and suggests that more work on this continent is required to test the generality of the hypothesis that long-term HA residents develop blunted HVR (Hochachka & Monge, 2000). The only published data of ventilatory sensitivity in African populations of which I am aware show that elderly Nigerians exhibit lower hypercapnic ventilatory responses compared to young Nigerians (Elegbeleye & Femi-Pearse, 1980), but these data are not directly comparable to those presented for the Xhosas. Forced vital capacity among Ethiopians has been measured (Harrison *et al.,* 1969), although this does not directly relate to my study.

HVR Components

The genetic influence on the control of ventilation and its components has been a topic of much interest (Neubauer, 2001). In rats there may be a strong genetic component underlying levels of hypoxic sensitivity (Strohl *et al.*, 1997; Weil *et al.*, 1998). Tankersley *et al.* (1994; 1997; 2000) observed different breathing patterns in different mouse populations during normoxia and exposed to hypoxia and similar results were obtained in different rat strains (Strohl *et al.*, 1997). Tankersley's results (e.g. 2000) suggested that ventilatory control in mice may be controlled by as few as two major genes. My data show a similar dichotomy in breathing patterns between two human populations responding to hypoxia. Therefore, in both mice and humans, marked differences in the two respiratory components of $\dot{V}_E$, namely the f_R and V_T, are evident. Although similar normoxic and hypoxic $\dot{V}_E$ values are achieved in both groups, the more rapid, shallower breathing in the Xhosas and the deeper, slower breathing of the

Caucasians represent two distinct breathing patterns during hypoxia and in normoxia. These similarities suggest the possibility that in humans the control of the two major respiration components (breathing frequency and tidal volume) may lie in the same two major genes proposed by Tankersley for mice. Further study of the human counterparts of these genes should provide important information regarding a genetic component in ventilatory control and chemosensitivity.

The two populations in my study display different hypoxic SaO_2 levels, but comparable data are not available from Tankersley's studies. Interestingly, estimations of alveolar ventilation showed that effective ventilation is similar in both populations, and it is likely that the differences in breathing patterns do not attribute to the observed saturation differences. The significantly lower SaO_2 values that I report for Xhosa subjects, in conjunction with their smaller hypoxic V_T, suggest that the two patterns of respiration differ in their ability to oxygenate the blood effectively during hypoxia. I explored this theory further since it is logical, that shallow breathing is less effective in increasing alveolar ventilation than is deep breathing, which reduces the relative contribution of respiratory dead-space to total $\dot{V}_E$, thereby increasing the fraction of fresh air reaching the alveoli, and ensuring slightly higher levels of P_AO_2. However, upon calculation of alveolar ventilation by means of Eq. 2.3 (page 46) the results indicated that alveolar ventilation was not significantly different between the two groups. However, these results do not preclude the possibility that the Xhosa subjects may have a decreased efficiency of pulmonary gas exchange either by reduced ventilation-perfusion matching or by diffusion limitations of haematological or alveolar wall origin. Another possibility may be that the

Xhosa subjects' erythrocytes are lower in Hb-concentration, but since the study was meant to be non-invasive in nature, we did not determine haematological parameters and these speculations require further testing. It is unlikely that the lower SaO_2 values obtained were as a result of measurement error in Xhosa subjects, as Bothma *et al.* (1996) presented evidence that skin pigmentation does not inhibit pulse oximetry performance.

The breathing circuit that we developed uses non-invasive techniques to quantify the acute isocapnic HVR. Since the HVR may be subjected to higher brain inputs such as psychological factors (Kawakami *et al.*, 1982), the measurement of ventilation as a response to hypoxia may be confounded. Non-invasive techniques do not permit comparisons of carotid-body stimulation at the site of the hypoxic stimulus. Nonetheless, inter-population differences in hypoxia tolerance, manifested as SaO_2 differences, beg the following question: are ventilatory differences between the two groups of phenotypic or genotypic origin?

Confounding factors

Unbalanced gender ratios in the two study populations may have complicated our findings, because of the anthropometric differences associated with gender. ANCOVA took gender and BMI into account, resolving this problem in the inter-group comparisons. The sample of Xhosa males is too small to be considered representative of the entire population therefore conclusions regarding inter-population gender comparisons are highly arbitrary at this point in time. Additional subjects may not alter the results, but it is impossible to know this until tested. Although all subjects had

sedentary lifestyles, inter-population differences in habitual exercise levels resulting from socio-economic differences between the two populations may have affected my results. Although Caucasian subjects practised recreational exercise more frequently than did the Xhosas, the latter performed more lifestyle-related physical exertion (such as walking to work) than did Caucasians, thus compensating partially for this difference, although exercise intensity may have been less among Xhosa subjects. Subjects with a history of respiratory or haematological disorders made up similar proportions for both groups, while there were more smokers and previous HA exposures among the Caucasians.

Intermittent hypoxia may be a more potent stimulus for adaptation than is continuous hypoxia (Prabhakar, 2001), in particular developmental plasticity (e.g. changes in neurotrophic factor enhances glutamatergic synaptic currents in phrenic motoneurons, increasing their responsiveness to bulbospinal inspiratory inputs (Mitchell *et al.*, 2001)), and carotid body sensitivity from hypoxic exposures occurring during developmental years (e.g. Okubo & Mortola, 1990) would further complicate our findings.

Activities such as regular breath-holding may not result in a significant difference between controls and elite breath-hold divers suggesting that regular breath-holding does not alter HVR (Grassi *et al.,* 1994) it remains difficult to account for effects of developmental plasticity on chemosensitivity. Furthermore, the effects of intermittent hypoxia upon intra-individual variability of HVR have not been studied, and understanding of this topic is required for its consequential effect on statistical analyses of ventilatory parameters. The effects of long-term moderate intermittent hypoxia in

humans remain unqualified, and studies of hypoxic sensitivity should take potential confounding factors into account, such as breath-holding, regular flying in un-pressurized aircraft or mountaineering, and matching of subjects with respect to timing and duration of such exposures should at least be attempted.

Allometry could also influence ventilation (Packard & Boardman, 1987). Smaller people do not have relatively larger lungs (Schmidt-Nielsen, 1984), and so a theory that shorter people have a relatively larger hypoxic dose than larger people (e.g. Smith *et al.*, 2001) does not seem applicable to my data. However, this does not account for possible size-related differences in anatomical dead-space. I report no correlations between height and SaO_2, and so conclude that there is no evidence for a size-dependent hypoxic dose, although this conclusion may be premature due to the group size and possible confounding variables such as gender. However, V_T and SaO_2 were related in my subjects. Comparison of these values across the published literature and for larger sample sizes is likely to be instructive.

Conclusion

To summarise, South-African sea-level populations exhibit normal ventilatory responses to isocapnic hypoxia, and hypoxic sensitivity among Caucasian and Xhosa peoples is similar. The differences in ventilation patterns between the two groups suggest that further study of African populations' ventilatory control may be of value to our understanding of hypoxic ventilatory chemosensitivity. Care should be taken to ensure well-balanced gender ratios and anthropometrically similar subject groups. Furthermore,

the observation that there were no differences between estimations of the two populations' alveolar ventilation despite arterial oxygen saturation differences, provokes questions regarding the effective differences of ventilation:perfusion mismatching during hypoxia and possible haematological differences affecting hypoxia. Finally, this is the first study of hypoxic sensitivity in African peoples, and provides useful baseline information for low altitude African populations while once again emphasizing the need for more research on high altitude African populations.

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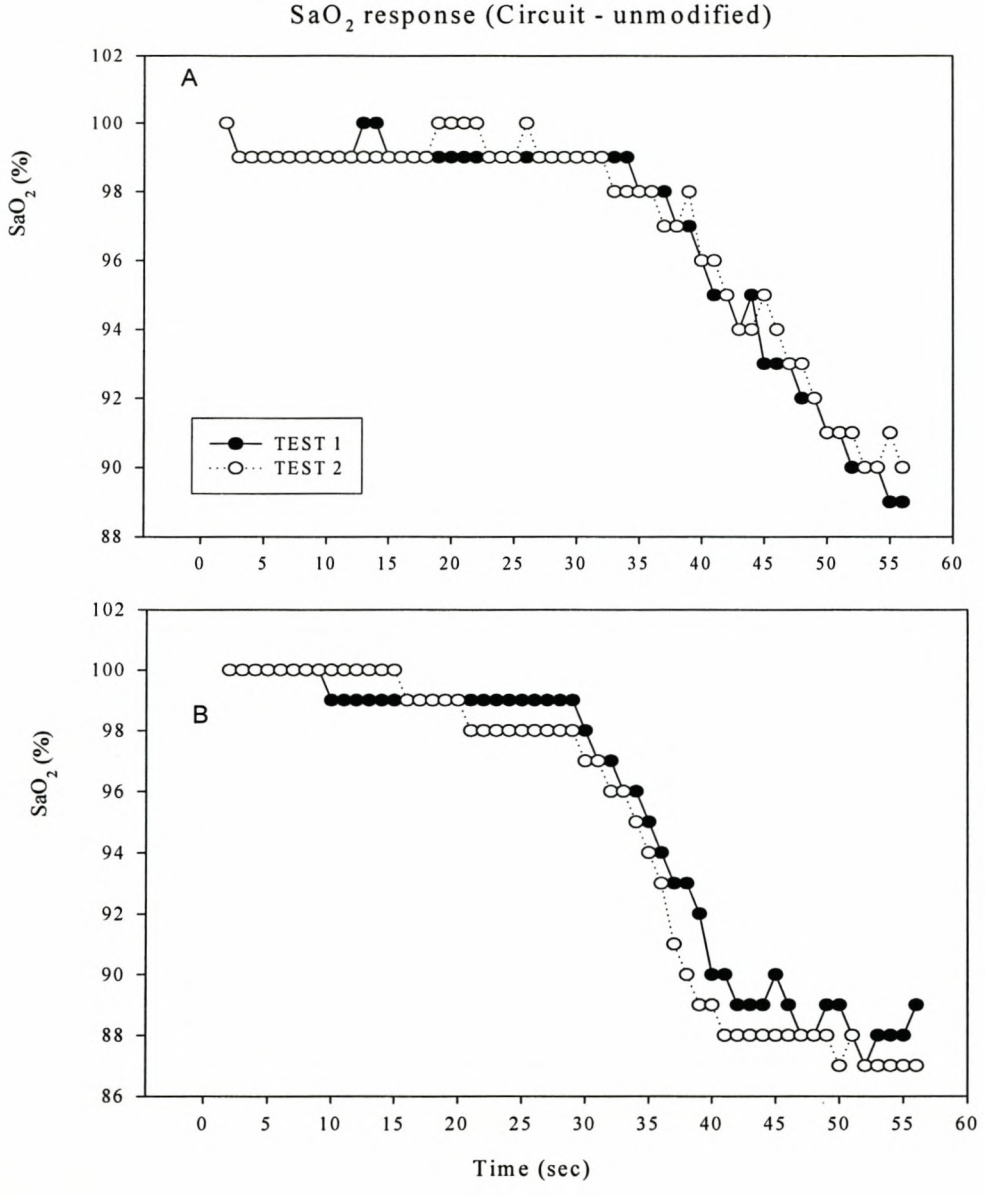
APPENDIX 1. The time course of the response of SaO_2 (%) to hypoxia before (A) and after (B) the reduction of dead-space in the circuit and relocation of the demand valve to a position nearer to the mouthpiece. All four tests were performed on the same subject.



Chapter 4

Repeatability of the ventilatory response to hypoxia in two South-African sea level populations

Abstract

High levels of intra-individual variability of the hypoxic ventilatory response (HVR) raise questions regarding the repeatability of this phenotypic trait and its potential use in experimental research in future. Knowledge of the repeatability of the HVR is of critical importance to the planning of ventilatory chemosensitivity studies. To understand the repeatability of the HVR, I undertook a family study with a 'single parent and two siblings' structure, using repeated measures to quantify the acute isocapnic HVR ($L \cdot min^{-1} \cdot \%^{-1}$) in twenty families from two populations (10 Caucasian, 10 Xhosa, total n of individuals = 60). The HVR was calculated from the change in minute ventilation ($\Delta \dot{V}_E$, $L \cdot min^{-1}$) divided by the change in arterial oxygen saturation (ΔSaO_2, %). Standard quantitative genetics were used to analyze the repeatability of the HVR within and between individuals in the two populations for each gender separately. Parent-sibling regression analysis assessed heritability of the HVR, its intra-individual variability (expressed as the CV (%), the hypoxic components of $\dot{V}_E$ ($L \cdot min^{-1}$) and the SaO_2 (%)), however the analysis revealed no significant heritability for any of the four selected components. Repeatability within populations was greater in the Caucasian than in the Xhosa group when separated into different gender (C Female: 98.1 %; C Male: 95.1 %; X Female: 77.3 %; X Male: 69.2 %). Repeatability within populations (genders pooled) of the HVR (86 %, one-way repeated measures ANOVA) was higher than expected on the basis of our current knowledge of high intra-individual variability. Repeatability of HVR, as well as that of hypoxic SaO_2, differed significantly between populations ($P < 0.05$) but not between families, probably because of the high variation between families within the

same population. In conclusion, the estimated repeatability levels, in conjunction with current knowledge of high innate intra-individual variability, indicates that the HVR requires repeated measurements but need not be precluded as a comparative tool from future research providing sufficient sample sizes are used.

Introduction

Evidence for heritability of the hypoxic ventilatory response (HVR)

The mechanisms underlying the ventilatory drive have been well-documented, yet the etiology remains unclear (Neubauer, 2001). The notion that hereditary factors have an important influence on ventilation responses appears consistently in the literature (Collins *et al.*, 1978; Scoggin *et al.*, 1978; Kawakami *et al.*,1982; Chatterjee & Das, 1995; Beall *et al.*, 1997; Hochachka & Monge, 2000; Neubauer, 2001; Fagan & Weil, 2001) but findings substantiating this idea are few, and many issues remain unresolved (Chatterjee & Das, 1995; Fagan & Weil, 2001; Neubauer, 2001).

Early attempts at linking ventilatory responses with hereditary factors in familial studies (Moore *et al.*, 1976; Collins *et al.*, 1978; Scoggin *et al.*, 1978) revealed the possibility that genetic factors play a major role in responses to hypoxia. Using comparisons of cellular or ventilatory responses to hypoxia in recent comparisons of inter-strain differences among inbred mice (Tankersley *et al.*, 1994, 1997, 2000; Soutiere & Tankersley, 2001) and rats (Strohl *et al.*, 1997; Weil *et al.*, 1998, Hodges *et al.*, 2002) support this theory, but human evidence remains insubstantial. Older literature may be outdated in terms of methodological advancements in this field (Powell *et al.*, 1998) and also in the understanding of the innate variability of this response (Zhang & Robbins, 2000; see also Chapter 2), and thus utilization of the conclusions that have been made could be unjustified. Use of quantitative genetics has recently been supplanted by the belief that modern rapidly advancing genetics could provide an answer by means of a

molecular analysis approach (Wenger, 2002). This does not necessarily mean that the issue has been resolved at the whole body response level, or even adequately dealt with, and many questions remain unanswered (Soutier & Tankersley, 2001; Wenger, 2002).

It has been proposed that the site and duration of the genetic influence on control of breathing are still to be determined (Yoshikazu *et al.*, 1982; Neubauer *et al.*, 2001). The genetic determinants of physiological responses to hypoxia are probably complex, involving both major genes with larger effects and minor genes with smaller effects (Neubauer *et al.*, 2001). Their exposition will probably require new analytical methods that explicitly take into account genotype-environment interaction, such as quantitative trait loci (QTL, Hartl, 2000; Neubauer, 2001). However, recent mouse studies have provided evidence that hypoxic breathing components may be controlled by as few as two genes (Tankersley *et al.*, 1997, Tankersley, 2000), and that aerobic capacity is largely attributable to genetic factors (Lightfoot *et al.*, 2000; Feitosa *et al.*, 2002), similar genetic control in humans has not yet been confirmed.

The best evidence that genetic determinants influence the HVR in humans has been derived from monozygotic and dizygotic twin studies (Arkinstall *et al.*, 1974; Collins *et al.*, 1978; Hubert *et al.*, 1982; Kawakami *et al.*, 1982), although these studies did not control for a "common-environment effect" (Falconer & Mackay, 1996; Hartl, 2000).

Repeatability estimation

Repeatability expresses the variance of a measurement that is equivalent to the proportion due to permanent differences between individuals due to both genetic (V_G) and environmental (V_E) effects (Falconer & Mackay, 1996). The intra-class correlation coefficient r (also known as repeatability) is the ratio of the inter-individual component to the total phenotypic variance (V_P). Repeatability analysis allows the separate estimation of the component of intra-individual variance (V_{Es}) due to the special environment arising from temporary circumstances as a fraction of the total, and the component of the inter-individual variance (or general environmental variance) (V_{Eg}) attributable to the environmental variance that contributes to the inter-individual component which arises from the permanent circumstances. The repeatability is the correlation between repeated measurements of the same individual. Total phenotypic variance is partitioned into two components and is expressed by the repeatability. The two components referred to are V_{Es} versus the sum of V_G and V_{Eg}, so that the repeatability is

$$r = \frac{V_G + V_{Eg}}{V_P} \qquad \text{(Eq. 4.1)}$$

This allows the separate estimation of the component V_{Es} due to the special environment, (as a proportion of the V_P). This is given by

$$\frac{V_{Es}}{V_P} = 1 - r \qquad \text{(Eq. 4.2)}$$

The variance components for the trait in question may also be partitioned within a population as follows:

$$\sigma_T^2 = \sigma_B^2 + \sigma_W^2 \qquad \text{(Eq. 4.3)}$$

where σ_T^2 is the total variance within the population, and σ_B^2 and σ_W^2 are the total variances between and within individuals within that population respectively. In other words repeatability can be given as

$$r = \frac{\sigma_B^2}{\sigma_T^2} \quad (0 \leq r \leq 1) \qquad \text{(Eq. 4.4)}$$

Repeatability differs considerably depending on the nature of the character in question, the genetic properties of the population, and the extent of the influence of the local environmental conditions experienced by that population's individuals. Repeatability estimates have become an important tool in evolutionary and ecological physiology in which the concept of repeatability has repercussions in the identification of traits. Significant repeatability may facilitate the study of selection acting on natural populations (Dohm, 2002). Repeatability can also set the upper limit to heritability (Falconer & Mackay, 1996); as long as certain assumptions are not violated (Dohm, 2002).

Heritability and Quantitative Genetics

The aim of quantitative genetics is "…to analyze the amount and nature of genetic variation within a population or a continuously varying phenotypic trait, and to partition total phenotypic variance into genetic and environmental components…"

Brutsaert, 2001

Most importantly, quantitative genetics (QG) can play a key role in the identification of an underlying genotype, should evidence for heritability exist in the phenotype. Gene mapping and segregation analyses can be used thereafter to determine the chromosomal locations of the genes. This approach has seen application in clinical research, and has by means of QTL, aided the discovery of genes such as the breast cancer (BRCA) genes that code for tumour suppressor proteins.

Heritability (h^2) is a statistic that describes the proportion of the total phenotypic variance that is due to genetic differences between individuals within a population (Falconer & Mackay, 1996). Heritability may have a broad and a narrow meaning. The broad meaning would refer to the total proportion of phenotypic variance attributable to all genetic effects, while heritability in the narrow sense would refer to the proportion of phenotypic variance due to additive genetic effects alone (Brutsaert, 2001). While QG strives to estimate heritability in the narrow sense, this is not always possible. Quantifying narrow sense heritability requires parent-sibling or half-sibling regression analysis and an understanding of the underlying genotype. In the case of HVR it is only possible to obtain a value for the broad sense heritability as the genotype is unknown (for more information on analysis of complex traits see Chapter 4 in Hartl, 2000).

The partitioning of variance in this manner is useful to plant and animal breeders (e.g. Butler & Dolling, 1992) because an understanding of heritability tells them *a priori* which traits are amenable to alteration through artificial selection. QG has also been embraced by evolutionary biologists over the past three decades and has provided

interesting comparisons in studies of the HVR in high altitude (HA) populations (Beall *et al.*, 1997; Hochachka & Monge, 2000). These studies have shown significant heritability for certain key phenotypes associated with HA populations, including chest dimensions, pulmonary function, hemoglobin concentration, and resting ventilation. QG has also been used to support a suggestion that a major gene explains the variance in resting SaO_2 levels among different high altitude populations (Beall *et al.*, 1997).

Limitations of Quantitative Genetics

The main limitations of a QG approach to analysis of the HVR that I am aware of, reside in the lack of information about the genetic structure of the trait, its inheritance patterns, and the presumed gene(s) position(s) on the chromosome(s), gene products, functional effects of the genes on the phenotype, and differences in the pattern of underlying genetic variation affecting the trait between population groups (Hartl, 2000). The last is of particular importance, and may emphasize the fact that the quantitative genetic analysis takes place *within* populations as opposed to *between* populations of interest (Falconer & Mackay, 1996). Many of the calculations for h^2 strongly rely on assumptions of Mendelian transfer, and also assume that the putative gene is not gender-linked.

Aims

The main aim of this chapter was to establish intra-individual repeatability levels in the hypoxic ventilatory response (HVR), by using repeated measurements of the HVR in two LA African populations residing in the same town. A second aim was the estimation of the heritability of the HVR and selected key components. Heritability assessment by

means of the 'parent-sibling' approach has proved successful in determining the heritability of many traits in domestic livestock (e.g. the quality and production of wool (Butler & Dolling, 1992)) and in exercise-related parameters such as the Family HERITAGE studies in America (e.g. Feitosa *et al.,* 2002).

Methods

Measurement and calculation of the acute isocapnic HVR and its associated components was executed precisely as described in Chapter 3.

Subjects

20 South African families participated voluntarily (10 Caucasian (C); 10 Xhosa (X); total individuals n = 60) in this study.

Family Criteria

The families invited to participate in the study had to comply with the following selection criteria.

a) The progeny were offspring of the parent(s) tested (although not objectively assessed, several lines of questioning were used to 'cross-check').
b) Two progeny and one parent were tested.
c) The youngest of the progeny was no younger than 15 years of age.
d) The oldest parent was less than 70 years of age.

e) All individuals had non-athletic lifestyles and had never participated in national or international sports.

f) All families lived at sea-level in the same town.

g) For Xhosa-speaking families, the family for at least two previous generations was of only Xhosa-speaking origin, i.e. they had not interbred with any people from another native South African tribe.

Where necessary, language differences between the investigators and subjects were overcome using translators. All experimental procedures were fully explained, verbally and in written form, before each subject signed a consent form. Under-age subjects signed a consent form in the presence of their parent. Participants understood that they were free to withdraw from the study at any time. Ethical approval for all procedures was granted by the Subcommittee C of the Research Committee of the University of Stellenbosch, which conforms to the internationally accepted ethical guidelines detailed in the Declaration of Helsinki.

Data Processing

HVR and ventilation parameters were measured according to the protocol fully described in Chapter 2. Data from the start of the experiment up to the last two minutes of the initial resting period (N_1) were discarded. Resting values for each subject were calculated as means of each variable for the final 120 seconds of N_1 (number of data points, $n = 22 \pm 1$), except in the case of $P_{ET}CO_2$ where the last 60 seconds were used (number of data points, $n = 20 \pm 1$). For the hypoxia exposure (H; number of data points, $n = 7 \pm 2$).

Statistical analysis

A mean of two siblings in one family and a value for the related siblings' one parent was calculated from the obtained repeated measures (as in Chapter 2). Siblings' means were compared to parents' means using regression analysis on the selected variables (NCSS, 2000) when nested for population and also weighted for test number. These regression analyses were also performed without nesting and without weighting. Regression coefficients thus obtained were used for the analysis of the heritability of HVR. Analyses of components such as coefficient of variation (CV, standard deviation/mean) of the HVR, expired minute ventilation volume ($\dot{V}_E$, $L \bullet min^{-1}$) and arterial oxygen saturation (SaO_2, %) were also performed. Further estimations were made using sibling data (i.e. parents excluded) by one-way repeated measures Analysis of Variance (ANOVA) for calculation of variance within populations, and used for determination of repeatability (Falconer & Mackay, 1996), while nesting the number of test repetitions within siblings within families and within population groups. The variance component within families was greater than that between families and therefore the grouping for families was discarded and data were pooled within each population.

Results

Estimation of variation represented by data of reduced mean squares is presented in Table 4.1. This data provides information relevant to the treatment of data in the next step of estimation of repeatability.

Table 4.1. Reduced Mean Squares of populations, individuals within populations and repetitions within individuals (families & siblings pooled due to greater variance between families than between siblings).

Groups	DF	HVR	SaO_2	$\dot{V}_E$
Populations	1	1.114	387.05	9.33
Individuals within Populations	38	0.222	45.42	68.91
Repetitions within Individuals	51	0.016	9.89	10.36

Statistical Analysis: One-way repeated measures ANOVA

The components of variance which contribute to repeatability and the estimated repeatability for all factors are given in Table 4.2. Larger values are greater contributors to that groups variance.

Table 4.2. Components of variance (average of 2.08 observations per individual) and repeatability values.

Groups	HVR	SaO_2	$\dot{V}_E$
Populations	0.043	16.425	0.000
Individuals within Populations	0.099	17.082	28.147
Repetitions within Individuals	0.016	9.893	10.364
Repeatability within populations (families & sibs pooled)	0.862	0.633	0.731

Statistical Analysis: One-way repeated measures ANOVA * ($P < 0.05$)

Table 4.3a Repeatability of Caucasian subjects calculated for Males and Females separately. The source refers to the type of variance, either among or within individuals, (SS: Sum of squares; MS: Mean Squares; DF: Degrees of Freedom; one- way repeated measures ANOVA).

Gender	Source	DF	SS	MS	Repeatability
Females	among	9	2.419725	0.268858	0.981
Females	within	19	0.038016	0.002001	
Males	among	21	10.41672	0.496034	0.951
Males	within	49	0.483163	0.00986	

Table 4.3b Repeatability of Xhosa subjects calculated for Males and Females separately. The source refers to the type of variance, either among or within individuals. (SS: Sum of squares; MS: Mean Squares; DF: Degrees of Freedom; one- way repeated measures ANOVA).

Gender	Source	DF	SS	MS	Repeatability
Females	among	24	9.638804	0.401617	0.773
Females	within	60	2.499832	0.041664	
Males	among	4	0.298043	0.074511	0.692
Males	within	14	0.155854	0.011132	

The overall means and correlations for HVR and additional selected components are displayed in Table 4.4.

Table 4.4. Overall means (diagonal) and correlations (off-diagonal) for HVR, hypoxic SaO_2 and hypoxic $\dot{V}_E$

Population		HVR	SaO_2	$\dot{V}_E$
Caucasian	**HVR**	-0.486	-0.584	-0.905
	SaO_2		82.065	0.463
	$\dot{V}_E$			19.002
Xhosa	**HVR**	-0.264	-0.176	-0.646
	SaO_2		77.920	-0.367
	$\dot{V}_E$			18.358

HVR

No significant correlation was found between HVR for the parent-sibling method (all subjects $P > 0.7$, $r^2 = 0.01$; X: $P > 0.68$, $r^2 = 0.03$; C: $P > 0.70$, $r^2 = 0.02$; Fig 4.1).

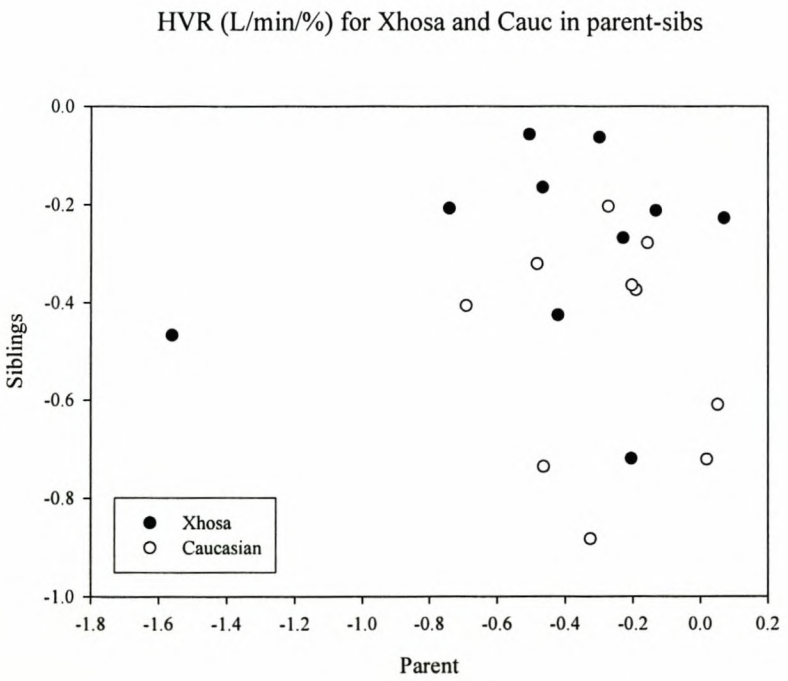


Figure 4.1 No relationship in parent-sibling comparison of mean HVR ($L \cdot min^{-1} \cdot \%^{-1}$) in Xhosa and Caucasian subjects. No relationship was found when all subjects were pooled.

Ventilation components

No significant correlation between parents and sibling pairs was found for the hypoxic $\dot{V}_E$ (X: $P > 0.44$, $r^2 = 0.03$; C: $P > 0.53$, $r^2 = 0.02$; Fig 4.2) and the change in $\dot{V}_E$ expressed as a %, and nor for the hypoxic SaO_2 (X: $P > 0.81$, $r^2 = 0.01$; C: $P > 0.25$, $r^2 = 0.16$; Fig. 4.3). There was no significant correlation in CV for either group, (X: $P > 0.55$, $r^2 = 0.05$; C: $P > 0.48$, $r^2 = 0.13$; Fig. 4.4).

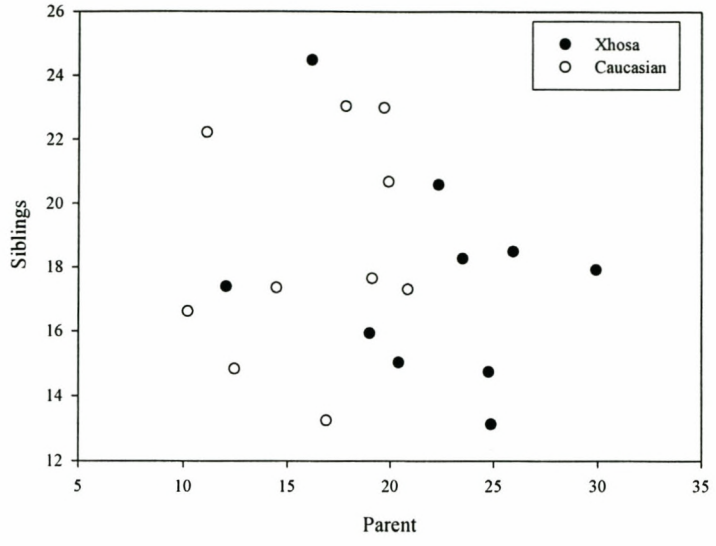


Figure 4.2 Relationship of parent-sibling hypoxic $\dot{V}_E$ ($L \cdot min^{-1}$) for Caucasian and Xhosa subjects.

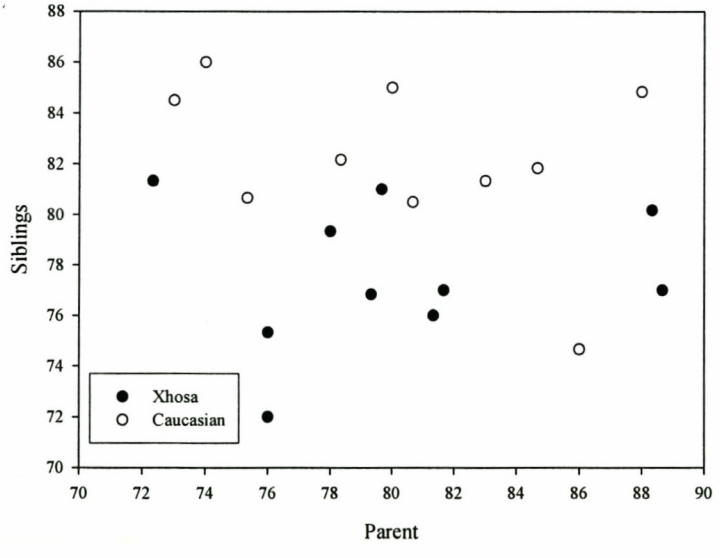


Figure 4.3 Relationship of parent-sibling hypoxic SaO_2 (%) for Caucasian and Xhosa subjects.

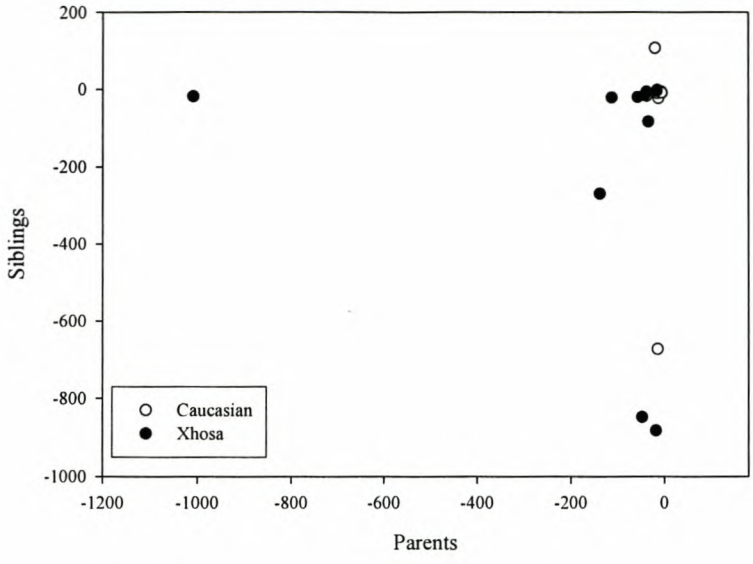


Figure 4.4 Relationship of parent-sibling intra-individual CV (%) of HVR in Xhosa and Caucasian subjects.

Discussion

The main finding in this study was the relatively high repeatability values obtained. The Xhosa subjects' HVR displayed lower repeatability than the Caucasian subjects when split into gender, in accordance with the higher variability (CV) obtained by the Xhosa group relative to the Caucasian group in Chapter 3. In both populations, HVR in females presented greater repeatability than in males. A relatively high repeatability estimate in conjunction with prior information exposing high variability (Chapter 2; Zhang & Robbins, 2000) revealed information indicating that the HVR may be highly variable but also highly repeatable. This is mainly as a result of the fact that the inter-individual variance is much greater than the within individual variance. Conclusions regarding the

HVR as a parameter suggest repeated measures should be used for determination of physiologically representative data, but based on the high inter-individual variability combined with high repeatability, the HVR need not be precluded from physiological research. With the estimation of repeatability, of primary concern in this relatively limited study are the unbalanced gender ratios and the possible interaction of gender with HVR estimation. In future, better matching of subjects for age, sex, and anthropometry should be considered a priority.

In addition, regression analysis of the HVR, hypoxic $\dot{V}_E$, hypoxic SaO_2, and the intra-individual CV for HVR using the parent-sibling approach showed no evidence for heritability of these as traits. A major concern from this aspect of the study is the indication for a lack of sufficient subjects for the determination of heritability.

The concept of repeatability and implications for heritability

Repeatability may set the upper limit to heritability should the trait in question be properly defined and measured (Dohm, 2002). This has potential interpretations that may be of use, since often the heritability estimation cannot be achieved. However, repeatability estimates may not set the upper limit to heritability when significant genotype-environment interaction is present or if the traits are influenced by maternal effects (Dohm, 2002). Since one or both of these factors may apply in the case of the HVR, I must caution the reader that in this study, it is not appropriate to use my repeatability estimates to set the upper limit to heritability of the HVR.

Repeatability

For traits with low repeatability within the same individual this information may lead the researcher to believe that there are practical problems with the measure such as high variability (Falconer & Mackay, 1996), or that some aspect of the testing is not accounting for consistency of the parameter in question (Arnold *et al.,* 1995), such as may occur with equipment or data capturing failure. Relatively high repeatability such as that which I found may have three implications. First, the repeatability is supposed to set the upper limit for the broad and narrow-sense heritability (see above) of a trait because it includes both the genetic and environmental basis of variation while heritability includes only the genetic differences among individuals (Falconer & Mackay, 1996; Dohm, 2002). Second, significant repeatability could be an important determinant of the efficacy of natural selection on the temporal moderation of the trait in question, due to its relationship with heritability (Boake, 1989; Dohm, 2002). Third, high levels of repeatability indicate that individuals perform relatively consistently and therefore there may be little practical reason to obtain multiple measurements (Falconer & Mackay, 1996; Dohm, 2002). This finding appears paradoxical, but may be explained simply in the following manner. Repeated measurements of the HVR have indicated that there is a high degree (26 %) of intra-individual variation (Sahn *et al.,* 1977; Zhang & Robbins, 2000, and see Chapter 2), while my data provide contrary information as obtained through repeatability estimation. What appears to be high intra-individual variability by comparison with other physiological and biochemical parameters is, in actuality, not particularly high when compared with the magnitude of inter-individual variability, which may be up to 10 times greater. In this situation, apparently high repeatability may

occur with simultaneously high variability. I propose this be termed the 'HVR variability paradox'. The conclusion drawn from this is that repeated measures with the appropriate statistical analyses (Bland & Altman, 1995) should still be used to account for intra-individual variability and obtain values of physiological accuracy, but that for the purposes of inter-population comparisons, the HVR is sufficiently consistent to support conclusions based primarily on high inter-individual variability. Caution should be used in ensuring data normality and sufficient sampling.

Comparisons with other studies

Although I report no significant correlations between respiratory parameters of parents and siblings, nevertheless there may be a heritable component to the HVR. Lack of parent-sibling correlations simply mean that the additive environmental influences on the parameters studied probably outweighed the genetic influences, making analysis of HVR by this method highly limited. Other HVR studies, such as those of twins (e.g. Collins *et al.*, 1978; Kawakami *et al.*, 1982; Chatterjee & Das, 1995) and of HA natives (Beall *et al.*, 1994; 1997), suggest that ventilatory responses have a strong genetic component, yet other variables related to lung function, such as $V_{O_{2max}}$ and running economy, appear to have minimal genetic influences (Rodas *et al.*, 1998). Small sample sizes, insufficiently homogenous subject groups, and different statistical methods (Bouchard *et al.*, 1992) may all lead to differing estimations of heritability for the same variable.

Possible Confounding Factors

Conclusions drawn from a pair-wise comparison of two sea-level populations, such as the one I present here, are limited because the study does not encompass comparisons of members of these same populations exposed to high altitude for different lengths of time, within and between generations. Such comparisons, combined with correction for the genetic distances between populations, are necessary for partitioning of environmental and genetic factors. Such studies may require a lifetime of dedication by the researcher (Brutsaert, 2001). Second, comparisons between two species, or two populations, may be insufficient to support conclusions about phenotypic adaptation (Garland & Adolph, 1994). Finally, it is very difficult to account for the environmentally-induced phenotypic plasticity inherent in long-term hypoxic exposure. This plasticity may occur over short periods of time (acclimatization), the growth and development period (developmental acclimatization), or even over generations, as when non-genetic maternal effects influence the first few generations of lowlanders recovering from altitude-induced low birth weights of founder members (Brutsaert, 2001). Respiratory plasticity (as referred to in Chapter 1) may be associated with hypoxia's ability to provoke changes in gene transcription (Kline *et al.,* 2002). Hypoxic exposures as shorts as 1 min can result in adaptation at the level of gene transcription, (which may therefore complicate any analysis), although no information is available regarding whether different tissues respond differently to continuous or intermittent hypoxia (Neubauer, 2001).

Different levels of daily activity between my two study groups may have confounded my analyses by influencing subjects' aerobic capacities, hence their ventilation. Traits

strongly correlated with physical fitness affect heritability estimations, and the HVR may be related to fitness levels, particularly when subject's train at high altitude (Levine *et al.*, 1992; Neubauer, 2001). However, all my subjects came from similarly sedentary backgrounds. Due to the possible relationship of HVR with fitness, the connection between the metric trait and fitness may be causal, and may confound heritability calculations. Heritability has been implied by familial aggregation reported in maximal and sub-maximal aerobic performance (Feitosa *et al.*, 2002; Rodas *et al.*, 1998). Torroni *et al.* (1994) showed that mutations in major genes (e.g. dwarfism in humans) are unlikely to play a major role in adaptation to HA.

Limitations of heritability studies

An important assumption of heritability analyses, probably met in most studies of the HVR, is that the trait studied does not result from a mutated gene. Furthermore, heritability studies do not provide information about the mode of gene transfer (e.g. dominant or recessive, mono- or polygenic; Falconer & Mackay, 1996), although this is often inferred from heritability calculations (e.g. Beall *et al.*, 1997), nor do they provide information about the locus/loci of the putative genetic factor/s, the time courses of and stimuli for their expression, and the mechanisms whereby they control ventilation.

There are conceptual, methodological and statistical issues that should be carefully considered when evolutionary presumptions are made from mean phenotypic comparisons between different population groups. While challenging, the approach of comparing phenotypes to isolate genetic adaptation have not yet been fully utilized

(Brutsaert, 2001). The relevance of continued study at the phenotypic level despite rapid advances in molecular biology should be emphasized, since natural selection acts on the phenotype only. Therefore, an integrative approach accounting for the interaction of the environment with the gene should be assessed relative to the production of a beneficial phenotype.

Summary

To summarise, the main finding in this study was the relatively high repeatability seen in the Caucasian subjects' HVR compared with those in the Xhosa when split into gender, or when both genders were pooled. In both populations, HVR in females presented greater repeatability than in males. Caucasians may perform more consistently in tests of ventilatory chemosensitivity to hypoxia than Xhosa, and females may perform more consistently than males.

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Chapter 5

Conclusions and Summary

5.1 High repeatability and high intra-individual variability in the HVR

Data presented in Chapter 2, Chapter 4 and also in the published literature (Sahn *et al.*, 1977; Zhang & Robbins, 2000) demonstrate high intra-individual variability of the HVR, making it difficult to obtain a single physiologically representative value for an individual. High levels of inter-individual variability in conjunction with *a priori* analyses suggest that larger sample sizes *and* repeated measures be used in future to support conclusions regarding ventilatory chemosensitivity to hypoxia.

5.2 Heritability of the HVR

There is strong evidence that a major gene controls lung function (Wilk *et al.*, 2000) and another resting SaO_2 in HA populations (Beall *et al.*, 1997). However, the location(s) and sequence(s) of these putative gene(s) remain elusive. Because of the large samples required to demonstrate heritability using a parent-sibling approach (see 5.1 above), HVR may not be a suitable research tool to explore hypoxic sensitivity. More reassuring were its high levels of repeatability within individuals, suggesting that it is heritable but that inter-individual variability may confound HVR studies if incorrect statistical analyses are used. Although the mechanisms are still unclear, some researchers have suggested that a gene-environment interaction may influence human HVR, and it's likely the physiological responses to a hypoxic challenge could be altered (Neubauer, 2001).

Differences in breathing patterns in rodents (e.g. Strohl *et al.*, 1997; Tankersley *et al.*, 2000) and humans (Chapter 3) suggest that a study exploring possible genetic differences in humans should be attempted in order to identify whether the mouse model for genetic

control of hypoxic ventilation and chemosensitivity is applicable to humans (Soutiere & Tankersley, 2001).

5.3 Inter-population differences in ventilation

Hypoxic sensitivity, measured as the acute isocapnic hypoxic ventilatory response is evident in low altitude South African populations. While the magnitudes of the HVR in the Xhosa and Caucasian groups were comparable, *a priori* analyses suggest that larger sample sizes are required before decisive conclusions about differences between these two groups can be drawn.

Blunted HVR in HA populations is accepted as an adaptation to hypoxia (e.g. Hochachka *et al.*, 1999 or Huang *et al.*, 1984). Natives to HA show acclimation abilities far superior to those of SL natives, attributable to various physiological mechanisms such as enlarged chest capacity and thus larger lung capacity, increased right cardiac capacity, greater capillary density, and higher oxygen delivery by the blood to the tissues (Hochachka *et al.*, 1999). Among the Bolivian Aymara, this improved O_2-carrying capacity is largely attributed to high Hb concentrations in the RBC's (Beall *et al.*, 1998). Furthermore, these HA natives utilize O_2 more efficiently (Matheson *et al.*, 1991) and display higher work efficiencies (Hochachka *et al.*, 1991) than do lowlanders.

Although breathing pattern differences within individuals forced to breath in a pattern unlike their normal resting pattern suggest that there are no differences in energy efficiency in normoxia (Mallios & Hodgson, 1994), the differences in unforced breathing

between populations that I report here may be attributed to different histories of residence at moderate altitude but this is unlikely. A difference in energy-efficiency of the two breathing patterns may be a cause of these underlying mechanisms (Milic-Emili & Orzalesi, 1998), and may have developed as a result of adaptations to specific altitudes of residence, but this theory remains to be explored in the future (see also MacIntyre & Leatherman, 1990). In ground squirrels, alterations in breathing patterns have been closely linked to reduced body temperature and changes in metabolic rate (Zimmer & Milsom, 2002). Oxygen extraction abilities may also differ between LA populations, but this too requires further scrutiny. A different explanation for the observed differences in respiratory patterns would be mechanoreceptor reflexes that are possibly related to anthropometric differences in the populations rather than an altitude history as such. Reflexes from respiratory muscle mechanoreceptors and airway slowly and rapidly adapting receptors have a much greater influence on breathing pattern than chemoreceptors (Smith *et al.*, 2001). In future, better matching of subjects for gender, age and anthropometrical data could resolve issues regarding the nature of conclusions that may be drawn from comparative studies, such as that reported in Chapters 3 and 4.

5.4 End-tidal PCO_2 is correlated with HVR

Although my findings contradict those of Reeves *et al.*, (1993), and while there is a large body of information available on the topic, the significant negative correlation I report between PET_{CO_2} and HVR suggests that further investigation of the HVR under experimentally varied PET_{CO_2} would be of value. Experiments testing this relationship should be informative. Also apparent from Chapter 3, was the fact that PET_{CO_2} can be a justifiable covariate for use in comparative studies such as altitude training studies (e.g. Levine *et al.*, 1992) or of HA and SL populations (e.g. Huang *et al.*, 1984) who may display differences in resting PET_{CO_2}.

5.5 The primary response: are we really measuring a physiological hypoxic response?

Carotid body stimulation can be quantified in rats (Weil *et al.*, 1998) through *in vivo* measurement of carotid sinus nerve (CSN) activity or *in vitro* using fluorimetry of cytosolic calcium, thereby eliminating confounding factors such as psychological effects (via nerve inputs), and improving the accuracy of measurement of the physiological response to hypoxia at the site of stimulus. Measurements of the ventilatory response to hypoxia are confounded by higher brain inputs other than simply the hypoxia in question (Kawakami *et al.,* 1982), and in my opinion, may form a critical point around which future physiological research should focus as this may be a cause of contradictory research in the existing HVR literature. Confounding effects of undesirable inputs could be eliminated by an approach such as that of Weil *et al.* (1998).

Intra-individual variability in the HVR may be reduced when subjects are all exposed to the same level of isocapnic hypoxia (i.e. to a fixed SaO_2 value) and their $\dot{V}_E$, fR and VT are directly compared. This approach effectively avoids the misuse of ratios such as the change in ventilation relative to the change in saturation used to calculate the HVR. Use of ratios to represent data that do not scale isometrically with body mass-related parameters, such as $\dot{V}_E$, leads to both type I and type II errors, and evolutionary physiologists have replaced such analyses with ANCOVAs (Packard & Boardman, 1987; Beaupre & Dunham, 1995; Hayes, 2002). I propose that human studies using body-mass related variables employ this approach, which by removing the confounding effects of body mass, may improve understanding of the remaining factors contributing to variability.

Heart rate during hypoxia may be a more precisely quantifiable response than is HVR (Sato *et al.*, 1996) and may therefore be more appropriate as a non-invasive tool for the measure of physiological adaptation to hypoxia. In the interests of science, future exploration into this parameter must be recommended. Furthermore, a test displaying lower inter-individual variability may prove financially and temporally more viable than the HVR systems currently in use. Future researchers should be aware of potential problems in the literature, in particular a lack of repeated measures designs in studies of chemosensitivity.

General Comments & Recommendations

A powerful and widely used tool in comparative physiology, ANCOVA is under-utilized in human physiology studies that often attempt to correct for body mass or height using ratios, thus risking the misinterpretation of data (Packard & Boardman, 1987), while whole-body values are still recommended as the best way to handle data that are correlated with body mass (Hayes, 2001).

The influence of intermittent hypoxic exposure on physiological plasticity (e.g. Prabhakar, 2001) and anatomical (e.g. Niermeyer *et al.* 2001) plasticity, and the potential complications arising from the poorly-studied effect of developmental plasticity (e.g. Okubo & Mortola, 1990) suggest that researchers measuring ventilatory chemosensitivity should consider their subjects' history of exposure to chronic or intermittent hypoxia when establishing exclusion criteria. Better understanding of the influence of developmental plasticity on HVR will improve our ability to control for this. However, exclusion criteria based on a subjects' lifetime history of hypoxic exposure may be impractical, because such histories are difficult to assess accurately.

Other methods for measuring a response to hypoxia should be considered, particularly those that avoid a psychological influence by operating on a cellular or enzymatic level. Optimal sample sizes may be so large that measuring HVR could prove to be a waste of time and money in future.

As a function of mammal maturity, the short- and long-term effects of hypoxia on the modulation of neurotransmitter release, receptor binding and expression, intracellular signalling cascades, transcription regulation, and gene expression are almost completely unknown (Gozal & Gozal, 2001).

5.6 Summary

In this thesis, I have successfully described a modified breathing circuit for measurement of the acute isocapnic hypoxic ventilatory response (HVR). The intra-individual variability of the HVR was estimated to be similar across days and within days and on average 27 % in magnitude either way. The comparison of two LA South African populations has provided novel information regarding the hypoxic sensitivity of African peoples, which can be used as a baseline in future comparative investigations of HA Africans, especially in hitherto unstudied East Africans. I am unaware of any published measurements of the hypoxic chemosensitivity in African populations such as those available for HA populations in the Himalaya and Andes. Data presented in this thesis also indicate that the HVR is evident in both the South African Caucasian and Xhosa sea-level populations, and that they are comparable in magnitude. Repeatability calculations have enhanced our understanding of the HVR. The Caucasians' HVR present greater repeatability than the Xhosa population, and males are more repeatable than females in either population. This study revealed that the repeatability of the HVR is relatively high within an individual relative to the inter-individual variation, which provides information related to the variability and heritability in not only the HVR, but components too. Heritability conclusions were limited since the data analyses indicated sample sizes were

inadequate, and were further confounded by the high variability both within and among individuals within a population. A priority for future studies is the better matching of populations for gender and anthropometry. Future HVR studies should employ repeated measurements, and samples greater than those generally used in the past are probably necessary to make conclusive deductions about hypoxic sensitivity.

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