CARDIOPULMONARY RESPONSES TO ACUTE HYPOXIA AND EXERCISE IN RELATION TO THE ANGIOTENSIN CONVERTING ENZYME INSERTION/DELETION GENE POLYMORPHISM

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SUMMARY

CARDIOPULMONARY RESPONSES TO ACUTE HYPOXIA AND EXERCISE IN RELATION TO THE ACE INSERTION/DELETION GENE POLYMORPHISM

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The physiological response to environmental hypoxia encountered at high altitude has a wide range of cardiovascular and pulmonary effects. The insertion allele of the angiotensin converting enzyme (ACE) gene polymorphism has been found to be more prevalent in endurance athletes and is associated with beneficial anabolic and functional responses in muscle. Furthermore, the insertion allele of this functional genetic polymorphism has been associated with enhanced physical performance at altitude, as defined by the successful ascent of peaks over 4800 metres. This thesis examines the cardiopulmonary responses during exercise and hypoxia in order to elucidate any genotype dependent differences in cardiopulmonary response that could explain this observation.

The main body of this work was carried out between August 1999 and September 2001. The studies involved 60 healthy subjects performing a maximal cardiopulmonary exercise test to determine ventilatory threshold (VT). At the second visit the subjects underwent a second set of steady state exercise tests, performed at 50% of the work rate attained at VT, under normoxic and hypoxic conditions (FiO_2 12.5%). Metabolic and ventilatory measurements were made during tests and the changes between normoxic and hypoxic response during rest and exercise were analysed. A second smaller study examined cardiac output response during hypoxia and exercise using bioimpedance cardiography. These studies were performed simultaneously with the cardiopulmonary exercise tests and included 31 subjects. Similar analyses were performed on cardiac output variables between normoxia and hypoxia. The repeatability of the steady state cardiopulmonary exercise experimental protocol was verified by repeat testing and analyses. Bioimpedance cardiography measurements were validated against simultaneous measurements during pulmonary catheter studies and thermodilution cardiac output measurement.

The results of the tests and the comparison of response demonstrated a larger increase in ventilation during exercise from normoxia to hypoxia in the insertion homozygous group. This was accompanied by a genotype dependent decrease in end-tidal carbon dioxide, suggesting a higher alveolar ventilation. There was no increase in oxygen saturations in the insertion homozygous group, which may have been due to the technical limitations of the oximeters. The cardiac output studies did not reveal any significant difference between genotype. The ventilatory study has demonstrated a response that may contribute to enhanced performance during prolonged hypoxic exposure, as experienced at high altitude.

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Sameer Patel

AUTHOR'S DECLERATION

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Glasgow. The work is original except where indicated by special reference in the text and no part of this dissertation has been submitted for any other degree.

Any views expressed in the dissertation are those of the author and in no way represent those of University of Glasgow.

The dissertation has not been presented at any other University for examination either in the United Kingdom or overseas.

Signed..... .... Date 23.11.07

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ABBREVIATIONS

2,3-DPG	2,3-diphosphoglycerate
2D	Two dimensional
5-HT	5-hydroxytryptamine
ACE	Angiotensin converting enzyme
Acetyl-CoA	Acetyl coenzyme A
ACTH	Adrenocorticotrophic hormone
ADP	Adenosine diphosphate
AMP	Aminopeptidase
AMS	Acute mountain sickness
Angiotensin-1	AT-1
Angiotensin-4	AT-4
ANOVA	Analysis of variance - statistical test
ANP	Atrial natriuretic peptide
AT	Anaerobic threshold
AT_1	Angiotensin II type I receptor
AT-2	Angiotensin-2
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BCE	Before the common era
BNP	Brain natriuretic peptide
bp	Base pairs
Ca^{2+}	Calcium
CaO_2	Arterial oxygen content (g/dl)
CHO	Carbohydrate
CI	Confidence interval
cm	Centimetres
cNOS	Constitutive nitric oxide synthase
CNS	Central nervous system
CO	Cardiac output
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease

CSF	Cerebrospinal fluid
CvO_2	Venous oxygen content (g/dl)
DA	Dopamine
D-Amp	Dipeptidyl-aminopeptidase
dl	Decilitres
DNA	Deoxyribonucleic acid
DRG	Dorsal respiratory group
dZ/dt	First time derivative of ΔZ
ECE	Endothelin converting enzyme
ECG	Electrocardiograph
EDTA	Ethylene-diamine-tetra-acetic acid
EIAA	Exercise induced arterial hypoxaemia
eNOS	Endothelial nitric oxide synthase
Epo	Erythropoietin
ET-1	Endothelin 1
ETC	Electron transport chain
$EtCO_2$	End-tidal CO_2
FAD^-	Flavin adenine dinucleotide
$FADH^+$	Reduced flavin adenine dinucleotide
FEV_1	Forced expiratory volume in one second
FFA	Free fatty acid
$FiCO_2$	Carbon dioxide fraction of inhaled gas
FiO_2	Oxygen fraction of inhaled gas
FVC	Forced vital capacity
g	grams
GABA	γ-aminobutyric acid
H^+	Hydrogen ion
HACE	High altitude cerebral oedema
HAPE	High altitude pulmonary oedema
Hb	Haemoglobin
HCO_3^-	Bicarbonate ion
HPVR	Hypoxic pulmonary vasoconstrictor response
HR	Heart rate
HRE	Hypoxia response element

Hz	Hertz
I	Current
ICP	Intracranial pressure
IFN	Interferon
Il-1	Interleukin 1
iNOS	Inducible nitric oxide synthase
K^+	Potassium
K^+_V	Voltage gated oxygen sensitive K^+ channels
kg	Kilograms
L-NMMA	N^G-monomethyl-L-arginine
LT	Lactate threshold
LV	Left ventricle
M	Molar concentration
MCLP	Myosin light chain phosphorylase
MLCK	Myosin light chain kinase
mls	Millilitres
mmHg	Millimetres of mercury
mRNA	Messenger ribonucleic acid
mV	millivolts
nA	Nucleus ambiguus
Na^+	Sodium
NAD^+	Nicotinamide adenine dinucleotide
$NADH^+$	Reduced nicotinamide adenine dinucleotide
nM	Nanometres
NO	Nitric oxide
Noradrenalin	NA
NOS	Nitric oxide synthase
nPa	Nucleus paraambigualis
nRA	Nucleus retroambigualis
nTS	Nucleus tractus solitarius
O_2	Oxygen
OCR	Optical character recognition
P	Pressure
P_A	Alveolar partial pressure

Pa	Arterial partial pressure
$P_{A\text{-}a}O_2$	Alveolar-arterial oxygen difference
$PaCO_2$	Arterial carbon dioxide partial pressures
PaO_2	Arterial oxygen partial pressure
PAP	Pulmonary artery pressure
PASMC	Pulmonary artery smooth muscle cells
PC	Personal computer
PCWP	Pulmonary capillary wedge pressure
$PetCO_2$	Partial pressure of end-tidal CO_2
pH	negative logarithm of hydrogen ion concentration
Pi	Inorganic phosphate
ppm	Parts per million
PRA	Plasma renin activity
pVHL	Von Hippel-Lindau protein
PVR	Pulmonary vascular resistance
Q	Pulmonary blood flow
R	Gas exchange ratio
RAS	Renin angiotensin aldosterone system
RBC	Red blood cell
REDOX	reduction/oxidation
ROS	Reactive oxygen species
rpm	Revolutions per minute
RQ	Respiratory quotient
RR	Respiratory rate
SaO_2	Oxygen saturation
SD	Standard deviation
SNA	Sympathetic nerve activity
SOCC	Store operated calcium channels
SV	Stroke volume
TBE	Tris/ Boric acid/ EDTA buffer
TCA	Tri-carboxylic acid cycle
T_E	Expiratory time
TH	Tyrosine hydroxylase
T_I	Inspiratory time

TNF	Tumour necrosis factor
TR	Tricuspid regurgitant (jet)
V	Voltage
V_A	Alveolar ventilation
VCO_2	Carbon dioxide production
V_D	Physiological dead space
V_E	Ventilation
V_ECO_2	Ventilatory equivalent of CO_2
VEGF	Vascular endothelial growth factor
V_EO_2	Ventilatory equivalent of O_2
VO_2	Oxygen uptake
VO_2 max	Maximal oxygen uptake
VOCC	Voltage operated calcium channels
VRG	Ventral respiratory group
V_T	Tidal volume
VT	Ventilatory threshold
Z	Impedance
Z_0	Baseline impedance
ΔZ	Change in impedance signal
µl	microlitre

CHAPTER ONE: INTRODUCTION

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Oxygen is one of the fundamental requirements for life. The basic requirement is the same whether we consider a unicellular organism or an evolved mammal such as ourselves. Food is metabolised using oxygen to produce energy, some of which maybe used to move the organism from one point to another. In a single cell organism the environment determines the availability of food and oxygen whereas eliminating the products of metabolism is simply a matter of diffusion into the environment. Land animals however have had to evolve complex systems which allow the same processes to occur. Physiological systems couple the uptake of oxygen from the atmosphere by the lungs, via the circulation to the tissues and in combination with food substrate produce energy. Yet other systems eliminate the products of metabolism and in doing so aid homeostasis. The mechanisms for energy production, irrespective of the site or purpose within the organism are the same, but during exercise there are dynamic changes in oxygen requirement as the intensity of exercise increases; these requirements are met by dynamic changes in circulatory and ventilatory systems. Furthermore in conditions of hypoxia the cost of work is maintained, whereas the availability of oxygen is reduced. This is overcome by hypoxic responses in the same physiological systems and act in concert to effect an observable adaptation.

Research in the field of hypoxic response is extremely active and broad in the disciplines it encompasses. In the last few decades the biochemical and molecular mechanisms that affect hypoxic responses have been elucidated and described. In the last few years the actual mechanisms of oxygen sensing have become clearer, though many details remain unresolved and contested. Research into the bioenergetics of exercise has elucidated many of the mechanisms of these cardiopulmonary responses. Finally, research at high altitude has furthered our understanding of physiology, hypoxic adaptation and high altitude diseases.

The purpose of this thesis is to present work which examines some of the cardiopulmonary responses to both acute and chronic hypoxia and relates them to a specific genetic polymorphism. This chapter will review: the fundamental mechanisms of cellular respiration; the physiological mechanisms of cardiopulmonary response to exercise and adaptations to hypoxia; the molecular and biochemical factors involved in these adaptations as well as the genetics of hypoxic response. The final section will review the work on high altitude adaptation and disease.

1.1 Definitions

The physiological response to hypoxia occurs at several points from the cellular level to entire homeostatic mechanisms affecting several organ systems. In examining these responses it is essential to distinguish the terms used to describe low oxygen environments and hypoxia at the organismal, cellular and molecular levels.

1.1.1 Hypoxia and hypoxaemia

In biological terms hypoxia can be defined as an 'inadequate supply of oxygen sufficient to compromise function'. This broad term is further defined by the point at which the insufficiency arises and offers further clarification. For example: the interruption to oxygen utilization at the cellular level due to toxins (e.g. cyanide) gives rise to cytotoxic hypoxia; changes in morphology can lead to diffusional hypoxia; disruption in blood supply either localised or systemic can cause ischaemic hypoxia; reduced haemoglobin levels or disrupted haemoglobin function causes anaemic hypoxia and reduced oxygen tension in arterial blood (due to primary pulmonary disease) causes hypoxaemic hypoxia. This list omits a fundamental cause for inadequate oxygen supply: Low ambient oxygen or environmental hypoxia. Low ambient oxygen concentrations or hypobaric hypoxia as encountered at high altitude is the most basic level of oxygen insufficiency and though it results in hypoxaemia it is important to draw a degree of distinction from hypoxaemic hypoxia as a result of pulmonary disease. Hypoxaemia is the stimulus to some of the physiological adaptations shared by both these situations, but adaptation to environmental hypoxia is distinguished by the absence of pathological processes that cause the hypoxaemic hypoxia of pulmonary disease. That being said, environmental hypoxia can cause disease in otherwise healthy individuals as a result of maladaptive hypoxic response.

The response to environmental hypoxia affects every level of biological function since it occurs at the source. As a consequence physiological adaptation is seen in every step of ventilation, gas exchange, pulmonary and systemic circulation, oxygen carriage and cellular metabolism.

1.1.2 Hypoxic environments

Hypoxia occurs at high altitude because of two fundamental physical properties of gases. Firstly, atmospheric pressure decreases as altitude increases. Secondly Dalton's Law of partial pressures states, that the pressure exerted by an individual gas in a mixture is the

product of the proportional concentration of that gas and the ambient pressure; therefore the actual oxygen concentration may remain the same, but the partial pressure oxygen exerts is reduced at high altitude since barometric pressure is reduced. This is crucial at the alveoli, where the reduction in oxygen partial pressure results in a reduction of oxygen transfer across the alveolar/ endothelial interface, hence the potential for hypoxaemia.

The effect of altitude on barometric pressure was first described by Blaise Pascal (1623-1662) in 1647. Though his experiments were simple and elegant, the implications of his discovery have had a profound influence on all forms of activity at high altitude, whether it is in the ascent of mountains or in any method of manned flight. The effect of altitude on barometric pressure is illustrated in figure 1.1.

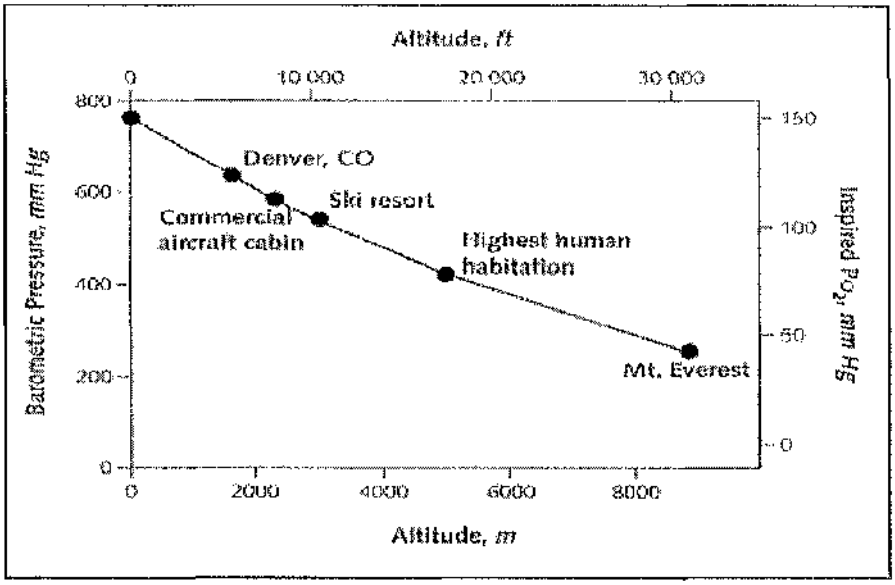

Figure 1.1 Relationship between barometric pressure and altitude. The right axis demonstrates the decline in PaO_2 [1].

As is shown above there is a negative relationship between altitude and barometric pressure which is slightly curvilinear. At the highest altitudes the degree of pressure drop is less than one would expect from a linear relationship such as the standard models used in the aviation industry; furthermore, there are variations in this relationship according to geography. This arises because of variations in the height of earth's atmosphere at different latitudes. Nearer the equator, where the atmosphere is at its thickest, barometric pressures for a particular altitude are higher than expected and coincides with the location of the highest mountain regions in the world.

The ascent of Mount Everest without supplementary oxygen would not have been possible without this variability between altitude and pressure at equatorial latitudes. The physiological stresses of hypoxia at the summit of Everest are such, that even seasonal variation in barometric pressures influences survival with such attempts.

1.1.3 Adaptations to acute and chronic hypoxia

The chronicity of hypoxic exposure is an important factor in the type and extent of physiological adaptation. Changes in ventilation, pulmonary circulation and cardiac output can be seen within the first minutes of exposure and develop as hypoxia persists. The effects these have on an individual's physiology can vary widely and are thought to determine how well that individual will perform in low oxygen environments and in some circumstances whether they will develop pathology associated with prolonged hypoxic exposure. The degree of hypoxia is also a crucial factor since many of the physiological effects are directly proportional to the oxygen deficit. What is becoming increasingly evident is the rate at which hypoxia increases is as important. High altitude disease, developing in the first few days of altitude exposure, is known to occur more frequently upon rapid ascents, when insufficient time is allowed for adaptive changes to take place and is classified as acute high altitude disease. Individuals exposed over a longer period, over months or years develop a different group of conditions associated with pathological cardiorespiratory adaptation and are classed as chronic high altitude disease. In terms of pathological disease obstructive and interstitial pulmonary diseases do result in persistent hypoxic states, with cardiopulmonary features that share similarities with chronic altitude diseases.

The focus of this thesis is the cardiopulmonary response to acute hypoxia, both at rest and during exercise. Furthermore, these responses are examined in relation to the angiotensin converting enzyme (ACE) gene polymorphism in order to determine how individual variations in these responses may influence the ability to cope in hypoxic environments and at altitude.

Before describing the control mechanisms and responses of cardiovascular and ventilatory systems, it is important to review the fundamental necessity of oxygen in energy generation which is the cause of adaptive changes in these systems in response to hypoxia. Furthermore recent research suggests that the basic mechanisms of energy generation are intrinsically linked to oxygen sensing in several organs and cell types.

1.2 Cellular respiration

Energy is vital for cellular activity and at the molecular level there is only one currency – adenosine triphosphate. The cleavage of the terminal high energy bond in this molecule produces energy for cellular processes. In skeletal myocytes, ATP is cleaved by myosin, enabling movement against actin molecules and elicit myofibril contraction. This system is dependent on calcium release stimulated by action potentials generated by an activated neuromuscular endplate. Calcium is released from the sarcoplasmic reticulum in close apposition to the myofibrils; however in order to allow myofibril relaxation the calcium is then removed by active uptake into the sarcoplasmic reticulum which also requires ATP. Therefore both muscular contraction and relaxation requires the cleavage of ATP. In smooth muscle cells there is a similar requirement for calcium influx into the cytoplasm, but in contrast to skeletal myocytes, there is a poorly developed sarcoplasmic reticulum and extracellular influx of calcium is an important factor. In smooth muscle various environmental stimuli can elicit contraction; whereas in skeletal muscle, contraction is dependent on neuronal stimulation of motor units. The features of hypoxic smooth muscle responses are discussed in detail further on, but for the purpose of the following review of cellular respiration, the skeletal myocyte is used as the model of the respiring cell. Irrespective of which cell is discussed the fundamental processes are the same.

1.2.1 Energy generation at the cellular level

Cellular respiration can occur in low oxygen conditions or with adequate oxygenation; this determines whether respiration terminates at the glycolytic pathway or proceeds, in the presence of oxygen, to the Tri-carboxylic acid cycle and oxidative phosphorylation. Both these pathways are discussed below, but terms such as 'anaerobic and aerobic respiration' have been used to describe these states. These terms can only provide a label for the overall state of respiration in a cell since both forms of respiration can occur simultaneously. It is the net state of the cell or tissue which determines whether it is classed as respiring 'aerobically or anaerobically'.

1.2.2 The glycolytic pathway

Glucose is a basic substrate for respiration, the initial process of ATP production is via the glycolytic pathway. The triose pathway of glycolysis, utilising triose molecules is illustrated in figure 1.2 and is called the Embden-Meyerhof pathway [2]. Glycolysis utilizes 2 molecules

of ATP to phosphorylate the early intermediaries in the pathway; each molecule of glucose is then cleaved to form two triose molecules: Glyceraldehyde-3-phosphate. Each molecule then undergoes reduction, isomerization and phosphoryl cleavage to produce pyruvate; therefore each glucose molecule produces two molecules of pyruvate. In the process, a total of 4 molecules of ATP are produced, giving a net gain of 2 ATP for each glucose molecule that enters the glycolytic pathway. Glycolysis also reduces 2 molecules of the electron transport molecule nicotinamide adenine dinucleotide (NAD^+), generating 2 molecules of NADH. In aerobic conditions NADH enters the electron transport chain and generates energy by oxidative phosphorylation; however in conditions of hypoxia the pyruvate is reduced by NADH, thereby regenerating itself and produces lactate.

Intermediate points in this pathway offer a means of introducing carbon skeletons from amino acids for the purpose of generating energy, equally the same intermediate molecules can be sequestered into amino acid synthesis. Similarly fatty acids can undergo metabolism to glycerol which can participate in the glycolytic pathway. In itself glycolysis is a relatively limited means of energy generation, with much of the potential energy of the carbohydrate substrate locked in either pyruvate or lactate. In order to fully metabolise substrate and maximise energy generation, pyruvate must enter the tri-carboxylic acid cycle. This pathway produces no ATP directly, but does allow the complete catabolism of carbohydrate whilst generating NADH for oxidative phosphorylation.

Figure 1.2 also shows the isomerization step for the production of 2,3-diphosphoglycerate, a molecule important in determining the affinity of haemoglobin at various oxygen tensions.

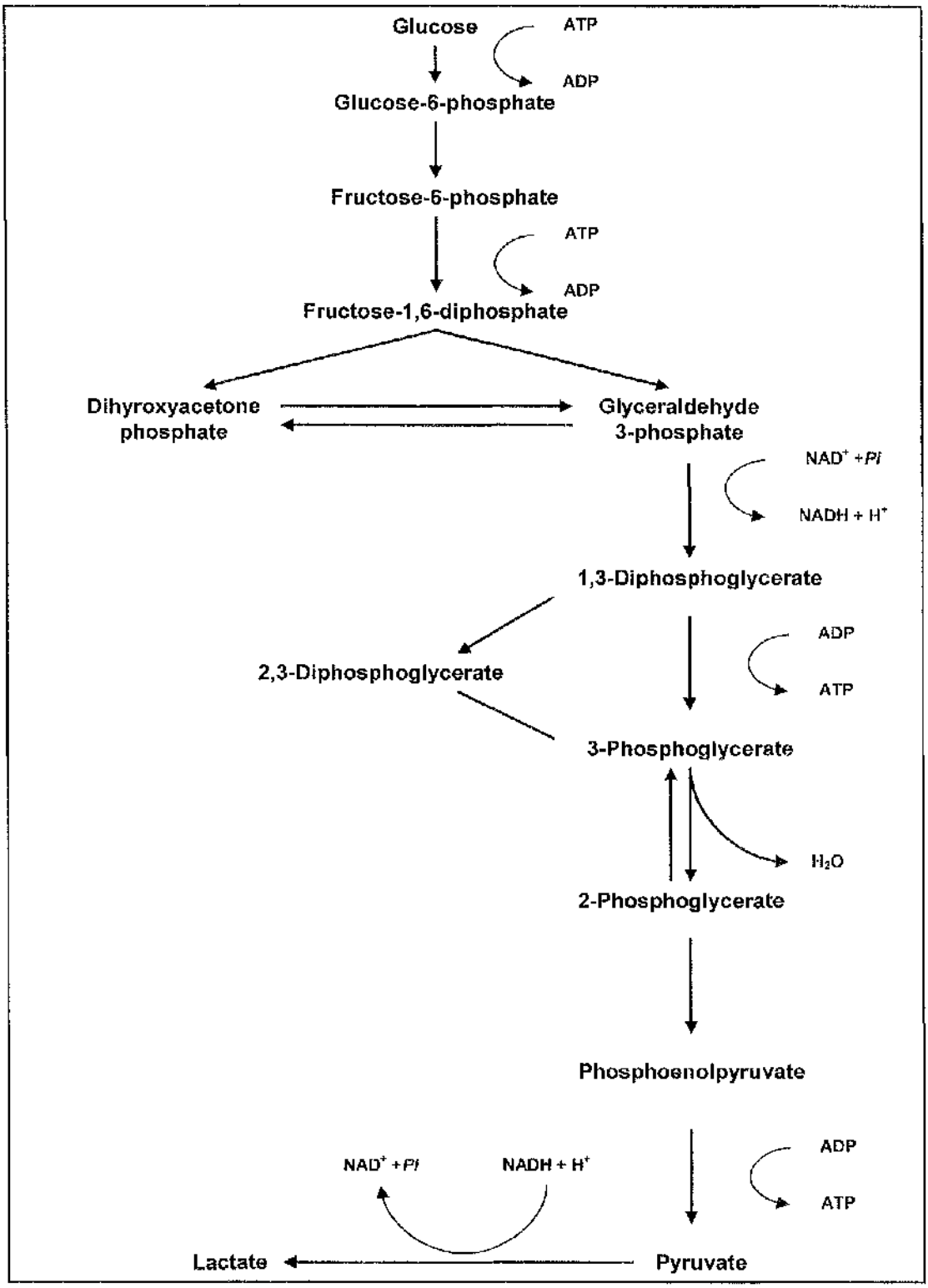

Figure 1.2 The Embden-Meyerhof pathway with sites of ATP utilisation and production. The pathway is discussed in detail in the text above.

1.2.3 Oxygen and respiration

The Tri-carboxylic acid cycle

Oxygen dependent respiration occurs in two parts, both within the mitochondria. The first part incorporates the pyruvate from glycolysis into the Tri-carboxylic acid cycle (TCA) (figure 1.3) which is located in the mitochondrial matrix. Pyruvate is converted into acetyl coenzyme A (acetyl-CoA) by pyruvate dehydrogenase, producing a molecule of reduced NAD ($NADH^+$). Acetyl-CoA then enters the TCA, replenishing it and allowing its continued function.

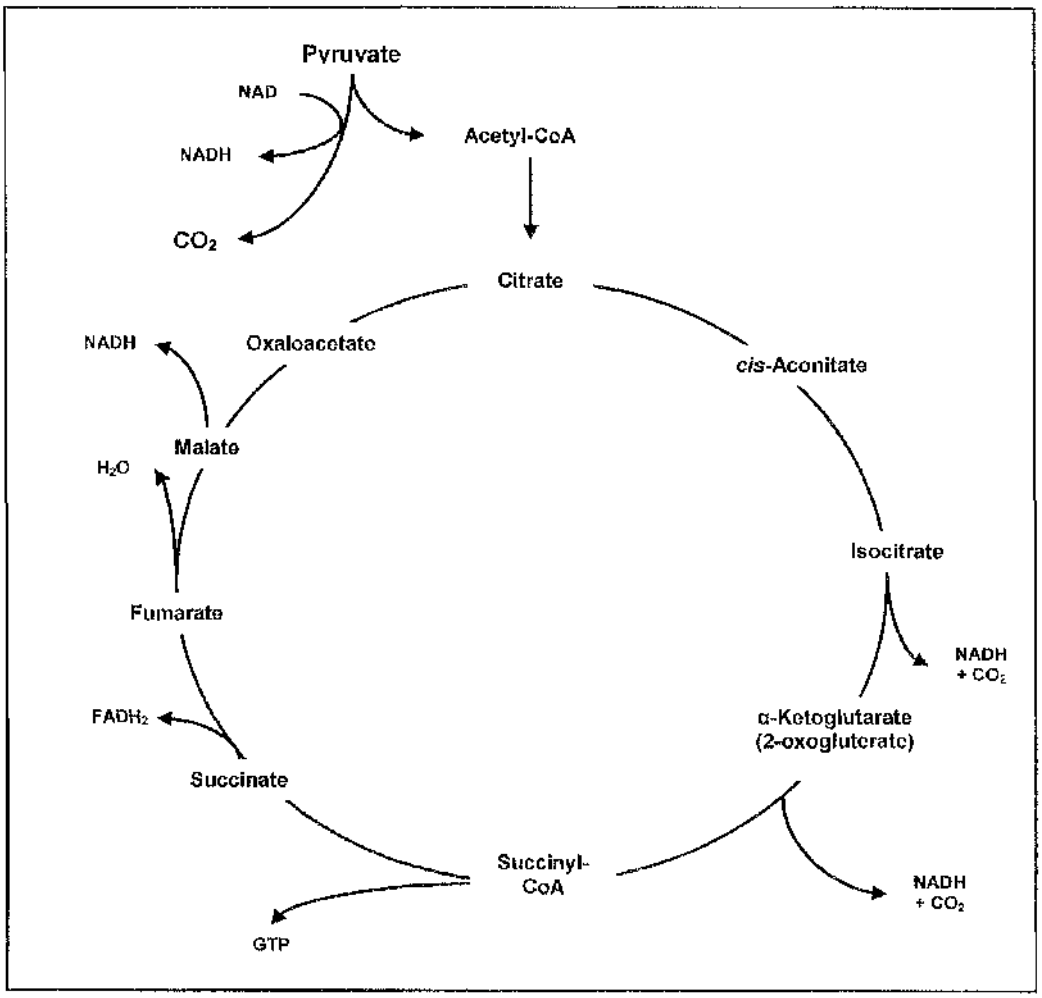

Figure 1.3 The Tri-carboxylic acid cycle, with constituent intermediate molecules and the points at which NADH and FADH are generated. Abbreviations as per the text.

The cycle then undergoes several enzymatic steps the products of which are 6 molecules of CO_2, 6 molecules of $NADH^+$, 2 molecules of reduced flavin adenine dinucleotide ($FADH^+$, another electron transport molecule) and 2 molecules of ATP for each molecule of glucose that enters the cycle (the guanidine triphosphate (GTP) produced by the hydrolysis of succinyl-CoA donates its phosphate to ADP to produce ATP).

At this point the direct net gain in terms of ATP is 4 molecules per molecule of glucose metabolised thus far; however the TCA cycle produces in total 8 molecules of $NADH^+$ and 2 molecules of $FADH^+$. On a molecular level, this burden on the reduction/oxidation (REDOX) state of the cell would be untenable; however the reduced state of these electron donors is resolved by entry into the electron transport chain (ETC) and further metabolism.

Oxidative phosphorylation

The electron transfer chain is located on the inner membrane of the mitochondrion. This membrane is relatively impermeable to molecules unless via specific transporter proteins. In contrast the outer membrane is permeable to most small molecules and ions via *porins*, large transmembrane proteins with a large central pore. In essence the electron transfer chain transfers electrons donated by NADH from the TCA cycle and as they are conveyed across the constituent complexes, the energy released is used to transport protons across the membrane. This creates a proton gradient which is used by ATP synthase to phosphorylate adenosine diphosphate (ADP) to ATP (figure 1.4). Complexes I to IV are NADH dehydrogenase, succinate dehydrogenase, cytochrome bc1 and cytochrome oxidase respectively. This description is of course simplified; the reality is that each complex has several constituent sub-units that rely on cofactors to perform their tasks. Complex I for example has between 42-43 subunits, one flavin mononucleotide, 8 different iron sulphide centres and is over 900 kilodaltons in size. The final complex in the chain is ATP synthase (complex V) and the final reduction reaction occurs with oxygen to produce water [3].

The energy generated per molecule of NADH was previously thought to be 3 ATP molecules; however this estimate seems to be overly generous. A more accurate figure is 2.5 molecules of ATP per $NADH^+$ oxidized and 1.5 molecules per $FADH^+$, allowing for variations in site of generation this gives a total of 26 molecules of ATP produced by aerobic respiration (table 1.1). This combined with the 2 molecules of ATP from glycolysis and the 2 molecules generated by the TCA cycle gives a sum total of 30 molecules of ATP per molecule of glucose [4].

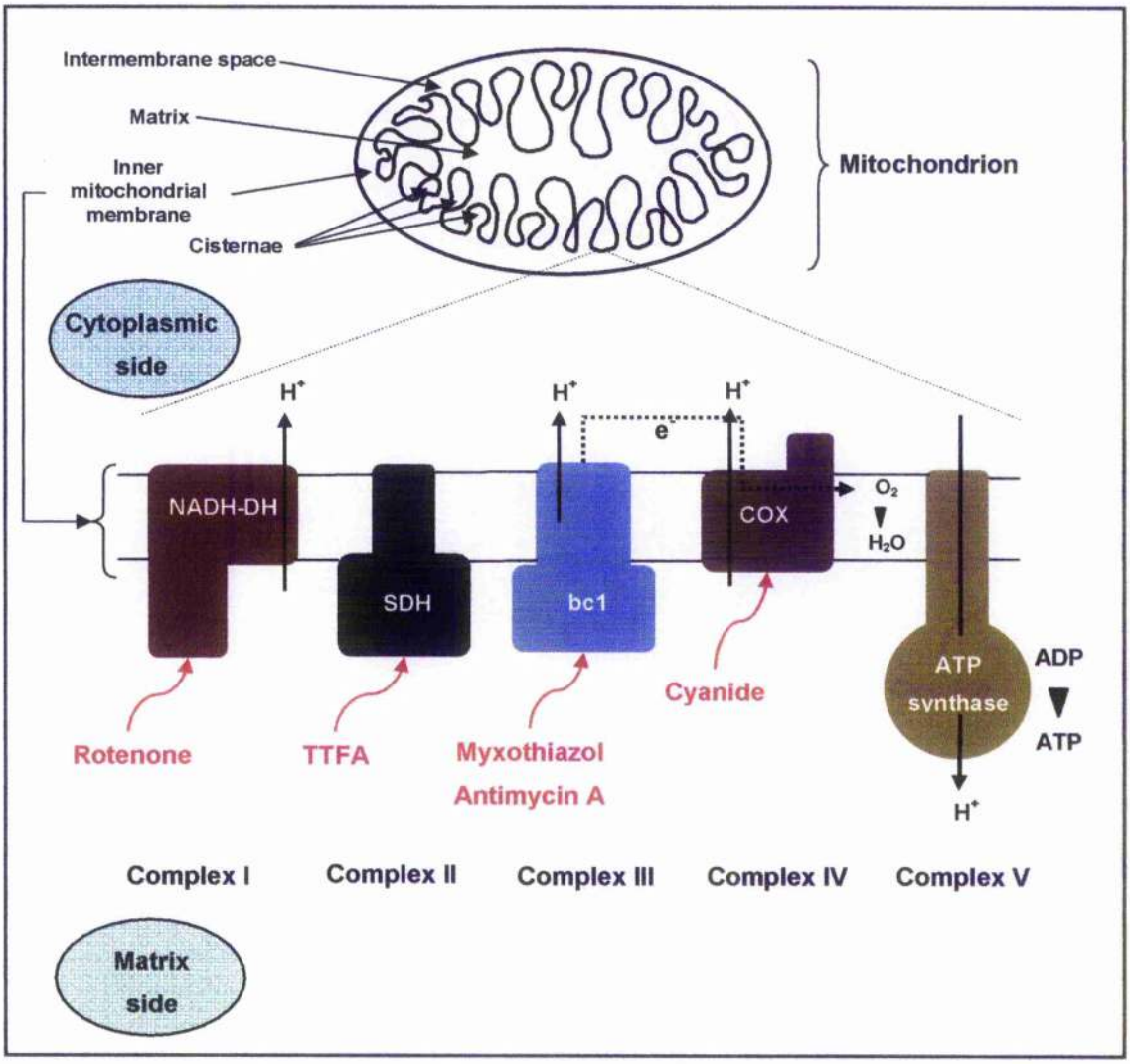

Figure 1.4. The electron transfer chain. NADH-DH: NADH dehydrogenase; SDH: succinyl dehydrogenase: bc1: cytochrome bc1; COX: cytochrome oxidase. The specific blocking agents for each complex are in red; TTFA: thenoyltrifluoroacetone. The final electron transfer between cytochrome bc1 and COX is illustrated by the dashed line.[3]

Oxidative phosphorylation (mitochondrial)	*ATP yield/glucose*
2 NADH formed in glycolysis; each yields 1.5ATP	+3
2NADH formed in the oxidative decarboxylation of pyruvate; each yields 2.5ATP	+5
2 $FADH_2$formed in the TCA; each yields 1.5ATP	+3
6 NADH formed in the TCA; each yields 2.5 ATP	+15
Net yield per glucose	+26

Table 1.1 ATP yield from oxidative phosphorylation of one glucose molecule

1.3 Ventilatory adaptations to hypoxia

The discussion thus far has established the need for oxygen at the cellular level, but in order to meet that requirement systems have evolved to extract oxygen from the environment and deliver it to respiring cells; furthermore these systems are dynamic as oxygen requirement is variable with activity and as ambient oxygen varies with environment. The first step in this process is the ventilation of air to the gas exchange surfaces.

Ventilatory control regulates oxygen uptake, carbon dioxide elimination and acid-base balance. At sea level it is one of many mechanisms which maintain homeostasis during exercise and ensures an optimum operating environment for the body tissues; at altitude it is a fundamental response to allow survival in hypobaric hypoxia. Sensory systems, both peripheral and central relay information on oxygen and carbon dioxide concentration as well as plasma pH to the ventilatory motor centres in the brainstem. In addition, the entire control mechanism can be overridden by higher cortical centres that enable controlled airflow for speech, but can also produce hyperventilation in response to emotional and physical stress. The sensory and motor components of control have been researched extensively, but despite this their precise mechanism and interaction is not fully understood.

1.3.1 The motor control of ventilation

Ventilation is mediated through skeletal muscle and is therefore entirely dependent on central nervous system (CNS) control. The brainstem motor neurones are situated in the pons and the medulla oblongata (figure 1.5). The pontine respiratory group is located around the parabrachial nuclei and is thought to play a role in the transition from inspiration to expiration. The medullary centres are divided into the dorsal and ventral respiratory groups

(DRG and VRG respectively). The DRG is in the dorsomedial medulla and is associated with nucleus of the tractus solitarius (nTS). The DRG contains mainly inspiratory neurones and receives afferent input from the lungs and airways via the vagus nerve. Slow adapting stretch receptors in lung airways inhibit the inspiratory neurones via this pathway and are thought to be part of the Hering-Breuer reflex. The VRG is located in the ventral medulla and comprises the Bötzinger complex, the nucleus paraambigualis (NPA), the nucleus retroambigualis (NRA) and the nucleus ambiguus (NA). The first three nuclei are within the nucleus retrofacialis, the NPA lies in parallel with the NA. These centres contain both inspiratory and expiratory neurones. Caudal to the Bötzinger complex are a group of neurones thought to be the site of ventilatory rhythm generation (pre-Bötzinger complex).

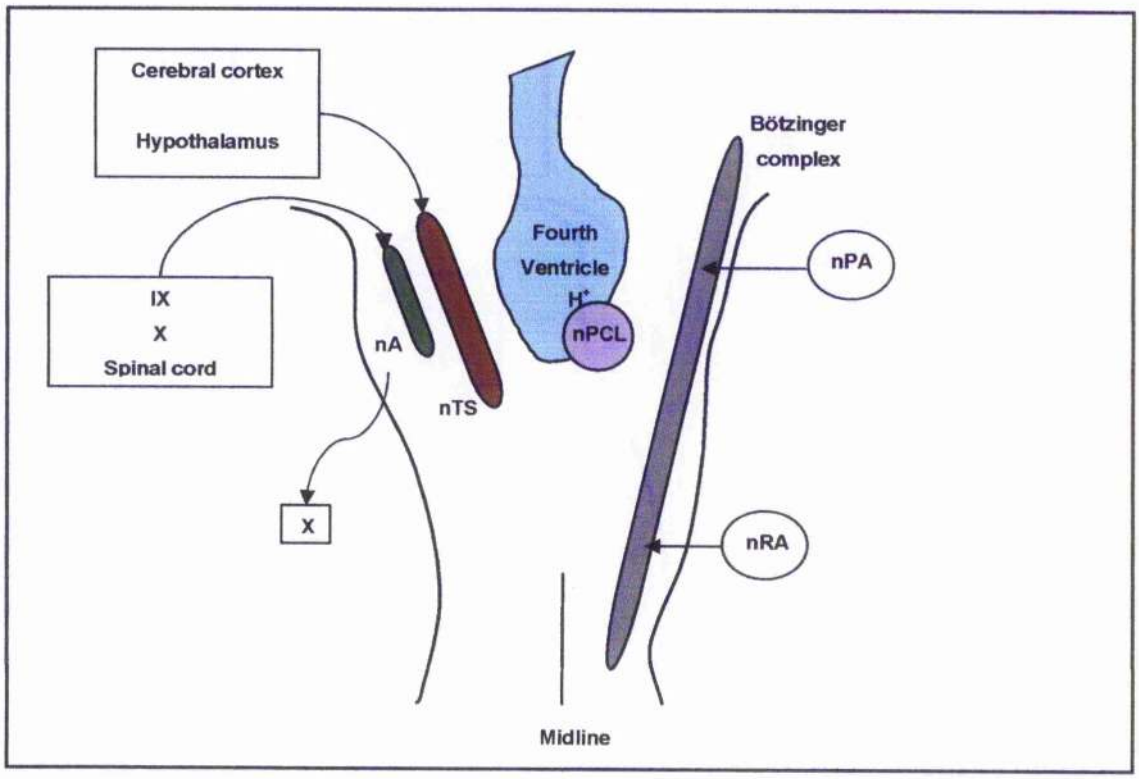

Figure 1.5. The ventral medullary nuclei. The nuclei lie in pairs on both sides of the midline, only some are demonstrated on each side for clarity. nA= nucleus ambiguous, nPA= nucleus paraambigualis, nRA= nucleus retroambigualis, nTS= nucleus tractus solitarius, nPCL= nucleus paragiganto cellularis lateralis.

The voluntary control of ventilation is transmitted via the corticospinal tract; however there are mechanisms of automatic ventilatory control that are transmitted in the bulbospinal tracts and are thought to mediate ventilation at rest [5]. The muscles of respiration under the control of these central motor centres are the diaphragm via the phrenic nerves and the intercostal muscles. Transection above the roots of the phrenic nerve at C3 results in the abolition of all ventilatory motor activity. The inspiratory phase of ventilation is mediated predominantly by the diaphragm and inspiratory intercostal muscles. Expiration is initially passive process (relying on the elastic recoil of the lung and chest wall) up to tidal volumes of around 50% of total vital capacity; thereafter expiratory intercostal muscle and abdominal wall contraction contribute to expiratory effort [6].

1.3.2 Central chemoreceptors

The classical model of central chemoreception places ventilatory sensing on the ventral surface of the medulla, [7]. This area responds to changes in blood CO_2 and pH, but demonstrates no direct response to changes in oxygen tension. Forty years has passed since it was first proposed and despite extensive work in this area, the precise identification of the chemoreceptor cells and their signalling pathways has yet to be described. The most recent evidence suggests that neurones utilising ATP as neurotransmitter are involved in CO_2 sensing [8]. An alternate hypothesis proposes that there is no cellular chemoreceptor on the ventral medulla, but rather dendritic projections of deeper neurones that perform the task [9]. Others posit the theory that the respiratory pattern generators in the ventral respiratory group themselves exhibit chemosensory properties and are responsible for chemoreception [10, 11]. Irrespective of the controversy surrounding the identity of the central chemoreceptor, its fundamental properties remain the same. The central chemoreceptors respond to changes in cerebrospinal fluid (CSF) pH; however the brain is never in direct contact with the blood and is protected by the blood brain barrier (BBB). Carbon dioxide can pass freely across the BBB and is ionised to form bicarbonate (HCO_3) and hydrogen ions (H^+) according to the Henderson-Hasselbach equation. Unlike blood, the CSF lacks a significant buffering capacity; therefore the hydrogen remains ionised and is the stimulus to central ventilation. The subsequent transmission of this signal to the respiratory groups seems to be via cholinergic neurones, since atropine can block this effect [12].

Hypoxia does not have a direct influence on central chemoreception; peripheral chemoreceptors fill the role of oxygen sensing and have an afferent input to the respiratory motor groups of the medulla (this is discussed in detail below). Hypoxia does have a global inhibitory affect on central ventilatory response. This effect has been attributed to a reduction in excitatory neurotransmitter such as acetyl choline (ACh), glutamate and glycine in favour of an increase in inhibitory neurotransmitters (γ-aminobutyric acid (GABA), adenosine and serotonin). Specific antagonists of these transmitters can partially ameliorate the effect suggesting a combined action of these transmitters or other unknown influences. Another theory is that hypoxia results in a reflex increase in cerebral blood flow that may wash out CO_2 and result in a depression of central chemoreceptor drive [6]. Central hypoxic depression is only evident in adults under circumstances of severe hypoxia, since peripheral hypoxic drive is dominant in these circumstances. Many details of the functioning of central ventilatory chemoreceptor remain unanswered. In contrast the work on peripheral chemoreceptors in the last few years has revealed some of the fundamental mechanisms of oxygen sensing and signal transduction.

1.3.3 Peripheral chemoreceptors: The carotid body

The peripheral chemoreceptors form the oxygen sensing limb of ventilatory control. The carotid body is the main oxygen sensor of ventilatory control and section of its afferent nerves results in abolition of hypoxic ventilatory response. The aortic body demonstrates some oxygen sensing capability, but has a negligible contribution to peripheral oxygen sensing; therefore it does not form part of this review. The carotid body is located at the bifurcation of the common carotid artery and receives its blood supply from a branch of the internal carotid artery. Each weighs around 1 gram, but in relation to their mass the carotid bodies have an abundant blood supply in the form of fenestrated sinusoidal capillaries organised into glomeruli. The generous blood supply means that most of the cellular oxygen requirement is extracted directly from soluble oxygen rather than O_2 bound to haemoglobin; therefore the carotid body response is to arterial oxygen tension and remains unaffected by anaemia. Each capillary tuft is surrounded by an arrangement of two cell types: type I and type II glomus cells. Glomus type I cells are responsible for oxygen sensing, whereas the type II cells seem to have a more sustentacular function; therefore the discussion, from this point on, will focus on the type I cells. Each glomus cell is rich in a variety of

neurotransmitter substances and has a receptor/neuronal synapse with branches of the carotid sinus nerve (a branch of the glossopharangeal nerve) [13]. Sensory afferents relay information to the respiratory groups of the medulla, predominantly to the nucleus of the *tractus solitarius* (nTS).

It is generally accepted that the type I glomus cell is the transducer for oxygen tension in blood; however the glomus cell releases a number of neurotransmitters in response to hypoxia. These fall into several categories: biogenic amines (ACh, dopamine (DA), noradrenaline (NA), 5-hydroxytryptamine (5-HT)); neuropeptides (enkephalins, substance P, endothelins); ATP and amino acids (GABA) [14-16]. The role of these transmitters is mixed and species specific: dopamine inhibits the activity of cat, but not rabbit carotid bodies; whereas acetyl choline is stimulatory in cats, but inhibitory in rabbits [13]. Currently, it is thought that dopamine and enkephalins are inhibitory neurotransmitters in humans, whereas substance P, ATP, noradrenaline and acetyl choline are excitatory [15].

Neurotransmitter release and membrane ion channels

The release of neurotransmitters in response to hypoxic stimulus is dependent on depolarisation of the glomus cell membrane and secretion of transmitters into the synaptic space between glomus cell and branches of the carotid sinus nerve [17]. The glomus cell has a resting negative membrane potential maintained by an electrochemical imbalance of ions across the membrane. Potassium (K^+) concentrations within the cell form a crucial part of this balance and efflux of K+ maintains the hyperpolarised state of the cell. Potassium flux in glomus cells is maintained and regulated by several different classes of potassium channel, those that exhibit sensitivity to hypoxia are: K_v channels (voltage gated), K_{Ca} channels (Ca^{2+} activated) and TASK like K^+ channels. K_v channels are activated usually by voltage changes or depolarisation across the membrane; whereas K_{Ca} channels open in response to an increase in intracellular Ca^{2+} (a result of membrane depolarisation). Two pore acid-sensitive (TASK) type K^+ channels are unlike the other channels in that they possess two transmembrane ion pores and remain open at negative membrane potentials.

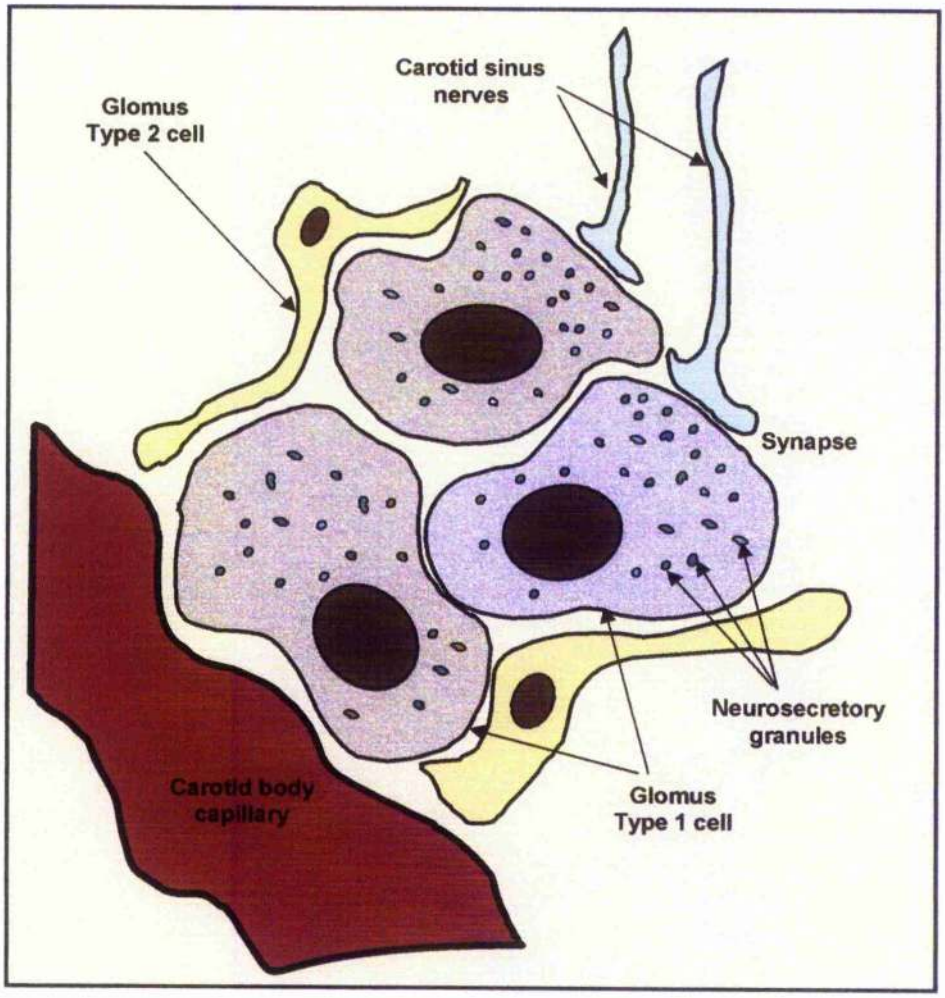

Figure 1.6. The carotid body with type 1 glomus cells in close apposition to the glomerular capillary and sustentacular type 2 cells. The type 1 cells contain neurosecretory particles that release into the synaptic cleft between carotid sinus nerve and glomus cell.

TASK channels appear to have a basal current of K^+ and maintain basal membrane potential. TASK channels are not usually sensitive to hypoxia; however in the carotid body, channels with similar properties to TASK channels have been identified [18]. Inhibition of these K^+ channels results in depolarisation of the membrane and influx of calcium through voltage gated L-type calcium channels (Long lasting Ca^{2+} channels). Calcium influx seems to be the critical event in glomus cell activation since calcium channel blockers inhibit the effect of hypoxia on glomus cells and activation of glomus cells is not possible without extracellular Ca^{2+} [19, 20]. Furthermore, dopaminergic release from glomus cells has been demonstrated in response to Ca^{2+} influx [21].

Which of these channels is responsible for the agonal depolarisation event in response to hypoxia is yet to be discovered, though it may be that each plays a role in membrane depolarisation; however the question that has generated much debate between researchers in the field is: how is oxygen sensed?

Oxygen sensing in the carotid body

The coupling of oxygen tension to the observed changes in glomus cell ion channels remains unknown; however intense research in this field has proposed several plausible models for oxygen sensing and transduction of this signal. The earliest of these is the role of the mitochondrion as an oxygen sensor [22], though alternative hypotheses of reactive oxygen species generation (ROS) and membrane bound haem-oxidases have been proposed as alternative mechanisms of oxygen sensing. This is a brief review of these different perspectives.

The evidence supporting mitochondrial involvement in oxygen sensing stems originally from the observation that uncoupling of the ETC by mitochondrial poisons (see figure 1.4) results in an increase of carotid sinus nerve discharge [22]. Furthermore these uncoupling molecules result in inhibition of K^+ channel flux [18]. The suggestion is that ATP generation is an integral feature of oxygen sensing; however work by López-Barneo *et al.* suggests a different explanation. Their experiments demonstrate a quantal increase in neurotransmitter release in response to hypoxia and the mitochondrial uncouplers: rotenone, antimycin A, myxothiazole and cyanide (figure 1.4). They hypothesised that hypoxia in addition to an uncoupling agent would give an additive signal if each agent acted at a separate site; this they found for all the uncoupling molecules, except rotenone. Rotenone did not demonstrate any additional effect with hypoxia, suggesting its action at complex one of the ETC; however the action of other complex I antagonists did not reproduce these results. Furthermore, 1-methyl-4-phenylpyridinium (MPP^+), a molecule which acts at the same site on complex I reproduced the effect. They surmised that another, yet unknown molecule, was crucial in glomus cell oxygen sensing and that this molecule was sensitive to rotenone [23]. This would seem to be supported by the fact that rotenone inhibits K^+ channels in cells with no mitochondria [24].

The second theory proposes a role for ROS in the oxygen sensing signal pathway; however there is disagreement as to whether increases in ROS are stimulatory or inhibitory. Reactive

oxygen species arise from either inefficiency in mitochondrial electron transfer or via the action of cytoplasmic molecules such as NADPH oxidase. The molecules formed are superoxide ions (O_2^-) which is transformed to hydrogen peroxide (H_2O_2) by superoxide dismutase. This in turn is converted to water by catalase or glutathione peroxidase [25]. The problems with this model are the inconsistencies in ROS generation. Proponents of the NADPH oxidase pathway postulate that in normoxic conditions, the availability of O_2 facilitates the production of ROS. In hypoxic conditions the production of ROS is diminished thereby altering the REDOX state of K^+ channels [26]. There is conflicting evidence to support this hypothesis, neuroepithelial cells demonstrate enhanced activity in knock out mice deficient in a NADPH oxidase subunit[27], but there are also reports that suggest either no effect or the opposite of NADPH oxidase knockout[28].

The alternate model proposes increases in mitochondrial ROS generation in response to stalling of the ETC at complex IV (due to the absence of O_2 for the final oxidation step). Reactive oxygen species can then form at complex I and complex III of the ETC and the use of antagonists at various points has demonstrated increased generation of ROS points before complex III of the ETC [29]. The role of increased ROS in the carotid body's responses to hypoxia is further supported by studies in intermittent hypoxia. Under these circumstances there appears to be an amplification of the hypoxic response which is inhibited by the effect of superoxide dismutase [30].

Research in the field of oxygen sensing at the tissue level is fertile as well as contentious, it is complicated by numerous factor, not least of which is the variability in response of different tissues, let alone different species. The two models reviewed are currently the most favoured theories for oxygen sensing, other theories include direct oxygen sensing by ion channels and the effects of nitric oxide and carbon monoxide (produced by haem-oxygenases) as intracellular second messengers [31], these have not been discussed here in detail since the purpose of this review is to highlight the possible mechanisms in a field that is continually changing with new discoveries. As will be described in the section reviewing hypoxic pulmonary vascular responses, similar mechanisms have been proposed as a model for oxygen sensing; however the body of work in this field is more extensive as is the controversy.

1.3.4 Ventilatory signal transduction in the brainstem

The peripheral and central mechanisms of chemosensation are integrated in the brainstem to affect response at the ventilatory motor centres located in close proximity. The relay pathways for central chemosensation remain unclear, though muscarinic neurones have been implicated in the transduction of this signal [12]. The peripheral chemoreceptors relay their afferent input to the nucleus of the tractus solitarius and the first synaptic relays from the carotid sinus nerve to the brainstem reside in the nTS [32]. Retrograde tracer studies have identified several areas of the nTS that receive dense innervation from peripheral chemoreceptors. Furthermore, microinjection studies using neurotoxins at the commissural area of the nTS demonstrated an attenuation of the hypoxic response whereas lesions induced in other areas had no effect [33]. The nTS response during the early part of the hypoxic ventilatory response appears to be mediated via the action of excitatory amino acids, glutamate in particular. Inhibition of N-methyl-D-aspartate (NMDA) glutamate receptors attenuates the hypoxic ventilatory response across several species [34-36]. The role of glutamate in the ventilatory response to hypoxia is also evident at points downstream from the nTS. The role of NMDA receptors in excitation of expiratory bulbospinal neurones has been demonstrated in dogs, and increases in phrenic nerve discharge seen in cat and rat models by both NMDA and non-NMDA mechanism [37-39]. The brainstem neural pathways have yet to be fully described; however the role of the nTS appears to be central in the acute response to hypoxia.

1.3.5 Hypoxic ventilatory response

Ventilation increases in response to hypoxia as a response to hypoxaemia, as arterial oxygen partial pressures fall below 60 mmHg the hypoxic ventilatory response (HVR) becomes the dominant drive to ventilation. The carotid bodies are responsible for this response, since in addition to hypoxia induced neurotransmitter release, the hypoxic response is abolished by carotid body denervation in a number of species [40] Hypoxic response eventually returns in these animals and this seems attributable to the aortic bodies assuming the role of the peripheral chemoreceptor [41]. The denervation of both vascular bodies does not abrogate the hypoxic response either and there is evidence that suprapontine structures have the capacity to take over hypoxic sensation [42]. This suggests that there are several sensory systems for oxygen homeostasis, 'hardwired' into the ventilatory control system and underlines the importance of maintaining adequate cerebral oxygenation.

The effect of hypoxic exposure can be seen within one breath of PaO_2 change at the carotid body by increases in phrenic nerve discharge [43]. The hypoxic drive to ventilation results in a lower CO_2 tension and a rise in pH as CO_2 elimination is increased as a consequence. This has two effects: firstly there is reduced ventilatory stimulus at the central chemoreceptors; secondly there is a relative inhibitory affect upon the carotid bodies themselves. The carotid bodies are sensitive to lower pH and higher CO_2; in combination with acute hypoxia, both have a synergistic effect and enhance the carotid response to hypoxia [40]. A decrease in this stimulus has the potential to attenuate the carotid body response to hypoxia. In addition, the hypoxic response demonstrates a biphasic response, with the initial rise in ventilation declining with time to a level above normoxic ventilation in adults. This is independent of the effects of CO_2, since isocapnic experiments have shown this to be a truly hypoxia dependent phenomenon [44, 45]. The time course of the decline occurs between 5 and 30 minutes of hypoxic exposure and has been attributed to mechanisms within the central nervous system [43, 46]. An attenuating effect on the hypoxic ventilatory response may result either from a decreased CO_2 stimulus or direct effects on the ventilatory centres; however the net effect is an increase in ventilation in response to acute hypoxia. Once dissected out using isocapnia, the relationship between ventilation and arterial oxygen tension is hyperbolic. In contrast the relationship between ventilation and oxygen saturation is linear down to saturations of 70%, mainly as a result of the sigmoid nature of the oxygen dissociation curve. These responses are seen in all mammals, including man. Furthermore, there is a marked reproducibility of the acute hypoxic ventilatory response for an individual, but a wide variation between individuals. [47].

With more prolonged exposure, over days and weeks there is a further increase in the hypoxic ventilatory response and enhancement of glomus cell responsiveness is thought to be contributory [48]. Inhibition of dopaminergic neurotransmission at the carotid body as a possible mechanism has also been examined, and though there is some evidence to support this model in cats [49], there is lack of evidence in humans [50]. The responses described are over the first few minutes then days and weeks of altitude exposure. It is of interest that a lifetime's exposure to altitude can result in a blunted response to hypoxia. The change is associated with a diminished hypercapnic drive and in some subjects a near absence of HVR [51].

1.4 Skeletal muscle function and adaptation

The previous sections have described the processes of energy generation at the cellular level and the influences that O_2 demand and CO_2 elimination impose on the control mechanisms of ventilation. Ventilatory control is tightly coupled to CO_2 production in normoxic conditions; whereas oxygen demand is the driving force to ventilation in hypoxic conditions. The dynamic nature of exercise both in terms of onset and variability in intensity has profound effects at both the cellular and the ventilatory level for these reasons. The following section considers the function and demands of respiring muscle, as well as the utilisation of substrate for energy generation, before considering the effects exercise has on ventilation under both conditions of normoxia and hypoxia.

1.4.1 Structure

Skeletal muscle mainly comprises of regimented arrays of muscle fibres or myocytes arranged in parallel, each fibre is a multinucleated cell encapsulated by a cell membrane or sarcolemma. Each muscle fibre consists of numerous myofibrils, the functional unit of which is the sarcomere. Myofibrils contain 4 main proteins: myosin, actin, tropomyosin and troponin. Myosin forms thick filaments which interdigitate with actin thin filaments. There are binding sites on actin for ATPase subunits of the larger myosin molecule; the interaction between the two is prevented at rest by tropomyosin, which covers these binding sites. Troponin is attached to tropomyosin; contraction depends on an influx of Ca^{2+} which binds to troponin and results in a shift in tropomyosin, revealing the myosin binding sites. The ATPase subunits on the myosin molecule then attach themselves and ATP is hydrolysed; this results in shortening the myofibril by moving thick filaments along the actin filaments. Once ATP is hydrolysed the myosin head uncouples from actin, the process is repeated as long as Ca^{2+} and ATP are available. Muscle contraction is initiated by depolarisation of the sarcolemma and conduction along T-tubules that penetrate from the sarcolemma, this results in the release of intracellular Ca^{2+} from stores held in the sarcoplasmic reticulum. The termination of contraction is dependent on the removal of Ca^{2+} into the sarcoplasmic reticulum and is an active process, requiring energy. Muscle fibre depolarisation and contraction is under the control of motor neurones, which synapse on the fibres at the motor end-plate. Multiple muscle fibres are innervated by a single motor neurone and constitute a motor unit.

1.4.2 Muscle fibre types

The basic architecture of myocytes is the same in every skeletal muscle, but there are different sub-populations of myocyte. There are two main types of muscle fibres in humans; type I and type II. They differ in the speed of contraction, oxidative capacity and substrate stores. Type I or 'slow twitch' fibres are characterised by a slower activity in myosin ATPase, troponin activity and calcium re-uptake to sarcolemma. They have greater stores of myoglobulin, lipid and higher levels of oxidative enzymes; hence they are geared for oxidative function and are slower to fatigue. Type II or 'fast twitch' fibres are more dependent on glycolysis for energy generation, as a consequence have fewer mitochondria and little myoglobulin stores. These cells posses a faster myosin heavy chain isoform than type I cells [52]. Furthermore, type II myocytes are further subdivided according to their speed of contraction and their dependence on oxygen for energy generation. These are identifiable by different myosin heavy chain isoform, types IIa, IIx and IIb, with varying oxidative capacity from high to low [53]. The slow twitch fibres are presumed to have a greater role to play in sustained work, maintenance of posture and have better endurance than the fast twitch fibres. In addition, the distribution of fibre type differs according to the location and function of the muscle. There is a capacity for switching between muscle fibre types and the transition between type II fibre types can be stimulated by physical training and muscle stretching; this process has been attributed to the degree of Ca^{2+} influx related to the frequency of depolarisation events [54]. The switch between type I and type II cell types in humans is less straight forward. Muscle fibre subclasses differ not only in their biochemical structure, but also in their innervation. It appears that the neurones that innervate a particular motor unit can determine the properties of those muscle fibres. The switch between slow and fast fibres by cross innervation was first demonstrated by Vrbová in 1963 [55], and there is a demonstrable difference in the motor neurone morphology innervating these types of fibre [56]. Nevertheless, the transition between type I and II suggested by animal experiments does seem to occur in humans [57, 58].

1.4.3 Muscle adaptations to hypoxia

The different types and subtypes of muscle fibre represents a level of individual variability in the response to exercise and the potential effects of hypoxia on exercise; furthermore the composition of muscle is amenable to the effects of physical training and therefore is not a fixed entity for an individual. Aside from the type of myosin chains expressed in these fibres

the concentrations of mitochondria, myoglobulin and capillary density have implications for oxidative capacity and endurance during exercise. These demonstrate plasticity in response to exercise and hypoxia. Endurance training results in an increase in muscle size, mitochondrial concentration and capillary density. The effects of activity at altitude are somewhat different. Animal experiments demonstrate loss of muscle mass on hypoxic exposure [59] which is also evident in humans [60, 61]. Exposure to high altitude over a period of 2 months, such as that encountered on mountaineering expeditions, resulted in a loss of body mass with a similar decrease in muscle volume. The loss in muscle mass was reflected in a decrease in muscle cross-sectional area of around 20% [62], a similar decrease in both type I and II fibres was reported during the hypobaric chamber study: Operation Everest II [63], but there was no evidence of transition from Type I to Type II fibres [64]. Capillary density increases with altitude exposure, but the same studies demonstrate the fibre to mitochondria ratio is preserved. The effect is thought to reflect muscle atrophy in hypoxia rather than neovascularisation. The muscle mitochondria are also diminished with similar altitude exposures, with a proportional loss of sub-sarcolemmal mitochondria in preference to interfibrillar populations [62]. The combined effect of this duration of exposure is a reduction in diffusion distance, but also a reduction in the oxidative capacity of muscle; however the maintained capillarity could reflect improved oxygenation of the remaining mitochondria.
Though the responses described are not a significant factor during short exposures to hypoxia, they are an important feature during longer exposures of hypoxia (weeks rather than minutes).

1.4.4 Muscle metabolism

The fundamental mechanisms for energy generation at the respiring muscle are the same as those utilised by all tissues, but there are some particular considerations in reference to muscle. Firstly, the energy requirement of muscular contraction at the onset of exercise is rapid; however the absolute quantities of ATP in the myocyte are relatively small and are insufficient to meet this demand. Muscle fibres contain a store of energy in the form of phosphocreatine a molecule present in myocytes which also possesses a high energy phosphate bond. Cleavage of this molecule provides the energy for the regeneration of ATP during initial muscular contraction. Again there are only limited intracellular stores of phosphocreatine which are quickly consumed on commencing exercise, but these are also regenerated by ATP once the processes of cellular respiration have increased to meet

muscular demand. Phosphocreatine is a weak acid in its phosphorylated state and its consumption at the onset of exercise results in a net increase in intracellular pH early in exercise.

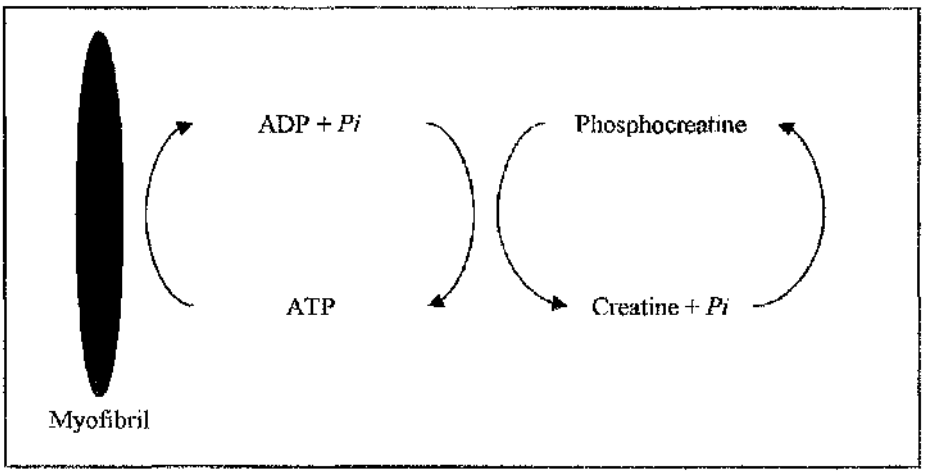

Figure 1.7. The phosphocreatine shuttle

Secondly, the mass of muscle used in physical activity can be considerable, with a sudden increase in oxygen demand and substrate utilisation; the net effect of these metabolic changes at the cellular level are reflected in the individual's oxygen consumption (VO_2) and carbon dioxide production (VCO_2). The type of substrate used for respiration, determines the stochiometric relationship between the amount of oxygen required for its complete metabolism and the CO_2 produced in the process. This can be described by the ratio of VO_2 to VCO_2 to give a respiratory quotient (*RQ*) for a particular substrate. In the earlier discussion, where glucose is metabolised, oxygen consumption and carbon dioxide production is stochiometrically equal; this gives an *RQ* of 1. In muscle, after stored glycogen, lipids form the next most abundant store of substrate for respiration. Palmitic acid is a free fatty acid which when metabolised aerobicall, liberates 16 molecules of CO_2, using 23 molecules of O_2 to give an *RQ* of 0.73. In oxygen terms, FFA is a costly substrate, but more efficient in terms of CO_2 production. In normoxia CO_2 tensions are the dominant stimulus to ventilation and therefore lipid metabolism can be viewed as less of a ventilatory burden; furthermore each molecule of palmitate gives rise to 130 molecules of ATP, which makes lipid an ideal substrate store for myocytes. In reality the average western diet results in an *RQ* of 0.8.

Measuring oxygen consumption and carbon dioxide at the cellular level is not usually performed, nor is it a feasible undertaking in an exercising system; therefore *RQ* is usually

inferred from the measurements of VO_2 and VCO_2 to give a gas exchange ratio (*R*). The implications of using R as a surrogate indicator of gas exchange at the cellular level are important when the body's capacity for CO_2 storage is taken into account. The body's storage capacity for CO_2 also carries implications for CO_2 coupling of ventilation to pulmonary gas exchange during exercise.

1.5 Dynamic changes in ventilation during exercise

1.5.1 Phase one & two of the 'on-transient'

At the onset of exercise there is an increase in oxygen demand and carbon dioxide production. Ventilation also increases at the onset of exercise, but the changes exhibited are not entirely coupled to metabolic change. During low to moderate intensity constant-load exercise VO_2, VCO_2 and ventilation (V_E) all increase before levelling out once steady state is achieved. This period, termed the 'on-transient phase', is further characterised by two phases. Phase one (or early dynamic phase) occurs immediately at the onset of exercise, with a near instantaneous increase in ventilation [65] and VO_2 (in response to a corresponding increase in cardiac output and pulmonary blood flow), the mechanism remains unclear since the time course is too rapid to be linked to muscle metabolism. Historically this effect has been attributed to neurogenic factors originating from exercising limbs and directly affecting the respiratory centres, but the degree of initial hyperpnoea is relatively constant and independent of the number of exercising muscle units since increasing load produces no demonstrable increase in initial hyperpnoea [66]. Furthermore there is an observed increase in ventilation on commencing unloaded exercise, but no additional hyperpnoea component when a load is subsequently imposed [67]. Phase one lasts from 10-15 seconds before the onset of phase two or late-dynamic phase which does demonstrate a relationship between muscle metabolism and gas exchange at the lung. VO_2 increases in a mono-exponential fashion, but increase in VCO_2 has a demonstrable lag, and since ventilation and arterial CO_2 are coupled there is also a lag in V_E. This is due to the capacity for CO_2 storage in muscle itself and the blood flowing from a respiring muscle [67]; therefore VCO_2 only demonstrates an increase once these stores are saturated. This means that the CO_2 drive to ventilation temporarily lags behind the increase in VO_2 during exercise and as a consequence there is a brief transient hypoxaemia at this point [68, 69].

1.5.2 Carotid body influences

Phase one occurs during the first few seconds of exercise, whereas the duration of phase two can demonstrate some variability. The carotid bodies have an influence on this time course, as demonstrated by experiments designed to influence carotid body input. Carotid body stimulation using hypoxia or metabolic acidosis (by ammonium chloride ingestion) can shorten phase two duration [70, 71]; whereas reducing peripheral chemosensitivity with hyperoxia, metabolic alkalosis (by sodium bicarbonate ingestion), dopamine infusion or bilateral carotid body resection can lengthen the duration [70-74]. At low and moderate exercise intensities (i.e. below anaerobic threshold) metabolic and ventilatory variables stabilise at a 'steady-state' level, this is labelled 'phase three' exercise. The transition through phase one and two on to steady state is usually complete in the first 3-4 minutes of exercise during low to moderate intensity exercise. During high intensity, exercise steady state is not achieved because of lactate accumulation. Identifying and correctly labelling the point at which lactate contributes significantly to the metabolic influence on ventilation-gas exchange coupling has been a topic of controversy.

1.5.3 Anaerobic, Lactate and Ventilatory Thresholds

Exercise at low to moderate intensity with a constant load eventually results in steady state; however serial increments in the work load would eventually reach a point where steady state could not be attained before exercise is terminated because of fatigue (figure 1.8). The reasons for muscle fatigue are manifold, but in the simplest of terms the oxygen supply to the muscle is outstripped by demand. The effects of cardiac output limitation and circulatory variations across the exercising muscle as a contribution to lactate build up are discussed in the review of cardiovascular considerations during exercise further in this chapter; however the importance of oxygen in the increased rate of lactate production is demonstrable by experiments with various inspired oxygen concentrations. Hyperoxia delays the onset of lactic acidosis for a given work rate, whereas hypoxia reduces the lactate threshold and increases the concentration of lactate for a given high intensity work rate [75]. The emphasis is on the rate of production, since lactate is utilised as an energy source in muscle (in the presence of oxygen) and by the liver for gluconeogenesis. Lactate accumulates when production outstrips demand; the point of increased lactate production can be measured directly by arterial blood sampling, either from an exercising muscle group or the whole body. This requires sampling of blood via an arterial or venous catheter and then

measurement of lactate biochemically. Modern blood gas analysers with lactate electrodes are capable of performing this measurement quickly and reliably. This makes real time measurement of lactate feasible, but there is an inherent time course from the onset of net anaerobic respiration at the muscle to a measurable increase in lactate. The point at which this occurs *was* called the anaerobic threshold (AT), but a more accurate description is the lactate threshold (LT).

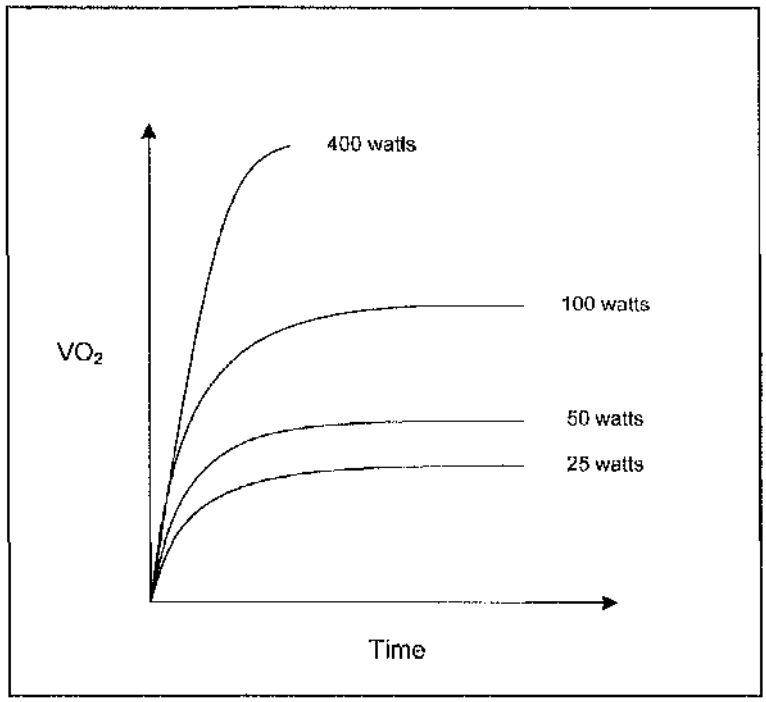

Figure 1.8. VO_2 increases with work loads in serial steady state exercise tests (25, 50 and 100 watts). In situations which are at the highest work intensity above anaerobic threshold, steady state is not achieved because exercise is terminated by fatigue (400 watts).

The increased rate of lactate production also has effects on ventilation and gas exchange. The ionised lactate liberates H^+ ions which are predominantly buffered by bicarbonate. This generates CO_2 which stimulates an increase in ventilation and eliminates the excess CO_2 in expired gas. Calculations based on the proportion of CO_2 produced for aerobic and anaerobic mechanisms of respiration during the production of the same amount of ATP, result in an estimated 2.5 fold increase in CO_2 production [76]. The term 'isocapnic buffering' is used to describe this portion of high intensity exercise, since arterial CO_2 tension is maintained at a constant level through the coupled increase in ventilation; however VO_2 is not coupled to

ventilation in normoxic conditions and there is a divergence of VO_2 from VCO_2 and ventilation. This point is evident during incremental exercise tests and is the central basis for non-invasive estimation of anaerobic threshold at high work intensity. The term used for the point of divergence is the ventilatory threshold (VT) and can be determined by a number of measured and calculated variables from a standard incremental exercise test. A plot of VO_2 against VCO_2 demonstrates an inflection point at the ventilatory threshold (Figure 1.9); monitoring end-tidal CO_2 (PetCO2) and end tidal O_2 demonstrates a similar divergence with an increase in PetCO2 at the VT. The degree of increased ventilation is reflected in the efficiency of ventilation to clear CO_2 and is determined by the alveolar CO_2 and arterial CO_2 partial pressure gradient or 'set-point' at the onset of exercise ($PaCO_2$ and P_ACO_2 respectively) and the amount of physiological dead space (V_D/V_T). Calculated values such as ventilatory equivalents for CO_2 and O_2 are useful indicator for this and represent ventilation in relation to CO_2 production and O_2 consumption (V_ECO_2 and V_EO_2 respectively).

$$V_EVCO_2 = V_E / VCO_2$$

$$V_EVO_2 = V_E / VO_2$$

As VCO_2 and V_E are coupled (allowing for the small variations in body CO_2 stores) then there is little discernable change in V_EVCO_2 when VT is reached, however the marked increase in ventilation is evident in the ventilatory equivalent of oxygen since VO_2 is not coupled to ventilation (in normoxia) there is a marked increase in V_EVO_2. Furthermore the effects of CO_2 storage in the body has to be taken into account since there is evidence that altering pre-test stores using measures such as hyperventilation can affect estimation of VT [77].

The use of gas exchange measurements to non-invasively estimate AT has limitations, but is useful as long as these limitations are taken into account, consequently it is important to make a clear distinction between the two and the term ventilatory threshold (VT) is used for this inferred measure of AT. Furthermore exercise at work rates below the anaerobic threshold (providing they are at constant load) allow the entire system of ventilation, oxygen delivery and consumption to reach metabolic homeostasis or 'steady state'; this is termed 'Phase three' exercise. For the purposes of the studies performed in this thesis measurement

of the VT was used to determine a level of exercise that would be high enough to provide a useful stressor, but not sufficient to result in anaerobic respiration and its effect on ventilation.

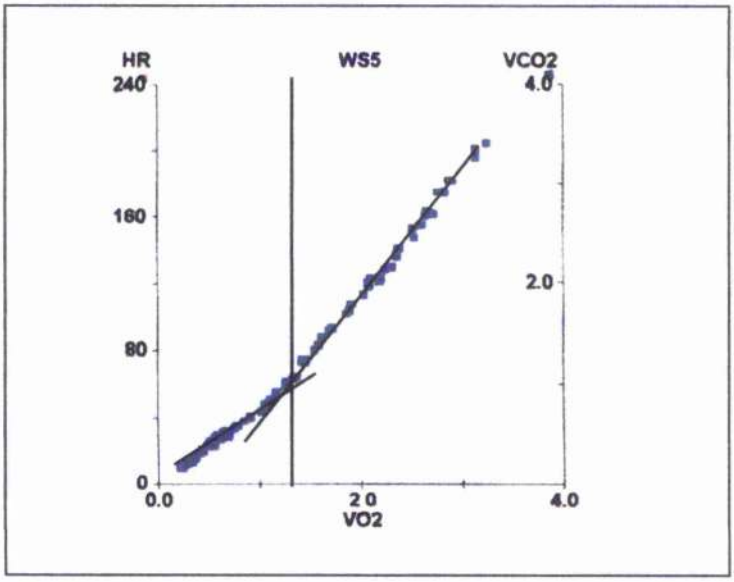

Figure 1.9. A panel from a standard incremental exercise test. The plots VO_2 against VCO_2 (right hand vertical axis), the vertical bar demonstrates the inflection point at the ventilatory threshold.

1.5.4. Hypoxic ventilatory responses during exercise

The discussion thus far has described the response of ventilation to hypoxia and exercise, as well as the interaction of $PaCO_2$ and PaO_2 at the central and peripheral chemoreceptors. Acute hypoxia during exercise further augments the increase in ventilation normally coupled to VCO_2, but under hypoxic conditions the increase in ventilation during exercise is mediated by the carotid bodies rather than CO_2 drive to the brainstem [78-80]. The effects of diffusion limitation at the lung and muscle under conditions of ambient hypoxia and the resultant hypoxaemia are discussed in the section dealing with cardiovascular response; however, exercise under such conditions increases the degree of hypoxaemia through increases in O_2 extraction, hence further stimulation of the carotid bodies. Carbon dioxide and pH changes during hypoxic exercise certainly have an influence on the response both in absolute terms and in the dynamic phases of exercise. The interpretation of incremental exercise under

conditions of hypoxia is complex given the variables, but the tests performed as part of the experiments described later were designed to exercise under constant load at low to moderate exercise intensity in order to avoid exercising above the VT, even under conditions of hypoxia. This was deliberate, in order to ensure steady state for measurements, but also to avoid the dynamic shifts in $PaCO_2$ and pH that might further influence carotid body drive to ventilation if anaerobic muscle metabolism were to become dominant. However at work rates below VT and at steady state the effect of an augmented carotid body stimulus increases total ventilation for a given work load, whilst reducing $PaCO_2$ and raising pH; indeed it is one compensatory measure to overcome the effects of pulmonary diffusion limitation [80].

A final point is that as during normoxia the use of lipid substrate stoichiometrically produces less CO_2 per molecule of O_2 than the use of glucose (23 O_2 & 16 CO_2 vs. 6 O_2 & 6 CO_2 for the metabolism of palmitate and glucose respectively). A consequence is a reduced burden on ventilation during normoxia; however ventilation is dependent on hypoxaemic drive during hypoxic exercise, hence palmitate would not be an ideal substrate in hypoxic environments because of the heavier oxygen burden it incurs. This suggests that carbohydrate, as a source of substrate, is a more efficient choice.

1.6 The pulmonary circulation: adaptations to hypoxia and exercise.

The fundamental necessity of oxygen in energy generation has been described, as have the mechanisms required to ventilate air from the environment to the gas exchange surfaces. The following section reviews the convective mechanisms that circulate blood across the lung and then deliver oxygen bound to haemoglobin in that blood to the respiring tissues. Each circulatory system is intrinsically bound to the other, but is very different in their responses to hypoxia. Both are reviewed in the following sections, with a particular emphasis on the pulmonary circulation and the cardiac output response to exercise and hypoxia.

1.6.1 The Pulmonary circulation

In contrast to the systemic circulation the role of the pulmonary circulation is to efficiently circulate deoxygenated blood from the body via the right side of the heart and through the pulmonary capillaries to allow oxygenation. The function of gas exchange necessitates the reduction of impedance to diffusion wherever possible; consequently, the lung has evolved a thin and attenuated interstitial space, minimizing the distance between the alveolar space and the pulmonary capillary lumen. In order to perfuse such a system without resulting in

haemodynamic stress and capillary damage, the pulmonary circulation has evolved into a low-pressure, low resistance system; this is not to say that it is not capable of accommodating high blood flows. The systemic circulation supplies the entire body, from the resting state up to and including exhaustive exercise; however the dramatic increases in cardiac output would not be possible without a matching preload at the left ventricle. This arrives at the left cardiac chambers after oxygenation by the lungs; therefore, even at maximal exercise the right sided cardiac output must match the left, and the pulmonary circulation must be able to accommodate this flow.

The pulmonary circulation is unique in its vasoconstrictor response to hypoxia (hypoxic pulmonary vasoconstrictor response or HPVR); this enables the matching of pulmonary blood flow to well ventilated segments of lung. The response is to alveolar hypoxia, as opposed to hypoxaemia, as seen in experiments using simultaneous, but separate ventilation of the left and right lung. A rise in pulmonary vascular resistance (PVR) is demonstrable in the hypoxic lung whilst normal flow is maintained in the hyperoxic lung (FiO_2 100%); this is in the absence of mixed venous oxygen desaturation during unilateral hypoxia [81]. The HPVR has a function in the transition from the in utero environment, where the foetus's oxygen requirements are met by the maternal circulation via the placenta. There is little need to ventilate the lungs in a fluid environment and the lungs remain in a collapsed state; consequently they have a high vascular resistance. Outflow from the right ventricle bypasses the lungs via the ductus arteriosus and into the aorta. At birth, with the first breath of life, the lungs expand with air causing the pulmonary vascular resistance to drop. In addition, the oxygen rich environment causes pulmonary vasodilatation and a further drop in vascular resistance; this causes right ventricular blood flow to enter the lungs, bypassing the ductus arteriosus, which closes [82]. In post-uterine life, the benefits of the HPVR are optimization of pulmonary perfusion to alveolar ventilation (V_A); this is especially relevant in humans due to the erect posture. Pulmonary blood flow favours the bases because of the effects of gravity, but despite the better ventilation (due to diaphragmatic contraction), blood flow is in excess of alveolar ventilation. Therefore, there is variation in ventilation and perfusion across the lung. The hypoxic pulmonary vascular response ameliorates this situation by diverting blood from poorly ventilated segments of lung to better ventilated segments; however, even in an optimized state of perfusion to ventilation, ratios are estimated at 0.8. A further adaptive benefit of the HPVR is implicit during illnesses that cause pulmonary atelectasis, such as acute asthma or pneumonia, where the shunting of blood from affected or diseased

segments of lung to well ventilated regions could influence recovery or survival. Animal models of such conditions confirm the shunting of pulmonary blood flow away from these poorly ventilated areas whilst maintaining oxygenation [83].

1.6.2 Pulmonary vascular responses to hypoxia

Isolated lung specimens demonstrate elevated pulmonary artery pressure (PAP) within seconds of hypoxic challenge [84] and vary according to the degree of hypoxia [85]. A rise in pulmonary artery pressure and vascular resistance are seen during right heart catheter studies in hypoxic conditions; this occurs in the absence of raised pulmonary capillary wedge pressure (PCWP) and indicates the effect is due vasoconstriction in the pre-capillary vessels [86-88]. In vitro pulmonary artery specimens [89, 90] and cultured pulmonary artery smooth muscle cells (PASMC) [91] contract in hypoxic conditions; however there is controversy surrounding the mechanism of pulmonary vasoconstriction and the role of the endothelium in this response.

Hypoxic pulmonary vasoconstriction is a rapid process, which demonstrates a biphasic response; an initial phase of vasoconstriction is seen that lasts between 10-15 minutes which then subsides, but not to baseline levels. A second slower phase then develops which reaches a maximum at 40 minutes [92]. Phase one is evident in isolated pulmonary artery smooth muscle cells (PASMC) and in specimens denuded of the endothelium; however the slower sustained phase two does require the presence of an intact endothelium. [93-95]. These findings suggest the importance of the endothelium in effecting phase two of the HPVR; however a more recent report by one group disputes this and suggests that there is a monophasic response which is independent of the endothelium [96]. The distinction this group makes is that hypoxic vasoconstriction is a property of PASMCs and that the endothelium acts by modulating this effect. This disagreement is yet to be resolved; however it does lead to the second point of controversy surrounding the HPVR: what is the mechanism of pulmonary artery vasoconstriction and how is hypoxia sensed in the pulmonary circulation?

Pulmonary arterial smooth muscle responses to hypoxia

Smooth muscle activity in common with all muscular contraction is dependent on cell membrane depolarisation and the subsequent influx of calcium ions. PASMCs have a resting membrane potential between -40 to -60 mV, the potential difference is the result of ion differences across the membrane. Key to maintaining this potential difference is membrane bound potassium channels and in this they share a similarity with carotid body glomus cells; however they do differ in the types of potassium channels. Whereas K_{Ca+} channels are an important factor in carotid bodies, they are not a major influence in maintaining PASMC trans-membrane potential in the adult [17, 97]. Voltage-gated K^+ (K_V) channels appear to be the oxygen responsive channel in PASMCs; there are several families of K_V channel, each with several isoforms (denoted by the nomenclature K_V 1.x - K_V 9.x) [98]. Of these, the K_V 1.5 and 2.1 appear to be responsible for the HPVR [17, 96]. These channels maintain an open state at resting membrane potentials, allowing K^+ efflux from the cell (down an intracellular: extracellular concentration gradient of 145mM to 5mM), thereby hyperpolarising the cell membrane. Hypoxia inhibits this effect and allows membrane polarity to rise, once the critical trans-membrane potential of -30mV is reached calcium channels are activated and allow the influx of Ca^{2+} into the cytoplasm. In addition to K_V channels, a population of TASK-like channels are also expressed in PASMCs (similar to those described in type I glomus cells). These have a demonstrable response to hypoxia and seem to form part of the HPVR seen in PASMCs [99].

There are points of controversy in this simplified pathway: what are the mechanisms of oxygen sensing; which K^+ channel is responsible for the agonal depolarisation seen during HPVR and does depolarisation release intracellular Ca^{2+} stores in addition to extracellular stores? The question of which channel is responsible for the HPVR remains hotly contested, whereas the question of oxygen sensing in PSMCs and the role of calcium in the HPVR is reviewed below.

Oxygen sensing in pulmonary artery smooth muscle.

The actual mechanism of oxygen sensing during hypoxic vasoconstriction remains disputed, K^+ channels possess domains that have potential oxygen sensing capabilities; however there is no direct evidence that they are solely responsible for signal transduction. The focus has direct towards the role of ROS and mitochondria, similar to the mechanisms discussed in carotid body signal transduction. Initial theories that mitochondria themselves are responsible

for HPVR through impaired production of ATP are not supported simply because the degree of hypoxia required to elicit the HPVR is not sufficient to limit mitochondrial ATP production significantly[100, 101]. The observed effects of ETC inhibitors that mimic HPVR [102] and attributed to this role have now been reinterpreted in light of the emerging role of ROS as intracellular second messengers for oxygen sensing [96]. Similar to the debate on carotid body oxygen sensing mechanisms, the controversy centres on whether an increase or decrease in ROS is responsible for oxygen sensing. The focus has been the role of mitochondria as a source of ROS, at complex I and III of the ETC. These issues have been recently reviewed at length and many discrepancies between conflicting studies have been attributed to differences in method; however the role of the ETC in the generation of ROS and their importance in oxygen sensing in PASMC is generally agreed [96, 98, 103].

Calcium influx/ release and PASMC contraction

Calcium is as essential for contraction in smooth muscle as it is in skeletal muscle. The source of calcium for contraction was thought to be predominantly extracellular through the action of voltage operated L-type Ca^{2+} channels (VOCC), in response to membrane depolarisation elicited by K^+ channel inhibition by hypoxia [104, 105]. This model of activation is supported by the observed attenuation of pulmonary hypertension in suffers of chronic pulmonary disease by L-type channel blockers (e.g. Nifedipine) [106]; however intracellular stores of Ca^{2+} are also an important contributory source for PASMC activation [107]. Depletion experiments have shown the importance of calcium release from the sarcoplasmic reticulum of PASMCs and their contribution to HPVR [108, 109]; furthermore depletion of these stores facilitates extracellular Ca^{2+} influx (whilst these stores remain deplete) and form a basis for sustained contraction during prolonged hypoxic exposures [110, 111]. The channels responsible for what is termed 'capacitative calcium entry' are labelled store operated Ca^{2+} channels (SOCC) and their contribution to HPVR has recently been described [112]; furthermore inhibition of these store operated Ca^{2+} channels inhibits HPVR by pulmonary artery smooth muscle [113].

Similar to skeletal muscle, calcium is required for contraction; however the mechanism of excitation-contraction coupling is different. Smooth muscle contains actin and myosin as in skeletal muscle, but contraction is modulated by calcium-calmodulin dependent myosin light chain kinases (MLCK) which phosphorylate myosin light chains, thereby enhancing myosin

ATPase activity and contraction [114]. Myosin phosphorylation therefore has a direct influence on contraction dependent on the balance between phosphorylation and dephosphorylation (mediated by myosin light chain phosphatase – MLCP). Rho-kinase is an inhibitor of MLCP and pushes the balance towards myosin light chain phosphorylation; therefore enhancing contraction. Rho-kinase is activated by the binding of RhoA, a small monomeric G protein. Rat lung models and isolated PASMC demonstrate inhibition of sustained contraction to hypoxia in the presence of Rho-kinase inhibitors [115]; furthermore Rho-kinase is activated by hypoxia in a RhoA dependent manner which can be inhibited by specific antagonists [116]. This suggests an integral role for Rho-kinase

The role of the various calcium channels in the HPVR adds a further level of complexity to the controversy surrounding oxygen sensing and K^+ channel contributions to the pulmonary vascular responses; however this is an area of intense research and as with carotid body mechanisms developments can only be anticipated.

1.6.3 Endothelial influences on the pulmonary circulation

The role of the endothelium has fallen from the forefront of research in hypoxic pulmonary vascular responses; however the endothelium does exhibit an effect in response to hypoxia. The release of mediators in response to hypoxia is a demonstrable fact, whether these play a direct role in the HPVR or modulate the effect is currently a point of disagreement. The following section reviews some of the mediator that have such a role, it is by no means exhaustive, but the molecules discussed have been studied in the context of hypoxic pulmonary vascular response.

Nitric oxide

Nitric oxide was first characterised as endothelium derived relaxing factor in 1987, but since then its role in numerous biological systems other than vasodilatation have become apparent. It is produced by nitric oxide synthase (NOS) which occurs in several different isoforms dependent on which role NO is filling. Two main forms of NOS occur, Ca^{2+}/calmodulin dependent or constitutive (cNOS) or Ca^{2+} independent or inducible form (iNOS). cNOS is stimulated by bradykinin, acetylcholine, histamine, leukotrienes and platelet activating factor. iNOS is regulated at the transcriptional level and iNOS mRNA production is a response to pro-inflammatory mediators such as interferon (INF), tumour necrosis factor α & β (TNF) and interleukin-1 (Il-1). In addition to smooth muscle relaxation, NO plays a role in

immunity, inflammation, neurotransmission and hormone release. In the lungs macrophages, neutrophils, mast cells, non-adrenergic non-cholinergic inhibitory (iNANC) neurones, fibroblasts, vascular smooth muscle cells, pulmonary arterial and venous endothelium and pulmonary epithelial cells are capable of producing NO.

Nitric oxide's role in hypoxic vasoconstriction has been demonstrated by attenuated hypoxic pulmonary vasoconstriction in response to inhaled NO in both animal models [117] and man. Experiments using infusions of N^{G}-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of NOS, produced vasoconstriction in normoxic and hypoxic conditions in the pulmonary circulation and diminished the systemic vasodilator response to hypoxia [118]. Transgenic mouse models deficient in endothelial NOS (eNOS) or nitric oxide synthase 3 (NOS-3) demonstrate raised PAP, increased pulmonary vascular resistance in response to hypoxia when compared to wild type mice or NOS deficient mice raised in normoxic conditions [119, 120]. These mice also demonstrate increased muscularisation of pulmonary arterial wall and increased right ventricular mass, changes which were preventable with the administration of inhaled nitric oxide. Therefore nitric oxide plays an integral part in the maintenance of basal vascular tone in the pulmonary circulation; however NO has numerous other roles, including second messenger functions and indirect influences on vasopressive mechanisms [121].

Endothelin

Endothelins are a family of potent vasoactive peptides produced in response to various stimuli included amongst these is hypoxia. Endothelin-1 (ET-1) was the first to be discovered [122] and the most widely studied. Endothelin-1 is produced by endothelin converting enzyme (ECE) from a peptide precursor, big ET-1. ET-1 mediates its effects through two types of endothelin receptor, endothelin A (ET_A) and endothelin B (ET_B). ET_A receptors are found on pulmonary smooth muscle cells and causes vasoconstriction and proliferation of cultured smooth muscle cells [123, 124]. Endothelin-1 is produced in the lung by endothelial cells, epithelial cells and tissue macrophages, but the secretion of ET-1 by endothelial cells is mainly abluminal [125], directed towards the muscular media, in vivo. Hypoxia is a potent stimulus for the production and release of ET-1 [126, 127] and levels of ET-1 are elevated in normal individuals exposed to hypoxia as well as in patients with hypoxic lung disease [128]. Furthermore other vasoactive mediators can modulate ET-1 expression and action. ET-1

expression in hypoxia is diminished by nitric oxide and L-NMMA potentiates the action of ET-1 [129]. Endothelin is a vasopressive mediator and has antagonistic actions to NO. Both are important mediators produced by the pulmonary endothelium, however the current thinking is that they play a modulatory role in HPVR and remodelling, influencing the direct effect of hypoxia on PASMC stated previously.

Atrial and Brain natriuretic peptides

Atrial natriuretic peptide (ANP) was discovered in 1981 by infusing extracts of atrial tissue into rats, producing a marked natriuresis [130]. Two further natriuretic peptides have since been discovered, Brain and C-type natriuretic peptides (BNP and CNP respectively). ANP and to lesser extent BNP have been studied in the context of hypoxic response. Both peptides are the product of cleavage from a larger precursor resulting in 28 and 32 amino acid biologically active carboxyl peptide fragments. ANP is produced by the cardiac atria and atrial wall distension is the strongest stimulus for secretion, though endothelins, arginine vasopressin (AVP) and catecholamines can directly stimulate ANP production. BNP is predominantly produced in the ventricles and ventricular hypertrophy or congestive cardiac failure stimulates secretion [131, 132]. The natriuretic properties of both peptides depend on increasing glomerular filtration by dilating afferent renal arterioles whilst vasoconstricting efferent ones. In addition they both inhibit the angiotensin II (AT-2) dependent sodium (Na^+) and water reabsorption from the proximal renal tubule as well as inhibit the anti-diuretic actions of AVP on the collecting ducts. ANP also inhibits the AT-2 stimulated release of aldosterone [133]. The mechanism is not entirely clear, but seems to be via a reduction in the conversion of endogenous cholesterol into pregnenolone [134], a process stimulated by AT-2 and adrenocorticotrophic hormone (ACTH). A reduction in aldosterone would reduce Na^+ and water reabsorption in the distal renal tubule, further reducing plasma volume and therefore atrial distension. This mechanism is described in detail below.

Acute hypoxia produces an increase in ANP secretion dependent on the intensity and duration of hypoxia [135], however the degree of response is also dependent on dietary salt and adrenocortical activation [136]. The acute rise in ANP and BNP concentration, before fluid changes have an opportunity to occur has been attributed to hypoxic pulmonary vascular responses [137]. The direct effect of natriuretic peptides in the pulmonary circulation is demonstrable in animal models. Exogenous BNP and ANP blunt the acute

hypoxic vascular response in rats and right ventricular hypertrophy in rats raised in a hypoxic environment [138]. Gene knockout mice deficient in ANP receptor develop pulmonary hypertension and the morphological changes associated with the condition [139]. Similar pulmonary vascular relaxing effects of ANP and BNP can be seen in humans [140]. ANP also rises rapidly in response to exercise during short duration [141] and prolonged exercise [142]. The combination of hypoxia and exercise on ANP levels remain unclear with conflicting results in the laboratory and the field. To date no clear relationship between natriuretic peptide levels and high altitude disease has been demonstrated.

The role of the endothelium in the hypoxic vascular response, though overshadowed by recent advances in the understanding of oxygen sensing and membrane channels, remains an important focus of research. The potential for developing novel pharmacological agents has implications in the treatment of human diseases, such as pulmonary hypertension and high altitude pulmonary oedema.

1.6.4 The pulmonary circulation during exercise and hypoxia

Pulmonary blood flow increases with the near instantaneous increase in cardiac output at the onset of exercise and the pulmonary vascular bed dilates; this has two effects: the first is the accommodation of the increased pulmonary blood flow ($\dot{Q}$) and secondly there is recruitment of previously poorly perfused units of lung in synergy with increased ventilation. This enables the near instantaneous rise in oxygen uptake and delivery during the early dynamic phase of exercise. The increase in $\dot{Q}$, results in a measurable rise in pulmonary artery pressure despite vasodilatation of the pulmonary arterioles [143-145]. In normal exercising humans at sea-level the pulmonary circulation can accommodate these haemodynamic stresses; however at the extremes of performance there is the potential for endothelial damage. This is evident in racehorses which can develop pulmonary haemorrhage at the peak of exertion during a race, and similarly elite athletes can have demonstrable red blood cells in the alveolar space after sustained maximal exercise [146].

Hypoxia adds a further point of stress to this model. Though the HPVR is beneficial in allowing perfusion to match alveolar ventilation across the lung, the effects of ambient hypoxia are less specific. Ambient hypoxia in animals causes a heterogeneous pattern of

vasoconstriction and blood flow [147, 148]; whereas in humans a similar response is suggested by the heterogeneous pattern of smooth muscle remodelling in the terminal arterioles of hypoxic lung [149]. High altitude pulmonary oedema is a disease of hypobaric hypoxia and an exaggerated HPVR is a prominent feature associated with its pathogenesis. Chest radiographs of sufferers demonstrate patchy infiltrate, again suggesting a non-uniform process. The consequences of a heterogeneous HPVR in the face of ambient hypoxia have been investigated in animal models; these suggest that areas of lung which are relatively vasodilated can be exposed to increased flow as blood is diverted from hypoxia vasoconstricted regions [150]. As a consequence, capillary beds which have lost the protection of pre-capillary arteriolar constriction are exposed to high pulmonary artery pressure, haemodynamic shear forces and capillary stress [151]. Animal models have demonstrated that there is sufficient disruption of pulmonary endothelium, basement membrane and epithelium to allow the leakage of red blood cells (RBC) and large molecular weight plasma proteins [152]; these are similar to the findings in HAPE. Though these are not an inevitable consequence of hypoxic exposure in humans, they may represent one extreme in the spectrum of response.

1.6.5 Diffusion limitation at the lung

After the increase in blood flow through the lungs the second consideration is the passage of oxygen; this needs to diffuse from the alveolar space across the alveolar epithelium, interstitial space, through plasma and the RBC cell membrane to haemoglobin. In normal humans exercising at sea level this does not pose a significant barrier to oxygen diffusion since the uptake of oxygen by deoxyhaemoglobin is rapid in comparison to the transit time across the pulmonary capillary bed. The exact time taken for oxygen uptake by RBC is somewhat disputed since factors such as RBC mixing, diffusion distances within the capillary/RBC and blood viscosity are variables that must be taken into account [153], but it can be assumed that haemoglobin saturation is near completion within 0.2 seconds [154]. The transit time, as estimated by using traditional physiological measurements and extrapolations is thought to be 0.75 seconds [155]. The increase in pulmonary blood flow that accompanies the increase in cardiac output is a further consideration since it reduces the transit time across the pulmonary capillary bed by up to two thirds. Modern radiolabelling techniques have demonstrated that cardiopulmonary transit time (a surrogate for pulmonary capillary transit

time) is variable with the exercise intensity up to a minimum value [156]; however there is still time for oxygenation of Hb to occur in normoxic conditions.

One major discrepancy is that systemic artery PO_2 is demonstrably lower than alveolar oxygen tensions when considering the whole lung (i.e. the alveolar-arterial oxygen difference ($P_{A-a}O_2$)). The main reason for this is the ventilation to perfusion (*V/Q*) inequality that occurs in normal humans at rest which becomes more evident with exercise [143]. Right-to-left shunts can also contribute to the $P_{A-a}O_2$ difference by allowing the admixture of deoxygenated and oxygenated blood; in normal humans this can occur in Thebesian vessel in the heart and bronchial vessels in the lung. A final reason for the $P_{A-a}O_2$ difference is the possibility of gas exchange inefficiencies through incomplete alveolar/airway gas mixing in some segments of lung. These factors are relatively trivial in normal individuals exercising at sea-level, but become more important as maximal exercise is reached especially in elite athletes. As mentioned diffusion limitation is not a feature of exercise by normal individuals at sea level; however there is an exception to be found in elite athletes. Individuals trained for peak physical performance can demonstrate exercise induced arterial hypoxaemia (EIAA). The causes for the $P_{A-a}O_2$ difference are thought to be contributory, but the main reason is the exceptionally low mixed venous oxygen tensions due to increased extraction of O_2 by these individuals at maximal exercise. In these circumstances, with the pulmonary capillary transit time at its minimum and *V/Q* inequality maximised, then there is insufficient time for complete oxygenation of blood entering the lungs and diffusion limitation can occur in normoxic conditions [157].

Ambient hypoxia affects this process in a number of ways: firstly, low ambient oxygen partial pressure decreases the oxygen diffusion gradient across the alveolar/endothelial interface. If the degree of hypoxia were severe enough then blood crossing the pulmonary capillary bed may not be fully oxygenated as a result of diffusion limitation. The PaO_2 of arterial blood under circumstances of acute hypoxia then equilibrates at a lower level. Secondly, the effects of hypoxia on the pulmonary circulation can result in heterogeneous pulmonary vasoconstriction and can exacerbate *V/Q* inequalities, short periods of hypoxia suggest this can be a major factor and widen the $P_{A-a}O_2$ [158]; however prolonged exposure over days or weeks results in an optimised *V/Q* [159]. During prolonged exposures to altitude deterioration in the $P_{A-a}O_2$ can develop in some individuals, but this is thought to become significant only in the presence of interstitial oedema [160]. Thirdly, if exercise is then introduced into this hypoxic system then oxygen extraction by respiring muscles would

further reduce the mixed venous oxygen tension returning to the lungs; thus resulting in a further decrease in arterial oxygen tension due to inadequate oxygenation by the lungs due to the initial effect of diffusion limitation. If steady state is attained (at sufficiently low enough exercise intensity for the given hypoxia) then PaO_2 equilibrates at a lower level. The resultant hypoxaemia drives ventilation via the carotid body mechanisms described; however the reduced oxygen diffusion gradient in combination with reduced pulmonary transit times offsets any benefit derived from increased ventilation. The most extreme example of this is at the summit of Mount Everest, where calculated values of arterial oxygen tension were 28mmHg, derived from a measured end-tidal CO_2 of 7.5mmHg [161].

1.6.6 The relationship between ventilation and pulmonary perfusion

The relationship between ventilation and pulmonary vascular response has been alluded to in the previous sections; gas exchange is dependent on the matching of alveolar ventilation to perfusion of pulmonary capillary beds. Hypoxia has effects upon response in both of these physiological systems that results in a matching of ventilation to perfusion; however the efficacy of *V/Q* matching is affected by both individual response and the effects of exercise. Total ventilation is the product of tidal volume and respiratory rate, but the alveolar ventilation is dependent on the effective tidal volume that reaches the gas exchange surfaces. A part of the tidal volume ventilates the airways, which are incapable of gas exchange and forms the anatomical deadspace; this is about 150mls in the average human. A further portion of tidal volume is 'wasted' by ventilating regions of lung which are poorly perfused and hence are less effective in gas exchange. This volume in combination with the anatomical deadspace constitutes the physiological deadspace (V_D). As reviewed previously, both hypoxia and exercise can influence *V/Q* matching and therefore V_D. One method of calculating the ratio of physiological deadspace to tidal volume is the Bohr method; this relies on differences in CO_2 concentration in the alveoli and expired gas. Since CO_2 excreted is produced by the alveoli then:

$$V_D/V_T = \frac{\text{alveolar } P_ACO_2 - \text{expired } P_ACO_2}{\text{alveolar } P_ACO_2}$$

Since alveolar P_ACO_2 is virtually identical to arterial $PaCO_2$ in normal subjects, then arterial CO_2 tensions can be substituted to give:

$$V_D/V_T = \frac{\text{arterial } PaCO_2 - \text{expired } P_ACO_2}{\text{arterial } PaCO_2}$$

Therefore the accurate calculation of physiological deadspace to tidal volume ratio requires the measurement of expired CO_2 and arterial CO_2 tensions. The use of this equation requires the measurement of arterial blood gas tensions and the siting of an arterial catheter during exercise testing. Jones et al. described an equation based on end-tidal CO_2 partial pressures, derived from multiple regression analyses, which seemed to provide a reliable estimate of alveolar CO_2 partial pressures [162]:

$$\text{Alveolar } P_ACO_2 = 5.5 + (0.90 \times PetCO_2) - 0.0021(V_T)$$

where $PetCO_2$ is the partial pressure of CO_2 in end tidal gas. This appeared to give a reliable measure of alveolar P_ACO_2 with a correlation coefficient of r=0.915 during steady state exercise in healthy subjects; however experiments during incremental exercise protocols demonstrated a widening degree of error between calculated V_D/V_T using arterial sampling and calculations based on measurements of $PetCO_2$ upon increased work rates. Furthermore, it appeared that estimations of V_D/V_T based on the Jones formula were no better than those based on $PetCO_2$ alone [163]. The reasons cited were the effects of impaired air flow in patients with obstructive airways disease, but more importantly the effect of increased V/Q inequalities, resulting in a widening of the difference between arterial and end-tidal CO2.

The effect of physiological deadspace ventilation has implications for the efficiency of ventilation and although the effects of hypoxia during exercise may preclude the use of extrapolations such as the Jones equation, $PetCO_2$ remains a useful indicator of alveolar ventilation.

1.6.7 Patterns of ventilation

The observed increases in ventilation elicited by hypoxia or exercise are the product of increased tidal volume and respiratory rate; furthermore, the respiratory rate can be further divided into the times for inspiration (T_I) and expiration (T_E). Hypoxia, hypercapnia and exercise cause an increase in tidal volume which is the result of increased airflow; however, the increase in respiratory rate is produced by a decrease in T_E, whilst T_I remains fairly constant up to maximal ventilatory rates. As tidal volume approaches 50% of the vital capacity, T_I decreases, due to the inhibitory effect of the Hering-Breuer reflex upon lung hyperinflation. During rest the pattern of ventilation is similar, irrespective of the stimulus; this suggests the presence of convergence of ventilatory signal from peripheral and central chemoreceptors on a central ventilatory pattern generator [164]. During the dynamic transient phase before steady state has been reached there is an observable difference in ventilatory patterns between different stimuli during rest. Hypercapnia produces the longest time course toward steady state ventilation during rest, taking up to 4 minutes. The increase in ventilation is mediated as in steady state by an increase in tidal volume, with a prolongation of the T_I and shortening of T_E; consequently respiratory rate does not change significantly during the initial phases of hypercapnia. The response to hypoxia is much more rapid, achieving steady state within a minute; interestingly the T_I is relatively constant, but the T_E is *increased* for the first few breaths before shortening and achieving a steady state pattern [165].

Respiratory flow cycles also have a demonstrable individuality and reproducibility, at rest [166, 167] and during exercise [168]; furthermore the similarity in breathing patterns in monozygotic twins suggests a genetic component [169]. The pattern of breathing has implications for pulmonary gas exchange that extend beyond the necessities of alveolar ventilation. As described previously, deadspace ventilation (V_D) is an important consideration during exercise and hypoxia; the pattern of ventilation has a significant effect upon alveolar ventilation in this context; alveolar ventilation is dead space subtracted from tidal volume. For example, to achieve a certain level of ventilatory change, increasing tidal volume is more efficient than increasing respiratory rate, in terms of alveolar ventilation, since less of the minute ventilation is wasted upon deadspace ventilation. This adds further value to V_D measurements in the interpretation of ventilatory responses in the context of altered V/Q matching or air flow limitation, which can significantly reduce the effective alveolar ventilation

1.7 Cardiovascular adaptations to hypoxia and exercise.

The heart and circulation provide the convective mechanism for the transport of oxygen and carbon dioxide to and from the respiring tissues and are intimately linked with the onset of exercise in a similar fashion to ventilatory responses. Blood oxygenated at the lung, must then be delivered to the respiring tissues; this is dependent on the saturation of haemoglobin, the concentration of that haemoglobin, but most importantly cardiac output. The systemic circulation and its responses are also crucial and by controlling blood flow in response to the changes in requirement it directs oxygen delivery. At the capillary level, local circulation in respiring muscle has implications for oxygen delivery, especially under hypoxic conditions. The following section reviews in detail the cardiovascular responses to upright exercise and how hypoxia influences them.

1.7.1 Cardiac output

Cardiac output (CO) is closely linked to VO_2 and increases in response to oxygen demand. Cardiac output increases almost instantaneously upon the onset of exercise with increases in stroke volume and heart rate. In combination with the increase in ventilation during Phase one exercise results in a rapid increase in VO_2. The increase in stroke volume is almost instantaneous and reaches a maximum up to the first third of the VO_2 max workload for an individual [170]; thereafter further increases in CO are dependent on increases in heart rate. Stroke volume increases in response to increase vascular return, as a consequence of the thoracic pump and muscular contraction in exercising limbs. It is determined by age, sex and size, as well as physical fitness [171].

As work rate increases a point is reached where demand outstrips the capacity of the cardiovascular system and anaerobic threshold is reached. Further increases in load results in the accumulation of lactate, muscle fatigue sets in and eventually exercise has to stop. The term used so far in the discussion for this point is VO_2 max; however this can only be accurately determined over a series of tests, is affected by the type of exercise and the proportion of total muscle exercised [171]. A more accurate description for the maximum attained oxygen consumption for a particular exercise test would be VO_2 peak. In order to avoid confusion the term VO_2 max will be used in this thesis when referring to maximal

exercise since the same form of exercise was used in the experiments described in the methods.

1.7.2 Circulatory limitation on exercise

The increased cardiac output during exercise is diverted to the exercising muscles with smaller fractional increase in skin circulation to allow thermoregulation during exercise, with a fractional decrease in CO to the splanchnic and renal circulations [172]. The increase in muscle circulation is almost instantaneous with the onset of exercise and is proportional to the strength of the contraction [173]. The mechanism is thought to be via the autonomic nervous system and local humoral effects of metabolism due to low oxygen tensions, raised CO_2, acidosis, adenosine and temperature. Increased muscle blood flow is undoubtedly crucial during exercise, but the local circulation through the muscle capillary bed is equally important. In order for oxygen to diffuse from haemoglobin to the respiring mitochondria there must be a sufficient gradient for this to occur. A reduction in the pH of respiring muscle due to CO_2 production with the associated rise in temperature during exercise both facilitate the release of O_2; however oxygen must then diffuse through the plasma, across the endothelium, the interstitial space into the myocyte and into the mitochondrion. Isolated mitochondria can function at very low oxygen tensions, between 0.5 and 1 mmHg [154, 174]. However, a sufficient capillary oxygen tension must be present to create a gradient across the physical barriers to oxygen diffusion and allow aerobic respiration; this is estimated to be between 15 and 20 mmHg [175]. The implications are twofold; firstly, myocytes at the arterial end of the capillary bed have sufficient oxygen to allow aerobic respiration whereas those at the venular end struggle to receive sufficient oxygen due to O_2 extraction proximally. During exercise, the increase in oxygen demand exacerbates the situation and although local humoral factors may offset this effect by increasing local blood flow there is the potential for a heterogeneous pattern of respiration, with both aerobic and anaerobic respiration occurring simultaneously within the muscle. Secondly, the concept of a critical capillary PaO_2 means that blood returning from an exercising muscle cannot drop below this value and increases in work load beyond this point result in an increase in lactate. This has been demonstrated in exercising humans with simultaneous sampling of the femoral vessels during cycle ergometry at work rates above and below LT; during high intensity the lowest PaO_2 is 20mmHg in the femoral venous effluent and does not fall lower despite increasing work loads[176]. Therefore, the muscle capillary represents the second point of diffusion limitation to oxygen

passage from the environment to the respiring mitochondria, but in contrast to the pulmonary circulation during hypoxia, occurs in normoxic conditions.

1.7.3 Circulatory limitations during acute hypoxia

The consequences of exercise under hypoxic conditions are diffusion limitation at the lung and the potential worsening of the diffusion gradient across the muscle capillary. The result is hypoxaemia due to these two effects; therefore, in order to maintain oxygen delivery CO must be higher for any given work load since the oxygen cost for that work is the same [143, 177, 178]. In contrast maximal cardiac output is reduced by hypoxic exposure as is VO_2 max; however CO at a specific VO_2 remains higher during acute hypoxia in comparison to normoxic values [143]. Several theories have been posited for this decline, one cause is based on the link between the effects of diffusion limitation and the burden of lactate build up. Up to the reduced VO_2 max, under hypoxic conditions, oxygen uptake matches exercise in a similar pattern to exercise in normoxia [160, 179]. Lactate builds up as a consequence of increasing anaerobic respiration and contributes to muscle fatigue. The reduction in oxygen at the arteriolar end of the muscle capillary means that the critical PaO_2 for diffusion from vascular lumen to mitochondria will be reached earlier in the passage of blood through the muscle capillary bed. Therefore the potential for anaerobic respiration is increased and occurs earlier during exercise during hypoxia. Cardiac output increases to offset this effect, but is only effective up to a certain level of hypoxaemia. The effects of diffusion limitation and the earlier onset of muscle fatigue have been cited as one cause of diminished VO_2 max under hypoxic conditions; however circulatory factors other than diffusion limitation are also thought to have a bearing. Recent work suggests that the blood flow to exercising muscle may not be as high in hypoxia despite identical work loads during normoxic exercise [180]. The fractional distribution of CO in normoxia favours the exercising muscle units; however in hypoxic conditions there is a minimal oxygen requirement of the organs which must be met. This is thought to result in a reduced fraction of CO flowing to exercising muscle; instead blood flow to critical organs is maintained by a higher fractional CO. This ensures a minimal oxygen delivery during hypoxia and therefore is a potential limitation to VO_2max. One site of muscular respiration seems to be spared the limiting effects of hypoxia, the myocardium. It was thought that cardiac dysfunction under conditions of hypoxia was a factor in VO_2 max limitation. This is not the case as several echocardiographic studies have

demonstrated that myocardial function is well preserved up to quite profound levels of hypoxia and during exercise [160, 181, 182].

The reduction in arterial oxygen tension and increasing hypoxaemia enhances the hypoxic ventilatory drive, but also influences the cardiovascular response to VO_2 during exercise. The effects on cardiac output and VO_2 limitation during prolonged hypoxia are discussed further in this chapter, but one immediate question remains: how does cardiac output increase in response to hypoxia?

1.7.4 Sympathetic activation during acute hypoxia

Sympathetic activity is exhibited by the release of endocrine mediators such as adrenaline or increased activity in the sympathetic nerves. In acute hypoxic conditions there is evidence of sympathetic stimulation, seen in the increase in cardiac output. Heart rate rises in response to brief exposures to hypoxia; however this is not accompanied by an increase in adrenaline or noradrenaline [183, 184]. Sympathetic nerve activity (SNA) is elevated in hypoxic conditions, as measured by SNA to skeletal muscle [184-186]. The stimuli that affect the level of response are the degree of hypoxia, arterial carbon dioxide partial pressures ($PaCO_2$) and ventilation. An increase in ventilation has a negative influence on SNA and this is independent of $PaCO_2$ [187], furthermore the inhibitory effect of increased ventilation on SNA seems to be reduced with breath-hold manoeuvres [188]. Many of these effects have been under conditions of isocapnic hypoxia, since elevated carbon dioxide tensions appear to intensify SNA in hypoxic conditions and normoxic conditions [186]. In healthy humans, ventilation rises and $PaCO_2$ falls in response to hypoxic exposure thereby potentially attenuating the effect of hypoxia, however in pathologies such as obstructive sleep apnoea there is increasing evidence that the effects of apnoeic episodes, hypoventilation and intermittent hypoxaemia combine to increase SNA with an increase in heart rate and systemic blood pressure [189-191]. These findings support the role of intermittent hypoxia in the pathogenesis of persistent hypertension during waking periods.

The mechanism of signal transduction seems to rely on peripheral chemoreceptors with modulating effects of pH and $PaCO_2$ exhibiting effects similar to those seen in ventilatory responses [192-194]. The role of the ventrolateral medulla in further sensing and then effecting the response to hypoxia has also been demonstrated by experiments in anaesthetised animals and direct measurement [193, 195]. The nucleus of the tractus solitarius is thought to play a role in cardiovascular sympathoreflexes; however the precise locations of the

brainstem centres responsible for sympathetic response during profound hypoxia remain unknown. The carotid body response has been discussed, but whether specific neurotransmitters play a role in hypoxic SNA tone is also unknown.

1.7.5 Sympathetic activation during prolonged hypoxia

The effects of more prolonged exposures to hypoxia on sympathetic activation differ from acute exposures. Brief exposure to hypoxia do not demonstrate any measurable rise in catecholamines such as adrenaline and noradrenalin; however, field studies have demonstrated elevated catecholamine levels at altitude with exposure over one weeks duration [196, 197]. Sympathetic activity is elevated at altitude; however the level of activation remains high despite oxygenation improving as acclimatisation occurs [198-200]. One explanation is that increased sympathetic activity may compensate for the loss in plasma volume that occurs at altitude by increasing vascular tone and offsets the vasodilator effects of hypoxia, thereby maintaining blood pressure [172, 185, 201].

The cardiac response to prolonged hypoxia differs from acute exposures. Cardiac output to work load relationships return to near sea-level values in well acclimatised individuals, but up to a reduced VO_2 max; however peak heart rate is reduced at altitude [202]. Cardiac parasympathetic tone appears to be responsible and cholinergic blockade restores maximal heart rate to sea-level values, but has no effect on maximal cardiac output or exercise capacity [203, 204]. Furthermore, there is evidence in animal models that prolonged hypoxic exposure down regulates β-adrenergic myocardial receptors and up regulates cholinergic receptors [205]. The effect in humans appears to be similar, with a decline in leucocyte β adrenergic receptors during prolonged exposures; β receptor blockade causes a fall in maximal heart rate, but does not affect VO_2 max at altitude [206]. Despite the demonstrable effects of hypoxia on adrenergic (and cholinergic) receptors at the heart and effects that antagonists have on heart rate, maximal cardiac output and exercise remain unchanged; increased stroke volume seems to be a compensatory mechanism that maintains CO under these circumstances [207]. A more controversial issue is the reason for the decrease in maximal exercise and CO in acclimatised individuals.

1.7.5 Cardiac output adaptations during prolonged hypoxic exposure

The discussion thus far has focussed on cardiac output response during acute hypoxic exposures. In healthy humans, outwith the laboratory, prolonged hypoxic exposure is only really experienced at altitude; although there are fundamental similarities with acute exposure

in terms of a VO_2max limitation, the responses change over time. Cardiac output increases upon initial exposure to hypoxia for the reasons explained, but then declines over a period of days to eventually return to sea level values for specific work rates [160, 202, 208]. The reason for the reduction in CO is thought to be due to the adaptations in various systems that effect the process of acclimatisation; these occur at several levels. Hypoxia stimulates ventilation, as an adaptive measure it improves alveolar ventilation to offset alveolar hypoxia, maximising the diffusion gradient between blood and air. In this way it ameliorates the effects of diffusion limitation at the lung. The oxygen carrying capacity of blood is increased by a combination of reduced plasma volume and increased haemoglobin synthesis through the effects of erythropoietin. At the muscle, diffusion distances are reduced by increases in capillary density; this is thought to be predominantly due to the effects of reduced muscle mass in response to chronic hypoxic exposure rather than neovascularisation. The question remains: why is maximal CO and VO_2 max reduced in acclimatised individuals?

The most commonly accepted theory is that diffusion limitation at the muscle in the context of hypoxia is the limiting factor, where increases in CO beyond a certain point are ineffectual, as described previously. There are however several other theories. The effect of autonomic changes at the heart during prolonged hypoxia is one, but the effects of receptor blockade demonstrate that there is no appreciable change in exercise capacity or CO in response to enhancing or inhibiting heart rate responses. Another possibility is the effect of increased plasma viscosity due to erythropoiesis. Polycythaemia does increase blood viscosity and can affect cardiac output, but experiments examining isovolumetric haemodilution demonstrate no effect on maximal cardiac output or VO_2 max [207]. The exact mechanism of VO_2 max and CO reduction in acclimatised individuals remains controversial; however the fact that VO_2 max and CO are restored to near normal levels by oxygen inhalation lends credence towards the diffusion limitation theory [179].

1.8 Haematological adaptations

The oxygen carrying capacity is a crucial step in the delivery of oxygen from lung to tissues; since oxygen is relatively poorly soluble in water, much of this is carried by haemoglobin molecules within red blood cells. Haematological adaptation to hypoxic conditions relies on the changes in concentration of haemoglobin and red blood cells and although it is not a

significant influence during acute hypoxic exposure, it is an important factor over prolonged exposures.

1.8.1 Haemoglobin

Over ninety nine percent of oxygen is carried in the blood attached to haemoglobin (Hb) within red blood cells (RBC). This molecule consists of four pyrole rings each with an iron ion in the ferrous state arranged within four separate globulin chains. Each molecule binds four oxygen molecules; the affinity of the molecule for O_2 is dependent on the binding state of each of its subunit, such that as oxygen affinity increases as successive O_2 molecules are bound. More molecules bind as the O_2 partial pressure (PaO_2) increases until saturation is reached (i.e. the lungs). As saturation approaches there is little increase in binding despite further increase in PaO_2 and the curve plateaus, explaining the sigmoid shape of the dissociation curve for haemoglobin; however there are several factors that influence the affinity of Hb for oxygen. The Bohr Effect describes the lowered affinity of Hb at low pH; this is due to the higher affinity deoxygenated haemoglobin demonstrates for H^+ ions in relation to O_2. Elevated CO_2 tensions (which liberate H^+ and reduce pH) and increased temperatures also reduce haemoglobin's affinity for O_2. All three factors are found in the exercising muscle and therefore facilitate the release of O_2 at the point of consumption. A further influence on Hb affinity is the molecule 2,3-diphosphoglygerate (2,3-DPG), a product of the Embden-Meyerhof glycolytic pathway. This highly charged anion binds to the β chains of deoxygenated haemoglobin, reducing its affinity for oxygen. 2,3-DPG; the concentration of 2,3-DPG is elevated on ascent to high altitude and returns to normal on descent to sea level. This reflects the 6 hour half life of the molecule

1.8.2 Carrying capacity of blood

Oxygen delivery is a function of cardiac output and the oxygen carrying capacity of blood; this fact is the basis of the Fick method of cardiac output measurement. The amount of oxygen carried for a given volume of blood can be calculated using the oxygen content equation:

$$CaO_2 = (SaO_2 \times Hb \times 1.34) + 0.003(PaO_2)$$

Where CaO_2 is the oxygen content in g/dl and SaO_2 is percentage oxygen saturation of blood, each gram of haemoglobin carries 1.34 mls of oxygen and 0.003 mls of oxygen are dissolved in every decilitre (dl) plasma *per* mmHg of PaO_2.

1.8.3 Red blood cell mass and plasma volume

Red blood cell mass is affected in two ways during prolonged hypoxic exposure, increased production by erythropoiesis and reduction in plasma volume. The effect of increased ventilation causes an alkalosis which is compensated by renal mechanisms; bicarbonate is eliminated in the renal tubules and results in a diuresis. In the absence of adequate fluid intake plasma volume is reduced and causes a rise in the haematocrit. Prolonged hypoxia can also have an effect on the renin-angiotensin system and maladaptive processes can cause fluid retention; this is discussed further in this chapter. The second and perhaps the most striking feature is the ability to increase the bloods carrying capacity by increasing the production of red blood cells. Polycythaemia at altitude was first described by Viault in 1890; however the mechanisms of this response were not fully understood until quite recently. Erythropoiesis is under the control of the hormone erythropoetin (Epo), a circulating glycoprotein made of 165 amino acids and 4 oligosaccharide side chains. It is secreted predominantly by renal tissue close to the juxtaglomerular apparatus; unlike the carotid body, anaemia in addition to ischaemia and hypoxia can produce stimulate secretion. Epo acts on erythroid precursor cells to increase production and accelerate maturation of red blood cells. The effects of increased Epo levels on RBC mass are not evident for some days at altitude and therefore have little effect on acute hypoxic challenges, however there are measurable increases in production of Epo within hours [209]. Hypoxia controlled gene transcription was first described in the Epo gene and has proved to be a milestone in the understanding of hypoxic response at the molecular level. The effects of hypoxia on gene transcription are described later in this chapter.

1.9 The Renin-angiotensin system

The renin-angiotensin system (RAS) has a number of physiological roles. At the systemic level it forms an integral part of the humoral control for sodium and water homeostasis; whereas at the cellular level the renin-angiotensin system is a prolific paracrine and cellular control pathway found in a wide range of tissues. This section describes the various roles of RAS in the response to hypoxia and exercise; however local RAS is a relatively recent discovery. The classical role of RAS in fluid homeostasis under hypoxic conditions had been the focus of research decades before the discovery of local systems; hence the review of RAS and hypoxia starts there.

1.9.1 Renin, Angiotensin and aldosterone

Aldosterone stimulates the resorption of sodium and water from the renal tubules in response to changes in intravascular volume, stress, low sodium intake and hypotension. Aldosterone is secreted by cells in the *zona glomerulosa* located in the adrenal cortex and is under the control of the endocrine RAS. Renin is secreted from the renal juxtaglomerular apparatus in response to changes in blood pressure and the direct neurohumoral effect of the sympathetic system. Renin then cleaves angiotensinogen to form angiotensin I; this in turn is cleaved by angiotensin converting enzyme (ACE) to angiotensin II (AT-2), predominantly by membrane bound ACE in the pulmonary endothelium. Angiotensin II stimulates the secretion of aldosterone via angiotensin type I receptors (AT_1) on cells in the *zona glomerulosa* activating phospholipase C; this produces an increase in protein kinase C. The result is an increase in the conversion of cholesterol to pregnenolone; this in turn is converted to aldosterone by aldosterone synthase. As described in the review of the effects of ANP, ACTH also stimulates the production of pregnenolone and enhances the effect of AT-2 on aldosterone production (Figure 1.10). The effect is an increase in plasma volume through the retention of sodium (Na^+) and water.

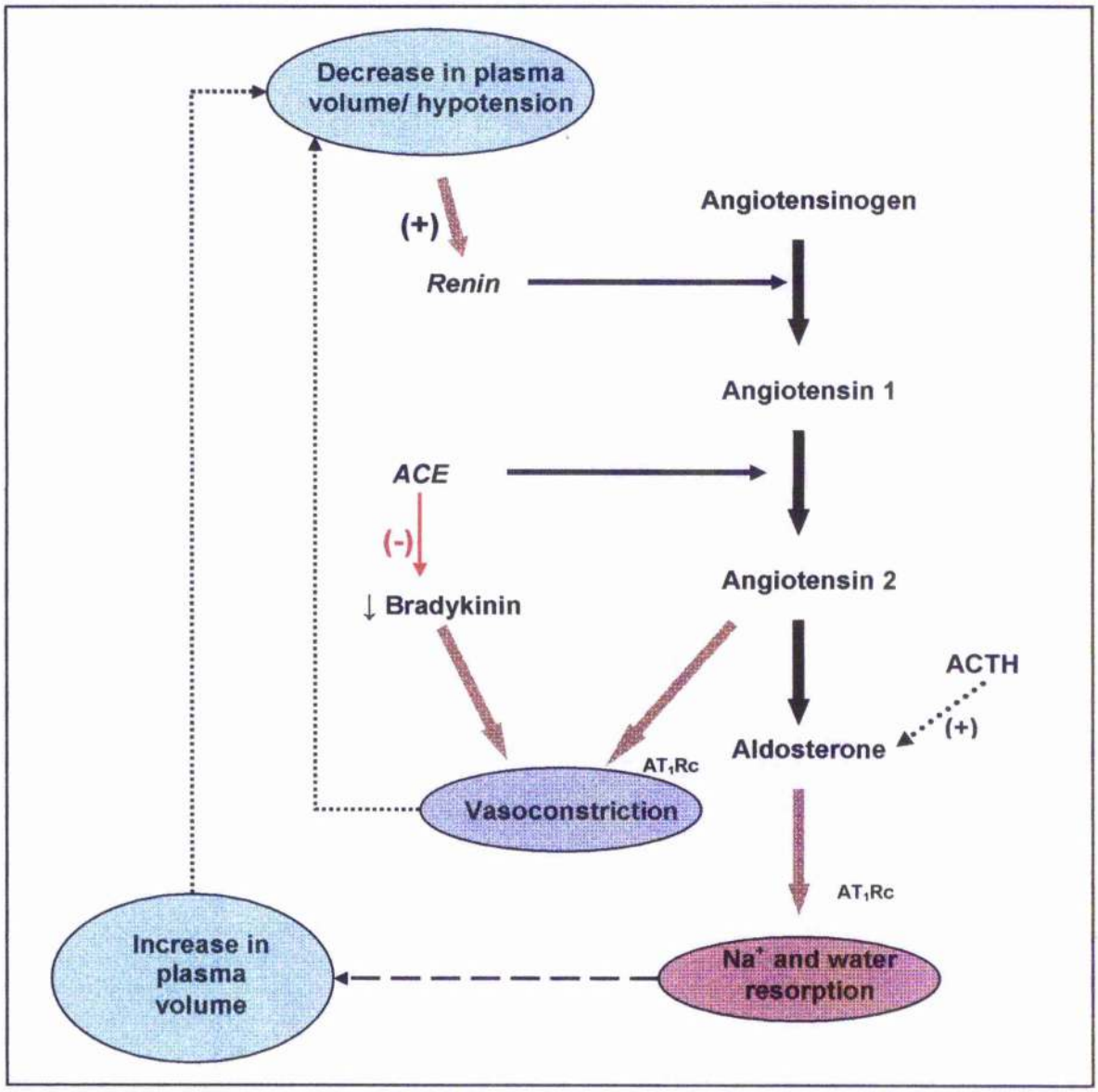

Figure 1.10 The Renin-angiotensin system and aldosterone mediated control of sodium and fluid homeostasis. Hypotension or hypovolaemia result in an increase in renin synthesis, with the subsequent resorption of fluid and vasoconstriction; this corrects the imbalance and acts as a negative feedback. This action is mediated via AT_1 receptors (AT_1Rc).

Renin secretion is measured as plasma renin activity (PRA); therefore sympathetic neural tone can increase PRA. Hypoxia increases SNA (as reviewed previously) and studies have demonstrated elevated PRA during hypoxia and at altitude [210, 211]; however at altitude the degree of aldosterone release does not match PRA [212]. A reduction in ACE activity does

not appear to be responsible for the uncoupling of PRA and aldosterone secretion since ACE activity is well maintained at high altitude [213]. The cause of dissociation from PRA seems to be a direct effect on aldosterone synthesis, in rat models there is a reduction in gene expression of enzymes in the aldosterone synthetic pathway [214]. As yet there is no direct evidence in humans that a similar pattern of reduced gene expression (since this would involve tissue sampling of the adrenal cortex). The increase in ANP at altitude could be a contributory factor; ANP can inhibit the aldosterone response to AT-2 and ACTH and could play a role in the uncoupling of aldosterone response from PRA during hypoxia. The responses described are during prolonged exposures to altitude; there is no evidence that acute hypoxia has any effect on aldosterone secretion [215]

1.9.2 Tissue and circulating RAS

Local tissue renin angiotensin systems, as characterised by the expression of ACE, have been identified in adipose tissue [216], skeletal muscle [217], the heart [218] and the lung [219]; furthermore ACE can also be cleaved from membrane sites to form part of a circulating RAS [220]. The discovery of the widespread distribution of RAS has been driven by research into the cardiovascular effects of the RAS, as well as intervention using ACE inhibitors and AT_1 receptor antagonists. The use of ACE inhibitors has a demonstrable influence on the outcome of disease. The HOPE study demonstrated a 25% reduction in death from cardiovascular disease; 20% reduction in myocardial infarction; 30% reduction in stroke; 22% reduction in heart failure and a 16% reduction in complications associated with diabetes mellitus [221]. The discovery of the ACE gene polymorphism is discussed in detail below, but similar incentives have driven research on the effects of this gene polymorphism. The result of this research is an understanding of RAS and its interaction with the kallikrien-kinin system that extends well beyond the role of an endocrine mediator of salt and fluid homeostasis. Figure 1.10 alludes to this interaction, but the interaction between RAS and the kinin system is more profound. This review examines the presence of RAS in some of the tissues involved in the response to exercise and hypoxia, including the pulmonary vasculature, the carotid bodies, the brainstem and skeletal muscle. Furthermore the discovery of active peptide fragments of angiotensin, the antagonistic nature of AT_1 and AT_2 receptors of AT-2, in addition to the ACE homologue – ACE-2 have clarified the RAS and revealed some of the autoregulatory mechanisms within this system.

1.9.3 Angiotensin peptides and bradykinin.

As described in the classical pathway; angiotensinogen, produced by the liver, is cleaved to the decapeptide – angiotensin-1 (AT-1) by renin. This in turn is cleaved to the octapeptide AT-2 by the dipeptidyl-carboxypeptidase – ACE. AT-2 is a potent vasoconstrictor in the systemic and pulmonary circulations [222, 223]; however there are several other peptide fragments of AT-1 that are biologically active. Angiotensin-4 (AT-4), angiotensin 3 (AT-3), angiotensin (1-9) (AT-(1-9)) and angiotensin (1-7) are fragments of AT-1 which are produced by a variety of peptidases, including ACE; however the homologue ACE-2 appears to be a key participant. ACE is not the only mechanism of AT-2 generation and cardiac mast cell chymase can also cleave AT-1 to AT-2 [224]. Angiotensin (1-9) (AT-(1-9)) is produced by proteolytic cleavage of AT-1 by the recently discovered homologue ACE2.

Angiotensin-2

Angiotensin-2 is perhaps the most widely studied peptide of the renin-angiotensin system; it has potent vasoconstrictor effects at the pulmonary and systemic circulations via the AT_1 receptor (AT_1Rc), but also has the potential to autoregulate via the action of the AT_2 receptor (AT_2Rc). In addition to eliciting vasoconstriction, the action of AT-2 on the AT_1Rc has demonstrable effects on cellular remodelling in blood vessels and tissues; in contrast the effect at the AT_2Rc appears to be the opposite with inhibition of proliferation and enhanced apoptosis [225, 226]. The distribution of angiotensin receptor subtypes does vary across the human lung; AT_1Rc appears to be localised to the pulmonary endothelium, whereas the AT_2Rc is mainly located in the alveolar epithelium (though there is a presence on the endothelium). The expression of both these receptors is increased under hypoxic conditions [227, 228]. The role of AT-2 is further supported by the effects on RAS in the lung during hypoxic exposures. ACE expression increases during hypoxia and hypoxia induces remodelling of the pulmonary circulation which is reversed by ACE inhibitors [229-231]. Acute hypoxic pulmonary vasoconstriction in humans is attenuated by ACE inhibitors and AT_1Rc antagonists whereas AT_2Rc blockade has no effect [232-234]. This suggests that the AT-2 effect under hypoxic conditions is predominantly mediated via the action on AT_1 receptors. The effects of RAS and AT-2 in systems relevant to the work presented in this thesis are described further in this chapter.

Angiotensin-4

Angiotensin-4 is produced by two pathways; the first is by the cleavage of AT-2 and angiotensin-3 (AT-3) by aminopeptidase (AMP) or dipeptidyl-aminopeptidase (D-Amp). The second is via an intermediate angiotensin fragment (AT-(2-10) - see figure 1.11). AT-4 is a NOS dependent vasodilator in the pulmonary and systemic circulation, as well as demonstrating activity in the heart and kidney [235]. The mechanism of action is receptor mediated and appears to be an insulin regulated aminopeptidase (IRAP) [236]. The receptor was first located in rat adipocytes, within vesicles containing the glucose transporter GLUT4 [237]. In response to insulin these vesicles translocate to the cell surface and enable increased cell glucose uptake by GLUT4. IRAP is a zinc dependent metalloproteinase capable of cleaving several peptides including vasopressin, lys-bradykinin, met-enkephalin, dynorphin A, somatostatin and cholecystokinin [238, 239]. One proposed mechanism of action for IRAP as the AT-4 receptor is by inhibition of the molecules peptidase action, enhancing the action of its usual peptide substrate.

Angiotensin-(1-7)

Angiotensin-(1-7) is formed in systemic vessels and the heart by a variety of pathways; the dominant mechanism is from AT-2 by the action of prolyl-carboxypeptidases (PCP), prolyl-endopeptidases (PEP) or ACE-2. The two lesser pathways are via the action of ACE or neprilysin (*aka.* neutral-endopeptidase 24.11) on AT-(1-9) and by the action of ACE-2 on AT-1. The breakdown of AT-(1-7) is ACE dependent. AT-(1-7) has demonstrable systemic vasodilator properties in animal models [240] and is thought to act via a combination of bradykinin potentiation [241] and NO release [242]. Furthermore cardiac infusions AT-(1-7) have a dose dependent effect and can be cardioprotective during ischaemia and even enhance cardiac output at low doses in rat models; whereas higher doses can result in reperfusion arrhythmias [243]. The tissue activity of AT-(1-7) has been suggested by the presence of AT-(1-7) mRNA, direct sampling and infusion studies in rat heart models, although the mechanism of action remains unclear; furthermore AT-(1-7) has diuretic properties, via alterations in renal blood flow and a direct tubular action [244]. The receptor(s) responsible for the action of AT-(1-7) remains unclear. A G-coupled receptor with a high affinity for AT-(1-7) has been described (Mas), but the mechanism of effect is not known [240]; furthermore AT_1 and AT_2 receptor antagonists block some of the effects of AT-(1-7), suggesting interaction with these receptors. The role of AT-(1-7) in the pulmonary circulation has yet to

be demonstrated and at least one study, examining responses in isolated rat lung, failed to show any effect of BK or neprilysin inhibition on pulmonary vasoconstrictor responses to AT-1 infusion [245].

Angiotensin converting enzyme-2

Angiotensin converting enzyme-2 is a homologue of ACE, which is similarly expressed on the endothelium and is also secreted by enzymatic cleavage. Previously, ACE-2 was thought to be limited to heart, kidney and testicular tissues in humans [246]; however it has since been described in the pulmonary endothelium and has been identified as the receptor for the severe acute respiratory syndrome-coronavirus (SARS Co-V) [247-249]. Unlike ACE, ACE-2 has only one active metallopeptidase domain and is unaffected by ACE inhibitors [250, 251]; however ACE-2 expression is increased by ACE inhibition and AT_1 receptor antagonists in the heart. ACE-2 degrades AT-2 to AT-(1-7) and acts as a counter effect to the vasoconstrictor effect of ACE and AT-2. The generation of AT-(1-7) from AT-1 by ACE-2 is controversial, since the kinetics of this reaction draws into question the significance of this pathway in vivo [252]; however, ACE-2 does generate angiotensin-(1-9) which is then cleaved by ACE and neprilysin to form AT-(1-7). The role of ACE-2 in the heart has been demonstrated by knockout mouse models and the increased expression in failing hearts [253, 254]; however the role in the lung is less clear. There is evidence from rescue experiments in models of acute lung injury in knock out mice that ACE-2 plays a protective role in the pathogenesis of ARDS [255]; however there is no direct evidence of a vasoregulatory role in the human pulmonary circulation.

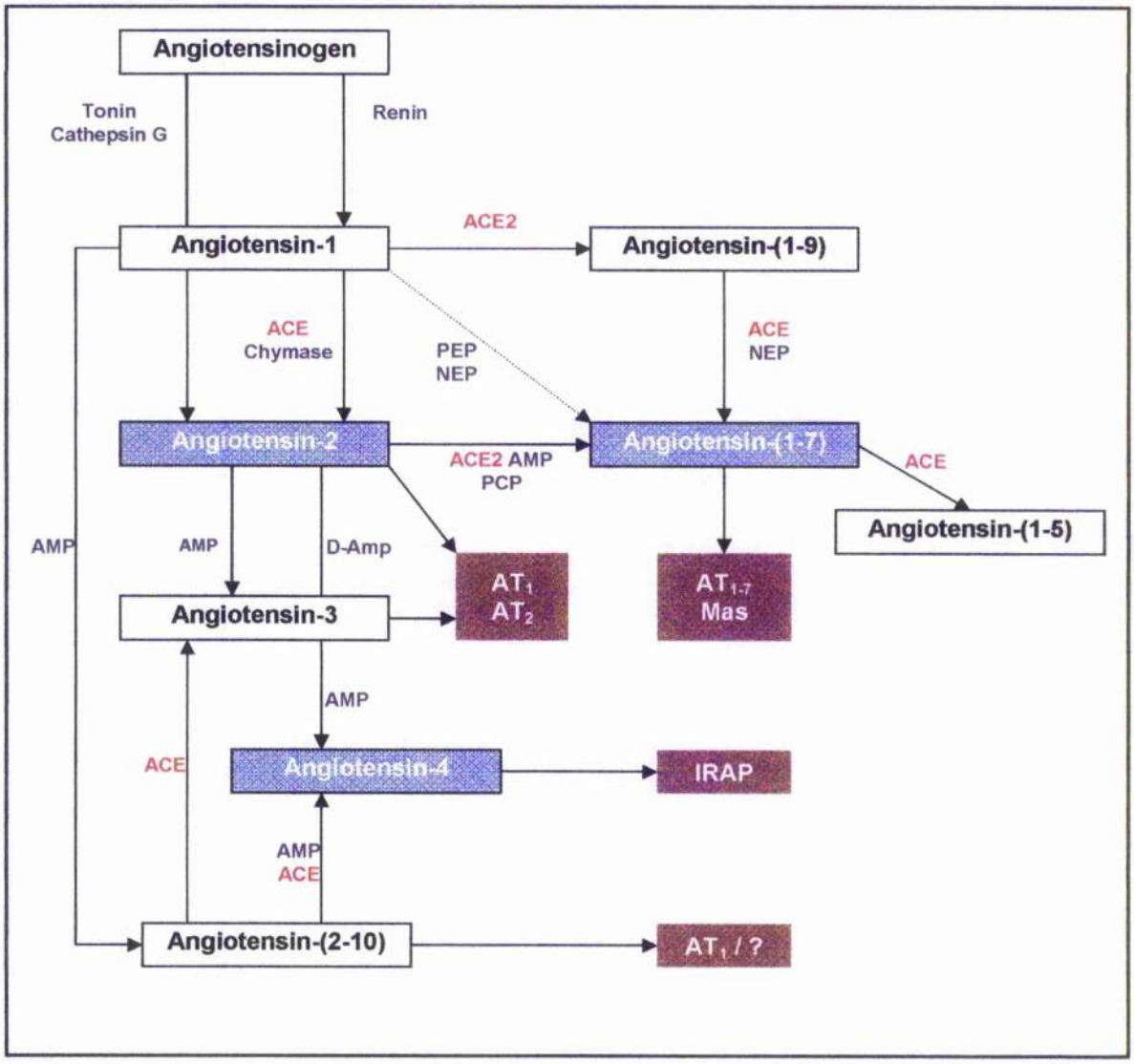

Figure 1.11 Angiotensin peptides and their generation. Angiotensin peptides discussed in the text are highlighted in blue; ACE and ACE2 are highlighted in red. ACE = angiotensin converting enzyme; ACE2 = angiotensin converting enzyme-2; PEP = prolylendopeptidase; NEP = neutral endopeptidase 24.11 (neprilysin); D-Amp = dipeptidyl-aminopeptidase; AMP = aminopeptidase; IRAP = insulin regulated aminopeptidase; Mas = AT-(1-7) receptor. [256]

Bradykinin

Bradykinin (BK) is a vasoactive nonapeptide liberated by cleavage of high and low molecular weight kininogens by kallikrein. Kallikrein in turn is activated by tissue inflammation and by prolyl-carboxypeptidase (PCP) which forms one of the degradation pathways of AT-2. Bradykinin is degraded by ACE in two dipeptide hydrolytic steps and is

also known as kinase II [257, 258]; furthermore ACE has a twenty fold greater affinity for BK than AT-1 [259]. In contrast, ACE-2 does not degrade BK; it does however degrade the related molecule Des-Arg^9-BK an inflammatory mediator which is released in response to inflammation and elevates blood pressure [260]. Bradykinin stimulates vasodilatation and NO formation; liberates tissue plasminogen activator; releases the vasodilator, prostacyclin and generates superoxide [261-263]. The interaction between AT-(1-7) and BK is suggested by the attenuation of vasodilator response of AT-(1-7) by the BK β_2 receptor antagonist HOE140 [241]; furthermore the action of AT-(1-7) on AT_2 receptors has the potential to release BK [264]. The vasodilator effect of BK on the pulmonary circulation has been demonstrated in cat models [265] and in humans [266].

The action of ACE inhibitors has been partly attributed to the effects of increased BK, via its anti-thrombotic and vasodilatatory actions [221, 241]; however it is apparent that the kallikrein-kinin system and RAS are linked at fundamental level, each with the ability to modulate the effects of the other.

1.9.4 Angiotensin-2 and local RAS

The prolific nature of local RAS suggests a role across multiple physiological systems; the following section examines the evidence of RAS in some of the systems reviewed in previous sections. The evidence for local RAS can be suggested by the expression of the various components of this system, the identification of receptors and the effect of intervention on the normal response of tissues. These points have been reviewed for the pulmonary circulation; however there is similar evidence of activity in the carotid body, the brainstem and skeletal muscle.

Carotid body RAS

As we have seen, carotid body responses are complex, demonstrating variation between species. Glomus type 1 cells are capable of releasing numerous neurotransmitter substances in response to hypoxia, some of which are capable of autoregulating their own response; in addition there is a further level of modulation by the carotid sinus nerve itself. Furthermore, the carotid body is a highly vascular tissue and autocrine/ paracrine factors produced at the endothelium can modulate blood flow, but potentially have an effect upon the glomus cells in their response to hypoxia, pH and circulating hormones. Autoradiographic studies have

demonstrated a high concentration of AT-2 receptors [267] and that chronic hypoxia upregulates the expression and function of AT-2 receptors, predominantly via the action of AT_1Rc [268].

The response appears to be biphasic with an initial inhibition followed by a marked stimulation of carotid sinus nerve activity. Infusion studies, using AT-2 in fresh ex vivo samples of carotid body suffused with bicarbonate buffer, demonstrate a two-fold increase in carotid sinus discharge up to a threshold concentration (which is within physiological levels). The AT_1Rc, losartan inhibits excitation, whereas AT_2Rc blockade (using the antagonist PD-123319) has no effect [267, 268]. The mechanism of action maybe an indirect effect modulating the release of neurotransmitter substance rather than a direct action,; however AT-2 can directly affect glomus cell activation, as demonstrated by increases in intracellular Ca^{2+} in response to AT-2 [269]. Again, this appears to be via the action of AT_1Rc, since losartan inhibits this effect whereas PD-123319 has no effect. AT_1Rc have been localised to glomus cells containing tyrosine hydroxylase and adds further weight to AT-2's role in chemosensitive cells; AT_2Rc mRNA has been located within type I cells, but as yet no functional effect has been demonstrated in ex vivo preparations [269].

The localisation of AT-2 receptors in glomus cells and the response to AT-2 infusion does suggest the role of RAS in modulating response to hypoxia; however direct evidence of a local carotid body RAS has been found by the expression and localisation of angiotensinogen and ACE within glomus cells [270]. The absence of renin within glomus cells is a factor against a functioning local RAS; however there is evidence that uptake of circulating RAS components can play a role in the action of local RAS [271]. Furthermore AT-2 can be generated by enzymes other that renin, including cathepsin G and tonin [272]. The direct effect of AT-2 on hypoxic sensitivity has recently been demonstrated in models of heart failure in rabbits. Li and Schultz have shown that AT-2/AT_1Rc effects increase hypoxic sensitivity of carotid body K_V channels and that high concentrations of AT-2 can inhibit intracellular K^+ currents; in this model, the effect was associated with a shift in K_V channel subtype from $K_V3.4$ to $K_V4.3$ [273].

The evidence supports the role of AT-2 in animal models of hypoxia at the carotid body, but as yet similar responses in humans have yet to be ascribed. Chronic hypoxia causes morphological change in the carotid bodies, including increased vascularisation, hypertrophy and hyperplasia of glomus cells [274, 275]. The proliferative effect of AT-2 at AT_1Rc,

similar to that seen in other tissues has been offered as a possible explanation for this, but currently lacks direct evidence.

Brainstem RAS

The ventilatory centres located in the brainstem are less well defined in terms of precise location and function. The research on the effects of RAS on the brainstem has mainly focussed on the blood pressure control and responses to baroreflexes in the pathogenesis of essential hypertension [276]. However, RAS has been demonstrated in the brain and direct injection of AT-2 in to the nucleus of the tractus solitarius (nTS) has demonstrable effects on ventilation in rats [277]. With the exception of the circumventricular organs, circulating RAS has limited access to the brain since RAS components do not cross the blood brain barrier [278]. RAS components have been located in various brain structures. Angiotensin peptides (AT-1, AT-2, AT-3 and AT-(1-7)) have all been identified in nerve terminals and neuronal cell bodies including the paraventricular nucleus and the nTS. Renin mRNA has been located in nerve cells, but the majority of angiotensin production is thought to be via the action of cathepsin G [279, 280]. Autoradiographic studies have mapped angiotensin receptors to many regions of the brain; AT_1Rc have been located in the NTS, the rostral and caudal ventrolateral medulla and midline raphe [281]; AT_2Rc distribution varies between species and in humans is located in cerebellum.

AT-4 receptor mapping has revealed a highly conserved pattern of distribution between species; the receptor has been located in the basal nucleus of Meynert, the hippocampus and throughout the neocortex. [282-284]. AT-4 receptors have been associated with cholinergic fibres, the enhancement of memory and motor control, but have yet to be associated with any of the brainstem centres linked with ventilatory control. ACE has been identified throughout the brain with the highest concentrations in the circumventricular organs. ACE has been localised to nigrostriatal structures, NTS and dorsal motor nuclei across several species, including man [285-288]. Brain RAS has been linked to blood pressure control, thirst sensation and vasopressin secretion [289]; however the function of RAS components in central ventilatory control is less defined. One study has demonstrated a link between AT-2 and ventilatory response. Microinjection studies of AT-2 into rat NTS has a negative effect on phrenic nerve discharge which exhibits some dose response [277]; therefore implies that brain RAS has the potential to modulate ventilation.

Skeletal muscle RAS

The evidence for skeletal muscle RAS originated from observed ACE activity in excess of serum ACE levels in muscle biopsy preparations [290]. Animal studies in muscle preparations and cultured myocytes demonstrate the presence of ACE, with demonstrable AT-2 production and bradykinin degradation [217, 291]. Studies in muscle, bereft of circulating ACE, continue to demonstrate vasoconstriction to AT-1 infusions that is blocked by ACE inhibitors [292]. Both subtypes of the AT-2 receptor are present in rat skeletal muscle, the receptors being distributed between the endothelium and the myocytes [293, 294]. The AT_1Rc mediates myocyte hypertrophy, whereas the AT_2Rc inhibits proliferation [295, 296]. The AT_2Rc has also a vasodilatatory function which appears to be mediated via the β2 BK receptor [264, 297], suggesting the involvement of AT-(1-7). Despite the wealth of evidence for AT_2Rc involvement in animal models of skeletal muscle function, only the AT_1Rc has been described in adult humans [298].

The identification of AT-2 receptors types sets the stage for RAS function at the skeletal muscle, but to what degree systemic RAS or local RAS stimulates these receptors is more difficult to differentiate. Whether muscle vascular endothelium or the myocytes themselves are the source of endogenously produced AT-2 also remains unknown. Irrespective of the uncertainty surrounding the exact source of AT-2 in muscle there is evidence of de novo synthesis of AT-2 from the conversion of intrinsic and circulating AT-1. Radiolabelled infusion studies have demonstrated that 67% of venous AT-1 and 55% of venous AT-2 are derived from skeletal muscle in pig models [299]. In humans, conversion of AT-1 to AT-2 across the forearm is 36% and this is virtually abolished by ACE inhibition [300].

The presence of skeletal muscle RAS has implications for exercise and performance; although no clear relationship has been demonstrated there are several possible areas of effect. Firstly, ACE inhibition attenuates overload stimulated muscle hypertrophy in rats and AT-2 infusion rescues this response. Furthermore, AT_1Rc blockade prevents the subsequent rescue effect of AT-2; this suggests a role for AT-2/AT_1Rc in muscle hypertrophy response [301]. Secondly, muscle blood flow also appears to be RAS dependent at least in the rat; there is evidence for redirection of blood flow from type 1 fibres to type 2 in isolated muscle preparations [302], supporting a model of enhanced power orientated performance with AT-2. Finally, RAS regulation of substrate utilisation at the muscle is implied by experiments using retrograde infusion of ACE inhibitors in human muscle; these experiments result in an increase in interstitial glucose and facilitated glucose transport [303, 304]. The effect appears

to be independent of AT-2, since AT_1Rc blockade has no effect in rat muscle; however the response does appear to be due to BK effects mediated by NO at the muscle, since the response is abolished by β2 BK receptor blockade or NOS inhibition [305].

Skeletal muscle RAS has been linked to muscle substrate utilisation. The translocation of the glucose transport molecule GLUT4 is found in muscle and adipose tissue; GLUT4 translocation can be stimulated by insulin dependent mechanisms, but also by insulin independent pathways such as BK [306]. Bradykinin increases GLUT4 translocation during exercise [307] and ACE inhibition has an enhancing effect, reversible by β2 BK receptor antagonists [308]; the effect is via translocation of GLUT4 rather than increased mRNA expression and transcription [309].

The roles of RAS and bradykinin in skeletal muscle have implications for exercise performance. ACE inhibitors appear to have a beneficial effect on muscle performance in heart failure patients and animal models, but no clear enhancement in healthy humans. The ACE gene polymorphism is a further point of endogenous ACE attenuation that has been extensively studied in relation to physical performance.

1.10 Angiotensin converting enzyme and genetic polymorphisms

Angiotensin converting enzyme is a dipeptidyl-carboxypeptidase responsible for many roles in the renin-angiotensin system and the degradation of bradykinin (see above). ACE exists in a membrane form, but can also be released by proteolytic cleavage to form a circulating ACE. The discovery of a physiologically significant polymorphism of the ACE gene resulted in a broad spectrum of research examining the effect of ACE insertion/ deletion polymorphism in both health and disease. The following section reviews some of the points of this research in reference to exercise physiology and hypoxic responses.

1.10.1 The link between phenotype and genotype

The information encoded within our genes determines our physical characteristics or phenotype. Genes are encoded within deoxyribonucleic acid (DNA) by a unique sequence of purine and pyrimidine bases linked covalently to give a highly conserved code (genotype) from which is transcribed messenger ribonucleic acid (mRNA), that in turn is translated into a polypeptide chain or protein. Genes have sections that are meaningful in terms of protein

production (exons) interspersed with non-transcribed segments (introns) which are excised from the final mRNA used for transcription.

Through the action of structural proteins, enzymes, signalling pathways and transcriptional activators, genes determine how we look, the diseases we are susceptible to and how we respond to the physical stimuli in our environment. This extends from the organismal through the tissue level down to the cellular environment. We share many of our genes not only with other humans, but other species, with the most fundamentally important genes found in common with almost all forms of life. The differences are in the fine details of genetic structure. In higher organisms genes are located in discrete organelles, the cellular nucleus, in the form of chromosomes. A healthy human possesses 46 chromosomes in each somatic cell, however 22 of these occur as duplicate or pairs, encoding the same genes. The remaining two determine sex of the individual and are heterologous in males and homologous in females.

The reason why the majority of our genetic make up is in duplicate lies in the need for variability. Each chromosome pair carries genes with subtle differences in the coding for the same proteins, these are termed alleles. Alleles for a certain gene are termed dominant if they have a demonstrable phenotype despite the presence of an alternate copy of the same gene which is termed recessive. In order for recessive genes to exhibit their phenotype two copies must be present on each chromosome, since transcription from one chromosome or another carries an equal probability. In the event that both alleles for a gene have an equal chance of affecting the phenotype then the genes are termed co-dominant, the result is an intermediate phenotype. During gametogenesis recombination occurs between the chromosome pairs and a re-assortment of alleles occurs whilst preserving the genes themselves. This occurs in both male and female, thereby increasing the genetic variability of the resulting offspring. This not only introduces variability in physical appearance, but raises the possibility of variability in the genes that encode for every aspect of life down to the cellular level. In the face of environmental change or disease subtle differences in phenotype may prove advantageous ensuring survival of the individual, successful mating and the conservation of the genes encoding the beneficial trait.

The term allele only has meaning at the organismal level describing the possible genetic re-assortment or phenotypical outcome of translation. Genes coding for a protein can exist in more than two forms, but only two forms are present in an individual as alleles. These variations are called genetic polymorphisms and constitute another level of individual variability.

1.10.2 ACE gene polymorphisms

Until recently, the ACE gene was thought to possess one functional polymorphism; however there is evidence that at least one other functional polymorphism exist [310]. The focus of much of the research to date has been on the insertion/deletion ACE polymorphism (ACE I/D polymorphism); this thesis presents work based on physiological response in relation to this gene polymorphism. The ACE I/D polymorphism comprises of two forms of the ACE gene, distinguished by the presence of a 287 bp *Alu* repeat sequence in intron 16 (insertion, I-allele) as opposed to its absence (deletion, D-allele). *Alu* repeat sequences are a common occurrence in the human genome and are associated with many gene polymorphisms, some of which are directly linked with disease [311]. The functional aspects of the ACE I/D polymorphism are a 47% reduction in enzyme activity associated with I-allele homogenicity in comparison with the D-allele homozygote. Furthermore, the ACE I/D polymorphism demonstrates co-dominance and heterozygotes exhibit an intermediate level of ACE activity. [312, 313].

The mechanism of variable ACE activity between individuals is not known. The I/D polymorphism is in a non-transcribed portion of the genome which makes it unlikely that it is directly responsible for the variation in activity. In addition to the I/D polymorphism there are a number of other single nucleotide polymorphisms (SNPs) in close proximity, of which some lie in exon segments of the gene; currently it is thought that one of these polymorphisms may be directly responsible or close to the exact loci responsible for the observed effect. There are a number of possible candidate loci that have been investigated; however the responsible locus or loci remain elusive [314-316].

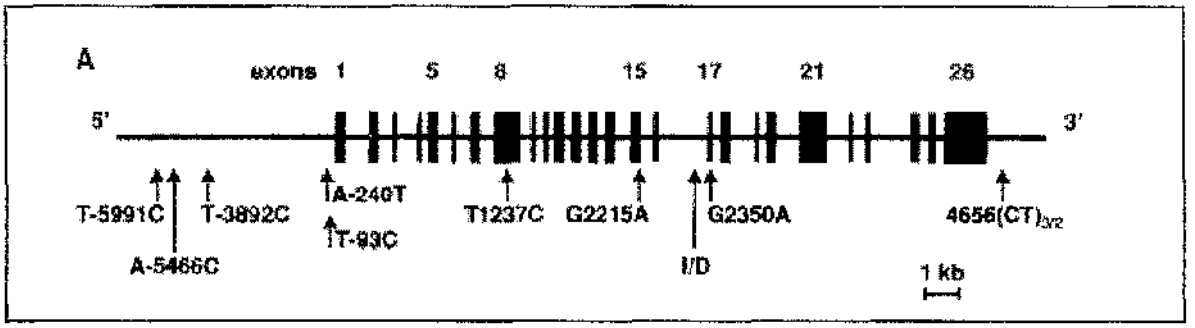

Figure 1.12 Diagram of the human ACE gene with 10 polymorphic sites labelled. Horizontal bars represent exons. The I/D polymorphism is labelled in intron 16 (From Keavney et al [315]).

The ACE I/D polymorphism has been studied in the context of disease epidemiology; in this context it is necessary to ensure that the gene frequency and genotype within a population are constant or in genetic equilibrium. This principle was described by the English mathematician G.H. Hardy, but previously outlined by the German physician Wilhelm Weinberg; consequently it is termed to Hardy-Weinberg equilibrium. In order for equilibrium to be reached the population must be allowed to breed freely, with no inter-breeding, mutation, migration or selection. In considering two alleles for a particular gene 'A' and 'a' with frequencies 'p' and 'q' respectively; then the expected frequency of genotypes can be calculated from the possible permutations of allele combination using a distribution square:

	A (p)	**a (q)**
A (p)	AA (p^2)	Aa (pq)
a (q)	Aa (pq)	aa (q^2)

The resulting frequencies for each genotype are:

$$AA = p^2$$
$$Aa = 2pq$$
$$aa = q^2$$

If we consider the allele frequency for the insertion and deletion polymorphisms to be equal, as one would expect in a Caucasian population, then the expected ratio of genotypes would be:

II : 2 ID: DD

In order to confirm whether a sample population conforms to the expected genotype frequency statistical analyses can be applied. Non-conformity can result from consanguinity, mutation, positive selection or negative selection on the basis of disease associated with a particular genotype.

1.10.3 ACE gene polymorphisms and physical performance

The variability of ACE activity across a population, in light of the widespread distribution of RAS in the body, has the potential to influence a wide variety of physiological systems. The point at which ACE I/D polymorphism may exert an influence is not limited to AT-2 generation and may include effects on other angiotensin peptides and bradykinin. The ACE I/D polymorphism has been widely studied in the context of a diverse range of pathological conditions and in epidemiological studies as a marker of cardiovascular risk; however, another facet of the insertion/ deletion polymorphism is the link between genotype and physical performance. This has proved a contentious area with conflicting results amongst investigators; however in studies examining elite athletes performing solely one endurance discipline, there is a significant excess frequency of the I-allele above that expected within the populations studied. [317-319]. The conflicting results produced by some investigators have been attributed to samples that include heterologous groups of athletes, with only a portion from endurance disciplines [320, 321].

The precise mechanism of the advantage conferred by the I-allele is not known, but the widespread presence of ACE raises several possibilities, from muscle substrate utilisation to central nervous system affects. In terms of measures of physical fitness there is disagreement as to the influence of ACE I/D polymorphism. One study examining VO_2 max in post-menopausal women demonstrated a higher VO_2 max in women with the II genotype in comparison to women with the DD genotype [322]; however the HERITAGE (health, risk factors, exercise training and genetics) study produced a conflicting result. The response of a standardised exercise program in 724 adults divided into: Caucasian and black subjects; parent and offspring groups, was assessed by ACE genotype. There was no difference in VO_2 max at baseline, but after 20 weeks of training the Caucasian offspring group demonstrated an enhanced metabolic response during exercise. VO_2 max and 80% of VO_2 max were increased by 14-38%; heart rate at 50 watts was decreased by 36%. A possible explanation is the close association between VO_2 and cardiac output; the ACE deletion allele is associated with increased left ventricular mass after a program of army basic training [323] and in athletes after training [324, 325]. Therefore, young Caucasian deletion homozygotes may exhibit the effect of increased left ventricular mass by an enhanced cardiac output response during exercise with improved oxygen delivery and VO_2.

The effects of ACE I/D polymorphisms on skeletal muscle have been examined in the context of performance enhancement. Studies examining military recruits have shown an

enhancement in muscle endurance [326] and efficiency after 11 weeks of physical training in the II individuals in comparison with the those possessing the DD genotype (there were no demonstrable differences at baseline) [327]. The study that reported an increase in VO_2max in postmenopausal women with the II genotype, also reported a greater arterio-venous oxygen difference across exercising muscle; this suggests local factors contribute to oxygen uptake [322]. Local ACE response to genotype is illustrated by the enhanced arterial contractile response to AT-I infusion into internal mammary artery preparations from DD individuals [328]. In vivo experiments confirm the variation in local RAS activity with infusional studies demonstrating higher AT-2 concentration in forearm venous blood following AT-I infusion in DD subjects. These experiments confirm the variable activity of ACE in muscle RAS, but do not confirm AT-2 as the mediating peptide. Bradykinin has demonstrable effects on muscle blood flow and substrate utilisation; furthermore, ACE I/D polymorphisms have a measurable effect on BK levels, with higher levels in I-allele homozygous individuals [329].

The debate over the relevance and mechanism of ACE I/D polymorphism influence on physical performance remains unresolved; however the observed association of the insertion allele with endurance performance is real and demands explanation.

1.10.4 ACE gene polymorphisms and hypoxia

ACE I/D polymorphism involvement in hypoxic physical performance was first indicated by frequency disequilibrium in elite mountaineers. Montgomery et al. reported an excess of the I-allele in a group of 33 elite mountaineers who had ascended above 7000m without supplementary oxygen; furthermore, of the 15 individuals who had ascended above 8000m without oxygen, none were homozygous for the D-allele [326]. A more recent study has confirmed this finding in a larger group of mountaineers ascending Mount Blanc (altitude: 4807m). In this study, 235 mountaineers were genotyped on the ascent; the I-allele frequency was 0.47 in those who completed the ascent in comparison to an I-allele frequency of 0.21 in those who were not successful [330]. The precise mechanism of these findings remains obscure; however a further study demonstrated enhanced oxygen saturations in insertion homozygous individuals during rapid ascent over 12 days to 5000m. Furthermore, the rate of ascent appears to be a crucial factor, since a second group of climbers ascending over 18 days did not demonstrate any significant difference in oxygen saturation at the same altitude [331]. This suggests enhanced performance at altitude may be attributable to differences in the

cardiorespiratory response to hypoxia by individuals of different ACE genotypes. The work presented in this thesis attempts to identify some of these differences by examining some of the cardiorespiratory responses during acute hypoxic exposure.

1.10.5 ACE genotype in high altitude disease

The association of ACE polymorphism with altitude performance has raised the question: Is there an association between the ACE genotype and high altitude disease that could account for the variation in performance? Several studies have examined the genotype frequency in groups of subjects susceptible to HAPE in comparison with controls; all failed to demonstrate any association with either allele [332, 333]. Furthermore, studies investigating a link between genotype and acute mountain sickness (AMS), showed either no association or inconclusive results [330, 334]. Therefore there appears to be no direct link between ACE I/D polymorphism and high altitude disease. One interesting feature noted in one of these studies was the increase in pulmonary vascular resistance during pulmonary catheter studies of HAPE patients with the D-allele; this suggests an enhanced HPVR in association with D-allele positivity, but no link with the development of HAPE [332]. The features of high altitude disease are described further in this chapter.

1.11 The Genetic response to hypoxia

The previous sections have reviewed the hypoxic physiological response and have touched on the relationship between ACE polymorphism and physical performance under conditions of hypoxia; however it is now apparent that the response to hypoxia is a more ubiquitous occurrence at the cellular level. The ACE polymorphism alludes to one aspect of genetic involvement in these responses, but in the last 15 years the discovery of oxygen dependent transcription from these genes has meant a whole new insight into hypoxic responses from the molecular perspective.

1.11.1 Hypoxia dependent gene expression

Erythropoietin was the first example of oxygen dependent gene expression to be described. EPO produces polycythaemia in response to hypoxia, through the accelerated maturation of red blood cell progenitor cells and by inhibition of progenitor cell apoptosis [335]. The resultant increase in the oxygen carrying capacity is one of the most fundamental responses

to prolonged hypoxia. An increase in EPO mRNA can be seen within one hour of hypoxic exposure which declines with a return to normoxic conditions [336]. If hypoxia is maintained then the increase in mRNA results in an increase in circulating EPO and erythropoesis. Transcription activation of the EPO gene is dependent on a 3' transcriptional activation sequence called the hypoxia response element (HRE) [337-339]. This sequence confers hypoxia sensitivity to genes if inserted into their promoter sequences and it is known that mutations within the HRE render it insensitive to hypoxic stimulation.

The hypoxia response element has been found in a wide range of genes which are sensitive to hypoxia. The genes responsible for glycolysis, glucose transport as well as cellular and vascular proliferation (vascular endothelial growth factor (VEGF), placental growth factor, angiopoetin -1 and -2 and platelet derived growth factor) demonstrate HREs and are oxygen sensitive [340-343]. In addition, the genes encoding vasoactive mediators such as NOS and endothelin possess HREs [344, 345]. Furthermore, the tyrosine hydroxylase gene possesses an HRE [346]; thus conferring hypoxia responsiveness on the crucial-rate limiting enzyme in the synthesis of ventilatory neurotransmitters [347].

1.11.2 Hypoxia inducible factor

Transcription of these 'oxygen sensitive' genes is promoted by binding of the HRE by a nuclear protein complex identified as hypoxia-inducible-factor-1 (HIF-1) [348]. HIF-1 consists of two basic helix-loop-helix subunits; both are required for binding to the HRE and activation of transcription. One subunit, HIF-1β is constitutively produced in normoxic cells and is also known as the aryl hydrocarbon receptor (ARNT). The second subunit, HIF-1α is an oxygen-regulated 826 amino acid peptide. HIF-1α is degraded rapidly under normoxic conditions, but has a prolonged half life in hypoxia with raised levels measurable within 2 minutes [349]. The rapid elevation in HIF-1α is most likely due to inhibited degradation since mRNA levels are unaffected by oxygen tensions. The peptide is hydroxylated at proline residues 402 and 564 in the oxygen dependent domain (ODD) by HIF-1α proline hydroxylase which is an oxygen dependent process [350]. Hydroxylation at these residues allows binding of von Hippel-Lindau tumour suppressor protein (pVHL) which then allows recognition by E3 ubiquitin-protein ligase and degrades HIF-1α [351, 352]. In hypoxic conditions the hydroxylation of proline residues does not occur and HIF-1α levels increase. The promoter function of HIF-1 is also sensitive to hypoxia. Oxygen dependent hydroxylation of the asparagine residue 803 by Factor inhibiting HIF-1 (FIH-1) blocks the

interaction of HIF-1α and coactivators required for HIF-1 mediated activation of transcription [353, 354]. HIF-2α is structurally homologous hypoxia inducible transcription regulator which shares similar properties and functions to HIF-1α and is similarly regulated by prolyl and asparginyl hydroxylation [350, 353, 355]. The difference lies in the distribution of HIF-2α since it appears to be limited to specific tissues in particular endothelial cells which may lend it a role in the response to hypoxic stress [356-358].

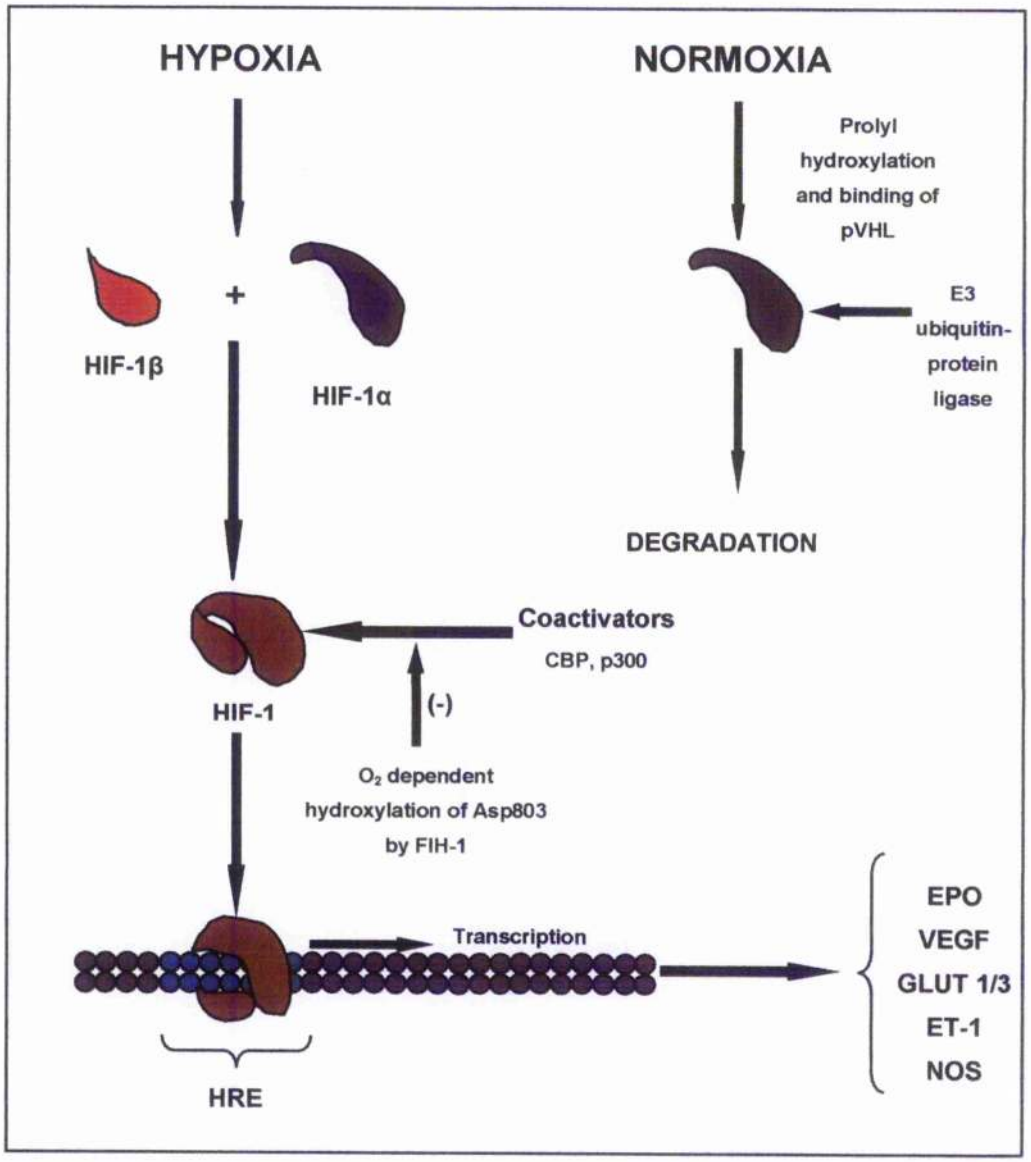

Figure 1.13 Oxygen dependent transcription by HIF-1α.

1.11.3 Oxygen sensing and gene expression

The demonstration of oxygen sensitive genes and HIF-1 mediated transcription has been a crucial step in the understanding of the body's response to hypoxia, but the exact mechanism of oxygen sensing and subsequent stabilisation and promotion of HIF-1α activity remained elusive for some years. Early evidence suggested a haem-based molecule as an oxygen sensor [359], however this was not supported by iron chelator or haem-synthesis inhibitor studies [348, 360]. The simplest model for an oxygen sensor is HIF-1α proline hydroxylase itself, since oxygen is the rate limiting substrate for its function [350]. In vitro studies have demonstrated 3 forms of HIF-1α proline hydroxylase [350, 361] which share a similar function and are expressed in similar tissues. Recombinant forms of these prolyl hydroxylases have affinities for oxygen near that of atmospheric concentrations [362] and are far in excess of oxygen tensions found in tissues. In vivo, assuming a similar oxygen affinity, these hydroxylases would be not be at equilibrium; therefore small fluctuations in oxygen concentration would have an affect on function, satisfying the primary prerequisite for an oxygen sensor. Furthermore mRNA of two of the prolyl hydroxylases involved accumulates in hypoxia with an increase in protein levels [350, 363, 364]. This has the potential to attenuate the effects of hypoxia, but most likely plays the important role of rapidly degrading HIF-1α upon reoxygenation, halting the hypoxic signal [365, 366].

HIF-1α prolyl hydroxylases appear to be the best candidate for oxygen sensing at the transcriptional level for genes possessing HREs, however this is a very active field of interest and further discoveries in this pathway can only be anticipated.

1.11.4 Carotid bodies and hypoxia induced gene regulation.

The transduction of oxygen chemoreception to sinus nerve activations by the carotid body glomus cell is dependent on catecholamine synthesis and release. Tyrosine hydroxylase (TH) is the rate limiting enzyme in the synthesis of dopamine and its gene contains a HRE; furthermore hypoxia induces the transcription of TH mRNA in a HIF dependent manner [367]. In rat carotid body glomus cells HIF-1α and HIF-2α are demonstrable in neonatal animals and adolescent rats exposed to hypoxia. The increased level of HIF-1α & 2α is accompanied by a measurable increase in TH; thus demonstrating a hypoxia induced activation of catecholamine pathway that participates in the hypoxic ventilatory response [368]. Similar expression in humans has yet to be described, but this animal model provides

an insight into a possible mechanism for hypoxic ventilatory adaptation in peripheral chemoreceptors at the genetic level.

1.12 High altitude disease

The topics reviewed thus far have focussed predominantly on acute hypoxic responses; however one of the most dramatic and relevant settings for hypoxic physiology is at altitude. The following is a brief review of the research into and the diseases encountered at high altitude.

1.12.1 History

There is some disagreement as to whether the earliest written record of altitude disease dates to the time of the Chinese Han dynasties in 30 BCE or 300 years earlier and are attributable to Aristotle [369]; in either case man has been aware of the travails of high altitude since ancient times. Unsurprisingly the source of these illnesses was tainted by superstitious belief and attributed to noxious vapours, maliferous spirits or toxic flora. It was not until the era of scientific enquiry that light was cast on the causes of altitude disease. Blaise Pascal's may have discovered the fall in barometric pressure at altitude, but it was the French Physiologist Paul Bert (1833-1886) who made the link between the low partial pressures of oxygen at altitude and the effects on human physiology (*La Pression Barométrique,* 1848).

Whilst scientists such as Bert were at work pushing forward the boundaries of physiology, so explorers were mapping every corner of the globe and the dangers posed by high altitude became all too real; it was not long before research moved from the laboratory into the field. Expeditions to the Alps and Rocky mountains preceded research at higher altitudes in the Andes and the Himalayas. Permanent research stations such as the Capanna Regina Margherita hut on the summit of Monte Rosa (4559m) have stood since 1893 and its facilities still permit sophisticated research in one of the world's most inhospitable environments. Whilst advances were being made in both laboratory and the field, mountaineers were ascending ever higher, culminating with the first ascent of Everest in 1953 by Hillary and Tensing. The work of figures such as Christian Bohr, John Scott Haldane, Joseph Barcroft and August Krogh at the beginning of the century is continued today, though extends beyond the remit of traditional physiology. Modern altitude research utilises all the disciplines of the biosciences, examining the biochemistry and genetics of hypoxic response in an attempt to

unravel the processes that belie the physiological responses that have been described previously. Some of the work described in this thesis utilises the 'new science' to examine the physiological responses at altitude.

1.12.2 Acute high altitude disease

The descriptive terms for high altitude diseases have developed over the last one hundred years, but a common theme amongst the varied nomenclatures has been the appreciation of duration of altitude exposure in the development of disease. As a consequence high altitude disease is divided into acute conditions that occur in the first few days of exposure and chronic, that occurs over weeks and months. Acute high altitude disease develops at altitudes over 2500m and is associated with rapid ascent in poorly acclimatised individuals. The conditions are directly attributable to hypoxia, though viral illness, dehydration and cold exposure can contribute. Descent to lower altitude is the treatment of choice and sometimes the only effective therapy. The current nomenclature divides acute altitude disease into three main entities; acute mountain sickness, high altitude cerebral oedema (HACE) and high altitude pulmonary oedema (HAPE); the first two conditions are considered to be opposite ends of the spectrum for the same disease process whereas HAPE is considered as a distinct pathology. The brief review that follows will concentrate on these acute conditions.

Acute mountain sickness and high altitude cerebral oedema.

Acute mountain sickness (AMS) is an almost universal occurrence at altitude and its principle symptom is headache (the Chinese writings mentioned refer to 'Big and Little headache mountains'). Other symptoms include anorexia, nausea and vomiting, fatigue, light-headedness and sleep disturbance. Signs maybe absent or manifest as peripheral oedema or low grade fever. The onset is usually after the first 6-12 hour at altitude, is at its worst on days 2 to 3, with complete resolution by day 5. In an attempt to categorise the symptomatic severity of AMS the Lake Louise score was devised and is still used as a subjective score of altitude illness [370]. AMS in itself is usually a benign self limiting condition; however it can progress to high altitude cerebral oedema (HACE), a much more serious and potentially fatal condition. HACE is characterised by ataxia, impaired consciousness, coma and eventual death; though it usually occurs in a progressive manner, HACE has been reported in conjunction with high altitude pulmonary oedema de novo. In

these cases, severe HAPE induced hypoxaemia is thought to precipitate the rapid development of HACE, without allowing time for the development of AMS symptoms.

AMS and HACE are thought to share a common pathophysiology; moderate to severe AMS is thought to be due to a mild form of cerebral oedema, HACE by definition *is* cerebral oedema. The precise cause for this oedema remains unclear and much debate exists whether the cause of raised intracranial pressure (ICP) is due to true oedema, fluid shift from extracellular to intracellular compartments or increased cerebral blood volume. Blood brain barrier (BBB) permeability is thought to play a crucial role in the pathology; due either mechanical stresses and/ or the action of mediators on the cerebral endothelium to produce a vasogenic oedema [371]. Another proposed factor is disturbance of the vascular endothelium by hypoxic angiogenesis [372], whereas the closed box theory suggests that abnormalities of cranial anatomy limits the flow of cerebrospinal fluid and thus facilitates the development of HACE [373].

The best preventative measure for any acute altitude illness is slow ascent with adequate time for acclimatisation. Acetazolamide has been used to facilitate acclimatisation and can be effective in the prevention of and treatment of AMS. HACE is a more serious condition and can be fatal within a matter of hours if no action is taken. As a consequence, immediate descent is the safest and optimum treatment, though dexamethasone and oxygen can be supportive in the short term.

1.12.3 High altitude pulmonary oedema

High altitude pulmonary oedema is potentially fatal. It initially manifests as excessive dyspnoea, dry cough and lethargy; AMS is invariably a feature. Symptoms precede signs that include pulmonary crackles; tachycardia is present and mild pyrexia is common; white frothy sputum develops, which eventually becomes blood stained. In a matter of hours the dyspnoea worsens with bubbling respirations, coma sets in and eventually death. Chest radiographs in those that receive medical attention demonstrate irregular patchy infiltrates in both lung fields, sometimes favouring the dependent side with prominent pulmonary arteries. X-ray findings tend to resolve within one week of descent [87, 374]. The condition is unpredictable in whom it affects. Young, fit individuals are susceptible and this may be more related to a higher level of physical activity; a known association with HAPE. Cold and rapid ascents are other contributory factors, though most intriguing is the individual susceptibility

towards HAPE; those that have been affected before are prone to subsequent episodes upon further ascents.

The pathophysiology of HAPE is dependent on an exaggerated HPVR as demonstrated by pulmonary artery catheterisation in HAPE susceptible individuals [86-88]. These studies confirmed a raised PAP, but a normal PCWP; thus demonstrating the non-cardiogenic nature of the oedema. The central role of hypoxia in the pathogenesis is demonstrated by amelioration of elevated PAP with oxygen inhalation [87]. As previously described HPVR in ambient hypoxia can lead to patchy pulmonary vasoconstriction and subsequent overperfusion of spared lung segments [150]. The effects of vascular shear forces can disrupt endothelial integrity causing a fluid leak and pulmonary oedema develops [375]. Inflammatory processes have been proposed as contributory to the development of HAPE since the oedematous fluid is rich in protein and cytokines [376]; however serial bronchoalveolar lavage in HAPE susceptible (HAPE-S) subjects demonstrated the absence of cytokines early in the development of HAPE and demonstrated clinically and radiographically. One possible explanation is the inflammatory effect of exposed basement membrane after haemodynamic shear effects [377] with subsequent platelet activation [378], which would explain the subsequent development of a cytokine rich exudate.

The humoral factors described in hypoxic pulmonary vascular responses have also been implicated in the pathogenesis of HAPE, but the response to sodium transport has been examined more closely in HAPE. Impaired sodium transport in HAPE-S individuals has been inferred using trans-nasal potential differences [379] and proposed as a contributory factor to the development of HAPE. Furthermore β-agonists are known to facilitate sodium pump activity and the clearance of lung water [380]. Trials of inhaled long acting β-agonists reduced the occurrence of HAPE in a susceptible group and improved transcutaneous oxygen saturations in HAPE-S subjects [381]. Nitric oxide levels are reduced in HAPE-S individuals [382] and this is due to a reduced activity of NOS as demonstrated by a reduction in L-citrulline (a stable by-product of NOS produced stochiometrically with NO) and cGMP [383]. Endothelin levels are demonstrably higher in HAPE-S individuals [384], but whether this is a primary pathological response or part of a wider 'hypoxic cascade' remains unclear. The link between humoral factors and HAPE remains one of the most intriguing areas of research not least because of the promise of new therapeutic strategies.

The hypoxic ventilatory response demonstrates individual variability and attenuation of the HVR has been associated with HAPE susceptibility [385-387]; however some individuals

exhibit a similar level of HVR as HAPE susceptible subjects, without developing HAPE. This suggests that HVR is not a central feature of HAPE pathogenesis, but might contribute by inducing a relative degree of alveolar hypoxia due to an inadequate HVR to hypobaric hypoxia. To date, there is no identifiable marker, either physiological or biochemical that reliably predicts the development of HAPE. This reflects the multiple physiological mechanisms that contribute to hypoxic adaptation and that the pathophysiology of HAPE is multifactorial.

1.13 Cardiopulmonary exercise testing

The previous sections have described the physiological and biochemical mechanisms behind the response to exercise and hypoxia. This section and the next reviews the practicalities of measuring the physiological variables described earlier, in this chapter. Cardiopulmonary exercise testing has come a long way from the use of Douglas bags for gas collection during exercise tests. Modern cardiopulmonary exercise tests (CPET) are performed on equipment utilising rapid gas analysers and flow meters to give breath-by-breath analysis during exercise. The different facets of CPET can influence the measured result; furthermore, the validity of these tests is reliant on stringent quality control and calibration. These aspects of cardiopulmonary exercise testing are briefly reviewed here.

1.13.1 The choice of exercise

The two commonest forms of exercise used in CPET are the treadmill and cycle ergometry, each has its own advantages and disadvantages. Treadmill exercise is a more natural form of exercise, utilises more of the major muscle groups and therefore is more likely to give a peak VO_2 close to VO_2max [171]. The disadvantages of treadmill exercise are: the bulkiness of the equipment; the potential of injury should the subject stumble; the increase in interference introduced into electrocardiographic measurements and that the actual power of exercise can only be estimated from calculation of incline, weight and the running speed. Cycle ergometry facilitates electrocardiographic measurement, since the upper body is relatively static and work rate can be measured directly. Ergometers can either be mechanically or electronically braked; the advantage of electronically braked ergometers is that they can maintain a constant work load despite variation in cycling speed. The rate of change in work load can also be increased in a smooth ramp by a computer attached to the cycle, enabling incremental

exercise testing without stepwise increase in work. Calibration of electronic ergometers requires the use of a dynamometer on a regular basis [388]. Electronically braked cycle ergometers are more expensive, but their flexibility during tests and the reliability of work rate (this is usually monitored during the test by the controlling computer) means that this form of exercise is the most appropriate for the studies in this thesis. One further consideration is that the subject is seated, therefore in the event of any adverse affect of exercising under hypoxic conditions the risk of injury is minimised.

1.13.2 Gas flow meters

Gas flow meters have replaced the Douglas bag to give expired gas volumes over time and there are several possible methods. Pneumotachometers, measure the pressure drop through a tube across a point of fixed resistance to air flow, from the differential in pressure gas flow can be calculated. Mass flow meters or anemometers rely on the cooling of a heated wire placed in the flow of gas. The degree of cooling and the current required to maintain the wire at the set temperature are a function of gas flow across the wire, thus enabling the calculation of flow. The final method of gas flow measurement are turbine spirometers; these give flow measurements according to the speed of rotation of the turbine located within the spirometer, the use of turbines is not commonly used in static CPET equipment, but is used in portable or even wearable units.

1.13.3 Gas analysis

There are several methods of gas analysis available, the method of choice for most physiologists is mass spectrometry, since it is rapid, accurate and is able to measure all the gases in the mixture simultaneously. The disadvantage lies in the expense of these systems and in some models mechanical failure. The principle is based on the acceleration of ionised gas molecules through an electromagnetic field; the degree of deflection is a property of mass and charge; the intensity of the beam of ions, as measured by sensors, gives the concentration. Commercially available metabolic carts sometimes utilise mass spectrometry, but usually employ rapid gas analysers, in order to minimise initial and maintenance costs. Whilst these were formerly limited by speed of response in combination with processing time, the vast improvements in the speed of standard desktop computers has shortened the gap between these two sensing modalities. There are two different types of oxygen sensor, but only one type of CO_2 sensor utilised in metabolic carts.

Oxygen sensors using zirconium electrochemical fuel cells utilise the semipermeable nature of zirconium oxide when heated; if placed in apposition to calcium, magnesium or yttrium oxides, zirconium oxide acts as a solid electrochemical cell. Exposure to oxygen results in the generation of a voltage in proportion to the concentration of oxygen. These cells are accurate, reliable and relatively cheap; the cell itself is a consumable item which requires regular replacement and is often performed as part of manufacturer servicing.

Paramagnetic oxygen analysers rely on the magnetic properties of the oxygen molecule; when O_2 enters a strong magnetic field, shifts in electrons within the molecules generate strong magnetic moments within the molecule. The paramagnetic sensors usually consist of two spheres arranged in a dumbbell, each containing nitrogen gas, suspended in a magnetic field. When oxygen enters the sensor, it becomes magnetised, enters the field and strengthens it; the nitrogen within the spheres has an opposing magnetic polarity and is forced out of the field, causing the dumbbell to spin. The rate of spin is proportional to the concentration of oxygen within the analyser. Paramagnetic analysers are relatively robust and resistant to movement; hence they are often used in portable CPET units.

Carbon dioxide analysers used in non-mass spectrometry metabolic carts are rapid infrared CO_2 analysers. As the name suggest they rely on the absorption of light at the infrared wavelength; this is proportional to the concentration of CO_2 absorption. Whether mass spectrometry or gas sensors are used, proper calibration is vital to maintain the fidelity of the measurements.

1.13.4 Quality control

The interpretation of any data from any test is only as reliable as the measuring system. Cardiopulmonary exercise testing integrates data from at least three different types of sensor; the integrity of test results is dependent on the validity of each of these measurements. Therefore, rigorous calibration of the metabolic cart is necessary to ensure reliable measurements. Gas flow is often calibrated using a standardised piston or syringe, connected to the flow meter; the software included with most commercially available metabolic carts enables calibration using this process. Gas analysers on modern metabolic carts use standardised calibration gases containing O_2 and CO_2 mixtures at precise concentrations. Prior to testing, the gas analysers are calibrated against two gas mixtures; one containing an O_2 concentration higher than atmospheric; the second often consists of CO_2 at a

concentration higher than that of expired air in combination with O_2 at concentrations lower than expired air. Sampling of each gas mixture enables the calibration of the analysers at gas concentration above and below that expected physiologically.

Calibration gases have been used in place of traditional Douglas bag calibration. This involves the collection of bag samples over one minute periods during exercise at 3 set work loads; these samples are then analysed and the volumes measured, to give a standardised value for minute ventilation, VO_2 and VCO_2 for comparison with the data produced by the CPET equipment. Many centres do not have the facilities to perform this type of calibration and rely on the standardised gases; however physiological validation is usually performed, where two or more subjects perform standard tests on both the CPET equipment being evaluated and in a centre capable of Douglas bag validation. The difference in measurements between the two centres should be within 6 percent [389].

Further quality control occurs during actual testing, through the monitoring of data produced during breath-by-breath tests; for example, variation in measurements such as VO_2 can give an early indication of error if they are higher than expected (e.g. air leak from mask or tubing). The duration of CPET should aim for exercise duration of 10-12 minutes, since longer durations can lead to erroneously high measurements of VO_2 and cardiac output; this occurs when there is a fractional increase in blood flow to the skin for thermoregulation, during prolonged exercise [171].

1.13.5 Oximetry and electrocardiographic data

The safety of CPET, as with all forms of exercise testing, requires the monitoring of cardiac function during the test. This is usually performed by electrocardiography (ECG) and is monitored during the test by trained personnel. Furthermore, the data from the ECG can be integrated into the test data and allow further analyses.

Oximetry

The oxygen saturation of the subject can be recorded during the test using a pulse oximeter, which works on the principle of the difference in absorption spectra between haemoglobin and oxyhaemoglobin. Two light emitting diodes, one red (660nM, absorbed by Hb) the other near infra-red (940nM, absorbed by oxyhaemoglobin) shine light through either the finger or the ear to a detector, within a specifically designed probe The absorption of these light spectra by tissues, venous and arterial blood result in a difference in absorption; the oximeter calculates the ratio of red:infra-red absorption and using algorithms gives a value of oxygen

saturation. The accuracy of pulse oximeters is around 2% between saturations of 70-100% [390-393]; however values lower than this are usually given by extrapolations; consequently, the accuracy of oximeters can vary by as much as 10% at values lower than 70-80% [391, 394, 395]. Oximeters made by different manufacturers can also give variable results, due to variations in the computer algorithms used by different makers [396]. Motion artefact, low temperatures and hypotension can all impair the accuracy of oximeter measurements [397, 398]. The choice of oximeter probe has an influence on accuracy, with finger oximetry demonstrating greater accuracy than ear or forehead probes [399]; furthermore, malpositioning of the probe can greatly reduce the accuracy of the measured SpO_2 [400]. Despite the limitations, pulse oximetry has become widely accepted in most clinical settings as a useful indicator of oxygenation; however the limitation of poor accuracy at low oxygen saturations is particularly relevant for these studies. Oximetry data is often integrated into the final test result and forms a vital part of interpretation of test in pathological conditions; in addition to providing test data during hypoxic testing, oximetry was a vital piece of safety equipment during the hypoxic studies described in this thesis.

Arterial catheter placement and exercise testing

Some CPET protocols necessitate the insertion of an arterial sampling catheter, thus enabling measurement of lactate and arterial O_2 and CO_2 tensions. The siting of these catheters are not without risk (though rare, embolic complications are recognised) and uncomfortable. Arterialised samples of blood can be acquired by the application of a vasodilator and the use of capillary samples via a pin prick, but again are a surrogate for arterial sampling and sometimes unacceptable due to discomfort. Modern gas analysers can rapidly give results for lactate and blood gas, but are expensive to buy and maintain; therefore availability is limited.

1.13.6 Breath-by-breath

The output from these various types of analysers gives O_2 and CO_2 concentrations of inspired and expired gas which are interfaced with a computer. This data is combined with flow meter data to enable breath-by-breath analysis. All variables are calculated from the data streams from these sensors and include: VO_2, VCO_2, end-expiratory CO_2 and O_2, tidal volume, respiratory rate and minute ventilation. Further calculations are possible, such as ventilatory equivalents, but are not essential to the requirements of most cardiopulmonary exercise tests. The output of breath-by-breath analysis incurs a variation in measurements between breaths, due to variations in ventilation, changes in functional residual capacity and the assumption

that inspired and expired nitrogen volumes are the same (mass spectrometry overcomes this by measuring O_2, CO_2 and N_2 simultaneously). Many commercially available metabolic carts allow averaging of the breath-by-breath data over a number of breaths or over a length of time. The duration of the averaging period can influence the variability of the results, with shorter durations resulting in greater variability. Breath-by-breath data averaged over 60 second reduces the degree of variation to that seen in non-automated systems [401, 402].

1.13.7 Variables that are measured and calculated during CPET

VO_2, VCO_2, respiratory rate, tidal volume and minute ventilation are considered as measured variables since they are directly derived from the measured gas flow and the difference between inspired and end tidal gas concentrations. Other variables are calculated from these 'measurements', ventilatory equivalents have been described previously, but the other major inferred ratio is the oxygen pulse. This is the ratio of VO_2 to heart rate and is sometimes used as surrogate measure for stroke volume. In addition to these basic measurements, modern metabolic carts are now capable of calculating a wide range of physiological variables. Irrespective of whether the variable is measured or calculated the gas volumes and concentrations must be standardised in order to allow comparison of results between different days of testing, let alone different centres. Measured gas concentrations and volumes are at ambient temperature and pressure and are standardised to either: body temperature and pressure, saturated with water vapour (BTPS); or standard temperature and pressure, dry gas (STPD, at 0°C and 760mmHg). By convention BTPS is used for ventilation and STPD for gas exchange; however many commercial systems can give all variable in either one standardised form or another.

The studies described in this thesis utilise the capabilities of a modern CPET metabolic cart to perform testing on healthy subjects at rest and during exercise under normoxic and hypoxic conditions.

1.14 Cardiac output monitoring

Cardiac output has been and remains an area of physiological interest in both health and disease. As a fundamental part of the convective mechanism between gas exchange at the

lung and oxygen consumption at the respiring tissues, cardiac output has been measured by a variety of methods for physiological study as well as the investigation and management of disease. The settings for measurement are varied, from the physiology laboratory to the intensive care setting. The subjects for measurement are variable too; from normal individuals to anaesthetised, critically ill patients. As a result of the broad range of circumstances for which CO measurements are necessary a variety of methods of measurement have been developed which are broadly divided into invasive and non-invasive techniques. The following is a brief overview of some of these methods focussing on bio-impedance cardiac output measurement as used in some of the studies described as part of this thesis.

1.14.1 'Gold standard' cardiac output measurement.

Fick first proposed a method of cardiac measurement based on the conservation of mass in 1870. The principle is that oxygen uptake at the lung is determined by the product of cardiac output and the difference in oxygen content between blood as it enters and as it leaves the lung:

$$VO_2 = CO \times (CaO_2 - CvO_2)$$

Where CaO_2 and CvO_2 represent the oxygen content of arterial blood and mixed venous blood respectively (the calculation for the oxygen content of blood is described in section A). The rearrangement of this equation gives:

$$CO = VO_2 / (CaO_2 - CvO_2)$$

Therefore simultaneous measurement of oxygen uptake, haemoglobin concentration and mixed venous and arterial O_2 saturation enables the determination of cardiac output. The practicality of such a measurement involves the insertion of a pulmonary artery catheter and arterial line into the subject to enable simultaneous sampling of blood whilst measuring oxygen uptake. Fick first described his principle in 1870, but it was another 70 years before this method was actually used in humans. Werner Forssmann was the first to insert a pulmonary catheter into a human (using himself!) in 1929; Cournand and Richards were the first to make measurements of chamber pressures and cardiac output in 1940 (on a New York

policeman). Their efforts earned them the Nobel prize for medicine in 1950 [403]. Since then modern pulmonary catheters and gas analysers have simplified the method for direct Fick cardiac output measurement and it remains the gold standard against which all other methods of CO measurement are compared. Out with research studies and the validation of other methods for CO, the 'direct Fick' method is rarely employed. As a consequence a number of methods have been devised to enable CO measurement both invasively and non-invasively.

1.14.2 Rebreathe or the 'indirect' Fick method for cardiac output measurement

The inability to sample mixed venous blood in humans made the use of the direct Fick method inapplicable for 70 years and even today the siting of a pulmonary catheter in subjects, especially during exercise is impractical at best. During the intervening years physiologists did not remain idle in devising novel and ingenious methods for the measurement of cardiac output. The forerunner of these was the 'indirect Fick method' where the mixed venous gas pressure is approximated by allowing equilibration with an inspired gas combined with a knowledge of the association-dissociation characteristics of that gas with blood at various gas tensions. If sampling of arterial blood gas tensions are use in the application of the Fick equation then the result reflects total cardiac output. If inferences of pulmonary end-capillary gas tensions from end expiratory gas concentrations are used then the result reflect the effective pulmonary blood flow. In normal lungs, in the absence of significant extra-pulmonary shunt or disease that impairs ventilation, then this is a reasonable measure of total blood flow.

For many years carbon dioxide rebreathe techniques were used for the 'indirect method', since it quickly equilibrates with blood. This involves the subject breathing in and out of a bag containing a known concentration of CO_2 at a higher partial pressure than mixed venous blood. As the CO_2 concentration in the bag plateaus so the mixed venous $PaCO_2$ tension can be viewed as 'fixed' and is higher than true mixed venous CO_2 (because of the Christiansen-Douglas-Haldane effect – where the CO_2 carrying capacity of Hb is reduced in the oxygenated state, i.e. the alveolar capillaries). The measurement of arterial $PaCO_2$ tension and knowledge of its dissociation curve enable the application of the Fick equation. This is applicable providing there is no recirculation of CO_2 from tissue stores and there is equilibration across the alveolar membrane. The effects of pH, Hb concentration and oxygen tension of the blood also affect the CO_2 content. Therefore measurements during exercise above the lactate threshold and the inherent pH shifts that occur makes this method of CO

measurement only feasible during low and submaximal exercise. Studies of cardiac output using CO_2 with the direct Fick method have demonstrated a greater degree of variability in comparison with oxygen; this was attributed to the complexity of CO_2 storage and dissociation from blood [404]. Despite this, CO_2 rebreathe measurements are still utilised predominantly because of the availability of rapid CO_2 analysers and cost.

In order to overcome the problems encountered with CO_2 and the indirect Fick method alternate gases have been tried. Of these, acetylene has become the most favoured, since it is rapidly soluble in blood and biologically inert. In essence, the method involves the inhalation of a known concentration of acetylene (since it is not endogenous it has zero concentration in mixed venous blood). The decline in concentration of gas as it is breathed from the bag is measured and is proportional to uptake of gas by blood circulating through the lung. From this the pulmonary blood flow can be calculated in a similar fashion to CO_2 rebreathing techniques. Of course this is a gross simplification; the initial alveolar concentration of acetylene has to be calculated to account for residual volume at the onset of measurement, also the quantity gas stored in the lung tissues has to be considered in the calculations. The use of non-absorbed gases as well as carbon monoxide in the inspired gas mix, in combination with rapid gas analysers has meant that these problems are now overcome and this technique is widely used in physiological study. There are now open circuit systems that deal with the problem of CO_2 rebreathing and ventilatory effects [405, 406], with at least one commercially available single-breath apparatus [407]. The last piece of equipment was evaluated in the development of methods described later in this thesis; however was deemed unsuitable due to the difficulties experienced in performing the single breath manoeuvre during hypoxic exercise.

1.14.3 Thermodilution methods

Swan and Ganz first described a method for measuring pulmonary blood flow using a thermodilution technique in 1971. The method utilises modified pulmonary artery catheter equipped with a thermistor at the tip; a known quantity of normal saline cooled to 4°C is injected into a proximal port of the catheter, as the injectate enters the pulmonary blood flow proximal to the thermistor cooling of blood. The temperature decline as it is measured at the thermistor when plotted against time, gives an inverted bell shaped curve; the area under this plot reflects blood flow across the thermistor and is calculated using the Steward-Hamilton equation.

$$Q= (V(Tb-Ti)K_1K_2) / (Tb(t)dt)$$

Where V is the volume of injectate; Tb is blood temperature; Ti is injectate temperature; K_1 is the catheter constant; K_2 is the apparatus constant and (Tb(t)dt) is the change in blood temperature over a given time.

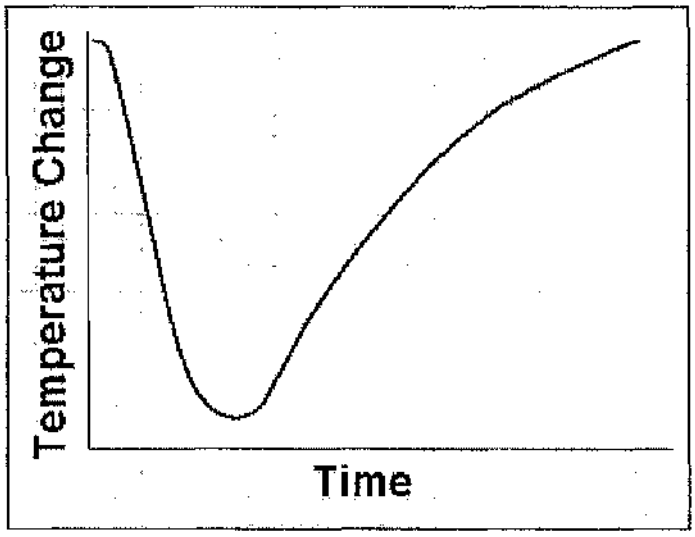

Figure 1.14 Temperature change curve of blood after injection of thermodilutant. Integration of this curve provides a value for cardiac output.

If the tip of the catheter is sited in the pulmonary artery then this value reflects pulmonary blood flow and CO. The thermodilution technique, though invasive has been used extensively in both medical and physiological studies, including measurements during exercise; however its validity has been drawn into question. Thermodilution measurements of CO have been used as a validation standard in lieu of the direct Fick technique in assessing the validity of other, 'non-invasive' methods of cardiac output measurement. This is understandable, since it removes the need for oxygen uptake measurement, and the need for a metabolic cart. The use of stringent quality control and dedicated hardware that monitors the thermistor output, injectate temperature and performs the necessary calculations has improved the reliability of this method. Though thermodilution measurement of cardiac output is widely accepted, it still requires the siting of a pulmonary artery catheter which is difficult to justify ethically when studying normal physiology. As a consequence physiologists have tended to rely on minimally invasive methods such as CO_2 re-breathe and

acetylene techniques; however technological developments have provided further techniques for non-invasive cardiac output measurement.

1.14.4 Echocardiography

Echocardiography has been used to measure cardiovascular variables in the diagnosis of pathology and response to therapies. Echocardiography has also been used to study physiological responses to stresses such as exercise and hypoxia. Two dimensional (2-D) and Doppler echocardiography have been used to measure CO; however Doppler is currently the preferred method since 2-D studies during exercise are technically demanding, and require measurement of left ventricular (LV) area and LV length in order to calculate volume. The Doppler method relies on measurement of the cross-sectional diameter of the aortic root by M-mode echocardiographic (which does not vary significantly during exercise) and the aortic blood velocity during systole, as determined by Doppler echocardiography. The product of aortic root surface area (as calculated from the measured diameter) and the blood velocity gives a value of stroke volume from which CO can be calculated [408]. Despite being easier to perform than 2-D echocardiography, trans-thoracic Doppler measurement of CO is nonetheless taxing on the operator, especially during maximal exercise and the reliability of these measurements were questioned, though studies to date demonstrate reasonable fidelity [409]. Trans-thoracic Doppler measurement of CO has been used at altitude to assess improvements in CO and the possible reduction in diffusion limitation to drugs such as Sildenafil [410]; however the major area of expansion in this measurement modality for CO has been in the intensive care setting. Trans-oesophageal Doppler probes are now enabling the continuous monitoring of intubated critically ill patients, providing invaluable information which is used to tailor therapy. Exercise echocardiography requires significant operator skill which makes it difficult to use in the absence of expertise and draws into question reliability. The placement of trans-oesophageal probes in exercising subjects is rarely acceptable and can interfere with the gathering data on ventilatory variables. Therefore further wholly non-invasive methods of measurement have been developed.

1.14.5 Bio-impedance cardiography

Bio-impedance cardiography relies on the variability of the thoracic cavity resistance during the cardiac cycle. In essence blood is mainly water which is an excellent electrical conductor. At the end of systole, blood leaves the heart through the aortic root, this alters the electrical resistance of the thoracic cavity; both in absolute terms, but also dynamically since there is a

waveform in the resistance trace. Measurement of these changes allows the calculation of stroke volume, integrating this with an ECG trace gives a value for cardiac output. Older measuring machines were cumbersome, requiring two bands to be placed around the subject's neck and a further two bands around the lower part of the thorax. A small alternating current is passed through the two outer bands (located on neck and thorax), whilst the change in the measured voltage across the inner two bands allows the calculation of thoracic resistivity according to Ohm's law:

$$R = V/I$$

Where R represents resistance; V represents voltage and I represents current. Electrical resistance when using an alternating current is termed 'impedance' (Z), hence 'bio-impedance'. Impedance has both a resistive component and a reactive component since it can vary with the frequency of the current applied. Bioimpedance monitoring utilizes a frequency that has a predominantly resistive component when applied to biological material (around 100Hz). The resultant impedance trace gives an absolute value of baseline impedance (Z_0) which varies with thoracic blood volume to give a variable wave form (ΔZ) similar to the aortic pressure wave and from this can be calculated the first time derivative dZ/dt.

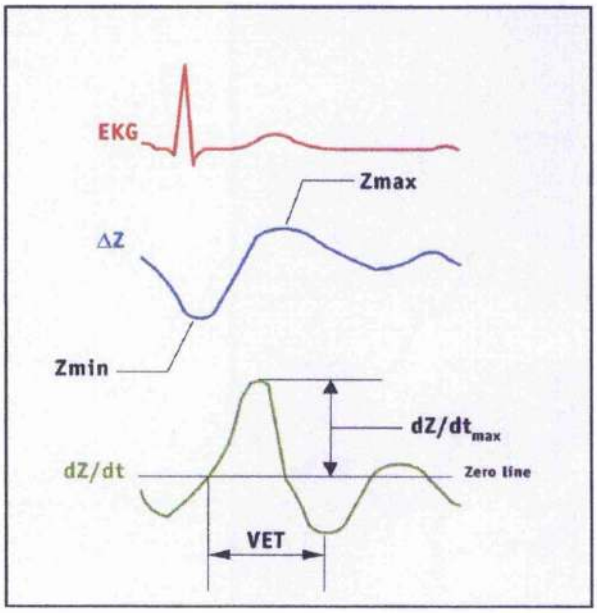

Figure 1.15 Bioimpedance waveform demonstrating the electrocardiogram (EKG), ΔZ and dZ/dt waveforms; VET is the ventricular ejection time.

The ΔZ waveform has a tendency to vary with respiratory oscillations and has made it less reliable; however the dZ/dt trace demonstrates clearly defined segments that facilitate automated calculations and are not as sensitive to respiratory change. Kubichek first described an equation that allowed calculation of SV based on the supposition that the thoracic cavity is a cylinder:

$$SV = \rho . [L^2]/[Z_0^2].dZ/dt.t$$

Where ρ is the electrical conductivity of blood and L is the mean distance between the two inner electrodes (thoracic height) [411]. This equation has been further modified, using the assumption that the thoracic cavity approximates to the shape of a truncated cone (frustrum) rather than a cylinder and eliminates the conductivity of blood, which can be assumed as a constant [411]. The thoracic length can be derived from the total height (H), thus simplifying the equation to:

$$SV = (0.17H)^3 / 4.2.dZ/dt.t/Z_0$$

The limitation of both these equations lies in the use of a baseline value for thoracic impedance (Z_0) which is variable with skin thickness, perspiration and electrode position. The latest generation of bioimpedance equipment removes this potential source of error by utilising the signal waveform to calculate SV [412]. This equipment (Physioflow PF-05) was assessed and then used for the experiments, described later in this thesis, for exercise cardiac output measurement. The PF-05 is light, portable and uses electrocardiography electrodes in lieu of metal bands. Bio-impedance signals can be inputted into a notebook computer, enhancing the portability of the equipment. Furthermore values of CO and SV can be averaged over a number of heart beats; this data then can be directly exported into a statistical program for a more detailed analysis.

The accuracy in terms of absolute values for CO has been questioned; however bioimpedance does seem to accurately reflect changes in CO. Moreover there is agreement that the values measured, are reliable and reproducible [413]. The methods section describes validation studies comparing the PhysioFlow equipment with thermodilution in the catheter laboratory setting; published studies comparing this equipment with the direct Fick method [412]

suggests an accurate and reliable technique for CO measurement. The Physioflow PF-05 was used for cardiac output measurements during some of the exercise test protocols described in this thesis; the equipment was not used for all tests, since it became available during the course of subject recruitment and testing. Echocardiography was evaluated and dismissed as a means of exercise cardiovascular measurement due to the lack of a suitable supine ergometer and dedicated echocardiographic expertise. These methods and those for CPET are described in detail in the next chapter.

Summary

This chapter has reviewed the physiological mechanisms underlying the cardiopulmonary response to exercise and hypoxia; the latest evidence and theories for oxygen sensing; the function of the renin-angiotensin system and ACE gene polymorphism in relation to hypoxia and exercise performance. The next chapter describes the methods used to investigate any possible link between the ACE I/D gene polymorphism and the cardiopulmonary response to exercise and acute hypoxia.

CHAPTER TWO: METHODS

LABORATORY STUDY METHODS

2.1 Study design

2.1.1 Overview

The purpose of these experiments was to examine whether the physiological responses to hypoxia during rest and exercise in comparison to measurements under normoxic conditions demonstrated any variation in relation to the ACE gene polymorphism. Particular attention was given to ventilatory and cardiovascular responses (in the form of cardiac output measurements) in addition to the metabolic measurements of VO_2 and VCO_2. To avoid exercising subjects at work rates above their ventilatory threshold (and therefore their anaerobic threshold), an initial maximal exercise test was performed. The subsequent steady state exercise periods were then performed at a load 50% of that required to reach ventilatory threshold as determined non-invasively by the V-slope method. Steady state exercise was performed in normoxic conditions and hypoxia (FiO_2 12.5%); thereby using normoxic measurements for each subject as a control for comparison with their hypoxic response during identical test conditions. Normoxic studies were performed first; this was to reduce the risk of lactate build up that might have occurred by performing hypoxic testing first. At least 15 minutes were allowed between steady state tests. Measurements were made toward the end of the resting phase, to allow familiarisation with the circuit and then during exercise when steady state was reached.

The aforementioned studies examining the feasibility of exercise echocardiography were performed by a technician in the Cardiac investigation department of the Western Infirmary. These studies were abandoned for several reasons. Firstly, the tricuspid regurgitant jet (TR jet) which would have enabled an estimation of systolic pulmonary artery pressure was not reliably detectable in the subject population. Secondly, a suitable supine ergometer was not available despite attempts to secure funding for the purchase of one; this may have facilitated exercise echocardiographic measurements. Finally, this line of investigation wsa abandoned after consultation with Dr Simon Cooper (Hammersmith Hospital, London) and Dr Adam Torbiki (University of Warsaw, Poland) both of whom have extensive experience of exercise echocardiography; in their opinion it was unlikely that sufficient number of subjects would demonstrate a significant TR jet in a subject group as young as ours.

2.1.2 Subject Selection

Subjects were recruited from the University of Glasgow Officer Training Corps, the Sports and recreation service of Glasgow University and the Department of Medicine at the Western Infirmary, Glasgow. A total of 60 volunteers were recruited all of whom had cardiopulmonary exercise testing and measurements of ventilatory and metabolic variables. Of these, 30 subjects had non-invasive cardiac output measurement by bio-impedance means during rest and exercise in normoxic and hypoxic conditions.

Ethical approval was granted by the West Glasgow Hospitals NHS trust Ethics Committee (Study number 99/191(2), Appendix A). Subjects were required to give written informed consent and complete a questionnaire for inclusion in the study.

Inclusion criteria

Inclusion criteria were regular exercise for at least 3 hours or more per week for most of the preceding year and age between 18 and 40 years.

Exclusion criteria

Exclusion criteria were:

1. A history or examination suggestive of cardiac or pulmonary disease.
2. No evidence of current illness of any description.
3. The use of prescription medication other than the oral contraceptive pill and simple analgesics.
4. The use of medicines prohibited by the International Olympic committee 1998.
5. A history of severe atopy or drug reaction.
6. Excursion to altitude in excess of 2000m in the previous 3 months.
7. Residence at altitude in excess of 200m for more than 3 months at anytime.
8. Cigarette smoking.
9. Pregnancy

In addition subjects were asked to abstain from caffeine and alcohol 12 hours prior to testing and to abstain from strenuous exercise in the 24 hours prior to tests, particularly prior to the maximal cardiopulmonary test.

2.1.3 Study structure

The laboratory study required two visits to the department. During the first visit subjects were required to read the information sheet provided and sign the consent form. They were then asked to complete a questionnaire to determine their suitability to participate in the study. Prior to testing calibration of the gas analysers and mass flow sensor was performed. A maximal exercise test in normoxic conditions was then conducted, primarily to determine the subject's ventilatory threshold and the work rate at which this was reached. Invariably subjects went on to perform a maximal effort test, mainly in order to determine their own fitness level as measured by VO_2max. At the second visit the steady state exercise tests were performed. The original protocol allowed a minimum of 6 hours between the maximal exercise test and the subsequent steady state tests. In reality the majority (all bar 8 subjects) attended the next day for the second phase of the test protocol.

2.1.4 Development of study design

The basic study structure was designed to give a reproducible measure of physiological response to hypoxia at rest and during exercise. A FiO_2 of 12.5% was chosen in order to elicit responses over short exposures whilst at the same time maintaining a degree of safety. Furthermore it was felt that exercise at lower FiO_2 subjects could reach ventilatory threshold during the hypoxic steady state test. Initial experiments performed on volunteers (including myself) using a lower FiO_2 of 11.5% certainly produced the required desaturation as measured by pulse oximetry, but did have symptomatic effects in terms of light-headedness during exercise. Initial tests had to be terminated prematurely because of adverse symptoms.

Previous studies that demonstrated enhanced performance in association with the I-allele of the ACE gene polymorphism were conducted in the field; hence, poikilocapnic hypoxia was used to reflect true environmental hypoxia. Pilot experiments were performed, using the available equipment, to establish the feasibility of isocapnic hypoxic challenge; this was in order to dissect out the pure hypoxic response in relation to ACE gene polymorphism. A 1000 litre Douglas bag filled with hypoxic mixture was used as a reservoir to be inhaled during the hypoxic phase of experiments. This was administered by a wide calibre, low impedance circuit. Attempts to introduce CO_2 into this circuit during the experiment resulted in an erratic $FiCO_2$ and produced variability into the measurements, probably as a result of

insufficient mixing within the circuit. Unfortunately suitable high flow rotameters which would have allowed a finer degree of control to the introduction of CO_2 to allow isocapnic hypoxic exercise testing were unavailable.

The metabolic cart used for these experiments (Sensormedics Vmax 29 series, Yorba Linda, CA, USA) measured inhaled FiO_2 and $FiCO_2$ allowing a further degree of quality control. The introduction of the circuit onto the mass flow sensor introduced a further 100 mls dead space, fortunately the software provided with the metabolic cart allowed adjustment of circuit dead space, and made allowances for this in the calculation of gas flow.

The use of hypoxic mixtures introduced lower gas exchange values than would normally be expected in normoxic conditions. This raised concerns regarding the reliability of these values, since the gases provided by the manufacturer for calibration of the metabolic cart were designed for measurements in room air. This query was raised with the manufacturer and assurances were given that the performance of the O_2 detector was reliable at the lower oxygen tensions, since it behaved in a direct relationship (straight line) with oxygen tension. Calibrating across part of the actual oxygen vs. measured oxygen plot would ensure fidelity of measurements across the entire range. Experiments using calibration gas with 0% FiO_2 compared with the usual 16% FiO_2 mixture provided, confirmed that there was no difference in the recorded values.

2.1.5 Statistical analysis

The biometric and physiological measurements and data derived from them were tested for normal distribution for the subject group. To allow for comparisons to be made between different subjects of varying size and degrees of fitness percentage change between normoxic and hypoxic variables during rest and exercise were calculated for each individual. These too were tested for a normal distribution between all three genotypes for the ACE gene. Statistical tests were then applied to determine whether there were any significant differences between the groups for each variable. One way analysis of variance (ANOVA) testing was used to determine any significant difference between the genotypes. The power calculation for the cardiopulmonary study was performed on the statistical software. Statistical analysis was performed on a generic PC running Minitab 12.1 (Minitab Inc., State College, PA., USA) on a Microsoft Windows XP operating system.

Normality testing

A normal population should have a symmetrical frequency distribution on either side of the mean, where 68% of the points lie within one standard deviation on either side of the mean and 95% within two standard deviations. The application of many powerful statistical analyses is dependent on establishing the normality of the groups being compared. There are a number of methods of establishing normality; frequency distribution plots can demonstrate a symmetrical bell shaped curve and plotting the standard deviation from the mean results in a straight-line which is characteristic of a normal sample population. There are statistical tests that offer quantitative and qualitative measure of normality. The Minitab statistical package offers a choice of tests. The Ryan-Joiner normality test was chosen because it relies on plotting the probability of data point proximity to the mean similar to the normal plot, but also gives a correlation coefficient. In this case the aim is not to refute the null hypothesis and a p value higher than the arbitrary threshold must be attained. Therefore, the Ryan-joiner test enables a qualitative and quantitative measure of straightness of the normal plot (Figure 2.1). In this way it is similar to the Shapiro-Wilk or Shapiro-Francia tests.

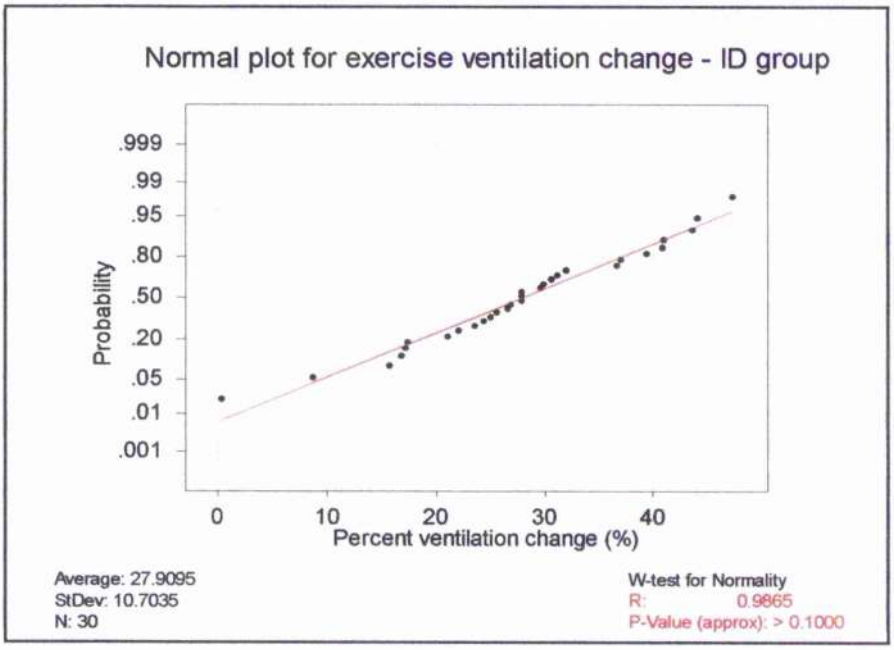

Figure 2.1 An example of a Ryan-joiner plot. The distribution of data points is plotted according to the probability of deviating from the mean. The straightness of the plot is verified by the correlation coefficient and p value in the bottom right hand corner (in red).

Parametric and non-parametric tests

Parametric statistical tests can be applied to data sets which demonstrate a normal distribution; in the event of a skewed distribution non-parametric tests are appropriate. In normal data sets, paired student t-tests were used to verify significant differences between normoxia and hypoxia. In data sets that failed to meet the criteria for normality, the Wilcoxon matched pairs signed sum rank test (the non-parametric equivalent to the student t-test) was used. Analysis of variance (ANOVA) was used to determine any significant difference between the ACE genotype groups. This test requires normality in the sample being compared; this was confirmed using the Ryan-Joiner test for each group. There was one occasion where the sample did not demonstrate normality, on this occasion the Kruskal-Wallis test was used which is the non-parametric equivalent of the ANOVA test.

Comparison of measuring methods and repeatability

The validation studies required the comparison of thermodilution and bioimpedance cardiac output measurement. This was verified, using the method described by Bland and Altman. This method plots the average results of simultaneous measurements using the two methods against the difference between the two measurements. An acceptable level of difference is set at two standard deviations (as recommended by British standard 5497 part 1) [414]. The same method was used to determine the repeatability of steady state cardiopulmonary exercise tests.

Correlation of ventilatory response with $etCO_2$

The minute ventilation, tidal volume and respiratory rate were compared to the end-tidal CO_2 as an index of alveolar ventilation. This was achieved using simple correlation plots, which included Pearson's correlation coefficients and regression lines in order to assess the presence of any relationship between ventilatory variables during the normoxic and hypoxic parts of the protocol. This in part was to confirm that the responses were in keeping with those expected physiologically, but also to ascertain whether there was any detectable evidence of hyperventilation, particularly during the resting phase of testing. Pearson correlation coefficient was calculated using the Minitab statistical package.

Conformity to the Hardy-Weinberg equilibrium

The genotype distributions of both the cardiopulmonary exercise group and the bioimpedance group were checked to ensure that the allele and genotype frequencies were in Hardy Weinberg equilibrium (HWE). This was achieved by calculating the allele frequencies in the group:

$$p = \frac{(2 \times \text{observed II}) + \text{observed ID}}{2 \times (\text{observed II} + \text{observed ID} + \text{observed DD})}$$

$$q = 1-p$$

Therefore expected genotype frequencies can be calculated as follows:

$II = p^2 \times n$ where n = subject number in sample
$ID = 2pq \times n$
$DD = q^2 \times n$

Once calculated these values can then be used to calculate the Chi square value (χ^2) against the observed genotype frequencies, thereby determining the goodness of fit to the HWE. In this case, the null hypothesis is that the population is in Hardy-Weinberg equilibrium. The degree of freedom is 1 (number of genotypes – number of alleles); therefore for a significance level of 0.05, χ^2 must be greater than 3.841 to reject the null hypothesis (from standard tables).

2.2 Cardiopulmonary exercise testing

Two visits to the department were necessary; during the first visit physical characteristics were measured and entered in to the computer database, before a maximal exercise test was performed. The steady state exercise tests in normoxic and hypoxic conditions were performed during the second test. The following is a description of the equipment used, the protocol for maximal effort exercise test and the steady state test performed at 50% of the work rate at which ventilatory threshold was reached. The first section describes the main exercise protocols. The latter sections describe the measured and calculated metabolic and

ventilatory variables. The final section describes the quality control measures and the validation studies performed to ensure the repeatability of the method.

2.2.1 Equipment

Experiments were performed on level 4 of the Glasgow Western Infirmary, in the Research Exercise laboratory of the Department of Respiratory Medicine.

Subject Biometrics

Subject height was measured on a scale mounted measuring rod (Seca 220, Seca, Hamburg, Germany) and weight was measured on digital scales (Seca 701, Seca).

Cardiopulmonary exercise testing

A commercially available metabolic cart (Sensormedics Vmax 29 series, Yorba Linda, CA, USA) was used for measurement of all spirometric, ventilatory and gas exchange variables. This equipment uses a mass flow sensor to measure gas flow. Sampling tubes were inserted into the sides of the sensor to allow gas to be extracted by the gas analyser; these were designed to dry the sampled gas before entering the analyser (Perma Pure, Nafion).
In addition to dynamic testing the same equipment was used to measure spirometric values, forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and flow volume loop data were measured. This was performed to ensure normal values for age and height, but to also exclude any air flow obstruction prior to dynamic testing.

Face mask and seals

The subjects were fitted with a reusable face mask with oronasal separator and disposable gelatinous seal (Universal seals, Hans Rudolph Inc., Kansas city, MO, USA). This was secured by elasticated straps and skull cap. The mass flow sensor could then be attached to the mask. In addition to the oronasal separator, the subjects wore nasal clips (similar to those used whilst swimming) to ensure oral breathing and reduce air leak.

Computer

Measured data from flow sensor and gas analysers were relayed via a serial interface into a generic personal computer (PC), running Microsoft Windows 1998 operating system and software provided by Sensormedics for use with the Vmax29 unit.

Exercise ECG and Pulse oximetery

A 12 lead exercise Electrocardiograph recorder (Marquette electronics, MAX-1, Milwaukee, WI, USA) allowed heart rate data to be recorded and a transcutaneous pulse oximeter (Ohmeda Biox 3700, Boulder, CO, USA) measured oxygen saturation, both data streams were interfaced into the PC and recorded by the Sensormedics software. Oximetry measurements were made with a finger probe; experiments with ear probes proved unreliable because of motion artefact.

Cycle Ergometer

An electronically braked upright cycle ergometer (Ergometrics, Ergoline 900, Bitz, Germany) was used to provide the exercise work load. This was connected to the PC, allowing control of the work load set by the Sensormedics software. During the maximal effort exercise test the work load was incremental, in the form of a continuous ramp at a set rate set by myself (e.g. 25 watts per minute). The incremental rate was estimated according to the subjects exercise history, with the aim of attaining between 8 and 12 minutes exercise before the subject fatigued to the point of stopping.

Hypoxic circuit

Hypoxic gas mixture was administered from a 1000 litre Douglas bag (Hans Rudolph Inc., Kansas city, MO, USA). This was first evacuated using a domestic vacuum cleaner. Air was then diluted using a modified flow rotameter originally designed for anaesthetic purposes to enrich air with oxygen to give a high FiO_2. This was adapted by connecting compressed air (Medical grade, Linde Gases Ltd. UK) as per the original design, but to introduce pure nitrogen via compressed cylinder (Diving grade, Linde Gases Ltd, Aberdeen, UK) instead of oxygen, thereby producing a hypoxic mixture. The percentage O_2 of the gas mix was measured using an oxygen analyser connected in series to the evacuated Douglas bag. The bag was filled with 12.5% O2, balance nitrogen via 35mm tubing and a three way tap attached to the bag (2100 series, Hans Rudolph Inc.). The subject was connected to the circuit during steady state tests by a non-rebreathe valve (2700 series, Hans Rudolph Inc.) and 35mm tubing. The normoxic phase of the test was performed with the three-way tap open to the atmosphere and during the hypoxic phase the subject breathed from the bag.

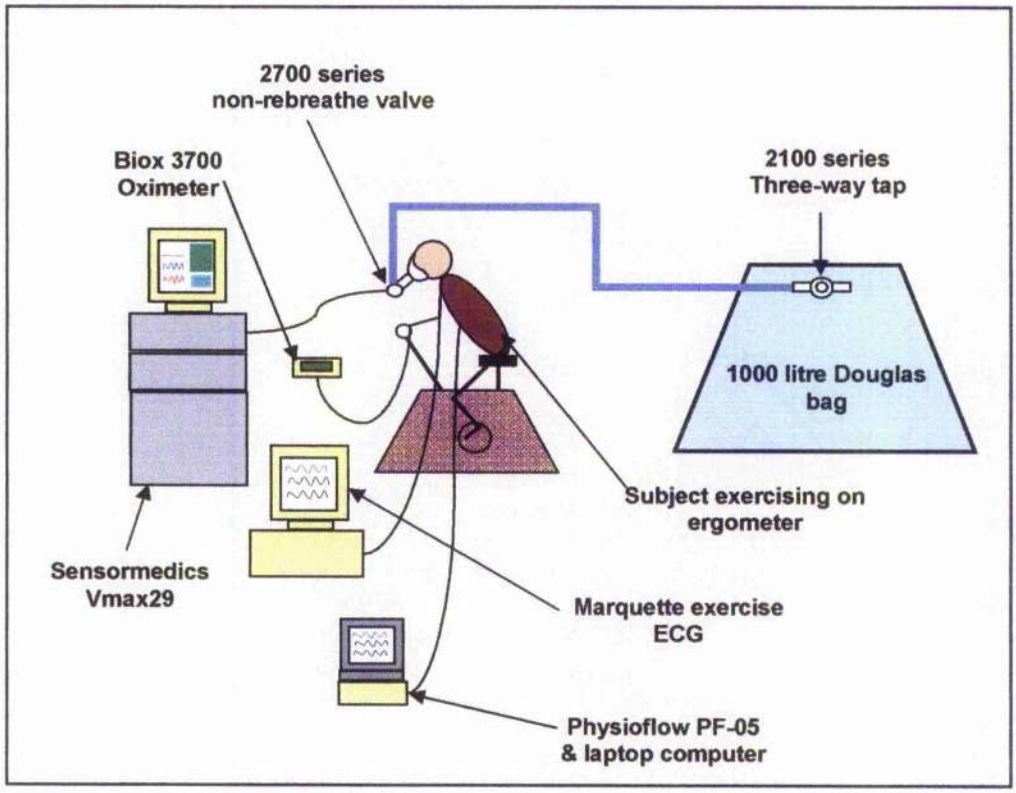

Figure 2.2 The layout of the various pieces of equipment during steady state exercise testing. During the normoxic part of the test, the three-way tap was turned to the atmosphere instead of the bag.

2.2.2 Maximal effort exercise protocol.

Prior to testing subjects were given the information sheet to read, consented and filled in the questionnaire to ensure they met the required criteria. Tests were carried out in room air. Two investigators one of whom was medically trained and familiar with the available resuscitation equipment were required to be present throughout testing (i.e me).

1. Height and weight were measured.
2. Subject biometric data, sex, and date of birth were entered into the Sensormedics software. This then calculated predicted values for variables to allow comparison [415].
3. Brachial artery pressures were measured using a stethoscope and portable sphygmomanometer.
4. Before each set of tests the mass flow sensor was calibrated using a standard volume 3 litre calibration syringe (Vitalograph Ltd., Buckingham, UK.) according to the manufacturer's instructions. The software's own quality control algorithms would reject the calibration process if the technique was inadequate or there was fault.
5. Gas sensors were then calibrated using standardized calibration gases provided by the manufacturer. These were 16% O_2:4% CO_2:balance N_2 and 24% O2:balance N_2 (Sensormedics, Yorba Linda, CA, USA)
6. Skin electrodes for 12-lead ECG were placed on the subject chest and proximal limbs.
7. The volume of dead space introduced into the circuit by the mass flow sensor was entered into the software (0.05 litres). The estimated work rate increment was entered into the software.
8. A nose clip was fitted to the subject and spirometric measurements (FEV_1, FVC and flow volume loop) were measured, the best effort of 3 attempts was recorded.
9. The face mask was attached to the subject, by elasticated straps and a skull cap.
10. The mass flow sensor was then attached and the subject transferred to the ergometer. The saddle height was then adjusted for optimal cycling position.
11. A 2 minute period of familiarisation was allowed before commencing a 3 minute period of unloaded pedalling at a cadence of 60 rpm.
12. The work load was commenced via the software without informing the subject. This increased at a smooth ramped rate as determined by the investigator (myself).

13. Subjects were reminded to monitor their cycling cadence, but only had one verbal encouragement towards the end of the exercise phase to ensure a maximal test was achieved.
14. The exercise test was deemed complete when the subject could not continue due to leg fatigue (no subjects stopped because of dyspnoea or other symptoms).
15. Blood pressure measurement was repeated and the subject was required to keep the mask on for a further 2 minutes or until heart rate had returned to within 20 beats of baseline.

2.2.3 Steady state exercise protocol

Ventilatory threshold (VT) was determined by analysis of the maximal effort exercise test using the Sensormedics software. The V-slope method was utilised by the software, but could be finely adjusted by the investigator in light of the other plots provided (e.g. $PetCO_2$). The work load at which VT was deemed to have been reached was then halved and this work load was used during the square wave steady state exercise test.
Similarly the presence of two investigators, one medical was required.

1. Subjects' data was retrieved from the computer database and entered automatically into the software.
2. Steps 3-9 of the maximal effort protocol were performed, with the exception of the dead space volume. The hypoxic circuit introduced 150mls of dead space and this value was entered into the software.
3. The mass flow sensor was attached and then the 2700 series non-rebreathe valve was attached, using a custom made adaptor. The weight of this circuit was supported by a head support worn by the subject. The circuit at this time was open to room air.
4. The subject then transferred to the ergometer and sat at rest for 5 minutes. This allowed familiarisation with the new circuit and baseline measurements.
5. They were then asked to cycle at a cadence of 60rpm.The calculated work load was applied from the onset of exercise as a square wave. Subjects exercised for 8 minutes.
6. The subject then dismounted the ergometer in order to rest and have repeat spirometry performed. Ten minutes were allowed for this.

7. The mask and circuit was reattached and the subject then returned to the ergometer, however early during the baseline measurements the valve was switched to the Douglas bag and the subject was breathing hypoxic mixture.
8. Steps 4 and 5 above were repeated.

2.2.4 Metabolic, ventilatory and cardiac measurements.

Measurements were made on breath-by-breath basis. The metabolic cart measured tidal volume (V_T), respiratory rate (RR), inhaled FiO_2 and $FiCO_2$, exhaled FiO_2 and $FeCO_2$. The difference between oxygen and carbon dioxide concentration during inhalation and exhalation in combination with the tidal volume (as measured by the mass flow sensor) allowed calculation of oxygen uptake (VO_2) and carbon dioxide production (VCO_2), end-tidal O_2 and CO_2 (PetO2 and PetCO2 respectively). All measurement were made using SI units, measurements of partial pressures were in kilopascals (kPa); a conversion factor of 7.501 is required for conversion to mmHg. Minute ventilation (V_E) was calculated from the product of tidal volume and respiratory rate. Further calculated variables available were ventilatory equivalents for O_2 and CO_2 (V_EO_2 and V_ECO_2), oxygen pulse and gas exchange ratio (R); ventilatory equivalents were used to confirm the VT inflection point during maximal tests.

Exercise ECG data provided monitoring during tests which satisfied safety concerns and pulse rate data which was incorporated into the data. Detailed examination of exercise ECG traces was not performed since they fell out with the aims of this project.

Steady state was considered attained by visual monitoring of the cardiopulmonary variables as the test progressed and confirmed by the software's own algorithms using a plot of 3 breath averages.

Dynamic data collected was averaged over the last 90 seconds of the rest period and exercise to give a value for steady state rest and exercise during normoxia and hypoxia to allow comparative analysis for each subject in these conditions (figure 2.3). Disruptions in normal ventilation during swallowing or attempted speech did result in a marked variation in ventilatory measurements; the breath-by breath measurements of all variables during disrupted ventilation were edited from the final data set before analysis.

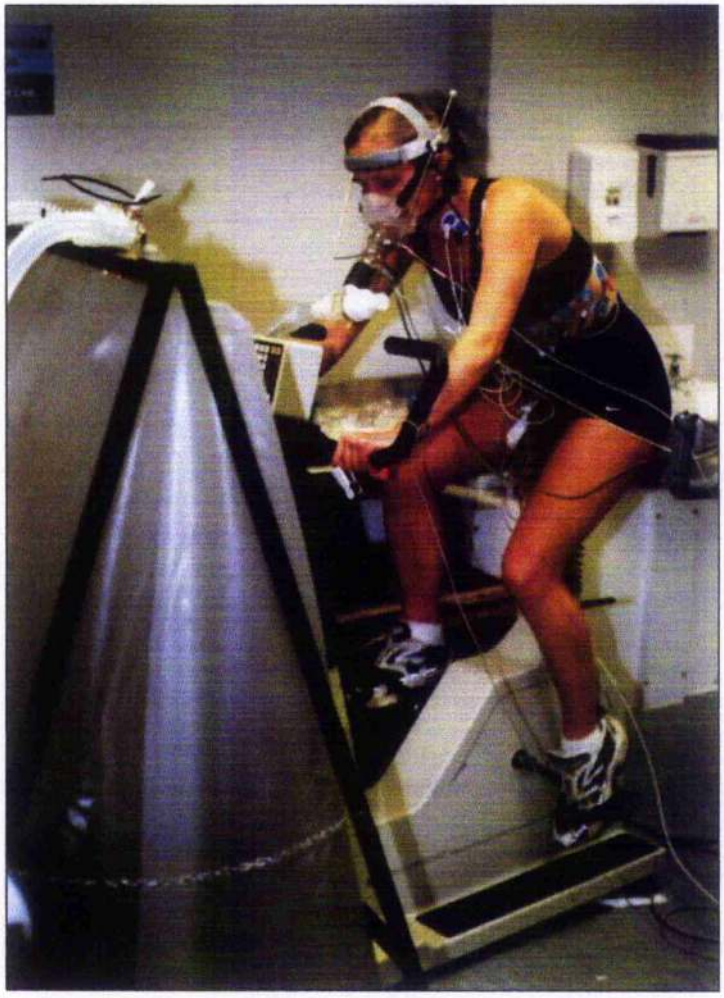

Figure 2.2 Subject on cycle ergometer performing a steady state test. The Douglas bag is in the foreground and the mask, flow meter and non-rebreathe valve connected to the hypoxic reservoir are attached to the subject.

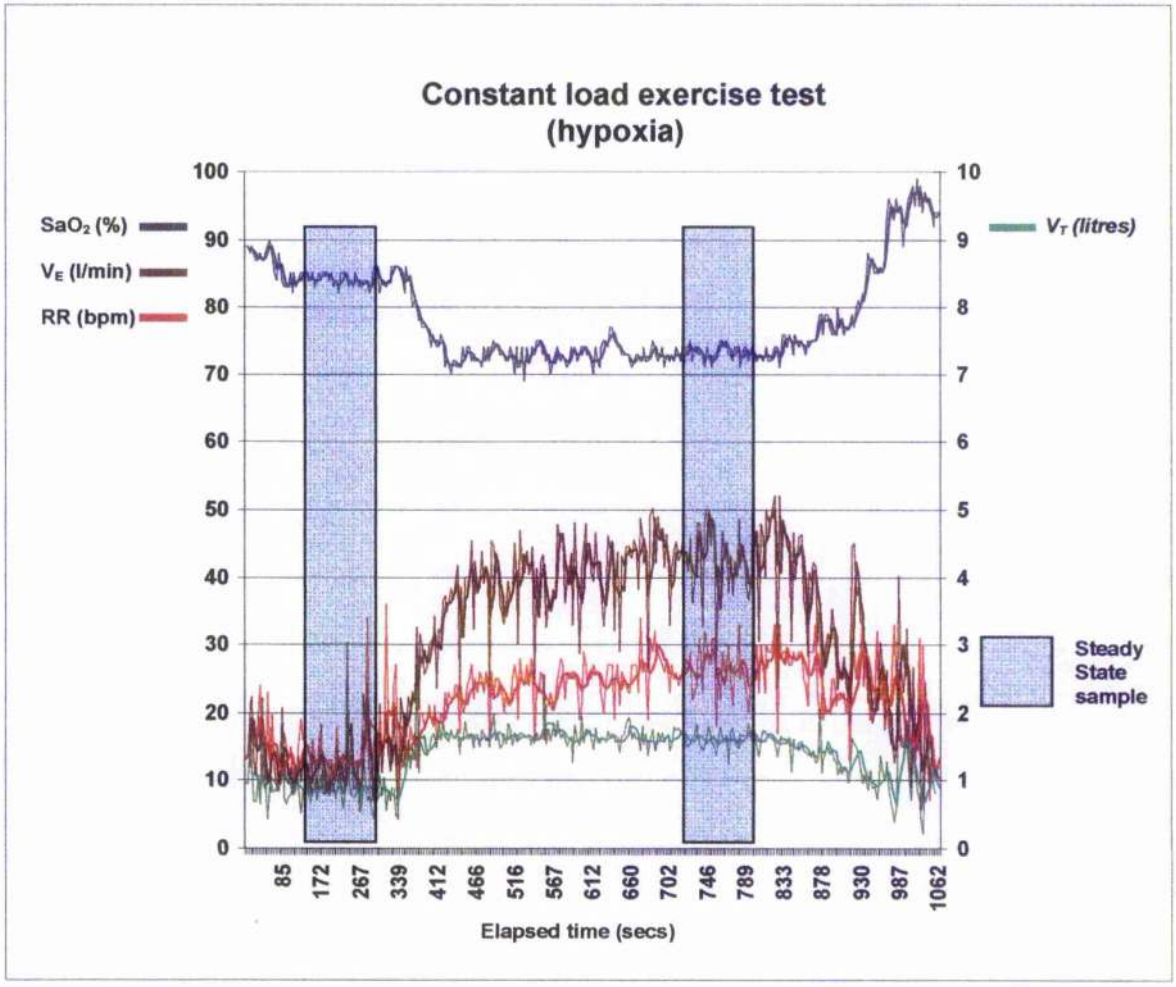

Figure 2.3 Constant load exercise test graph. This plot shows minute ventilation, tidal volume, respiratory rate and oxygen saturation during a constant load exercise test in hypoxic conditions. The areas highlighted in blue represent the 90 seconds during which mean values of each variable were taken. The graph also demonstrates that steady state has been achieved.

2.2.5 Quality control measures and validation studies

Quality control

The Sensormedics Vmax29 metabolic cart was central to the measurement of cardiopulmonary responses to exercise and static spirometric data. Therefore it was essential to ensure the equipment was in good working order and properly calibrated to ensure the

fidelity of the data collected. This was achieved in several ways. First the equipment was serviced regularly as part of the agreement with the supplier (Sensormedics UK.), this included gas analysers, interfaces and software updates. Mass flow sensors and gas sampling tubes were also provided to replace old or worn units. Secondly, regular calibration was performed. The protocols describe the calibration process for both ventilatory and gas exchange measuring systems. If these were not within the tolerances designated by the manufacturer, they were rejected by the software and had to be repeated. The software that analysed the data from the sensors also required updates of local barometric pressure to compensate for daily variations; this was measured from a mercury barometer (situated on the same floor) and inputted into the software. Periodic calibration of the ergometer was performed to ensure the fidelity of work load. The entire system was then checked by biological calibration using a volunteer again on a periodic basis. Thirdly, between tests drying tubes were changed and allowed to dry before being reused. This ensured that gases sampled were free of water vapour and would not interfere with gas measurement (the infra-red method of CO_2 measurement is particularly susceptible to this). Finally three sizes of mask were available and in combination with the disposable gelatinous seals ensured a good fit. This was checked by an inspiratory manoeuvre with the mask attached and the orifice occluded.

Disinfection and cleaning

Mass flow sensors, masks, nose clips, mouthpieces, saliva traps (the last two items were used for spirometric measurements) were disinfected in a 1% solution of Virkon® (DuPont, France), rinsed in sterile water and allowed to air dry. Each subject had equipment that had been sterilized by this method for each test.

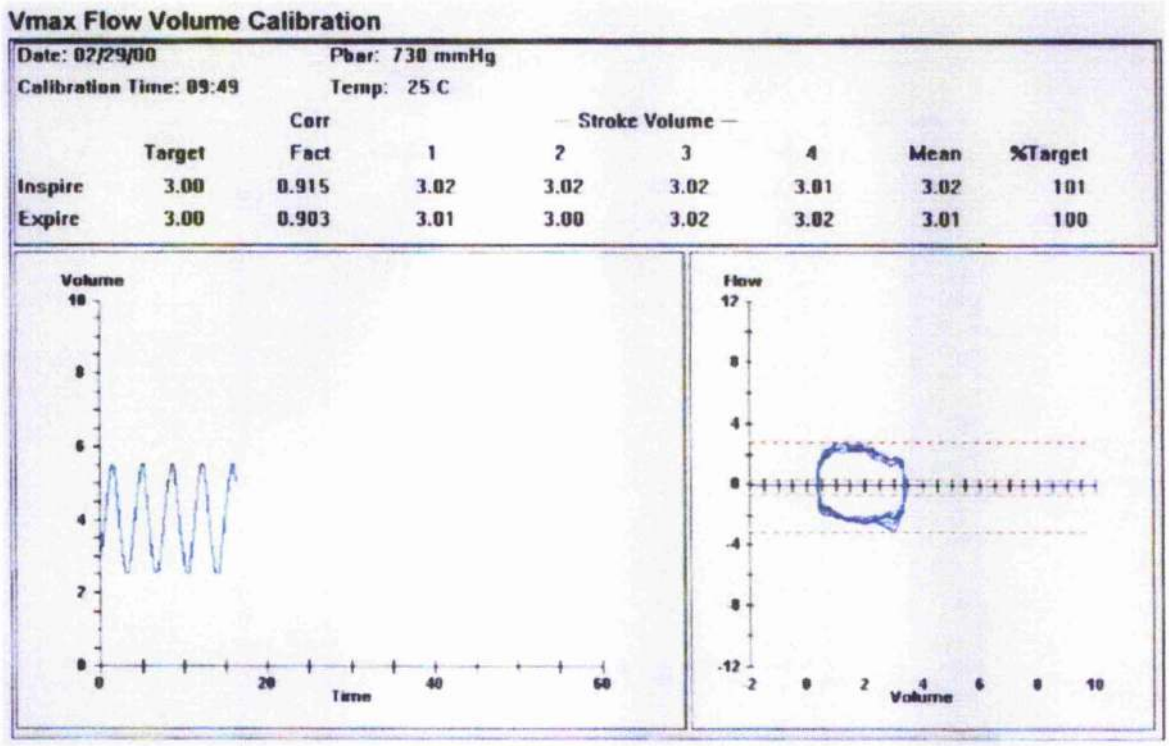

Figure 2.4 Screen shot of the flow meter calibration sequence.

Validation Studies

The repeatability of the tests was verified by repeating the steady state exercise protocol in 5 subjects. These tests examined the repeatability of VO_2 and minute ventilation measurements between repeated tests. The data is presented in the results section. Of the five subjects, 4 completed two sets of normoxic and hypoxic steady state protocol. The final subject repeated only the normoxic steady state protocol. The results were analysed by direct percent difference between the two tests. Furthermore, Bland-Altman analyses of the results from the repeated tests were plotted. For this purpose the resting and exercise results were pooled, irrespective of whether they were performed under normoxic or hypoxic conditions; however the results of tests were always compared like for like (i.e. normoxic results with the results of the repeated normoxic test etc.).

2.2.6 Data collection

The Sensormedics software allowed steady state averages to be analysed on screen and stored. It did not allow a detailed analysis. Therefore data was exported into a Microsoft Excel spreadsheet and then into the statistical analysis program. Unfortunately the version of the Vmax 29 software we were working with would not allow the direct export of the data in this fashion. To overcome this, a hardcopy of the breath-by-breath data was printed out in 12pt Arial font. This was then scanned into the spreadsheet using an optical flat bed scanner with automatic paper feed (Epson Perfection 1640SU, Seiko Epson Corp., Japan) using Optical character recognition (OCR) software (Omnipage Pro-version 10, Caere corp., Los Gatos, CA, USA). The fidelity of this transfer process was then proof read and checked by me. Statistical analysis has been discussed in the section dealing with study structure. Once data had been imported into a Microsoft Excel spreadsheet it was exported into a Minitab worksheet for analysis.

2.3 Cardiac output testing

The following is a description of the development of methods and protocols to measure cardiovascular variables in relation to exercise and hypoxia.

2.3.1 Development of study design

Systemic cardiovascular variables measured during these experiments took the form of pulse measurement during the entire protocol as outlined above and blood pressure measurement at rest and after exercise. The main focus of these experiments was to measure the pulmonary cardiovascular responses to hypoxia and exercise. Potential areas of focus include pulmonary artery pressure and cardiac output responses to hypoxic and exercise stimuli. The choice of technique was limited by availability of equipment and acceptability.

Pulmonary artery catheters with pressure transducers are capable of measuring pulmonary artery pressures and inferring cardiac output by thermodilution methods. These certainly give a direct measurement of the variables, but would have involved exposing healthy subjects to

an invasive procedure with potentially hazardous effects. This was felt to be unacceptable on ethical grounds and to the subjects themselves.

Non-invasive measurements of pulmonary artery pressure are possible using echocardiography, however these methods are dependent on operator skill and the availability of a supine cycle ergometer that would allow reproducible exercise and leave the subject in a suitable position for echocardiography. The development and availability of harmonic echocardiography had produced a vast improvement in resolution and Doppler measurements and in combination with injected contrast (either agitated saline with microbubbles or commercially available contrast) was hoped to provide the best hope of achieving reproducible measurements. However a suitable ergometer was not available and initial experiments were performed on an old supine ergometer (of unknown vintage or origin) to assess the feasibility of studies. Despite the availability of an experienced operator, the use of the most up to date harmonic echocardiograph equipment and trials of agitated saline, we were unable to achieve results with any degree of fidelity. The main problem were in identifying the tricuspid regurgitant jet (TR jet) in young healthy subjects and a failure rate of up to 50% meant that this technique would prove unfeasible. The possible use of commercially available contrast media was proposed and dismissed because the media itself was derived from human albumin there was the small risk of hypersensitivity reaction and in light of the concerns over prion disease, the risk of possible infection were deemed unacceptable to healthy normal subjects.

Single breath inhalation of acetylene was next investigated as a means of measuring cardiac output. This was available from the manufacturer of the metabolic cart in the form of an add-on module. Sensormedics were kind enough to loan such a unit for a trial period. In order to produce reliable measurements a controlled smooth exhalation manoeuvre was necessary. This was problematic since it was a difficult manoeuvre to perform during exercise (especially during hypoxic exercise) and tended to interfere with the ventilatory measurements during exercise. In addition several volunteers felt the need to cough, again introducing further variability and was unpleasant for the subjects (two subjects stated they would not wish to repeat the procedure). This method was therefore abandoned.

The bio-impedance method provide a non-invasive measurement of cardiac output, using a new means of measuring the wave form of the bio impedance signal provides a more accurate reflection of stoke volume and in combination with ECG pulse recordings a value for cardiac output. A PhysioFlow PF05 Lab 1 non-invasive cardiac output unit (Manatec biomedical, Paris, France) was assessed for accuracy in validation experiments comparing measurements of cardiac output during pulmonary catherisation of patients undergoing investigation of pulmonary arterial hypertension. As part of their investigations patients would have cardiac output measured by thermodilution method, concurrent measurements of cardiac output were made with the PhysioFlow equipment. Once satisfied with the fidelity of measurements, cardiac output measurement by bio-impedance was incorporated into the steady state exercise protocols.

2.3.2 Validation studies using bio-impedance and thermodilution methods

Patients with pulmonary arterial hypertension underwent right heart catheterisation as part of their initial investigations in order to establish the severity of their disease, responses to exercise and effect of vasodilator challenge. Exercise was in the form of straight leg raises (patients with a femoral site for intravenous access were excluded). Vasodilator challenges were in the form of inhaled oxygen (FiO_2 60%), adenosine infusion or nitric oxide inhalation (at 40ppm). Patient selection was limited by ability to exercise and site of catheter access. In total 4 patients had concurrent bioimpedance and thermodilution methods for measuring cardiac performed. This data is presented in the results chapter

The protocol for the validation studies is as follows:

1. Patient details were entered into the PhysioFlow software.
2. Chest and neck electrodes (Blue Sensor R – stud fixing, Ambu Inc., Linthicum, MD., USA) were applied and connected to the unit.
3. Blood pressure was measured with a sphygmomanometer and stethoscope. The values were entered into the software.
4. The automatic calibration cycle was commenced and lasted 30 seconds.
5. A venous sheath was inserted into the internal jugular vein under aseptic technique with anaesthetic (Lignocaine 2%) instilled locally.

6. The right heart catheter was inserted and the tip sited in one of the pulmonary arteries under radiographic guidance.
7. Cardiac output measurement by thermodilution was then performed. Sterile isotonic saline chilled to 4°C (a thermistor had been placed in the cooling bath and was connected to the thermodilution computer) was injected into the proximal port of the catheter. The distal thermistor records the temperature and relays this to the thermodilution computer. This then calculates the cardiac output.
8. At least 3 measurements were taken by thermodilution with simultaneous bioimpedance values recorded.
9. The patient was then asked to raise and lower their legs in turn in order to cause a heart rate rise.
10. Steps 7 and 8 were repeated.

The comparison of measurements during vasodilator studies were not made since the effect of pharmacologically altering the intra-thoracic fluid compartment and therefore bioimpedance was thought to be a possible confounding factor. These measurements, however interesting fell out with the remit of these studies.

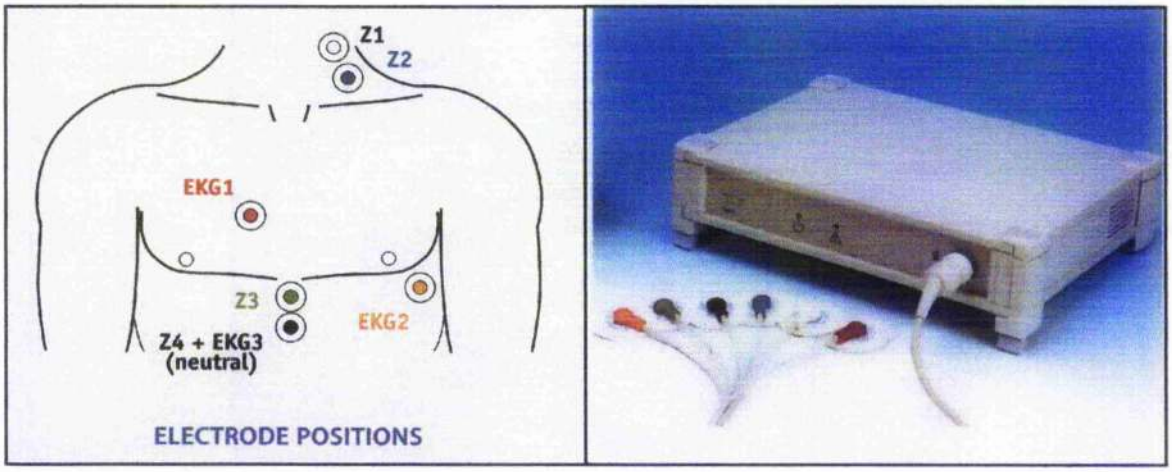

Figure 2.5. PhysioFlow equipment and chest electrode positions

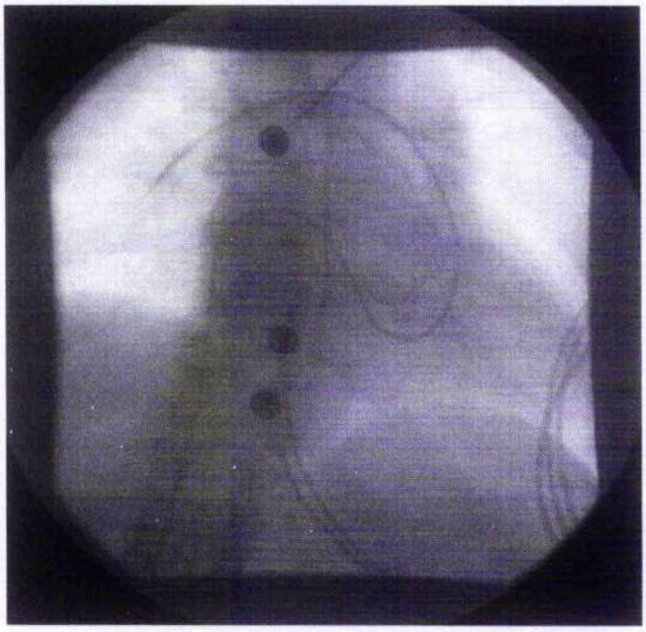

Figure 2.6 Fluoroscopic image demonstrating catheter placement in the right main pulmonary artery. Some of the PhysioFlow leads are visible in the image. (This patient was not included in the validation studies due to the femoral access used for catheter access)

2.3.3 Steady state exercise cardiac output protocol

Measurement of cardiac output was performed concurrently during the steady state exercise protocols during normoxia and hypoxia. No measurement of cardiac output was made during maximal effort exercise testing.

The equipment used for measurements was a PhysioFlow PF05 bioimpedance unit interfaced with a laptop computer (Toshiba Satellite Pro 3000, Toshiba, Tokyo, Japan) running the PhysioFlow software on a Microsoft Window 98 operating system. Blue senor R electrodes (Ambu Inc., Linthicum, MD., USA) were used throughout the experiments.

The protocol was entirely based on the exercise protocol as described in section 2.2.3. The only addition at the onset of testing was the application of electrodes to the neck and chest as described above and the recorded blood pressure was inputted into the PhysioFlow software. The only difference was the semi-supine position adopted by subjects during the automated calibration period (this was unnecessary in the validation studies since the patients were supine). Measurement of cardiac output continued throughout the test period. Points of note during the testing (rest, onset of hypoxia, exercise etc.) were recorded using the software.

The data was then electronically exported into an excel spread sheet, cardiac output, stroke volume (SV), heart rate (HR) and cardiac output (CO) were plotted against time. Measurements over 90 seconds were averaged at the same points as those used for cardiopulmonary variables, to give steady state values for rest and exercise in conditions of normoxia and hypoxia. These values were entered into a Minitab worksheet. Statistical analyses similar to those performed on the ventilatory and metabolic variables were performed (e.g. normality plots and ANOVA).

2.4 Genetic analysis

ACE genotyping was performed at the University College London in the centre for Cardiovascular Genetics in the Rayne Institute by our collaborators (Drs Hugh Montgomery and Dr David R Woods). The method used has been published [416] and used widely. The extraction of DNA and the subsequent ACE genotyping were not performed by me.

2.4.1 Specimen collection

Genetic material was primarily isolated from buccal washings. These were collected by asking subjects to rinse their mouths vigorously with 10mls of sterile saline for 30 seconds and then collect the mouthwash in a 20mls sterile universal container. Specimens were then transported by courier to The Rayne institute for analysis. In the rare occasions where genotyping was unsuccessful by this method, blood samples were collected from the subject. The S-Monovette® system of venesection was used for this, the sample was collected in 7.5ml potassium EDTA container, frozen to minus 70°C and transported in dry ice by courier to the Rayne institute. Seven subjects had ambiguous results from buccal washing and a further blood sample was sent ot confirm the genotyping of these individuals.

2.4.2 DNA extraction protocol

From saliva

1. Centrifuge universal container for 4 minutes at 3000rpm.
2. Decant saliva leaving buccal cells behind.
3. Add 500µl lysis buffer to remaining buccal cells and mix (cell lysis).
4. Transfer contents to a large eppendorf.
5. Add 150µl 5M Sodium Perchlorate. Then add 500µl chloroform.

6. Agitate vigorously for 3-4 minutes before centrifuging again at 14000rpm for 4 minutes in micro-centrifuge.
7. Draw off 500µl of supernatant and place in fresh Eppendorf.
8. Add 1000µl of 100% ethanol and invert tubes gently 5 mins. (DNA precipitation).
9. Centrifuge at 14000rpm for 3 minutes in microfuge.
10. Decant ethanol, aspirate remainder and allow DNA pellet to air dry for 10 minutes.
11. Resuspend DNA in 1000µl sterile water

From blood

1. Blood was anticoagulated with 5mM potassium EDTA and stored in 0.5 ml aliquots at -20°C.
2. Specimens were thawed and 50µl added to 50µl methanol in deep welled Beckham titre plate and evaporated to dryness using a hot air dryer (no exceeding 55°C)
3. 100µl sterile distilled water was added; the plate was covered and then heated in a water bath at 100°C for 25 minutes.
4. The aqueous DNA extract was withdrawn with a multichannel pipette and 4µl transferred to a 96 well Omniplate anmd allowed to air dry prior to PCR.

2.4.3 Genotyping protocol

The insertion/ deletion polymorphism was identified by polymerase chain reaction amplification (PCR) and subsequent electrophoretic separation of fragments. Two priming oligonucleotides flank the insertion (Alu) sequence in intron 16 (ACE1, 5' or left hand D specific oligonucleotide and ACE3, 3' or right hand common oligonucleotide) and a third oligonucleotide is specifically within the insertion sequence (ACE2, the insertion specific oligonucleotide). Misclassification of heterozygotes as being D homozygotes [417] was prevented by the use of an insertion allele-specific third primer as described by Evans [418] but with a modified protocol as subsequently described below.

1. 5µl DNA solution was pipetted onto a microsatellite array plate and dried at 80°C for 10mins.
2. 20µl PCR mix and then 20µl mineral oil was added to each well. Plates were then sealed with a plastic film and centrifuged at 3000rpm for 1 minute.

3. DNA samples were then amplified on an Omnigene® (Hybaid, Middlesex, UK) PCR block.
4. All 96 wells were always filled with reagents to ensure constant thermal mass on the block.
5. 6ul of amplification product was mixed with 2ul formamide dye and then 6ul pipetted onto a Microtitre Array Diagonal gel (MADGE) in an electrophoresis bath of 1xTBE.
6. Electrophoresis was at 150 volts for 40mins before amplification products were visualised by ultraviolet light.

Constituents of the PCR mix used in step 2 above:

PCR mix for 4mls (192 wells):

1. 10xpolmix-400µl.
2. W1Buffer -200µl.
3. 50mM Magnesium Chloride–120µl.
4. Primer mix :30µl ACE 1, 4µl ACE 2, 20µl ACE 3.
5. 8µl Taq (*Thermus acquaticus*) DNA polymerase (Gibco BRL, Paisley, U.K.).
6. 3218µl sterile water.

Primer sequences:

ACE 1: 5' CAT CCT TTC TCC CAT TTC TC (FH76)

ACE 2: 5' TGG GAT TAC AGG CGT GAT ACA G (FH77)

ACE 3: 5' ATT TCA GAG CTG GAA TAA AAT T (FH78)

A= adenine T=thymidine C=cystine G=guanine

Amplification conditions:

1. 95°C 5 min1 cycle for initial denaturation.
2. 95°C 1 min.
3. 50°C 1 min.
4. 72°C 30secs.
5. Repeat steps 2-4 for 32 cycles.

7.5% polyacrylamide MADGE (microtitre array diagonal gel electrophoresis) production (per 6 MADGE gels).

1. Glass plates cleaned thoroughly with detergent and left to air dry.
2. Clean 1 side with 100% ethanol and again air dry.
3. Apply sticky silane (1.25mls 0.5% glacial acetic acid, 1.25mls gamma methacryloxypropyltrimethyl silane made up to 250mls with 100% ethanol) to same surface and air dry in fume cupboard.
4. Wash plate with distilled water and dry.
5. Wash and dry MADGE formers (template).
6. Pour 40mls into beaker, stir, add 150µl 25% Ammonium Persulphate solution, stir and immediately pour onto MADGE former.
7. Place glass plate onto MADGE former and solution, and weight down.
8. Leave for 30 minutes and remove new MADGE plate from template and store in 1xTBE mixed with 10µl per 200mls of Ethidium Bromide stain.

Constituents of 10xTBE (Tris-borate-EDTA)buffer used as 1 in 10 dilution to give 1xTBE

1. 108gms Tris base (trishydroxymethylaminomethane)
2. 55 gms Boric acid
3. 40mls 0.5M Sodium EDTA (pH 8.0)
4. Dilute to 1 litre with sterile deionised water, the final pH is 8.3.

Constituents of 10xpolmix

1. 500 mmol/l potassium chloride
2. 100mmol/l Tris, pH8.3
3. 0.1 g/l gelatine
4. 2 mmol/l of each dNTP

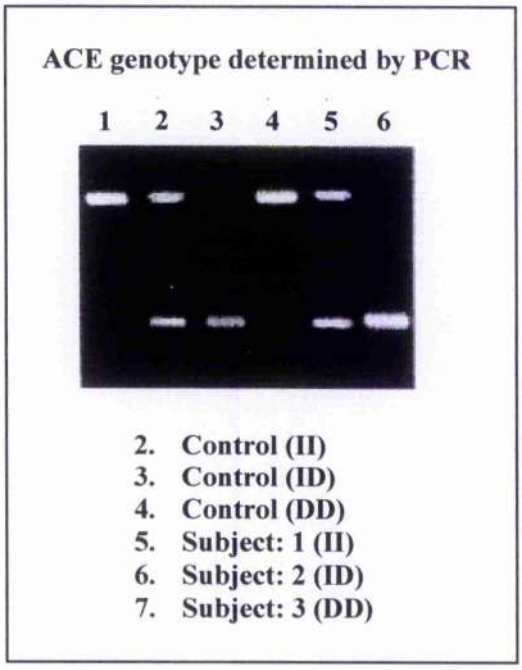

Figure 2.7 The amplification products are 84bp for allele ACE D and 65bp for allele ACE I which are separated by electrophoresis and interpreted by the naked eye as 2 clearly distinct bands. Positive control samples of II, ID and DD samples were always amplified and electrophoresed concurrently.

CHAPTER THREE: RESULTS

3.1 Subject characteristics

The subject group comprised mainly of students attending Glasgow University recruited from the Officer training corp. and the Glasgow University Sports and recreational faculty. There were some subjects recruited from the medical staff of the Western Infirmary and Gartnavel General Hospital in Glasgow. This is reflected in the biometric data presented. Sixty Caucasian subjects were included in the laboratory study, 38 male.

3.1.1 Age

The mean age of the entire group was 23.7 years (SD ± 4.8 years, range 18-37 years). Individual ages are presented on table 3.2 and figure 3.1.

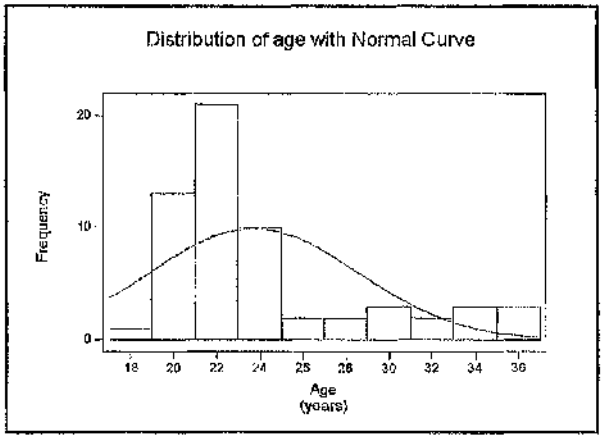

Figure 3.1 Frequency distribution of ages within the subject group with normal distribution curve superimposed (red). Note the skewed distribution towards the younger end of the range.

3.1.2 Heights and weights of subject group

The average subject height was 177.8cm (SD ± 7.96, range 157-193cm). Summary data is presented on table 3.1 and individual data is on table 3.2 and figure 3.2.

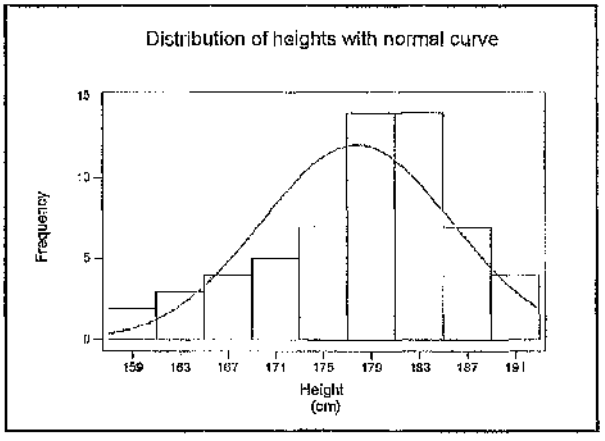

Figure 3.2 Frequency distribution of heights with normal distribution curve superimposed (red). The distribution of heights is skewed toward the taller end of the range.

The mean weight of the whole group was 73.8kg (SD ± 10.43, range 53.0-100.0kg). Summary data is presented on table 3.1 and individual data is on table 3.2 and figure 3.3.

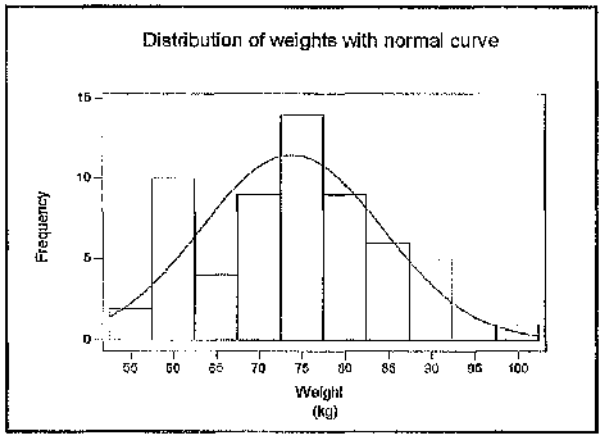

Figure 3.3 Frequency distribution of weights with normal curve superimposed (red). The weight distribution of the subject group demonstrated normality.

n=60	*Age (years)*	*Height (cm)*	*Weight (kg)*
Range	18-37	157-193	53.0-100.0
Mean (±SD)	23.7 (±4.8)	177.8 (±7.96)	73.8 (±10.4)

Table 3.1 Summary descriptive statistics of biometric data for the whole subject group

3.1.3 Maximal oxygen uptake and ventilatory threshold

The mean VO_2 max was 50.9 mls/kg/min (SD 10.8, range 30.4-85.5 mls/kg/min). The mean VO_2 at ventilatory threshold was 30.5 mls/kg/min (SD 8.6, range 17.9-54.8 mls/kg/min). The mean maximum work load achieved was 287.8 watts (SD 84.6, range 104-444 watts). The mean work load at ventilatory threshold was 186.4 (SD 20.7, range 100-242 watts) summary and individual data is on table 3.3 and 3.4 respectively.

Subject	*Age*	*Sex*	*Height (cm)*	*Weight (kg)*	*Subject*	*Age*	*Sex*	*Height (cm)*	*Weight (kg)*
1	24	M	185	70.8	**31**	21	M	186	82.2
2	22	M	182	74.5	**32**	27	M	185	76.6
3	19	M	157	58.0	**33**	20	M	184	84.3
4	24	M	189	100.0	**34**	24	M	178	79.0
5	21	M	189	82.1	**35**	21	M	184	85.0
6	21	M	178	70.0	**36**	23	F	165	58.2
7	22	M	180	83.2	**37**	33	F	180	72.4
8	21	M	181	75.0	**38**	32	F	185	74.6
9	23	M	181	90.0	**39**	21	F	171	53.0
10	20	M	179	66.8	**40**	30	F	183	72.0
11	29	M	177	80.7	**41**	19	F	175	58.3
12	20	M	180	80.0	**42**	20	F	174	71.4
13	31	M	184	76.6	**43**	21	F	178	74.3
14	21	M	193	91.5	**44**	22	F	163	60.5
15	23	M	183	89.1	**45**	22	F	168	58.5
16	22	M	189	83.0	**46**	23	F	168	58.0
17	22	M	180	76.5	**47**	19	F	173	70.0
18	21	M	175	75.6	**48**	20	F	160	63.6
19	20	M	180	72.4	**49**	20	M	183	80.0
20	37	M	175	67.9	**50**	21	F	169	67.0
21	25	M	185	80.4	**51**	21	M	175	75.6
22	35	M	184	78.9	**52**	21	M	177	88.8
23	18	F	176	74.0	**53**	27	F	163	62.0
24	20	M	188	84.5	**54**	28	M	179	71.0
25	23	F	171	60.2	**55**	37	M	183	92.3
26	22	F	179	63.3	**56**	20	M	182	82.7
27	21	F	171	56.2	**57**	24	M	186	76.8
28	23	F	169	59.4	**58**	26	M	174	74.3
29	34	M	182	78.6	**59**	20	F	163	73.4
30	21	F	165	57.8	**60**	21	M	182	76.2

Table 3.3 Individual biometric data for the entire subject group. The individual's gender is denoted by 'M' for male and 'F' for female.

Subject	*ACE genotype*	*VO_2 max (mls/min/kg)*	*VO_2 at VT (mls/min/kg)*	*Work at VT (watts)*	*Maximum work (watts)*
1	*II*	69.4	44.2	191	373
2	*DD*	41.6	20.0	181	270
3	*ID*	47.9	35.2	183	204
4	*ID*	39.2	18.8	185	368
5	*DD*	30.4	19.1	183	350
6	*II*	48.5	30.6	189	240
7	*ID*	65.9	35.6	196	409
8	*DD*	56.1	30.9	185	307
9	*DD*	57.5	33.8	185	354
10	*ID*	75.9	54.8	192	371
11	*ID*	50.3	40.0	192	326
12	*DD*	46.2	22.5	195	291
13	*II*	68.2	46.5	155	284
14	*ID*	45.3	27.7	191	345
15	*ID*	54.6	35.9	222	238
16	*II*	57.0	40.8	194	434
17	*ID*	71.6	43.0	197	389
18	*DD*	54.7	30.9	190	267
19	*ID*	54.7	37.7	192	348
20	*ID*	47.4	32.1	184	305
21	*II*	52.7	27.0	173	337
22	*DD*	60.8	36.3	170	444
23	*II*	52.3	35.6	153	208
24	*DD*	52.2	29.9	197	362
25	*ID*	38.3	25.2	209	142
26	*ID*	43.6	21.7	199	269
27	*ID*	41.1	20.6	191	104
28	*ID*	40.0	29.2	175	138
29	*DD*	56.5	37.3	242	239
30	*DD*	41.3	19.6	192	182
31	*II*	85.5	46.8	192	435
32	*DD*	47.1	30.6	221	196
33	*ID*	35.8	22.3	180	292
34	*ID*	58.8	28.8	194	373
35	*ID*	46.1	25.2	171	386
36	*DD*	34.4	21.5	180	166
37	*ID*	65.9	51.6	166	354
38	*II*	68.2	47.7	174	436
39	*ID*	50.7	23.9	189	272
40	*II*	41.6	24.0	173	337
41	*ID*	50.8	31.5	181	261
42	*ID*	55.7	26.7	183	210
43	*II*	42.9	26.1	176	251
44	*II*	43.3	26.3	179	202
45	*II*	49.9	27.2	100	151
46	*ID*	46.8	28.2	190	123
47	*II*	54.3	29.8	185	275
48	*DD*	41.7	24.1	187	214
49	*DD*	49.2	28.8	224	312
50	*ID*	52.1	37.2	192	284
51	*DD*	54.7	30.9	190	267
52	*ID*	50.3	25.9	196	419
53	*ID*	32.1	17.9	173	213
54	*II*	51.0	29.7	166	306
55	*DD*	38.3	26.7	176	276
56	*ID*	54.5	23.6	160	196
57	*ID*	45.9	25.0	172	268
58	*ID*	41.2	19.4	180	246
59	*ID*	61.6	37.4	242	320
60	*ID*	45.5	21.6	208	328
Mean	-	**50.95 ±10.8**	**30.5 ±8.6**	**186.4 ±20.7**	**287.8 ±84.6**

Table 3.4 Summary metabolic data from maximal cardiopulmonary exercise tests.

3.1.4 Physical activity of the subject group

The physical activity of each individual was recorded on the pre-test questionnaire as part of the consent procedure and assessment of altitude exposure. None of the subject group had been to high altitude in the 6 months preceding the test protocol. None of the group had been born or lived at high altitude. The summary of physical activity is shown on table 3.5.

Subject	*Activity*	*Subject*	*Activity*
1	Long distance running (competitive)	31	Rowing, sailing (both recreational
2	Football, badminton	32	Cycling, running (competitive), rock-climbing
3	Squash, Fell running (recreational)	33	Swimming, cycling
4	Running, swimming, kick-boxing (all recreational)	34	Rowing, rugby, running (all recreational)
5	Fell running (competitive), cycling, canoeing, sailing	35	Rock-climbing, skiing, running (all recreational)
6	Running, football	36	Football (competitive), cycling
7	Swimming, running, cycling - triathlon (competitive)	37	Swimming (recreational)
8	Running (recreational)	38	Judo (competitive), running, volleyball
9	Running, skiing (competitive)	39	Swimming, cycling
10	Running, swimming, mountain biking	40	Adventure racing (competitive), rowing (competitive), cycling, swimming, sailing
11	Running (recreational)	41	Rowing (competitive), running (recreational)
12	Running, cycling	42	Rugby (competitive), running
13	Football, skiing	43	Cycling, running
14	Football, badminton (competitive), running	44	Cycling, running (recreational)
15	Long distance running (competitive)	45	Swimming, squash, cycling
16	Swimming, running (both recreational)	46	Swimming, running
17	Running, weights	47	Long distance running (competitive)
18	Football, squash, running	48	Rowing, running (recreational)
19	Football (competitive)	49	Rock climbing, running, highland dancing
20	Orienteering (competitive), cycling	50	Cycling
21	Cycling, running (competitive)	51	Cycling, running, rock-climbing
22	Distance running (competitive), adventure racing	52	Skiing, swimming (both recreational)
23	Swimming, cycling, running (all recreational)	53	Rowing (competitive), running
24	Running, orienteering, cycling	54	Running, Highland dancing
25	Highland dancing	55	Running, football (recreational)
26	Running, canoeing, hockey, rock-climbing	56	Running, cycling (recreational)
27	Running, swimming	57	Tennis (recreational)
28	Running (recreational)	58	Swimming, running, cycling (all recreational)
29	Rock-climbing, cycling	59	Running, cycling
30	Rowing, sailing (both recreational)	60	Skiing (competitive), running

Table 3.5 The physical activities of the subject group. There is a wide range in physical exercise activity within the subject group, which is also variable in the degree of participation in each sport (recreational vs. competitive).

3.1.4 Spirometry

The individual spirometric data is displayed on table 3.6. The mean FEV_1 was 4.18 litres (SD 0.74; range 2.55-5.70 litres). The mean FVC was 5.10 litres (SD 0.91; range 2.97-7.49 litres). All subjects were within predicted values for height, age and sex. There was no evidence of significant airflow limitation.

Subject	*FEV_1 (litres)*	*FVC (litres)*	*Subject*	*FEV_1 (litres)*	*FVC (litres)*	*Subject*	*FEV_1 (litres)*	*FVC (litres)*	*Subject*	*FEV_1 (litres)*	*FVC (litres)*
1	4.80	6.16	**16**	4.33	5.42	**31**	5.01	6.21	**46**	3.36	4.65
2	4.28	5.67	**17**	4.10	4.58	**32**	4.95	5.88	**47**	3.56	4.52
3	3.74	4.09	**18**	5.70	7.49	**33**	4.78	5.58	**48**	3.02	3.81
4	5.62	7.31	**19**	5.30	5.99	**34**	4.22	5.10	**49**	4.42	5.01
5	5.37	6.36	**20**	4.34	5.07	**35**	5.38	6.05	**50**	3.44	4.35
6	4.10	5.01	**21**	5.16	6.39	**36**	2.55	2.97	**51**	4.29	5.10
7	4.63	5.84	**22**	4.30	5.36	**37**	3.14	3.58	**52**	4.48	5.26
8	4.10	5.85	**23**	4.15	4.76	**38**	3.18	4.43	**53**	3.18	3.87
9	5.38	6.80	**24**	4.95	5.70	**39**	3.13	3.70	**54**	4.31	5.23
10	4.98	6.21	**25**	3.66	4.51	**40**	4.22	5.08	**55**	4.17	4.94
11	4.30	5.21	**26**	3.75	4.72	**41**	3.70	4.31	**56**	4.38	5.37
12	4.92	5.24	**27**	3.56	4.50	**42**	3.07	4.30	**57**	3.98	4.79
13	4.21	4.80	**28**	3.38	4.18	**43**	3.77	4.24	**58**	3.25	3.92
14	4.69	5.75	**29**	4.48	5.26	**44**	3.38	4.10	**59**	4.49	5.18
15	4.94	6.15	**30**	3.33	4.18	**45**	3.50	4.48	**60**	4.21	5.24

Table 3.6. Summary spirometry data for the subject group.

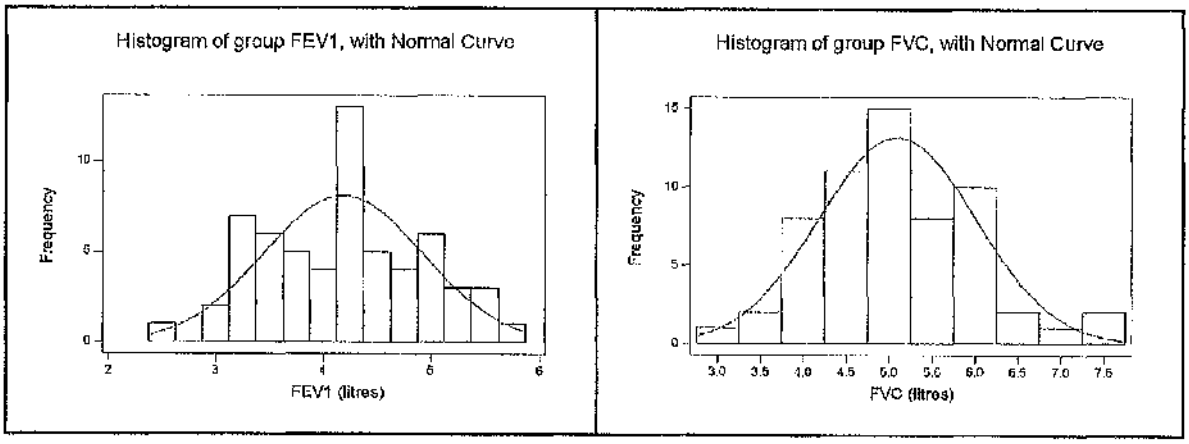

Figure 3.4 Frequency distributions of FEV_1 (left panel) and FVC (right panel); both the distribution of FEV_1 and FVC demonstrated normality (normality curves are superimposed in red).

3.2 Cardiopulmonary exercise validation results

The repeatability of steady state CPET for VO_2 and VE was verified in 5 subjects, by repeating the steady state protocol during normoxia and hypoxia. The variability of results during rest was more noticeable than exercise results; therefore, resting and exercise variables from normoxic and hypoxic steady state tests are pooled to give values for comparison. Tables 3.7 and 3.8 show the absolute values and the percent difference between tests for VO_2 and VE respectively; tests 1 to 5 were performed in normoxic conditions, test 6-9 were during hypoxia (one subject only repeated the normoxic test). Individual work loads for repeated steady state test were the same. Repeatability of tests is demonstrated by Bland-Altman plots in Figures 3.5 and 3.6.

Test	*Resting VO_2 (l/min)*			*Exercise VO_2 (l/min)*		
	Test a	*Test b*	*% difference*	*Test a*	*Test b*	*% difference*
1	0.47	0.43	7.7	1.76	1.69	3.8
2	0.33	0.32	4.4	2.46	2.41	2.0
3	0.37	0.34	8.7	1.25	1.26	-0.3
4	0.47	0.48	-1.7	2.19	2.20	-0.7
5	0.26	0.28	-7.6	1.12	1.10	1.7
6	0.43	0.45	-4.2	1.70	1.76	-3.6
7	0.43	0.42	1.5	2.43	2.33	4.1
8	0.46	0.43	5.3	2.08	2.07	0.4
9	0.30	0.29	3.6	1.09	1.14	-4.1

Table 3.7 Oxygen uptake results of repeated steady state exercise tests. Test 1-5 were during normoxia, test 6-9 were during hypoxia. The work load between *test a* and *test b* was the same.

Test	*Resting VE (l/min)*			*Exercise VE (l/min)*		
	Test a	*Test b*	*% difference*	*Test a*	*Test b*	*% difference*
1	10.80	9.91	8.3	35.61	36.83	-3.4
2	10.57	10.57	-0.0	44.19	43.81	0.8
3	11.32	10.66	5.8	32.37	32.50	-0.4
4	10.95	11.18	-2.1	39.73	38.93	2.0
5	8.88	8.78	1.1	26.93	28.53	-6.0
6	13.14	13.73	-4.5	45.10	46.62	-3.4
7	14.48	14.48	-0.0	67.39	65.24	3.2
8	12.88	13.60	-5.6	54.70	54.23	0.9
9	9.39	9.80	-4.3	33.54	31.56	5.9

Table 3.8 Minute ventilation results of repeated steady state exercise tests. Test 1-5 were during normoxia, test 6-9 were during hypoxia. The work load between *test a* and *test b* was the same.

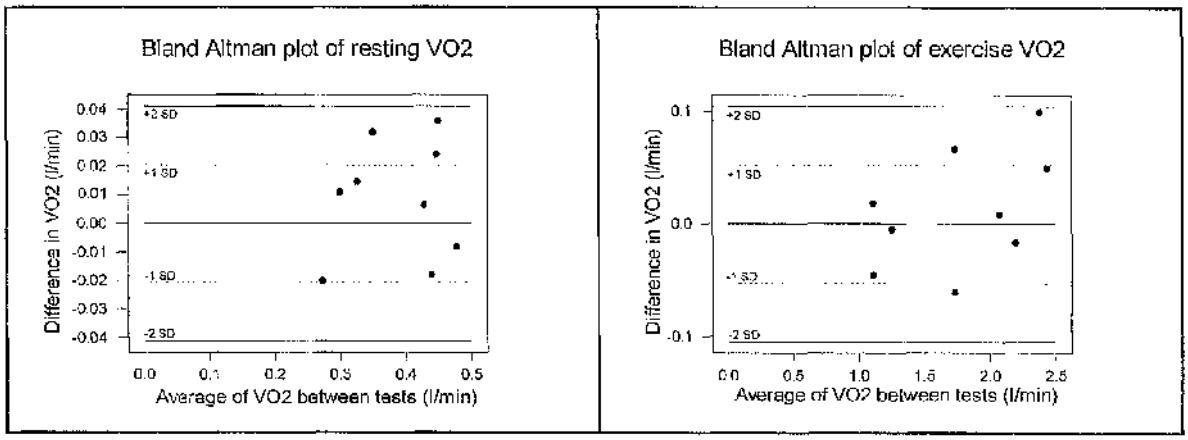

Figure 3.5a Bland-Altman plots of oxygen uptake (VO_2) measurements in 5 subjects. The difference in measurements between tests is plotted against the mean VO_2 measurement for the two tests. The horizontal bars are placed at one and two standard deviations of the differences in VO_2 (=1 SD and ±2 SD respectively). The left panel demonstrates the repeatability of VO_2 during rest (SD 0.021), the right during exercise (SD 0.052).

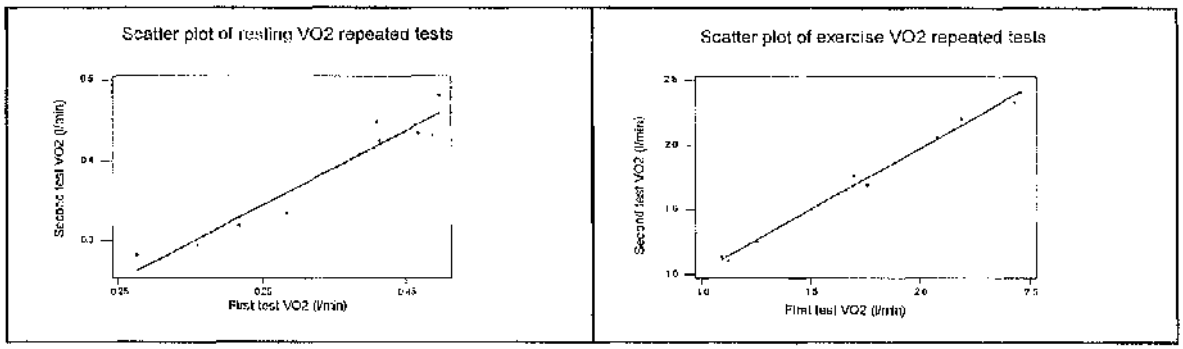

Figure 3.5b Scatter plots of oxygen uptake (VO_2) measurements in 5 subjects. The left panel demonstrates resting VO2 results from the repeated tests plotted together; the right panel plots the exercise VO2 results.

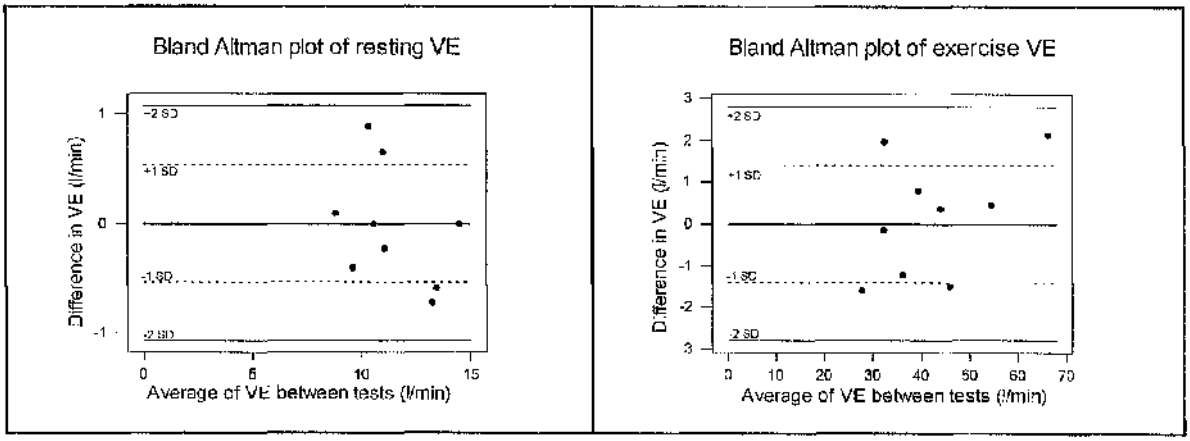

Figure 3.6a Bland-Altman plots of minute ventilation (VE) measurements in 5 subjects. The difference in measurements between tests is plotted against the mean VE measurement for the two tests. The horizontal bars are placed at one and two standard deviations of the differences in VE (±1 SD and ±2 SD respectively). The left panel demonstrates the repeatability of VE during rest (SD 0.54), the right during exercise (SD1.40).

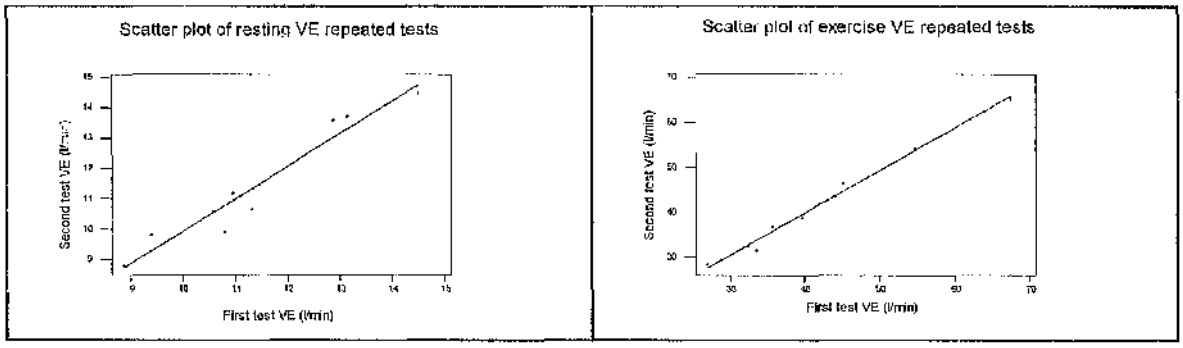

Figure 3.6b Scatter plots of minute ventilation (VE) measurements in 5 subjects. The left panel demonstrates resting VE results from the repeated tests plotted together; the right panel plots the exercise VE results.

3.3 Cardiopulmonary exercise test results

The following section presents data for each individual and the entire group. The tables are set out in a similar fashion. Each table presents data during rest and exercise under conditions of normoxia and hypoxia (normoxic rest, normoxic exercise, hypoxic rest and hypoxic exercise respectively). Data is presented as the mean measurements during breath-by-breath cardiopulmonary exercise testing over a period of 90 seconds at the end of the rest and exercise periods. The average measurement during exercise is after 4 minutes to ensure steady state had been attained. Furthermore, the change in variables between normoxia and hypoxia during rest and exercise are presented, both as absolute value and as a percent change of normoxic measurements.

3.3.1 Oxygen uptake

Oxygen uptake data for the whole subject group is displayed in table 3.9. Complete data points for the entire subject group were measured (n=60). The mean VO_2 during normoxic rest and exercise was; 0.411 l/min (SD 0.10; range 0.23-0.69 l/min) and 1.63 l/min (SD 0.40; range 0.95-2.46 l/min) respectively. The mean VO_2 during hypoxic rest and exercise was 0.411 l/min (SD 0.08; range 0.26-0.64 l/min) and 1.62 l/min (SD 0.36; range 1.01-2.43 l/min) respectively. The absolute change in VO_2 at *rest* between conditions of normoxia and hypoxia was 0.002 l/min (SD 0.06; range -0.15-0.18 l/min); the percent change was 2.76% (SD 18.6; range -32.3-66.0%). The absolute change in VO_2 during *exercise* between conditions of normoxia and hypoxia was -0.01 l/min (SD 0.13; range -0.44-0.21 l/min); the percent change was 0.33% (SD 7.6; range -14.8-15.8).

Oxygen uptake during rest and exercise under conditions of normoxia and hypoxia demonstrated a normal distribution. The values for VO_2 would not be expected to vary significantly between normoxia and hypoxia; therefore paired student t-tests were performed. VO_2 change at rest and exercise from normoxia to hypoxia did not demonstrate any significant difference (p values: 0.89 and 0.53 respectively). Furthermore, Bland Altman plots of VO_2 measurements at rest and exercise, between conditions of normoxia and hypoxia were plotted. These are presented in figure 3.7; there is a higher level of variability in the resting values than during exercise. The majority of the recordings lie within two standard deviations of the difference between normoxic and hypoxic measurement at rest; all exercise measurements are within 2 standard deviations.

Table 3.9. Individual oxygen uptake data and changes between normoxia and hypoxia.

Subject	$\dot{V}O_2$ *Normoxic Rest (l/min)*	$\dot{V}O_2$ *Normoxic Exercise (l/min)*	$\dot{V}O_2$ *Hypoxic Rest (l/min)*	$\dot{V}O_2$ *Hypoxic Exercise (l/min)*	$\Delta\dot{V}O_2$ *(Rest)*		$\Delta\dot{V}O_2$ *(Exercise)*	
					l/min	*%*	*l/min*	*%*
1	0.473 ±0.12	2.05 ±0.27	0.450 ±0.16	1.93 ±0.38	-0.02	-5.0	-0.12	-5.9
2	0.429 ±0.21	2.15 ±0.29	0.425 ±0.17	1.90 ±0.20	-0.00	-1.0	-0.25	-11.7
3	0.563 ±0.22	2.42 ±0.24	0.507 ±0.10	2.26 ±0.18	-0.06	-9.9	-0.17	-6.9
4	0.533 ±0.25	2.25 ±0.25	0.537 ±0.20	2.21 ±0.41	0.00	0.7	-0.03	-1.5
5	0.528 ±0.32	1.98 ±0.27	0.448 ±0.14	1.74 ±0.29	-0.08	-15.2	-0.24	-12.0
6	0.398 ±0.13	1.49 ±0.20	0.395 ±0.15	1.50 ±0.14	-0.00	-0.8	0.01	0.6
7	0.490 ±0.13	2.15 ±0.29	0.491 ±0.15	1.97 ±0.24	0.00	0.2	-0.18	-8.2
8	0.373 ±0.14	1.58 ±0.34	0.387 ±0.26	1.59 ±0.33	0.01	3.9	0.02	1.0
9	0.453 ±0.13	1.97 ±0.37	0.438 ±0.20	1.73 ±0.19	-0.02	-3.3	-0.24	-12.0
10	0.488 ±0.19	2.08 ±0.29	0.345 ±0.12	2.06 ±0.35	-0.14	-29.3	-0.02	-1.0
11	0.560 ±0.32	2.19 ±0.32	0.507 ±0.16	2.20 ±0.22	-0.05	-9.5	0.02	0.7
12	0.454 ±0.18	1.49 ±0.25	0.307 ±0.06	1.40 ±0.18	-0.15	-32.3	-0.10	-6.6
13	0.447 ±0.17	1.54 ±0.41	0.382 ±0.14	1.50 ±0.15	-0.07	-14.6	-0.04	-2.7
14	0.548 ±0.28	1.89 ±0.16	0.554 ±0.18	1.87 ±0.17	0.01	1.0	-0.02	-1.0
15	0.519 ±0.16	2.10 ±0.31	0.536 ±0.13	2.14 ±0.33	0.02	3.2	0.04	1.8
16	0.691 ±0.28	2.39 ±0.43	0.590 ±0.16	2.40 ±0.33	-0.10	-14.7	0.01	0.5
17	0.529 ±0.31	2.10 ±0.18	0.439 ±0.14	2.15 ±0.15	-0.09	-17.2	0.05	2.5
18	0.548 ±0.18	1.91 ±0.32	0.516 ±0.15	1.82 ±0.21	-0.03	-5.7	-0.09	-4.7
19	0.515 ±0.19	1.96 ±0.41	0.575 ±0.17	2.05 ±0.34	0.06	11.7	0.08	4.2
20	0.516 ±0.20	1.69 ±0.43	0.454 ±0.23	1.54 ±0.18	-0.06	-12.0	-0.15	-9.7
21	0.611 ±0.16	2.46 ±0.27	0.638 ±0.12	2.43 ±0.43	0.03	4.4	-0.03	-1.3
22	0.474 ±0.25	2.37 ±0.22	0.450 ±0.26	2.11 ±0.15	-0.02	-5.1	-0.26	-10.9
23	0.345 ±0.19	1.19 ±0.14	0.305 ±0.10	1.24 ±0.10	-0.04	-11.7	0.05	4.4
24	0.318 ±0.18	1.44 ±0.12	0.349 ±0.13	1.62 ±0.22	0.03	9.9	0.18	12.5
25	0.260 ±0.12	1.30 ±0.18	0.364 ±0.10	1.42 ±0.13	0.10	40.3	0.12	9.3
26	0.315 ±0.18	0.97 ±0.18	0.275 ±0.14	1.01 ±0.23	-0.04	-12.8	0.04	3.7
27	0.228 ±0.12	1.24 ±0.21	0.269 ±0.08	1.31 ±0.16	0.04	17.9	0.07	5.5
28	0.436 ±0.25	1.20 ±0.21	0.386 ±0.10	1.27 ±0.22	-0.05	-11.5	0.08	6.3
29	0.449 ±0.29	1.92 ±0.19	0.404 ±0.10	2.04 ±0.28	-0.05	-10.1	0.12	6.2
30	0.283 ±0.17	0.95 ±0.13	0.302 ±0.19	1.04 ±0.22	0.02	6.6	0.09	9.5
31	0.419 ±0.09	1.83 ±0.32	0.431 ±0.09	1.73 ±0.36	0.01	2.9	-0.10	-5.4
32	0.452 ±0.26	1.46 ±0.31	0.428 ±0.26	1.64 ±0.26	-0.02	-5.3	0.18	12.5
33	0.325 ±0.28	1.26 ±0.22	0.418 ±0.36	1.34 ±0.29	0.09	28.6	0.09	6.9
34	0.270 ±0.10	1.40 ±0.18	0.448 ±0.05	1.54 ±0.11	0.18	66.0	0.14	10.2
35	0.456 ±0.18	1.55 ±0.17	0.441 ±0.17	1.55 ±0.22	-0.01	-3.2	0.00	0.1
36	0.399 ±0.10	1.60 ±0.33	0.374 ±0.08	1.46 ±0.21	-0.03	-6.3	-0.14	-8.7
37	0.351 ±0.16	1.33 ±0.15	0.295 ±0.08	1.38 ±0.25	-0.06	-15.9	0.04	3.4
38	0.347 ±0.09	1.22 ±0.17	0.258 ±0.05	1.04 ±0.13	-0.09	-25.8	-0.18	-14.8
39	0.468 ±0.10	1.76 ±0.32	0.425 ±0.13	1.70 ±0.29	-0.04	-9.2	-0.07	-3.7
40	0.439 ±0.24	1.51 ±0.17	0.439 ±0.24	1.47 ±0.18	0.00	0.0	-0.04	-2.7
41	0.332 ±0.13	1.46 ±0.25	0.280 ±0.09	1.40 ±0.21	-0.05	-15.6	-0.07	-4.7
42	0.344 ±0.11	1.79 ±0.24	0.385 ±0.15	1.63 ±0.19	0.04	11.9	-0.16	-9.0
43	0.450 ±0.10	1.50 ±0.21	0.400 ±0.14	1.38 ±0.12	-0.05	-11.0	-0.12	-7.8
44	0.250 ±0.14	0.95 ±0.13	0.297 ±0.08	1.10 ±0.13	0.05	18.5	0.15	15.8
45	0.291 ±0.16	1.19 ±0.40	0.407 ±0.25	1.27 ±0.22	0.12	39.8	0.08	6.5
46	0.353 ±0.09	1.07 ±0.39	0.371 ±0.13	1.11 ±0.45	0.02	5.2	0.04	4.1
47	0.306 ±0.25	1.27 ±0.14	0.351 ±0.12	1.26 ±0.11	0.05	14.8	-0.01	-0.9
48	0.319 ±0.09	1.36 ±0.20	0.311 ±0.14	1.44 ±0.21	-0.01	-2.5	0.08	6.3
49	0.512 ±0.32	1.59 ±0.17	0.470 ±0.28	1.57 ±0.32	-0.04	-8.2	-0.02	-1.2
50	0.332 ±0.15	1.55 ±0.22	0.448 ±0.29	1.76 ±0.26	0.12	35.2	0.21	13.3
51	0.374 ±0.19	1.26 ±0.21	0.391 ±0.20	1.31 ±0.13	0.02	4.7	0.05	4.0
52	0.388 ±0.10	1.68 ±0.22	0.482 ±0.22	1.74 ±0.17	0.09	24.2	0.06	3.3
53	0.267 ±0.10	1.10 ±0.17	0.334 ±0.09	1.23 ±0.17	0.07	25.4	0.14	12.4
54	0.358 ±0.08	1.57 ±0.12	0.375 ±0.11	1.57 ±0.14	0.02	4.8	-0.00	-0.1
55	0.353 ±0.14	1.55 ±0.36	0.420 ±0.19	1.66 ±0.22	0.07	19.0	0.11	6.8
56	0.291 ±0.12	1.37 ±0.30	0.379 ±0.29	1.34 ±0.22	0.09	30.2	-0.03	-2.0
57	0.374 ±0.28	1.30 ±0.28	0.473 ±0.32	1.38 ±0.27	0.10	26.5	0.08	6.4
58	0.236 ±0.11	1.17 ±0.23	0.265 ±0.12	1.24 ±0.25	0.03	12.5	0.07	6.2
59	0.407 ±0.36	1.87 ±0.33	0.468 ±0.35	1.92 ±0.31	0.06	15.0	0.05	2.6
60	0.400 ±0.21	1.64 ±0.20	0.420 ±0.12	1.64 ±0.22	0.02	5.3	-0.01	-0.0
Group Mean	**0.411 ±0.10**	**1.63 ±.40**	**0.411 ±0.08**	**1.62 ±0.36**	**0.002 ±0.06**	**2.8 ±18.6**	**-0.01 ±0.13**	**0.33 ±7.6**

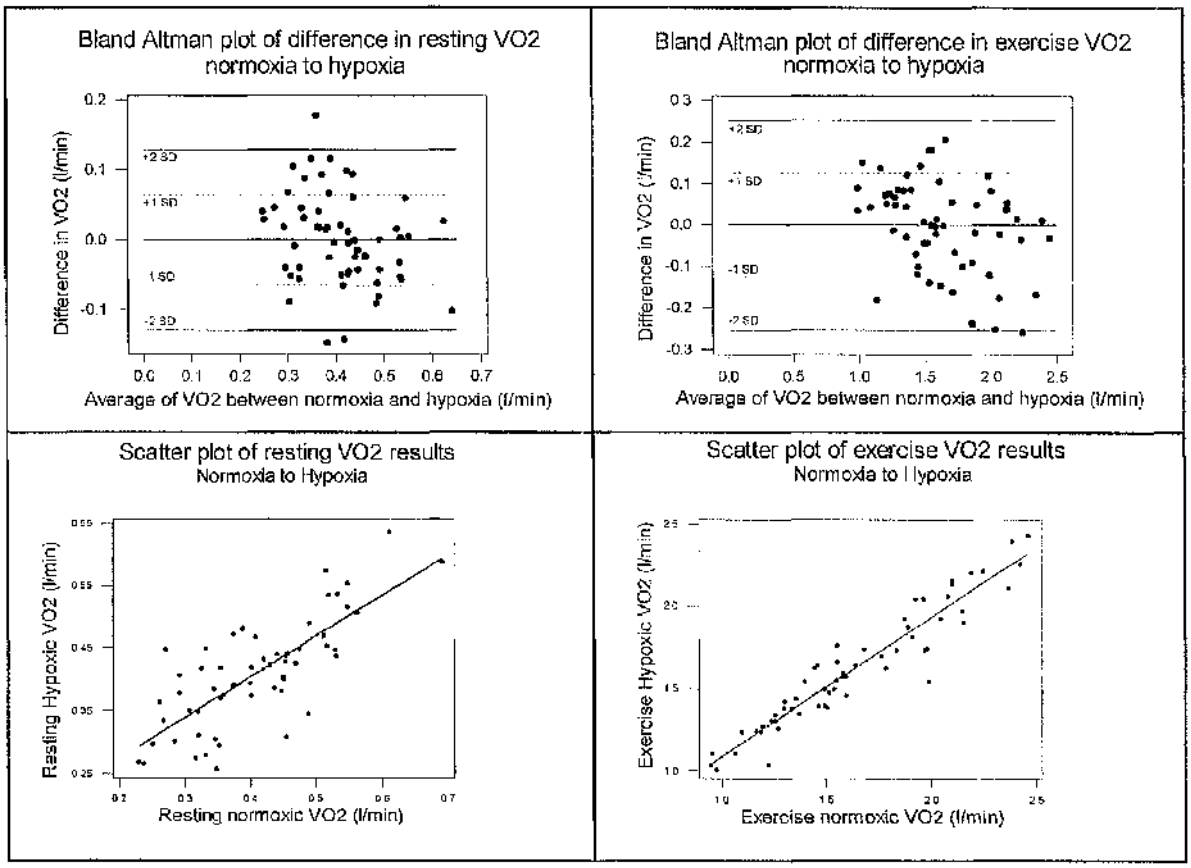

Figure 3.7 Bland-Altman plots and scatter plots of oxygen uptake (VO_2) measurements for the entire subject group. The difference in measurements between normoxic and hypoxic tests is plotted against the mean VO_2 measurement for the two tests. The horizontal bars are placed at one and two standard deviations of the differences in VO_2 (±1 SD and ±2 SD respectively). The upper left panel compares measured VO_2 between normoxia and hypoxia during rest (SD 0.065), the upper right panel during steady state exercise (SD 0.13). The lower panels are scatter plots of the data; the left panel is of resting results, the right is from the exercise data. The resting values demonstrate a wider degree of variability in relation to measured VO_2 in comparison to exercise results. Three subjects were outwith 2 standard deviations of observed difference in VO_2 between normoxia and hypoxia during rest; however 95% of subjects did demonstrate consistency in VO_2. All the exercise results were within 2 standard deviations.

3.3.2 Carbon dioxide production

Carbon dioxide production (VCO_2) data for the whole subject group is displayed in table 3.10 and figure 3.8. Complete data points for the entire subject group were measured (n=60). The mean VCO_2 during normoxic rest and exercise was; 0.340 l/min (SD 0.09; range 0.18-0.54 l/min) and 1.41 l/min (SD 0.32; range 0.90-2.18 l/min) respectively. The mean VCO_2 during hypoxic rest and exercise was 0.408 l/min (SD 0.11; range 0.23-0.85 l/min) and 1.58 l/min (SD 0.36; range 0.95-2.39 l/min) respectively. The absolute change in VCO_2 at *rest* between conditions of normoxia and hypoxia was 0.069 l/min (SD 0.06; range -0.03-0.32 l/min); the mean percent change was 21.95% (SD 19.0; range -10.6-66.0%). The absolute change in VCO_2 during *exercise* between conditions of normoxia and hypoxia was 0.176 l/min (SD 0.12; range -0.16-0.65); the mean percent change was 12.3% (SD 0.02; range -10.2-37.3%). Paired student t-tests demonstrated a significant difference between normoxia and hypoxia at rest and during exercise (both $p<0.0001$).

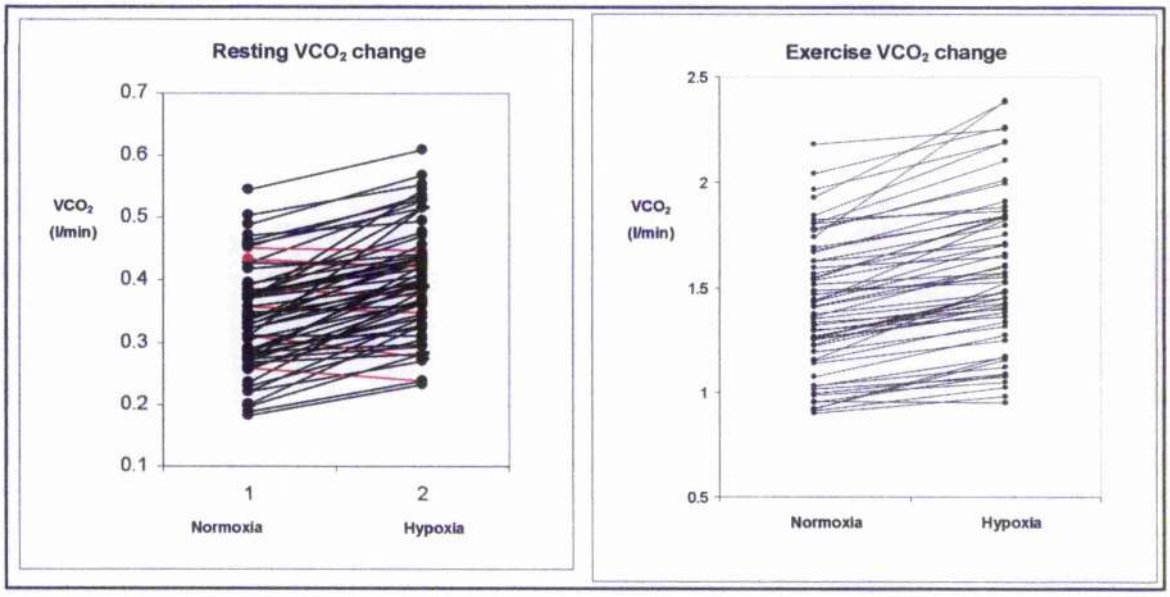

Figure 3.8 The change in VCO_2 from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during hypoxia. These plots demonstrate the increase in VCO_2 from normoxia to hypoxia; due to increased CO_2 elimination during hypoxia. This is attributable to the increase in ventilation during hypoxia due to hypoxic ventilatory drive. Furthermore, there is a wide variation in individual responses. The red data sets demonstrate a decrease in VCO_2 in 6 individuals from normoxia to hypoxia during rest; this maybe explicable by a degree of hyperventilation during the normoxic resting phase.

Table 3.10. Individual VCO_2 data and changes between normoxic and hypoxic conditions.

Subject	*Normoxic Rest (l/min)*	*Normoxic Exercise (l/min)*	*Hypoxic Rest (l/min)*	*Hypoxic Exercise (l/min)*	*ΔVCO_2 (Rest)*		*ΔVCO_2 (Exercise)*	
					l/min	*%*	*l/min*	*%*
1	0.393 ±0.09	1.81 ±0.24	0.476 ±0.18	1.89 ±0.36	0.08	21.1	0.08	4.4
2	0.372 ±0.19	1.69 ±0.24	0.429 ±0.17	1.84 ±0.21	0.06	15.5	0.14	8.5
3	0.490 ±0.20	2.18 ±0.25	0.569 ±0.10	2.26 ±0.47	0.08	16.2	0.07	3.3
4	0.429 +0.19	2.04 ±0.22	0.528 ±0.21	2.26 ±0.43	0.10	23.2	0.22	10.8
5	0.452 +0.27	1.63 ±0.23	0.449 ±0.14	1.71 ±0.30	-0.00	-0.8	0.08	5.0
6	0.321 +0.11	1.27 ±0.17	0.396 ±0.15	1.45 ±0.13	0.08	23.4	0.19	14.7
7	0.464 ±0.14	1.82 ±0.25	0.529 ±0.16	1.86 ±0.23	0.07	14.2	0.04	2.2
8	0.339 ±0.13	1.42 ±0.30	0.431 ±0.30	1.60 ±0.36	0.09	27.4	0.19	13.1
9	0.336 ±0.09	1.57 ±0.29	0.456 ±0.21	1.75 ±0.18	0.12	35.6	0.19	11.8
10	0.386 ±0.15	1.78 ±0.27	0.374 ±0.14	1.99 ±0.36	-0.01	-3.0	0.21	12.0
11	0.473 ±0.28	1.84 ±0.27	0.494 ±0.16	2.19 ±0.22	0.02	4.4	0.35	19.1
12	0.358 ±0.14	1.30 ±0.22	0.350 ±0.06	1.36 ±0.23	-0.01	-2.4	0.06	4.8
13	0.357 ±0.14	1.35 ±0.36	0.395 ±0.15	1.45 ±0.14	0.04	10.5	0.10	7.2
14	0.504 ±0.25	1.63 ±0.15	0.552 ±0.18	1.80 ±0.18	0.05	9.6	0.17	10.4
15	0.369 ±0.11	1.55 +0.23	0.546 +0.13	1.84 ±0.28	0.18	47.9	0.29	18.5
16	0.544 ±0.21	1.93 ±0.39	0.608 ±0.17	2.38 ±0.32	0.06	11.7	0.45	23.4
17	0.428 ±0.25	1.80 ±0 16	0.426 ±0.14	2.11 +0.16	-0.00	-0.6	0.30	16.7
18	0.455 ±0.15	1.68 ±0 29	0.535 ±0.14	1.85 +0.31	0.08	17.6	0.17	10.0
19	0.369 ±0.14	1.55 ±0.33	0.514 ±0.18	1.84 ±0.33	0.15	39.5	0.30	19.3
20	0.372 ±0.15	1.56 ±0.33	0.408 ±0.21	1.40 ±0.16	0.04	9.5	-0.16	-10.2
21	0.527 ±0.15	1.97 ±0.21	0.848 ±0.16	2.19 ±0.36	0.32	61.2	0.22	11.3
22	0.377 ±0.20	1.77 ±0.18	0.471 ±0.28	2.01 ±0.15	0.09	24.8	0.24	13.7
23	0.285 ±0.285	1.03 ±0.13	0.295 ±0.10	1.17 ±0.10	0.01	3.6	0.14	13.8
24	0.275 ±0.16	1.36 ±0.12	0.347 ±0.13	1.61 +0.23	0.07	26.3	0.25	18.1
25	0.198 ±0.09	1.30 ±0.18	0.328 ±0.09	1.41 +0.14	0.13	65.4	0.11	8.5
26	0.309 +0.19	0.90 ±0 18	0.277 ±0.15	0.98 ±0.24	-0.03	-10.6	0.08	8.7
27	0.183 ±0.10	1.04 ±0.17	0.233 ±0.07	1.12 ±0.15	0.05	27.1	0.09	8.6
28	0.311 ±0.17	0.99 ±0.17	0.312 ±0.09	1.05 ±0.18	0.00	0.4	0.06	6.2
29	0.434 ±0.28	1.67 ±0.17	0.421 ±0.11	1.91 ±0.27	-0.01	-2.9	0.24	14.5
30	0.221 ±0.13	0.91 ±0.13	0.268 ±0.19	1.03 ±0.22	0.05	21.5	0.11	12.3
31	0.458 ±0.10	1.74 ±0.43	0.517 ±0.11	2.39 ±0.36	0.06	12.8	0.65	37.3
32	0.348 ±0.22	1.23 ±0.27	0.361 ±0.25	1.49 ±0.25	0.01	3.8	0.26	20.9
33	0.281 ±0.26	1.19 ±0.24	0.412 ±0.40	1.32 ±0.28	0.13	46.6	0.13	10.5
34	0.235 ±0.08	1.16 ±0.15	0.385 ±0.05	1.53 ±0.11	0.15	63.8	0.37	32.0
35	0.396 ±0.16	1.47 ±0.18	0.439 ±0.17	1.55 ±0.24	0.04	10.9	0.09	5.8
36	0.344 ±0.09	1.43 +0.28	0.391 ±0.07	1.82 ±0.26	0.05	13.5	0.39	27.5
37	0.273 +0.12	1.15 ±0.13	0.294 ±0.08	1.34 ±0.24	0.02	7.8	0.18	16.0
38	0.259 ±0.07	0.96 ±0.13	0.239 ±0.05	0.95 ±0.11	-0.02	-7.8	-0.00	-0.5
39	0.379 ±0.08	1.44 ±0.25	0.436 ±0.14	1.66 ±0.31	0.06	14.9	0.23	15.7
40	0.375 ±0.19	1.26 ±0.14	0.435 ±0.23	1.48 ±0.19	0.06	16.0	0.22	17.5
41	0.291 ±0.11	1.26 ±0.23	0.322 ±0.11	1.39 ±0.39	0.03	10.8	0.13	10.4
42	0.269 ±0.09	1.52 ±0.21	0.365 ±0.14	1.57 ±0.19	0.10	35.5	0.06	3.7
43	0.383 +0.08	1.26 ±0.17	0.419 ±0.14	1.39 ±0.13	0.04	9.6	0 13	10.3
44	0.229 ±0.13	0.92 ±0.13	0.305 ±0.09	1.16 ±.014	0.08	33 5	0.23	25.4
45	0.219 ±0.11	1.02 ±0.35	0.339 ±0.23	1.08 ±0.20	0.12	54 9	0.06	5.9
46	0.277 ±0.07	0.92 ±0.33	0 339 ±0 13	1.17 ±0.41	0.06	22.3	0.25	27.1
47	0.261 ±0.21	1.08 ±0.13	0.328 ±0.13	1.28 +0.13	0.07	25.5	0.20	18.5
48	0.267 ±0.08	1.38 ±0.22	0.282 ±0.14	1.48 ±0.23	0.01	5.4	0.10	7.2
49	0.418 ±0.26	1.49 ±0.16	0.441 ±0.29	1.53 ±0.34	0.02	5.6	0.04	2.9
50	0.255 ±0.12	1.26 ±0.17	0.362 ±0.26	1.48 ±0.22	0.11	42.3	0.22	17.8
51	0.304 ±0.15	1.14 ±0.19	0.365 ±0.19	1.25 ±0.13	0.06	20.1	0.11	9.7
52	0.327 ±0.08	1.54 ±0.20	0.441 ±0.23	1.71 ±0.21	0.11	34.7	0.17	10.8
53	0.197 ±0.08	0.95 ±0.14	0.281 ±0.08	1.08 +0.15	0.08	42.6	0.13	13.7
54	0.289 ±0.06	1.34 ±0.11	0.370 +0.11	1.40 ±0.14	0.08	28.3	0.07	5.0
55	0.288 ±0.12	1.45 +0.34	0.396 ±0.19	1.71 ±0.24	0.11	37.6	0.26	18.2
56	0.273 ±0.12	1.32 ±0.29	0.426 ±0.37	1.43 ±0.26	0.15	56.2	0.11	8.1
57	0.310 ±0.24	1.22 ±0.27	0.514 ±0.37	1.42 ±0.30	0.20	66.0	0.20	16.2
58	0.187 ±0.08	1.00 ±0.21	0.237 ±0.11	1.09 ±0.24	0.05	26.7	0.09	9.3
59	0.323 ±0.33	1.60 ±0.27	0.383 ±0.27	1.65 ±0.29	0.06	18.8	0.05	3.2
60	0.330 ±0.09	1.41 ±0.14	0.400 ±0.11	1.57 ±0.21	0.07	20.9	0.16	11.3
Group Mean	*0.340 ±0.09*	*1.41 ±0.32*	*0.408 ±0.11*	*1.58 ±0.36*	*0.07 ±0.06*	*22.0 ±19.0*	*0.176 ±0.12*	*12.3 ±0.02*

3.3.3 End-tidal CO_2

End-tidal CO_2 ($PetCO_2$) data for the whole subject group is displayed in table 3.11 and figure 3.9. Complete data points for the entire subject group were recorded (n=60). Data is presented in SI units (kPa, to convert to mmHg apply a conversion factor of 7.501). The mean $PetCO_2$ during normoxic rest and exercise was 4.96 kPa (SD 0.38) and 5.82 kPa (SD 0.45) respectively. The mean $PetCO_2$ during hypoxic rest and exercise was 4.60 kPa (SD 0.41) and 4.90 kPa (SD 0.40) respectively. The mean absolute change in $PetCO_2$ at *rest* between conditions of normoxia and hypoxia was -0.37 kPa (SD 0.31); the mean percent change was -7.3% (SD 6.2). The mean absolute change in $PetCO_2$ during *exercise* between conditions of normoxia and hypoxia was -1.04 kPa (SD 1.0); the mean percent change was -16.5% (SD 7.7). End-tidal CO_2 measurements demonstrated a normal distribution; paired t-tests showed a significant change in $PetCO_2$ during rest and exercise between normoxia and hypoxia ($P < 0.0001$ for both rest and exercise).

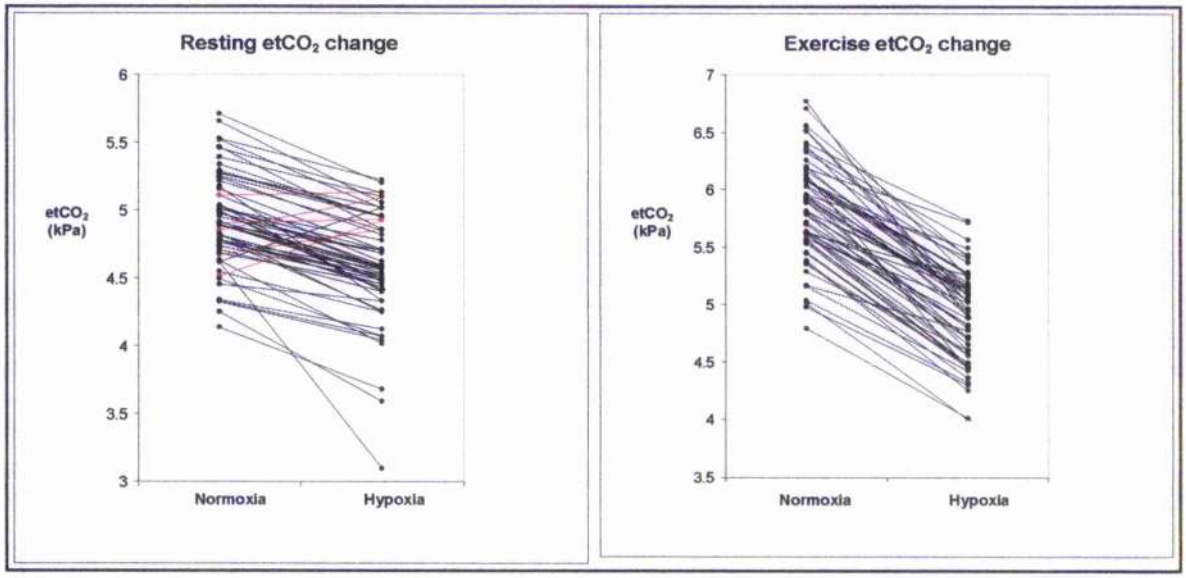

Figure 3.9 The change in $PetCO_2$ from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during exercise. These plots demonstrate the decrease in $PetCO_2$ from normoxia to hypoxia. This reflects the increase in alveolar ventilation during hypoxia due to hypoxic ventilatory drive. There is a wide variation in individual responses. The red data sets demonstrate an increase in $PetCO_2$ in 5 individuals from normoxia to hypoxia during rest; these were 5 of the 6 subjects that demonstrated a greater increase in VCO_2, during rest. Similarly this could be attributable to a degree of hyperventilation during the normoxic resting phase.

Table 3.11. Individual $etCO_2$ data and changes between normoxic and hypoxic conditions.

Subject	*Normoxic Rest (kPa)*	*Normoxic Exercise (kPa)*	*Hypoxic Rest (kPa)*	*Hypoxic Exercise (kPa)*	*$\Delta ETCO_2$ (Rest)*		*$\Delta ETCO_2$ (Exercise)*	
					kPa	*%*	*kPa*	*%*
1	4.76 ±0.2	5.70 ±0.1	4.53 +0.3	4.72 ±0.3	-0.23	-4.9	-0.97	-17.1
2	4.67 ±0.4	6.25 ±0.2	4.85 ±0.2	5.36 ±0.1	0.19	4.0	-0.89	-14.2
3	4.81 ±0.2	5.71 +0.2	4.43 ±0.1	4.82 ±0.1	-0.38	-7.9	-0.89	-15.6
4	5.01 ±0.2	6.08 ±0.2	4 78 ±0.2	5.14 +0.2	-0.23	-4.6	-0.93	-15.4
5	5.34 ±0.3	6.07 ±0.2	4 95 ±0.1	5.28 ±0.2	-0.39	-7.3	-0.79	-13
6	4.77 ±0.2	5.93 ±0.1	4.50 ±0.1	5.04 ±0.1	-0.26	-5.5	-0.89	-15.1
7	4.78 ±0.3	5.91 ±0.2	4.56 ±0.2	5.09 ±0.2	-0.21	-4.5	-0.82	-13.8
8	5.15 ±0.2	5.88 ±0.3	4.71 ±0.3	5.09 ±0.3	-0.45	-8.7	-0.79	-13.5
9	5.22 ±0.2	6.20 ±0.2	4.85 ±0.3	5.03 +0.1	-0.36	-6.9	-1.17	-18 9
10	5.03 ±0.1	6.17 ±0.2	4 57 ±0.4	5.16 +0.2	-0.46	-9.1	-1	-16.3
11	4.85 ±0.5	6.55 ±0.2	5.09 +0.2	5.41 ±0.1	0.24	5.0	-1.14	-17.4
12	4.95 +0.3	5.57 ±0.2	4.68 ±0.8	5.02 ±0.2	-0.27	-5.5	-0.55	-9.8
13	4.90 +0.2	5.54 ±0.4	4.50 ±0.2	4.56 ±0.1	-0.39	-8.0	-0.99	-17.8
14	4.68 ±0.4	5.62 ±0.2	4.46 ±0.3	4.82 ±0.1	-0.23	-4.8	-0.8	-14.2
15	4.63 ±0.1	5.62 ±0.2	4.01 ±0.3	4.56 ±0.1	-0.61	-13.3	-1.06	-18.8
16	5.52 ±0.2	6.77 ±0.2	5.23 ±0.2	4.88 ±0.2	-0.30	-5.4	-1.89	-27.9
17	5.03 ±0.2	5.90 ±0.1	4.59 ±0.1	4.70 ±0.1	-0.43	-8.6	-1.20	-20.4
18	4.78 ±0.2	5.62 ±0.3	4.26 ±0.3	4.45 ±0.3	-0.52	-10.9	-1.17	-20.8
19	5.01 ±0.2	5.78 ±0.3	4.71 ±0.2	4.65 ±0.1	-0.30	-6.0	-1.13	-19.6
20	5.00 ±0.2	5.80 ±0.3	4.41 +0.2	5.15 ±0.2	-0.59	-11.7	-0.65	-11.2
21	4.61 ±0.2	6.13 +0.2	3.10 ±0.2	4.43 ±0.1	-1.52	32.9	-1.70	-27.7
22	4.89 ±0.1	5.38 ±0.1	4.53 ±0.3	4.24 ±0.1	-0.36	-7.3	-1.13	-21.1
23	4.89 ±0.3	5.81 ±0.4	4.57 ±0.1	5.12 ±0.1	-0.32	-6.6	-0.69	-11.9
24	5.11 ±0.2	5.94 ±0.2	4.62 ±0.2	5.12 ±0.1	-0.49	-9.5	-0.83	-13.9
25	4.89 ±0.2	5.63 ±0.2	4.58 ±0.1	4.87 ±0.1	-0.31	-6 3	-0.75	-13.4
26	4.62 ±0.5	5.62 +0.2	5.02 ±0.3	5.25 ±0.2	0.40	8.6	-0.36	-6.5
27	4.33 +0.2	4.98 ±0.1	4.07 ±0.1	4.29 ±0.1	-0.26	-6.0	-0.68	-13.7
28	4.33 ±0.5	5.01 ±0.1	4.04 ±0.1	3.99 +0.1	-0.29	-6.6	-1.02	-20.3
29	5.11 ±0.5	6.70 ±0.2	5.14 ±0.2	5.23 +0.2	0.03	0.6	-1.48	-22.0
30	4.78 ±0.3	5.17 ±0.2	4.45 ±0.3	4.77 ±0.2	-0.33	-6.8	-0.40	-7.6
31	4.13 ±0.1	5.56 ±0.3	3.67 ±0.1	4.46 ±0.2	-0.46	-11.1	-1.10	-19.8
32	4.71 ±0.3	5.35 ±0.2	4 40 ±0.3	4.49 ±0.2	-0.31	-6.6	-0.86	-16.1
33	4.91 ±0.4	5.63 ±0.2	4.58 ±0.4	5.14 ±0.2	-0.33	-6.8	-0.49	-8 7
34	4.91 ±0.3	5.95 ±0 1	4.93 ±0.1	5.49 +0.1	0.02	0.5	-0.46	-7.7
35	5.25 ±0.2	6.03 ±0 2	4 95 ±0.2	5.28 +0.2	-0.30	-5.7	-0.76	-12.6
36	5.46 +0.2	6.18 ±0.2	5.13 ±0.1	5.71 ±0.2	-0.34	-6.1	-0.47	-7.6
37	5.03 ±0.2	5.28 ±0.1	4.40 ±0.1	4.60 ±0.2	-0.63	-12.6	-0.68	-12.9
38	4.34 ±0.2	5.03 ±0.3	4.12 ±0.1	4.32 ±0.2	-0.22	-5.0	-0.71	-14 2
39	5.52 ±0.1	6.51 ±0.2	5.01 ±0.2	5.07 ±0.2	-0.51	-9.2	-1.44	-22.1
40	5.27 ±1.2	6.50 ±0.1	4.96 ±0.3	5.02 ±0.2	-0.32	-6.0	-1.48	-22.8
41	5.39 ±0.2	6.40 ±0.3	5.10 ±0.2	5.72 ±0.2	-0.29	-5.4	-0.67	-10.5
42	4.83 ±0.2	5.95 ±0.2	4.25 ±0.7	4.59 ±0.2	-0.58	-12.0	-1.36	-22.8
43	4.97 +0.1	5.78 ±0.2	4.68 ±0.1	4.94 ±0.1	-0.29	-5.9	-0.84	-14.5
44	4.25 ±0.2	4.79 ±0.1	3.59 ±0.1	4.01 +0.2	-0.66	-15.5	-0.78	-16 3
45	4.45 ±0.4	5.16 ±0.3	4.33 +0.3	4.42 ±0.1	-0.12	-2.6	-0 74	-14.3
46	5.66 ±0.1	6.09 ±0.3	5.05 ±0.2	5.18 ±0.3	-0.60	-10.7	-0.90	-14.9
47	5.24 ±0.4	6.38 ±0.2	4.82 ±0.1	4.96 ±0.1	-0.43	-8.1	-1.42	-22.2
48	5.47 ±0.1	6.03 ±0.3	4 86 ±0.3	4.95 ±0.2	-0.61	-11.1	-1.08	-17.9
49	5.71 ±0.4	6.08 ±0.2	5 20 ±0.9	5.26 ±0.3	-0.51	-8.9	-0.82	-13.4
50	4.82 ±0.2	5.44 ±0.2	4.49 ±0.2	4.44 +0.2	-0.33	-6.8	-1.00	-18.4
51	5.27 ±0.1	6.35 ±0.2	5.02 +0.3	5.56 ±0.7	-0.25	-4.7	-0.79	-12.5
52	4.74 ±0.1	5.45 ±0.2	4.48 ±0.1	4.46 ±0 1	-0.25	-5.3	-0.99	-18.1
53	4.98 ±0.1	5.61 ±0.2	4.47 ±0.1	4 70 ±0 1	-0.51	-10.3	-0.91	-16.1
54	4.88 ±0.2	6.11 ±0.3	4.71 ±0.3	4.9 0±0.2	-0.18	-3.7	-1.21	-19.8
55	4.55 ±0.1	5.53 ±0.3	4.25 ±0.4	4.48 ±0.2	-0.30	-6.6	-1.05	-19.0
56	4.51 ±0.5	6.33 ±0.2	4.92 ±0.9	5.43 ±0 2	0.41	9.1	-0.90	-14.2
57	5.18 ±0.2	5.81 ±0.4	4.33 ±0.2	4.79 ±0 3	-0.84	-16.3	-1.01	-17.5
58	4.49 ±0.4	5.36 ±0.2	4.07 ±0.3	4.35 ±0.1	-0.42	-9.4	-1.00	-18.7
59	5.29 ±0.2	6.08 ±0.3	4.96 ±0.1	5.40 ±0.2	-0.34	-6.3	-0.67	-11.1
60	4.76 ±0.2	5.70 ±0.1	4.53 ±0.3	4 72 ±0.3	-0.23	-4.9	-0.97	-17.1
Group Mean	***4.96 ±0.38***	***5.82 ±0.45***	***4.60 ±0.41***	***4.90 ±0.40***	***-0.37 ±0.31***	***-7.3 ±6.2***	***-1.04 ±1.0***	***-16.5 ±7.7***

3.3.4 Oxygen saturation.

Oxygen saturation (SaO_2) data for the whole subject group is displayed in table 3.12 and figure 3.10. Complete data points for 58 subjects are recorded (n=58). The missing data points were due to a fault in the oximetry-metabolic cart interface. The mean SaO_2 during normoxic rest and exercise was; 95.9% (SD 1.4) and 94.8% (SD 1.6) respectively. The mean SaO_2 during hypoxic rest and exercise was 84.4% (SD 2.1) and 69.8% (SD 2.5) respectively. The mean absolute change in SaO_2 at *rest* between conditions of normoxia and hypoxia was -11.1% (SD 3.5); the mean percent change was -11.6% (SD 3.7). The mean absolute change in SaO_2 during *exercise* between conditions of normoxia and hypoxia was -25.8 (SD 11.4); the mean percent change was -26.0% (SD 7.1). Exercise measurements demonstrated normal distributions; however normoxic and hypoxic resting SaO_2 did not. A Wilcoxon signed rank sum test was performed on the differences between normoxia and hypoxia during rest; this demonstrated a significant difference ($p<0.0001$). Exercise measurements were compared using student t-test and showed a significant difference ($p<0.0001$). Lower than expected normoxic exercise resultrs were noted; the cause of this was individuals gripping the handle bars of the ergometer too tightly, despite instructions not to do so.

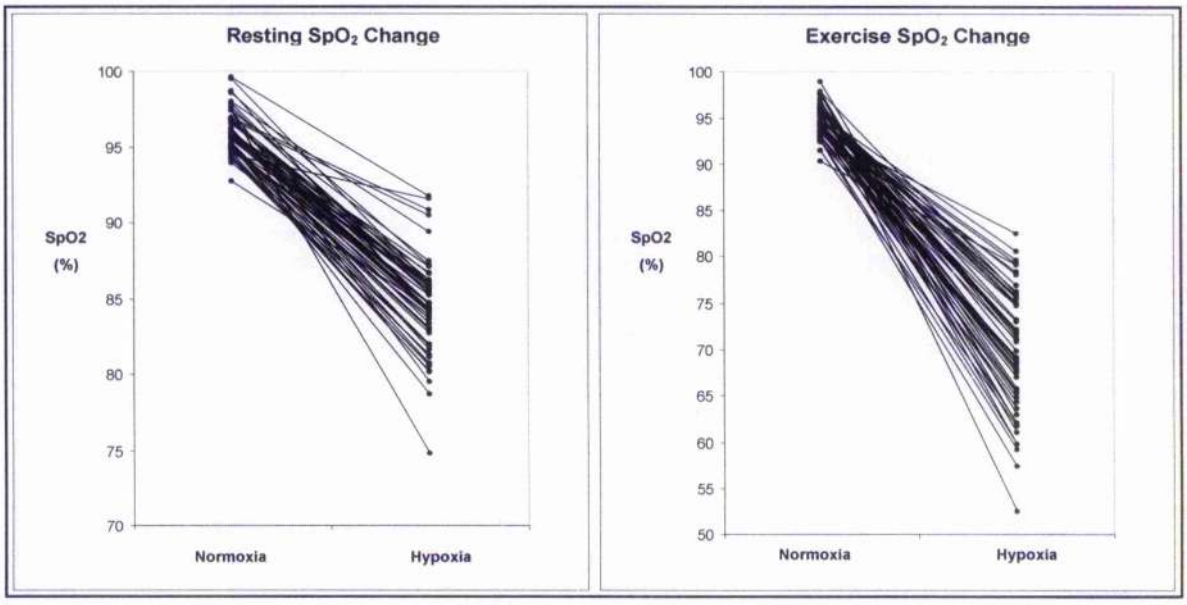

Figure 3.10 The change in SaO_2 from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during exercise. These plots demonstrate the decrease in SaO_2 from normoxia to hypoxia, reflecting the hypoxaemia during hypoxic exposure which intensified during exercise.

Table 3.12. Individual oxygen saturation data and changes between normoxia and hypoxia.

Subject	*Normoxic Rest % saturation*	*Normoxic Exercise % saturation*	*Hypoxic Rest % saturation*	*Hypoxic Exercise % saturation*	*ΔSaO_2 (Rest)*		*ΔSaO_2 (Exercise)*	
					Sats.	*%*	*Sats.*	*%*
1	95.6 ±1.1	95.7 ±1.1	85.8 ±1.0	76.9 ±1	-9.8	-10.3	-18.8	-19.6
2	95.3 ±1.3	93.5 ±1.0	82.0 ±2.5	68.5 ±1.5	-13.3	-14.0	-25.0	-26.8
3	94.9±1.1	94.7 ±1.0	86.2 ±1.1	74.9 ±0.8	-8.7	-9.2	-19.8	-20.9
4	95.8 ±1.0	95.4 ±1.0	84.2 ±1.2	76.1 ±1.5	-11.6	-12.1	-19.3	-20.2
5	97.8 ±1.2	97.1 ±1.0	85.3 ±1.9	52.5 ±1.5	-12.5	-12.8	-44.6	-46.0
6	94.9 ±1.1	94.3 ±1.2	85.2 ±1.0	71.8 ±1.1	-9.6	-10.2	-22.4	-23.8
7	95.6 ±1.0	94.5 ±1.3	84.2 ±2.7	68.8 ±1.1	-11.4	-11.9	-25.7	-27.2
8	96.1 ±1.1	96.2 +1.0	85.9 ±1.7	71.9 ±1.0	-10.2	-10.6	-24.3	-25.3
9	95.7 ±1.1	95.4 ±1.1	86.0 ±1.6	75.7 ±1.2	-9.7	-10.1	-19.7	-20.7
10	95.8 ±0.9	95.2 ±1.3	84.7 ±1.8	67.7 ±1.2	-11.2	-11.7	-27.5	-28.9
11	94.7 ±0.9	94.0 ±1.0	80.2 +2.0	65.2 ±1.4	-14.5	-15.3	-28.8	-30.6
12	94.9 ±0.8	94.5 ±1.1	83.7 ±1.2	79.2 ±1.3	-11.2	-11.8	-15.3	-16.2
13	95.4 ±1.3	93.3 ±1.2	85.6 ±1.7	78.3 ±1.3	-9.8	-10.3	-14.9	-16.0
14	95.9 ±1.2	93.6 ±1.6	85.5 ±2.0	74.7 ±1.4	-10.4	-10.8	-18.9	-20.2
15	94.8 ±2.7	91.4 ±1.7	84. ±2.67	61.7 ±1.3	-10.2	-10.7	-29.7	-32.5
16	94.2 ±1.0	92.6 ±1.1	80.6 +1.9	59.3 ±1.1	-13.6	-14.5	-33.4	-36.0
17	96.0 ±1.2	95.9 ±1.3	86.0 ±2.0	74.7 ±1.5	-10.0	-10.5	-21.2	-22.1
18	95.1 +1.2	94.2 ±1.3	83.1 ±2.3	73.0 ±1.4	-12.1	-12.7	-21.2	-22.5
19	94.3 ±1.6	94.1 ±1.1	81.2 ±1.2	66.9 +1.9	-13.1	-13.9	-27.2	-28.9
20	94.5 ±0.8	94.1 ±1.1	80.5 ±1.9	59.8 ±1.2	-13.9	-14.8	-34.3	-36.4
21	94.5 ±0.9	92.9 ±1.1	91.5 ±1.5	68.9 ±1.6	-2.9	-3.1	-24.0	-25.8
22	97.4 ±1.0	97.5 ±0.8	79.5 ±1.9	59.8 ±1.1	-17.9	-18.4	-37.8	-38.7
23	96.7 ±1.2	95.4 ±1.1	84.1 ±1.4	65.4 ±1.5	-12.6	-13.1	-30.1	-31.5
24	96.0 ±1.3	95.1 ±1.3	86.0 ±1.3	75.8 ±0.9	-10.0	-10.4	-19.3	-20.3
25	96.0 ±1.2	96.1 ±1.1	84.3 ±0.7	73.2 +1.3	-11.6	-12.1	-22.9	-23.9
26	95.0 ±1.3	94.4 ±1.3	80.1 ±1.7	72.0 ±1.6	-14.9	-15.7	-22.4	-23.7
27	96.8 ±1.3	95.4 +1.0	90.5 ±1.4	-	-6.3	-6.5	-95.4	-
28	98.5 ±1.4	97.8 ±1.5	87.1 ±2.2	75.9 ±1.4	-11.4	-11.6	-21.9	-22.4
29	99.6 ±0.8	97.8 ±1.2	91.7 ±2.5	61.9 ±1.5	-7.8	-7.9	-35.9	-36.7
30	95.3 ±1.7	95.1 ±0.9	81.1 ±2.1	71.7 ±1.5	-14.1	-14.8	-23.4	-24.6
31	96.9 ±1.4	94.7 ±1.1	90.8 ±1.0	79.5 ±1.5	-6.1	-6.3	-15.2	-16.0
32	97.6 ±2.2	94.5 ±3.2	85.9 ±2.8	78.0 ±2.6	-11.7	-12.0	-16.5	-17.4
33	95.1 ±1.6	96.5 ±1.4	84.1 ±1.9	71.3 ±1.3	-11.0	-11.6	-25.2	-26.1
34	95.2 +1.3	92.6 ±1.7	82.8 ±1.8	57.4 ±1.3	-12.4	-13.0	-35.1	-38.0
35	94.2 +1.3	94.6 ±1.6	82.0 ±1.3	67.7 ±1.6	-12.2	-12.9	-26.9	-28.4
36	96.2 ±1.3	94.5 ±1.3	81.6 ±0.9	62.0 ±2.0	-14.6	-15.2	-32.5	-34.4
37	96.9 ±1.5	93.5 ±1.5	85.7 ±2.3	71.0 ±1.3	-11.2	-11.6	-22.4	-24.0
38	96.5 ±1.1	96.1 ±1.1	86.6 ±1.8	75.0 ±0.8	-9.9	-10.2	-21.1	-22.0
39	94.7 ±0.9	93.5 ±1.3	85 3 ±1.0	68.4 ±2.7	-9.4	-10.0	-25.2	-26.9
40	94.2 ±1.5	93.0 ±0.7	81.3 ±1.8	68.0 ±1.6	-12.9	-13.7	-25.1	-26.9
41	96.8 ±0.7	97.7 ±1.1	84 7 ±1.5	61.0 ±1.5	-12.1	-12.5	-36.6	-37.5
42	96.0 ±1.2	94.5 ±1.2	83.4 +1.4	67.4 ±1.6	-12.6	-13.2	-27.1	-28.6
43	95.2 ±0.8	94.3 ±1.0	83.0 ±1.3	63.6 ±1.4	-12.2	-12.8	-30.6	-32.5
44	95.9 ±1.2	95.3 ±1.1	87.1 ±2.3	80.5 ±1.0	-8.8	-9 2	-14.8	-15.5
45	97.9 ±1.7	93.4 ±2.4	89.4 ±2.2	82.4 ±1.6	-8.6	-8.8	-11.0	-11.8
46	98.7 ±1.0	93.0 ±2.3	84.1 ±2.0	64.2 ±1.1	-14.6	-14.8	-28.8	-30.9
47	95.1 ±1.5	94.5 ±0.6	78.7 ±1.4	64.7 ±2.1	-16.4	-17.3	-29.7	-31.5
48	97.4 ±1.2	96.8 ±1.0	74.8 ±2.4	65.7 ±2.9	-22.5	-23.2	-31.1	-32.1
49	94.9 ±1.4	96.0 ±1.1	81.9 ±1.5	69.8 ±1.8	-13.0	-13.7	-26.2	-27.3
50	96.8 ±1.0	95.9 ±1.0	-	-	-	-	-	-
51	97.4 ±1.8	95.1 ±2.5	87.4 ±3.0	72.2 ±2.5	-9.9	-10.2	-22.9	-24.1
52	95.2 ±1.3	95.7 ±1.3	80.7 ±1.6	68.4 ±1.1	-14.5	-15.2	-27.3	-28.6
53	96.6 ±1.3	96.1 ±2.0	85.9 ±1.8	70.8 ±1.4	-10.7	-11.1	-25.3	-26.3
54	96.1 ±1.5	94.6 ±1.0	84.7 ±1.4	62.0 ±1.1	-11.3	-11.8	-32.6	-34.4
55	96.6 ±1.4	90.3 1.8	85.4 ±1.6	79.1 ±3.3	-11.2	-11.6	-11.1	-12.3
56	96.8 ±1.1	95.6 ±1.1	86.2 ±3.9	75.4 ±1.2	-10.6	-10.9	-20.1	-21.1
57	93.9 ±1.8	94.7 ±1.3	83.9 ±3.4	76.2 ±1.1	-10.0	-10.7	-18.5	-19.5
58	95.6 ±2.8	93.7±1.0	86.7 ±3.3	71.8 ±3.6	-9.0	-9.4	-21.9	-23.3
59	99.5 ±0.7	98.9 ±1.3	86.0 ±2.7	63.0 ±2.0	-0.6	-0.6	-21.9	-25.8
60	92.7 ±1.2	92.4 ±1.1	84.4 ±1.8	69.1 ±2.1	-0.0	-0.0	-15.3	-18.1
Group Mean	*95.9 ±1.4*	*94.8 ±1.6*	*84.4 ±2.1*	*69.8 +2.5*	*-11.1 ±3.6*	*-11.6 ±3.7*	*-25.8 ±11.4*	*-26.0 ±7.0*

3.3.5 Tidal volume

Tidal volume data (V_T) data for the whole subject group is displayed in table 3.13 and figure 3.11. Complete data points for the entire subject group were measured (n=60). The mean V_T during normoxic rest and exercise was 0.87 litres (SD 0.40) and 1.78 litres (SD 0.47) respectively. The mean V_T during hypoxic rest and exercise was 0.99 litres (SD 0.40) and 2.03 litres (SD 0.57) respectively. The mean absolute change in V_T at *rest* between conditions of normoxia and hypoxia was 0.12 litres (SD 0.38); the mean percent change was 18.9% (SD 26.5). The mean absolute change in V_T during *exercise* between conditions of normoxia and hypoxia was 0.25 litres (SD 0.24); the mean percent change was 14.3 litres (SD 12.3). Resting measurements of V_T did not demonstrate a normal distribution; Wilcoxon sign sum rank analysis showed a significant change between normoxia and hypoxia ($p<0.0001$). Exercise measurements were in a normal distribution and significant change between normoxia and hypoxia was demonstrated using a paired student t-test ($p<0.0001$).

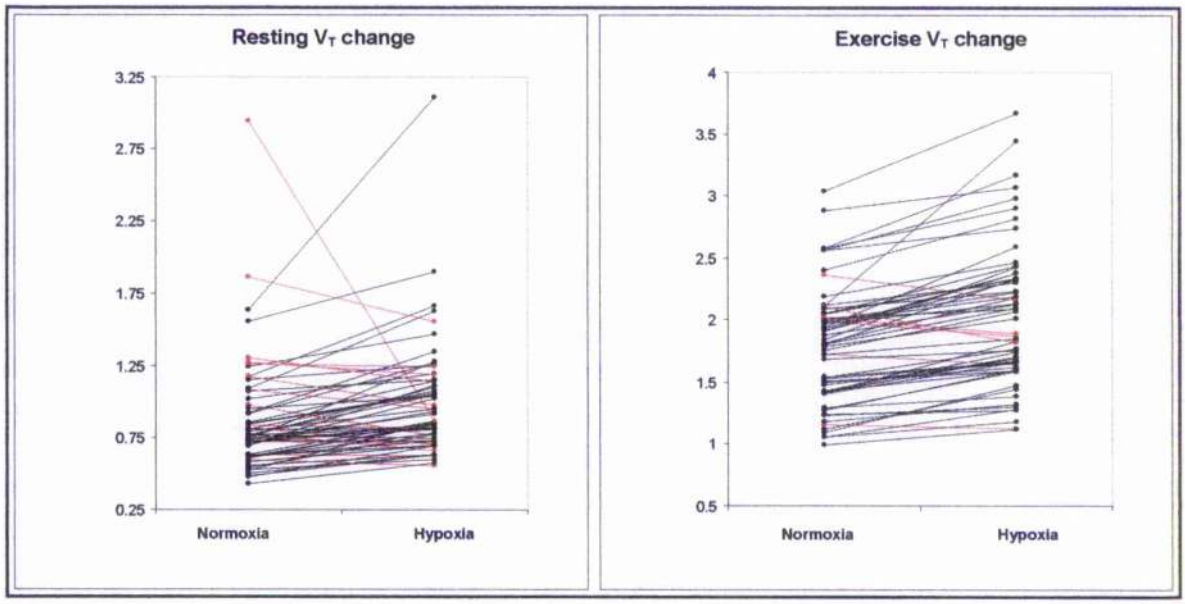

Figure 3.11 The change in tidal volume from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during exercise. These plots demonstrate the wide range of responses of tidal volume during rest and exercise during hypoxic exposure. Some individuals demonstrated a rise in V_T, whilst others had a decrease in V_T (in red) during hypoxic exposure. The majority of subjects demonstrated a rise in V_T during exercise, but of a variable degree. Two subjects exhibited clear evidence of hyperventilation at rest, one during normoxia, the second during hypoxia.

Table 3.13. Individual tidal volume data and changes between normoxia and hypoxia

Subject	*Normoxic Rest (litres)*	*Normoxic Exercise (litres)*	*Hypoxic Rest (litres)*	*Hypoxic Exercise (litres)*	*ΔV_T (Rest)*		*ΔV_T (Exercise)*	
					litres	*%*	*litres*	*%*
1	1.17 ±0.48	2.56 ±0.54	1.66 ±0.67	2.98 ±0.47	0.49	41.8	0.42	16.3
2	1.24 ±0.93	2.56 ±0.60	1.47 ±0.38	2.74 ±0.42	0.22	18.1	0.18	6.9
3	1.07 ±0.82	3.04 ±0.74	1.15 ±0.21	3.66 ±0.58	0.08	7.0	0.63	20.7
4	1.02 ±0.49	2.88 ±0.35	1.20 ±0.75	3.06 ±0.41	0.18	17.6	0.18	6.3
5	1.08 ±0.78	1.81 ±0.43	0.95 ±0.3	2.18 ±0.41	-0.13	-12.1	0.37	20.4
6	0.60 ±0.20	1.43 ±0.25	0.84 ±0.32	1.74 ±0.18	0.24	40.1	0.31	21.8
7	1.55 ±0.69	2.05 ±0.49	1.90 ±0.86	2.33 ±0.63	0.35	22.8	0.28	13.5
8	0.77 ±0.34	1.51 ±0.42	1.05 ±0.64	1.59 ±0.43	0.28	36.6	0.09	5.9
9	0.70 ±0.18	1.84 ±0.21	0.85 ±0.66	2.42 ±0.49	0.15	20.7	0.59	31.8
10	0.75 ±0.31	1.91 ±0.35	1.10 ±0.64	2.38 ±0.40	0.35	46.8	0.47	24.6
11	0.92 ±0.29	2.40 ±0.49	1.35 ±0.65	2.82 ±0.28	0.43	46.5	0.42	17.6
12	0.97 ±0.68	1.99 ±0.55	0.76 ±0.14	1.86 ±0.42	-0.21	-21.8	-0.12	-6.2
13	0.77 ±0.43	1.68 ±0.46	0.82 ±0.45	1.76 ±0.17	0.05	6.5	0.07	4.4
14	1.27 ±0.93	2.04 ±0.18	1.19 ±0.59	2.44 ±0.30	-0.08	-6.6	0.40	19.4
15	0.80 ±0.26	2.04 ±0.47	1.08 ±0.53	2.34 ±0.55	0.27	34.0	0.29	14.3
16	0.85 ±0.21	2.37 ±0.43	1.06 ±0.51	2.16 ±0.29	0.21	25.0	-0.21	-8.9
17	0.95 ±0.52	2.19 ±0.28	1.04 ±0.53	2.46 ±0.22	0.09	9.5	0.27	12.4
18	1.09 ±0.45	2.12 ±0.51	1.63 ±0.59	2.30 ±0.32	0.54	49.1	0.18	8.7
19	1.15 ±0.69	1.91 ±0.31	1.25 ±0.45	2.32 ±0.33	0.11	9.2	0.42	21.8
20	1.17 ±0.72	1.94 ±0.34	0.98 ±0.44	2.59 ±0.33	-0.20	-16.8	0.65	33.7
21	1.63 ±0.41	2.57 ±0.42	3.11 ±0.63	3.16 ±0.31	1.48	90.7	0.59	22.8
22	0.73 ±0.26	1.93 ±0.17	1.28 ±0.74	2.37 ±0.20	0.55	74.2	0.45	23.2
23	0.81 ±0.44	1.43 ±0.33	0.70 ±0.22	1.59 ±0.19	-0.11	-13.6	0.16	11.3
24	0.91 ±0.39	1.88 ±0.33	1.27 ±0.49	2.12 ±0.35	0.36	39.8	0.25	13.2
25	0.52 ±0.15	1.73 ±0.28	0.86 ±0.15	1.64 ±0.13	0.34	65.2	-0.09	-4.9
26	0.77 ±0.48	1.15 ±0.39	0.66 ±0.34	1.12 ±0.35	-0.11	-14.7	-0.03	-2.8
27	0.50 ±0.32	1.23 ±0.24	0.63 ±0.25	1.30 ±0.24	0.13	25.9	0.07	5.7
28	0.63 ±0.39	0.99 ±0.24	0.62 ±0.23	1.12 ±0.23	-0.01	-1.2	0.13	12.9
29	1.30 ±0.92	1.76 ±0.24	1.14 ±0.36	2.07 ±0.31	-0.17	-12.7	0.31	17.8
30	0.52 ±0.22	1.05 ±0.15	0.60 ±0.24	1.18 ±0.20	0.08	14.5	0.12	11.8
31	1.86 ±0.34	2.11 ±0.50	1.55 ±0.27	3.44 ±0.36	-0.32	-16.9	1.33	63.2
32	0.84 ±0.61	1.80 ±0.65	0.81 ±0.65	2.10 ±0.47	-0.02	-2.6	0.30	16.4
33	0.72 ±0.65	1.54 ±0.39	0.81 ±0.21	1.77 ±0.39	0.09	13.1	0.23	14.9
34	0.73 ±0.20	1.80 ±0.41	0.91 ±0.31	2.34 ±0.15	0.18	24.1	0.54	30.0
35	0.85 ±0.21	1.97 ±0.44	1.14 ±0.29	2.13 ±0.36	0.29	34.1	0.16	8.4
36	0.48 ±0.10	1.09 ±0.18	0.63 ±0.11	1.47 ±0.18	0.15	30.6	0.39	35.6
37	0.48 ±0.15	1.11 ±0.16	0.69 ±0.21	1.44 ±0.26	0.21	43.9	0.33	29.3
38	0.59 ±0.09	1.41 ±0.23	0.56 ±0.09	1.71 ±0.21	-0.04	-5.9	0.29	20.7
39	0.63 ±0.08	1.50 ±0.33	0.87 ±0.39	1.62 ±0.27	0.24	38.3	0.13	8.4
40	0.62 ±0.25	1.41 ±0.17	0.72 ±0.24	1.83 ±0.21	0.11	17.3	0.42	29.4
41	0.61 ±0.19	1.72 ±0.41	0.83 ±0.46	1.84 ±0.33	0.22	35.9	0.12	7.3
42	0.72 ±0.16	2.10 ±0.17	0.82 ±0.21	1.82 ±0.15	0.10	13.7	-0.28	-13.3
43	0.72 ±0.11	1.28 ±0.17	0.71 ±0.22	1.59 ±0.21	-0.01	-1.2	0.30	23.7
44	0.80 ±0.79	1.48 ±0.22	0.81 ±0.19	1.67 ±0.18	0.01	1.0	0.19	13.2
45	0.72 ±0.47	1.24 ±0.40	0.72 ±0.44	1.28 ±0.28	0.00	0.2	0.04	2.9
46	0.58 ±0.08	1.18 ±0.44	0.69 ±0.23	1.32 ±0.30	0.12	20.2	0.15	12.5
47	0.62 ±0.27	1.42 ±0.16	0.69 ±0.26	1.69 ±0.13	0.07	11.8	0.27	18.9
48	0.56 ±0.11	1.29 ±0.27	0.63 ±0.28	1.38 ±0.24	0.07	12.7	0.09	7.0
49	0.74 ±0.42	1.53 ±0.20	0.80 ±0.32	1.66 ±0.42	0.07	9.1	0.13	8.4
50	0.61 ±0.26	1.41 ±0.22	0.78 ±4.26	1.66 ±4.29	0.17	27.8	0.25	17.9
51	1.26 ±0.20	2.58 ±0.61	1.24 ±0.5	2.90 ±0.45	-0.02	-1.6	0.32	12.4
52	0.69 ±0.10	2.08 ±0.47	1.11 ±0.92	2.23 ±0.49	0.42	61.1	0.15	7.0
53	0.42 ±0.07	1.05 ±0.11	0.57 ±0.2	1.28 ±0.12	0.15	34.4	0.23	21.5
54	0.84 ±0.13	2.01 ±0.21	1.05 ±0.27	2.08 ±0.21	0.20	24.2	0.07	3.7
55	0.69 ±0.24	2.00 ±0.67	1.02 ±0.43	2.22 ±0.32	0.33	47.5	0.23	11.3
56	0.76 ±0.25	2.02 ±0.53	0.75 ±0.4	1.89 ±0.36	-0.02	-2.1	-0.13	-6.2
57	0.71 ±0.55	1.27 ±0.37	0.93 ±0.59	1.60 ±0.43	0.23	31.9	0.33	25.5
58	0.54 ±0.13	1.54 ±0.39	0.77 ±0.68	1.66 ±0.45	0.24	44.4	0.12	8.0
59	2.95 ±1.01	1.98 ±0.62	0.86 ±0.47	2.19 ±0.47	-2.08	-70.7	0.20	10.2
60	0.79 ±0.08	1.78 ±0.22	0.95 ±0.23	2.01 ±0.18	0.16	20.3	0.23	12.9
Group Mean	*0.87 ±0.40*	*1.78 ±0.47*	*0.99 ±0.40*	*2.02 ±0.57*	*0.12 ±0.38*	*19.0 ±26.5*	*0.25 ±0.24*	*14.3 ±12.3*

3.3.6 Respiratory rate

Respiratory rate (RR) data for the whole subject group is displayed in table 3.14 and figure 3.12. Complete data points for the entire subject group were measured (n=60). The mean RR during normoxic rest and exercise was 15.0 bpm (SD 3.1) and 20.9bpm (SD 4.2) respectively. The mean RR during hypoxic rest and exercise was 15.4bpm (SD 3.3) and 23.7 bpm (SD 4.8) respectively. The mean absolute change in RR at *rest* between conditions of normoxia and hypoxia was 0.4 bpm (SD 2.4); the mean percent change was 4.0% (SD 17.9). The mean absolute change in RR during *exercise* between conditions of normoxia and hypoxia was 2.8 bpm (SD 2.8); the mean percent change was 14.5% (SD 15.7). Respiratory rate during rest and exercise demonstrated normality under conditions of normoxia and hypoxia; paired student t-tests demonstrated significant change between normoxia and hypoxia for exercise ($p<0.0001$), but not during rest ($p=0.182$).

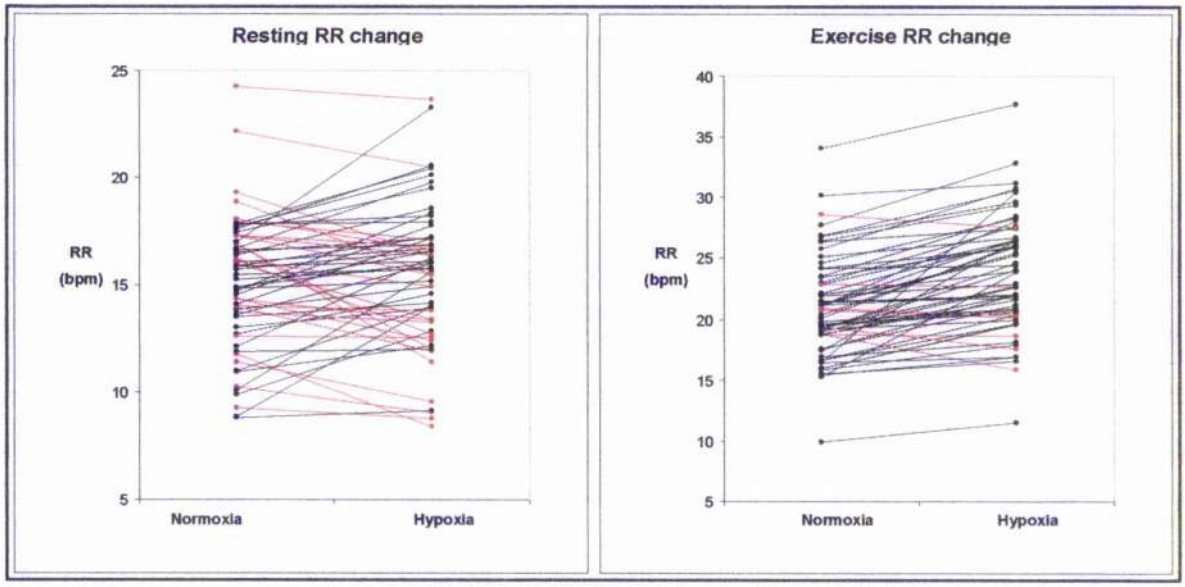

Figure 3.12 The change in respiratory rate from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during exercise. Similar to the responses seen with tidal volume, there is a wide range of response in RR, with both increases and decrease (in red) evident. The high RR evident in some of the subjects a degree of hyperventilation.

Table 3.14. Individual respiratory rate data and changes between normoxia and hypoxia.

Subject	*Normoxic Rest (bpm)*	*Normoxic Exercise (bpm)*	*Hypoxic Rest (bpm)*	*Hypoxic Exercise (bpm)*	*ΔRR (Rest)*		*ΔRR (Exercise)*	
					bpm	*%*	*bpm*	*%*
1	11.4 ±4.0	17.0 ±2.9	9.5 ±3.4	18.1 ±3.3	-1.9	-16.3	1.2	7.0
2	11.8 ±4.7	15.4 ±4.1	8.4 ±2.9	17.0 ±1.8	-3.4	-28.6	1.6	10.1
3	16.0 ±3.6	18.7 ±4.8	16.7 ±2.8	18.7 ±2.9	0.6	4.0	-0.0	-0.3
4	13.1 ±2.9	16.0 ±1.4	14 ±2.1	19.7 ±2.5	0.9	6.9	3.7	23.0
5	13.5 ±4.3	20.7 ±2.9	14.2 ±1.9	20.2 ±2.6	0.7	4.8	-0.5	-2.6
6	19.3 ±4.3	21.9 ±3.2	16.3 ±3.7	22.9 ±2.2	-2.9	-15.3	1.0	4.7
7	9.3 ±1.5	21.2 ±3.8	8.8 ±2.5	22.1 ±4.7	-0.5	-5.0	0.8	4.0
8	13.7 ±3.4	23.5 ±5.2	13.3 ±5.4	28.5 ±5.4	-0.4	-3.2	5.0	21.1
9	14.7 ±2.7	19.2 ±3.1	18.4 ±3.9	19.9 ±4.5	3.7	25.0	0.7	3.6
10	16.0 ±3.5	21.4 ±2.9	13.4 ±5.7	22.0 ±3.0	-2.6	-16.1	0.6	2.8
11	16.9 ±6.8	16.6 ±2.4	11.4 ±3	19.7 ±2.2	-5.5	-32.4	3.1	18.5
12	12.7 ±3.2	16.6 ±2.8	14.6 ±1.9	20.4 ±3.6	1.9	14.8	3.8	22.7
13	16.5 ±5.6	21.2 ±4.3	17.2 ±2.5	25.4 ±2.4	0.7	4.3	4.1	19.5
14	13.9 ±2.6	19.6 ±2.0	14.9 ±3.9	20.8 ±2.5	1.1	7.6	1.2	6.0
15	16.7 ±4.4	19.2 ±2.2	19.8 ±2.6	24.6 ±4.6	3.1	18.5	5.3	27.7
16	17.3 ±3.7	16.5 ±2.7	16.8 ±3.8	30.5 ±3.2	-0.5	-3.2	14.0	84.9
17	14.0 ±3.4	19.3 ±1.5	13.8 ±2.6	24.0 ±1.8	-0.2	-1.4	4.8	24.7
18	14.4 ±3.1	21.2 ±3.8	12.4 ±3.2	26.5 ±4.7	-2.0	-13.7	5.3	25.0
19	10.9 ±3.9	19.0 ±2.7	12.2 ±2.3	22.6 ±2.3	1.2	11.2	3.6	18.8
20	10.1 ±3.3	18.8 ±2.7	15.6 ±5	15.9 ±2.4	5.5	55.0	-2.9	-15.6
21	9.9 ±2.0	17.6 ±3.5	12.9 ±3	21.2 ±2.6	3.0	30.1	3.6	20.4
22	16.9 ±2.9	24.6 ±2.1	12.0 ±1.3	28.3 ±2.7	-4.9	-28.9	3.7	15.0
23	13.6 ±8.3	19.8 ±5.4	16.0 ±5.3	20.9 ±3.2	2.4	17.6	1.1	5.6
24	10.3 ±5.7	18.8 ±2.8	9.1 ±1.6	22.0 ±3.1	-1.2	-11.5	3.3	17.4
25	14.3 ±3.6	20.1 ±2.7	13.4 ±3.1	26.1 ±3.0	-1.0	-6.6	6.1	30.2
26	15.4 ±2.9	23.0 ±4.6	15.7 ±3.4	27.6 ±4.8	0.3	1.9	4.6	20.2
27	17.9 ±4.5	26.9 ±4.0	17.1 ±2.6	30.6 ±3.1	-0.8	-4.7	3.7	14.0
28	24.3 ±12.9	34.1 ±6.2	23.7 ±4.2	37.7 ±6.3	-0.6	-2.5	3.6	10.6
29	12.6 ±3.7	22.8 ±3.8	12.6 ±2.1	26.5 ±2.7	-0.1	-0.4	3.6	15.9
30	17.8 ±5.9	26.3 ±4.2	18.2 ±7.6	27.4 ±5.7	0.4	2.3	1.1	4.1
31	8.9 ±1.6	22.9 ±6.6	14.0 ±2.6	22.7 ±3.7	5.1	57.9	-0.1	-0.5
32	17.7 ±7.9	22.1 ±9.9	20.1 ±6.7	23.9 ±4.0	2.5	14.0	1.8	8.4
33	16.1 ±3.3	21.5 ±3.3	14.9 ±4.5	21.8 ±3.6	-1.2	-7.6	0.3	1.3
34	11.0 ±1.1	16.4 ±3.3	14 ±2.4	16.9 ±1.6	3.0	27.3	0.5	2.9
35	16.2 ±2.9	19.6 ±3.4	12.7 ±2.5	20.6 ±2.0	-3.5	-21.4	1.1	5.4
36	22.2 ±3.7	30.1 ±4.7	20.5 ±4.6	31.1 ±4.6	-1.7	-7.6	1.0	3.3
37	18.9 ±3.2	28.6 ±3.6	15.5 ±5.1	27.5 ±4.2	-3.4	-17.8	-1.1	-3.8
38	15.9 ±2.4	19.4 ±3.5	16.1 ±1.4	17.6 ±2.5	0.2	1.3	-1.9	-9.6
39	17.3 ±2.3	21.2 ±5.1	15.4 ±2.6	28.1 ±5.3	-1.8	-10.6	6.9	32.7
40	17.8 ±3.1	18.8 ±1.1	17.9 ±2.9	21.6 ±2.6	0.1	0.7	2.8	15.1
41	14.0 ±3.4	15.9 ±2.8	11.9 ±2.7	17.9 ±2.7	-2.0	-14.4	2.1	12.9
42	12.1 ±2.8	17.4 ±2.4	16.3 ±4.1	26.4 ±4.3	4.2	34.3	9.0	51.6
43	17.6 ±5.2	24.2 ±4.6	20.6 ±5.9	24.4 ±3.6	3.0	16.9	0.2	0.9
44	14.6 ±3.2	20.4 ±4.0	17.8 ±3.2	26.0 ±3.3	3.2	21.9	5.6	27.2
45	17.8 ±8.0	25.8 ±5.3	20.5 ±10.6	30.8 ±6.3	2.6	14.9	5.0	19.3
46	15.9 ±2.7	23.4 ±9.7	17.2 ±3.6	26.0 ±8.4	1.3	8.3	2.5	10.7
47	14.1 ±5.6	17.5 ±2.5	17.2 ±2.9	22.1 ±2.1	3.1	21.9	4.6	26.2
48	15.7 ±3.2	27.7 ±4.7	16.6 ±5.0	32.8 ±4.9	0.9	5.5	5.1	18.5
49	17.2 ±4.3	24.2 ±2.2	16.4 ±4.9	25.9 ±2.7	-0.9	-5.1	1.7	6.9
50	16.4 ±4.7	26.4 ±3.8	18.5 ±4.3	29.4 ±1.5	2.2	13.4	3.0	11.2
51	8.8 ±3.4	9.9 ±3.2	9.1 ±2.0	11.5 ±2.0	0.3	3.7	1.6	15.8
52	18.0 ±2.3	21.2 ±3.9	16.6 ±4.1	25.3 ±4.1	-1.4	-7.7	4.1	19.3
53	17.4 ±4.1	25.1 ±4.4	19.5 ±4.1	26.7 ±4.1	2.1	11.9	1.6	6.4
54	11.9 ±1.9	16.0 ±2.9	12 ±1.8	19.6 ±2.3	0.1	0.9	3.7	23.0
55	16.6 ±3.0	22.2 ±3.8	16.8 ±3.9	26.4 ±4.3	0.2	1.5	4.3	19.2
56	14.9 ±2.1	15.5 ±2.6	16.5 ±4.8	16.5 ±4.8	1.6	11.1	1.0	6.7
57	17.0 ±9.2	26.8 ±5.4	23.3 ±20.5	29.7 ±9.0	6.3	37.2	2.9	10.7
58	14.9 ±4.3	19.4 ±5.3	16.0 ±3.7	22.7 ±5.6	1.2	8.0	3.3	17.3
59	14.8 ±2.7	20.9 ±3.0	15.2 ±3.9	20.3 ±2.4	0.3	2.3	-0.6	-2.9
60	15.3 ±1.1	15.3 ±4.6	15.8 ±2.5	23.9 ±2.8	0.5	3.3	8.6	56.6
Group Mean	***15.0 ±3.1***	***20.9 ±4.2***	***15.4 ±3.3***	***23.7 ±4.8***	***0.42 ±2.4***	***4.0 ±17.9***	***2.8 ±2.8***	***14.5 ±15.7***

3.3.7 Minute ventilation

Minute ventilation (VE) data for the whole subject group is displayed in table 3.15 and figure 3.13. Complete data points for the entire subject group were measured (n=60). The mean VE during normoxic rest and exercise was 11.32 litres (SD 2.26) and 35.28 litres (SD 6.80) respectively. The mean VE during hypoxic rest and exercise was 13.65 litres (SD 2.74) and 46.17 (SD 10.20) respectively. The absolute change in VE at *rest* between conditions of normoxia and hypoxia was 2.33 (SD 1.92); the percent change was 21.9% (SD 18.2). The mean absolute change in VE during *exercise* between conditions of normoxia and hypoxia was 10.88 litres (SD 5.02); the mean percent change was 30.6% (SD 12.4). Minute ventilation measurements during normoxia demonstrated normality, but hypoxic VE data during rest and exercise were not. Wilcoxon sign rank sum analysis demonstrated a significant change between normoxia and hypoxia during rest and exercise (both p values<0.0001).

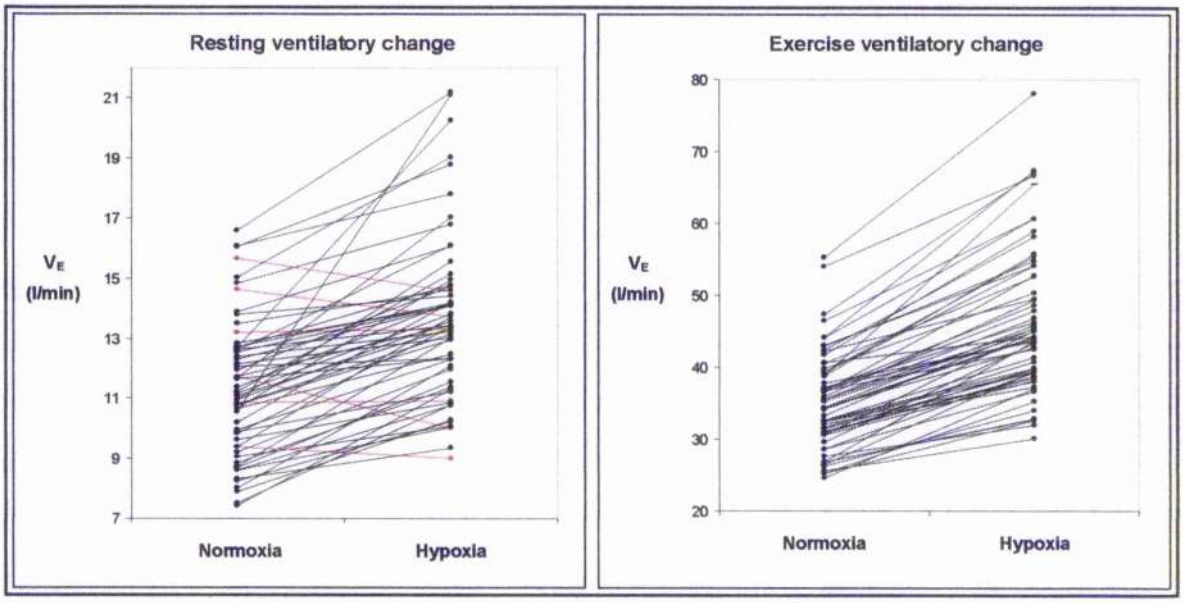

Figure 3.13 The change in minute ventilation from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during exercise. The wide variation in response is seen as with the other plots. The resting plot shows 6 subjects that demonstrated a higher V_E during normoxic rest in comparison to hypoxia; these subjects also had a decrease in VCO_2 and increase in $PetCO_2$ on exercise. This suggests that hyperventilation during this portion of the protocol was present.

Table 3.15. Individual minute ventilation and changes between normoxia and hypoxia.

Subject	*Normoxic Rest (l/min)*	*Normoxic Exercise (l/min)*	*Hypoxic Rest (l/min)*	*Hypoxic Exercise (l/min)*	*ΔVE (Rest)*		*ΔVE (Exercise)*	
					l/min	%	*l/min*	%
1	11.95 ±2.8	43.06 ±6.5	14.77 ±5.9	54.16 ±11.7	2.82	23.6	11.1	25.8
2	12.06 ±6.2	37.08 ±5.5	12.34 ±5.1	46.24 ±6.2	0.28	2.3	9.16	24.7
3	16.04 ±7.4	53.99 ±7.6	18.80 ±3.0	66.65 ±6.2	2.76	17.2	12.66	23.5
4	12.58 ±4.8	46.45 ±7	16.11 ±6.8	60.65 ±13	3.53	28.1	14.2	30.6
5	13.21 ±7.9	37.07 ±5.9	13.32 ±3.6	43.7 ±8.2	0.11	0.9	6.63	17.9
6	11.06 ±2.8	30.85 ±4.1	13.36 ±4.6	39.81 ±3.9	2.30	20.8	8.96	29
7	13.88 ±5.3	42.3 ±6.9	16.05 ±6.0	49.56 ±7.5	2.18	15.7	7.26	17.2
8	9.84 ±3.1	34.18 ±7.6	13.39 ±8.8	44.1 ±9.2	3.55	36.0	9.92	29
9	10.19 ±2.4	35.5 ±7.3	14.21 ±5.7	46.86 ±4.5	4.02	39.4	11.36	32
10	11.65 ±3.9	40.54 ±7.3	12.32 ±4.3	52.63 ±11.1	0.67	5.7	12.09	29.8
11	15.66 ±8.9	39.3 ±6.4	14.59 ±4.9	55.37 ±6.7	-1.08	-6.9	16.08	40.9
12	10.87 ±4.4	32.18 ±5.9	10.92 ±1.7	37.22 ±6.5	0.06	0.5	5.04	15.7
13	11.73 ±4.1	30.61 ±7.6	13.44 ±5.2	45.1 ±4.4	1.71	14.6	14.49	47.3
14	16.07 ±8.2	34.2 ±4.5	17.81 ±6.1	44.28 ±5.7	1.74	10.8	10.08	29.5
15	12.73 ±3.1	39.71 ±6.8	20.28 ±5.1	50.35 ±8.9	7.55	59.3	10.65	26.8
16	14.83 ±5.2	38.79 ±8.6	16.80 ±4.7	55.71 ±9.9	1.97	13.2	16.92	43.6
17	12.61 ±6.7	38.74 ±4.6	13.44 ±4.1	65.45 ±5.8	0.83	6.6	26.71	68.9
18	15.01 ±4.9	41.77 ±8.3	19.05 ±4.9	58.88 ±11.8	4.03	26.9	17.11	40.9
19	11.74 ±5.2	44.13 ±8.6	15.56 ±6.7	60.61 ±10.6	3.82	32.5	16.47	37.3
20	10.97 ±5.0	36.58 ±8.7	14.03 ±5.2	52.71 ±4.7	3.06	27.9	16.13	44.1
21	10.57 ±4.4	36.65 ±5.3	14.48 ±8.1	36.77 ±10.8	3.91	37.0	0.12	0.3
22	12.69 ±5.7	44.18 ±5.7	14.78 ±7.8	67.39 ±6.8	2.09	16.5	23.21	52.5
23	9.63 ±4.6	47.38 ±0.2	10.86 ±3.5	67.02 ±3.5	1.23	12.8	19.63	41.4
24	8.85 ±4.9	26.87 ±3.5	11.37 ±4.0	32.8 ±5.3	2.52	28.5	5.93	22.1
25	7.41 ±2.6	34.33 ±5.2	11.32 ±2.9	45.91 ±5.1	3.91	52.7	11.58	33.7
26	11.73 ±6.9	34.38 ±5.1	10.01 ±4.4	42.76 ±6.8	-1.72	-14.7	8.37	24.4
27	8.25 ±3.1	25.62 ±5.3	10.23 ±2.4	30.06 ±4.9	1.98	24.0	4.44	17.3
28	12.83 ±5.4	32.53 ±5.1	14.11 ±3.1	39.38 ±6.7	1.27	9.9	6.85	21.1
29	14.63 ±8.0	32.67 ±4.2	13.75 ±2.7	41.33 ±6.5	-0.88	-6.0	8.66	26.5
30	8.61 ±3.9	39.29 ±4.7	10.77 ±7.1	54.18 ±7.1	2.16	25.0	14.88	37.9
31	16.57 ±3.1	27.62 ±9.1	21.20 ±4.0	31.97 ±12.4	4.63	27.9	4.35	15.7
32	13.51 ±8.2	55.19 ±7.6	14.62 ±8.8	78.03 ±9.5	1.11	8.2	22.84	41.4
33	10.66 ±7.9	35.91 ±7.2	12.22 ±4.3	49.33 ±7.6	1.55	14.6	13.42	37.4
34	8.02 ±2.3	32.5 ±3.8	12.58 ±1.7	37.97 ±3.1	4.56	56.9	5.47	16.8
35	13.79 ±4.4	28.63 ±5.6	14.42 ±4.7	39.23 ±7	0.63	4.6	10.6	37
36	10.65 ±2.6	37.74 ±6.7	12.49 ±2.4	43.65 ±7.8	1.84	17.3	5.91	15.6
37	9.06 ±3.2	32.65 ±3.5	10.08 ±2.5	39.9 ±7.7	1.03	11.3	7.25	22.2
38	9.40 ±2.1	31.52 ±3.3	9.00 ±1.6	39.4 ±2.6	-0.40	-4.3	7.88	25
39	10.79 ±2.0	26.93 ±5.4	13.14 ±4.8	43.67 ±9.9	2.36	21.8	16.74	62.2
40	10.76 ±4.0	26.53 ±2.9	12.96 ±5.2	39.47 ±5.8	2.19	20.4	12.94	48.8
41	8.32 ±2.5	26.93 ±6.4	9.38 ±3.5	32.87 ±6.2	1.06	12.7	5.93	22
42	8.75 ±2.8	36.52 ±5.8	13.01 ±4.3	47.88 ±6.8	4.26	48.7	11.36	31.1
43	12.30 ±3.0	30.61 ±4.7	14.09 ±4.4	38.14 ±4.2	1.79	14.6	7.53	24.6
44	11.38 ±9.2	29.57 ±4.4	14.20 ±3.4	43.37 ±5.8	2.82	24.8	13.8	46.7
45	12.72 ±9.8	31.11 ±4.1	14.19 ±9.2	38.48 ±7.5	1.47	11.5	7.36	23.7
46	9.22 ±1.9	25.28 ±9.2	11.97 ±4.3	35.25 ±7	2.74	29.7	9.97	39.4
47	8.71 ±5.9	24.66 ±3.7	11.56 ±3.2	37.06 ±4.3	2.85	32.7	12.4	50.3
48	8.62 ±2.1	35.21 ±6.5	10.05 ±4.3	44.99 ±7.4	1.44	16.7	9.77	27.8
49	12.38 ±6.5	36.78 ±4.1	13.24 ±8.3	42.44 ±9.5	0.86	7.0	5.66	15.4
50	9.41 ±2.4	36.83 ±5.5	13.73 ±0.2	48.62 ±0.2	4.32	46.0	11.79	32
51	9.91 ±3.6	25.26 ±5	11.21 ±4.7	32.64 ±3.9	1.31	13.2	7.38	29.2
52	12.53 ±2.8	42.86 ±5.3	16.20 ±7.9	54.8 ±7.1	3.67	29.3	11.94	27.9
53	7.51 ±2.6	26.25 ±4.5	10.81 ±2.6	34.02 ±5.4	3.31	44.0	7.77	29.6
54	9.96 ±18	31.64 ±3.8	12.31 ±3.0	40.73 ±5	2.35	23.6	9.1	28.7
55	11.68 ±4.0	42.39 ±9.7	17.04 ±7.1	58.1 ±9.2	5.36	45.9	15.71	37.1
56	11.22 ±3.7	30.82 ±7.3	11.22 ±3.7	38.7 ±7.5	-0.01	-0.1	7.88	25.6
57	10.55 ±7.2	33.27 ±7.5	17.71 ±12.3	45.45 ±9.3	7.15	67.8	12.19	36.6
58	7.90 ±2.9	28.69 ±7	10.28 ±4.0	36.66 ±8.9	2.38	30.2	7.98	27.8
59	12.15 ±9.0	40.67 ±7.2	13.02 ±7.6	44.19 ±8.5	0.88	7.2	3.52	8.7
60	11.15 ±2.0	35.53 ±5.6	18.84 ±3.5	45.4 ±5.7	7.69	69.0	9.87	27.8
Group Mean	*11.32 ±2.3*	*35.28 ±6.8*	*13.65 ±2.7*	*46.17 ±10.2*	*2.33 ±1.9*	*21.9 ±18.2*	*10.9 ±5.0*	*30.8 ±12.4*

3.3.8 Patterns of ventilation and $etCO_2$

The increase in ventilation evident upon hypoxic exposure demonstrated a wide variation in respiratory rate and tidal volume response between individuals. In the absence of measurements of physiological deadspace, end-tidal CO_2 was used as an index of alveolar ventilation. Correlation plots of minute ventilation, respiratory rate and tidal volume were plotted against PetCO2. Results are given in SI units (kilopascals – kPa). In addition the Pearson correlation coefficient was calculated and regression lines were included in the plots.

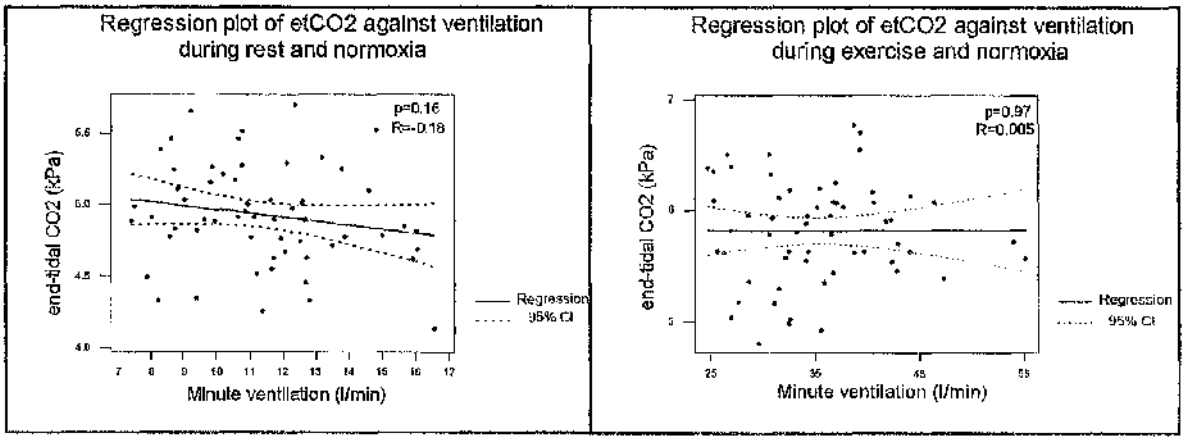

Figure 3.14 Regression plots of minute ventilation and $PetCO_2$ in normoxic conditions. The left panel shows resting response; the right panel shows the exercise response. The red dashed lines are the 95% confidence intervals. There is no significant correlation between $PetCO_2$ and V_E during rest or exercise (p=0.16 and 0.97 respectively). The resting plot demonstrates a trend toward a negative correlation that might belie a degree of hyperventilation during rest whilst connected to the circuit. The lack of correlation during normoxic exercise is to be expected since exercise ventilation is closely coupled to CO_2 production and elimination.

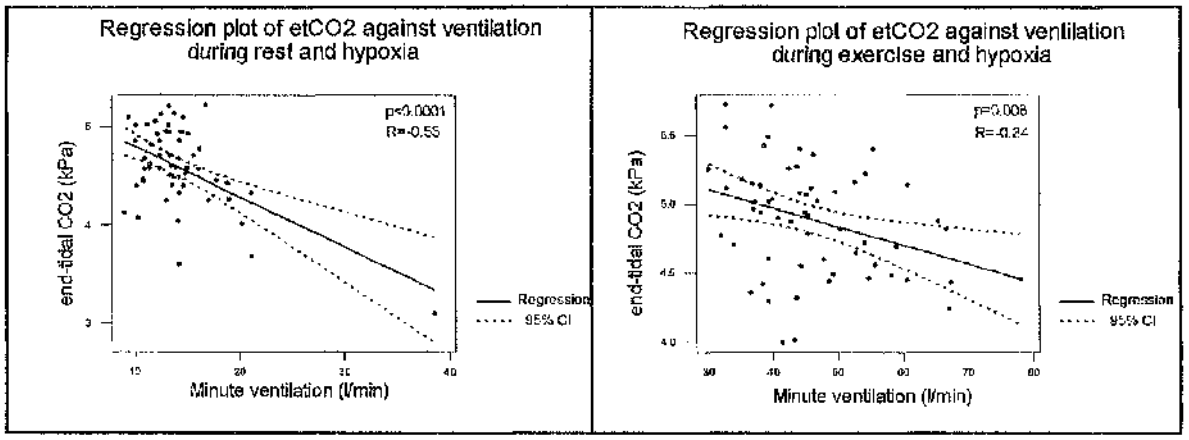

Figure 3.15 Regression plots of minute ventilation and $etCO_2$ in hypoxic conditions. The left panel shows resting response; the right panel shows the exercise response. The red dashed lines are the 95% confidence intervals. There is a highly significant correlation between $PetCO_2$ and V_E during rest and exercise ($p<0.0001$ and $p=0.008$ respectively). This is expected, since ventilation in hypoxic conditions is driven by hypoxia as opposed to CO_2. The resting response is made more significant by one individual that may have exhibited a degree of hyperventilation; however removal of this subject retained the significance of this correlation ($p=0.03$)

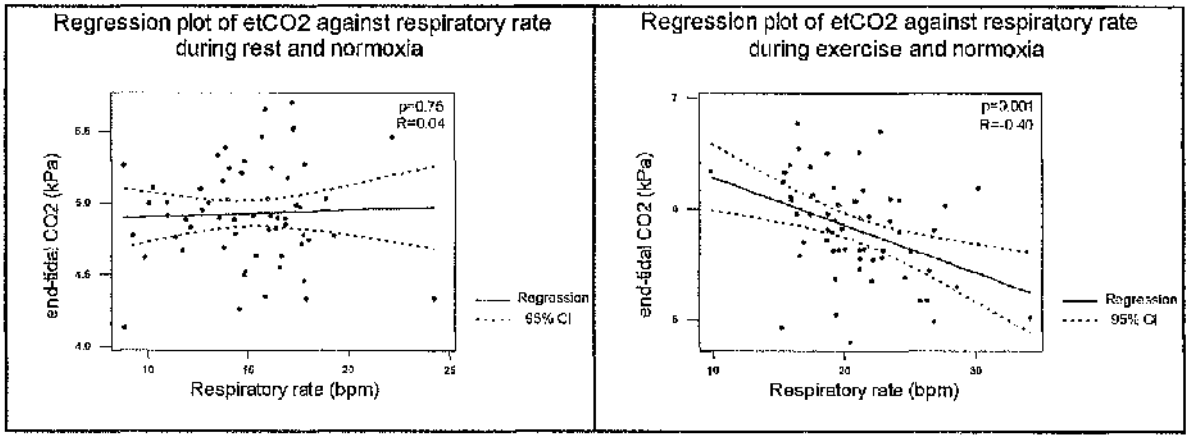

Figure 3.16 Regression plots of respiratory rate and $etCO_2$ in normoxic conditions. The left panel shows resting response; the right panel shows the exercise response. The red dashed lines are the 95% confidence intervals. There was no significant correlation between RR and $PetCO_2$ during rest ($p=0.75$), but a significant correlation during exercise ($p=0.001$).

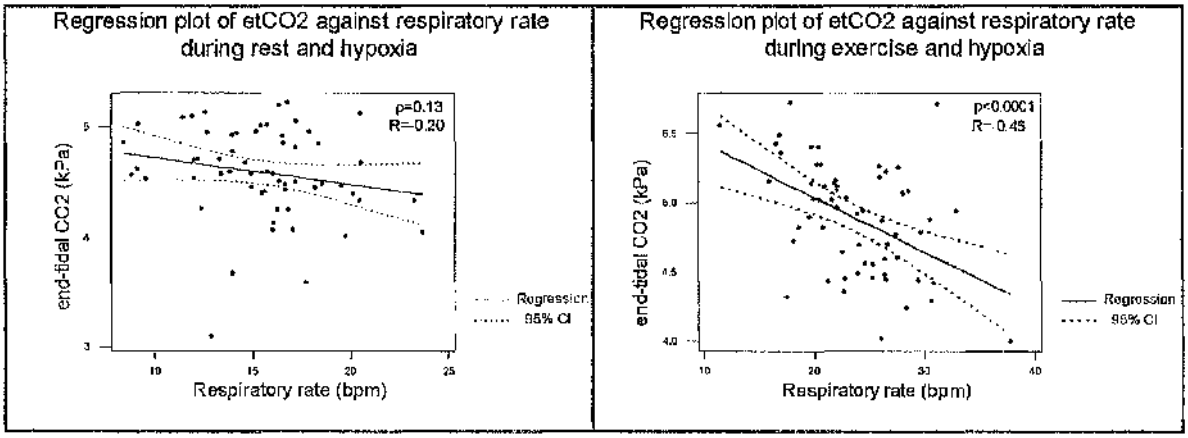

Figure 3.17 Regression plots of respiratory rate and $PetCO_2$ in hypoxic conditions. The left panel shows resting response; the right panel shows the exercise response. The red dashed lines are the 95% confidence intervals. There was no significant correlation between RR and $PetCO_2$ during rest (p=0.13), but a significant correlation during exercise (p<0.0001).

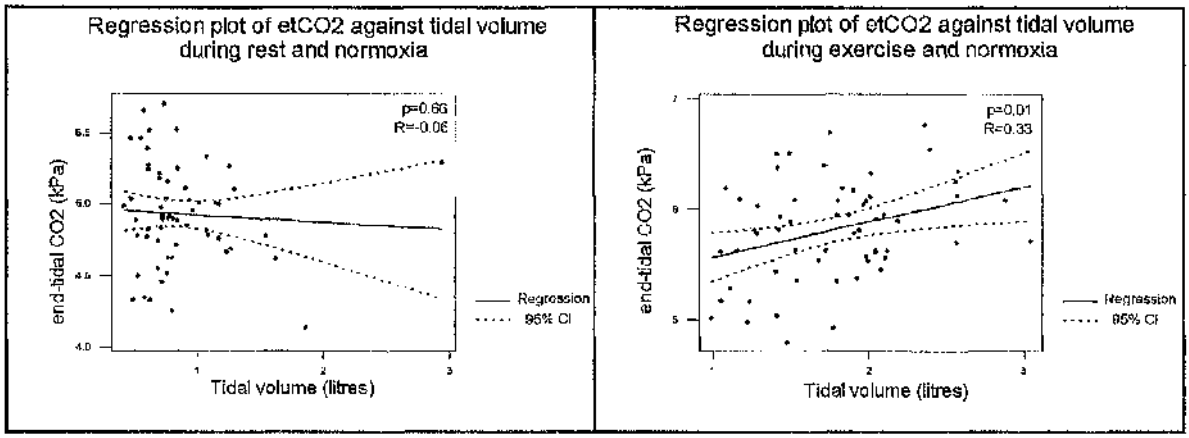

Figure 3.18 Regression plots of tidal volume and $PetCO_2$ in normoxic conditions. The left panel shows resting response; the right panel shows the exercise response. The red dashed lines are the 95% confidence intervals. There was no significant correlation between V_T and $PetCO_2$ during rest (p=0.66), but a significant correlation during exercise (p=0.01).

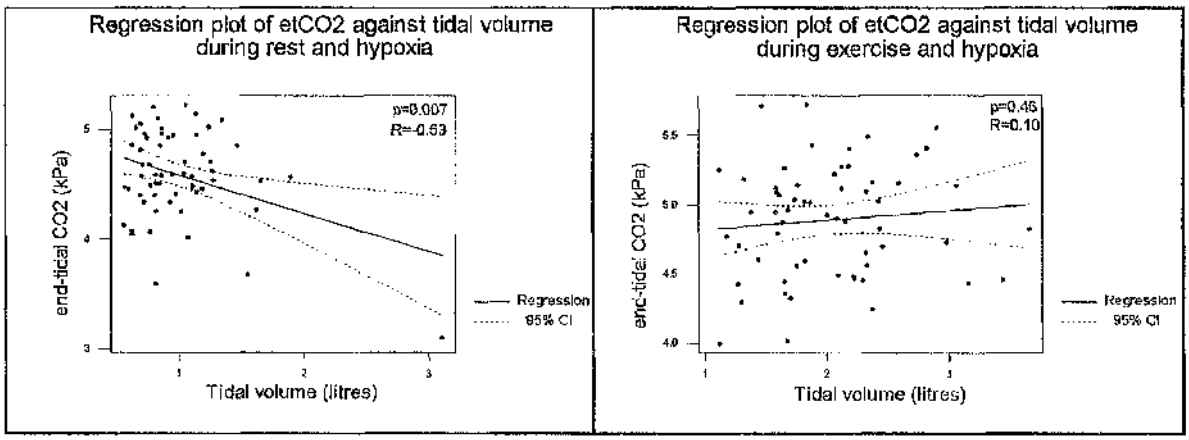

Figure 3.19 Regression plots of tidal volume and $PetCO_2$ in hypoxic conditions. The left panel shows resting response; the right panel shows the exercise response. The red dashed lines are the 95% confidence intervals. The resting plot suggests a significant negative correlation between $PetCO_2$ and V_T during hypoxic rest; however this appears to be the effect of one outlier, who demonstrated a significant degree of hyperventilation during this portion of the protocol (the same subject increased the significance of the minute ventilation plot during hypoxic rest). The removal of this subject from the correlation calculation produced a non-significant result (p=0.85). There was no significant correlation between V_T and $PetCO_2$ during exercise (p=0.66).

3.4 Bio-impedance cardiac output results

In addition to the study data, validation studies were performed comparing bioimpedance with invasive cardiac output measurement.

3.4.1 Catheter laboratory validation of bioimpedance cardiac output monitoring

The validity of the Physioflow PF-05 bioimpedance cardiography unit was verified by comparison with pulmonary catheter studies and thermodilution measurement of cardiac output during diagnostic studies in four patients. The results are shown in table 3.16, figure 3.20 and figure 3.21.

Subject number (Test)	*CO#*	*Catheter CO (l/min)*	*Catheter Mean CO (l/min)*	*Bioimpedance CO (l/min)*	*Bioimpedance mean CO (l/min)*
Subject 1 (rest)	*1*	3.25	3.06	2.85	3.11
	2	3.01		3.28	
	3	3.03		3.20	
	4	2.97		3.10	
Subject 1 (exercise)	*1*	3.3	3.23	3.441	3.50
	2	3.3		3.627	
	3	3.1		3.42	
Subject 2 (rest)	*1*	3.89	3.91	3.876	3.80
	2	3.79		3.696	
	3	4.05		3.84	
Subject 2 (exercise)	*1*	4.47	4.52	4.042	4.14
	2	4.36		4.089	
	3	4.72		4.284	
Subject 2 (exercise)	*1*	3.8	4.13	3.84	4.02
	2	4.33		4.134	
	3	4.1		3.95	
	4	4.28		4.16	
Subject 3 (rest)	*1*	5.61	5.66	5.85	5.91
	2	5.9		6.2	
	3	5.48		5.7	
Subject 3 (exercise)	*1*	7.37	7.33	8.1	8.07
	2	7.16		7.9	
	3	7.46		8.2	
Subject 4 (rest)	*1*	5.72	5.70	4.9	4.77
	2	5.85		4.6	
	3	5.55		4.8	
Subject 4 (exercise)	*1*	9.01	9.06	9.3	9.57
	2	9.29		10	
	3	8.89		9.4	

Table 3.16 The results of simultaneous thermodilution and bioimpedance cardiac output measurement in 4 patients. At least 3 measurements of cardiac output were performed; the mean of these results is show in the relevant column. Subject 2 underwent two periods of exercise as shown.

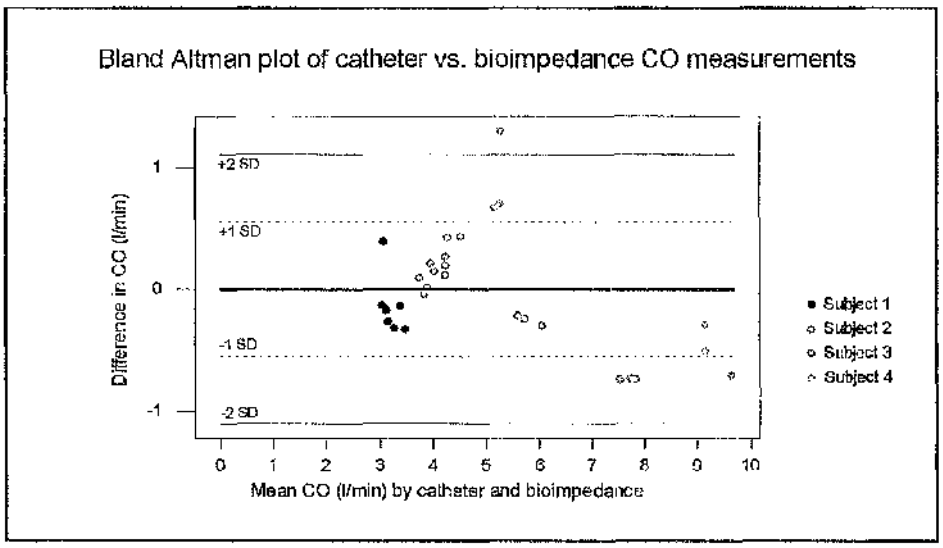

Figure 3.20a Bland Altman plot comparing raw cardiac output measurements using bioimpedance and thermodilution simultaneously. Measurements were made during rest and exercise and have been pooled. The two methods show reasonable agreement, with the exception of one outlier.

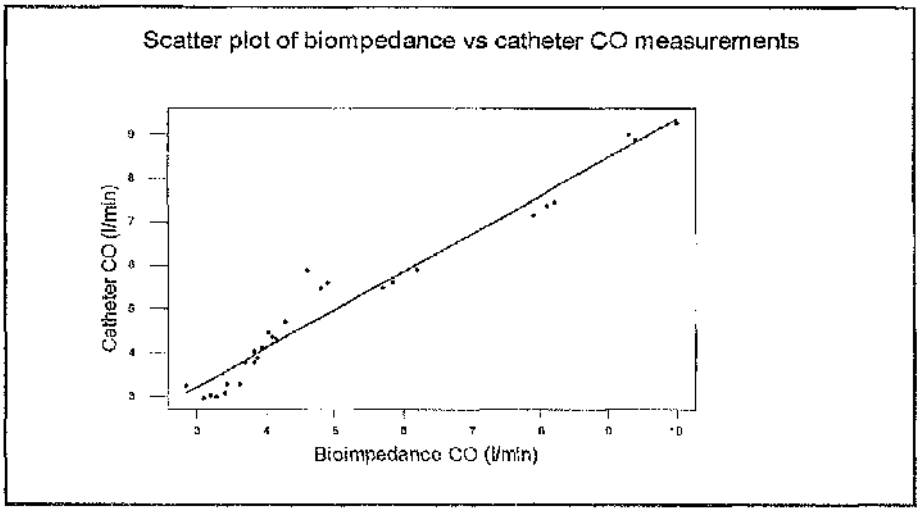

Figure 3.20b Scatter plot of raw cardiac output measurements using bioimpedance and thermodilution simultaneously.

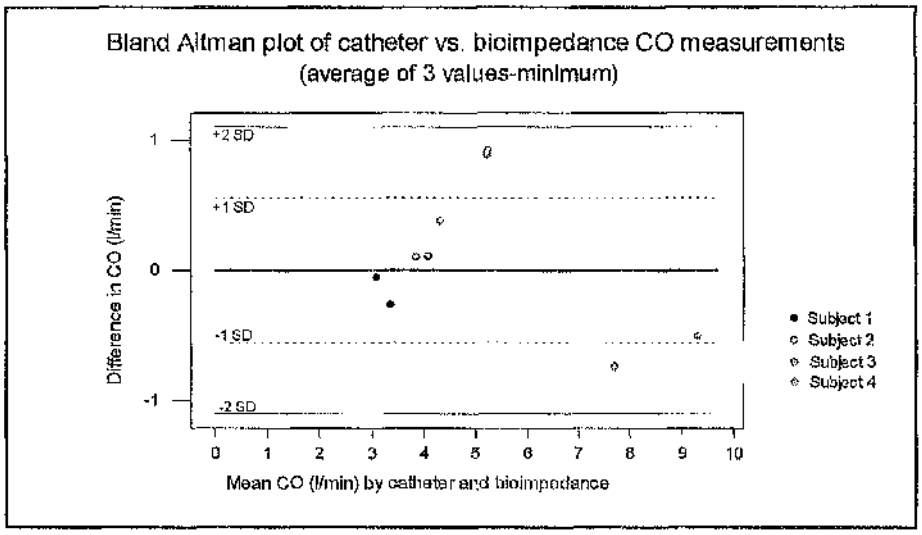

Figure 3.21a Bland Altman plot comparing the mean of 3-4 measurements made in succession. The measurements are of pooled rest and exercise recordings. The degree of agreement is much improved by averaging several results.

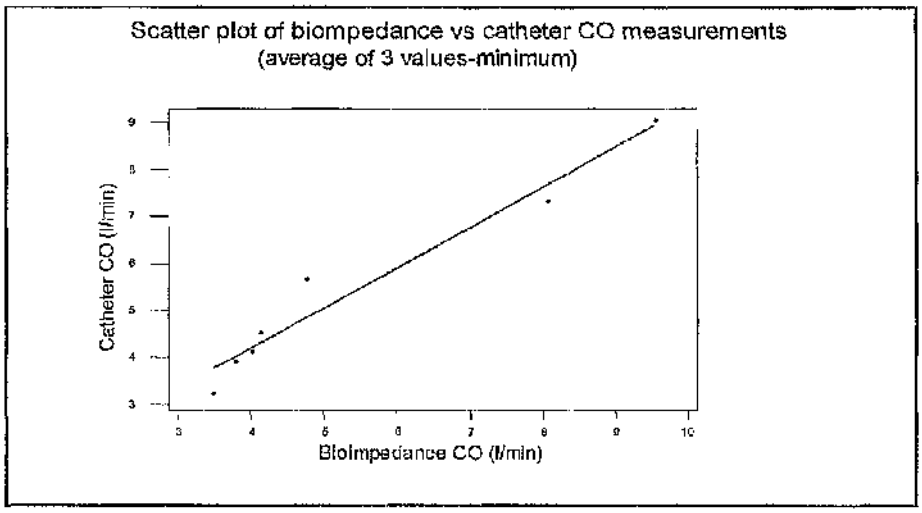

Figure 3.21b Scatter plot of the mean of 3-4 measurements made in succession, using bioimpedance and thermodilution simultaneously. The measurements are of pooled rest and exercise recordings.

3.4.2 Subject group biometric data

The majority of the subjects presented in this aspect of the study were a subset the previous group; except for two subjects denoted '*A*' and '*B*', the subjects retain the subject numbers from the first study for continuity. A brief description of this group is provided. Thirty one subjects were included in these studies, 15 male: 16 female. Biometric data is presented below in table 3.17. The mean age of the group was 23.0 years (SD 4.1 years; range 18-37); the mean height was 174.6 cm (SD 7.9 cm; range 160-186 cm); the mean weight was 71.0 kg (SD 10.9 kg; range 53-92). The whole group demonstrated normality for height and weight.

Subject	*ACE gene*	*Age*	*Sex*	*Height (cm)*	*Weight (kg)*	*Subject*	*ACE gene*	*Age*	*Sex*	*Height (cm)*	*Weight (kg)*
21	II	25	M	185	80.4	45	II	22	F	168	58.5
23	II	18	F	176	74.0	46	II	23	F	168	58.0
25	ID	23	F	171	60.2	47	II	19	F	173	70.0
26	ID	22	F	179	63.3	48	DD	20	F	160	63.6
27	ID	21	F	171	56.2	49	DD	20	M	183	80.0
28	ID	23	F	169	59.4	50	ID	21	F	169	67.0
29	DD	34	M	182	78.6	*A*	ID	21	M	179	70.6
30	DD	21	F	165	57.8	52	ID	21	M	177	88.8
31	II	21	M	186	82.2	53	ID	27	F	163	62.0
32	DD	27	M	185	76.6	26	ID	28	M	179	71.0
33	ID	20	M	184	84.3	55	DD	37	M	183	92.3
34	ID	24	M	178	79.0	56	ID	20	M	182	82.7
35	II	21	M	184	85.0	58	ID	26	M	174	74.3
36	DD	23	F	165	58.2	59	ID	20	F	163	73.4
39	ID	21	F	171	53.0	60	ID	21	M	182	76.2
44	II	22	F	163	60.5						

Table 3.17 Biometric data for cardiac output subject group.

The following sections present the bioimpedance cardiography results in the form of tables and graphs and are laid out in a similar fashion as presented in the previous section. Measurements of CO during rest and exercise are over a period of 90 seconds toward the end of the resting and exercise segments of the protocol. Exercise measurements were made after 4 minutes to ensure steady state.

3.4.3 Heart rate

Heart rate data for the subgroup is displayed in table 3.18 and figure 3.22. Complete data points were measured for the entire subject group (n=31). The mean heart rate during normoxic rest and exercise was; 73.3 bpm (SD 12.4) and 117.0 bpm (SD 16.7) respectively. The mean heart rate during hypoxic rest and exercise was; 85.6 bpm (SD 13.5) and 142.3 bpm (SD 18.5) respectively. The mean absolute change in heart rate at *rest* between conditions of normoxia and hypoxia was 12.3 bpm (SD 5.8); the mean percent change was 17.3% (SD 8.0). The mean absolute heart rate change during *exercise* between conditions of normoxia and hypoxia was 25.2 bpm (SD 6.1); the mean percent change was 21.9 (SD 5.7). All heart rate responses demonstrated normality; paired student t-test analysis showed a highly significant difference between normoxia and hypoxia during rest and exercise (both p values <0.0001).

Subject	*Normoxic rest (bpm)*	*Normoxic Exercise (bpm)*	*Hypoxic Rest (bpm)*	*Hypoxic exercise (bpm)*	*Δ Heart rate (Rest)*		*Δ Heart rate (Exercise)*	
					bpm	*%*	*bpm*	*%*
1	89.5 ±3.3	127.8 ±1.8	109.8 ±2.6	146.9 ±1.8	20.3	22.7	19.1	15.0
2	87.8 ±4.6	142.1 ±2.5	96.9 ±5.7	168.6 ±2.2	9.1	10.4	26.6	18.7
3	81.1 ±5.2	110.5 ±2.7	88.9 ±4.7	139.2 ±2.6	7.9	9.7	28.7	25 9
4	66.2 ±4.0	116.6 ±2.0	76.5 ±3.5	141.3 ±1 2	10.3	15.5	24.7	21.2
5	64.4 ±4.9	101.7 ±1.8	74.7 ±3.9	129.5 ±1.9	10.3	16.0	27.8	27.3
6	55.8 ±2.5	104.1 ±1.9	67.4 ±5.4	134.8 ±1.1	11.6	20.9	30.7	29.5
7	84.6 ±4.1	135.9 ±3.1	95.3 ±4.5	160 .0±1.3	10.6	12.6	24.0	17.7
8	64.3 ±4.3	100.3 ±2.9	70.5 ±5.2	123.2 ±1.8	6.1	9.6	22.9	22.8
9	67.2 ±3.6	116.7 ±2.1	79.2 ±4.0	143.9 ±2.0	12.0	17 9	27.2	23.3
10	77.7 ±2.8	112.5 ±1.4	93.1 ±1.8	134.2 ±1.0	15.4	19 9	21.7	19.3
11	60.5 ±4.8	89.6 ±1.4	67.9 ±2.6	103.5 ±1.6	7.4	12.2	13.9	15.5
12	87.5 ±1.9	123.5 ±2.2	82.1 ±7.0	148.8 ±0.9	-5.4	-6.2	25.3	20.5
13	75.6 ±5.1	113.0 ±3.4	88.9 ±4.2	146 7 ±3.3	13.3	17.6	33.7	29.8
14	85.8 ±4.5	127.7 ±2.3	106.8 ±5.4	160 9 ±4.8	21.0	24.5	33.1	25.9
15	91.7 ±5.3	135.8 ±2.5	111.6 ±3.1	175.6 ±4.2	19.9	21.7	39.8	29.3
16	78.2 ±3.0	115.9 ±1.0	92.9 ±3.8	144.1 ±1.1	14 8	18.9	28.2	24.3
17	73.7 ±3.2	134.4 ±2.5	95.9 ±4.5	156.9 ±9.2	22.3	30.2	22.4	16.7
18	55.0 ±3.6	96.4 ±2.7	69 6 ±6.7	117.9 ±1.3	14.6	26 5	21.5	22.3
19	72.2 ±3.7	109.1 ±2	83.2 ±2.7	126.8 ±1.3	11.1	15 3	17.7	16.2
20	80.8 ±3.0	154.8 ±1 6	98.1 ±3.1	175.4 ±1.3	17.2	21.3	20.6	13.3
21	53.5 ±1.6	80.8 ±1.5	66.9 ±4.0	104.8 ±3.3	13.4	25 1	24.0	29.7
22	66.3 ±3.8	97.5 ±6.9	80.0 ±3.3	125.0 ±4.0	13.7	20.7	27.4	28.2
23	82.0 ±6.2	130.8 ±2.6	87.5 ±4.0	146 0 ±0.9	5.4	6.6	15.2	11.6
24	95.3 ±1.4	130.4 ±1.8	100.4 ±3.3	153.5 ±1.1	5.0	5.3	23.2	17.8
25	64.2 ±3.4	112.5 ±1.9	77.9 ±3.9	135.8 ±3 5	13.7	21.3	23.3	20.7
26	82.9 ±3.0	135.3 ±1.5	101.1 ±4.3	164.8 ±1.3	18.1	21.8	29.5	21.8
27	76.9 ±3.9	129.1 ±2.6	91.5 ±3.7	166.3 ±2.1	14.6	19	37.2	28.8
28	53 6 ±2.3	104.4 ±1.0	70.6 ±2.0	124.0 ±2.1	17.0	31.8	19.7	18.9
29	81 4 ±2.3	114.9 ±1.7	88.3 ±1.4	135.1 ±1.3	7.0	8.6	20.2	17.6
30	55.6 ±4.0	101.0 ±2.2	62.3 ±2.7	134.2 ±3.1	6.7	12.1	33.2	32.9
31	62.8 ±4.3	123 2 ±3.3	78.5 ±4.6	142.9 ±1.3	15.7	25	19.7	16.0
Group mean	*73.4 ±12.4*	*117.0 ±16.7*	*85.6 ±13.5*	*142.3 ±18.5*	*12.3 ±5.6*	*17.2 ±8.0*	*25.2 ±6.1*	*21.9 ±5.7*

Table 3.18. Individual heart rate data and changes between normoxia and hypoxia

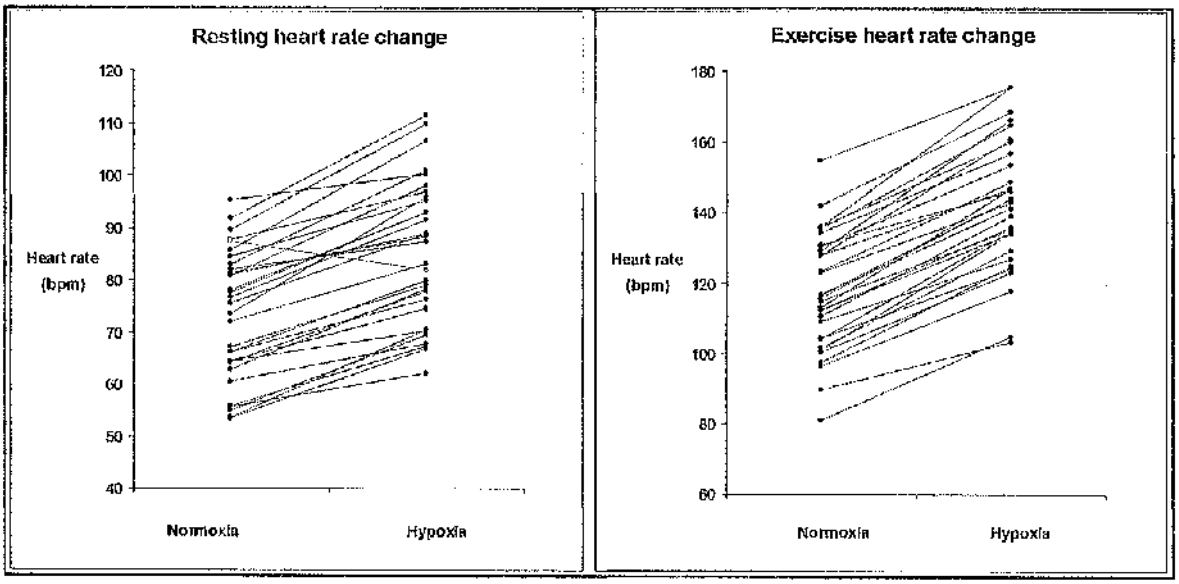

Figure 3.22 The change in heart rate from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during exercise. The wide variation in response is seen as with the other plots. One subject had a higher heart rate during normoxic rest in comparison to hypoxic rest (data point in red), this subject did not have concurrently elevated ventilation during this part of the protocol; therefore it is difficult to assess whether this was due to anxiety. All subjects increased their heart rate from normoxia to hypoxia during exercise.

3.4.4 Stroke volume

Stroke volume data for the subgroup is displayed in table 3.19 and figure 3.23. Complete data points were measured for the entire subject group (n=31). The mean stroke volume during normoxic rest and exercise was; 89.3 mls (SD 25.6) and 113.7 mls (SD 29.0) respectively. The mean stoke volume during hypoxic rest and exercise was; 91.7 mls (SD 26.3) and 121.0 mls (SD 34.6) respectively. The mean absolute change in stroke volume at *rest* between conditions of normoxia and hypoxia was 2.4 mls (SD 5.4); the mean percent change was 2.8% (SD 6.7). The mean absolute stroke volume change during *exercise* between conditions of normoxia and hypoxia was 7.2 mls (SD 8.5); the mean percent change was 5.9% (SD 5.8). All stroke volume responses demonstrated normality; paired student t-test demonstrated a highly significant difference during exercise ($p<0.0001$) and a significant difference at rest from normoxia to hypoxia ($p=0.023$).

Subject	*Normoxic rest (mls)*	*Normoxic Exercise (mls)*	*Hypoxic Rest (mls)*	*Hypoxic exercise (mls)*	*Δ Stroke volume (Rest)*		*Δ Stroke volume (Exercise)*	
					mls	*%*	*mls*	*%*
1	68.2 ±1.0	95.1 ±4.1	73.6 ±2.9	102.5 ±4.0	5.3	7.8	7.4	7.8
2	101.2 ±2.9	147.3 ±4.9	111.8 ±3.0	162.5 ±11.6	10.6	10.5	15.2	10.3
3	67.7 ±1.8	82.6 ±7.8	71.0 ±1.7	82.9 ±5.0	3.3	4.9	0.4	0.4
4	39.2 ±0.9	40.7 ±1.9	43.9 ±1.1	44.8 ±1.9	4.7	12.0	4.0	10.0
5	97.4 ±5.5	115.9 ±12.5	83.2 ±18.5	115.2 ±8.2	-14.2	-14.6	-0.6	-0.5
6	130.5 ±2.4	142.5 ±1.5	135.1 ±5.0	146.1 ±2.5	4.7	3.6	3.6	2.5
7	74.4 ±2.2	87.2 ±3.3	76.7 ±5.1	88.8 ±2.4	2.3	3.1	1.6	1.8
8	95.1 ±4.1	139.6 ±7.7	98.4 ±4.2	158.1 ±11.2	3.3	3.4	18.5	13.3
9	114.6 ±1.7	125.5 ±2.4	117.8 ±1.6	130.2 ±1.2	3.1	2.7	4.7	3.8
10	76.7 ±6.4	126.9 ±3.9	85.3 ±6.5	134.0 ±7.8	8.5	11.1	7.0	5.6
11	97.3 ±7.9	137.6 ±23.2	93.3 ±5.3	149.7 ±20.3	-4.0	-4.1	12.1	8.8
12	83.9 ±7.9	99.2 ±11.8	91.1 ±6.6	98.2 ±7.3	7.2	8.5	-0.9	-1.0
13	107.0 ±2.0	107.2 ±4.4	109.1 ±2.0	107.5 ±4.0	2.1	2.0	0.3	0.3
14	62.8 ±2.9	86.7 ±6.2	66.8 ±5.1	97.5 ±10.1	4.0	6.4	10.8	12.4
15	118.1 ±2.5	116.6 ±1.9	116.6 ±0.9	119.3 ±1.1	-1.5	-1.2	2.8	2.4
16	75.9 ±2.7	92.2 ±3.1	76.1 ±4.1	93 ±4.9	0.2	0.3	0.7	0.8
17	69.2 ±2.2	107.2 ±4.6	73.1 ±2.0	112.3 ±7.0	3.9	5.6	5.1	4.8
18	141.7 ±2.5	173.1 ±7.5	144.9 ±3.7	206.8 ±13.0	3.2	2.3	33.7	19.5
19	73.6 ±1.5	100.5 ±4.9	72.0 ±3.4	115 ±5.7	-1.7	-2.3	14.5	14.5
20	95.5 ±2.5	96.2 ±2.2	93.7 ±1.8	98.0 ±2.6	-1.9	-1.9	1.8	1.9
21	83.5 ±4.2	98.2 ±3.2	86.1 ±3.2	108.6 ±5.8	2.6	3.2	10.4	10.6
22	82.3 ±6.8	110.7 ±11.8	85.8 ±4.3	115.5 ±9.4	3.5	4.3	4.8	4.4
23	134.4 ±1.7	142.5 ±1.0	134.9 ±0.8	144.9 ±1.4	0.5	0.4	2.5	1.7
24	57.6 ±6.7	103.9 ±4.7	47.7 ±8.6	99.8 ±13.1	-9.9	-17.2	-4.1	-4.0
25	85.2 ±2.6	115.2 ±4.6	90.0 ±4.9	113.2 ±16.1	4.8	5.6	-2.1	-1.8
26	44.9 ±1.8	63.4 ±2.1	46.0 ±1.6	68.5 ±3.9	1.0	2.3	5.1	8.0
27	101.1 ±3.5	137.5 ±10.8	117.4 ±6.3	151.4 ±22.6	16.2	16.0	13.9	10.1
28	126.4 ±2.5	179.3 ±17.3	124.4 ±4.2	206.2 ±26.9	-1.9	-1.5	26.9	15.0
29	72.2 ±4.1	111.8 ±6.4	75.1 ±3.4	120.2 ±6.8	2.9	4.1	8.4	7.5
30	76.0 ±1.8	114.4 ±8.1	80.2 ±4.1	128.7 ±15.8	4.2	5.5	14.4	12.6
31	115.5 ±2.0	129.2 ±2.4	121.8 ±2.5	129.9 ±2.9	6.4	5.5	0.7	0.5
Group mean	***89.3 ±25.6***	***113.7 ±29.0***	***91.7 ±26.3***	***121.0 ±34.6***	***2.7 ±5.4***	***2.8 ±6.7***	***7.2 ±8.5***	***5.9 ±5.8***

Table 3.19. Individual stroke volume data and changes between normoxia and hypoxia

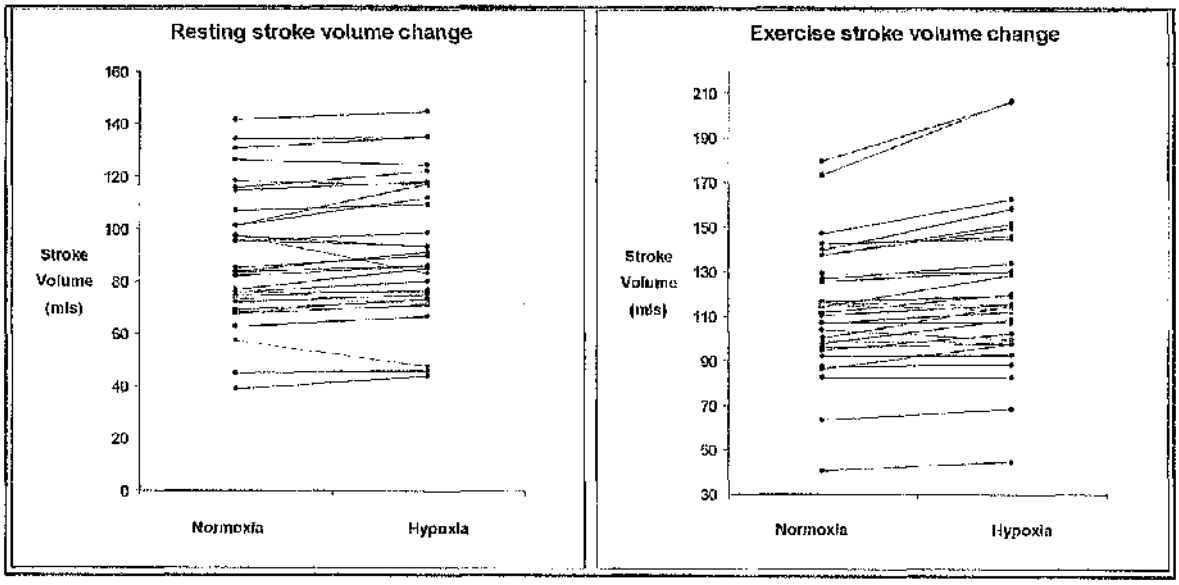

Figure 3.23 The change in stroke volume from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during exercise. The wide variation in response is seen as with the other plots. There were 6 subjects that demonstrated a decrease in SV from normoxia to hypoxia during rest and 4 different subjects decreased their SV during exercise (data sets in red).

3.4.5 Cardiac output

Cardiac output data for the subgroup is displayed in table 3.20 and figure 3.24. Complete data points were measured for the entire subject group (n=31). The mean CO during normoxic rest and exercise was; 6.44 l/min (SD 1.81) and 13.19 l/min (SD 3.40) respectively. The mean CO during hypoxic rest and exercise was; 7.74 l/min (SD 2.21) and 16.99 l/min (SD 4.56) respectively. The mean absolute change in CO at *rest* between conditions of normoxia and hypoxia was 1.30 (SD 0.79); the mean percent change was 20.6% (SD 11.7). The mean absolute CO change during *exercise* between conditions of normoxia and hypoxia was 3.90 l/min (SD 1.65); the mean percent change was 29.1% (SD 9.4). All CO responses demonstrated normality; paired student t-tests showed a highly significant difference at rest and during exercise, between normoxia and hypoxia (both p values <0.0001)

Subject	Normoxic rest (l/min)	Normoxic Exercise (l/min)	Hypoxic Rest (l/min)	Hypoxic exercise (l/min)	Δ Cardiac output (Rest)		Δ Cardiac output (Exercise)	
					l/min	*%*	*l/min*	*%*
1	6.11 ±0.3	12.15 ±0.6	8.09 ±0.5	15.07 ±0.7	1.98	32.38	2.91	23.97
2	8.9 ±0.7	20.92 ±0.8	10.84 ±0.7	27.4 ±2	1.94	21.85	6.47	30.94
3	5.48 ±0.2	9.11 ±0.8	6.31 ±0.3	11.54 ±0.7	0.83	15.15	2.42	26.61
4	2.6 ±0.2	4.75 ±0.3	3.36 ±0.1	6.32 ±0.3	0.76	29.25	1.57	33.15
5	6.26 ±0.4	11.79 ±1.4	6.23 ±1.4	14.91 ±0.9	-0.03	-0.53	3.12	26.44
6	7.28 ±0.4	14.84 ±0.4	9.13 ±1.0	19.7 ±0.4	1.85	25.37	4.86	32.77
7	6.29 ±0.2	11.87 ±0.6	7.29 ±0.3	14.21 ±0.4	1.00	15.98	2.34	19.72
8	6.12 ±0.5	14.0 ±0.9	6.95 ±0.8	19.48 ±1.4	0.83	13.59	5.48	39.11
9	7.7 ±0.5	14.65 ±0.4	9.33 ±0.6	18.74 ±0.4	1.63	21.14	4.10	27.96
10	5.95 ±0.4	14.28 ±0.5	7.94 ±0.7	17.98 ±1.1	1.99	33.45	3.70	25.90
11	5.87 ±0.4	12.33 ±2.1	6.33 ±0.5	15.5 ±2.2	0.47	7.98	3.17	25.76
12	7.34 ±0.7	12.23 ±1.3	7.46 ±0.5	14.61 ±1.1	0.12	1.59	2.38	19.45
13	8.08 ±0.5	12 12 ±0.8	9.7 ±0.4	15.77 ±0.6	1.62	20.00	3.65	30.12
14	5.39 ±0.5	11.08 ±0.8	7.12 ±0.5	15.7 ±1.9	1.73	32.10	4.62	41.71
15	10.82 ±0.5	15.83 ±0.3	13.02 ±0.4	20.96 ±0.6	2.19	20.25	5.13	32.41
16	5.93 ±0.2	10.69 ±0.4	7.07 ±0.5	13.39 ±0.7	1.14	19.28	2.70	25.28
17	5.1 ±0.2	14.41 ±0.7	7.01 ±0.4	17.62 ±1.7	1.92	37.56	3.22	22.32
18	7.79 ±0.6	16.7 ±0.9	10.09 ±1.1	24.39 ±1.4	2.29	29.4.0	7.69	46.05
19	5.32 ±0.3	10.97 ±0.6	5.99 ±0.4	14.59 ±0.7	0.68	12.72	3.62	33.01
20	7.72 ±0.3	14.89 ±0.4	9.19 ±0.4	17.18 ±0.5	1.46	18.95	2.30	15.43
21	4.47 ±0.3	7.94 ±0.3	5.77 ±0.5	11.39 ±0.8	1.30	29.09	3.45	43.50
22	5.45 ±0.6	10.84 ±1.7	6.87 ±0.6	14.46 ±1.5	1.42	25.99	3.62	33.39
23	11.03 ±0.9	18.64 ±0.4	11.8 ±0.6	21.16 ±0.2	0.77	6.97	2.52	13.51
24	5.49 ±0.6	13.55 ±0.7	4.77 ±0.8	15.33 ±2.1	-0.71	-12.99	1.78	13.14
25	5.48 ±0.4	12.96 ±0.4	7.01 ±0.5	15.38 ±2.4	1.53	27.89	2.43	18.72
26	3.73 ±0.3	8.57 ±0.2	4.65 ±0.3	11.28 ±0.6	0.92	24.66	2.71	31.61
27	7.78 ±0.5	17.74 ±1.7	10.74 ±0.8	25.17 ±3.6	2.96	38.11	7.43	41.90
28	6.77 ±0.3	18.71 ±1.8	8.78 ±0.4	25.58 ±3.5	2.02	29.80	6.88	36.76
29	5.87 ±0.4	12.83 ±0.6	6.64 ±0.4	16.23 ±1.0	0.77	13.09	3.40	26.53
30	4.22 ±0.3	11.57 ±1.0	4.98 ±0.2	17.09 ±2.1	0.76	18.00	5.52	47.72
31	7.26 ±0.6	15.92 ±0.5	9.58 ±0.7	18.57 ±0.3	2.32	31.93	2.65	16.62
Group mean	*6.44 ±1.81*	*13.19 ±3.40*	*7.74 ±2.21*	*16.99 ±4.56*	*1.30 ±0.79*	*20.6 ±11.7*	*3.80 ±1.65*	*29.1 ±9.4*

Table 3.20. Individual cardiac output data and changes between normoxia and hypoxia.

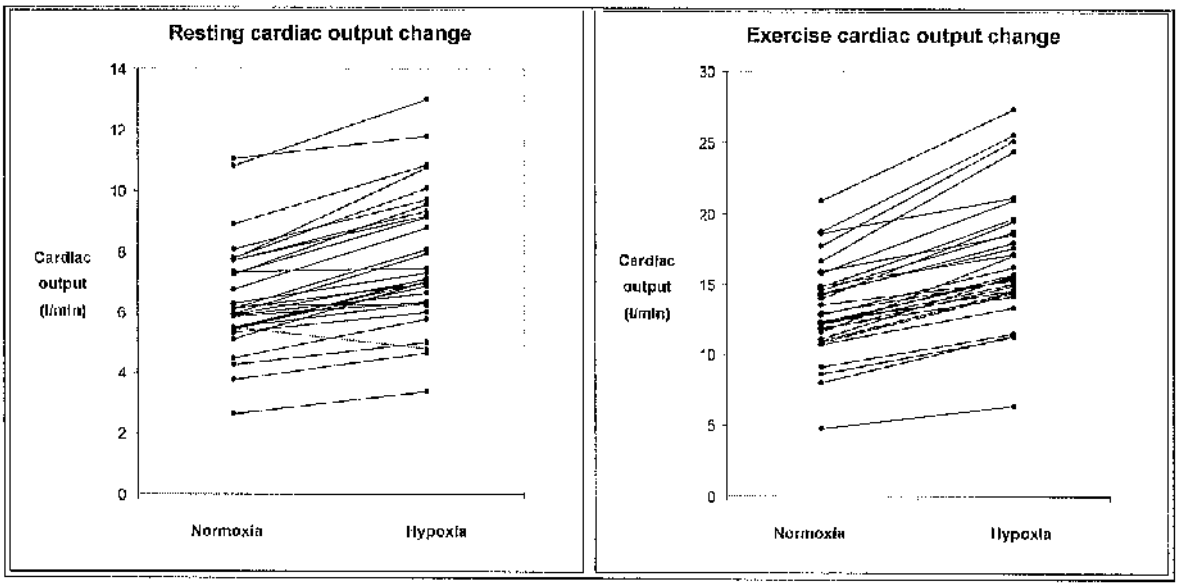

Figure 3.24 The change in cardiac output from normoxia to hypoxia. The panel on the left shows changes during rest; the panel on the right shows changes during exercise. The wide variation in response is seen as with the other plots. There were 3 subjects that demonstrated a decrease in CO from normoxia to hypoxia during rest (data sets in red), this may have been due to a degree of anxiety when being initially connected to the exercise equipment and circuit. All subjects demonstrated a rise in CO from normoxia to hypoxia during exercise.

3.4 ACE gene polymorphism and cardiopulmonary exercise results

The following section compares the CPET results previously presented by the ACE I/D genotype for each subject. The distribution of genotypes (displayed for each individual in table 3.2) was 14: II, 30: ID and 16: DD; this distribution is in Hardy-Weinberg equilibrium (χ^2=0.005). Table 3.20 summarises the biometric data for each subject group; furthermore, the distribution of age, weight and oxygen uptake between groups is similar. This was verified using ANOVA. The power calculation for the ANOVA analysis of the exercise ventilation results from the three groups allowing for a maximal standard deviation of 15.4 and the mean percent changes from normoxia to hypoxia from the three groups is 0.99.

Genotype	*II*	*ID*	*DD*
Number	14	30	16
Age (years)	23.6 (±4.7)	23.2 (±4.3)	24.7 (±6.1)
Height (cm)	178.3 (±8.3)	177.1 (±8.14)	178.5 (±7.9)
Weight (kg)	71.9 (±8.3)	73.4 (±11.7)	75.9 (±10.4)
VO_2 max (ml/kg)	55.9 (±13.2)	49.3 (±11.0)	52.8 (±11.6)
VO_2 at VT (ml/kg)	33.9 (±7.6)	29.3 (±9.1)	32.1 (±10.4)

Table 3.21 The physical characteristics of the subject groups by ACE genotype.

The following sections present the cardiopulmonary and bioimpedance results according to ACE genotype. The response to hypoxia during rest and exercise is compared between groups. The insertion homozygous group is denoted by the abbreviation II; the heterozygous and deletion homozygous groups are denoted by ID and DD respectively. The following sections have a similar layout and present the data in both graphic and tabulated form.

3.5.1 Oxygen uptake

The group oxygen data is displayed by ACE genotype in table 3.22 and figure 3.25. The absence of any significant change in percent VO_2 from normoxia to hypoxia during rest and exercise was verified by ANOVA analysis (p values are displayed on table 3.20 and graphically on figure 3.25).

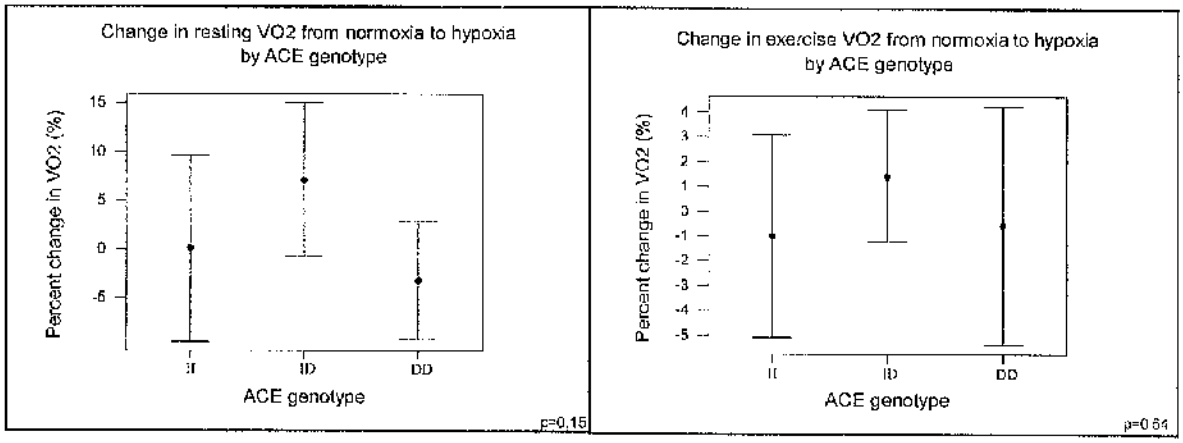

Figure 3.25 ANOVA analysis of change in VO_2 from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. Neither the response during rest or exercise demonstrates any significant difference between normoxia and hypoxia (ANOVA p values: rest p= 0.15, exercise p= 0.54).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting VO_2 (l/min)	Normoxia	0.42 ±0.12	0.40 ±0.10	0.42 ±0.08	**0.15**
	Hypoxia	0.41 ±0.10	0.42 ±0.09	0.40 ±0.06	
	Absolute change	-0.008 ±0.06	0.02 ±0.07	-0.02±0.05	
	Percent change (Δ%)	0.11 ±16.6	7.2 ±21.0	-3.2 ±11.5	
Exercise VO_2 (l/min)	Normoxia	1.59 ±0.45	1.64 ±0.41	1.66 ±0.36	**0.54**
	Hypoxia	1.56 ±0.43	1.64 ±0.36	1.63 ±0.27	
	Absolute change	-0.02 ±0.09	0.007 ±0.12	-0.03 ±0.16	
	Percent change (Δ%)	-1.0 ±7.1	1.42 ±7.16	-0.6 ±9.0	

Table 3.22 Oxygen uptake results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is denoted by the ± symbol. ANOVA p values are in the far right column.

3.5.2 Carbon dioxide production

Group data for VCO_2 is displayed by ACE genotype in table 3.23, and graphically on figure 3.26. There was no significant difference in VCO_2 from normoxia to hypoxia during rest or exercise by ACE genotype (ANOVA p values 0.24 and 0.67, respectively).

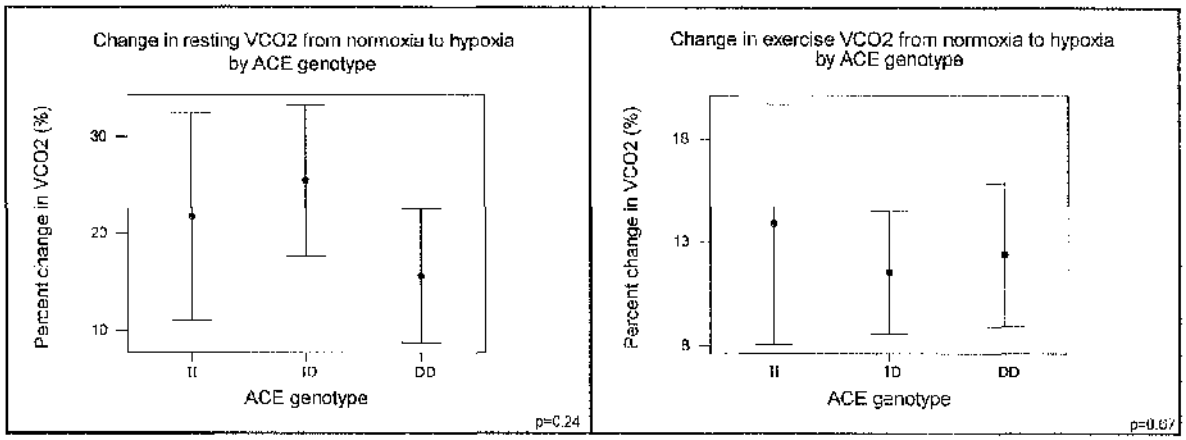

Figure 3.26 ANOVA analysis of change in VCO_2 from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. Neither the response during rest or exercise demonstrates any significant difference between normoxia and hypoxia (ANOVA p values: rest p= 0.24, exercise p= 0.67).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting VCO_2 (l/min)	Normoxia	0.35 ±0.10	0.33 ±0.09	0.35 ±0.07	**0.24**
	Hypoxia	0.426 ±0.155	0.40 ±0.09	0.40 ±0.07	
	Absolute change	0.08 ±0.08	-0.07 ±0.06	-0.05 ±0.04	
	Percent change (Δ%)	21.7 ±18.7	25.5 ±21.2	15.6 ±13.1	
Exercise VCO_2 (l/min)	Normoxia	1.35 ±0.10	1.41 ±0.34	1.44 ±0.23	**0.67**
	Hypoxia	1.55 ±0.48	1.60 ±0.36	1.62 ±0.26	
	Absolute change	0.20 ±0.17	0.17 ±0.11	0.18 ±0.09	
	Percent change (Δ%)	13.9 ±10.0	11.5 ±7.9	12.4 ±6.5	

Table 3.23 Carbon dioxide production results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is denoted by the ± symbol. ANOVA p values are in the far right column.

3.5.3 Oxygen saturation

Group data for oxygen saturation is displayed by ACE genotype in table 3.24, and graphically on figure 3.27. There was no significant difference in SaO_2 from normoxia to hypoxia during rest or exercise by ACE genotype (ANOVA p values 0.24 and 0.67, respectively).

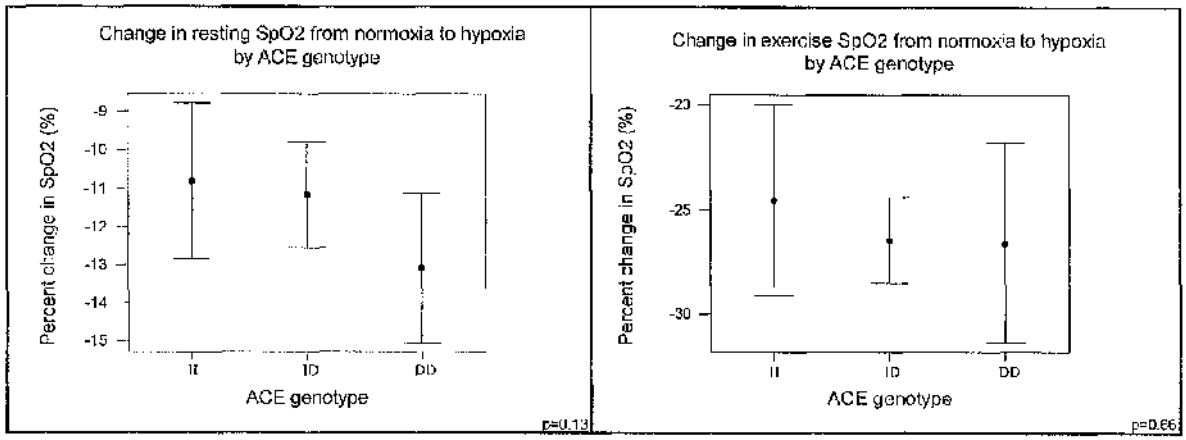

Figure 3.27 ANOVA analysis of change in SaO_2 from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. Neither the response during rest or exercise demonstrates any significant difference between normoxia and hypoxia (ANOVA p values: rest p= 0.13, exercise p= 0.66).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting SaO_2 (%)	Normoxia	95.7 ±1.1	95.8 ±1.5	96.5 ±1.3	**0.13**
	Hypoxia	85.3 ±1.1	84.3 ±2.3	83.8 ±3.8	
	Absolute change	-10.4 ±3.3	-10.7 ±3.5	-12.6 ±3.6	
	Percent change (Δ%)	-10.8 ±3.5	-12.3 ±3.6	-13.1 ±3.7	
Exercise SaO_2 (%)	Normoxia	94.3 ±1.1	94.8 ±1.6	95.2 ±1.8	**0.66**
	Hypoxia	71.2 ±7.6	69.1 ±5.3	69.8 ±7.7	
	Absolute change	-23.1 ±7.4	-27.33 ±14.	-25.4 ±8.9	
	Percent change (Δ%)	-24.6 ±7.9	-26.5 ±5.4	-26.6 ±9.0	

Table 3.24 Oxygen saturation results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is denoted by the ± symbol. ANOVA p values are in the far right column.

3.5.4 End-tidal carbon dioxide

End tidal carbon dioxide data is presented by ACE genotype on table 3.25 and figure 3.28. There was a significant difference by ANOVA analysis, with the insertion homozygous group demonstrating a greater decrease in $etCO_2$ during exercise (p=0.003)

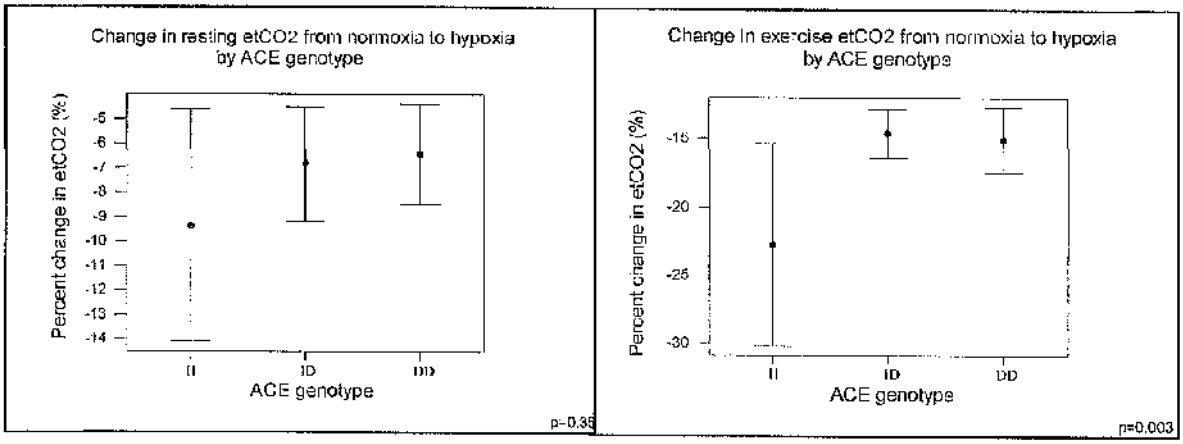

Figure 3.28 ANOVA analysis of change in $etCO_2$ from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. During exercise the II group demonstrated a greater fall in $etCO_2$ (p=0.003), no significant decrease was evident at rest (p=0.38).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting $etCO_2$ (kPa)	Normoxia	4.79 ±0.41	4.95 ±0.38	5.08 ±0.33	**0.38**
	Hypoxia	4.38 ±0.58	4.60 ±0.32	4.75 ±0.31	
	Absolute change	-0.42 0.37	-0.35 ±0.33	-0.33 ±0.20	
	Percent change (Δ%)	-9.0 ±7.7	-6.80 ±6.26	-15.1 ±4.6	
Exercise $etCO_2$ (kPa)	Normoxia	5.60 ±0.57	5.77 ±0.43	5.90 ±0.42	**0.003**
	Hypoxia	4.70 ±0.33	4.92 ±0.41	5.01 ±0.42	
	Absolute change	-1.62 ±1.87	-0.85 ±0.29	-0.89 ±0.28	
	Percent change (Δ%)	-22.2 ±12.1	-14.6 ±4.8	-15.1 ±4.5	

Table 3.25 End-tidal CO_2 results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is denoted by the ± symbol. ANOVA p values are in the far right column.

3.5.5 Respiratory rate

Group data for respiratory rate is displayed by ACE genotype in table 3.26, and graphically on figure 3.29. There was no significant difference in RR from normoxia to hypoxia during rest or exercise by ACE genotype (ANOVA p values 0.15 and 0.71 respectively); however there appears to be a trend towards a higher ventilation rate in the II-group.

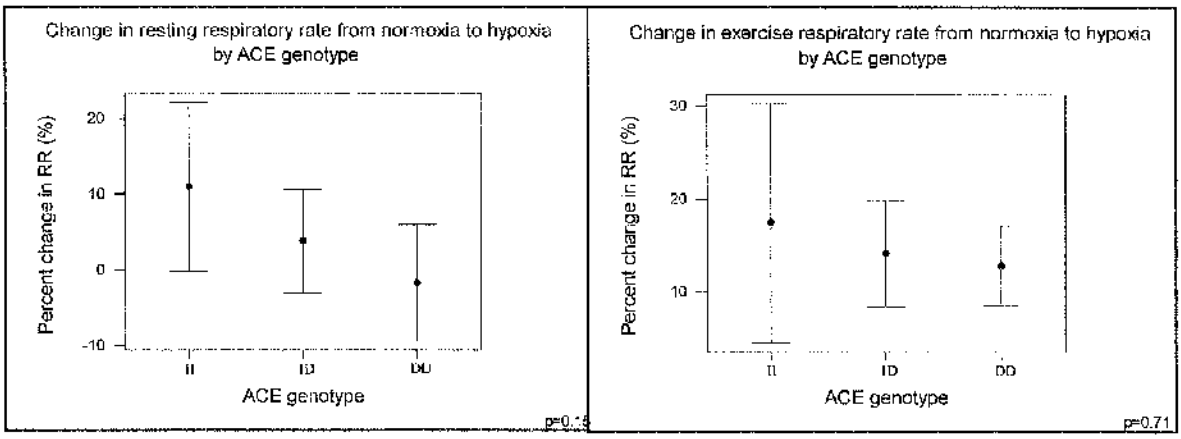

Figure 3.29 ANOVA analysis of change in respiratory rate from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. There appears to be trend toward a higher RR in the II group but this is not significant (rest: p=0.15, exercise p=0.71).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting RR (bpm)	Normoxia	14.7 ±3.2	15.3 ±2.9	14.8 ±3.3	**0.15**
	Hypoxia	16.0 ±3.1	15.7 ±3.2	14.5 ±3.8	
	Absolute change	1.3 ±19.4	0.4 ±2.6	-0.24 ±2.1	
	Percent change (Δ%)	11.0 ±19.4	3.8 ±18.3	-1.7 ±14.5	
Exercise RR (bpm)	Normoxia	19.9 ±3.0	21.0 ±4.0	21.6 ±5.0	**0.71**
	Hypoxia	23.1 ±4.0	23.7 ±4.8	24.2 ±5.5	
	Absolute change	3.2 ±3.8	2.8 ±2.7	2.7 ±1.8	
	Percent change (Δ%)	17.4 ±22.4	14.2 ±18.3	12.8 ±8.1	

Table 3.26 Respiratory rate results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is denoted by the ± symbol. ANOVA p values are in the far right column.

3.5.6 Tidal volume

Group data for tidal volume is displayed by ACE genotype in table 3.27, and graphically on figure 3.30. There was no significant difference in V_T from normoxia to hypoxia during rest or exercise by ACE genotype (ANOVA p values 0.86 and 0.55 respectively).

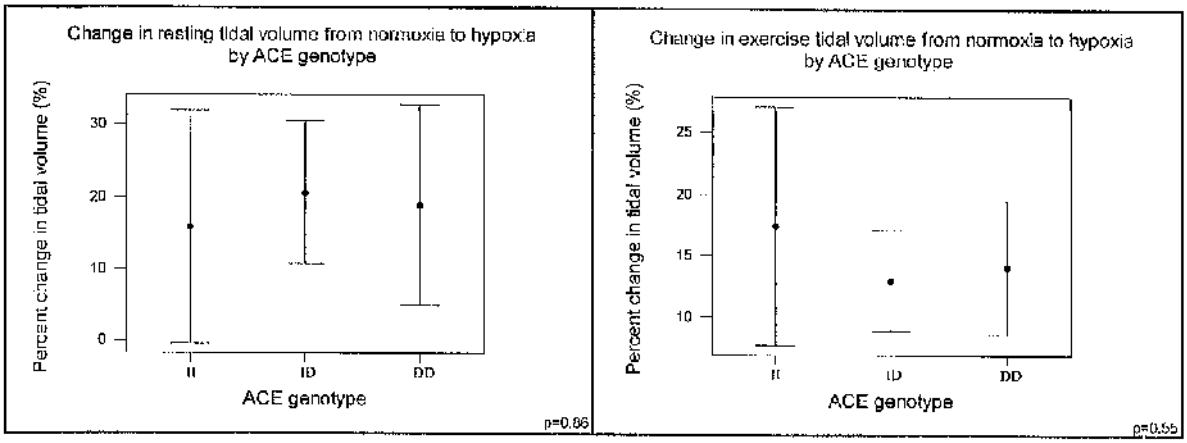

Figure 3.30 ANOVA analysis of change in tidal volume from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. Neither the response during rest or exercise demonstrates any significant difference between normoxia and hypoxia (ANOVA p values: rest p= 0.86, exercise p= 0.55).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting V_T (litres)	Normoxia	0.90 ±0.39	0.86 ±0.47	0.87 ±0.27	**0.86**
	Hypoxia	1.07 ±0.67	0.95 ±0.27	1.01 ±0.31	
	Absolute change	0.17 ±0.42	0.09 ±0.44	0.14 ±0.23	
	Percent change (Δ%)	15.8 ±25.1	20.5 ±26.7	18.9 ±26.3	
Exercise V_T (litres)	Normoxia	1.74 ±0.48	1.78 ±0.50	1.80 ±0.44	**0.55**
	Hypoxia	2.05 ±0.66	2.01 ±0.60	2.07 ±0.43	
	Absolute change	0.30 ±0.36	0.23 ±0.21	0.24 ±0.17	
	Percent change (Δ%)	17.4 ±16.8	13.0 ±10.9	14.0 ±10.2	

Table 3.27 Tidal volume results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is given after the ± symbol. ANOVA p values are in the far right column.

3.5.7 Minute ventilation

Minute volume data is presented by ACE genotype on table 3.28 and figure 3.31. There was a significant difference by ANOVA analysis, with the insertion homozygous group demonstrating a greater increse in V_E during exercise fron normoxia to hypoxia (p=0.003). There is no evidence of a co-dominant effect, since the heterozygous response is lower than the deletion homozygous group.

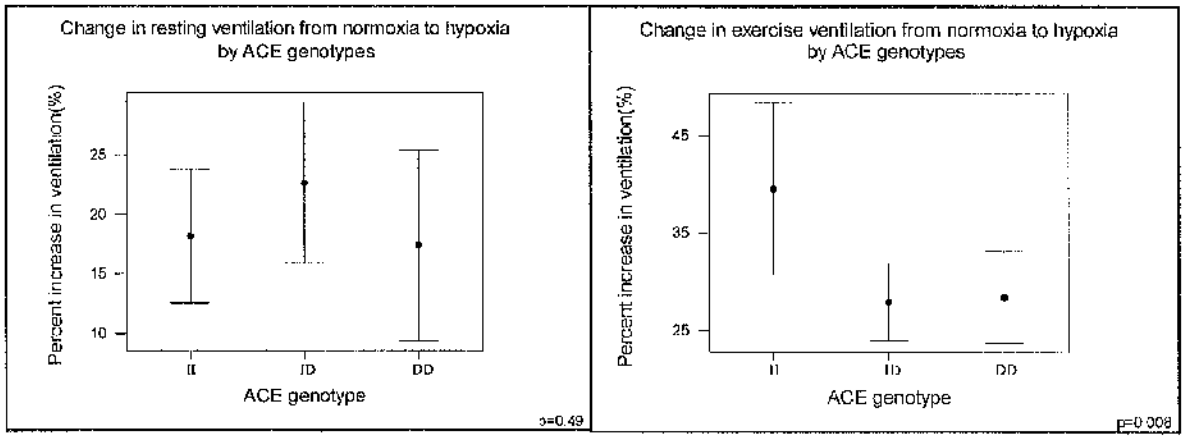

Figure 3.31 ANOVA analysis of change in V_E from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. During exercise the II group demonstrated a greater increase in V_E (p=0.008), no significant decrease was evident at rest (p=0.49). In addition, there is no evidence of a co-dominant effect with the heterozygous group.

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting V_E (l/min)	Normoxia	11.92±2.40	11.15 ±2.47	11.42 ±2.05	**0.49**
	Hypoxia	15.50 ±7.22	13.85 ±2.95	13.28 ±2.37	
	Absolute change	2.13 ±1.46	2.70 ±2.36	1.86 ±1.70	
	Percent change (Δ%)	18.2 ±9.3	23.6 ±17.7	17.4 ±15.2	
Exercise V_E (l/min)	Normoxia	33.87 ±8.58	35.53 ±6.60	36.10 ±5.59	**0.008**
	Hypoxia	47.35 ±13.60	45.40 ±9.98	46.57 ±9.60	
	Absolute change	13.50 +6.50	9.87 -4.15	10.51 +4.50	
	Percent change (Δ%)	39.6 ±15.4	27.9 ±10.7	28.4 ±8.9	

Table 3.28 Minute ventilation results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is given after the ± symbol. ANOVA p values are in the far right column.

3.5 ACE genotype and bioimpedance cardiac output results

The following section compares the bioimpedance cardiography results for the subgroup previously presented by the ACE I/D genotype for each subject. The distribution of genotypes (displayed for each individual in table 3.2) was 8: II, 16: ID and 7: DD; this distribution is in Hardy-Weinberg equilibrium ($\chi^2 = 0.03$). Table 3.29 summarises the age, heights and weight. The small size of the subject groups resulted in non-normal distribution; therefore the Kruskal-Wallis test, a non-parametric equivalent of ANOVA was used to confirm the absence of significant difference between the genotype groups for age, height and weight.

Genotype	*II*	*ID*	*DD*
Number	8	16	7
Age (years)	20.8 ±1.4	23.7 ±4.4	23.7 ±5.2
Height (cm)	173.0 ±8.42	173.9 ±7.6	178.1 ±8.1
Weight (kg)	67.56 ±12.56	71.98 ±10.6	72.8 ±10.4

Table 3.29 The physical characteristics of subjects included in the cardiac output studies, grouped by ACE genotype.

The results of the bioimpedance studies are laid out in a similar fashion to the previous sections. The normality of heart rate, stroke volume and cardiac output hypoxic response for each genotype group was verified during resting and exercise measurements, before the application of ANOVA testing.

3.6.1 Heart rate

Heart rate data for each genotype group is presented on table 3.30 and figure 3.32. There was no significant difference in heart rate response between the genotype groups during resting and exercise measurements by ACE genotype (ANOVA p values 0.94 and 0.75 respectively).

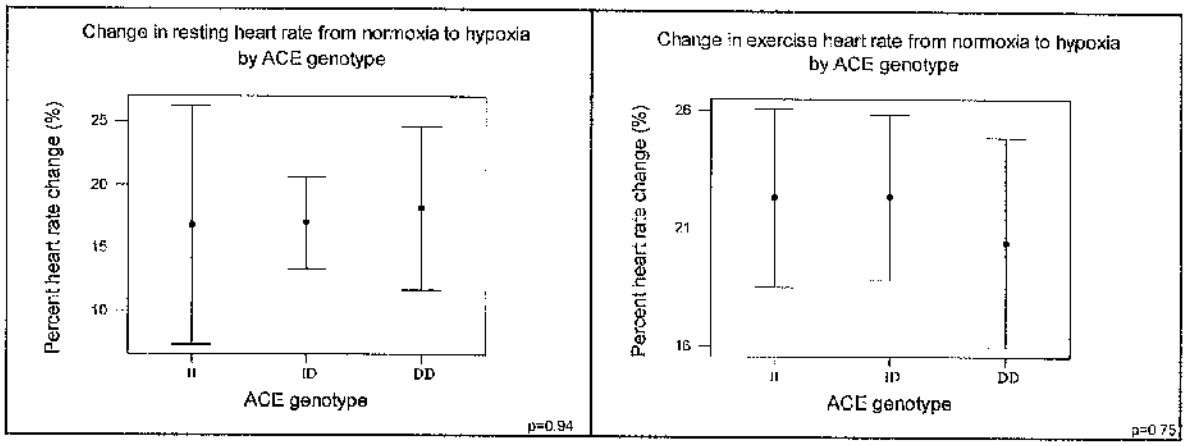

Figure 3.32 ANOVA analysis of change in heart rate from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. Neither the response during rest or exercise demonstrates any significant difference between normoxia and hypoxia (ANOVA p values: rest p= 0.94, exercise p= 0.75).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting Heart rate (bpm)	Normoxia	75.1 ±12.8	72.2 ±13.6	74.0 ±10.2	**0.94**
	Hypoxia	87.3 ±8.7	84.1 ±14.1	87.2 ±11.6	
	Absolute change	12.2 ±8.7	11.9 ±4.5	13.2 ±5.2	
	Percent change (Δ%)	16.8 ±11.4	17.0 ±6.8	18.2 ±7.0	
Exercise Heart rate (bpm)	Normoxia	116.1 ±10.2	115.5 ±20.6	121.5 ±13.5	**0.75**
	Hypoxia	142.0 ±12.7	140.8 ±22.7	146.0 ±14.6	
	Absolute change	25.8 ±5.5	25.2 ±7.1	24.5 ±4.8	
	Percent change (Δ%)	22.3 ±4.5	22.3 ±6.6	20.4 ±4.8	

Table 3.30 Heart rate results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is given after the ± symbol. ANOVA p values are in the far right column.

3.6.2 Stroke volume

Stroke volume data for each genotype group is presented on table 3.31 and figure 3.33. There was no significant difference in heart rate response between the genotype groups during resting and exercise measurements by ACE genotype (ANOVA p values 0.60 and 0.70 respectively).

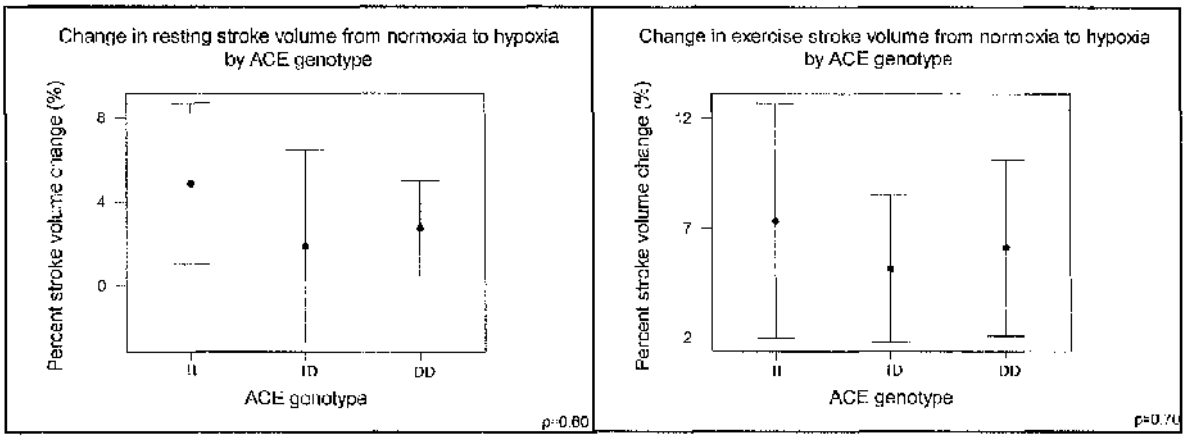

Figure 3.33 ANOVA analysis of change in stroke volume from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. Neither the response during rest or exercise demonstrates any significant difference between normoxia and hypoxia (ANOVA p values: rest p= 0.60, exercise p= 0.70).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting Stroke volume (mls)	Normoxia	82.3 ±27.2	95.7 ±23.1	82.8 ±29.4	**0.60**
	Hypoxia	85.4 ±25.6	97.7 ±25.0	85.1 ±30.5	
	Absolute change	3.1 ±2.9	2.0 ±7.4	2.3 ±2.1	
	Percent change (Δ%)	4.9 ±4.6	1.9 ±8.6	2.7 ±2.4	
Exercise Stroke volume (mls)	Normoxia	105.5 ±40.5	121.7 ±29.9	105.4 ±25.6	**0.70**
	Hypoxia	113.5 ±48.4	128.8 ±29.9	116.6 ±25.8	
	Absolute change	8.4 ±9.9	7.0 ±9.4	6.15 ±4.23	
	Percent change (Δ%)	7.3 ±6.4	5.2 ±6.3	6.1 ±4.4	

Table 3.31 Stroke volume results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is given after the ± symbol. ANOVA p values are in the far right column.

3.6.3 Cardiac output

Cardiac output data for each genotype group is presented on table 3.32 and figure 3.34. There was no significant difference in heart rate response between the genotype groups during resting and exercise measurements by ACE genotype (ANOVA p values 0.85 and 0.76 respectively).

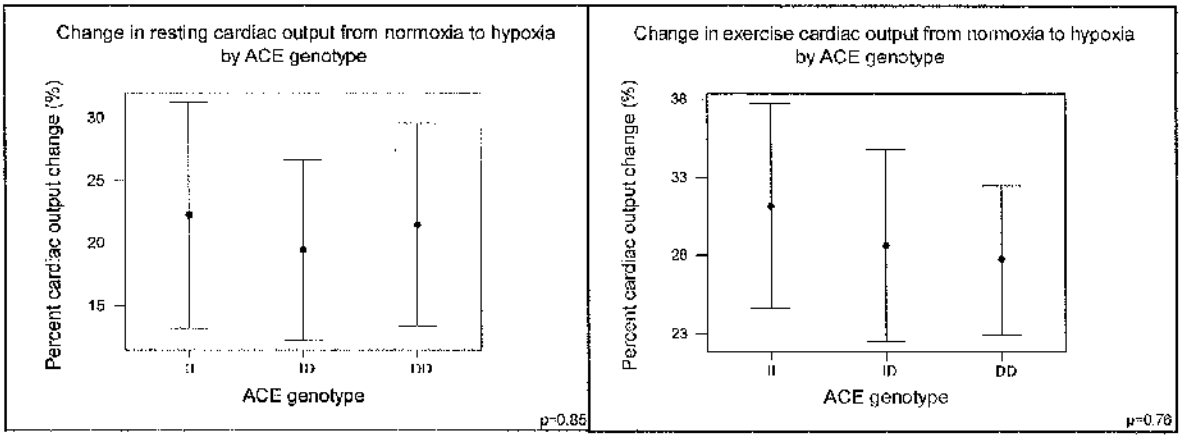

Figure 3.34 ANOVA analysis of change in cardiac output from normoxia to hypoxia. The left panel shows the response during rest; the right panel during exercise. The central point marks the group mean, the bars represent the 95% confidence interval for each group. Neither the response during rest or exercise demonstrates any significant difference between normoxia and hypoxia (ANOVA p values: rest p= 0.85, exercise p= 0.76).

Rest/ exercise		*II*	*ID*	*DD*	*ANOVA p value (Δ%)*
Resting Cardiac output (l/min)	Normoxia	6.04 ±1.63	6.87 ±2.04	5.90 ±1.35	**0.85**
	Hypoxia	7.31 ±1.87	8.22 ±2.56	7.15 ±1.66	
	Absolute change	1.27 ±0.68	1.34 ±0.96	1.25 ±0.53	
	Percent change (Δ%)	22.3 ±10.8	19.5 ±13.4	21.5 ±8.8	
Exercise Cardiac output (l/min)	Normoxia	11.97 ±3.86	12.59 ±2.30	7.31 ±1.86	**0.76**
	Hypoxia	15.74 ±5.44	18.02 ±4.68	16.05 ±2.93	
	Absolute change	3.77 ±1.77	3.96 ±1.89	3.46 ±0.84	
	Percent change (Δ%)	31.2 ±7.9	28.6 ±11.6	27.7 ±5.2	

Table 3.32 Cardiac output results grouped by ACE genotype. Group means are expressed as absolute values of baseline data and change from normoxia to hypoxia, in addition to percent change; standard deviation is given after the + symbol. ANOVA p values are in the far right column.

CHAPTER FOUR: DISCUSSION

CHAPTER FOUR: DISCUSSION

The studies in this thesis have examined the ventilatory and cardiac responses to acute hypoxia during rest and exercise in relation to the ACE insertion/deletion genotype. The larger study, examining the ventilatory response, has demonstrated a significantly higher ventilatory response to hypoxia during exercise in the insertion homozygote group; this finding may offer a possible explanation for the excess of insertion alleles in elite mountaineers and the higher oxygen saturation associated with the insertion allele during rapid ascents to altitude. However, the higher level of exercise ventilation in the insertion homozygous group was not matched by an intermediate level of ventilation in the heterozygous group, which demonstrated hypoxic ventilatory responses during exercise similar to those seen in the deletion homozygous group. The concurrent decrease in end-tidal CO_2 suggests that the ventilatory responses across the genotype groups are accompanied by an increase in alveolar ventilation. There was a diverse range of response in the patterns of breathing between individuals, which did not demonstrate any significant difference between genotype groups. Cardiac output responses to hypoxia did demonstrate expected patterns of response to hypoxia, with an increase in cardiac output, mediated predominantly by increase in heart rate under hypoxic conditions. An increased cardiac output was evident at rest and at equal work loads performed in hypoxic conditions in comparison with normoxic measurements, as expected; however, there was no significant difference in heart rate, stroke volume and cardiac output response between the three ACE genotype groups.

The higher hypoxic exercise ventilatory response seen in the insertion homozygous group (II-group) raises several questions: First, what was the mechanism of the increased response? Second, why was there no concurrent increase in oxygen saturation with the increase in ventilation seen in the II-group? Third, why was there no evidence of co-dominance in the hypoxic exercise ventilatory response seen between the three ACE genotype groups? Fourth, why was there no evidence of a similar ventilatory response during rest? And finally, why was there no evidence of a significant cardiac output response between the genotype groups? This chapter considers each of these questions, but begins by considering the important points in the study design, the subject characteristics and how the physiological responses in these studies compare with established models of physiological response to both hypoxia and exercise.

4.1 Study design and cardiopulmonary exercise test validation

4.1.1 Study design

The object of these studies was to compare the ventilatory and cardiac output response in normal healthy individuals under conditions of normoxia and then hypoxia during rest and exercise in relation to the insertion/deletion ACE gene polymorphism. Therefore the study was designed to minimise the possible interference with ventilatory measurements by lactate accumulation under hypoxic conditions, hence a constant load of 50% of the work at ventilatory threshold during maximal testing was chosen. This offered a reasonable level of work intensity under hypoxic conditions, whilst ensuring the subject did not pass the hypoxic ventilatory threshold. This was confirmed during the steady state exercise tests by real time monitoring of heart rate and ventilatory variables, including ventilatory equivalents for oxygen and carbon dioxide. Other studies, investigating hypoxic response during exercise have used different work loads: aimed toward a specific heart rate response [419, 420] or a percentage of maximal heart rate [421]; at low intensity, that would not induce lactate accumulation [422, 423]; at work loads 50% of that attained during maximum exercise [424] or based on predicted VO_2 max [425]. These studies were designed to examine different aspects of physiological response to hypoxia, including ventilation; however the aim of these studies was to examine the physiological responses in relation to a genetic polymorphism. From the outset, it was appreciated that the differences in physiological response between ACE genotype groups may have been quite subtle; therefore a precise measure of steady state exercise work load was deemed essential. For similar reasons, the physiological responses were compared in terms of percentage changes, in order to express the individual response in terms suitable for comparison; therefore establishing the ventilatory threshold and exercising at 50% work loads of the work at VT, meant that each individual was exercising within their own range. A possible criticism is that the VT represents a variable percentage of total work capacity as measured by VO_2 max; and that the actual work performed in hypoxic conditions may constitute a different level of work intensity between subjects, with some exercising at low rather than moderate intensities. The impact of this is difficult to assess, but hypoxia does have the effect of increasing the intensity of exercise when compared to normoxic conditions if the same degree of physiological stress is elicited (e.g. similar exercise heart rate responses) [426]. Therefore exercising at the same work load in hypoxic conditions would then place a work rate that might be perceived as low intensity in normoxia into the

moderate intensity. Any further increase in normoxic work load may push the hypoxic work intensity into the high range and lactate could accumulate. The method used ensured that individual subjects did not exercise above their *hypoxic* ventilatory threshold.

The choice of poikilocapnic hypoxia was also deliberate. The original reports of enhanced performance with the ACE I/D gene polymorphism under hypoxic conditions were at high altitude [326, 330, 331]; therefore, poikilocapnic hypoxic challenge was chosen to emulate the effects of altitude. This had the potential to interfere with the pure hypoxic response, but the aim of these studies was to examine any possible link between hypoxia, physiological response and the ACE genotype, including the effect of variable $PaCO_2$ upon hypoxic response.

4.1.2 The reproducibility of steady state tests and validation

Establishing the reproducibility of the steady state tests was also essential, in order to appreciate the small difference in physiological response that might otherwise be lost in poorly conducted tests with a wide margin of inter-test variability. The resting portion of the protocol, during both normoxia and hypoxia, did demonstrate a wider degree of variation in measured variables, both during the test and between repeated testing. This in part could be attributed to subject awareness and possible anxiety during rest; however the difference in measured response at rest during the validation studies was within 10%. The repeatability of ventilatory and VO_2 measurement during the exercise portion of the protocol was much closer with differences between tests to within 6%, which is the degree of acceptable inter-test variability for calibration purposes of cardiopulmonary exercise equipment [389]. The degree of variability during exercise was less than resting measurements; this is an expected finding since interference from distracting factors or hyperventilation is much less once attention is fixed during exercise. The repeatability of tests was also demonstrated by the Bland-Altman plots, which show inter-test variability within 2 standard deviations for both VO_2 and minute ventilation during exercise with the majority of responses during rest demonstrating a similar degree of agreement. Familiarisation with the test surroundings and equipment are strategies used to reduce the degree of subject anxiety during studies; this was achieved by conducting the hypoxic testing in the same surroundings as the maximal test (performed during the first visit) and allowing a period of familiarisation with the hypoxic circuit during the resting period.

A further measure of quality control is demonstrated by the relatively small degree of variation in VO_2 between the normoxic and hypoxic exercise tests for all the subjects. Since both the steady state tests were performed at the same work load, the VO_2 should theoretically be the same. The majority of subjects demonstrate similar VO_2 responses between the two parts of the protocol, with a reduced degree of variation during exercise, as seen in the repeatability studies. The degree of variation falls largely within the acceptable limits of repeatability, but there are two other considerations that might have had an influence. Firstly, the two steady state tests were conducted at the same visit, consecutively with a short period between the tests, this would entail 16 minutes of exercise in total, albeit at low to moderate exercise intensity. This raises the possible of increased VO_2 during the second test due to a raised body temperature and increased blood flow to the skin for thermoregulation [171]. The second consideration is the increased ventilation during hypoxia and the increased oxygen demands of the respiratory muscles, one study examining the effects of hypoxia on VO_2 suggested the effect was appreciable, but difficult to quantify [427]. Both of these factors could contribute to the variation in VO_2 between tests; however, the rest period between steady state test should have allowed sufficient cooling time and the sub-maximal intensity of exercise should have reduced the difference between normoxic and hypoxic oxygen uptake by respiratory muscles.

4.2 Subject group

The subject group was mainly comprised of students from the University of Glasgow with some members of staff from the medical department in the Glasgow Western Infirmary and officers from the Glasgow University Officer training corp. This is reflected in the skewed distribution of ages towards the younger end of the range. The height range was slightly skewed towards the higher end of the range; this may reflect the disproportionate number of male subjects. The weight distribution demonstrated a normal distribution. The mean VO_2 max in the subject group reflected the higher degree of fitness in a group of motivated individuals that undertake regular exercise; this was one prerequisite of the study, due to safety and ethical considerations of exercise under hypoxic conditions. The fitness of the subject group is also evident from the participation across a broad range of sports activities; furthermore, the degree of participation varied, with some individuals competing at a national level in some sports. The spirometric data did not reveal any cases of undiagnosed asthma or

air flow limitation, the wide range of measured spirometric values were within predicted values for each individual, with many subjects demonstrating supra-normal values.

The subject group was entirely Caucasian in ethnicity; this was intentional since both the distribution and effects of the ACE gene polymorphism can differ with ethnicity. The original work describing the variation in ACE activity between genotype was measured in a Caucasian population [312] and subsequent studies have demonstrated less of a difference in ACE activity between genotype groups in black populations [428]. Furthermore, the distribution of ACE genotype in different ethnic groups may not be in Hardy-Weinberg equilibrium [429]. The variation in response to ACE inhibition in different ethnic populations is also well documented, with Afro-American and Afro-Caribbean populations demonstrating a reduced response to ACE inhibitors [430-432]; furthermore, the decreased response has been linked with reduced vascular reactivity and response to bradykinin [433]. Therefore the choice of a Caucasian population was designed to maximise any effect ACE genotype may exert on cardiorespiratory response.

The distribution of ACE genotypes was in Hardy-Weinberg equilibrium. These individuals demonstrated a similar distribution of baseline physical characteristics between genotype groups; furthermore the level of fitness, as measured by VO_2 max was also similar. Previous studies examining the association of the ACE gene polymorphism and physical performance have shown an increased frequency of the insertion allele in endurance athletes, suggesting a link between the I-allele and endurance performance. There was no ACE allele disequilibrium in the VO_2 max distribution of the sample group with the distribution of VO_2 max demonstrating a similar distribution between genotype groups. This might reflect the wide range of physical activities undertake by the subject group, at differing degrees of involvement, with only some activities classed as endurance sports. The original link between enhanced performance during hypoxia was in a group of mountaineers. Mountaineering requires endurance and power (as well as mental stamina); therefore is not strictly a homogenous group in terms of endurance activity. Furthermore, comparisons between mountaineers, sedentary controls and long distance runners have shown that the VO_2 max in climbers was between that of sedentary individuals and runners. In addition, the maximum anaerobic power in climbers was the same as sedentary controls and 40% less than long distance runners [434]. This would suggest that mountaineers are distinct from endurance athletes in terms of physical performance and that any observed benefit from possessing the I-allele is only evident at altitude and under conditions of hypoxia. This might

explain why the differences in the physiological response between genotype groups seen in these studies were only apparent under hypoxic conditions.

4.3 Responses in metabolic variables during hypoxia and exercise

The responses in individual VO_2 between normoxia and hypoxia, at rest and during exercise, have been discussed previously. This section examines the response of VCO_2, $etCO_2$ and SpO_2 during the steady state tests and the responses from normoxia to hypoxia.

4.3.1 Carbon dioxide production

The production of CO_2 as measured during the tests reflects the metabolic production of CO_2, but also the degree of ventilation that eliminates CO_2 from the body. As reviewed previously, ventilation is closely coupled to arterial CO_2 tensions and ventilatory changes are coupled to CO_2 production during exercise in normoxia. In hypoxic conditions it is hypoxaemia and the effects on the carotid body that are the dominant stimulus to ventilation; consequently, CO_2 elimination is increased and is in excess of metabolic production, with a concomitant reduction in arterial CO_2 tensions. Therefore VCO_2 is increased during acute hypoxic exposure. This is what essentially happened across the whole subject group, except during the resting period, where 6 individuals demonstrated a higher VCO_2 during normoxic rest in comparison to hypoxic conditions. Hyperventilation during the initial stages of the steady state protocol could explain the higher than expected ventilation during normoxia, since higher cortical stimulation of ventilation may have provided unexpectedly high ventilation because of mild anxiety; the ventilatory measurements confirmed the presence of a relative hyperventilation in these subjects. There was wide range of individual responses to hypoxia during both rest and exercise, with a wider degree of variability during resting measurements as compared to exercise measurements

4.3.2 End-tidal CO_2

End-tidal CO_2 is dependent on the rate of CO_2 production and the alveolar ventilation. The use of $etCO_2$ as a measure of alveolar ventilation has been reviewed previously and though it is not as accurate as invasive determination of alveolar ventilation, it is a useful indicator. The majority of subjects demonstrated a decrease in $etCO_2$ from normoxic to hypoxic conditions, as expected when ventilatory drive became dependent on hypoxia. This was the case for all exercise measurements, but 5 subjects demonstrated a lower $etCO_2$ during

normoxia than during hypoxia. These subjects demonstrated a degree of hyperventilation as described previously, the 6th individual did not decrease his $etCO_2$ the reason for this may have been due to a higher level of initial V/Q inequality that may have impeded the elimination of CO_2.

4.3.3 Oxygen saturation

Finger pulse oximetry demonstrated a reduction in oxygen saturation upon hypoxic exposure that intensified during exercise. This is to be expected as oxygen extraction from exercising muscles further reduces the degree of hypoxia at rest, induced by diffusion limitation in the lung. There was a wide range of resting and exercise hypoxic responses with marked inter-subject variability. The oximetry values were slightly lower than anticipated during exercise (resting 95.9%, SD 1.4; exercise 94.8%, SD 1.6). The reasons for this may have been due to motion artefact or malpositioning of the oximeter during exercise (despite the best efforts of the investigator). Another factor was the circulation to the finger probe during exercise; some subjects insisted on gripping the handle bars of the ergometer very tightly (despite being encouraged to relax), thus constricting the circulation in the fingers. The finger probe was chosen because of better accuracy [399]; however experiments with ear probes did not overcome these problems because of motion artefact. The range of desaturation during exercise also draws into question the accuracy of the oximetry data, since over half the subjects dropped their SpO_2 to less than 70%, below the range of reliable measurement by non-invasive methods (rest 84.4%, SD 2.1; exercise 69.8%, SD 2.5) [394, 395].

4.4 Ventilatory responses to hypoxia and exercise

One of the primary objectives of the studies presented in this thesis was to examine the ventilatory response to hypoxia during rest and exercise. The measured minute ventilation, respiratory rate and tidal volume demonstrated a wide range of individual variation across rest and exercise during normoxia and hypoxia. Furthermore, the degree of change between normoxia and hypoxia was also varied between subjects. The increase in ventilation was mediated by a mixed pattern of increased respiratory rate and tidal volume. The features of the absolute response to hypoxia are discussed in this section, in addition to the effects of altered patterns of ventilation on end-tidal CO_2.

4.4.1 Minute ventilation

Qualitatively there was a wider variation in resting minute ventilation values in comparison to exercise values and was reflected in the higher ventilation seen in six subjects during normoxic rest in comparison to resting hypoxic minute ventilation. This could only be attributed to hyperventilation during this phase, despite familiarisation with the surroundings during the first visit and a period of familiarisation with the circuit prior to data collection. A possible explanation is a degree of self awareness during the initial part of the protocol which abated once distracted by the onset of exercise. Whether the resting hypoxic response is consistent with other studies is problematic, since there is a wide inter-subject variation in HVR and the ventilatory response varies with the degree of hypoxia. Similarly, during exercise the degree of ventilatory response is variable with the degree of hypoxia and the intensity of exercise. Therefore, it is difficult to make comparisons between the various combinations of different levels of hypoxia and exercise used by previous studies [419-424, 435].

4.4.2 Tidal volume, respiratory rate and patterns of breathing

A wide range of individual response was evident for both tidal volume and respiratory rate across the subject group, with a mixture of respiratory rate and tidal volume increases. The resting values demonstrated a wider range of variation as seen with the other variables (V_T: normoxic mean 0.87 litres, SD 0.40; hypoxic mean 0.99 litres, SD 0.40. RR: normoxic mean 15.0 bpm, SD 3.1; hypoxic mean 15.4 bpm, SD 3.3). Twenty four of the sixty subjects had a higher respiratory rate during normoxic rest than during hypoxia; similarly, 14 subjects had a higher tidal volume during normoxic vs. hypoxic rest. The range of variability was reduced during exercise measurements (V_T: normoxic mean 1.78 litres, SD 0.47; hypoxic mean 2.02 litres, SD 0.57. RR: normoxic mean 20.9 bpm, SD 4.2; hypoxic mean 23.7 bpm, SD 4.8). Seven subjects demonstrated a higher exercise respiratory rate response during normoxia in comparison to hypoxia; in addition, 7 different subjects demonstrated a higher tidal volume during normoxic exercise than during normoxic exercise. As described previously, the overall ventilation during exercise was invariably higher during hypoxic exposure; therefore it appears the contribution of increased RR and V_T to increased V_E had a demonstrable individual variability. Dead space ventilation is increased by the effects of exercise and hypoxia on V/Q inequality; however, in the absence of reliable measures of V_D, it is difficult

to assess the effect that individual variability of V_T and RR might have had upon the efficiency of ventilation.

The use of $etCO_2$ as an index of alveolar ventilation has been reviewed and was used to examine any relationship between ventilatory variables and alveolar ventilation. The total minute ventilation during normoxia did not demonstrate any correlation at rest or during exercise with $etCO_2$, as one would expect. In normoxia, ventilation and CO_2 production are closely coupled, and maintains a stable intravascular CO_2 tension and pH during the dynamic changes in CO_2 production caused by exercise; this would explain this finding. The responses under hypoxic conditions demonstrated a significant negative correlation between hypoxic ventilation and $etCO_2$ during both rest and exercise, again this is expected. Under hypoxic conditions, ventilatory drive is dominated by arterial O_2 tension; therefore the higher ventilatory rate is reflected in increased CO_2 excretion, lower arterial CO_2 and since $etCO_2$ is dependent on $PaCO_2$, a reduction in $etCO_2$. There was no relationship between tidal volume and $etCO_2$ during normoxic rest; furthermore, the removal of one outlier (who appeared to have a degree of hyperventilation) from the resting hypoxic data produced an insignificant correlation between V_T and $etCO_2$. The response to exercise was somewhat different; the normoxic exercise V_T had a significant positive correlation with $etCO_2$, whereas the hypoxic exercise V_T did not demonstrate any correlation. This is an unexpected finding which is difficult to explain. One possible explanation may lie with the respiratory rate response to exercise and hypoxic exercise. The respiratory rate during both normoxic and hypoxic exercise demonstrated a significant negative correlation with $etCO_2$, with the hypoxic exercise response demonstrating a stronger negative correlation ($R= -0.40$ vs. $R= -0.46$, normoxia and hypoxia respectively). Exercise in normoxia, results in an increase both V_T and RR from resting values, stimulated by CO_2 produced as a consequence of increased metabolism; in addition, CO_2 tensions tend to increase tidal volume by changes in both T_I and T_E. The positive correlation between etCO2 and V_T seen during normoxic exercise may reflect these factors. During hypoxic exercise, the drive to ventilation is dependent on hypoxaemia and arterial CO_2 tensions decrease. The diminished contribution of $PaCO_2$ to ventilatory drive may explain the reduced correlation between $etCO_2$ and V_T during hypoxic exercise. A further factor is the steeper response in respiratory rate seen during hypoxic exercise in comparison with normoxic measurements, which may reflect a stronger stimulus to RR change due to the combined effects of hypoxia and exercise.

The correlation analyses of ventilatory variable and $etCO_2$ do not provide any clear indication of the contribution of increased V_D, due to increased V/Q inequalities, upon alveolar ventilation. This may be due to the confounding effects of altered V/Q states with hypoxic exposure upon $etCO_2$ as a marker of alveolar ventilation or that the hypoxic stimulus to ventilation masks any negative affects of increased V/Q mismatch upon $etCO_2$ and alveolar ventilation.

4.5 Responses in cardiac variables during hypoxia and exercise

The investigation of cardiovascular responses to hypoxia and exercise in relation to the ACE gene polymorphism was one of the primary objectives of this thesis; however, the practicalities of implementing and evaluating a useful physiological measurement modality meant that bioimpedance cardiography was introduced into the study protocol after the ventilatory studies had commenced. As a consequence 31 subjects were included in the study of cardiac response to hypoxia and exercise.

4.5.1 Bioimpedance validation studies

Prior to using the Physioflow bioimpedance equipment for exercise testing, the validity of measured cardiac output (CO) response was validated against thermodilution measurements of CO in 4 patients undergoing right heart catheterisation. The results demonstrated a reasonable degree of agreement for individual measurements of cardiac output; the degree of agreement between the two methods was greatly improved by taking the average of at least 3 measurements. The steady state measurements of cardiac output variables during the different parts of the test protocol were mean measurements over a 90 second period in order to provide a steady state measurement of CO, but also to improve the fidelity of CO measurements as much as possible.

4.5.2 Heart rate, stroke volume and cardiac output

Cardiac output increased upon hypoxic exposure during rest in 28 of the 31 subjects. Three subjects had an elevated heart rate during normoxic rest and this was reflected in a cardiac output higher than hypoxic rest values. This may have been due to a degree of anxiety; however these subjects did not exhibit any evidence of hyperventilation during simultaneous measurement of their ventilatory response. The cardiac output response during exercise was invariably positive, but as with the other measured variables demonstrated a wide range of individual response. The increase in cardiac output was expected, as a means of maintaining

oxygen delivery in the face of reduced oxygen content of blood (due to the combined effects of diffusion limitation at the lung and increased O_2 extraction during exercise). The rise in CO was predominantly due to increases in heart rate response during rest and exercise from normoxia to hypoxia (Δ rest: 12.3bpm SD 5.6: Δ exercise: 25.2bpm SD 6.1). Stroke volume had a variable degree of change between normoxic and hypoxic conditions during both rest and exercise with some individuals decreasing their stroke volume during hypoxic exposure; however, despite the statistical significance of the difference, the actual difference between normoxia and hypoxia was comparatively small (Δ rest: 2.7 mls SD 5.4; Δ exercise: 7.2 mls SD 8.5) and could be accounted for by the variation in measurement. Two subjects during measured exercise response from normoxia to hypoxia had a marked increase in SV, but the remaining 29 subjects had little demonstrable change; this is expected since SV increases early in the range of capable exercise, with heart rate sustaining further increases in CO as oxygen demand increases.

4.5.3 Summary

The mechanisms underlying the response to hypoxia and exercise involve the interaction between ventilatory response, gas exchange, the convective processes of circulation and oxygen carriage and utilisation at the tissue level. The basic results of the ventilatory and cardiac studies highlight the individual variability in response to the hypoxic stimulus across several physiological variables; furthermore, many of the responses in one system are inherently dependent upon the response in another (c.f. cardiac output and oxygen uptake). In addition to variables that are examined during these studies there are many other factors that have a bearing on hypoxic response. Muscle fibre composition has a profound effect on the stamina of a muscle and has a direct bearing upon the onset of lactate accumulation. Substrate utilisation has a direct effect on the burden of oxygen demand of exercise, with carbohydrate proving more efficient in terms of oxygen usage. Certain physiological responses have a reproducible pattern (such as ventilation), distinct to an individual, which may be encoded within their genes. Any genetic influence on the observed physiological response to hypoxia is almost certainly polygenic and involves multiple gene loci; some of the crucial biochemical pathways involved have hypoxia dependent transcription of proteins crucial to their function. Furthermore, the identification of a functional genetic polymorphism in the ACE gene has been linked with endurance performance. The studies described in this thesis have demonstrated a significant difference in the ventilatory response to hypoxia and

exercise that may contribute to the observed benefit of the insertion allele of the ACE gene polymorphism under hypoxic conditions encountered at high altitude.

4.6 Cardiopulmonary responses to hypoxia and the ACE gene polymorphism

The ventilatory studies demonstrated an enhanced ventilatory response to hypoxic exercise in a group of individuals homozygous for the insertion ACE gene polymorphism. However, the studies also demonstrate two conflicting responses; there was no evidence of increased oxygenation in the insertion group in comparison to the ID or DD- groups and there was no evidence of codominance. These points are discussed in detail in addition to the most important question: what is the mechanism of increased ventilatory response in the insertion homozygous groups?

4.6.1 The possible mechanisms of enhanced ventilation in the II group

The control of ventilation, as we have seen, is dependent on sensation at the peripheral and central chemoreceptors and the subsequent relay of neural afferents from these areas to the ventilatory motor areas in the ventral medulla. The nucleus of the tractus solitarius in the dorsomedial medulla is the first synaptic relay between the peripheral chemoreceptors and the ventilatory pattern generators centres. During normoxia, CO_2 is the dominant stimulus to ventilation and the production of CO_2 is closely coupled to ventilation during exercise. However, under conditions of hypoxia, hypoxaemia is the dominant stimulus and ventilation is closely linked to the intensity of both exercise and hypoxia in these circumstances. A further point of complexity is the effects of changing CO_2 during hypoxic exercise and that reduced $PaCO_2$ might attenuate the hypoxic ventilatory response. There is a wide range in the individual hypoxic ventilatory response, both at rest [47, 436] and during exercise [437]. Furthermore, studies in monozygotic twins suggests there is a genetic component that underlies the individual hypoxic ventilatory response [438-440].

The studies presented in this thesis demonstrate an enhanced ventilatory response in the insertion homozygous group in comparison to the other genotypes. The increase in ventilation was seen during hypoxic exercise, but was not accompanied by an increase in oxygen saturation. This may have been due to technical issues or to the possibility of increased V/Q inequality in the II-group, these points are discussed in detail further in this chapter; however, the increased ventilation does suggest increased hypoxic ventilatory sensitivity during exercise in the II-group. The increase in ventilation was accompanied by a

decrease in $etCO_2$ which suggests increase in alveolar ventilation; this has two implications. First, due to the effects of Dalton's law of partial pressures, the reduced alveolar P_ACO_2 implies an increase in the alveolar P_AO_2; furthermore, the reduction in alveolar P_ACO_2 as a consequence of hypoxia induced hyperventilation is one factor that made the Ascent of Mount Everest without supplementary oxygen physiologically possible by allowing a higher P_AO_2 than was previously thought. The second consideration is that the reduced $etCO_2$ implies a reduction in arterial $PaCO_2$; therefore, the increase in ventilation in the II-group occurred against a relative hypocapnia and a reduction in the CO_2 contribution to ventilatory response; this was while there was no significant difference in CO_2 production between genotype groups.

The precise mechanisms underlying this response are more difficult to elucidate; both the carotid body glomus cells and the nTS have local renin-angiotensin systems, as demonstrated by the presence of RAS components. The local RAS in the carotid bodies has a stimulatory function on the glomus cells. Angiotensin-2 causes Ca^{2+} influx into glomus cells via effects on AT_1Rc [269] and can heighten the hypoxic sensitivity of glomus cells by inhibiting K^+ efflux and facilitating depolarisation [273]. The reduction in ACE activity evident in II individuals should attenuate this response and have negative effect on hypoxic ventilatory response. In contrast, the nTS appears to have a negative response to AT-2, as demonstrated by an inhibition of phrenic nerve discharge with the direct microinjection of AT-2; therefore, a reduction in ACE activity would have an enhancing effect upon hypoxic ventilatory response at the nTS (figure 4.1). Of course the ventilatory control system has many other constituent centres and pathways, many of which have yet to be elucidated, and the effects of local RAS at any other putative ventilatory pathways remains to be identified; however, these studies do highlight the difference in AT-2 response in at least two points in the ventilatory control mechanism. Furthermore, the effect of exercise in this system appears to have an amplifying effect on the response since there was no evidence of a similar effect during rest. This could have been due to the wider variability evident in the resting measurements, which may have obscured a smaller response during rest; the increased variability may have reduced the power of these studies and resulted in failure to detect any significant difference in response between the genotype groups. There was a trend towards a higher respiratory rate in relation to the I-allele; however, this failed to reach significance due to wide inter-subject variability. Increasing the sample size in combination with further measures to reduce

hyperventilation during the resting part of the studies may prove the presence of a similar hypoxic ventilatory response during rest.

The absence of a co-dominant effect on ventilatory response

The differences in response to AT-2 at different points in the ventilatory control pathway may explain the absence of a co-dominant effect, since the conflicting effects of ACE gene polymorphism upon hypoxic ventilatory response may have altered the expected occurrence of an intermediate response in the heterozygous group. The difference in site specific ACE response may have been further modified by the effect of other neurotransmitters at both the carotid body and the brainstem.

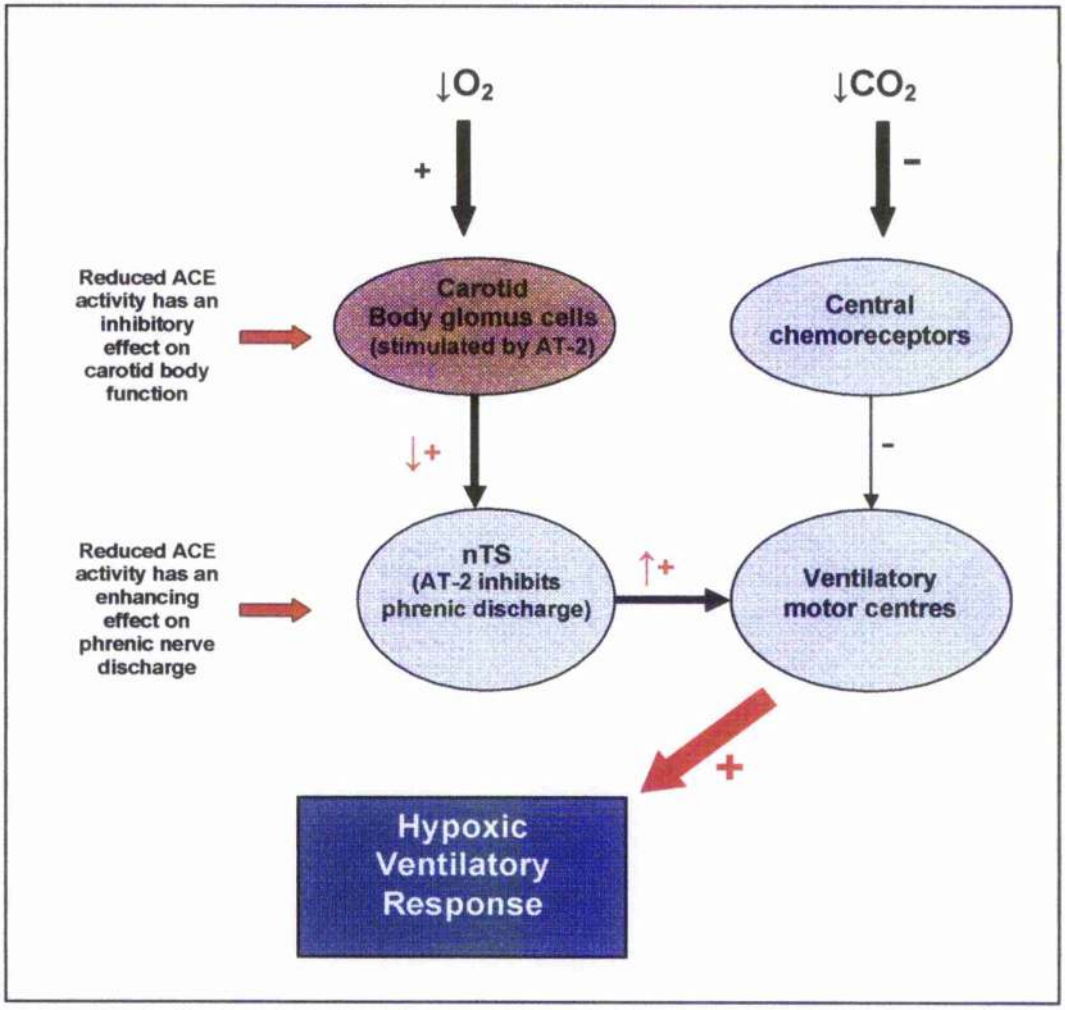

Figure 4.1 The interaction of the peripheral and central chemoreceptors in response to hypoxia, hypocapnia and the effects of local RAS. The proposed effects of a reduction in

ACE activity, as seen in insertion homozygotes are shown by the red arrows and notation. The reduced stimulatory effect at the carotid body is shown by the ↓+ symbol; the enhanced phrenic nerves discharge by the ↑+ symbol. The effect of reduced CO_2 is also shown.

A second possible explanation is the tissue dependent variability in the co-dominant effect seen in some cells; an exclusively homozygous dependent increase in ACE activity has been described in monocytes, with high ACE activity only seen in deletion homozygotes [441]. Although, no direct influence of such a mechanism in the brain exists the occurrence of this example does set a precedent. A third possibility is that the observed effect may not be linked to ACE genotype, but to an unknown genetic factor that is in linkage disequilibrium with the ACE gene. To-date no other candidate gene has been located in proximity to the ACE gene locus on chromosome 17 that might explain this effect.

Until the mechanisms of carotid body function in man and the functional aspects of central ventilatory control are clarified it is difficult to establish with any certainty the mechanism of enhanced ventilatory response hypoxic exercise response in insertion homozygotes.

4.6.2 Ventilatory and oxygen saturation responses between ACE genotypes

The increase in hypoxic ventilation during exercise seen in the insertion homozygous group was not accompanied by an increase in oxygen saturation. Enhanced ventilation should have improved alveolar oxygen partial pressures and this is certainly supported by the decrease in end-tidal CO_2; therefore, the question remains: why was there no concurrent increase in oxygen saturation? There are two possible explanations: firstly, the fidelity of pulse oximetry at the oxygen saturations experienced during hypoxic exercise may have fallen below the technical limits of the oximeter (SpO_2: II 71.2% SD 7.6; ID 69.1% SD 5.3; DD 69.8 SD7.7). The use of arterial blood sampling would have been a preferable measure of oxygenation, but informal discussions during the study development determined that this would be ethically dubious in the context of healthy subjects and that permission would be denied. The second explanation, assuming the fidelity of the oximetry measurements, was a relative increase in ventilation to perfusion mismatch in the II-group, such that there was no detectable benefit of increased ventilation on oxygenation. This seems less likely: firstly, the increase in alveolar P_AO_2 by enhanced ventilation would optimise gas exchange by matching alveolar ventilation to pulmonary blood flow. Secondly, the genotype dependent decrease in $etCO_2$, which was

commensurate with increases in ventilation suggest that changes in total ventilation were matched by an increase in alveolar ventilation. Finally, the evidence in the field, does not support such a fundemental impedance to function at high altitude. The original association between ACE gene polymorphism and high altitude performance demonstrated significant excess frequency of the insertion allele in climber who had successfully summited some of the highest mountains in the world [326]. Furthermore, a more recent study has confirmed the excess in I-allele in climbers at lower altitudes, suggesting that this is a fundamental effect evident over a range of hypoxic exposures [330]. The direct effect of ACE genotype on physiological response was demonstrated by Woods et al; they showed an insertion allele dependent improvement in oxygenation in a group of climbers during a rapid ascent to 5100m; these measurements were at rest, but represent the first direct evidence of advantageous physiological response in association with the I-allele during hypoxia. Although these studies support the advantageous influence of the ACE insertion allele, the possible effect of V/Q inequality as a factor in the acute response to hypoxia needs to be excluded by further investigation. Proper invasive measurement of oxygenation may reveal improved oxygenation masked by the poor accuracy of oximeter readings. Arterial sampling would also permit the direct measurement of deadspace ventilation and with sufficient resolution to elucidate differences in V/Q inequalities between genotype groups. Non-invasive measures of V_A and therefore V_D using extrapolations from $etCO_2$ are unreliable in situations where there may be a wide V/Q mismatch; this has been demonstrated in patients with various cardiopulmonary conditions during exercise [163].There are other non-invasive methods for ascertaining the degree of ventilation to perfusion inequality: multiple inert gas elimination techniques (MIGET) utilise the differential dissociation of 6 inert gases from solution into the gaseous phase. Infusions of these gases in solution are introduced into the subject and the varying rates of elimination in the expired gas are measured, usually by mass spectrometry [442]. This technique has been used to assess V/Q inequalities in several pulmonary conditions, including pulmonary embolic disease [443] and could shed light on any differences in V/Q matching by genotype; furthermore, MIGET has been used to examine response during hypoxia and exercise [419, 444]. The requirement of peripheral venous access makes this less attractive for study in large groups of healthy individuals and the limited availability of suitable equipment is a further restriction on the use of this technique.

4.6.3 ACE gene polymorphism and pulmonary vascular effects

The effect of reduced ACE activity in the pulmonary endothelium is difficult to assess, not least because of the complexity of the RAS and the interaction with bradykinin. Angiotensin-2 is generated by ACE and is a potent pulmonary vasoconstrictor and pulmonary vascular ACE expression is increased in response to hypoxia [229, 230]; furthermore ACE inhibition and AT_1Rc antagonists attenuate the hypoxic pulmonary vascular response in humans. This suggests the possible benefit of reduced ACE activity and AT-2 generation could be via the ameliorating effect on the HPVR and the disparate vasoconstrictor response that is evident during ambient exposure. An exaggerated hypoxic pulmonary vascular response is one factor in the pathogenesis of HAPE and though there is no evidence that ACE genotype is associated with the development of HAPE it is possible that impairment of gas diffusion as a consequence of subclinical pulmonary oedema could occur. One study in Japanese HAPE patients reported higher pulmonary vascular resistance in deletion homozygotes when compared to insertion homozygotes during pulmonary catheterisation shortly after evacuation from high altitude [332]; this suggests a hyperresponsive pulmonary vascular response in deletion homozygotes. Exercise elicits further increases pulmonary artery pressures during hypoxia, which are also exaggerated in HAPE susceptible individuals [445]. There have been no studies that have demonstrated any association with ACE polymorphism, HPVR and hypoxic exercise; however, one study examining the effect in chronic obstructive pulmonary disease (COPD) has shown an association with heightened pulmonary artery pressure in patients homozygous for the deletion allele during exercise.

The studies in this thesis were not structured to examine these elements of hypoxic response, but the physiological responses to hypoxia and demonstrable effects of the ACE polymorphism on pulmonary vascular responses in HAPE sufferers and in patients with COPD suggest a link between the two. This could provide a benefit at altitude that contributed to the observed disequilibrium in ACE genotypes, with the association of the I-allele and enhanced altitude performance.

4.6.4 Cardiac output response and ACE genotype

The response in bioimpedance cardiac output results at rest and during exercise from normoxia to hypoxia did not demonstrate any significant difference between genotype

groups. A similar lack of response was seen in stroke volume and heart rate variables. The increase in cardiac output was predominantly due to an increase in heart rate with small increases in stroke volume. The resting response between normoxia and hypoxia demonstrated a smaller degree of change in HR, SV and CO; furthermore, the resting SV response was equivocal, with some subjects demonstrating a slight decrease in SV from normoxia to hypoxia. The increase in cardiac output between conditions of normoxia and hypoxia was to be expected since this is one of the compensatory mechanisms that allow the maintenance of oxygen delivery in the face of reduced oxygen content of blood; this is a consequence of reduced oxygen saturation caused by diffusion limitation at the lung and increased oxygen extraction during exercise. The dominance of heart rate change in this response is also expected since stoke volume increases to maximum early in the range of exercise and the changes in intravascular volume seen with prolonged hypoxic exposure at altitude would not have had time to develop during an acute challenge. The increase in heart rate between normoxic and hypoxic conditions during rest and exercise did not demonstrate any significant difference between the genotype groups. The increase in stroke volume during exercise could reflect the increase in sympathetic nerve activity seen during hypoxic exposure; this produces a vasoconstrictor effect that offsets the vasodilator effect of hypoxia in the systemic circulation and maintains blood pressure during hypoxic exposure. It is possible that increased vasomotor tone in the capacitance vessels could have produced a marginal, but significant increase in venous return and preload to the ventricle; however there was no significant difference between the genotype groups.

The absence of significant differences in stoke volume and heart rate response between the groups suggests that there was no significant difference in sympathetic nervous activity in response to hypoxia as a result of the ACE gene polymorphism. To date, there have been no published reports of enhanced hypoxia induced sympathetic nerve activation in relation to the ACE gene polymorphism. There was a small trend towards a higher cardiac output response during exercise in the insertion homozygous group (Δ exercise cardiac output: II 31.2%, SD 7.9: ID 28.6%, SD 11.6: DD 27.7%, SD 5.2); this raises the possibility of a significant difference, which was overlooked because of the limited size of this study and lack of power. Based on the degree of change in CO between the three genotype groups and the maximal variation within the groups a study aimed at a power level of 0.95 using ANOVA analysis would require a minimum of 316 subjects in each genotype group. This is of course assuming there is an underlying association between the insertion allele and enhanced cardiac output

response during hypoxic exercise. The evidence supports the opposite position in normoxic conditions with increased left ventricular mass in deletion homozygotes and increased VO_2 max in Caucasian subjects after a regimented course of physical training [323, 446]. As previously discussed the subjects had a wide range of physical activities and did not have a standardised exercise regimen; therefore, a lack of variation in cardiac output response is not unexpected. However, these two studies were conducted under normoxic conditions and as we have seen, the effects of hypoxia have elucidated ventilatory changes in a group of subjects with a background of heterogeneous physical activity; therefore, it may be that there *is* an underlying ACE associated effect that the current study is underpowered to detect. A further consideration is the utilisation of oxygen by muscle itself and the effects this might have on cardiac output.

4.6.5 Cardiac output and muscle

Cardiac output is closely coupled to oxygen consumption, as reviewed previously; however the fibre composition of muscle has a direct bearing on the oxidative capacity of muscle and oxygen utilisation. Muscle displays marked plasticity in response to exercise and hypoxia both in terms of switching of fibre type and changes in size and oxidative apparatus. The duration of hypoxic exposure and exercise is too brief to allow any such changes to influence the outcome of these experiments; however muscle substrate utilisation is one site amenable to acute change. ACE inhibition appears to enhance glucose utilisation in exercising muscle via a bradykinin/ NO dependent mechanism [303] and glucose metabolism has a reduced oxygen cost in stochiometric terms. This provides a possible mechanism for insertion genotype associated decrease in ACE activity to exert a beneficial effect during acute hypoxia. This is purely theoretical and has yet to be demonstrated experimentally.

The ACE polymorphism has other demonstrable influences on muscle function; an enhanced anabolic response in response to exercise regimens [447]. Furthermore, the ACE insertion allele is associated with increased muscle efficiency in response to army basic training [327]. Muscle efficiency is expressed as delta efficiency, which is the percentage ratio of change in work performed per minute to the change in energy expenditure (usually above that required for unloaded exercise) i.e.

$$\text{Delta efficiency} = \frac{\text{Change in work accomplished (joules/min)}}{\text{Change in energy expended (joules/min)}} \times 100$$

This is calculated by performing serial steady state exercise regimens whilst measuring work load and oxygen uptake. The change in energy expenditure can then be calculated using a theoretical-thermodynamic approach based on a ratio of 11.0 kilocalories per mole of ATP hydrolysed. The original ratio of 3 ATP per molecule of oxygen consumed was used by Gaesser and Brooks when the method was first proposed [448]: however this is thought to be an over estimate and the ratio is now thought to be 2.5 [449]. The work performed is a known variable since it is set on the ergometer. The effect demonstrated was after a training program with no prior genotype dependent difference in efficiency.

4.6.6 Study findings in relation to prolonged hypoxia

The discussion thus far has focused on the results of studies conducted in acute hypoxic conditions; this has inherent implications in terms of the mechanisms that are involved in the physiological response. The time scale of these experiments would have excluded factors that are important considerations when examining physiological responses during prolonged exposures to hypoxia at altitude. The effects on muscle function and metabolism; cardiac output adaptation; increase in oxygen carriage capacity of blood through the effects of erythropoesis; adaptation in the pulmonary circulation to optimise V/Q matching and the changes in ventilation over time are all effects that influence performance at altitude. These studies have demonstrated a direct link between the insertion homozygous genotype of the ACE gene and a physiological response to ambient hypoxia of any duration. It remains to be seen whether the increase in exercise ventilation is a fundamental feature of insertion homozygosity or whether this response fades in intensity over prolonged periods of hypoxic exposure. However, a feature of successful performance at altitude is the rate of acclimatisation during rapid ascents; furthermore, many of the pathological conditions at altitude are precipitated by going too high, too quickly. The work by Woods et al. has demonstrated that ACE insertion allele dependent improvement in oxygen saturation is only seen during rapid ascents. Therefore, the enhanced ventilatory response seen in the insertion

homozygous group could be perceived as one factor in favour of enhanced performance from the earliest point of hypoxic exposure and activity.

4.6.7 Study limitations

The limitations of the studies performed in this thesis have been mentioned in the course of the discussion and include: the lack of reliable oximetry measurement; the lack of arterial blood sampling, which would have given accurate PaO_2 measurements and a method of measuring V_D/V_T; a more controlled familiarisation with the equipment to avoid increased variability resting measurements and an increase in subject numbers to improve the power of bioimpedance cardiac output studies.

Further important limitations of the studies performed centre on the size of the subject group in the context of a genetic study. The lack of significant differences in the other physiological variables studied, most notably the cardiac output, may reflect a lack of sufficient power to elucidate any significant differences and would require a much larger sample size. Furthermore, a different study design may have offset some of the limitations of sample size. The sample group demonstrated a quite varied pursuit of physical activities, not all of them in the endurance disciplines. The beneficial effect of the ACE insertion allele at altitude may differ from its normoxic effects, but to date the beneficial effects of the ACE insertion allele has been seen in endurance atheletes and in response to a period of physical training. A case control study comparing the effects of hypoxic exercise in endurance atheletes versus controls by genotype may offer a better insight into the mechanism of the beneficial effects of the ACE genotype. Another factor which could be addressed by an alternate study design is the effect of ACE genotype on muscle efficiency at altitude and the effect this might have on enhanced performance during rapid altitude ascents. A longitudinal study at altitude examining the effect of training at a set altitude could offer an insight into these effects.

The limited size of the sample group also raises the possibility that the observed effect was a product of chance and a larger sample would refute the findings; this is always a consideration in small genetic studies, particularly when comparisons are being made between more than two groups. The p-values for these studies were not corrected for multiple comparisons and this is another limitation of these studies. A final point is that the original study was designed in 1999, at that time there were relatively limited numbers of genotypes that were available for study (Nitric oxide synthase was of particular interest at that time), unfortunately we neither had the ability to study these genotypes nor the manpower to

perform further genotyping. Modern techniques allow the genotyping of hundreds of genes simultaneously. DNA microarrays offer an insight into RNA expression at the cellular level, but have been adapted to the genotyping of single nucleotide polymorphisms. In combination with the increasing numbers of genetic polymorphisms and the completed Human Genome project there is now the potential to apply techniques to investigate variation in physiological responses that to date have been used to investigate the genetic basis of diseases such cancer and systemic hypertension.

4.7 Future work

The experiments performed during this thesis were during acute hypoxic exposures in poikilocapnic conditions. Future studies should aim to confirm these responses during prolonged hypoxic exposure, preferably at high altitude during the first few days of hypoxic exposure designed to examine the longitudinal effects over time. Furthermore, experiments in isocapnic hypoxia should be performed, to elucidate the peripheral chemoreceptor element to the ventilatory response seen during exercise in isolation from central chemoreceptor effects. Modification of the protocol to address the limitations identified would clarify some of the experimental findings and an increase in study numbers may reveal any cardiac output response in association with the ACE genotype. Finally there is now the scope for examining numerous genes simultaneously in combination with further candidate genes that offer an insight into who will cope well with the stresses of altitude or who might succumb to the effects of high altitude disease.

4.8 Conclusion

The experiments in this thesis have demonstrated repeatability and agreement with recognised measurement modalities during validation studies. The subject responses are in accordance with expected patterns of cardiopulmonary response to hypoxia and exercise. In relation to the ACE gene polymorphism, an increased ventilatory response has been demonstrated in a group possessing the insertion homozygous genotype during an individually standardised exercise test from conditions of normoxia to hypoxia. This could offer an advantage during initial exposure through an enhanced ventilatory response during exertion in hypoxic conditions; furthermore the increased exercise ventilation may contribute to the enhanced performance at altitude attributed to the insertion allele, as evident by the excess I-allele in those successful in ascending to the highest summits in the world.

REFERENCES

1. West, J.B., *The physiologic basis of high-altitude diseases.* Ann Intern Med, 2004. **141**(10): p. 789-800.
2. Ganong, W.F., *Review of medical physiology.* 2005, Lange.: Los Altos, Calif.,. p. v.
3. Saraste, M., *Oxidative phosphorylation at the fin de siecle.* Science, 1999. **283**(5407): p. 1488-93.
4. Hinkle, P.C., et al., *Mechanistic stoichiometry of mitochondrial oxidative phosphorylation.* Biochemistry, 1991. **30**(14): p. 3576-82.
5. Laghi, F. and M.J. Tobin, *Disorders of the respiratory muscles.* Am J Respir Crit Care Med, 2003. **168**(1): p. 10-48.
6. Cotes, J.E., D.J. Chinn, and M.R. Miller, *Lung function : physiology, measurement and application in medicine.* 6th ed. 2006, Malden, Mass.: Blackwell Pub. xi, 636 p.
7. Loeschcke, H.H., *Central chemosensitivity and the reaction theory.* J Physiol, 1982. **332**: p. 1-24.
8. Gourine, A.V., et al., *ATP is a mediator of chemosensory transduction in the central nervous system.* Nature, 2005. **436**(7047): p. 108-11.
9. Mulkey, D.K., et al., *Respiratory control by ventral surface chemoreceptor neurons in rats.* Nat Neurosci, 2004. **7**(12): p. 1360-9.
10. Richerson, G.B., *Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis.* Nat Rev Neurosci, 2004. **5**(6): p. 449-61.
11. Nattie, E.E., *Central chemosensitivity, sleep, and wakefulness.* Respir Physiol, 2001. **129**(1-2): p. 257-68.
12. Nattie, E.E., et al., *Rostral ventrolateral medulla muscarinic receptor involvement in central ventilatory chemosensitivity.* J Appl Physiol, 1989. **66**(3): p. 1462-70.
13. Prabhakar, N.R., *Oxygen sensing by the carotid body chemoreceptors.* J Appl Physiol, 2000. **88**(6): p. 2287-95.
14. Fitzgerald, R.S., M. Shirahata, and H.Y. Wang, *Acetylcholine release from cat carotid bodies.* Brain Res, 1999. **841**(1-2): p. 53-61.
15. Kumar, G.K., J.L. Overholt, and N.R. Prabhakar, *Oxygen sensing : responses and adaptation to hypoxia.* Lung biology in health and disease ; v. 175, ed. S. Lahiri, G.L. Semenza, and N.R. Prabhakar. 2003, New York: Marcel Dekker. pp421-438.
16. Rong, W., et al., *Pivotal role of nucleotide P2X2 receptor subunit of the ATP-gated ion channel mediating ventilatory responses to hypoxia.* J Neurosci, 2003. **23**(36): p. 11315-21.
17. Weir, E.K., et al., *Acute oxygen-sensing mechanisms.* N Engl J Med, 2005. **353**(19): p. 2042-55.
18. Buckler, K.J., et al., *The role of TASK-like K+ channels in oxygen sensing in the carotid body: Signalling pathways in acute oxygen sensing.* Novartis Foundation symposium ; 272. 2006, Chichester: Wiley. pp73-85.
19. Buckler, K.J. and R.D. Vaughan-Jones, *Effects of hypoxia on membrane potential and intracellular calcium in rat neonatal carotid body type I cells.* J Physiol, 1994. **476**(3): p. 423-8.
20. Pardal, R., et al., *Secretory responses of intact glomus cells in thin slices of rat carotid body to hypoxia and tetraethylammonium.* Proc Natl Acad Sci U S A, 2000. **97**(5): p. 2361-6.
21. Montoro, R.J., et al., *Oxygen sensing by ion channels and chemotransduction in single glomus cells.* J Gen Physiol, 1996. **107**(1): p. 133-143.

22. Mills, E. and F.F. Jobsis, *Mitochondrial respiratory chain of carotid body and chemoreceptor response to changes in oxygen tension.* J Neurophysiol, 1972. **35**(4): p. 405-28.
23. Lopez-Barneo, J., et al., *Oxygen-sensing by ion channels and mitochondrial function in caortid body glomus cells: Signalling pathways in acute oxygen sensing.* Novartis Foundation symposium ; 272. 2006. Chichester: Wiley. pp54-64.
24. Searle, G.J., et al., *Lack of contribution of mitochondrial electron transport to acute O(2) sensing in model airway chemoreceptors.* Biochem Biophys Res Commun, 2002. **291**(2): p. 332-7.
25. Halliwell, B. and J.M. Gutteridge, *Role of free radicals and catalytic metal ions in human disease: an overview.* Methods Enzymol, 1990. **186**: p. 1-85.
26. Wang, D., et al., *NADPH-oxidase and a hydrogen peroxide-sensitive K+ channel may function as an oxygen sensor complex in airway chemoreceptors and small cell lung carcinoma cell lines.* Proc Natl Acad Sci U S A, 1996. **93**(23): p. 13182-7.
27. Fu, X.W., et al., *NADPH oxidase is an O2 sensor in airway chemoreceptors: evidence from K+ current modulation in wild-type and oxidase-deficient mice.* Proc Natl Acad Sci U S A, 2000. **97**(8): p. 4374-9.
28. Lopez-Barneo, J., et al., *Regulation of oxygen sensing by ion channels.* J Appl Physiol, 2004. **96**(3): p. 1187-95; discussion 1170-2.
29. Chandel, N.S. and P.T. Schumacker, *Cellular oxygen sensing by mitochondria: old questions, new insight.* J Appl Physiol, 2000. **88**(5): p. 1880-1889.
30. Prabhakar, N.R., et al., *Reactive oxygen species facilitate oxygen sensing: Signalling pathways in acute oxygen sensing.* Novartis Foundation symposium ; 272. 2006, Chichester: Wiley. xi, 288 p.
31. Prabhakar, N.R. and J.L. Overholt, *Cellular mechanisms of oxygen sensing at the carotid body: heme proteins and ion channels.* Respir Physiol, 2000. **122**(2-3): p. 209-21.
32. Jordan, D. and K.M. Spyer, *Brainstem integration of cardiovascular and pulmonary afferent activity.* Prog Brain Res, 1986. **67**: p. 295-314.
33. Housley, G.D. and J.D. Sinclair, *Localization by kainic acid lesions of neurones transmitting the carotid chemoreceptor stimulus for respiration in rat.* J Physiol, 1988. **406**: p. 99-114.
34. Ang, R.C., B. Hoop, and H. Kazemi, *Role of glutamate as the central neurotransmitter in the hypoxic ventilatory response.* J Appl Physiol, 1992. **72**(4): p. 1480-7.
35. Mizusawa, A., et al., *In vivo release of glutamate in nucleus tractus solitarii of the rat during hypoxia.* J Physiol, 1994. **478 (Pt 1)**: p. 55-66.
36. Lin, J., et al., *Effect of N-methyl-D-aspartate-receptor blockade on hypoxic ventilatory response in unanesthetized piglets.* J Appl Physiol, 1996. **80**(5): p. 1759-63.
37. Dogas, Z., et al., *NMDA receptor-mediated transmission of carotid body chemoreceptor input to expiratory bulbospinal neurones in dogs.* J Physiol, 1995. **487 (Pt 3)**: p. 639-51.
38. Chitravanshi, V.C. and H.N. Sapru, *NMDA as well as non-NMDA receptors in phrenic nucleus mediate respiratory effects of carotid chemoreflex.* Am J Physiol, 1997. **272**(1 Pt 2): p. R302-10.
39. Nattie, E.E., M. Gdovin, and A. Li, *Retrotrapezoid nucleus glutamate receptors: control of CO2-sensitive phrenic and sympathetic output.* J Appl Physiol, 1993. **74**(6): p. 2958-68.

40. Dempsey, J.A. and H.V. Forster, *Mediation of Ventilatory Adaptations.* Physiol Rev, 1982. **62**(1): p. 262-346.
41. Bisgard, G.E., et al., *Hypoventilation in ponies after carotid body denervation.* J Appl Physiol, 1976. **40**(2): p. 184-90.
42. Gautier, H. and M. Bonora, *Possible alterations in brain monoamine metabolism during hypoxia-induced tachypnea in cats.* J Appl Physiol, 1980. **49**(5): p. 769-77.
43. Powell, F.L., W.K. Milsom, and G.S. Mitchell, *Time domains of the hypoxic ventilatory response.* Respir Physiol, 1998. **112**(2): p. 123-34.
44. Vizek, M., C.K. Pickett, and J.V. Weil, *Interindividual variation in hypoxic ventilatory response: potential role of carotid body.* J Appl Physiol, 1987. **63**(5): p. 1884-9.
45. Eden, G.J. and M.A. Hanson, *Maturation of the respiratory response to acute hypoxia in the newborn rat.* J Physiol, 1987. **392**: p. 1-9.
46. Gozal, D., E. Gozal, and N.U.-h.w.s.c.s.a.B.T.J.-D.K.-C.f.d.d.b.a.f.f. Simakajornboon, *Signaling pathways of the acute hypoxic ventilatory response in the nucleus tractus solitarius.* Respiration Physiology, 2000. **121**(2-3): p. 209-221.
47. Weil, J.V., et al., *Hypoxic Ventilatory Drive in Normal Man.* The Journal of Clinical Investigtion, 1970. **49**: p. 1061-1072.
48. Vizek, M., C.K. Pickett, and J.V. Weil, *Increased carotid body hypoxic sensitivity during acclimatization to hypobaric hypoxia.* J Appl Physiol, 1987. **63**(6): p. 2403-10.
49. Tatsumi, K., C.K. Pickett, and J.V. Weil, *Decreased carotid body hypoxic sensitivity in chronic hypoxia: role of dopamine.* Respir Physiol, 1995. **101**(1): p. 47-57.
50. Pedersen, M.E., K.L. Dorrington, and P.A. Robbins, *Effects of dopamine and domperidone on ventilatory sensitivity to hypoxia after 8 h of isocapnic hypoxia.* J Appl Physiol, 1999. **86**(1): p. 222-9.
51. Weil, J.V., et al., *Acquired attenuation of chemoreceptor function in chronically hypoxic man at high altitude.* J Clin Invest, 1971. **50**(1): p. 186-95.
52. Goldspink, G., et al., *Gene expression in skeletal muscle in response to stretch and force generation.* Am J Physiol, 1992. **262**(3 Pt 2): p. R356-63.
53. Goldspink, G., *Gene expression in muscle in response to exercise.* J Muscle Res Cell Motil, 2003. **24**(2-3): p. 121-6.
54. Chin, E.R., *The role of calcium and calcium/calmodulin-dependent kinases in skeletal muscle plasticity and mitochondrial biogenesis.* Proc Nutr Soc, 2004. **63**(2): p. 279-86.
55. Vrbova, G., *The Effect of Motoneurone Activity on the Speed of Contraction of Striated Muscle.* J Physiol, 1963. **169**: p. 513-26.
56. Freund, H.J., *Motor unit and muscle activity in voluntary motor control.* Physiol Rev, 1983. **63**(2): p. 387-436.
57. Thayer, R., et al., *A decade of aerobic endurance training: histological evidence for fibre type transformation.* J Sports Med Phys Fitness, 2000. **40**(4): p. 284-9.
58. Baumann, H., et al., *Exercise training induces transitions of myosin isoform subunits within histochemically typed human muscle fibres.* Pflugers Arch, 1987. **409**(4-5): p. 349-60.
59. Banchero, N., *Long term adaptation of skeletal muscle capillarity.* Physiologist, 1982. **25**(4): p. 385-9.
60. Cerretelli, P., et al., *After effects of chronic hypoxia on cardiac output and muscle blood flow at rest and exercise.* Eur J Appl Physiol Occup Physiol, 1984. **53**(2): p. 92-6.

61. Boutellier, U., et al., *Human muscle adaptations to chronic hypoxia.* Prog Clin Biol Res, 1983. **136**: p. 273-85.
62. Hoppeler, H., et al., *Morphological adaptations of human skeletal muscle to chronic hypoxia.* Int J Sports Med, 1990. **11 Suppl 1**: p. S3-9.
63. MacDougall, J.D., et al., *Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated altitude.* Acta Physiologica Scandinavica, 1991. **142**(3): p. 421-7.
64. Green, H.J., et al., *Operation Everest II: adaptations in human skeletal muscle.* Journal of Applied Physiology, 1989. **66**(5): p. 2454-61.
65. Krogh, A. and J. Lindhard, *The regulation of respiration and circulation during the initial staged of muscular work.* J. Physiol (Lond), 1913. **47**: p. 112-136.
66. Jensen, J.I., *Neural ventilatory drive during arm and leg exercise.* Scand J Clin Lab Invest, 1972. **29**(2): p. 177-84.
67. Whipp, B.J., et al., *Parameters of ventilatory and gas exchange dynamics during exercise.* J Appl Physiol, 1982. **52**(6): p. 1506-13.
68. Oldenburg, F.A., et al., *A comparison of exercise responses in stairclimbing and cycling.* J Appl Physiol, 1979. **46**(3): p. 510-6.
69. Young, I.H. and A.J. Woolcock, *Changes in arterial blood gas tensions during unsteady-state exercise.* J Appl Physiol, 1978. **44**(1): p. 93-6.
70. Griffiths, T.L., I.C. Henson, and B.J. Whipp, *Influence of inspired oxygen concentration on the dynamics of the exercise hyperpnoea in man.* J Physiol, 1986. **380**: p. 387-403.
71. Oren, A., B.J. Whipp, and K. Wasserman, *Effect of acid-base status on the kinetics of the ventilatory response to moderate exercise.* J Appl Physiol, 1982. **52**(4): p. 1013-7.
72. Casaburi, R., et al., *Ventilatory control characteristics of the exercise hyperpnea as discerned from dynamic forcing techniques.* Chest, 1978. **73**(2 Suppl): p. 280-3.
73. Boetger, C.L. and D.S. Ward, *Effect of dopamine on transient ventilatory response to exercise.* J Appl Physiol, 1986. **61**(6): p. 2102-7.
74. Wasserman, K., et al., *Effect of carotid body resection on ventilatory and acid-base control during exercise.* J Appl Physiol, 1975. **39**(3): p. 354-8.
75. Yoshida, T., et al., *Effect of hypoxia on lactate variables during exercise.* J Hum Ergol (Tokyo), 1987. **16**(2): p. 157-61.
76. Whipp, B.J. and S.A. Ward, *Determinants and control of breathing during muscular exercise.* Br J Sports Med, 1998. **32**(3): p. 199-211.
77. Ozcelik, O., S.A. Ward, and B.J. Whipp, *Effect of altered body CO2 stores on pulmonary gas exchange dynamics during incremental exercise in humans.* Exp Physiol, 1999. **84**(5): p. 999-1011.
78. Rausch, S.M., et al., *Role of the carotid bodies in the respiratory compensation for the metabolic acidosis of exercise in humans.* J Physiol, 1991. **444**: p. 567-78.
79. Lugliani, R., et al., *Effect of bilateral carotid-body resection on ventilatory control at rest and during exercise in man.* N Engl J Med, 1971. **285**(20): p. 1105-11.
80. Ward, S.A., *Peripheral and central chemoreceptor control of ventilation during exercise in humans.* Can J Appl Physiol, 1994. **19**(3): p. 305-33.
81. Hambraeus-Jonzon, K., et al., *Hypoxic pulmonary vasoconstriction in human lungs. A stimulus-response study.* Anesthesiology, 1997. **86**(2): p. 308-15.
82. Morin, F.C., 3rd and K.R. Stenmark, *Persistent pulmonary hypertension of the newborn.* Am J Respir Crit Care Med, 1995. **151**(6): p. 2010-32.

83. Brimioulle, S., P. LeJeune, and R. Naeije, *Effects of hypoxic pulmonary vasoconstriction on pulmonary gas exchange.* J Appl Physiol, 1996. **81**(4): p. 1535-43.
84. Jensen, K.S., et al., *Rapid onset of hypoxic vasoconstriction in isolated lungs.* J Appl Physiol, 1992. **72**(5): p. 2018-23.
85. Bergofsky, E.H. and S. Holtzman, *A study of the mechanisms involved in the pulmonary arterial pressor response to hypoxia.* Circ Res, 1967. **20**(5): p. 506-19.
86. Hultgren, H.N., et al., *Physiologic studies of pulmonary oedema at altitude.* Circulation, 1964. **29**: p. 393-408.
87. Penazola, D. and F. Sime, *Circulatory dynamics during high altitude pulmonary edema.* Am J Cardiol., 1969. **23**: p. 369-378.
88. Roy, S.B., et al., *Haemodyanmic studies in high alttiude pulonary oedema.* Br. Heart. J., 1969. **31**: p. 52-58.
89. Harder, D.R., J.A. Madden, and C. Dawson, *Hypoxic induction of Ca2+-dependent action potentials in small pulmonary arteries of the cat.* J Appl Physiol, 1985. **59**(5): p. 1389-93.
90. Madden, J.A., C.A. Dawson, and D.R. Harder, *Hypoxia-induced activation in small isolated pulmonary arteries from the cat.* J Appl Physiol, 1985. **59**(1): p. 113-8.
91. Murray, T.R., et al., *Hypoxic contraction of cultured pulmonary vascular smooth muscle cells.* Am J Respir Cell Mol Biol, 1990. **3**(5): p. 457-65.
92. Ward, J.P. and T.P. Robertson, *The role of the endothelium in hypoxic pulmonary vasoconstriction.* Exp Physiol, 1995. **80**(5): p. 793-801.
93. Hoshino, Y., K.J. Morrison, and P.M. Vanhoutte, *Mechanisms of hypoxic vasoconstriction in the canine isolated pulmonary artery: role of endothelium and sodium pump.* Am J Physiol, 1994. **267**(2 Pt 1): p. L120-7.
94. Leach, R.M., et al., *Hypoxic vasoconstriction in rat pulmonary and mesenteric arteries.* Am J Physiol, 1994. **266**(3 Pt 1): p. L223-31.
95. Zhang, F. and A.H. Morice, *Effect of levcromakalim on hypoxia-, KCl- and prostaglandin F2 alpha-induced contractions in isolated rat pulmonary artery.* J Pharmacol Exp Ther, 1994. **271**(1): p. 326-33.
96. Moudgil, R., E.D. Michelakis, and S.L. Archer, *Hypoxic pulmonary vasoconstriction.* J Appl Physiol, 2005. **98**(1): p. 390-403.
97. Kemp, P.J., et al., *Functional proteomics of BK potassium channels: defining the acute oxygen sensor: Signalling pathways in acute oxygen sensing.* Novartis Foundation symposium ; 272. 2006, Chichester: Wiley. pp141-150.
98. Archer, S.L., et al., *A central role for oxygen-sensitive K+ channels and mitochondria in the specialised oxygen-sensing system: Signalling pathways in acute oxygen sensing.* Novartis Foundation symposium ; 272. 2006, Chichester: Wiley. pp157-171.
99. Gurney, A.M. and S. Joshi, *The role of twin pore domain and other K+ channels in hypoxic pulmonary vasoconstriction: Signalling pathways in acute oxygen sensing.* Novartis Foundation symposium ; 272. 2006, Chichester: Wiley. pp218-228.
100. Leach, R.M., et al., *Divergent roles of glycolysis and the mitochondrial electron transport chain in hypoxic pulmonary vasoconstriction of the rat: identity of the hypoxic sensor.[see comment].* Journal of Physiology, 2001. **536**(Pt 1): p. 211-24.
101. Leach, R.M., et al., *Divergent roles of glycolysis and the mitochondrial electron transport chain in hypoxic pulmonary vasoconstriction of the rat: identity of the hypoxic sensor.* J Physiol, 2001. **536**(Pt 1): p. 211-24.

102. Rounds, S. and I.F. McMurtry, *Inhibitors of oxidative ATP production cause transient vasoconstriction and block subsequent pressor responses in rat lungs.* Circ Res, 1981. **48**(3): p. 393-400.
103. Waypa, G.B. and P.T. Schumaker, *Role for mitochondrial reactive oxygen species in hypoxic pulmonary vasoconstriction: Signalling pathways in acute oxygen sensing.* Novartis Foundation symposium ; 272. 2006, Chichester: Wiley. pp176-192.
104. Post, J.M., et al., *Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction.* Am J Physiol, 1992. **262**(4 Pt 1): p. C882-90.
105. Yuan, X.J., et al., *Hypoxia reduces potassium currents in cultured rat pulmonary but not mesenteric arterial myocytes.* Am J Physiol, 1993. **264**(2 Pt 1): p. L116-23.
106. Burghuber, O.C., *Nifedipine attenuates acute hypoxic pulmonary vasoconstriction in patients with chronic obstructive pulmonary disease.* Respiration, 1987. **52**(2): p. 86-93.
107. Olschewski, A., et al., *Graded response of K+ current, membrane potential, and [Ca2+]i to hypoxia in pulmonary arterial smooth muscle.* Am J Physiol Lung Cell Mol Physiol, 2002. **283**(5): p. L1143-50.
108. Salvaterra, C.G. and W.F. Goldman, *Acute hypoxia increases cytosolic calcium in cultured pulmonary arterial myocytes.* Am J Physiol, 1993. **264**(3 Pt 1): p. L323-8.
109. Dipp, M., P.C. Nye, and A.M. Evans, *Hypoxic release of calcium from the sarcoplasmic reticulum of pulmonary artery smooth muscle.* Am J Physiol Lung Cell Mol Physiol, 2001. **281**(2): p. L318-25.
110. Gonzalez De La Fuente, P., J.P. Savineau, and R. Marthan, *Control of pulmonary vascular smooth muscle tone by sarcoplasmic reticulum Ca2+ pump blockers: thapsigargin and cyclopiazonic acid.* Pflugers Arch, 1995. **429**(5): p. 617-24.
111. Ng, L.C. and A.M. Gurney, *Store-operated channels mediate Ca(2+) influx and contraction in rat pulmonary artery.* Circ Res, 2001. **89**(10): p. 923-9.
112. Wang, J., et al., *Acute hypoxia increases intracellular [Ca2+] in pulmonary arterial smooth muscle by enhancing capacitative Ca2+ entry.* Am J Physiol Lung Cell Mol Physiol, 2005. **288**(6): p. L1059-69.
113. Weigand, L., et al., *Inhibition of hypoxic pulmonary vasoconstriction by antagonists of store-operated Ca2+ and nonselective cation channels.* Am J Physiol Lung Cell Mol Physiol, 2005. **289**(1): p. L5-L13.
114. Somlyo, A.P. and A.V. Somlyo, *Signal transduction and regulation in smooth muscle.* Nature, 1994. **372**(6503): p. 231-6.
115. Wang, Z., et al., *Rho-kinase activation is involved in hypoxia-induced pulmonary vasoconstriction.* Am J Respir Cell Mol Biol, 2001. **25**(5): p. 628-35.
116. Robertson, T.P., et al., *Inhibition of sustained hypoxic vasoconstriction by Y-27632 in isolated intrapulmonary arteries and perfused lung of the rat.* Br J Pharmacol, 2000. **131**(1): p. 5-9.
117. Frostell, C., et al., *Inhaled Nitric Oxide: A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction.* Circulation, 1991. **83**(6): p. 2038-47.
118. Blitzer, M.L., et al., *Endothelium-derived nitric oxide regulates systemic and pulmonary vascular resistance during acute hypoxia in humans.* J. Amer. Coll. Cardiol., 1996. **28**(3): p. 591-596.
119. Steudel, W., et al., *Sustained pulmonary hypertension and right ventricular hypertrophy after chronic hypoxia in mice with congenital deficiency of nitric oxide synthase 3.* J Clin Invest, 1998. **101**(11): p. 2468-77.

120. Fagan, K.A., et al., *The pulmonary circulation of homozygous or heterozygous eNOS-null mice is hyperresponsive to mild hypoxia.* Journal of Clinical Investigation, 1999. **103**(2): p. 291-9.
121. Gaston, B., et al., *The Biology of Nitrogen Oxides in the Airways. (State of the Art.).* Amer. J. Resp. Crit. Care. Med., 1994. **149**: p. 538-551.
122. Yanagisawa, M., et al., *A novel potent vasoconstrictor peptide produced by vascular endothelial cells.* Nature, 1988. **332**(411-415).
123. Hassoun, P.M., et al., *Endothelin-1:mitogenic activity on pulmonary artery smooth muscle cells and release from hypoxic endothelial cells.* Proc. Soc. Exp. Biol. Med., 1992. **199**(165-170).
124. Janakidevi, K., et al., *Endothelin-1 stimulates DNA synthesis and peoliferation of pulmonary artey smooth muscle cells.* Am. J. Physiol., 1992. **263**(C1295-C1301).
125. Yoshimoto, S., et al., *Effect of carbon dioxide and oxygen on endotheln production by cultured porcine cerebral endothelial cells.* Stroke, 1991. **22**: p. 378-383.
126. Elton, T.S., et al., *Normobaric hypoxia stimulates endothelin-1 gene expression in the rat.* American Journal of Physiology, 1992. **263**(6 Pt 2): p. R1260-4.
127. Kourembanas, S., et al., *Hypoxia induces endothelin gene expression and secretion in cultured human endothelium cells.* J. Clin. Invest., 1991. **88**: p. 1054-57.
128. Cargill, R.I., et al., *Hypoxaemia and release of endothelin-1.* Thorax, 1995. **50**: p. 1308-1310.
129. Kourembanas, S., et al., *Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia.* Journal of Clinical Investigation, 1993. **92**(1): p. 99-104.
130. de Bold, A.J., et al., *A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats.* Life Sci, 1981. **28**(1): p. 89-94.
131. McDonagh, T.A., et al., *Biochemical detection of left-ventricular systolic dysfunction [see comments].* Lancet, 1998. **351**(9095): p. 9-13.
132. Murdoch, D.R., et al., *Brain natriuretic peptide is stable in whole blood and can be measured using a simple rapid assay: implications for clinical practice.* Heart, 1997. **78**(6): p. 594-7.
133. Anderson, J.V., et al., *Atrial natriuretic peptide inhibits the aldosterone response to angiotensin II in man.* Clin Sci (Lond), 1986. **70**(5): p. 507-12.
134. Elliott, M.E. and T.L. Goodfriend, *Inhibition of aldosterone synthesis by atrial natriuretic factor.* Fed Proc, 1986. **45**(9): p. 2376-81.
135. Kawashima, A., et al., *Hemodynamic responses to acute hypoxia, hypobaria, and exercise in subjects susceptible to high-altitude pulmonary edema.* J Appl Physiol, 1989. **67**(5): p. 1982-9.
136. Lawrence, D.L., J.B. Skatrud, and Y. Shenker, *Effect of hypoxia in atrial natriuretic factor and aldosterone regulation in humans.* American journal of physiology., 1990. **258**(Endocrine and Metabolism 21): p. E243-E248.
137. Kawashima, A., et al., *Hypoxia-induced ANP secretion in subjects susceptible to high-altitude pulmonary edema.* Respir Physiol, 1992. **89**(3): p. 309-17.
138. Klinger, J.R., et al., *Brain natriuretic peptide inhibits hypoxic pulmonary hypertension in rats.* Journal of Applied Physiology, 1998. **84**(5): p. 1646-52.
139. Klinger, J.R., et al., *Targeted disruption of the gene for natriuretic peptide receptor-A worsens hypoxia-induced cardiac hypertrophy.* American Journal of Physiology - Heart & Circulatory Physiology, 2002. **282**(1): p. H58-65.

140. Cargill, R.I. and B.J. Lipworth, *The role of the renin-angiotensin and natriuretic peptide systems in the pulmonary vasculature.* British Journal of Clinical Pharmacology, 1995. **40**(1): p. 11-8.
141. Somers, V.K., et al., *Atrial natriuretic peptide is released by dynamic exercise in man.* Horm Metab Res, 1986. **18**(12): p. 871-2.
142. Milledge, J.S., S. McArthur, and A.H. Morice, *Atrial natriuretic peptide and exercise-induced fluid retention in man.* J. Wilderness Med, 1991. **2**: p. 94-101.
143. Wagner, P.D., et al., *Pulmonary gas exchange in humans exercising at sea level and simulated altitude.* J Appl Physiol, 1986. **61**(1): p. 260-70.
144. Groves, B.M., et al., *Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen.* Journal of Applied Physiology, 1987. **63**(2): p. 521-30.
145. Reeves, J.T., et al., *Operation Everest II: cardiac filling pressures during cycle exercise at sea level.* Respiration Physiology, 1990. **80**(2-3): p. 147-54.
146. West, J.B., *Vulnerability of pulmonary capillaries during exercise.* Exerc Sport Sci Rev, 2004. **32**(1): p. 24-30.
147. Fowler, K.T. and J. Read, *Effect of alveolar hypoxia on zonal distribution of pulmonary blood flow.* J Appl Physiol, 1963. **18**: p. 244-50.
148. Lehr, D., M. Triller, and L. Fisher, *Induced changes in the pattern of pulmonary blood flow in the rabbit.* Circulation research, 1963. **13**: p. 119-131.
149. Reid, L., *The pulmonary circulation: remodelling in growth and disease.* American Review of Respiratory Disease., 1979. **119**: p. 531-546.
150. Hultgren, H.N., M.C. Robinson, and R.D. Wuerflein, *Over perfusion pulmonary oedema.* Circulation, 1966. **34**(Supp 3): p. 132-3.
151. West, J.B. and O. Mathieu-Costello, *Vulnerability of pulmonary capillaries in heart disease.* Circulation, 1995. **92**(3): p. 622-31.
152. West, J.B., K. Tsukimoto, and O. Mathieu-Costello, *Stress failure in Pulmonary capillaries.* J. App. Physiol., 1991. **70**: p. 1731-1742.
153. Coin, J.T. and J.S. Olson, *The rate of oxygen uptake by human red blood cells.* J Biol Chem, 1979. **254**(4): p. 1178-90.
154. Wasserman, K., et al., *Principles of exercise testing and interpretation.* Third ed. 1999, Baltimore: Lippincort Williams and Wilkins. 556.
155. Roughton, F.J., *Average time spent by blood in human lung capillary and its relation to the rates of CO uptake and elimination in man.* Am. J. Physiol., 1945. **143**: p. 621-633.
156. Zavorsky, G.S., K.R. Walley, and J.A. Russell, *Red cell pulmonary transit times through the healthy human lung.* Exp Physiol, 2003. **88**(2): p. 191-200.
157. Dempsey, J.A. and P.D. Wagner, *Exercise-induced arterial hypoxemia.* J Appl Physiol, 1999. **87**(6): p. 1997-2006.
158. Reeves, J.T., et al., *Increased alveolar-arterial oxygen difference during simulated high-altitude exposure.* J Appl Physiol, 1969. **27**(5): p. 658-61.
159. Grover, R.F., J.V. Weil, and J.T. Reeves, *Cardiovascular adaptation to exercise at high altitude.* Exercise & Sport Sciences Reviews, 1986. **14**: p. 269-302.
160. Reeves, J.T., et al., *Operation Everest II: preservation of cardiac function at extreme altitude.* Journal of Applied Physiology, 1987. **63**(2): p. 531-9.
161. West, J.B., et al., *Pulmonary gas exchange on the summit of Mount Everest.* J Appl Physiol, 1983. **55**(3): p. 678-87.
162. Jones, N.L., D.G. Robertson, and J.W. Kane, *Difference between end-tidal and arterial PCO2 in exercise.* J Appl Physiol, 1979. **47**(5): p. 954-60.

163. Zimmerman, M.I., et al., *Estimated vs actual values for dead space/tidal volume ratios during incremental exercise in patients evaluated for dyspnea.* Chest, 1994. **106**(1): p. 131-6.
164. Hey, E.N., et al., *Effects of various respiratory stimuli on the depth and frequency of breathing in man.* Respir Physiol, 1966. **1**(2): p. 193-205.
165. Gardner, W.N., *The pattern of breathing following step changes of alveolar partial pressures of carbon dioxide and oxygen in man.* J Physiol, 1980. **300**: p. 55-73.
166. Benchetrit, G., et al., *Individuality of breathing patterns in adults assessed over time.* Respir Physiol, 1989. **75**(2): p. 199-209.
167. Dejours, P., Kelloggrh, and N. Pace, *Regulation of respiration and heart rate response in exercise during altitude acclimatization.* J Appl Physiol, 1963. **18**: p. 10-8.
168. Eisele, J.H., et al., *Individuality of breathing patterns during hypoxia and exercise.* J Appl Physiol, 1992. **72**(6): p. 2446-53.
169. Shea, S.A., et al., *The breathing patterns of identical twins.* Respir Physiol, 1989. **75**(2): p. 211-23.
170. Loeppky, J.A., et al., *Beat-by-beat stroke volume assessment by pulsed Doppler in upright and supine exercise.* J Appl Physiol, 1981. **50**(6): p. 1173-82.
171. Rowell, L.B., *Human cardiovascular adjustments to exercise and thermal stress.* Physiol Rev, 1974. **54**(1): p. 75-159.
172. Rowell, L., *Human circulation regulation during physical stress.* 1986, New York: Oxford Universtiy Press. 215.
173. Corcondilas, A., G.T. Koroxenidis, and J.T. Shepherd, *Effect of a Brief Contraction of Forearm Muscles on Forearm Blood Flow.* J Appl Physiol, 1964. **19**: p. 142-6.
174. Gnaiger, E., et al., *Mitochondrial oxygen affinity, respiratory flux control and excess capacity of cytochrome c oxidase.* J Exp Biol, 1998. **201**(Pt 8): p. 1129-39.
175. Wittenberg, B.A. and J.B. Wittenberg, *Transport of oxygen in muscle.* Annu Rev Physiol, 1989. **51**: p. 857-78.
176. Stringer, W., et al., *Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise.* J Appl Physiol, 1994. **76**(4): p. 1462-7.
177. Honig, C.R. and S.M. Tenney, *Determinants of the circulatory response to hypoxia and hypercapnia.* Am Heart J, 1957. **53**(5): p. 687-98.
178. Kontos, H.A., et al., *Comparative circulatory responses to systemic hypoxia in man and in unanesthetized dog.* J Appl Physiol, 1967. **23**(3): p. 381-6.
179. Pugh, L.G., et al., *Muscular Exercise at Great Altitudes.* J Appl Physiol, 1964. **19**: p. 431-40.
180. Calbet, J.A., et al., *Determinants of maximal oxygen uptake in severe acute hypoxia.* Am J Physiol Regul Integr Comp Physiol, 2003. **284**(2): p. R291-303.
181. Suarez, J., J.K. Alexander, and C.S. Houston, *Enhanced left ventricular systolic performance at high altitude during Operation Everest II.* American Journal of Cardiology, 1987. **60**(1): p. 137-42.
182. Boussuges, A., et al., *Operation Everest III (Comex '97): modifications of cardiac function secondary to altitude-induced hypoxia. An echocardiographic and Doppler study.* Am J Respir Crit Care Med, 2000. **161**(1): p. 264-70.
183. Richardson, D.W., et al., *Modification by beta-adrenergic blockade of the circulatory respones to acute hypoxia in man.* J Clin Invest, 1967. **46**(1): p. 77-85.
184. Rowell, L.B., et al., *Hypoxemia raises muscle sympathetic activity but not norepinephrine in resting humans.* J Appl Physiol, 1989. **66**(4): p. 1736-43.

185. Saito, M., et al., *Responses in muscle sympathetic activity to acute hypoxia in humans.* J Appl Physiol, 1988. **65**(4): p. 1548-52.
186. Somers, V.K., et al., *Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans.* J Appl Physiol, 1989. **67**(5): p. 2101-6.
187. Seals, D.R., N.O. Suwarno, and J.A. Dempsey, *Influence of lung volume on sympathetic nerve discharge in normal humans.* Circ Res, 1990. **67**(1): p. 130-41.
188. Somers, V.K., et al., *Influence of ventilation and hypocapnia on sympathetic nerve responses to hypoxia in normal humans.* J Appl Physiol, 1989. **67**(5): p. 2095-100.
189. Cutler, M.J., et al., *Hypoxia-mediated prolonged elevation of sympathetic nerve activity after periods of intermittent hypoxic apnea.* J Appl Physiol, 2004. **96**(2): p. 754-61.
190. Leuenberger, U., et al., *Surges of muscle sympathetic nerve activity during obstructive apnea are linked to hypoxemia.* J Appl Physiol, 1995. **79**(2): p. 581-8.
191. Leuenberger, U.A., et al., *Effects of intermittent hypoxia on sympathetic activity and blood pressure in humans.* Auton Neurosci, 2005. **121**(1-2): p. 87-93.
192. Marshall, J.M., *Peripheral chemoreceptors and cardiovascular regulation.* Physiol Rev, 1994. **74**(3): p. 543-94.
193. Guyenet, P.G., *Neural structures that mediate sympathoexcitation during hypoxia.* Respir Physiol, 2000. **121**(2-3): p. 147-62.
194. Smith, M.L. and N.K.U.-h.w.s.c.s.a.B.T.J.-D.K.-c.a.c.e.e.c.d.e.c. Muenter, *Effects of hypoxia on sympathetic neural control in humans.* Respiration Physiology, 2000. **121**(2-3): p. 163-171.
195. Sun, M.K., *Pharmacology of reticulospinal vasomotor neurons in cardiovascular regulation.* Pharmacol Rev, 1996. **48**(4): p. 465-94.
196. Rostrup, M., *Catecholamines, hypoxia and high altitude.* Acta Physiol Scand, 1998. **162**(3): p. 389-99.
197. Antezana, A.M., et al., *Adrenergic status of humans during prolonged exposure to the altitude of 6,542 m.* J Appl Physiol, 1994. **76**(3): p. 1055-9.
198. Bartsch, P., et al., *Enhanced exercise-induced rise of aldosterone and vasopressin preceding mountain sickness.* J. Appl. Physiol., 1991. **71**: p. 136-43.
199. Reeves, J.T., et al., *Increased arterial pressure after acclimatization to 4300 m: possible role of norepinephrine.* Int J Sports Med, 1992. **13 Suppl 1**: p. S18-21.
200. Mazzeo, R.S., et al., *Acclimatization to high altitude increase muscle sympathetic activity both at rest and during exercise.* Am J Physiol, 1995. **269**(1 Pt 2): p. R201-7.
201. Asano, K., et al., *Relation of sympathetic activation to ventilation in man at 4300 m altitude.* Aviation Space & Environmental Medicine, 1997. **68**(2): p. 104-10.
202. Sutton, J.R., et al., *Operation Everest II: oxygen transport during exercise at extreme simulated altitude.* Journal of Applied Physiology, 1988. **64**(4): p. 1309-21.
203. Boushel, R., et al., *Parasympathetic neural activity accounts for the lowering of exercise heart rate at high altitude.* Circulation, 2001. **104**(15): p. 1785-91.
204. Bogaard, H.J., et al., *Role of the autonomic nervous system in the reduced maximal cardiac output at altitude.* J Appl Physiol, 2002. **93**(1): p. 271-9.
205. Favret, F., et al., *Myocardial adrenergic and cholinergic receptor function in hypoxia: correlation with O(2) transport in exercise.* Am J Physiol Regul Integr Comp Physiol, 2001. **280**(3): p. R730-8.
206. Moore, L.G., et al., *Propranolol does not impair exercise oxygen uptake in normal men at high altitude.* J Appl Physiol, 1986. **61**(5): p. 1935-41.
207. Wagner, P.D., *Reduced maximal cardiac output at altitude--mechanisms and significance.* Respiration Physiology, 2000. **120**(1): p. 1-11.

208. Cerretelli, P., *Limiting factors to oxygen transport on Mount Everest.* J Appl Physiol, 1976. **40**(5): p. 658-67.
209. Eckardt, K.U., et al., *Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia.* J Appl Physiol, 1989. **66**(4): p. 1785-8.
210. Tuffley, R.E., et al., *Serum renin activity during exposure to hypoxia.* J Endocrinol, 1970. **48**(4): p. 497-510.
211. Frayser, R., et al., *Hormonal and electrolyte response to exposure to 17,500 ft.* J Appl Physiol, 1975. **38**(4): p. 636-42.
212. Milledge, J.S., et al., *Effect of prolonged exercise at altitude on the renin-aldosterone system.* J Appl Physiol, 1983. **55**(2): p. 413-8.
213. Milledge, J.S. and D.M. Catley, *Angiotensin converting enzyme activity and hypoxia.* Clin Sci (Lond), 1987. **72**(1): p. 149.
214. Raff, H., et al., *Hypoxia in vivo inhibits aldosterone synthesis and aldosterone synthase mRNA in rats.* J Appl Physiol, 1996. **81**(2): p. 604-10.
215. Ashack, R., et al., *Renal and hormonal responses to acute hypoxia in normal individuals.* J Lab Clin Med, 1985. **106**(1): p. 12-6.
216. Jonsson, J.R., et al., *The expression and localisation of the angiotensin-converting enzyme mRNA in human adipose tissue.* Blood Press, 1994. **3**(1-2): p. 72-5.
217. Dragovic, T., et al., *Kininase II-type enzymes. Their putative role in muscle energy metabolism.* Diabetes, 1996. **45 Suppl 1**: p. S34-7.
218. Neri Serneri, G.G., et al., *Evidence for the existence of a functional cardiac renin-angiotensin system in humans.* Circulation, 1996. **94**(8): p. 1886-93.
219. Pieruzzi, F., Z.A. Abassi, and H.R. Keiser, *Expression of renin-angiotensin system components in the heart, kidneys, and lungs of rats with experimental heart failure.* Circulation, 1995. **92**(10): p. 3105-12.
220. Zisman, L.S., *Inhibiting tissue angiotensin-converting enzyme: a pound of flesh without the blood?* Circulation, 1998. **98**(25): p. 2788-90.
221. Yusuf, S., et al., *Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators.* N Engl J Med, 2000. **342**(3): p. 145-53.
222. Segel, N., P. Harris, and J.M. Bishop, *The effects of synthetic hypertensin of the systemic and pulmonary circulations in man.* Clin Sci, 1961. **20**: p. 49-61.
223. Lipworth, B.J. and K.D. Dagg, *Vasoconstrictor effects of angiotensin II on the pulmonary vascular bed.* Chest, 1994. **105**(5): p. 1360-4.
224. Urata, H., H. Nishimura, and D. Ganten, *Chymase-dependent angiotensin II forming systems in humans.* Am J Hypertens, 1996. **9**(3): p. 277-84.
225. Lyall, F., et al., *Angiotensin II increases proto-oncogene expression and phosphoinositide turnover in vascular smooth muscle cells via the angiotensin II AT1 receptor.* J Hypertens, 1992. **10**(12): p. 1463-9.
226. Carey, R.M. and H.M. Siragy, *Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation.* Endocr Rev, 2003. **24**(3): p. 261-71.
227. Bullock, G.R., et al., *Distribution of type-1 and type-2 angiotensin receptors in the normal human lung and in lungs from patients with chronic obstructive pulmonary disease.* Histochem Cell Biol, 2001. **115**(2): p. 117-24.
228. Chassagne, C., et al., *Modulation of angiotensin II receptor expression during development and regression of hypoxic pulmonary hypertension.* Am J Respir Cell Mol Biol, 2000. **22**(3): p. 323-32.

229. King, S.J., et al., *Hypoxia stimulates endothelial cell angiotensin-converting enzyme antigen synthesis.* American Journal of Physiology, 1989. **256**(6 Pt 1): p. C1231-8.
230. Morrell, N.W., et al., *Angiotensin converting enzyme expression is increased in small pulmonary arteries of rats with hypoxia-induced pulmonary hypertension.* Journal of Clinical Investigation, 1995. **96**(4): p. 1823-33.
231. Nong, Z., et al., *Inhibition of Tissue Angiotensin-Converting Enzyme With Quinalapril Reduces Hypoxic Pulmonay Hypertension and Pulmonary Vascular remodelling.* Circulation, 1996. **94**(8): p. 1941-1947.
232. Cargill, R.I. and B.J. Lipworth, *Lisinopril attenuates acute hypoxic pulmonary vasoconstriction in humans.* Chest, 1996. **109**(2): p. 424-29.
233. Kiely, D.G., R.I. Cargill, and B.J. Lipworth, *Angiotensin II receptor blockade and effects on pulmonary hemodynamics and hypoxic pulmonary vasoconstriction in humans [see comments].* Chest, 1996. **110**(3): p. 698-703.
234. Kiely, D.G., R.I. Cargill, and B.J.U.-h.w.s.c.s.a.B.T.-Y.-e.c.c.f.f.b.a. Lipworth, *Acute hypoxic pulmonary vasoconstriction in man is attenuated by type I angiotensin II receptor blockade.* Cardiovascular Research, 1995. **30**(6): p. 875-880.
235. Patel, J.M., et al., *Angiotensin IV receptor-mediated activation of lung endothelial NOS is associated with vasorelaxation.* American Journal of Physiology, 1998. **275**(6 Pt 1): p. L1061-8.
236. Albiston, A.L., et al., *Evidence that the angiotensin IV (AT(4)) receptor is the enzyme insulin-regulated aminopeptidase.* J Biol Chem, 2001. **276**(52): p. 48623-6.
237. Keller, S.R., et al., *Cloning and characterization of a novel insulin-regulated membrane aminopeptidase from Glut4 vesicles.* J Biol Chem, 1995. **270**(40): p. 23612-8.
238. Herbst, J.J., et al., *Insulin stimulates cell surface aminopeptidase activity toward vasopressin in adipocytes.* Am J Physiol, 1997. **272**(4 Pt 1): p. E600-6.
239. Matsumoto, H., et al., *Expression of placental leucine aminopeptidase/oxytocinase in neuronal cells and its action on neuronal peptides.* Eur J Biochem, 2001. **268**(11): p. 3259-66.
240. Santos, R.A., M.J. Campagnole-Santos, and S.P. Andrade, *Angiotensin-(1-7): an update.* Regul Pept, 2000. **91**(1-3): p. 45-62.
241. Schmaier, A.H., *The kallikrein-kinin and the renin-angiotensin systems have a multilayered interaction.* Am J Physiol Regul Integr Comp Physiol, 2003. **285**(1): p. R1-13.
242. Heitsch, H., et al., *Angiotensin-(1-7)-Stimulated Nitric Oxide and Superoxide Release From Endothelial Cells.* Hypertension, 2001. **37**(1): p. 72-76.
243. Sampaio, W.O., A.A. Nascimento, and R.A. Santos, *Systemic and regional hemodynamic effects of angiotensin-(1-7) in rats.* Am J Physiol Heart Circ Physiol, 2003. **284**(6): p. H1985-94.
244. Ferrario, C.M. and M.C. Chappell, *Novel angiotensin peptides.* Cell Mol Life Sci, 2004. **61**(21): p. 2720-7.
245. Bartus, J.B. and S. Chlopicki, *Effect of neutral endopeptidase inhibition on vascular response induced by exogenous angiotensin I in the isolated rat lung.* Pol J Pharmacol, 2003. **55**(6): p. 1071-8.
246. Donoghue, M., et al., *A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9.* Circ Res, 2000. **87**(5): p. E1-9.
247. Wang, P., et al., *Expression cloning of functional receptor used by SARS coronavirus.* Biochem Biophys Res Commun, 2004. **315**(2): p. 439-44.

248. Li, W., et al., *Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus.* Nature, 2003. **426**(6965): p. 450-4.
249. Prabakaran, P., X. Xiao, and D.S. Dimitrov, *A model of the ACE2 structure and function as a SARS-CoV receptor.* Biochem Biophys Res Commun, 2004. **314**(1): p. 235-41.
250. Towler, P., et al., *ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis.* J Biol Chem, 2004. **279**(17): p. 17996-8007.
251. Ferrario, C.M., et al., *Effects of renin-angiotensin system blockade on renal angiotensin-(1-7) forming enzymes and receptors.* Kidney Int, 2005. **68**(5): p. 2189-96.
252. Ferrario, C.M., et al., *Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2.* Circulation, 2005. **111**(20): p. 2605-10.
253. Crackower, M.A., et al., *Angiotensin-converting enzyme 2 is an essential regulator of heart function.* Nature, 2002. **417**(6891): p. 822-8.
254. Zisman, L.S., et al., *Increased angiotensin-(1-7)-forming activity in failing human heart ventricles: evidence for upregulation of the angiotensin-converting enzyme Homologue ACE2.* Circulation, 2003. **108**(14): p. 1707-12.
255. Imai, Y., et al., *Angiotensin-converting enzyme 2 protects from severe acute lung failure.* Nature, 2005. **436**(7047): p. 112-6.
256. Ferreira, A.J. and R.A. Santos, *Cardiovascular actions of angiotensin-(1-7).* Braz J Med Biol Res, 2005. **38**(4): p. 499-507.
257. Yang, H.Y., E.G. Erdos, and Y. Levin, *Characterization of a dipeptide hydrolase (kininase II: angiotensin I converting enzyme).* J Pharmacol Exp Ther, 1971. **177**(1): p. 291-300.
258. Yang, H.Y., E.G. Erdos, and Y. Levin, *A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin.* Biochim Biophys Acta, 1970. **214**(2): p. 374-6.
259. Jaspard, E., L. Wei, and F. Alhenc-Gelas, *Differences in the properties and enzymatic specificities of the two active sites of angiotensin I-converting enzyme (kininase II). Studies with bradykinin and other natural peptides.* J Biol Chem, 1993. **268**(13): p. 9496-503.
260. Ni, A., et al., *Overexpression of kinin B1 receptors induces hypertensive response to des-Arg9-bradykinin and susceptibility to inflammation.* J Biol Chem, 2003. **278**(1): p. 219-25.
261. Palmer, R.M.J., A.G. Ferrige, and S. Monacada, *Nitric oxide release accounts for the biological activity of endothelium-deived relaxing factor.* Nature, 1987. **327**: p. 524-526.
262. Holland, J.A., et al., *Bradykinin induces superoxide anion release from human endothelial cells.* J Cell Physiol, 1990. **143**(1): p. 21-5.
263. Smith, D., M. Gilbert, and W.G. Owen, *Tissue plasminogen activator release in vivo in response to vasoactive agents.* Blood, 1985. **66**(4): p. 835-9.
264. Tsutsumi, Y., et al., *Angiotensin II type 2 receptor overexpression activates the vascular kinin system and causes vasodilation.* J Clin Invest, 1999. **104**(7): p. 925-35.
265. DeWitt, B.J., et al., *Analysis of responses to bradykinin in the pulmonary vascular bed of the cat.* Am J Physiol, 1994. **266**(6 Pt 2): p. H2256-67.

266. Sangsree, S., et al., *Kininase I-type carboxypeptidases enhance nitric oxide production in endothelial cells by generating bradykinin B1 receptor agonists.* Am J Physiol Heart Circ Physiol, 2003. **284**(6): p. H1959-68.
267. Allen, A.M., *Angiotensin AT1 receptor-mediated excitation of rat carotid body chemoreceptor afferent activity.* J Physiol (Lond), 1998. **510**(3): p. 773-781.
268. Leung, P.S., S.Y. Lam, and M.L. Fung, *Chronic hypoxia upergulates the expression and function of AT_1 receptors in rat cartid body.* J. Endocrin., 2000. **167**: p. 517-24.
269. Fung, M.L., et al., *Functional expression of angiotensin II receptors in type-I cells of the rat carotid body.* Pflugers Arch, 2001. **441**(4): p. 474-80.
270. Lam, S.Y. and P.S. Leung, *A locally generated angiotensin system in rat carotid body.* Regul Pept, 2002. **107**(1-3): p. 97-103.
271. Danser, A.H., et al., *Is there a local renin-angiotensin system in the heart?* Cardiovasc Res, 1999. **44**(2): p. 252-65.
272. Lippoldt, A., et al., *The brain renin-angiotensin system: molecular mechanisms of cell to cell interactions.* Clin Exp Hypertens, 1995. **17**(1-2): p. 251-66.
273. Li, Y.L. and H.D. Schultz, *Enhanced sensitivity of Kv channels to hypoxia in the carotid body in heart failure: role of angiotensin II.* J Physiol (in press), 2006.
274. Bee, D., D.J. Pallot, and G.R. Barer, *Division of type I and endothelial cells in the hypoxic rat carotid body.* Acta Anat (Basel), 1986. **126**(4): p. 226-9.
275. McGregor, K.H., J. Gil, and S. Lahiri, *A morphometric study of the carotid body in chronically hypoxic rats.* J Appl Physiol, 1984. **57**(5): p. 1430-8.
276. Veerasingham, S.J. and M.K. Raizada, *Brain renin-angiotensin system dysfunction in hypertension: recent advances and perspectives.* Br J Pharmacol, 2003. **139**(2): p. 191-202.
277. Paton, J.F.R. and S. Kasparov, *Differential effects of angiotensin II on cardiorespiratory reflexes mediated by nucleus tractus solitarii - a microinjection study in the rat.* J Physiol (Lond), 1999. **521**(1): p. 213-225.
278. Ganten, D., et al., *The iso-renin angiotensin systems in extrarenal tissue.* Clin Exp Pharmacol Physiol, 1976. **3**(2): p. 103-26.
279. Chappell, M.C., et al., *Identification of angiotensin-(1-7) in rat brain. Evidence for differential processing of angiotensin peptides.* J Biol Chem, 1989. **264**(28): p. 16518-23.
280. Chappell, M.C., et al., *Characterization by high performance liquid chromatography of angiotensin peptides in the plasma and cerebrospinal fluid of the dog.* Peptides, 1987. **8**(5): p. 939-42.
281. Lenkei, Z., et al., *Expression of angiotensin type-1 (AT1) and type-2 (AT2) receptor mRNAs in the adult rat brain: a functional neuroanatomical review.* Front Neuroendocrinol, 1997. **18**(4): p. 383-439.
282. Chai, S.Y., et al., *Distribution of angiotensin IV binding sites (AT4 receptor) in the human forebrain, midbrain and pons as visualised by in vitro receptor autoradiography.* J Chem Neuroanat, 2000. **20**(3-4): p. 339-48.
283. Moeller, I., et al., *Localization of angiotensin IV binding sites to motor and sensory neurons in the sheep spinal cord and hindbrain.* Brain Res, 1995. **701**(1-2): p. 301-6.
284. Roberts, K.A., et al., *Autoradiographic identification of brain angiotensin IV binding sites and differential c-Fos expression following intracerebroventricular injection of angiotensin II and IV in rats.* Brain Res, 1995. **682**(1-2): p. 13-21.
285. Chai, S.Y., et al., *Angiotensin converting enzyme in the human basal forebrain and midbrain visualized by in vitro autoradiography.* J Comp Neurol, 1990. **291**(2): p. 179-94.

286. Chai, S.Y., et al., *Angiotensin converting enzyme in the monkey (Macaca fascicularis) brain visualized by in vitro autoradiography.* Neuroscience, 1991. **42**(2): p. 483-95.
287. Chai, S.Y., F.A. Mendelsohn, and G. Paxinos, *Angiotensin converting enzyme in rat brain visualized by quantitative in vitro autoradiography.* Neuroscience, 1987. **20**(2): p. 615-27.
288. Rogerson, F.M., et al., *Localization of angiotensin converting enzyme by in vitro autoradiography in the rabbit brain.* J Chem Neuroanat, 1995. **8**(4): p. 227-43.
289. McKinley, M.J., et al., *The brain renin-angiotensin system: location and physiological roles.* Int J Biochem Cell Biol, 2003. **35**(6): p. 901-18.
290. Reneland, R. and H. Lithell, *Angiotensin-converting enzyme in human skeletal muscle. A simple in vitro assay of activity in needle biopsy specimens.* Scand J Clin Lab Invest, 1994. **54**(2): p. 105-11.
291. Ward, P.E., J.S. Russell, and P.L. Vaghy, *Angiotensin and bradykinin metabolism by peptidases identified in skeletal muscle.* Peptides, 1995. **16**(6): p. 1073-8.
292. Vicaut, E. and X. Hou, *Arteriolar constriction and local renin-angiotensin system in rat microcirculation.* Hypertension, 1993. **21**(4): p. 491-7.
293. Linderman, J.R. and A.S. Greene, *Distribution of angiotensin II receptor expression in the microcirculation of striated muscle.* Microcirculation, 2001. **8**(4): p. 275-81.
294. Nora, E.H., et al., *Localization of the ANG II type 2 receptor in the microcirculation of skeletal muscle.* Am J Physiol, 1998. **275**(4 Pt 2): p. H1395-403.
295. Matsubara, H., *Pathophysiological role of angiotensin II type 2 receptor in cardiovascular and renal diseases.* Circ Res, 1998. **83**(12): p. 1182-91.
296. Yang, Z., et al., *Angiotensin II type 2 receptor overexpression preserves left ventricular function after myocardial infarction.* Circulation, 2002. **106**(1): p. 106-11.
297. Munzenmaier, D.H. and A.S. Greene, *Opposing actions of angiotensin II on microvascular growth and arterial blood pressure.* Hypertension, 1996. **27**(3 Pt 2): p. 760-5.
298. Malendowicz, S.L., et al., *Angiotensin II receptor subtypes in the skeletal muscle vasculature of patients with severe congestive heart failure.* Circulation, 2000. **102**(18): p. 2210-3.
299. Danser, A.H., et al., *Production of angiotensins I and II at tissue sites in intact pigs.* Am J Physiol, 1992. **263**(2 Pt 2): p. H429-37.
300. Saris, J.J., et al., *Functional importance of angiotensin-converting enzyme-dependent in situ angiotensin II generation in the human forearm.* Hypertension, 2000. **35**(3): p. 764-8.
301. Gordon, S.E., et al., *ANG II is required for optimal overload-induced skeletal muscle hypertrophy.* Am J Physiol Endocrinol Metab, 2001. **280**(1): p. E150-9.
302. Rattigan, S., et al., *Perfused skeletal muscle contraction and metabolism improved by angiotensin II-mediated vasoconstriction.* Am J Physiol, 1996. **271**(1 Pt 1): p. E96-103.
303. Frossard, M., et al., *Paracrine effects of angiotensin-converting-enzyme- and angiotensin-II-receptor- inhibition on transcapillary glucose transport in humans.* Life Sci, 2000. **66**(10): p. PL147-54.
304. Muller, M., et al., *Inhibition of paracrine angiotensin-converting enzyme in vivo: effects on interstitial glucose and lactate concentrations in human skeletal muscle.* Eur J Clin Invest, 1997. **27**(10): p. 825-30.
305. Henriksen, E.J., et al., *ACE inhibition and glucose transport in insulinresistant muscle: roles of bradykinin and nitric oxide.* Am J Physiol, 1999. **277**(1 Pt 2): p. R332-6.

306. Jessen, N. and L.J. Goodyear, *Contraction signaling to glucose transport in skeletal muscle.* J Appl Physiol, 2005. **99**(1): p. 330-7.
307. Taguchi, T., et al., *Involvement of bradykinin in acute exercise-induced increase of glucose uptake and GLUT-4 translocation in skeletal muscle: studies in normal and diabetic humans and rats.* Metabolism, 2000. **49**(7): p. 920-30.
308. Shiuchi, T., et al., *ACE inhibitor improves insulin resistance in diabetic mouse via bradykinin and NO.* Hypertension, 2002. **40**(3): p. 329-34.
309. Wong, V., et al., *Enhancement of muscle glucose uptake by the vasopeptidase inhibitor, omapatrilat, is independent of insulin signaling and the AMP kinase pathway.* J Endocrinol, 2006. **190**(2): p. 441-50.
310. Sayed-Tabatabaei, F.A., et al., *ACE polymorphisms.* Circ Res, 2006. **98**(9): p. 1123-33.
311. Batzer, M.A. and P.L. Deininger, *Alu repeats and human genomic diversity.* Nat Rev Genet, 2002. **3**(5): p. 370-9.
312. Rigat, B., et al., *An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels.* J Clin Invest, 1990. **86**(4): p. 1343-6.
313. Danser, A.H.J., et al., *Angiotensin-Converting Enzyme in the Human Heart : Effect of the Deletion/Insertion Polymorphism.* Circulation, 1995. **92**(6): p. 1387-1388.
314. Farrall, M., et al., *Fine-mapping of an ancestral recombination breakpoint in DCP1.* Nat Genet, 1999. **23**(3): p. 270-1.
315. Keavney, B., et al., *Measured haplotype analysis of the angiotensin-I converting enzyme gene.* Hum Mol Genet, 1998. **7**(11): p. 1745-51.
316. Zhu, X., et al., *Localization of a small genomic region associated with elevated ACE.* Am J Hum Genet, 2000. **67**(5): p. 1144-53.
317. Myerson, S., et al., *Human angiotensin I-converting enzyme gene and endurance performance.* J Appl Physiol, 1999. **87**(4): p. 1313-1316.
318. Gayagay, G., et al., *Elite endurance athletes and the ACE I allele - the role of genes in atheletic performance.* Hum Genet, 1998. **103**: p. 48-50.
319. Alvarez, R., et al., *Genetic variation in the renin-angiotensin system and athletic performance.* Eur J Appl Physiol, 2000. **82**(1-2): p. 117-20.
320. Taylor, R.R., et al., *Elite athletes and the gene for angiotensin-converting enzyme.* J Appl Physiol, 1999. **87**(3): p. 1035-7.
321. Rankinen, T., et al., *No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status.* J Appl Physiol, 2000. **88**(5): p. 1571-5.
322. Hagberg, J.M., et al., *VO2 max is associated with ACE genotype in postmenopausal women.* J Appl Physiol, 1998. **85**(5): p. 1842-1846.
323. Montgomery, H., et al., *Association of angiotensin-converting enzyme gene I/D polymorphism with*
change in left ventricular mass in response to physical training. Circulation., 1997. **96**(3): p. 741-7.
324. Fatini, C., et al., *RAS genes influence exercise-induced left ventricular hypertrophy: an elite athletes study.* Med Sci Sports Exerc, 2000. **32**(11): p. 1868-72.
325. Hernandez, D., et al., *The ACE/DD genotype is associated with the extent of exercise-induced left ventricular growth in endurance athletes.* J Am Coll Cardiol, 2003. **42**(3): p. 527-32.
326. Montgomery, H.E., et al., *Human gene for physical performance [letter].* Nature, 1998. **393**(6682): p. 221-2.

327. Williams, A.G., et al., *The ACE gene and muscle performance.* Nature, 2000. **403**(6770): p. 614.
328. Buikema, H., et al., *The deletion polymorphism of the angiotensin-converting enzyme gene is related to phenotypic differences in human arteries.* Eur Heart J, 1996. **17**(5): p. 787-94.
329. Murphey, L.J., et al., *Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism Modulates the Human In Vivo Metabolism of Bradykinin.* Circulation, 2000. **102**(8): p. 829-832.
330. Tsianos, G., et al., *Performance at altitude and angiotensin I-converting enzyme genotype.* Eur J Appl Physiol, 2005. **93**(5-6): p. 630-3.
331. Woods, D.R., et al., *Insertion/deletion polymorphism of the angiotensin I-converting enzyme gene and arterial oxygen saturation at high altitude.* Am J Respir Crit Care Med, 2002. **166**(3): p. 362-6.
332. Hotta, J., et al., *Polymorphisms of renin-angiotensin system genes with high-altitude pulmonary edema in Japanese subjects.* Chest, 2004. **126**(3): p. 825-30.
333. Kumar, R., et al., *Renin angiotensin aldosterone system and ACE I/D gene polymorphism in high-altitude pulmonary edema.* Aviat Space Environ Med, 2004. **75**(11): p. 981-3.
334. Dehnert, C., et al., *No association between high-altitude tolerance and the ACE I/D gene polymorphism.* Med Sci Sports Exerc, 2002. **34**(12): p. 1928-33.
335. Silva, M., et al., *Erythropoetin can promote erythroid progenitor survival by repressing apoptosis through bcl-xl and bcl-2.* Blood, 1996. **88**(1576-1582).
336. Schuster, S.J., et al., *Stimulation of erythropoietin gene transcription during hypoxia and cobalt exposure.* Blood, 1989. **73**: p. 13-16.
337. Beck, I., et al., *Enhancer element at the 3'-flanking region controls transcriptional response to hypoxia in the human erythropoietin gene.* Journal of Biological Chemistry, 1991. **266**(24): p. 15563-6.
338. Pugh, C.W., et al., *Functional analysis of an oxygen-regulated transcriptional enhancer lying 3' to the mouse erythropoetin gene.* Proc. Nat. Acad. Sci. U.S.A., 1991. **88**: p. 10553-10557.
339. Semenza, G.L., et al., *Cell-type-specific and hypoxia-inducible expression of the human erythropoietin gene in transgenic mice.* Proceedings of the National Academy of Sciences of the United States of America, 1991. **88**(19): p. 8725-9.
340. Firth, J.D., B.L. Ebert, and P.J. Ratcliffe, *Hypoxic Regulation of Lactate Dehydrogenase A.* J. Biol. Chem., 1995. **270**(36): p. 21021-21027.
341. Levy, A.P., et al., *Transcriptional Regulation of the Rat Vascular Endothelial Growth Factor Gene by Hypoxia.* J. Biol. Chem., 1995. **270**(22): p. 13333-13340.
342. Blancher, C., et al., *Relationship of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha expression to vascular endothelial growth factor induction and hypoxia survival in human breast cancer cell lines.* Cancer Research, 2000. **60**(24): p. 7106-13.
343. Semenza, G.L., et al., *Hypoxia Response Elements in the Aldolase A, Enolase 1,and Lactate Dehydrogenase A Gene Promoters Contain Essential Binding Sites for Hypoxia-inducible Factor 1.* J. Biol. Chem., 1996. **271**(51): p. 32529-32537.
344. Palmer, L.A. and R.A. Johns, *Hypoxia upregulates inducible (Type II) nitric oxide synthase in an HIF-1 dependent manner in rat pulmonary microvascular but not aortic smooth muscle cells.* Chest, 1998. **114**(1 Suppl): p. 33S-34S.

345. Yamashita, K., et al., *Molecular regulation of the endothelin-1 gene by hypoxia. Contributions of hypoxia-inducible factor-1, activator protein-1, GATA-2, AND p300/CBP.* Journal of Biological Chemistry, 2001. **276**(16): p. 12645-53.
346. Czyzyk-Krzeska, M.F., *Molecular aspects of oxygen sensing in physiological adaptation to hypoxia.* Respiration Physiology, 1997. **110**(2-3): p. 99-111.
347. Bisgard, G.E., *Carotid body mechanisms in acclimatization to hypoxia.* Respiration Physiology, 2000. **121**(2-3): p. 237-246.
348. Wang, G. and G. Semenza, *Characterization of hypoxia-inducible factor 1 and regulation of DNA binding activity by hypoxia.* J. Biol. Chem., 1993. **268**(29): p. 21513-21518.
349. Jewell, U.R., et al., *Induction of HIF-1alpha in response to hypoxia is instantaneous.* Faseb J, 2001. **15**(7): p. 1312-4.
350. Epstein, A.C., et al., *C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation.* Cell, 2001. **107**(1): p. 43-54.
351. Ivan, M., et al., *HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing.* Science, 2001. **292**(5516): p. 464-8.
352. Jaakkola, P., et al., *Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation.* Science, 2001. **292**(5516): p. 468-72.
353. Lando, D., et al., *FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor.* Genes Dev, 2002. **16**(12): p. 1466-71.
354. Mahon, P.C., K. Hirota, and G.L. Semenza, *FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity.* Genes Dev, 2001. **15**(20): p. 2675-86.
355. Maxwell, P.H., et al., *The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis.* Nature, 1999. **399**(6733): p. 271-5.
356. Park, S.K., et al., *Hypoxia-induced gene expression occurs solely through the action of hypoxia-inducible factor 1alpha (HIF-1alpha): role of cytoplasmic trapping of HIF-2alpha.* Mol Cell Biol, 2003. **23**(14): p. 4959-71.
357. Tian, H., S.L. McKnight, and D.W. Russell, *Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells.* Genes Dev, 1997. **11**(1): p. 72-82.
358. Scortegagna, M., et al., *Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1-/- mice.* Nat Genet, 2003. **35**(4): p. 331-40.
359. Goldberg, M.A., S.P. Dunning, and H.F. Bunn, *Regulation of the erythropoeitn gene:evidence thaht tha oxygen sensor is a haem protein.* Science, 1988. **242**: p. 1412-1415.
360. Horoguchi, H. and H.F. Bunn, *Erythropoetin induction in Hep3B cells is not affected by inhibition of heme biosynthesis.* Biochemica et Biophysica Acta, 2000. **1495**: p. 231-236.
361. Bruick, R.K. and S.L. McKnight, *A conserved family of prolyl-4-hydroxylases that modify HIF.* Science, 2001. **294**(5545): p. 1337-40.
362. Hirsila, M., et al., *Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor.* J Biol Chem, 2003. **278**(33): p. 30772-80.
363. Cioffi, C.L., et al., *Differential regulation of HIF-1 alpha prolyl-4-hydroxylase genes by hypoxia in human cardiovascular cells.* Biochem Biophys Res Commun, 2003. **303**(3): p. 947-53.

364. Metzen, E., et al., *Intracellular localisation of human HIF-1 alpha hydroxylases: implications for oxygen sensing.* J Cell Sci, 2003. **116**(Pt 7): p. 1319-26.
365. Berra, E., et al., *HIF-1-dependent transcriptional activity is required for oxygen-mediated HIF-1alpha degradation.* FEBS Lett, 2001. **491**(1-2): p. 85-90.
366. Berra, E., et al., *HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia.* Embo J, 2003. **22**(16): p. 4082-90.
367. Schnell, P.O., et al., *Regulation of tyrosine hydroxylase promoter activity by the von Hippel-Lindau tumor suppressor protein and hypoxia-inducible transcription factors.* J Neurochem, 2003. **85**(2): p. 483-91.
368. Roux, J.C., et al., *Developmental changes in HIF transcription factor in carotid body: relevance for O2 sensing by chemoreceptors.* Pediatr Res, 2005. **58**(1): p. 53-7.
369. West, J.B. and American Physiological Society (1887-), *High life : a history of high-altitude physiology and medicine.* 1998, New York ; Oxford: Published for the American Physiological Society by Oxford University Press. xv,493 p.
370. Roach, R.C., et al., *The Lake Louise acute mountain sickness scoring system.* Hypoxia and mountain medicine, ed. J.R. Sutton, C.s. Houston, and G. Coates. 1993, Burlington, VT: Queen city printers. 272-4.
371. Hackett, P.H., *High Altitude Cerebral Oedema and Acute Mountain sickness. A pathophysiological update.*, in *Hypoxia into the next millenium.*, R.C. Roach, P.D. Wagner, and P.H. Hackett, Editors. 1999, Kluwer Academic/ Plenum: New York. p. 23-45.
372. Severinghaus, J.W., *Hypothetical roles of angiogenesis, osmotic swelling and ischaemia in high-altitude cerebral oedema.* Jouirnal of applied physiology, 1995. **79**: p. 375-9.
373. Ross, R.T., *The random nature of cerebral mountain sickness.* Lancet, 1985. **1**: p. 990-991.
374. Vock, P., et al., *High-altitude pulmonary edema: Findings at High-altitude Chest radiography and physical examination.* Radiology, 1989. **170**: p. 661-6.
375. West, J.B., et al., *Pathogenesis of high-altitude pulmonary oedema: direct evidence of stress failure of pulmonary capillaries.* European Respiratory Journal, 1995. **8**(4): p. 523-9.
376. Schoene, R.B., et al., *The lung at high altitude: bronchoalveolar lavage in acute mountain sickness and pulmonary edema.* Journal of Applied Physiology, 1988. **64**(6): p. 2605-13.
377. Tsukimoto, K., et al., *Ultrastructural appearances of pulmonary capillaries at high transmural pressures.* Journal of Applied Physiology, 1991. **71**(2): p. 573-82.
378. Tsukimoto, K., et al., *Protein, cell, and LTB4 concentrations of lung edema fluid produced by high capillary pressures in rabbit.* Journal of Applied Physiology, 1994. **76**(1): p. 321-7.
379. Knowles, M.R., et al., *Measurement of nasal transepithelial electric potential differences in normal human subjects in vivo.* Am. Rev. Resp. Dis., 1981. **124**: p. 484-90.
380. Sakuma, T., et al., *Alveolar fluid clearance in the resected human lung.* Am. J. Respir. Crit. Care. Med., 1994. **150**: p. 305-10.
381. Sartori, C., et al., *Prevention of high -altitude pulmonary edema by bet-adrenergic stimulation of the alveolar transepithelial sodium transport. (Abstract).* Am. J. Resp. Crit. Care Med., 2000. **161**(3): p. A415.

382. Duplain, H., et al., *Exhaled Nitric Oxide in High-Altitude Pulmonary Edema . Role in the Regulation of Pulmonary Vascular Tone and Evidence for a Role against Inflammation.* Am. J. Respir. Crit. Care Med., 2000. **162**(1): p. 221-224.
383. Schneider, J.-C., et al., *Response of nitric oxide to L-arginine infusion at the altitude of 4,350m.* Eur. Resp. J., 2001. **18**: p. 286-92.
384. Sartori, C., et al., *Exaggerated Endothelin Release in High-Altitude Pulmonary Edema.* Circulation, 1999. **99**(20): p. 2665-2668.
385. Burki, N.K., *Effects of acute exposure to high altitude on ventilatory drive and respiratory pattern.* Journal of Applied Physiology, 1984. **56**(4): p. 1027-1031.
386. Hackett, P.H., et al., *Abnormal control of ventilation in high-altitude pulmonary oedema.* Journal of Applied Physiology, 1988. **64**(3): p. 1268-1272.
387. Matsuzawa, Y., et al., *Blunted hypoxic ventilatory drive in subjects susceptible to high-altitude pulmonary edema.* Journal of Applied Physiology, 1989. **66**(3): p. 1152-1157.
388. Russell, J.C. and J.D. Dale, *Dynamic torquemeter calibration of bicycle ergometers.* J Appl Physiol, 1986. **61**(3): p. 1217-20.
389. Zeballos, R.J. and I.M. Weisman, *Behind the scenes of cardiopulmonary exercise testing.* Clin Chest Med, 1994. **15**(2): p. 193-213.
390. Jensen, L.A., J.E. Onyskiw, and N.G. Prasad, *Meta-analysis of arterial oxygen saturation monitoring by pulse oximetry in adults.* Heart Lung, 1998. **27**(6): p. 387-408.
391. Nickerson, B.G., C. Sarkisian, and K. Tremper, *Bias and precision of pulse oximeters and arterial oximeters.* Chest, 1988. **93**(3): p. 515-7.
392. Morris, R.W., M. Nairn, and T.A. Torda, *A comparison of fifteen pulse oximeters. Part I: A clinical comparison; Part II: A test of performance under conditions of poor perfusion.* Anaesth Intensive Care, 1989. **17**(1): p. 62-73.
393. Choc, H., et al., *Comparison of recorded values from six pulse oximeters.* Crit Care Med, 1989. **17**(7): p. 678-81.
394. Severinghaus, J.W. and K.H. Naifeh, *Accuracy of response of six pulse oximeters to profound hypoxia.* Anesthesiology, 1987. **67**(4): p. 551-8.
395. Hannhart, B., et al., *Reliability of six pulse oximeters in chronic obstructive pulmonary disease.* Chest, 1991. **99**(4): p. 842-6.
396. Langton, J.A. and C.D. Hanning, *Effect of motion artefact on pulse oximeters: evaluation of four instruments and finger probes.* Br J Anaesth, 1990. **65**(4): p. 564-70.
397. Plummer, J.L., et al., *Evaluation of the influence of movement on saturation readings from pulse oximeters.* Anaesthesia, 1995. **50**(5): p. 423-6.
398. Wilkins, C.J., M. Moores, and C.D. Hanning, *Comparison of pulse oximeters: effects of vasoconstriction and venous engorgement.* Br J Anaesth, 1989. **62**(4): p. 439-44.
399. Clayton, D.G., et al., *Pulse oximeter probes. A comparison between finger, nose, ear and forehead probes under conditions of poor perfusion.* Anaesthesia, 1991. **46**(4): p. 260-5.
400. Barker, S.J., et al., *The effect of sensor malpositioning on pulse oximeter accuracy during hypoxemia.* Anesthesiology, 1993. **79**(2): p. 248-54.
401. Matthews, J.I., B.A. Bush, and F.M. Morales, *Microprocessor exercise physiology systems vs a nonautomated system. A comparison of data output.* Chest, 1987. **92**(4): p. 696-703.
402. Myers, J., et al., *Effect of sampling on variability and plateau in oxygen uptake.* J Appl Physiol, 1990. **68**(1): p. 404-10.

403. Laszlo, G., *Respiratory measurements of cardiac output: from elegant idea to useful test.* J Appl Physiol, 2004. **96**(2): p. 428-37.
404. Sun, X.G., et al., *Comparison of exercise cardiac output by the Fick principle using oxygen and carbon dioxide.* Chest, 2000. **118**(3): p. 631-40.
405. Johnson, B.D., et al., *Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick.* J Appl Physiol, 2000. **88**(5): p. 1650-8.
406. Barker, R.C., et al., *Measurement of cardiac output during exercise by open-circuit acetylene uptake.* J Appl Physiol, 1999. **87**(4): p. 1506-12.
407. Zenger, M.R., et al., *Measurement of cardiac output by automated single-breath technique, and comparison with thermodilution and Fick methods in patients with cardiac disease.* Am J Cardiol, 1993. **71**(1): p. 105-9.
408. Christie, J., et al., *Determination of stroke volume and cardiac output during exercise: comparison of two-dimensional and Doppler echocardiography, Fick oximetry, and thermodilution.* Circulation, 1987. **76**(3): p. 539-47.
409. Rowland, T. and P. Obert, *Doppler echocardiography for the estimation of cardiac output with exercise.* Sports Med, 2002. **32**(15): p. 973-86.
410. Richalet, J.P., et al., *Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension.* Am J Respir Crit Care Med, 2005. **171**(3): p. 275-81.
411. Bernstein, D.P., *A new stroke volume equation for thoracic electrical bioimpedance: theory and rationale.* Crit Care Med, 1986. **14**(10): p. 904-9.
412. Charloux, A., et al., *A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: comparison with the "direct" Fick method.* European Journal of Applied Physiology, 2000. **82**: p. 313-320.
413. Newman, D.G. and R.U.-h.w.s.c.s.a.B.T.G.-X.C.J.-N.c.e.e.a.a.d.e.d. Callister, *The non-invasive assessment of stroke volume and cardiac output by impedance cardiography: A review.* Aviation Space and Environmental Medicine, 1999. **70**(8): p. 780-789.
414. Bland, J.M. and D.G. Altman, *Statistical methods for assessing agreement between two methods of clinical measurement.* Lancet, 1986. **1**(8476): p. 307-10.
415. Glindmeyer, H.W., et al., *Blue-collar normative spirometric values for Caucasian and African-American men and women aged 18 to 65.* Am J Respir Crit Care Med, 1995. **151**(2 Pt 1): p. 412-22.
416. O'Dell, S.D., S.E. Humphories, and I.N.M. Day, *Rapid methods for population-scale analysis for gene polymorphisma: the ACE gene as an example.* Br. Heart. J., 1995. **73**(368-371).
417. Shanmugam, V., K.W. Sell, and B.K. Saha, *Mistyping ACE heterozygotes.* PCR Methods Appl, 1993. **3**(2): p. 120-1.
418. Evans, A.E., et al., *Polymorphisms of the angiotensin-converting-enzyme gene in subjects who die from coronary heart disease.* Q J Med, 1994. **87**(4): p. 211-4.
419. Gale, G.E., et al., *Ventilation-perfusion inequality in normal humans during exercise at sea level and simulated altitude.* J Appl Physiol, 1985. **58**(3): p. 978-88.
420. Torre-Bueno, J.R., et al., *Diffusion limitation in normal humans during exercise at sea level and simulated altitude.* J Appl Physiol, 1985. **58**(3): p. 989-95.
421. Chronos, N., L. Adams, and A. Guz, *Effect of hyperoxia and hypoxia on exercise-induced breathlessness in normal subjects.* Clin Sci (Lond), 1988. **74**(5): p. 531-7.
422. Bechbache, R.R., et al., *The effects of hypercapnia, hypoxia, exercise and anxiety on the pattern of breathing in man.* J Physiol, 1979. **293**: p. 285-300.
423. Flenley, D.C., et al., *Ventilatory response to steady-state exercise in hypoxia in humans.* J Appl Physiol, 1979. **46**(3): p. 438-46.

424. Schirlo, C., et al., *Characteristics of the ventilatory response in subjects susceptible to high altitude pulmonary edema during acute and prolonged hypoxia.* High Alt Med Biol, 2002. **3**(3): p. 267-76.
425. Ricart, A., et al., *Acclimatization near home? Early respiratory changes after short-term intermittent exposure to simulated altitude.* Wilderness & Environmental Medicine, 2000. **11**(2): p. 84-8.
426. Friedmann, B., et al., *Exercise with the intensity of the individual anaerobic threshold in acute hypoxia.* Med Sci Sports Exerc, 2004. **36**(10): p. 1737-42.
427. Benoit, H., et al., *Oxygen uptake during submaximal incremental and constant work load exercises in hypoxia.* Int J Sports Med, 1997. **18**(2): p. 101-5.
428. Bloem, L.J., A.K. Manatunga, and J.H. Pratt, *Racial difference in the relationship of an angiotensin I-converting enzyme gene polymorphism to serum angiotensin I-converting enzyme activity.* Hypertension, 1996. **27**(1): p. 62-6.
429. Sagnella, G.A., et al., *A population study of ethnic variations in the angiotensin-converting enzyme I/D polymorphism: relationships with gender, hypertension and impaired glucose metabolism.* J Hypertens, 1999. **17**(5): p. 657-64.
430. Menon, S., et al., *Racial differences are seen in blood pressure response to fosinopril in hypertensive children.* Am Heart J, 2006. **152**(2): p. 394-9.
431. Wu, J., et al., *A summary of the effects of antihypertensive medications on measured blood pressure.* Am J Hypertens, 2005. **18**(7): p. 935-42.
432. Brewster, L.L., J. Kleijnen, and G.A. van Montfrans, *Effect of antihypertensive drugs on mortality, morbidity and blood pressure in blacks.* Cochrane Database Syst Rev, 2005(1): p. CD005183.
433. Gainer, J.V., et al., *Interactive Effect of Ethnicity and ACE Insertion/Deletion Polymorphism on Vascular Reactivity.* Hypertension, 2001. **37**(1): p. 46-51.
434. Oelz, O., et al., *Physiological profile of world-class high-altitude climbers.* J Appl Physiol, 1986. **60**(5): p. 1734-42.
435. Ricart, A., et al., *Effects of sildenafil on the human response to acute hypoxia and exercise.* High Alt Med Biol, 2005. **6**(1): p. 43-9.
436. Hirshman, C.A., R.E. McCullough, and J.V. Weil, *Normal values for hypoxic and hypercapnic ventilaroty drives in man.* J Appl Physiol, 1975. **38**(6): p. 1095-8.
437. Benoit, H., et al., *Influence of hypoxic ventilatory response on arterial O2 saturation during maximal exercise in acute hypoxia.* Eur J Appl Physiol Occup Physiol, 1995. **72**(1-2): p. 101-5.
438. Collins, D.D., et al., *Hereditary aspects of decreased hypoxic reponse.* J. Clin. Invest., 1978. **62**: p. 105-110.
439. Kawakami, Y., et al., *Control of breathing in young twins.* J Appl Physiol, 1982. **52**(3): p. 537-42.
440. Kawakimi, Y., et al., *Chemical and behavioral control of breathing in adult twins.* Am. Rev. Respir. Dis., 1984. **129**: p. 703-707.
441. Costerousse, O., et al., *Angiotensin I-converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes.* Biochem J, 1993. **290 (Pt 1)**: p. 33-40.
442. Wagner, P.D., et al., *Estimation of ventilation-perfusion inequality by inert gas elimination without arterial sampling.* J Appl Physiol, 1985. **59**(2): p. 376-83.
443. Rodriguez-Roisin, R. and P.D. Wagner, *Clinical relevance of ventilation-perfusion inequality determined by inert gas elimination.* Eur Respir J, 1990. **3**(4): p. 469-82.
444. Hammond, M.D., et al., *Pulmonary gas exchange in humans during normobaric hypoxic exercise.* J Appl Physiol, 1986. **61**(5): p. 1749-57.

445. Grunig, E., et al., *Stress Doppler echocardiography for identification of susceptibility to high altitude pulmonary edema.* J Am Coll Cardiol, 2000. **35**(4): p. 980-7.
446. Rankinen, T., et al., *Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study.* J Appl Physiol, 2000. **88**(3): p. 1029-1035.
447. Montgomery, H., et al., *Angiotensin-converting-enzyme gene insertion/deletion polymorphism and response to physical training.* Lancet, 1999. **353**(9152): p. 541-5.
448. Gaesser, G.A. and G.A. Brooks, *Muscular efficiency during steady-rate exercise: effects of speed and work rate.* J Appl Physiol, 1975. **38**(6): p. 1132-9.
449. Ferguson, R.A., et al., *Muscle oxygen uptake and energy turnover during dynamic exercise at different contraction frequencies in humans.* J Physiol, 2001. **536**(Pt 1): p. 261-71.

BIBLIOGRAPHY

1. Ganong, W.F., *Review of medical physiology*. 2005, Lange.: Los Altos, Calif.,. p. v.

2. Wasserman, K., J.E. Hansen, D.Y. Sue, R. Casaburi, and B.J. Whipp, *Principles of exercise testing and interpretation*. Third ed. 1999, Baltimore: Lippincort Williams and Wilkins. 556.

3. Whipp, B.J. and K. Wasserman, *Exercise: Pulmonary physiology and pathophysiology*. Lung biology in heath and disease, ed. C. Lenfant. 1991.

4. West, J.B., *Respiratory physiology : the essentials*. 7th ed. ed. 2005, Philadelphia, Pa. ; London: Lippincott Williams & Wilkins. ix, 186 p.

5. Stryer, L., *Biochemistry*. 4th ed. 1995, New York: W.H. Freeman. [1248]p.

6. Novartis Foundation., *Signalling pathways in acute oxygen sensing*. Novartis Foundation symposium ; 272. 2006, Chichester: Wiley. xi, 288 p.

7. Cotes, J.E., D.J. Chinn, and M.R. Miller, *Lung function : physiology, measurement and application in medicine*. 6th ed. 2006, Malden, Mass.: Blackwell Pub. xi, 636 p.

8. Ward, M., J.S. Milledge, and J.B. West, *High altitude medicine and physiology*. 3rd ed. 2000, London: Arnold. xiv, 434 p.

9. Stedman, T.L., *Stedman's medical dictionary illustrated*. 24th ed ed. 1982, Baltimore London: Williams & Wilkins. xlvii,1678p,24p of plates.