PULMONARY OEDEMA FOLLOWING EXERCISE IN HUMANS

by

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Abstract

In order to determine if transient pulmonary oedema occurs after strenuous exercise, 10 well trained male athletes were challenged in normoxic and hypoxic conditions. To determine the minimal tolerable F_IO_2 for hypoxia, ten aerobically trained male athletes (VO_2max = 57.2 ± 7.95mL·kg^{-1}·min^{-1}) performed graded cycling work to maximal effort under four conditions of varying F_IO_2 (21%, 18%, 15%, 12%). Mean VO_2max was significantly reduced while breathing 15 and 12% oxygen (VO_2max = 48.2 ± 7.9 and 31.5 ± 7.4 mL·kg^{-1}·min^{-1} respectively). In the 12% oxygen condition, the majority of the subjects were not able to complete maximal exercise without SaO_2 falling below 70%. Ten highly trained males (VO_2max = 65.0 ± 7.5mL·kg^{-1}·min^{-1}) then underwent assessment of lung density by quantified magnetic resonance imaging prior to and 54.0 ± 17.2 and 100.7 ± 15.1 min following 60 min of cycling exercise (61.6 ± 9.5% VO_2max). The same subjects underwent an identical measure prior to and 55.6 ± 9.8 and 104.3 ± 9.1 min following 60 min cycling exercise (65.4 ± 7.1% hypoxic VO_2max) in hypoxia (F_IO_2 = 15.0%). Two subjects demonstrated mild exercise-induced arterial hypoxaemia (EIAH) ($minSaO_2$ = 94.5 & 93.8%), and 7 demonstrated moderate EIAH ($minSaO_2$ = 91.4 ± 1.1%) during a preliminary VO_2max test in normoxia. No significant differences ($p<0.05$) were found in lung density following exercise in either condition. Mean lung densities, measured once pre- and twice post-exercise, were 0.177 ± 0.019, 0.181 ± 0.019 and 0.173 ± 0.019g·mL^{-1} in the normoxic condition, and 0.178 ± 0.021, 0.174 ± 0.022 and 0.176 ± 0.019g·mL^{-1} in hypoxic condition. These results indicate that transient interstitial pulmonary oedema does not occur following sustained steady-state cycling

exercise in normoxia or hypoxia. This diminishes the likelihood of transient oedema as a mechanism for changes in SaO_2 during sustained exercise.

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List of symbols, nomenclature and abbreviations

ATP	adenosine triphosphate
a-vO_2diff.	arterial-venous oxygen difference
BAL	broncho-alveolar lavage
CT	computerized tomography
DL_{CO}	diffusing capacity of carbon monoxide
D_M	diffusing capacity of the alveolar-capillary membrane
ECG	electrocardiograph
EIAH	exercise induced arterial hypoxaemia
EVLW	extravascular lung water
FEV_1	forced expiratory volume in one second
F_IO_2	fraction of inspired oxygen
FVC	forced vital capacity
HAPE	high altitude pulmonary oedema
HRCT	high-resolution computerized tomography
HR_{max}	maximal heart rate
MIGET	multiple inert gas elimination technique
MRI	magnetic resonance imaging
$PaCO_2$	arterial partial pressure of carbon dioxide
PaO_2	arterial partial pressure of oxygen
PAP	pulmonary artery pressure
PEFR	peak expiratory flow rate
Q_C	cardiac output
R_f	respiratory frequency
ROI	region of interest
RV	residual volume
SaO_2	arterial oxyhaemoglobin saturation
TE	time to echo
TLC	total lung capacity
TR	repetition time
T_{vent}	ventilatory threshold
V_A	alveolar ventilation
V_A/Q_C	ventilation / perfusion ratio
V_E	ventilation
V_C	pulmonary capillary blood volume
VO_2	oxygen consumption
VO_2max	maximal oxygen consumption
V_T	tidal volume

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CHAPTER ONE: General Introduction

1.1 INTRODUCTION

The membrane separating the pulmonary capillaries from the alveoli is a remarkable structure in the human lung. The exceptionally thin yet strong nature of this membrane allows gas exchange while maintaining structural integrity under the mechanical force of the pulmonary circulation. Normally, in healthy humans, this structure functions throughout life to allow maintenance of arterial PO_2 and PCO_2 within the ranges appropriate to normal physiological function.

There are, however, circumstances such as disease and exposure to altitude, in which the normal function of this membrane is impaired, in particular due to disruption of the structural integrity of the membrane, or accumulation of fluid within the interstitial space (interstitial pulmonary oedema). The focus of this manuscript is the examination of pulmonary oedema in relation to one particular circumstance known as exercise-induced arterial hypoxaemia (EIAH), which sometimes occurs during exercise in aerobically fit individuals.

Chapter two provides a brief review of the literature to date on the topics relevant to this dissertation including exercise-induced arterial hypoxaemia and transient pulmonary oedema following exercise in animals and humans. Chapter three describes an experiment involving the examination of incremental cycling exercise under several

conditions of hypoxia. This was necessary to determine the tolerable level of hypoxia that would allow sustained high-intensity exercise. Chapter four describes an experiment designed to assess the effects of exercise, both in normoxia and hypoxia, on the development of EIAH and extravascular lung water measured as lung density by magnetic resonance imaging. Chapter five provides a summary and conclusion of the dissertation.

1.2 STATEMENT OF THE PROBLEM

Transient pulmonary oedema leading to diffusion limitation represents a possible mechanism for EIAH. Examination of transient pulmonary oedema in humans has been difficult due to the indirect nature of the measures used, and has led to inconclusive results following exercise intervention. In recent years magnetic resonance imaging (MRI) technology has allowed indirect assessment of lung density to a reasonably sensitive level. This technology allows detection of changes in lung density likely to be reflective of transient pulmonary oedema if it occurs during and following exercise. Before making the case for pulmonary oedema as a mechanism for EIAH, the first step is to establish whether it occurs in a predictable and quantifiable manner. Therefore, the purpose of the following studies is to examine the effects of exercise on transient pulmonary oedema as measured by the most sensitive tool available to date.

[1]CHAPTER TWO: Review of Literature

During continuous intense exercise the ability of the skeletal locomotor muscles to perform work is limited by the regeneration of ATP through oxidative phosphorylation. The proposed limiting factors in this process have been a focus of research during the past century, and have included pulmonary diffusing capacity, cardiac output, skeletal muscle capillary proliferation, and skeletal muscle mitochondrial capacity. As a result of experimental research over the past 30 years, cardiac output has become the most commonly accepted limit to continuous whole-body skeletal muscle work in healthy athletic individuals[8, 10, 21, 102, 107] and the pulmonary system has been accepted as a non-limiting system in healthy humans during exercise at sea level.[23]

2.1 EXERCISE-INDUCED ARTERIAL HYPOXAEMIA

Oxyhaemoglobin saturation is maintained within 2 – 4% of resting values during strenuous aerobic exercise in normal healthy human subjects. However, arterial hypoxaemia has been observed during exercise in aerobically trained athletes. This phenomenon has been observed almost half a century ago[53, 101] and well described over the past 15 years, and is termed exercise-induced arterial hypoxaemia (EIAH). The incidence of EIAH is often accepted as approximately 50% of elite male endurance athletes,[24, 90] but may occur more frequently and at lower aerobic work rates in females.[50, 99] There is variation between individuals in the degree of severity of EIAH experienced during exercise and, although EIAH is often considered to be a phenomenon that occurs

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near maximal work loads, it has also been demonstrated during moderate work.[98] Dempsey and Wagner[25] have proposed three levels of EIAH as defined by the degree of arterial haemoglobin saturation: mild (93-95%), moderate (88-93%), and severe (< 88%). Despite extensive research the mechanism responsible for EIAH has not been completely established. Those most likely to contribute are right to left shunts, relative alveolar hypoventilation, ventilation/perfusion (V_A/Q_C) mismatch, and diffusion limitation.[25] A significant amount of research has been dedicated to the study of EIAH in the past two decades with advances in the understanding of the nature of this phenomenon and the mechanisms involved.

Right to left, or venoarterial shunts allow the passage of venous blood into the arterial system causing a slight drop in the arterial oxygen content. Venoarterial shunts are the result of bronchial and Thebesian venous drainage into the left heart and represent 1 – 3% of cardiac output at rest.[22] It has been found that breathing 100% O_2 during exercise generally eradicates EIAH suggesting that venoarterial shunts are not the mechanism responsible.[24, 44, 117, 123] Recently, however, two studies involving the use of agitated saline contrast echocardiography have provided new evidence of intrapulmonary shunting of blood during exercise, in the form of contrast bubbles appearing in the left heart following injection in a systemic vein.[31, 114]

Relative alveolar hypoventilation is defined as ventilation below the rate required to maintain arterial blood gases at normal values[93] and is generally accompanied by an increased $PaCO_2$. Dempsey et al[24] have noted that little or no hyper-ventilatory response

occurred in the most hypoxemic subjects in their study. Powers et al[92] have investigated the role of inadequate hyperventilation in EIAH by examining arterial blood gases and pulmonary gas exchange in cyclists during incremental and sustained exercise while varying the fraction of inspired oxygen (F_IO_2) and concluded that relative hypoventilation is not the major cause of EIAH. However, there is evidence that relative hypoventilation may contribute to EIAH in some athletes during heavy and submaximal exercise.[28, 51] It is likely that another mechanism contributes to EIAH even if relative hypoventilation is a factor.

During exercise there is an increased A-aDO_2, typically caused by V_A/Q_C mismatch, right to left shunting of blood, or diffusion limitation.[25] V_A/Q_C mismatch may be responsible for 50% of the A-aDO_2 at rest[94], and increases with exercise increases[123] with exercise intensity to explain 60% of the A-aDO_2 during moderate to severe exercise.[44, 56] It is speculated that pulmonary diffusion limitation has an increased contribution to A-aDO_2 near maximal exercise.[94] However, the mechanism of increase in V_A/Q_C mismatch with exercise remains unclear. The investigation of the contribution of V_A/Q_C to a widening A-aDO_2 during exercise is best assessed with the multiple inert gas elimination technique (MIGET), in which inert gases are infused into the blood and the pattern of removal through the lungs is observed and recorded. In their review of EIAH, Dempsey and Wagner[25] have outlined several possible mechanisms for an increased V_A/Q_C mismatch during exercise including minor structural differences in airways and blood vessels, bronchoconstriction, airway secretions, variations in the modulation of airway and vascular tone, and mild interstitial oedema.

A limitation in the diffusion of oxygen from the alveoli to the red blood cell could contribute to EIAH, and could occur primarily in one of two ways: inadequate red blood cell transit time in the pulmonary capillaries, or an increase in the thickness of the alveolar pulmonary capillary membrane. Hopkins et al[54] have given evidence of a relationship between shortened pulmonary transit time (2.91 ± 0.3 seconds during maximal exercise vs. 9.32 ± 1.42 seconds at rest) diffusion limitation in subjects with evidence of diffusion disequilibrium from MIGET. In contrast, Warren et al[124] examined pulmonary capillary blood volume (V_C), A-aDO_2, and mean transit time during varying exercise intensities and concluded that the decrease in PaO_2 is not due to a plateau in pulmonary capillary blood volume and reduction in pulmonary transit time. Clearly examination of the relationship between pulmonary transit time and diffusion limitation is not precise and the conclusions are at least partly speculative. Powers et al[91] have investigated the effects of mildly hyperoxic (26% oxygen) exercise on two groups of athletes defined according to aerobic fitness and demonstration of EIAH with the finding that VO_2max was increased only in the more highly trained group. This is taken as evidence that pulmonary gas exchange may contribute to the limitation of VO_2max in subjects with EIAH. In females, an increase in VO_2max has been observed in a majority of subjects (22 of 25) when hyperoxic gas (26% oxygen) was administered during exercise, with approximately a 2% increase in VO_2max for every 1% decrease in the arterial saturation below resting values.[49] Hopkins et al have found that the integrity of the pulmonary blood-gas barrier is impaired during intense[57] but not during sustained submaximal[58] exercise. This is consistent with pulmonary capillary stress failure theory discussed below which may lead to diffusion limitation during exercise. Edwards et al[29]

concluded that alveolar epithelial integrity is maintained during exercise suggesting that any compromise of the alveolar-pulmonary capillary membrane occurs on the capillary side. Two further studies by Hopkins et al[55, 56] have produced results consistent with diffusion limitation as a contributor to EIAH.

EIAH has been shown to have an affect on maximal oxygen uptake (VO_2max) during exercise[68, 77, 91] and therefore, although the pulmonary system does not normally limit exercise performance, it may do so with the presence of EIAH. As described above, pulmonary diffusion limitation has been proposed as one of the contributing mechanisms to EIAH, but the nature of this limitation has remained elusive due to the difficult and invasive nature of investigation in this area. While it is possible that a combination of the mechanisms discussed above contribute to EIAH in different subjects and under different circumstances, a single mechanism has yet to be isolated as the major contributor to EIAH in highly trained athletes during severe exercise. Given the research conducted over the past several years, diffusion limitation has not been ruled out and remains a likely candidate.

2.2 PULMONARY OEDEMA

Excluding blood, the lung is approximately 80% water by weight. Between 30 – 50% of this water is extra cellular consisting of interstitial fluid and lymph.[109] Interstitial pulmonary oedema is the development of additional extravascular lung water (EVLW) caused by an increase in the filtration of fluid from the pulmonary capillaries into the interstitial space between the alveolar epithelium and capillary endothelium. Pulmonary

oedema may occur through changes in the Starling forces (pulmonary capillary leakage) and/or changes in the permeability of the capillary membrane (pulmonary capillary stress failure).[2]

Clinically, pulmonary oedema is associated with raised left atrial pressure, intravascular coagulation, or the release of vasoactive substances associated with shock. Microvascular permeability and lymphatic flow may also contribute to pulmonary oedema, but the proposed mechanism during intense exercise is increased pulmonary pressure (> 20 mmHg). A larger increase (> 30 mmHg) in pulmonary pressure may lead to fluid accumulation in the alveoli space (alveolar oedema).[69] Clinical symptoms of pulmonary oedema include coughing, cyanosis, and dyspnoea.

2.3 PULMONARY CAPILLARY LEAKAGE

The hydrostatic and osmotic pressures in the pulmonary capillaries and the interstitial space control fluid movement across the capillary alveolar membrane according to the Starling equation:[113]

$$Q_f = K_f [(P_{mv} - P_{pmv}) - \sigma (\Pi_{mv} - \Pi_{pmv})]$$

Where Q_f is net water flow out of the vascular compartment, K_f is the membrane filtration coefficient, P_{mv} is the hydrostatic pressure in the microvasculature, P_{pmv} is the interstitial hydrostatic pressure, σ is the microvascular membrane coefficient for plasma proteins, Π_{mv} is the osmotic pressure in the microvasculature, and Π_{pmv} is the interstitial

osmotic pressure.[109] Thus, net water flow is governed by the differences in hydrostatic and osmotic pressures.

Estimates of the hydrostatic pressures of the pulmonary capillaries and the interstitial space are 10 mm Hg and –3 mm Hg respectively, while the corresponding osmotic pressures are estimated as 25 mm Hg and 19 mm Hg.[2, 60] It is likely that under normal resting circumstances, Q_f is positive[109] indicating some filtration of fluid out of the pulmonary capillaries at an estimated rate of 20 $ml \cdot hr^{-1}$ in normal conditions.[126] An added complication in the practical use of the Starling equation is the discrepancy in pressures between the apices and bases of the lungs.[126]

Clearance of alveolar pulmonary oedema is a more complicated process than either a simple reversal of the Starling forces associated with alveolar epithelial leakage or a reduction in pulmonary capillary pressure associated with pulmonary capillary stress failure. Clearance of interstitial oedema involves drainage through lymphatic ducts but alveolar oedema clearance is achieved through an active pumping process involving a sodium-potassium ATPase pump in alveolar epithelial cells.[126]

2.4 PULMONARY CAPILLARY STRESS FAILURE

Pulmonary capillary stress failure is a condition that occurs as a result of structural changes in the capillary alveolar membrane.[125] Stress failure may occur in humans as a result of increased pulmonary capillary pressure in a number of circumstances: severe exercise, altitude exposure, and disease such as left ventricular dysfunction and mitral

stenosis. The capillary alveolar membrane is an exceptionally thin structure (0.2 – 0.3 μm) comprised of three layers: capillary endothelium, alveolar epithelium, and a basement membrane or extra cellular matrix between the endothelial and epithelial layers. There is some evidence that the basement membrane provides a large portion of the strength of the entire membrane structure,[118] indicating that structural changes to the basement membrane may alter the behaviour of the entire structure. Type IV collagen fibres synthesized by epithelial and endothelial cells in the basement membrane likely provide the strength of this structure.[128] The Type IV collagen fibres are arranged in lattice-shaped structures that may be temporarily altered upon introduction of stress forces. This temporary change in the collagen structure may account for a change in permeability of the membrane with raised pulmonary capillary pressure.[32] The strength of the capillary alveolar membrane is demonstrated by its ability to maintain integrity during all but the most severe physiological stresses including maximal exercise in highly trained humans and animals.

2.5 ASSESSMENT OF PULMONARY OEDEMA

Clinically, pulmonary oedema is observed with radiography (Figure 2.1) or computerized tomography (CT) scan (Figure 2.2). Several methods have been used in an attempt to detect and quantify transient pulmonary oedema including wet/dry lung weight ratio, chest radiography, presence of rapid shallow breathing, and MRI. Of these, MRI provides

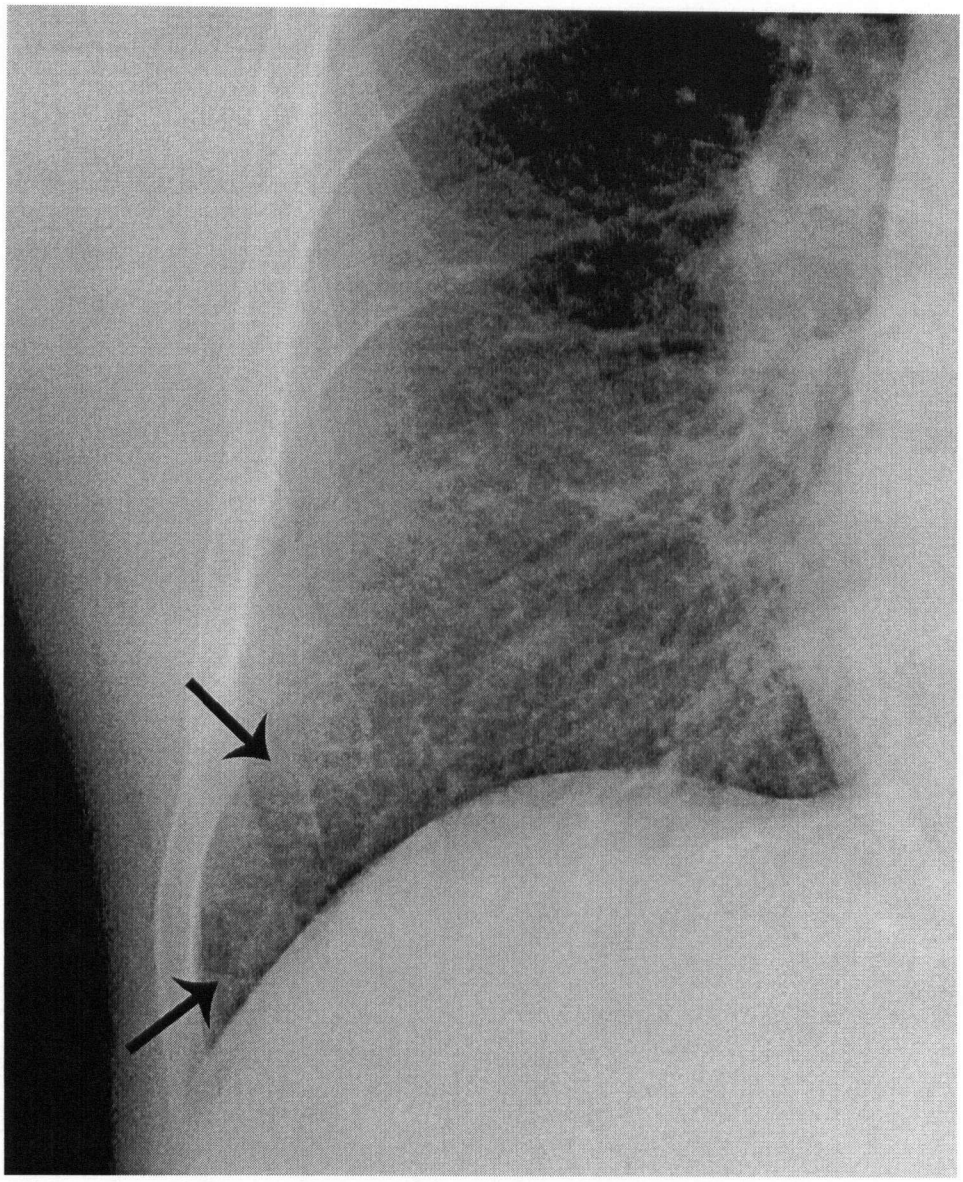

Figure 2.1. Radiograph of pulmonary oedema.
Thickened lymphatic channels in the interlobular septa (arrows) can only be seen in tangent in the periphery of the lung on this chest radiograph of a 61 year old male with mild cardiac failure. The 2 dimensional viewing perspective and the limited soft tissue contrast of the chest radiograph make it impossible to appreciate thickened interlobular septa in other regions of the right lung base.

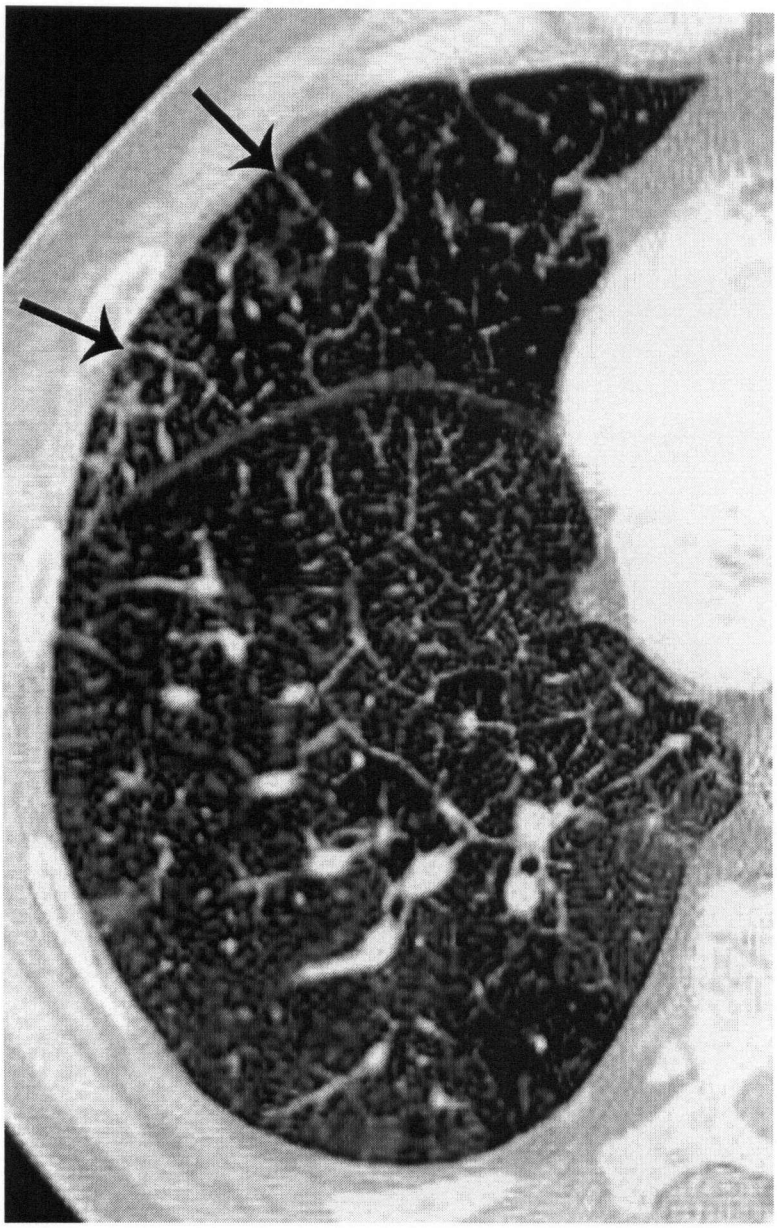

Figure 2.2. CT of pulmonary oedema.
Thin section (1mm) CT scan of the same patient as Figure 2.1 within 1 hour of radiography better demonstrates distended lymphatic channels in the interlobular septa (arrows) all around the right lung base. The cross sectional perspective and improved soft tissue contrast provided by CT imaging enhances visualization of the increased interstitial fluid in the lung.

the greatest potential for detection of the limited oedema likely to be present following severe exercise. High-resolution CT (HRCT) has been used in the evaluation of exercise-induced pulmonary oedema in cardiac patients.[12] HRCT was used before and following symptom limited exercise (Bruce treadmill protocol)[13] in 2 groups of 10 patients with chronic congestive heart failure and 10 healthy controls. Significantly more visual abnormalities were seen in the heart failure patients than controls and it was concluded that HRCT was a useful tool in the assessment of interstitial pulmonary oedema.

Comparison of three-dimensional MRI and conventional methods (multiple-indicator dilution) of assessing lung water was undertaken by Caruthers et al[19] following lung injury induction in dogs. Results suggested that the MRI technique used was sensitive to changes in lung water and that the method may be used to assess the time course of oedema formation. MRI has also been used to examine pulmonary inflammation in rats due to allergen exposure.[9, 116] It was found that the MRI signals correlated significantly with inflammatory parameters determined by broncho-alveolar lavage (BAL) and the authors conclude that MRI provides a measure of lung oedema and the pulmonary inflammatory response following allergen challenge.

MRI has been used specifically to measure water content in the lung in a study by Estilaei et al.[35] Nineteen juvenile pigs were scanned and the in vitro water content measured by MRI was strongly correlated ($r^2 = 0.98$) with gravimetric measurements.

Using the same MRI scanner and sequence, McKenzie et al[84] found a significant increase in EVLW following exercise in 8 human subjects.

There is a moderate amount of research that contributes to the literature on diffusion limitation following exercise. The remainder of this review is divided into two main areas: pulmonary diffusing capacity, and pulmonary oedema and the effects of exercise on each. The former area has been the focus of precisely controlled experiments that have come to a reasonable consensus, while the latter has remained a more difficult area to study with no clear conclusions yet on the existence or nature of oedema following exercise.

2.7 PULMONARY DIFFUSING CAPACITY

The rate of diffusion for a gas (Vgas) across the capillary alveolar membrane is determined by the following Fick equation and is controlled by the concentration gradient ($P_1 - P_2$), membrane area (A), membrane thickness (T), and a diffusion constant (D):[126]

$$Vgas = (A / T) \times D \times (P_1 - P_2)$$

During exercise these factors may be influenced by changes in alveolar or capillary gas concentrations, V_A/Q_C inequalities, and the development of pulmonary oedema respectively. In the lung, diffusing capacity (D_L) takes area, thickness, and the diffusion constant into account[126] and may be partitioned into the diffusing capacity of the alveolar-capillary membrane (D_M) and capillary blood volume (V_C).[61] Total resistance

($1/D_L$) is the sum of membrane resistance (1/DM) and red cell resistance ($1/\theta V_C$) such that:

$$1 / D_L = 1 / D_M + 1 / \theta V_C \quad [61]$$

The measurement of pulmonary diffusing capacity is a relatively easy and non-invasive procedure in the laboratory. Carbon monoxide (CO) is a gas that has a high affinity for haemoglobin and therefore is diffusion limited,[126] which makes it useful for the measurement of pulmonary diffusing capacity. The single breath method involves the inhalation of a trace amount of CO, followed by a 10 s breath hold. Upon exhalation the rate of diffusion is calculated from the difference in CO concentration in the inhaled and exhaled breaths.

2.8 PULMONARY DIFFUSING CAPACITY & EXERCISE

Normally the diffusion rate for CO (DL_{CO}) is 25 $ml \cdot min^{-1} \cdot mmHg^{-1}$ at rest, and may increase by 2 or 3 fold during exercise[126] primarily as a result of changes in the V_A/Q_C relationship and pulmonary capillary recruitment and distension. Diffusion limitation has been proposed as a contributing mechanism to EIAH,[25] but the role of pulmonary oedema in this limitation remains unclear. While diffusing capacity is reduced following exercise,[46, 47, 73, 83, 95-97, 108] this reduction has been associated with a decreased V_C rather than a reduction in D_M, which would be more indicative of pulmonary oedema[46, 48, 84]. A number of studies have examined pulmonary diffusing capacity following high intensity exercise.

Rasmussen et al have reported a 6.7% decline in D_L 2.1 hr following a short maximal bout of arm exercise.[96] A reduction in D_L of 15% has been demonstrated independent of exercise mode (maximal arm cranking, treadmill running, or ergometer rowing) 2 – 3 hr post-exercise.[97] The decreased D_L has been shown to persist for 2.5 days following intense rowing exercise.[95]

A study involving 12 elite handball players examined the effects of maximal exercise (incremental cycling) on membrane diffusing capacity (D_M).[73] Pulmonary capillary blood volume (V_C) and D_M were determined with the single breath method prior to and for 30 minutes following progressive maximal exercise. DL_{CO} was found to decrease significantly up to 30 minutes post-exercise, and at 30 minutes returned to the control resting value.

Two studies by Hanel et al[46, 47] suggest that the drop in diffusion capacity post-exercise is the result of a change in central blood volume rather than pulmonary oedema. In the first study, DL_{CO} was measured following sub-maximal rowing and maximal rowing. Diffusion capacity was decreased by 6% and 10% following 6 minutes of exercise at 61% and 76% VO_2max respectively, and by 7%, 8%, and 7% following maximal rowing for one, two, and three minutes respectively. The authors concluded that the decrease in DL_{CO} following even a short-term exercise bout is unlikely due to change in the pulmonary capillary membrane integrity. The second study involved the measurement of DL_{CO} following exercise in 21 subjects divided into three groups. The first group

performed two all-out rows on an ergometer for six min, the second group performed one all-out rowing bout followed by the administration of a diuretic (10 mg furosemide) 150 min post-exercise, and the third group performed one all-out rowing bout and served as controls. It was found that DL_{CO} was reduced following exercise from a median of 37 to 34 $ml \cdot min^{-1} \cdot mmHg^{-1}$. Both D_M and V_C were reduced, and a second bout of exercise did not change DL_{CO} or D_M. Administration of furosemide did not affect DL_{CO}.

McKenzie et al[83] examined the effects of repeated exhaustive cycling on EIAH in 13 athletic male subjects. DL_{CO} was 36.3 ± 4.6, 32.4 ± 6.0, and 30.4 ± 5.4 $ml \cdot min^{-1} \cdot mmHg^{-1}$ at rest and following the first and second bouts of exercise respectively. D_M and V_C were also progressively reduced with each bout of exercise. No significant difference was found between the minimum SaO_2 in the two tests, and therefore the authors concluded that, if pulmonary oedema developed during the initial exercise bout, it was of no clinical significance. Further, the authors suggest that the changes in DL_{CO} may reflect changes in blood flow rather than oedema.

Hanel et al[48] hypothesized that the decreased DL_{CO} following exercise is partly the result of a redistribution of blood away from the central vascular bed. DL_{CO} measures were made in nine male oarsmen prior to and 120 minutes following six minutes of maximal rowing exercise, and in six male controls with no exercise intervention. Blood volume and thoracic and thigh blood flow activity was imaged by labelling with ^{99m}TC pertechnetate. DL_{CO} was decreased by 6% post-exercise and was unchanged in the

control group, and a shift in fluid from the thoracic to peripheral area occurred confirming the hypothesis.

A study by Sheel et al[108] examined the time course of DL_{CO} 1,2,4,6, and 24 h post-exercise in three groups of subjects defined according to aerobic fitness (group one ≥ 65, group two 50 – 60, group three ≤ 50 ml·kg^{-1}·min^{-1}). The exercise intervention consisted of high intensity cycling. DL_{CO} was decreased one hour post-exercise, with a minimal value of 88% of baseline values at 6 h post-exercise. DL_{CO} following 24 h was not different than baseline values. The authors conclude that the decreased DL_{CO} following intense exercise is largely due to a decreased V_C.

2.9 PULMONARY OEDEMA & EXERCISE

During exercise there is an observed increase in the alveolar-arterial oxygen difference $(A\text{-}aDO_2)$[4] resulting from V_A/Q_C mismatch[56] and diffusion disequilibrium.[117] It has been hypothesized that pulmonary oedema may contribute to the V_A/Q_C mismatch during exercise.[75, 104]

Pulmonary oedema has been demonstrated following exercise in animals.[103] There is evidence that during severe exercise in Thoroughbred horses, the high pulmonary capillary pressures cause stress failure and interstitial oedema.[129] Evidence in humans is less consistent and, while there is evidence of membrane damage and/or pulmonary oedema following both severe[57] and prolonged exercise,[82, 84] it is not clear that all

humans demonstrate this phenomenon nor, when present, is it clear the degree to which it may contribute to impairment of gas exchange.

There is limited data on the study of pulmonary oedema in animals and humans following exercise. The most accurate measures involve invasive techniques that often limit study of this phenomenon in humans. (See Table 2.1 for a summary of the following findings).

2.10 ANIMAL STUDIES

Marshall et al[75] examined canine lungs for changes in lung water by weighing of lung tissue drained of blood. Four dogs performed maximal treadmill exercise (running at 10 $km \cdot h^{-1}$ for 20 min) and four dogs served as resting controls. Immediately following exercise the animals were sacrificed and the lungs were removed and prepared for weighing. No significant difference was found in lung water between exercised and control animals.

In a study on the detection of pulmonary oedema in dogs through the use of radiography, densitometry and lung water, Snashall et al[110] found a positive correlation between radiological grade and lung water. Pulmonary oedema was induced in three groups of dogs with the intention of determining the ability to detect extravascular lung water by chest radiography. Intravenous injection of Alloxan to increase micro vascular permeability was used in one group, extra cellular fluid volume expansion with Hartmann's solution was used in a second group, and the third group underwent

pulmonary angiography with 70 ml of sodium iothalamate. A fourth group of dogs acted as controls. In addition to radiography, the lungs were removed and the extravascular water/dry lung weight ratio was measured. Further, change in the opacity of the radiograph films was assessed. In the Alloxan group, perivascular cuffs were observed in all dogs and peribronchial cuffs in cases of severe oedema, and mean extravascular water / dry lung weight ratio was 8.9 ± 3.8. In the angiography group, one dog developed severe oedema in one lung, three of the dogs were normal, and small perivascular cuffs were visible in the final subject. The mean extravascular water / dry lung weight ratio was 4.8 ± 0.6 in this group. In the Hartmann's solution group perivascular cuffing was seen in all dogs and two dogs had peribronchial cuffs. Mean extravascular water / dry lung weight ratio was 7.3 ± 4.6. In the control group macroscopic examination of the frozen lungs was normal in all animals. Microscopically, perivascular cuffs were seen in two animals and the mean extravascular water / dry lung weight ratio was 4.1 ± 0.6 in the upper lobes. The authors conclude that the radiograph is a sensitive method for the detection of acute pulmonary oedema with an increase in extravascular water of greater than 35% being detectable as oedema by chest radiography. With any measure that relies on the subjective analysis of an observer, the reproducibility of the measures are important. In this study, there were no statistically significant differences between the two radiologists' assessments of the lower zones of the lungs, but their upper zone assessment did differ significantly, which may call into question the reliability of this conclusion. However, the authors concluded that this method may be appropriate for the detection of clinical pulmonary oedema in humans.

Examination of swine has provided evidence of pulmonary oedema following heavy exercise. In a study involving high-intensity treadmill exercise,[103] five pigs ran at the highest speed maintainable for 6 – 7 minutes while five pigs acted as controls. Control and exercised animals had identical previous treadmill training. Upon completion of the exercise period, the animals were sacrificed and four lung tissue blocks from each animal were prepared for microscopic examination and documentation of perivascular or peribronchial cuffing. The exercised animals showed significantly more periarterial cuffing in general, as well as a higher percentage of periarterial cuffing in the lower lobes and ventral areas of the lung than the control animals. Interstitial oedema is characterized by peribronchial and perivascular engorgement,[126] and the authors suggest this, and the observation of cuffing primarily around the larger vessels, provides evidence of early stage interstitial oedema and conclude that mild pulmonary oedema can occur during heavy exercise in the pig.

Manohar has extensively researched the effects of exercise on Thoroughbred horses. In a study involving repeat treadmill exercise bouts in seven Thoroughbred horses, Manohar et al[74] reported significantly higher PaO_2 during the second exercise performed at the same workload (galloping at 14 $m \cdot s^{-1}$ on a 3.5% uphill grade) following six minutes of rest. These results suggest that a structural change is not the mechanism for EIAH in these animals, or at least that there is no worsening of oedema during subsequent exercise.

2.11 HUMAN STUDIES

Evidence of increases in lung water following exercise in humans is inconclusive. While there is evidence suggesting that transient pulmonary oedema may occur, direct measurement has proven difficult and studies have drawn conclusions in both directions about the occurrence of oedema following exercise in humans.

One of the earliest studies on pulmonary oedema in humans involved the case studies of two ultra-marathon runners following completion of a 90-km race.[82] Both athletes demonstrated bilateral pulmonary consolidation, upper lobe venous congestion and cardiomegaly with no evidence of hypertrophic cardiomyopathy, mitral stenosis, or aortic valve disease. The authors suggest that the radiological findings, in conjunction with the lack of disease, are likely due to cardiogenic pulmonary oedema following exercise of an extreme duration (9.4 and 9.9 hrs for athletes one and two respectively).

Buono et al[15] have conducted a series of studies to examine the changes in residual volume and total lung capacity following maximal exercise shown in an earlier study.[14] In the first study, trans-thoracic electrical impedance was decreased following 30 minutes of recovery from exercise suggesting an increase in intra-thoracic fluid. The second study examined the effects of G-suit use on central blood volume and post-exercise RV and found that the post-exercise increase in RV was not due to increased thoracic blood volume. In the third study, no decrease in DL_{CO} / V_A was observed despite an increased post-exercise heart rate. The authors conclude that pulmonary oedema is present following exercise and may contribute to the post-exercise increase in RV and TLC.

Schaffartzik and colleagues[104] examined the V_A/Q_C relationship in 13 male humans at rest, during exercise, and during recovery in an attempt to explain the changes in V_A/Q_C through the occurrence of pulmonary oedema. It was hypothesized that a V_A/Q_C mismatch that remained elevated for some time following exercise would be indicative of pulmonary oedema, whereas resolution of a V_A/Q_C mismatch that followed the return of cardiac output and ventilation to resting values would be indicative of changes in ventilation and blood flow per se. V_A/Q_C mismatch was assessed by the measurement of Q_C through indocyanine green dye dilution and the measurement of V_A through the multiple inert gas elimination technique. Two groups of subjects were classified according to the degree of V_A/Q_C mismatch observed from rest to heavy exercise. Group one demonstrated an increased V_A/Q_C mismatch during exercise while group two did not. During recovery, both groups initially showed a decrease in V_A/Q_C mismatch consistent with decreases in ventilation and blood flow during recovery. Over the initial 20 min of recovery there was a resolution of the differences in V_A/Q_C mismatch between the two groups consistent with oedema formation in group one. The authors conclude that, while there is no proof of pulmonary oedema, the findings are consistent with the formation of pulmonary interstitial oedema.

Exercise in hypoxic conditions provides a unique circumstance for the study of pulmonary oedema due to the accentuating role of hypoxia in the development of oedema. Anholm et al[3] have examined cyclists at altitude for radiographic evidence of pulmonary oedema . Chest radiographs were obtained before and following various

distances ranging from 70 – 131.5 km of road cycling at altitude (range 2,097 – 3,369 m), and analyzed by three radiologists for signs of pulmonary oedema (loss of sharp definition of pulmonary vascular markings, hilar blurring, Kerley A, B, or C lines, peribronchial cuffing, thickening of the fissures, diffuse opacification, and pleural effusion). Scores were given according to the degree of oedema observed, and subtle but significant signs of oedema were found (loss of definition of vascular markings). The authors concluded that the findings provide evidence of pulmonary oedema as a result of increased cardiac output and increased filtration beyond clearance ability, rather than as a result of capillary stress failure, which they conclude would appear radiographically more similar to the signs of high altitude pulmonary oedema.

Caillaud and colleagues [16] have provided further indirect evidence of pulmonary interstitial oedema following exercise. Eight male trained subjects (triathletes) and eight untrained subjects performed an incremental cycling VO_2max test followed by measures of ventilation and arterial blood gases during recovery. Rapid shallow breathing was defined as a positive ΔV_T, where ΔV_T is the difference in exercise V_T and recovery V_T. Both groups of subjects developed rapid shallow breathing during recovery and the trained group did so to a significantly greater degree than the untrained group.

A recent study by McKenzie et al[84] provides evidence of extravascular lung water following sustained exercise in humans. Eight male cyclists performed a 45-minute cycling test followed by measures of DL_{CO}, D_M, V_C, and MRI of the chest. DL_{CO} and V_C were significantly decreased post exercise while D_M was unchanged. There was a

significant increase in extravascular lung water post exercise (0.223 ± 0.0225 vs. 0.244 ± 0.0506 $g \cdot ml^{-1}$ pre- and post-exercise respectively). A previous study has shown that extravascular water increases should be greater than 35% to be detected by chest radiography,[110] but the detection of an increase of less than 10% in this study provides promise that MRI technology may be more effective in this area. This use of MRI technology may provide a new technique for quantitative assessment of extra-vascular lung water in humans.

There is indirect evidence of pulmonary oedema as a mechanism for EIAH in the form of pulmonary capillary stress failure as shown by increased concentration of red blood cells in BAL fluid following intense exercise.[57] As indicated earlier in the study of Thoroughbred horses,[74] in theory exercise-induced pulmonary oedema should have an effect on arterial saturation during subsequent exercise if the oedema is of the severity to affect diffusion, and if it persists through the rest period between exercise bouts. Two studies have investigated such effects of repeated exercise bouts on EIAH in humans.[74, 83, 112] St. Croix et al[112] studied the effects of two high-intensity treadmill exercise bouts separated by 20 minutes on EIAH (measured by arterial blood samples) in females of various fitness levels. Contrary to the authors' hypothesis, the results showed a slightly less severe EIAH during the second exercise bout. This is taken as evidence of a "functionally based mechanism" which does not persist beyond the exercise, rather than a mechanism resulting from a temporary change in structure of the gas barrier. Further, the authors suggest that the structural change of stress failure in the pulmonary capillaries caused by high pulmonary pressures is not the mechanism responsible for EIAH. In a

similar study, McKenzie et al[83] found no significant difference in minimum arterial saturation (measured by pulse oximetry at the ear lobe) between two progressive cycling VO_2max tests separated by 60 minutes of rest. The authors suggest that the mechanism for EIAH is not aggravated by repeat exercise, and these results suggest that any pulmonary oedema that was present either cleared prior to the second exercise bout, or was not significant enough to interfere with pulmonary gas exchange.

Upon examination with CT, mean lung density has been shown to increase (0.21 ± 0.009 to 0.25 ± 0.01 $g \cdot cm^{-3}$) following triathlon in male athletes.[16] Manier et al[72] studied nine trained runners in an attempt to verify and quantify the mechanism of this change. Specifically, the previous study by Caillaud et al[16] examined only a few slices of the lungs, while Manier et al[72] examined the distribution of density in the whole lung to provide a measure of lung mass. In this study, no significant changes were found in measured lung density or lung mass following two hours of running at 75% VO_2max. No visual observations associated with pulmonary oedema (increase in the observed pulmonary vessels) were present. The authors concede that any change in extravascular lung water could have been below the CT resolution.

In a study involving nine well-trained male subjects, extravascular lung water measured by the double indicator dilution technique, and pulmonary capillary blood volume measured by the carbon monoxide diffusing capacity method were compared between rest and following 10 and 50 min of bicycle ergometer work.[120] A significant increase in extravascular lung water was found between rest and following 10 min of exercise ($178 \pm$

37 to 219 ± 46 ml), with no significant change between 10 and 50 minutes. Pulmonary capillary blood volume also increased significantly from 140 ± 42 to 220 ± 106 ml from rest to 10 min, with no significant difference after 50 min. It was concluded that the increase from rest to exercise signalled the redistribution of alveolar wall blood flow, and that the lack of difference between the two exercise durations signalled a lack of accumulation of lung fluid.

Marshall et al[76] found an increase in pulmonary extravascular water (126 ± 15 vs.155 ± 8 ml), as measured with the double indicator-dilution technique, when male subjects changed posture from sitting to lying supine. A further increase in extravascular water (229 ± 22 ml) was found with supine cycling exercise at a workload of 150 $kg \cdot m^{-1}$. The double indicator-dilution technique is dependant on lung perfusion since the measure of extravascular lung water depends upon the almost instantaneous equilibrium of indicator (tritiated water in this study) with pulmonary extravascular water. Therefore, it cannot be concluded that an increase in extravascular water as measured by this technique necessarily represents pulmonary oedema.

Radiographic examination of five male subjects following maximal exercise (cycling VO_2max test) failed to show evidence of pulmonary oedema.[39] Subjects cycled to fatigue on a ramp protocol starting at 50 W and increasing by 50 W every three minutes, immediately followed by a chest radiograph (within two minutes of the end of exercise). Radiographs were examined for signs of pulmonary oedema including redistribution of pulmonary blood flow, loss of sharp definition of pulmonary vascular markings, hilar

blurring, and perivascular and peribronchial cuffing. A densitometer was used to measure the radiographic density of six areas of the lungs. No evidence of pulmonary oedema was found, and the authors therefore concluded that any increase in extravascular lung water must have been trivial.

Measure	Model	EVLW Change	Reference
Lung weighing	Canine	No	Marshall et al[75]
Radiography & lung	Canine	Yes	Snashall et al[110]
Microscopic	Swine	Yes	Schaffartzik et al[103]
Radiography	Human	Yes	McKechnie et al[82]
Double indicator-	Human	No	Marshall et al[76]
Double indicator-	Human	No	Vaughan et al[120]
Trans-thoracic electrical	Human	Yes	Buono et al[15]
CT	Human	Yes	Caillaud et al[16]
CT	Human	No	Manier et al[72]
Radiography	Human	No	Gallagher et al[39]
Radiography	Human	Yes	Anholm et al[3]
MRI	Human	Yes	McKenzie et al[84]

Table 2.1. Summary of major extravascular water studies.

2.12 EXERCISE IN ACUTE HYPOXIA

Any sojourner to altitude has experienced the detrimental effects of hypoxia on exercise, and these have been well documented. With exposure to altitude or decreased F_IO_2, there is a decrease in arterial PO_2, and arterial oxyhaemoglobin saturation falls according to the

sigmoidal oxyhaemoglobin dissociation curve. The decrease in exercise capacity is demonstrated as a curvilinear[81] decrease in VO_2max with increasing altitude or isobaric hypoxia. Upon exposure to moderate altitudes of approximately 4,000m, this drop in VO_2max may appear more linear.[70, 87] The exact response to hypoxic exercise is somewhat variable in the literature. Fulco et al[38] have reviewed a number of studies involving exercise testing during exposure to hypoxia and listed potential sources of variation in the responses including: differences in fitness levels, resident altitude prior to study, gender, age, hypoxic ventilatory response, and duration of exposure.

Upon acute exposure to hypoxia during exercise, ventilation is significantly increased compared to sea-level exercise of comparable intensities. Fifty years ago Astrand[5, 6] performed studies on the ventilatory response to hypoxia and demonstrated that ventilation is significantly increased during exercise at altitude, but that this increased ventilation is mitigated by breathing oxygen rather than normal air. Maximal exercise values for V_E, and V_E/VO_2 have been shown to increase linearly with exposure to decreased F_IO_2.[87] Nevertheless, the increased ventilation during exercise in hypoxia is not adequate to maintain SaO_2 or VO_2max at sea-level values. According to Calbet et al,[17] the decrease in VO_2max in moderate hypoxia can be explained by the decrease in arterial oxygen content but in severe hypoxia this only partially explains the decrease in VO_2max. Other factors in severe hypoxia ($F_IO_2 \leq 10.5\%$) include impaired pulmonary gas exchange and decreased cardiac output (and therefore decreased skeletal muscle blood flow).

2.13 HIGH ALTITUDE PULMONARY OEDEMA

High altitude pulmonary oedema (HAPE) is a condition that may accompany exposure to altitude (2,500m and greater) or hypoxia, particularly when exposure is acute and without adequate acclimatization, or in combination with exercise. Prevalence of HAPE is <0.2% with a slow ascent and up to 10% with a rapid ascent.[7] There are many intricately connected mechanisms involved in the development of HAPE, some of which may be a factor in the development of oedema during exercise in hypoxia. According to Bartsch[7] these include pulmonary vasoconstriction, increased endothelin release[27] and decreased nitric oxide (NO) synthesis,[105] structural damage,[122] increased pulmonary capillary permeability,[43, 64, 106] and changes in alveolar fluid clearance.[71, 89] In general a high pulmonary pressure is associated with the development of HAPE.[7] Pulmonary pressure is increased above resting values during exercise in normoxia, and in combination with hypoxia the effect may be accentuated.

2.14 MAGNETIC RESONANCE IMAGING (MRI)

MRI or nuclear magnetic resonance (NMR) is a non-invasive medical imaging technique that involves placing the patient within the coil of a strong magnet. Three strengths of magnetic field are commonly used with MRI: low field (0.035-0.3 T), mid field (0.5-1.0 T), and high field (1.0-1.5 T). Field strengths of over 2.0 T are often used during research imaging. The large magnetic field causes the protons of hydrogen atoms in the body to align the poles of their spin in a uniform direction. The protons are then bombarded with electromagnetic radiation of radio frequency, which causes a momentary change in the proton orientation. Upon return to the uniform orientation the protons emit a detectable

radio signal reflecting the number of protons in a particular volume of tissue. Typically MRI is used clinically for the visual examination of soft tissues, particularly cerebral areas.

Because MRI technology depends on a proton signal, a water phantom may be used to provide a baseline measure of the signal generated by a known volume of water. In this way MRI may be adapted for research use to examine the quantity of water in a volume of tissue.

2.15 CONCLUSION

It may be concluded from these studies that diffusing capacity generally declines following exercise in humans. However, it should not be concluded that the commonly observed reduction in D_L following exercise could be definitively attributed to interstitial pulmonary oedema.[45] The studies directly examining diffusing capacity suggest that a redistribution of blood flow is the more likely explanation. Those studies that have attempted to measure pulmonary oedema following exercise do not offer such a distinct conclusion. It appears from this review that transient pulmonary oedema following exercise is possible in certain circumstances, but more research is needed before a definitive conclusion may be drawn.

CHAPTER THREE: Incremental Exercise Under Varying Hypoxic Conditions

3.1 INTRODUCTION

Continuous high-intensity exercise such as that undertaken by endurance athletes requires a dramatic increase in the oxygen demand of the aerobic metabolic processes that occur in skeletal muscle mitochondria. In accordance with the Fick equation [$VO_2 = Q_C \cdot (a\text{-}vO_2)diff.$], both cardiac output and mean arterial-venous oxygen difference increase during continuous exercise to meet the increased demand for oxygen. For this reason, any interference with these changes stands to influence maximal oxygen consumption. The alteration of F_IO_2 during exercise has a number of effects on the normal physiological response to exercise, most notably increased ventilation for a given workload in an attempt to maintain PaO_2. During heavy exercise in moderate hypoxia, however, PaO_2 inevitably falls below values normally seen during exercise of comparable intensity in normoxia.

A number of studies have documented the performance[1, 26, 30, 111] and physiological[17, 33, 36, 59, 62, 63, 66, 77, 87, 88, 100, 127, 130] responses to maximal exercise in hypoxia, but the degree of hypoxia in which subjects are capable of completing maximal exercise without SaO_2 dropping below acceptable levels, remains unclear. In our lab we have set a minimum SaO_2 of 70% as the cut-off threshold, dictated mainly by the specifications of the pulse oximeters in use. During exercise, mean pulmonary arterial pressure (PAP) may rise above 30 mmHg[40] from resting values of approximately 15 mmHg[126]. Although the mechanisms of increased PAP are different with exercise and exposure to hypoxia, both

provide a useful means to examine the effects of raised mean PAP on pulmonary blood flow and the development of interstitial oedema. Of interest is the level of hypoxia that subjects may tolerate without prohibiting them from performing high intensity exercise.

Therefore the purpose of this study was to examine maximal cycling exercise in varying levels of hypoxia and to establish the greatest level of hypoxia while still allowing subjects to perform high intensity cycling work. To elicit a significant physiological response to simulated altitude, the lowest possible F_IO_2 was desirable for subsequent portions of this thesis (Chapter 4). Therefore in this study, four conditions of varying levels of F_IO_2 (21, 18, 15, and 12% O_2) were used while performing cycling exercise of increasing intensity to maximal effort on several visits to the laboratory. It was hypothesized that VO_2max and SaO_2 would be significantly different between each condition, and that not all subjects would manage to achieve volitional fatigue during the F_IO_2 = 12% condition without SaO_2 falling below 70%.

3.2 METHODS

Subjects

Ten healthy, habitually active males (age = 29.6 ± 5.8 y, height = 181.1 ± 8.3 cm, mass = 79.4 ± 5.6 kg) with no history of respiratory disease participated in this study. Written informed consent was obtained from all subjects prior to participation as approved by The University of British Columbia Committee on Human Experimentation.

Exercise Testing

A randomized, blinded design was used with four conditions corresponding to normoxia and three levels of hypoxia during exercise. Under each condition, subjects performed a graded cycle test to exhaustion (VO_2max test) on an electronically braked cycle ergometer (Quinton Excalibur, Lode, Groningen, Netherlands). Subjects started at 0 W and increased at a rate of 30 $W \cdot min^{-1}$. Expired gases were collected and analyzed and ventilation was measured (True One, Parvomedics, Sandy, UT) and averaged every 15 seconds. VO_2max was calculated as the mean of the four highest consecutive readings. Heart rate was measured by telemetry (Polar Vantage XL, Kemple, Finland) and averaged every 15 seconds, and SaO_2 was measured every 15 seconds using an ear pulse oximeter (Biox 3740, Ohmeda, Madison, WI). The test stopped when SaO_2 dropped below 70%, or when the subject experienced volitional fatigue and could not maintain a constant pedalling rate. There are certain considerations to be taken into account when using pulse oximetry to assess SaO_2. These are discussed in the methods section of Chapter 4.

Gas Delivery

The four conditions included 21, 18, 15, and 12% F_IO_2, with the balance nitrogen. Following delivery from a compressed gas tank, the inspired air was humidified and subjects breathed through a two-way valve (Hans Rudolph, Kansas City, MO). Due to limited flow through the tank regulator, a Douglas bag was used to store the humidified air prior to delivery to the subject to allow adequate ventilation during high-intensity exercise. Compressed gas from a tank was used in all four conditions, including

normoxia, in order to preserve the blinding effect. Prior to beginning exercise, subjects remained seated on the cycle for five minutes of rest while breathing the selected gas concentration. For safety reasons, medical oxygen was readily available throughout the testing.

Statistics

Repeated measures analysis of variance was used to examine the differences between VO_2max, peak power, SaO_2, HR, V_E, V_T, R_f, V_E/VO_2, and V_E/VCO_2 under each condition. Tukey's Honest Significant Difference post-hoc test was performed where a significant difference was found. For all analyses, significance was set at $\alpha = 0.05$. Mean decline in VO_2max for all conditions was calculated for each subject and correlated to normoxic VO_2max and to minimum SaO_2 in the normoxic condition.

3.3 RESULTS

There were significant differences in VO_2max, $F(3,36) = 24.5$, peak power $F(3,36) = 28.4$, and minimum SaO_2, $F(3,36) = 142.1$ between the four conditions (see figures 3.1, 3.2, and 3.3).

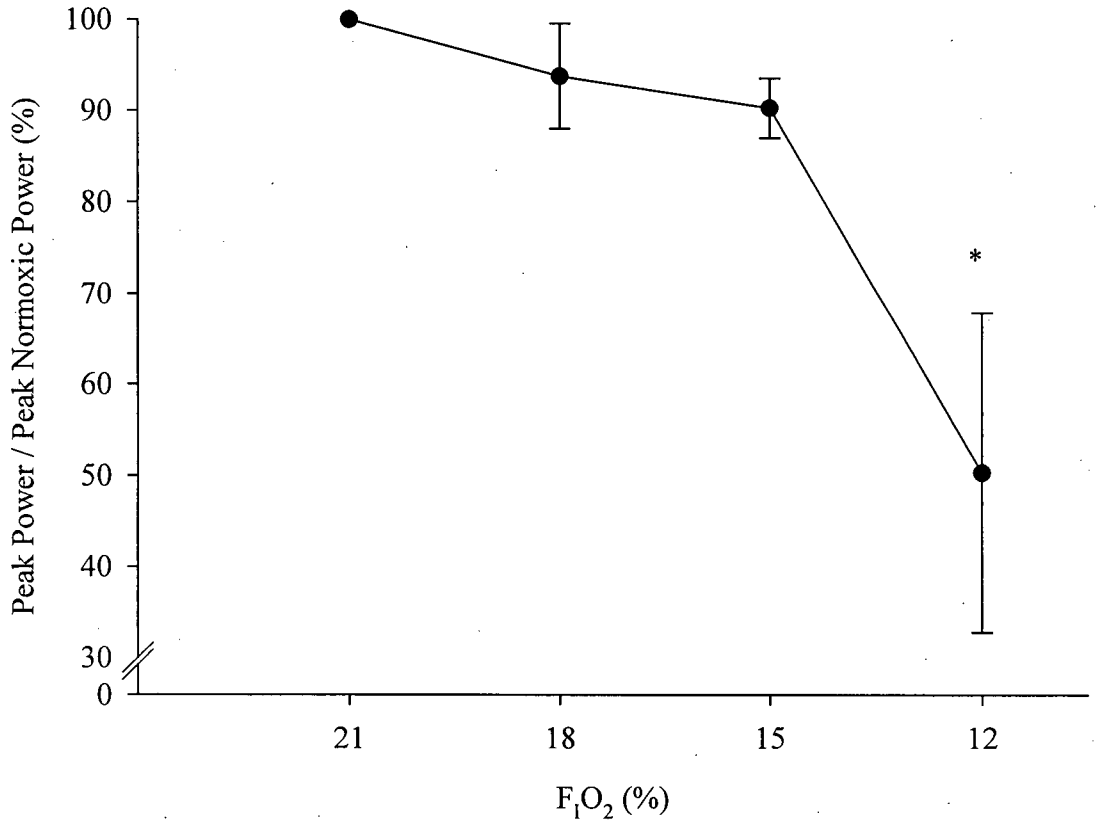

Figure 3.1. Peak power / peak normoxic power (mean ± SD); N = 10.
* Significantly different from the normoxic values ($p<0.05$).

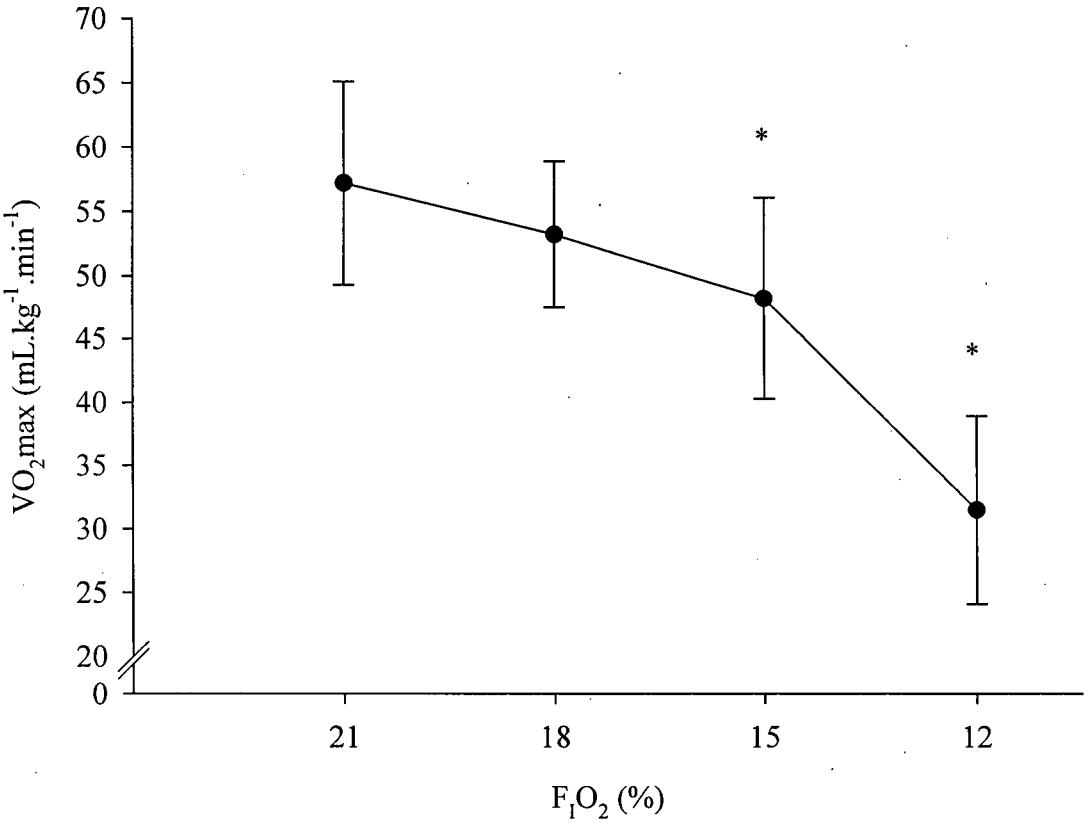

Figure 3.2. VO_2max with varying F_IO_2 (mean ± SD); N = 10.
* Significantly different from the normoxic values ($p<0.05$).

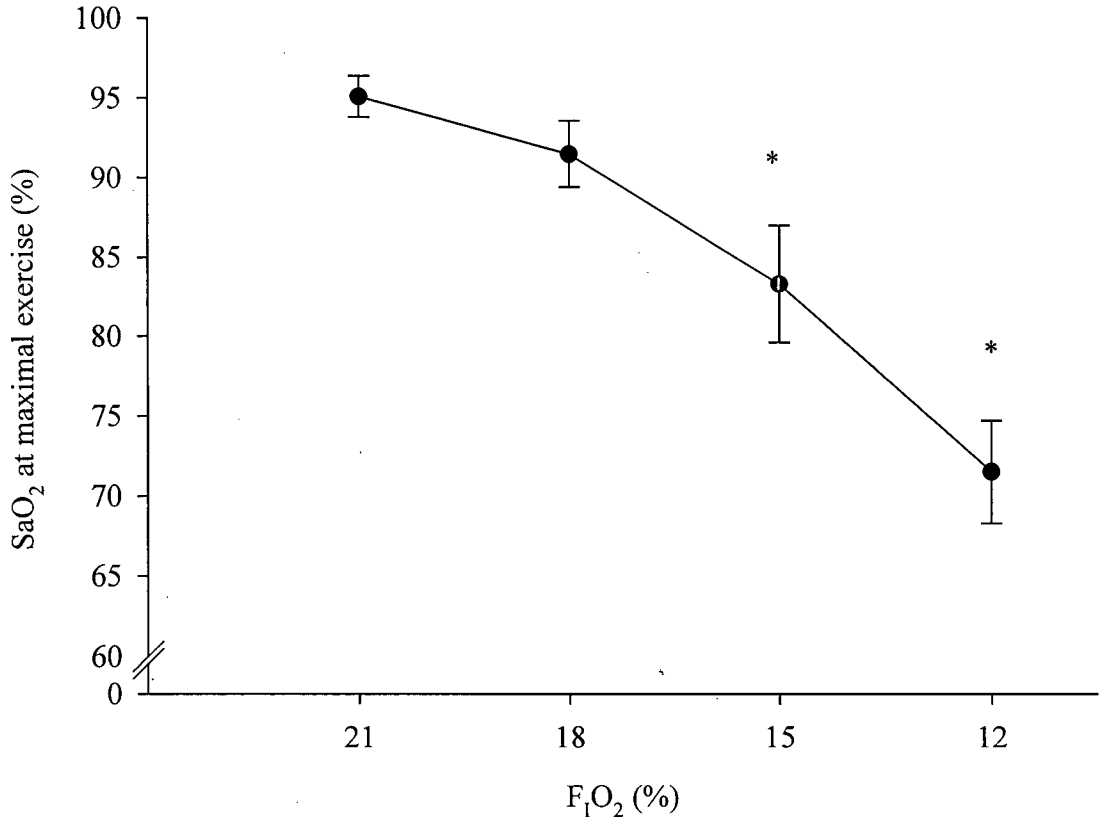

Figure 3.3. Minimal SaO_2 with varying F_IO_2 (mean ± SD); N = 10.
* Significantly different from the normoxic values ($p<0.05$).

Post-hoc analyses revealed a significant difference in VO_2max from the normoxic condition in the 12% ($p = 0.00016$) and 15% ($p = 0.035$) conditions. VO_2max was not significantly decreased in the 18% condition. Peak power was significantly reduced in the 12% ($p = 0.00016$) condition, but not in the 15% or 18% condition. SaO_2 was significantly lower in the 12% ($p = 0.00016$), and the 15% ($p = 0.00016$) conditions than the normoxic condition. Tables 3.1 and 3.2 summarize the results for each condition in this study.

F_IO_2 (%)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	HR ($beat \cdot min^{-1}$)	Peak Power (W)	Minimum SaO_2 (%)
21	57.2 ± 7.9‡•	178.0 ± 18.6•	401.4 ± 47.9•	95.1 ± 1.3‡•
18	53.2 ± 5.7•	179.7 ± 11.6•	375.7 ± 42.1•	91.5 ± 2.1‡•
15	48.2 ± 7.9*•	176.6 ± 10.5•	362.2 ± 41.4•	83.3 ± 3.7*†•
12	31.5 ± 7.4*†‡	153.0 ± 20.4*†‡	201.8 ± 75.1*†‡	71.5 ± 3.2*†‡

Table 3.1. VO_2max, heart rate, peak power, and minimum SaO_2 during maximal exercise in various conditions of hypoxia (means ± SD); N = 10.
* Significantly different from F_IO_2 = 21% ($p < 0.05$).
† Significantly different from F_IO_2 = 18% ($p < 0.05$).
‡ Significantly different from F_IO_2 = 15% ($p < 0.05$).
• Significantly different from F_IO_2 = 12% ($p < 0.05$).

F_IO_2 (%)	V_E ($L \cdot min^{-1}$)	V_T ($L \cdot br^{-1}$)	R_f ($br \cdot min^{-1}$)	V_E/VO_2	V_E/VCO_2
21	140.9 ± 19.3•	3.0 ± 0.6•	58.1 ± 10.6•	31.3 ± 2.1	24.8 ± 1.2
18	132.6 ± 18.7•	2.9 ± 0.6•	56.8 ± 9.5•	31.5 ± 1.2	24.9 ± 1.2
15	134.2 ± 17.9•	3.0 ± 0.5•	55.8 ± 9.2•	35.5 ± 5.8	26.8 ± 1.6
12	74.8 ± 34.7*†‡	2.5 ± 0.5*†‡	32.9 ± 11.2*†‡	30.4 ± 6.3	25.6 ± 3.5

Table 3.2: Ventilatory data during maximal exercise in various conditions of hypoxia (means ± SD); N = 10.
* Significantly different from F_IO_2 = 21% ($p < 0.05$).
† Significantly different from F_IO_2 = 18% ($p < 0.05$).
‡ Significantly different from F_IO_2 = 15% ($p < 0.05$).
• Significantly different from F_IO_2 = 12% ($p < 0.05$).

With an F_IO_2 of 12%, only one of ten subjects was able to achieve 70% of peak normoxic power, and mean peak power during the 12% condition was 50.3 ± 17.5% of peak normoxic power (Figure 3.1). With an F_IO_2 of 15%, however, all ten subjects achieved at least 85% of peak normoxic power, and mean peak power during the 15% condition was 90.3 ± 3.3% of that in normoxia. During interpretation of these data it is important to note that several subjects did not achieve volitional fatigue during the 12% condition due

to termination of the test when SaO_2 reached 70%. This had a significant effect on the data recorded in this condition and reported as maximal exercise data. Nonetheless, this data adds important value to the study, in particular with reference to Chapter 4, and therefore all data is reported and included in analyses.

As demonstrated in Figure 3.4, there were no significant correlations between mean decline in VO_2max and normoxic VO_2max ($R = 0.41$, $p = 0.23$), or between mean decline in VO_2max and minimum SaO_2 in the normoxic condition ($R = 0.48$, $p = 0.16$) (Figure 3.5).

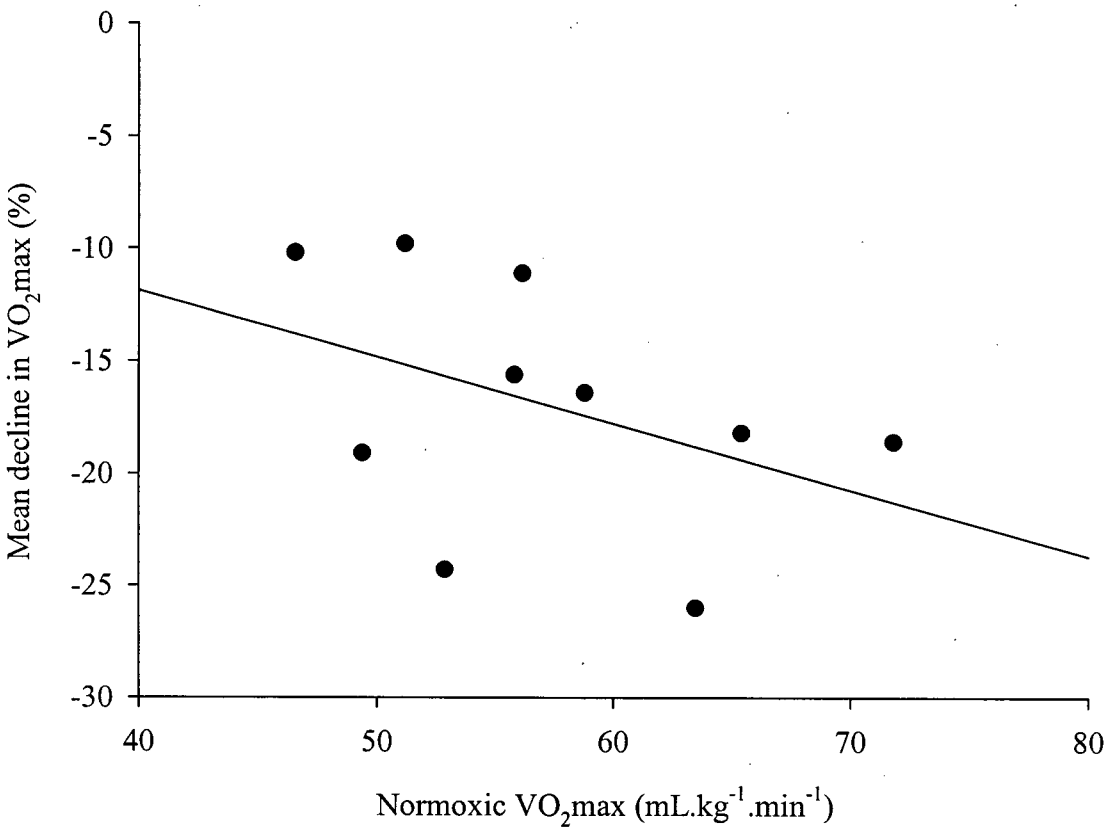

Figure 3.4. Mean decline in VO_2max (for all levels of hypoxia) vs. normoxic VO_2max, R = - 0.41, p = 0.23.

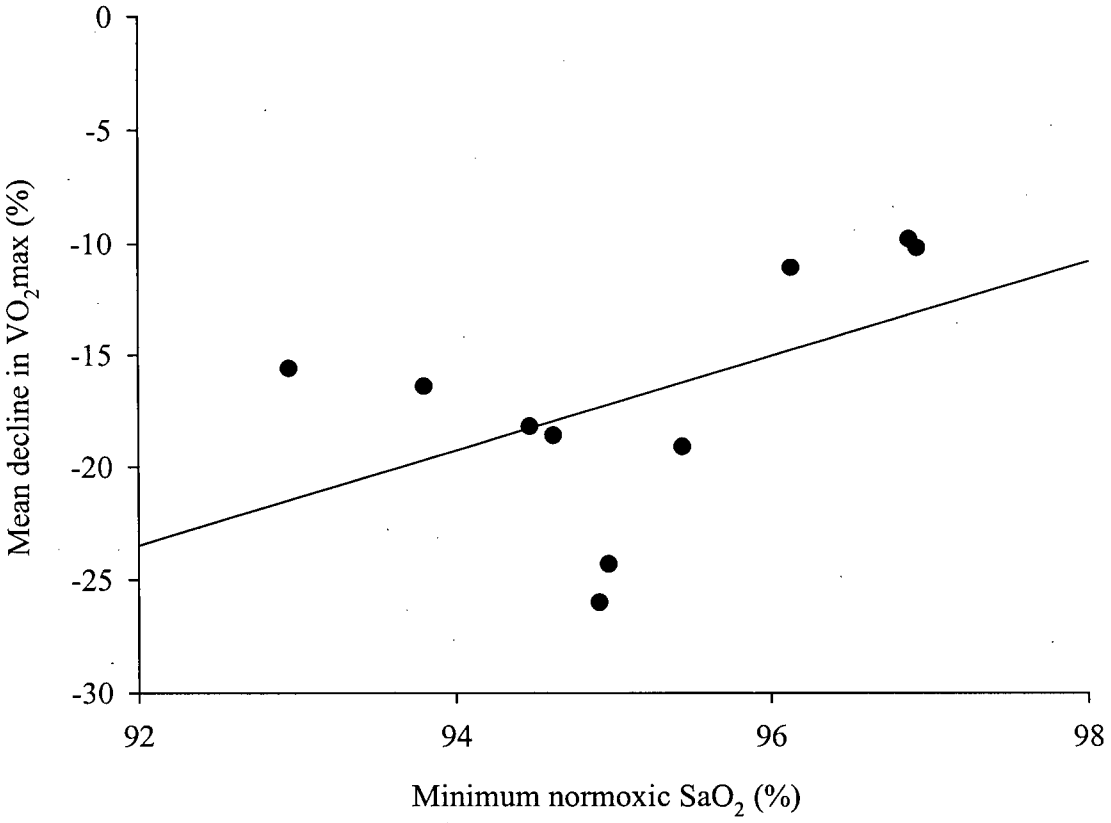

Figure 3.5. Mean decline in VO_2max (for all levels of hypoxia) vs. normoxic SaO_2, R = 0.48, p = 0.16.

3.4 DISCUSSION

The purpose of this study was to examine maximal cycling exercise in varying levels of hypoxia and to establish the greatest degree of hypoxia that subjects could tolerate while maintaining sustained high intensity cycling exercise.

Exercise in Hypoxia

The results of this study show the expected declines in VO_2max, peak power, and SaO_2 with decreased F_IO_2 during incremental exercise on a cycle ergometer. This decline in VO_2max has been well documented in past work, and the results of this study complement the existing data. Figure 3.6 demonstrates the comparison between mean values of VO_2max (as a percentage of VO_2max in normoxia) of a number of previous studies[18, 20, 36, 59, 66, 70, 77, 86-88, 111, 130] with similar values in the present study.

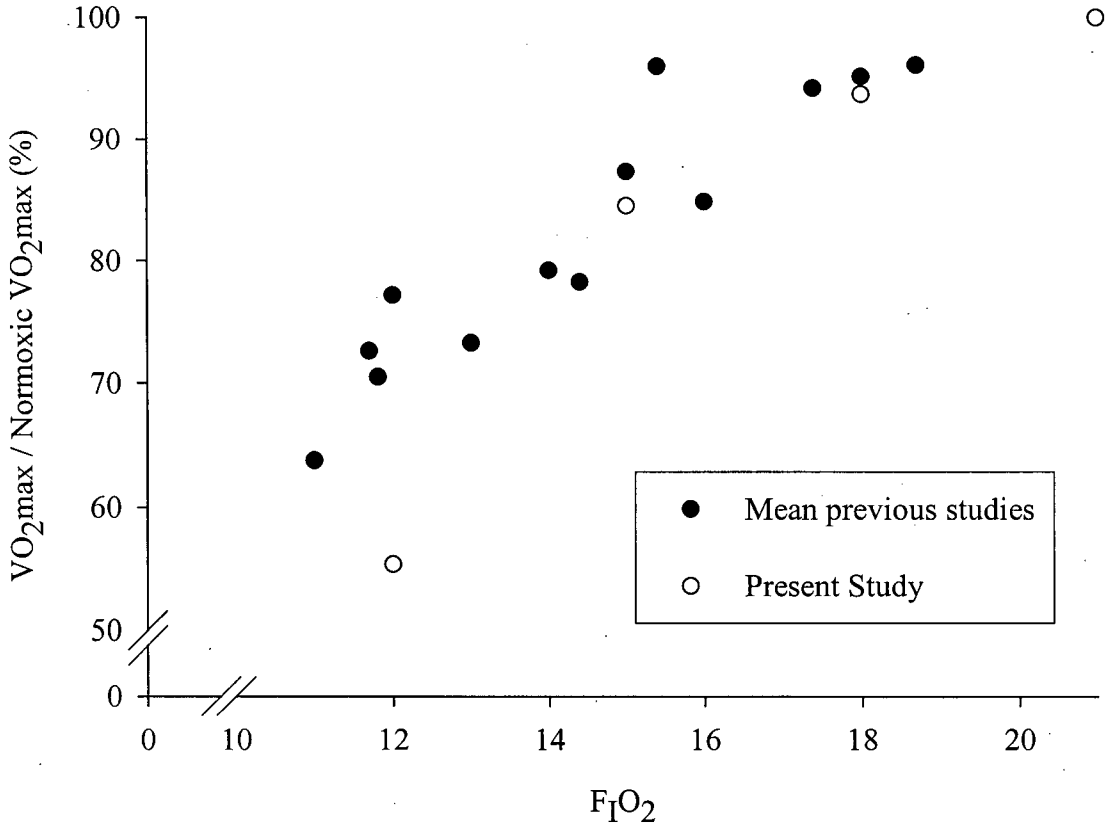

Figure 3.6. Hypoxic VO_2max / normoxic VO_2max vs. F_IO_2.

The values in the present study decline in a slightly more curvilinear fashion than do the values in previous studies, and this is likely the result of the failure of subjects in this study to achieve a true VO_2max while breathing 12% oxygen. Thus, the values presented for VO_2max in the most severe hypoxic condition are lower than expected (as they do not represent true volitional fatigue or maximal exercise exertion). A cut-off value of SaO_2 = 70% may not have been used in the previous studies. Martin & O'Kroy[77] report a minimum $SaO_2 = 67.0 \pm 7.1\%$ in a group of trained athletes, Ferretti et al[36] report a minimum $SaO_2 = 66.2 \pm 2.9\%$, and Hughes et al[59] report multiple individual values of $SaO_2 < 70\%$. Nonetheless, the general pattern of decline in VO_2max is similar and falls within the expected range of the previous studies. Of the previous studies referred to, three[20, 87, 111] involved treadmill running as the mode of exercise testing, while the remainder involved cycle ergometer work similar to this study.

Several previous studies have examined the relationships between $\%\Delta VO_2$max in hypoxia, and normoxic VO_2max in normoxia and minimum SaO_2 values. Lawler et al[70] specifically studied the relationship between VO_2max and VO_2max decrement during exposure to acute hypoxia (F_IO_2 = 14%) and found that trained subjects demonstrated significantly lower SaO_2 during exercise in hypoxia than untrained subjects. Further, a significant linear correlation (r = 0.94) was found between $\%\Delta VO_2$max in hypoxia and normoxic VO_2max. Martin and O'Kroy also found a significant negative correlation (r = -0.91) between $\%\Delta VO_2$max in hypoxia and normoxic VO_2max (data presented inversely to Lawler et al,[70] but with the same direction of correlation), and a significant negative

correlation (r = -0.84) between %ΔVO_2max in hypoxia and SaO_2 at maximal exercise in hypoxia. In a study of six ice-hockey players and six cross-country skiers[66] a similar significant correlation (r = -0.61) was found between %ΔVO_2max in hypoxia and normoxic VO_2max, but a study of seven endurance trained and seven sedentary women,[130] showed significance in this relationship only amongst trained (VO_2max = 56.3 ± 4.7 $mL \cdot kg^{-1} \cdot min^{-1}$) females (r = 0.8) but not amongst untrained (VO_2max = 34.8 ± 5.6 $mL \cdot kg^{-1} \cdot min^{-1}$) females (r = 0.13). This indicates that those subjects with the highest aerobic capacity, suffer the greatest decline in VO_2max in hypoxia, and that those subjects with the greatest degree of arterial desaturation in hypoxia also suffer the greatest decline in VO_2max in hypoxia. The latter point falls in line with what is known about the relationship between exercise performance and arterial saturation. Similarly, in normoxia exercise performance is related to the degree of exercise-induced arterial hypoxaemia.[91] Contrary to some of the previous findings, the results of this study do not show a significant correlation between %ΔVO_2max in hypoxia and either normoxic VO_2max or minimum SaO_2. In this study, even the degree of arterial desaturation at an F_IO_2 = 15% and the % ΔVO_2max at this level of hypoxia are not significantly correlated (R = -0.17). Figure 3.4 demonstrates that the data in this study do follow the pattern of correlation reported in the previous studies, but the correlation did not achieve statistical significance. It is also possible that the range of data (particularly the range of SaO_2) reported in this study did not lend itself to statistically powerful correlational analyses, in which case a larger group of subjects may have been helpful for these particular analyses of the data. Statistical calculations demonstrate that powers of 0.38 and 0.44 were achieved for the correlations of decrease in F_IO_2 with decline in VO_2max and SaO_2

respectively, with $\alpha = 0.05$. Therefore, a greater sample size would be desirable before making bold claims about the correlations reported. Finally, the group of subjects used in this study were moderately trained individuals ($VO_2max = 57.2 \pm 7.9$ mL·kg^{-1}·min^{-1}), and as evidenced in the study by Woorons et al,[130] these relationships may hold true only for more highly trained subjects.

Maximal Exercise Tolerance in Hypoxia

It was decided that an appropriate level of hypoxia would be indicated by the ability of subjects to reach 70% peak normoxic power. A peak hypoxic power lower than this would likely lead to sustained hypoxic exercise at a level too low to stimulate the desired physiological and pulmonary responses to exercise that were to be studied in Chapter 4. It is clear from the results that an F_IO_2 of 12% is too severe for subjects to maintain this level of exercise. Indeed, in the 12% condition, only one of the subjects involved managed to achieve 70% of peak normoxic power. In the 15% condition, all ten subjects achieved at least 85% of peak normoxic power. Further, when examining the peak power in each condition as a percentage of peak power in normoxia, the standard deviation increased substantially from 3.3% with $F_IO_2 = 15\%$ to 17.5% with $F_IO_2 = 12\%$. This would seem to indicate that there is less inter-individual variation and unpredictability in the physiological response to intense exercise with an F_IO_2 of 15% than 12%, although this increased variation is most likely due to the fact that the subjects were unable to complete exercise to volitional fatigue during the 12% condition without SaO_2 falling below 70%. This sharp change at 12% oxygen is very evident when the values are displayed graphically as in figure 3.1. It is worth noting that while the F_IO_2 of 18% was

included in the study, it is clear from the results that the physiological response to exercise at 18% is only marginally different from that in normoxia. Indeed, in 18% hypoxia, VO_2max, peak power, and SaO_2 were not significantly different than in normoxia. Minimum SaO_2 at 18% oxygen was only 91.5 ± 2.1% compared to 95.1 ± 1.3% in normoxia. One of the purposes of Chapter 4 is to provide the greatest hypoxic perturbation possible while preserving the ability of the subjects to perform sustainable exercise of a moderately high intensity. Therefore it is appropriate that, to elicit the maximal physiological response from hypoxia while preserving the ability of subjects to perform intense sustainable exercise, an F_IO_2 of 15% be selected as the hypoxic condition in Chapter 4.

CHAPTER FOUR: Effects of Prolonged Exercise on Extravascular Lung Water

4.1 INTRODUCTION

During intense continuous exercise in humans, oxygen consumption may increase 20-fold or more from rest, placing large demands on the pulmonary system to maintain arterial PO_2 and PCO_2 within normal tolerable values. Typically, in humans, arterial PO_2 is effectively maintained near resting values of approximately 100 mmHg during exercise by a pulmonary system that effectively meets this increase in metabolic demand. However, in approximately 50% of trained aerobic athletes (VO_2max > 150% predicted) exercise-induced arterial hypoxaemia (EIAH) occurs during strenuous exercise.[24, 90] Dempsey and Wagner[25] have proposed three levels of hypoxaemia as defined by the degree of arterial haemoglobin saturation: mild (93-95%), moderate (88-93%), and severe (< 88%). Despite extensive research the mechanisms responsible for EIAH have not been completely established. Those most likely to contribute are relative alveolar hypoventilation, ventilation/perfusion (V_A/Q_C) mismatch diffusion limitation,[25] and right to left shunts. A significant amount of research has been dedicated to the study of EIAH in the past two decades with advances in the understanding of the nature of this phenomenon and the mechanisms involved.

Interstitial pulmonary oedema is the development of additional extravascular lung water caused by an increase in the filtration of fluid from the pulmonary capillaries into the interstitial space between the alveolar epithelium and capillary endothelium, and may interfere with gas exchange across the alveolar-capillary membrane. Pulmonary oedema

may occur through changes in the Starling forces (pulmonary capillary leakage) and/or changes in the permeability of the capillary membrane (pulmonary capillary stress failure)[2]. Mean pulmonary artery pressure (PAP) in humans at rest is typically 15 mmHg (systolic = 25 mmHg, diastolic = 8 mmHg).[40, 126] Mean PAP rises with exercise and near-maximal exercise values have been measured at 33 mmHg.[40] Hypoxia also alters mean PAP with resting and near-maximal exercise values reaching 34 and 54 mmHg respectively.[40]

Transient interstitial oedema during exercise could partly explain the A-aO_2 difference observed during exercise in hypoxaemic subjects. Whether transient pulmonary oedema occurs or not has been the subject of a number of studies and remains an unresolved issue. In animals, evidence of pulmonary oedema following heavy exercise has been demonstrated in a study by Schaffartzik et al[103] using swine. Five pigs ran at the highest speed maintainable for 6 – 7 minutes while five pigs acted as controls. Lung tissue blocks from each animal were prepared for microscopic examination and documentation of perivascular or peribronchial cuffing, and the exercised animals showed significantly more periarterial cuffing in general, as well as a higher percentage of periarterial cuffing in the lower lobes and ventral areas of the lung than the control animals. In humans, evidence of pulmonary oedema (bilateral pulmonary consolidation, upper lobe venous congestion and cardiomegaly) was observed in two subjects following very long duration exercise.[82] Assessment of pulmonary oedema with modern imaging techniques including radiography, CT, and MRI has led several authors to conclude that transient pulmonary oedema was present in subjects following exercise.[3, 16, 84] However, a number of studies

both in animals and humans have found no evidence of transient pulmonary oedema following exercise including a study involving treadmill exercise in dogs,[75] and several exercise studies in humans.[39, 72, 76, 120]

The first step to understanding the role of transient pulmonary oedema as a mechanism for diffusion limitation leading to EIAH in highly trained athletes is to establish whether oedema occurs in the lung in exercising humans, and to quantify it. Direct measure of lung density is not possible in humans, and indirect measures are generally only practical or valid following rather than during exercise. Therefore, the purpose of this study was to assess measurement of *in vivo* lung density by MRI and to describe transient pulmonary oedema, through this measure, following exercise in healthy athletic humans while breathing normoxic and hypoxic air. The hypoxic condition was included in an attempt to increase mean PAP as high as possible during intense exercise. With increasing severity of hypoxia exercise capacity decreases, and a moderate degree of hypoxia (F_IO_2 = 15%) was selected to combine the effects of hypoxia and heavy exercise on mean PAP. It was hypothesized that following sustained exercise in normoxia, mean lung density would increase over baseline values, and that a similar but greater magnitude of increase would occur in the hypoxic condition. Additionally, it was hypothesized that two hours post-exercise, lung density would not be different than the baseline values in each condition.

4.2 METHODS

Subjects

With a minimum difference of 0.03 g·mL-1 being considered a meaningful change in lung density, and an expected standard deviation of 0.02 $g \cdot mL^{-1}$, the required number of subjects were 9.3, given a power value of 0.8 and alpha set at $\alpha = 0.05$. Therefore, ten male subjects (age = 25.9 ± 4.7 y, height = 184.1 ± 8.2 cm, mass = 79.4 ± 9.5 kg) were used in this study. Subjects reported for testing on four separate testing days. The first two involved maximal cycling (VO_2max) tests under normoxic and hypoxic conditions in a randomized order, and a pulmonary function test to assess FVC, FEV_1, and PEFR. The second two days involved MRI assessment of lung density prior to and following an exercise intervention in normoxic and hypoxia. In the hypoxic condition, F_IO_2 was 15% oxygen and balance nitrogen as determined during the study explained in Chapter 3. Figure 4.1 illustrates the order of each of the second two days involving sustained exercise and MRI assessment of lung density. The order of these days was also randomized.

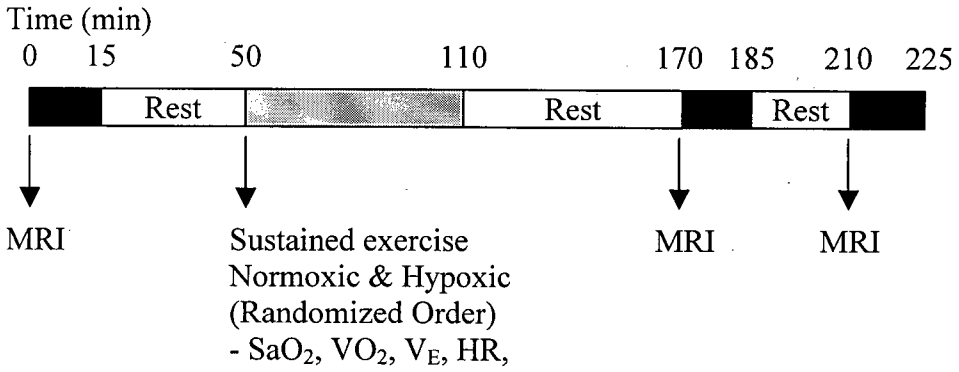

Figure 4.1. Timeline of experimental protocol for each condition (normoxia and hypoxia).

Exercise Testing

Subjects were requested to avoid exercise, alcohol, and caffeine for 24 h prior to each testing session. The VO_2max tests were performed on an electronically braked cycle ergometer (Quinton Excalibur, Lode, Groningen, Netherlands) starting at 0 W and increasing at a rate of 30 $W \cdot min^{-1}$ until the subject experienced volitional fatigue and could not maintain a constant pedalling rate. Expired gases were collected and analyzed and ventilation was measured (True One, Parvomedics, Sandy, UT) and averaged every 15 seconds. Heart rate was measured by telemetry (Polar Vantage XL, Kemple, Finland) and averaged every 15 seconds, and SaO_2 was measured using an earlobe pulse oximeter (Biox 3740, Ohmeda, Madison, WI), and averaged every 15 seconds. During all exercise testing in this study, SaO_2 was monitored by a second finger-tip oximeter (Nonin 8500, Nonin Medical Inc., Plymouth, MN) to ensure that inadequate earlobe perfusion was not an issue in assessment of SaO_2. VO_2max was calculated as the average of the four highest

consecutive readings. In the hypoxic condition, following delivery from a compressed gas tank, the inspired air was humidified and subjects breathed through a two-way valve (Hans Rudolph, Kansas City, MO). Due to limited flow through the tank regulator, a Douglas bag was used to store the humidified air prior to delivery to the subject to allow adequate ventilation during high-intensity exercise. In this condition, arterial oxyhaemoglobin saturation was monitored continuously and the exercise was terminated if SaO_2 fell below 70%. Following each VO_2max test, the results were examined for the following criteria to determine whether maximal exercise was achieved: a plateau in VO_2 with increasing exercise intensity, achievement of a maximal heart rate within 5% of age-predicted HR_{max}, and a respiratory exchange ratio of ≥ 1.10.

For measurement of lung density, subjects reported to the Radiology Department of the University of British Columbia Hospital for MRI assessment. Approximately one hour (62.3 ± 9.6 min normoxic condition; 62.0 ± 7.5 min hypoxic condition) following the completion of the baseline lung MRI, subjects began the exercise intervention at the Allan McGavin Sports Medicine Centre Exercise Physiology Laboratory at the University of British Columbia. This consisted of 60 min cycling exercise while breathing either normal room air or hypoxic gas of 15% O_2 and balance N_2 by volume. The cycling was performed on the same ergometer, and with the same metabolic equipment and gas delivery system as the previous VO_2max tests. Initial workloads were set at between 55 – 60% of peak power achieved in the VO_2max tests, and subjects had control of the workload on the cycling ergometer and were instructed to perform as much work as possible in 60 min. Workload intensities were monitored throughout the exercise

and feedback was given to the subjects in an attempt to maximize work accomplished. Approximately 50 min (54.0 ± 17.2 min normoxic condition; 55.6 ± 9.8 min hypoxic condition) and 100 min (100.7 ± 15.1 min normoxic condition; 104.3 ± 9.1 min hypoxic condition) following the exercise intervention subjects underwent identical MR scans as prior to exercise. Therefore, for each condition, each subject underwent three MR scans (one pre-exercise and two post-exercise scans) for a total of six scans per subject. Two of the subjects (subjects 3 and 7) were unable to complete the measure of resting lung density under both conditions. Therefore, for these two subjects data is shown for the hypoxic condition only.

Although subjects were given time to perform a self-selected warm-up prior to the sustained exercise intervention, there was some delay in set-up following warm-up and prior to the start of the exercise. Therefore the initial minutes of the exercise do not represent steady-state exercise at a high workload. For this reason, the exercise data are averaged over the last 50 min of exercise, with the exception of total work which includes all 60 min of exercise.

Subjects were weighed before the first and second MR scans, and immediately prior to and following the exercise interventions in each condition. Following exercise and prior to the first post-exercise MR scan, subjects were required to drink at least the volume of water corresponding to the mass loss during the exercise intervention. Heart rates were recorded at the initiation of each MR scan.

Methodological Considerations

Pulse oximetry provides a non-invasive measure of SaO_2, and is therefore a convenient tool for use during exercise in human subjects. Validation of SaO_2 measured by pulse oximetry and by direct arterial blood analysis has been performed by Martin et al.[78] Specifically, a mean difference of $0.52 \pm 1.36\%$, and a correlation of $r = 0.98$ was found between examination of SaO_2, as measured by 3 Ohmeda oximeters and direct arterial blood, in eleven aerobically fit cyclists while performing constant load high-intensity exercise and incremental exercise. These findings involved 232 observations of SaO_2 ranging from 72 – 99%. However, pulse oximetry provides only a representation of true arterial oxyhaemoglobin saturation, and several issues should be noted when using pulse oximetry. Firstly, pulse oximetry measures SaO_2 at one peripheral site on the body. During exercise this is normally on the finger tip, ear lobe, or forehead and any change in normal perfusion of these areas may affect the pulse oximeter representation of true arterial saturation. Secondly, normally pulse oximetry does not allow for the correction of values to blood temperature and pH. Nonetheless, in this study the use of direct arterial blood for analysis of blood gases was not included because precise measurement of changes in arterial PO_2 during both incremental and sustained exercise were not critical to the findings. The main issue was the determination of whether transient pulmonary oedema occurred during and persisted following exercise. The primary reason for measuring SaO_2 was to describe EIAH and changes in SaO_2 during sustained exercise in normoxia and hypoxia rather than to draw any conclusion from the degree of hypoxaemia. Therefore it was decided that arterial blood gas assessment was an unnecessarily invasive technique on the subjects.

Magnetic Resonance Imaging

During assessment of lung density, subjects were imaged with the body coil on a 1.5 Tesla General Electric Horizon Echospeed MR scanner (General Electric Medical Systems, Milwaukee, WI, 5.7 software release) while lying supine and during normal tidal volume breathing. Three water phantoms were placed over the right lung immediately below the clavicle. An eight-echo pulse sequence was used with an echo spacing of 10 msec and a repetition time (TR) of between 3,000 and 4,000 msec. (time to echo (TE) 10, 20, 30, 40, 50, 60, 70, 80). In this single slice 8-echo sequence, the 90° radiofrequency (rf) pulse was slice selective and the 180° rf pulses were non-selective composite pulses. Multiple gradient lobes of alternate sign and incrementally decreasing amplitude were applied in the slice selection direction to eliminate stimulated echo artefacts. The single slice sequence yielded 8 images of a single slice that provided pixel-by-pixel data on lung T_2 relaxation times. For each scan, multi-exponential T_2 analysis was used with extrapolation to TE = 0. A T_1 relaxation time correction was included. Each scan was cardiac-gated after the R wave peak to limit heart and blood flow artefact. The images were obtained using a 320 mm field of view and 256 x 128 mm matrix.

Output was eight images of each sagittal section, which were transferred to Matlab software (MathWorks, Milwaukee, WI). After extrapolation to TE = 0, a water content map was generated on a Linux workstation using multi-exponential T_2 analysis. Each scan was then analysed for density as compared to the known density of one of the water phantoms. A region of interest (ROI) was drawn manually on the water phantom. The

phantom pixel intensity was calibrated to the known density of the phantom (1.0 $g \cdot mL^{-1}$), and an ROI of the sagittal section of the right lung was drawn manually including as much lung tissue as possible, while definitively excluding any non-lung tissues at the margin of the ROI to avoid partial voluming. Pixel thickness, height, and width were 10.0, 1.0, and 1.0 mm respectively providing a slice thickness of 10.0 mm.

To account for vascular components of the lung, all pixels above a set density threshold were removed during the calculation of lung density. Mean lung slice density values were calculated and recorded at density thresholds of 0.25, 0.30, 0.35, 0.4, 0.45, and 1.0 $g \cdot mL^{-1}$. This technique has been used in a previous study,[84] in which pixels above a density of 0.30 $g \cdot mL^{-1}$ were excluded, however in this study it was decided to record data on a range of threshold densities for comparison purposes (see Appendix C). For each scan, the area removed was compared visually with areas of high density to verify the total area removed (see figure 4.8). Data are presented for each threshold level in Appendix C (Table C.1 – C.6); however, it was observed that a threshold of 0.3 $g \cdot mL^{-1}$ corresponded to the removal of major blood vessels while avoiding the removal of other tissues. Further, it was noted that for each threshold value, the lung density for each segment of lung on an anterior-posterior scale did not exceed 0.3 $g \cdot mL^{-1}$. For these reasons, a threshold value of 0.30 $g \cdot mL^{-1}$ was used for analyses. Ultimately the measurements were performed at the wide variety of threshold densities to ensure that increased extravascular water following exercise was not missed as a result of discarding certain densities of tissues, and as is evident in the data (Appendix C), it can be stated with confidence that this objective was met.

In order to assess lung density gradient, lung density was measured at 3 regions anterior – posterior: the anterior 5 cm; the middle 5 cm; and the posterior 5 cm of the lung. For each image, an equation of the lung density gradient was calculated by regression using these three density values. The slope of this equation represented the density gradient for the particular image, and was expressed as $mg \cdot mL^{-1} \cdot cm^{-1}$.

All analyses described above were performed in an identical manner by the same observer. For intra-observer reliability purposes, twenty of the images were analysed twice and a correlation was calculated between the mean slice densities of each group of analyses. Twenty images were analyzed by a second observer and inter-observer reliability between two observers was calculated in an identical manner on these twenty different scans.

Statistics

Repeated measures analysis of variance was used to examine the differences between the following variables during maximal exercise and during sustained exercise in each condition: VO_2max, power, SaO_2, HR, V_E. A repeated measures 2X3 ANOVA was used to analyse the density data between the pre- and the two post-measures and between normoxia and hypoxia. Several variables were recorded prior to the MR scans in an attempt to avoid extraneous factors that could have an effect on the measure of lung density. Heart rates were monitored by ECG throughout the scans and were recorded at the beginning of each scan. Subject masses were recorded prior to the first scan of each

day, and the first post-exercise scan of each day. The time between the pre-exercise scan and the two post-exercise scans was recorded and controlled. An ANOVA with Tukey's Honest Significant Difference post-hoc test was performed on these data. No control of heart rates was possible during the study, but the mass of each subject and the scan times were controlled to maintain as much uniformity as possible. Paired one-tailed t-tests were performed on the data of mass loss during exercise for each of the two conditions.

4.3 RESULTS

Exercise

In the hypoxic condition, VO_2max, peak power, and minimum SaO_2 during maximal exercise were significantly lower than in the normoxic condition (Table 4.1). The individual patterns of oxyhaemoglobin desaturation are shown in Figures 4.2 and 4.3.

Condition	HR ($b \cdot min^{-1}$)	VE ($L \cdot min^{-1}$)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	Peak Power (W)	Minimum SaO_2 (%)
Normoxic	186.7 ± 5.9	155.2 ± 23.9	65.0 ± 7.5	456.9 ± 66.6	92.3 ± 1.8
Hypoxic	178.4 ± 7.6	141.1 ± 26.6	54.1 ± 7.0*	377.2 ± 29.3*	79.8 ± 4.5*

Table 4.1. Maximal exercise data (means ± SD); N = 10.
* Significantly different from $F_IO_2 = 21\%$ ($p < 0.05$).

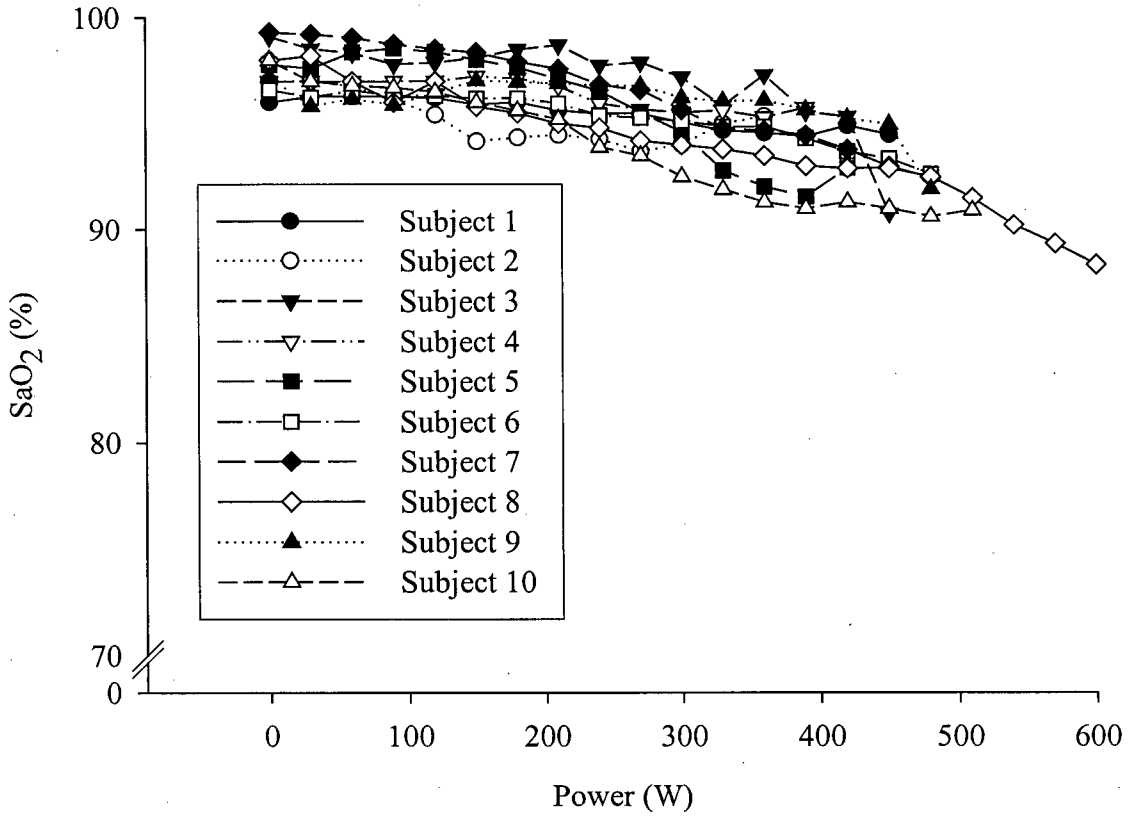

Figure 4.2. Arterial oxyhaemoglobin saturation during normoxic VO_2max test.

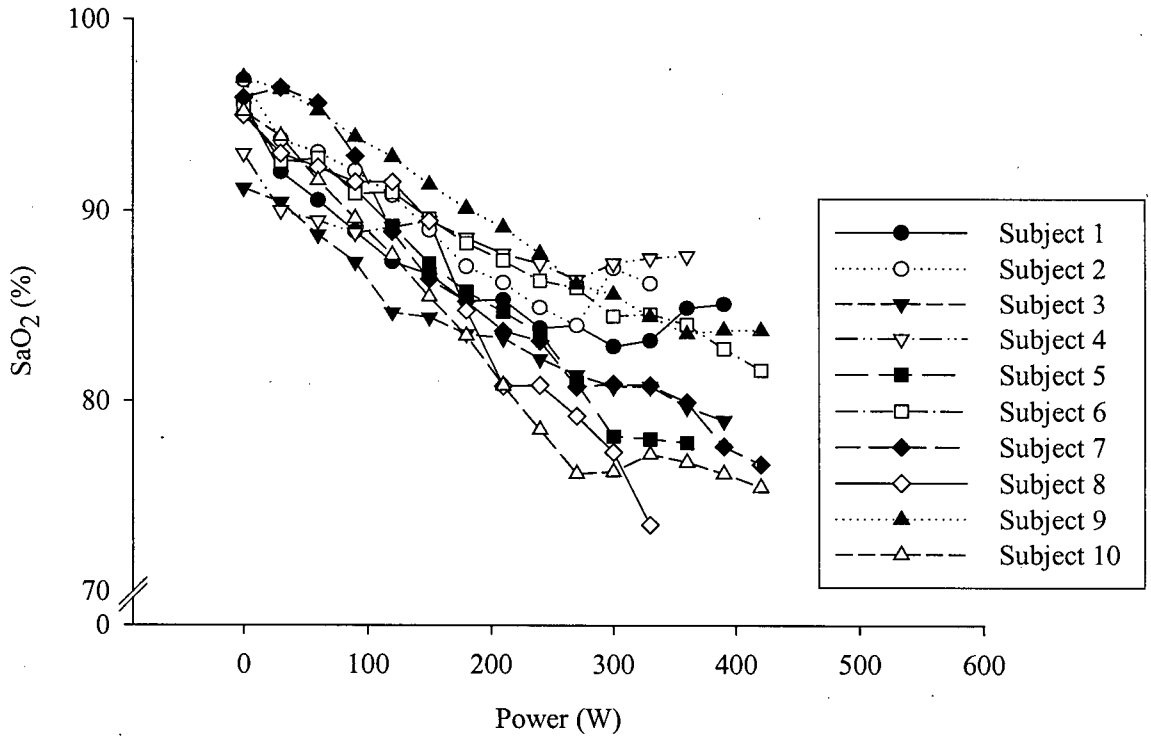

Figure 4.3. Arterial oxyhaemoglobin saturation during hypoxic VO_2max test.

Two subjects demonstrated mild EIAH (minSaO_2 = 94.5 & 93.8%), and seven demonstrated moderate EIAH (minSaO_2 = 91.4 ± 1.1%) during the normoxic VO_2max test. During the exercise intervention, subjects cycled at 61.6 ± 9.5 and 65.4 ± 7.1% VO_2max (56.6 ± 4.1 and 57.2 ± 6.9% peak power) in the normoxic and hypoxic conditions respectively (Table 4.2). One subject (subject 8) was unable to complete the hypoxic VO_2max test without SaO_2 falling below 70%, and therefore the test was terminated prematurely in the 12th minute of exercise. For In the hypoxic condition, mean VO_2, SaO_2, power, and total work during the sustained exercise were significantly lower than in the normoxic condition. Mean power and total work performed in each

condition is demonstrated in Table 4.3. Heart rate, VO_2, and SaO_2 data in the normoxic and hypoxic exercise intervention are displayed in Figures 4.4, 4.5, and 4.6.

Condition	HR ($b \cdot min^{-1}$)	VE ($L \cdot min^{-1}$)	VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	SaO_2 (%)
Normoxic	156.9 ± 5.7	63.3 ± 4.0	39.9 ± 1.3	95.2 ± 0.1
Hypoxic	156.3 ± 5.3	64.6 ± 3.4	35.4 ± 0.9*	82.6 ± 1.2*

Table 4.2. Exercise intervention data averaged over the last 50 min (means ± SD); N = 10.
* Significantly different from $F_IO_2 = 21\%$ ($p < 0.05$).

Condition	Power (W)	Total Work (kJ)
Normoxia	260.6 ± 4.1	926.0 ± 199.3
Hypoxia	215.2 ± 3.3*	753.9 ± 88.9*

Table 4.3: Total work performed in 60 minutes (means ± SD); N = 10.
* Significantly different from $F_IO_2 = 21\%$ ($p < 0.05$).

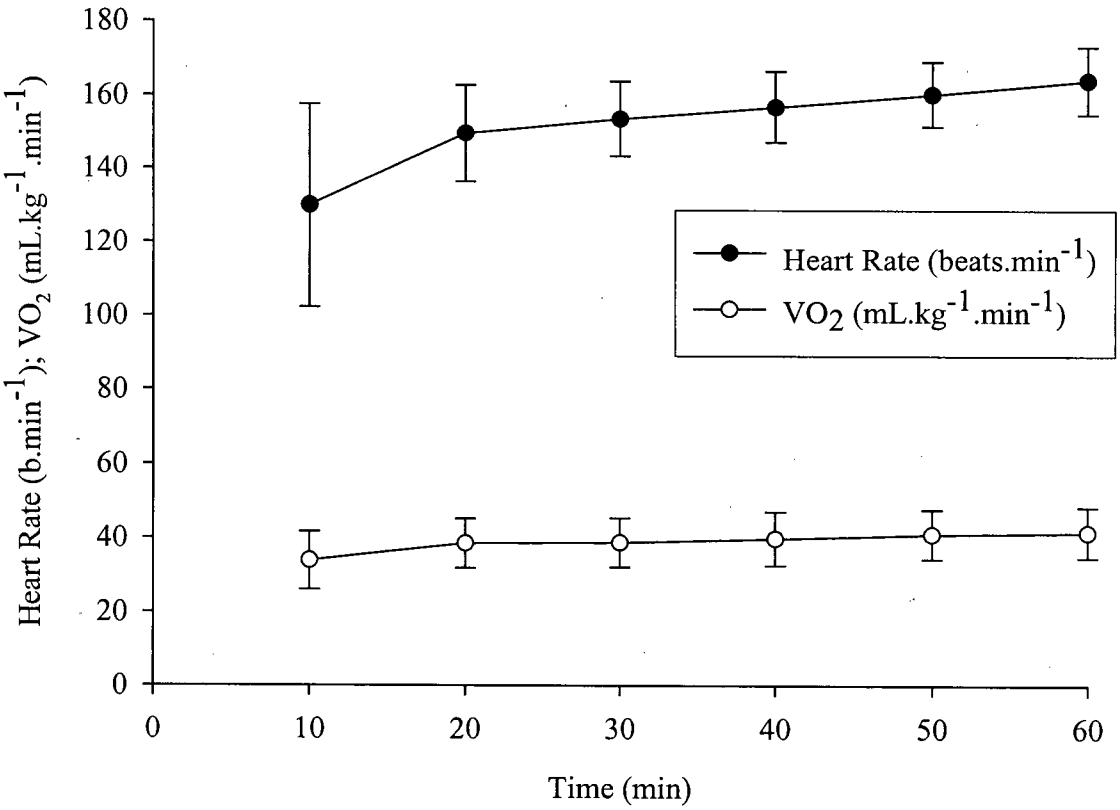

Figure 4.4. Normoxic exercise data. (Means ± SD); N = 10.

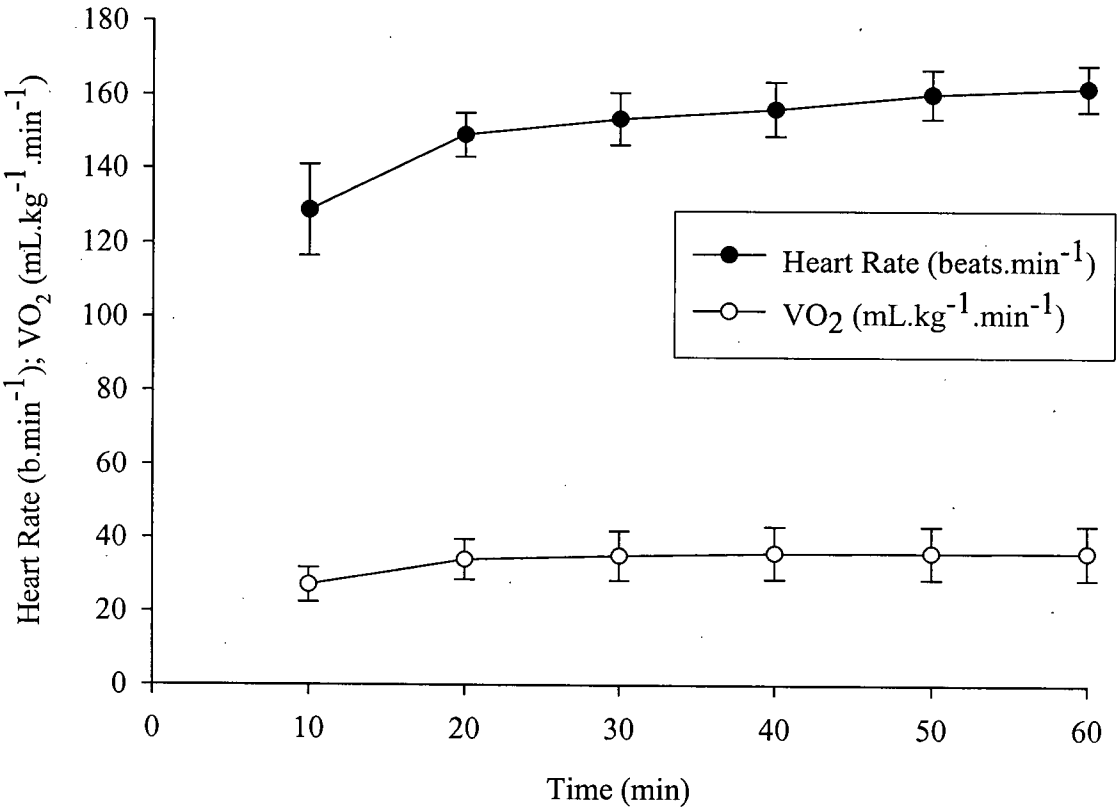

Figure 4.5. Hypoxic exercise data. (Means ± SD); N = 10.

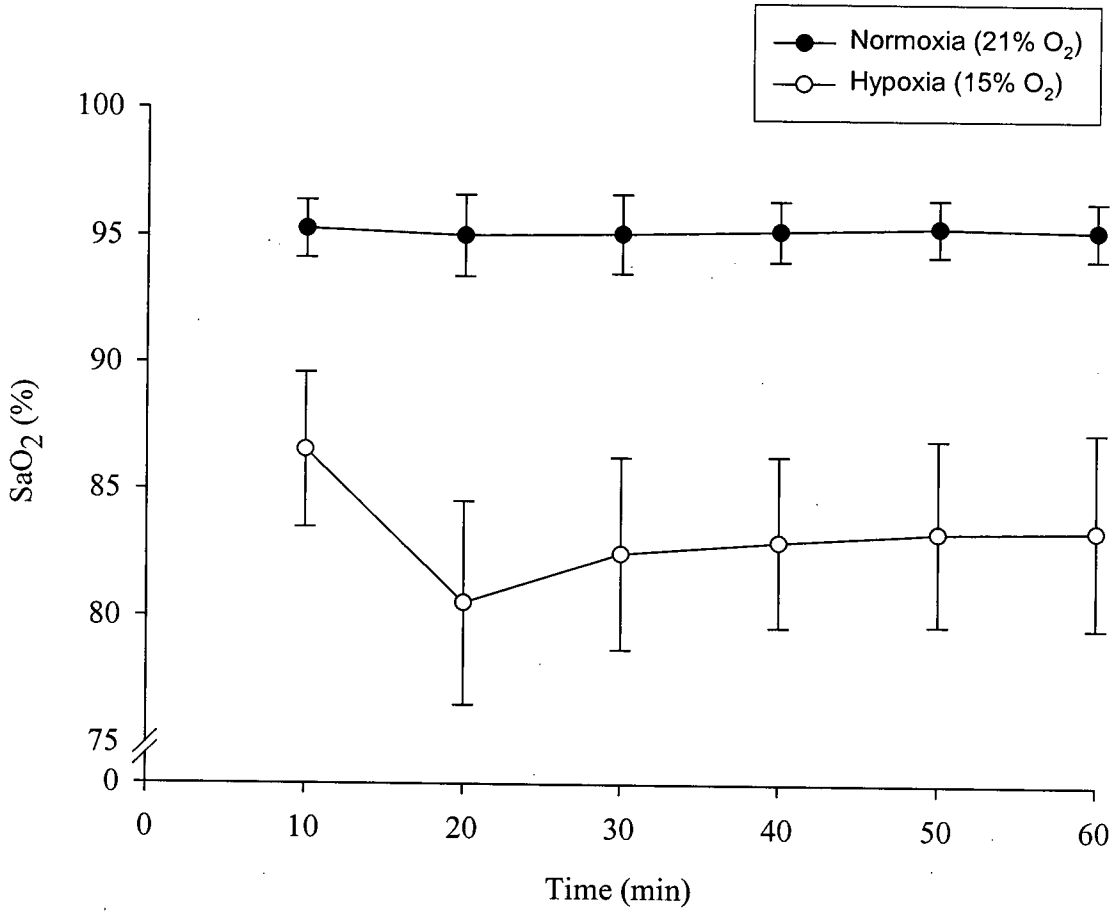

Figure 4.6. SaO_2 during the exercise intervention. (Means ± SD); N = 10.

Lung Density

There were no significant differences in lung density between conditions $F(1,54) = 0.13$; $p = 0.72$ or scan times $F(2,54) = 0.23$; $p = 0.80$, nor was there an interaction effect between condition and scan time $F(2,54) = 0.37$; $p = 0.70$ (see Table 4.4 and Figure 4.7). For clarity, standard deviations are not presented in Figure 4.7, rather individual lung densities are provided in Appendix B (Figures B.1 & B.2).

Condition	Pre Density ($g \cdot mL^{-1}$)	Post1 Density ($g \cdot mL^{-1}$)	Post2 Density ($g \cdot mL^{-1}$)
Normoxic	0.177 ± 0.019	0.181 ± 0.019	0.173 ± 0.019
Hypoxic	0.178 ± 0.021	0.174 ± 0.022	0.176 ± 0.019

Table 4.4: Lung densities (density threshold = 0.3 $g \cdot mL^{-1}$) pre and post-exercise intervention (means ± SD); N = 10.

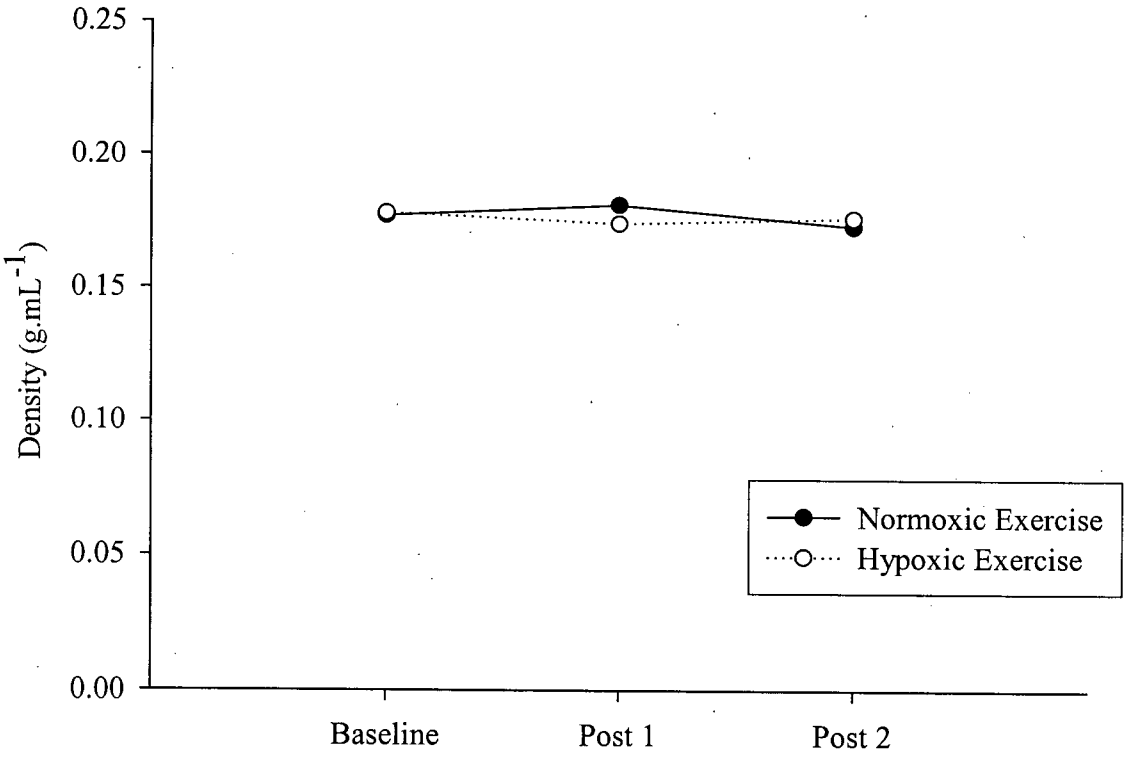

Figure 4.7 Lung densities (density threshold = 0.3 $g \cdot mL^{-1}$). (Means); N = 10.

During the exercise intervention there were significant ($p < 0.05$) decreases in mass of 1.4 ± 0.5 and 1.1 ± 0.5 kg in the normoxic and hypoxic conditions respectively. Heart rate was significantly ($p < 0.05$) increased at the initiation of the first post-exercise scan

compared to the pre-exercise scan in both the normoxic (59.7 ± 8.5 vs. 53.3 ± 5.2 beats·min^{-1}) and hypoxic (58.3 ± 7.0 vs. 51.7 ± 2.1 beats·min^{-1}) conditions. No other significant differences in heart rate or mass were found between scans. There were no significant differences between the two conditions in the time between the pre-exercise and the two post-exercise scans. Mean heart rate, mass, and time data are summarized in Table 4.5.

Scan	HR (b·min^{-1})	Mass (kg)	Time (min)
Normoxic Pre	53.3 ± 5.2	79.4 ± 9.5	0
Normoxic Post1	*59.7 ± 8.5	78.6 ± 10.3	54.0 ± 17.2
Normoxic Post2	62.5 ± 6.4	--	100.9 ± 18.3
Hypoxic Pre	51.7 ± 2.1	79.0 ± 10.3	0
Hypoxic Post1	*58.3 ± 7.0	78.8 ± 10.3	55.6 ± 9.8
Hypoxic Post2	54.0 ± 6.6	--	104.3 ± 9.1

Table 4.5: Heart rate (HR), mass, and time data at initiation of each MR scan (means ± SD); N = 10 .
* Significantly different than resting baseline (pre) measure ($p<0.05$).

As described above, for each scan a range of threshold densities were used, above which density all pixels were discarded before calculation of mean slice density. A sample MR image of a right lung sagittal slice from this study is presented in Figure 4.8, along with images of computer representations of the scan showing the discarded pixels at each threshold used.

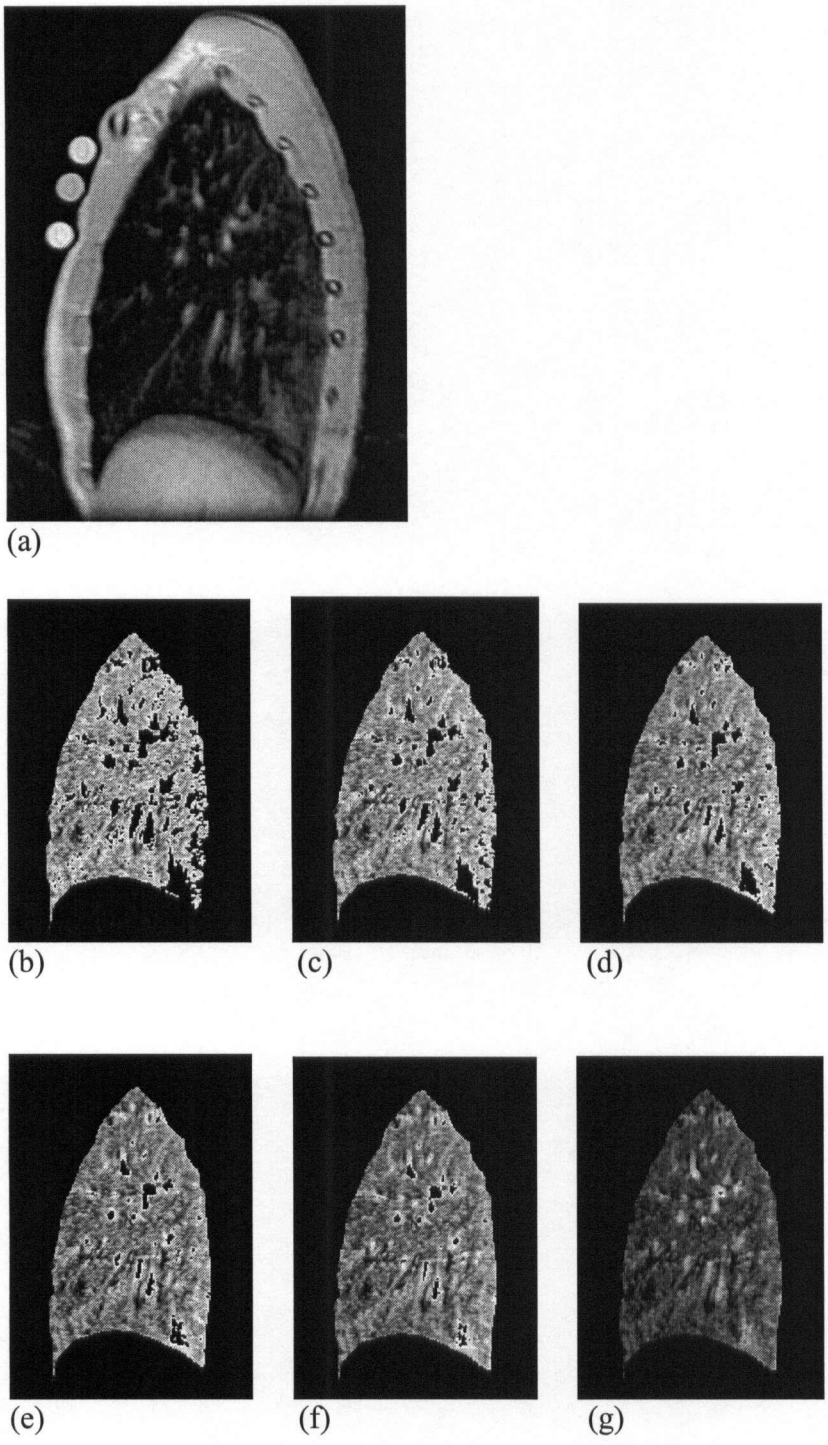

Figure 4.8. (a) Sample MR image of right sagittal lung slice. (b) – (g): Computerized image of the lung scan showing areas of pixel removal. Dark areas within the lung represent areas of pixel removal using a threshold of (b) 0.25 $g \cdot mL^{-1}$ (c) 0.3 $g \cdot mL^{-1}$ (d) 0.35 $g \cdot mL^{-1}$ (e) 0.4 $g \cdot mL^{-1}$ (f) 0.45 $g \cdot mL^{-1}$ (g) 1.0 $g \cdot mL^{-1}$.

Mean lung densities (0.177 ± 0.019 g·mL^{-1}) are consistent with established values in the literature.[80, 84] The variation between subjects was relatively small (coefficient of variation = 8.0%) and, importantly, the repeatability of resting slice density within subjects gave a significant Casewise correlation of $r^2 = 0.73$ ($p=0.007$). The Casewise correlations for intra-observer and inter-observer repeatability on 20 randomly selected scans were both $r^2 = 0.98$ ($p<0.05$) (see Appendix C Figures C.1 and C.2). The mean areas of the ROI used in each MR scan are presented in Table 4.6.

Condition	Pre Area (mm^2)	Post Area (mm^2)	Post Area (mm^2)
Normoxic	18,158 ± 3,729	16,227 ± 3,779	16,365 ± 4,010
Hypoxic	16,670 ± 3,603	16,423 ± 4,066	16,313 ± 3,853

Table 4.6. Lung slice areas (density threshold = 0.3 g·mL^{-1}) pre and post-exercise intervention (means ± SD); N = 10.

Lung Density Gradient

Mean lung density gradient (anterior to posterior) in the normoxic condition was 5.7 ± 1.8, 5.6 ± 1.9, and 6.9 ± 1.4 mg.mL^{-1}.cm^{-1} in the resting, first, and second post-exercise scans respectively. In the hypoxic condition, the corresponding lung density gradients were 6.5 ± 1.2, 6.5 ± 1.6, and 6.8 ± 1.5 mg.mL^{-1}.cm^{-1} (Table 4.7).

Condition	Pre Gradient ($mg \cdot mL^{-1} \cdot cm^{-1}$)	Post1 Gradient ($mg \cdot mL^{-1} \cdot cm^{-1}$)	Post2 Gradient ($mg \cdot mL^{-1} \cdot cm^{-1}$)
Normoxic	5.7 ±1.8	5.6 ± 1.9	6.9 ± 1.4
Hypoxic	6.5 ± 1.2	6.5 ± 1.6	6.8 ± 1.5

Table 4.7. Lung density gradients (density threshold = 0.3 $g \cdot mL^{-1}$) pre and post-exercise intervention (means ± SD); N = 10.

Individual values for lung density gradient at each threshold level are presented in Appendix C Tables C.7 – C.12. In the normoxic condition, the mean anterior – posterior distances used in the pre-exercise scans were 5.0 ± 0.0, 73.8 ± 8.3, and 144.5 ± 16.3 mm with corresponding tissue densities of 0.137 ± 0.025, 0.179 ± 0.020, and 0.216 ± 0.023 $g \cdot mL^{-1}$. These data are illustrated in Figure 4.9, which is included as a sample of lung density gradient for one of six scans for each subject.

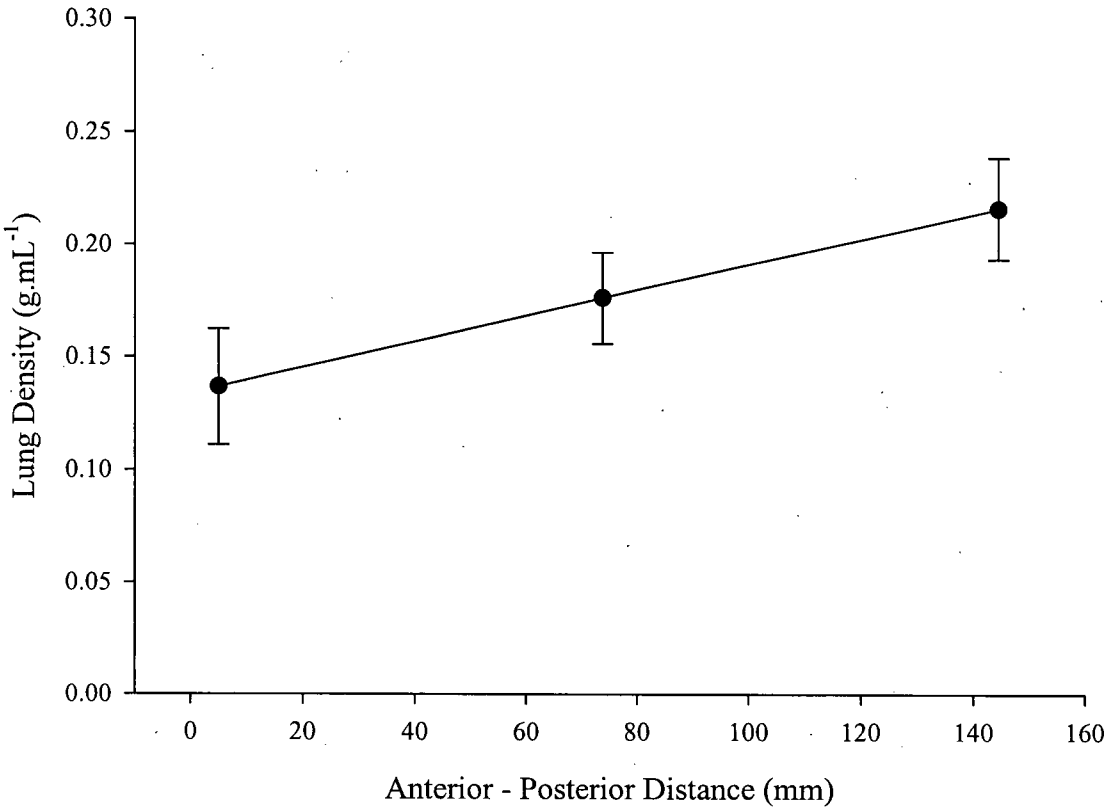

Figure 4.9. Sample lung density gradient for the resting normoxic condition with a threshold density of 0.3 $g \cdot mL^{-1}$.

4.4 DISCUSSION

The purpose of this study was to assess measurement of *in vivo* lung density by magnetic resonance imaging and to describe transient pulmonary oedema, through this measure, following exercise in healthy athletic humans while breathing normoxic and hypoxic air.

Lung Density

Lung density varies with inspiration and age of subjects. Use of CT allows measurement of lung density at specific lung volumes, and values of 0.0715 ± 0.017 and 0.272 ± 0.067

$g \cdot mL^{-1}$ are reported at TLC and RV respectively in healthy subjects while supine.[85] Values obtained in 50 subjects of mean age 50 years in the upper, middle, and lower lung were 0.123 ± 0.46, 0.121 ± 0.033, and 0.154 ± 0.057 $g \cdot mL^{-1}$ during inspiration and 0.215 ± 0.058, 0.228 ± 0.066, and 0.260 ± 0.078 $g \cdot mL^{-1}$ during expiration.[65] Van Dyk et al[119] have reported lung density in five year-olds of 0.36 and 0.20 $g \cdot mL^{-1}$, and in 80 year-olds of 0.22 and 0.16 $g \cdot mL^{-1}$ during inspiration and expiration respectively. Further findings of mean lung density by CT are 0.235 and 0.199 $g \cdot mL^{-1}$ for the right and left lungs respectively.[42] Mayo et al[80] measured lung density by MRI at TLC as 0.21 ± 0.03 and 0.20 ± 0.03 $g \cdot mL^{-1}$ in the prone and supine positions respectively, and McKenzie et al[84] measured resting lung density by MRI as 0.223 ± 0.023 $g \cdot mL^{-1}$. The findings and variation of lung density during normal quiet breathing in this study (resting lung density $= 0.177 \pm 0.019$ $g \cdot mL^{-1}$) compare with these previous measurements made with both CT and MRI, and the consistency of repeated measurements of lung density in this study lends confidence to the validity and reliability of MRI assessment of lung density.

Previously, MRI technology has been used to measure lung density both *in vitro* in animals[34, 35, 80] and *in vivo* in humans[52, 80, 84] with good results when compared to known values for lung density. Estilaei et al[34, 35] have conducted two validation studies involving *in vitro* measurement of porcine lung tissue density by MRI and gravimetric means. In the first study the ratio of wet/dry lung density as measured by NMR and gravimetric means was 1.00 ± 0.08 and 1.00 ± 0.05 respectively. The second study showed a linear correlation between MRI and gravimetric measure of water content of $R^2 = 0.98$. A study by the same group has found a ratio of lung water measured by MR and gravimetric

means was 0.95 ± 0.03, and concluded that MRI allows measurement of lung water content.[80] The methods and equipment used to measure lung density used in this study were very similar to those mentioned above by Mayo et al,[80] and as such the measure of lung density in this study is further validated.

Lung Density Gradient

It was found that the mean resting lung density gradient was 5.7 ± 1.8 $mg \cdot mL^{-1} \cdot cm^{-1}$. Lung density is gravity dependant: while standing upright, the inferior portions of the lung are more dense than the inferior, and while lying supine the posterior portions are more dense that the anterior.[80, 85] Figure 4.9 demonstrates this pattern with a very linear relationship between anterior – posterior distance and lung density ($r^2 = 0.999994$). As a result, the mid-lung density is almost identical to the reported overall mean lung density. Most importantly, the consistency of the mean lung density gradient values across all scans indicates that changes in mean lung density were not missed simply through variance in the pattern of density across each scan slice.

EVLW and Exercise

Table 2.1 summarizes previous findings of changes in EVLW, as measured in a variety of techniques, following various interventions – generally exercise. There is no clear consensus on change in EVLW following exercise, with five[3, 15, 16, 82, 84] of these studies in humans indicating that some increase is observed or likely, and three[39, 76, 120] of them indicating no such change. At first observation, the results of this study could simply be added to the group of studies demonstrating no change in EVLW following exercise.

However, this study is different than the majority of these previously cited studies in that the measure of lung density by MRI is presently the most direct measure available for *in vivo* studies. This study also specifically used exercise as the intervention in an attempt to investigate the link between pulmonary oedema and EIAH. Further, it is imperative to compare these results with those of McKenzie et al[84] in which an increase in lung density following exercise was observed using the same technology as this study.

Several possibilities arise when attempting to explain the discrepancy in results between this study and the previous one using MRI assessment of lung density in humans.[84] Firstly, the exercise intervention was different between these two studies. McKenzie et al[84] used an intervention of 45 min cycling exercise at 77% of VO_2max. For three minutes at the end of the exercise, subjects were encouraged to sprint and increased power outputs to only 35 W below peak values obtained in a VO_2max test, and increased heart rate to within 1 beat·min^{-1} of maximal values. By contrast, in this study subjects cycled for 60 min at a workload of 61% of VO_2max during the normoxic condition with no sprinting. Theoretically, the greater exercise intensity (for a shorter duration) could produce greater cardiac output and pulmonary capillary pressures leading to increased pulmonary leakage and/or stress failure and increased EVLW, while the lower intensity exercise (for a longer duration) may not have provided these stimuli. However, the inclusion of a hypoxic condition in this study should have adequately provided for this contingency by providing an added effect of raising PAP further than with sustained exercise alone. Further, the results of a recent unpublished study[41] from our lab involving severe short-term exercise (five bouts of approximately four minutes each) in hypoxia

also failed to demonstrate increased EVLW as measured by CT scan. Thus, if exercise mode is the key to the different findings, it appears that the very specific mode of 45 min ending with a sprint was the only mode to induce pulmonary oedema.

Secondly, it is possible that demonstration of increased EVLW is an individual response not uniform across the population of aerobically trained human subjects. Interestingly, McKenzie et al[84] found increased EVLW in only four of eight subjects with the remaining four showing no change. A significant increase in mean EVLW was found as a result of the changes in these four subjects, but the lack of change in 50% of the subjects is physiologically relevant. Perhaps importantly, the four subjects in whom there was an increase in lung density following exercise had a significantly higher resting lung density than those who showed no change (this significant difference was not reported in McKenzie et al,[84] but was calculated from the data reported in the results). It is possible that a segment of this population demonstrates increased EVLW following exercise while the remainder does not, and McKenzie et al[84] happened to include some of these "responder" subjects while the present study did not. This possibility cannot be accounted for in the statistical power calculations made in either study, as there is no estimate of what percentage of this population may fall into this "responder" category. If this is the case, however, there is no way of discriminating these subjects through any physiological measure used in the study: no relationship was found between VO_2max or minimum SaO_2 and degree of increased EVLW by McKenzie et al.[84] That fact alone discredits this theory of "responder" and "non-responder" subjects, at least in as much as it refers to pulmonary oedema as a mechanism for EIAH, and further the fact that no

"responders" were found in the present study or the subsequent unpublished study[41] involving severe hypoxic exercise suggests they would be a very small proportion of the population indeed. Given that approximately 50% of aerobically trained male subjects demonstrate EIAH, it is very unlikely indeed that pulmonary oedema is the sole mechanism responsible for EIAH, even if the theory of "responders" and "non-responders" were true. And if that were the case, it is unlikely that McKenzie et al[84] would have found 50% "responders", while this study found none. This argument is less convincing for the fact that the present study did not include a subset of "responders" and therefore, in short, the "responder" and "non-responder" scenario is statistically unlikely. Nonetheless, it is possibly worth further examination and comparison of physiological variables of the two groups in further studies, if this pattern is ever reproducible.

This hypothesis of responders and non-responders is worth examining in light of recent evidence of intrapulmonary shunting of blood during exercise. In two recent studies using agitated saline contrast echocardiography, evidence of intrapulmonary shunting in the form of contrast bubbles visible in the left heart following injection in a vein occurred in approximately 90% of subjects (21 of 23 subjects in a study by Eldridge et al,[31] and seven of eight subjects in a study by Stickland et al[114]). While merely speculative, it is possible that the development of intrapulmonary shunts provides a protective effect. Therefore the possibility that those subjects who do not demonstrate intrapulmonary shunting of blood during exercise are more likely to develop transient pulmonary oedema due to increased PAP, is worth further investigation.

One of the limitations of lung density measures in human is that they are necessarily indirect. Further, the techniques that offer the most sensitive quantitative measure of lung density, including CT and MRI, generally require a period of time between the end of exercise and measurement of lung density to allow pulmonary blood flow to return to normal resting values. As a result there is uncertainty whether transient pulmonary oedema occurred and resolved prior to the MR scan, or whether no transient pulmonary oedema occurred during exercise. Clearance of alveolar oedema is a complicated process involving active transepithelial sodium transport by sodium-potassium ATPase[11, 115] and is likely to take an extended period of time to occur. But interstitial oedema, which is perhaps the more likely level of oedema to result from the perturbations used in this study given that none of the clinical signs of alveolar oedema were observed, may clear much more quickly. Furthermore, exercise-induced hyperpnoea has been shown to increase lymph clearance of interstitial oedema in sheep[67] indicating that the exercise these subjects performed may have paradoxically assisted in the clearance of any oedema that did occur through post-exercise hyperpnoea. A recent study of horses has shown increased transvascular fluid flux in the pulmonary vasculature during exercise when compared to rest,[121] suggesting that, in these animals at least, fluid movement across the blood gas barrier is increased during exercise even if there is no accumulation of oedema. Unfortunately, at least in human subjects, the limitations involved in direct measurement of pulmonary oedema are unlikely to be resolved in the near future.

The main questions raised in this study are whether sustained exercise can cause increased EVLW, and whether this represents sub-clinical pulmonary oedema that may

be a mechanism of EIAH. The first question is answered quite decisively by the lack of difference in lung density between any of the MR scans. Arterial oxyhaemoglobin saturation during exercise in the hypoxic condition was in the range of 80 – 85 (Figure 4.6), indicating that the perturbations of exercise in combination with hypoxia met the conditions associated with raised mean pulmonary arterial pressure. But even following 60 min of sustained intense exercise in hypoxia, no subject in this study demonstrated an increase in lung density of any significance. The greatest increase in lung density by any single subject was 0.011 $g \cdot mL^{-1}$ which is much less than the range of changes reported by McKenzie et al[84] of 0.02 – 0.071 $g \cdot mL^{-1}$ for the four subjects that showed increased EVLW, and the change of 0.04 $g \cdot mL^{-1}$ (as measured by CT) reported by Caillaud et al.[16] in subjects following a triathlon. Furthermore, in the present study, the greatest decrease in lung density by a single subject was 0.024 $g \cdot mL^{-1}$ which exceeds the greatest single increase. As presented in the results, the variation between subjects was relatively small and the repeatability of resting slice density within subjects gave a significant Casewise correlation of $r^2 = 0.73$ ($p=0.007$). Therefore, in this study no change in lung density was found in either condition. The second question is slightly more complex. While this study does not add any strength to the possibility of pulmonary oedema as a mechanism for EIAH, it may not so clearly rule it out. The exercise intervention used was the highest intensity exercise that subjects could sustain for 60 min. EIAH often presents during exercise of short duration and very high intensity, but has also been observed during submaximal exercise of intensities as low as 40% VO_2max.[98] While the possibility remains that pulmonary oedema is an integral part of the mechanisms of EIAH during more intense exercise that would elicit higher intrapulmonary capillary pressures, this is

not a likely scenario as this study included hypoxic exercise to specifically raise these pressures. The present study provided a strong stimulus for pulmonary capillary leakage to occur over time, during the 60 min of exercise. Increased intrapulmonary pressures during very intense exercise over a short period of time are unlikely to result in more capillary leakage. However, capillary stress failure theory remains a possibility in this situation. If steady-state exercise over a long period of time does not produce an increase in EVLW (as shown in this study), but evidence of EVLW exists following more intense exercise, then stress failure may be the more logical explanation. Signs of stress failure have been observed in horses,[129] dogs,[79] rabbits,[37, 118] and in humans following intense,[57] but not sustained[58] exercise. The findings of these last two studies of humans by Hopkins and colleagues are confirmed to some degree by the present finding of no change in lung density following sustained exercise.

CHAPTER FIVE: General Summary and Conclusions

Exercise-induced arterial hypoxaemia (EIAH) is an established and well-studied response to exercise that occurs in approximately 50% of aerobically trained male athletes. The mechanisms involved have been extensively examined to the point that it is commonly accepted that there are four possibilities: right to left intrapulmonary shunts, relative alveolar hypoventilation during exercise, ventilation/perfusion (V_A/Q_C) mismatch, and diffusion limitation.[25] The focus of this manuscript was to examine diffusion limitation in the lung as a mechanism for EIAH. Specifically, these studies examined the effects of exercise on lung density. The significance of an exercise-induced change in lung density is that it represents an increase in fluid in the lung and, providing the assumption that intravascular fluid is constant at each measure, this fluid represents increased EVLW indicative of sub-clinical pulmonary oedema.

There is evidence of pulmonary oedema following exercise in animals[103, 129] and humans[57, 82, 84] but, as discussed in Chapter 2, there is no consistency in the development of oedema following exercise in humans. Initially, the present studies may simply be added to the list of work done on pulmonary oedema following either an exercise or pharmacological intervention. But the results of these studies add to the existing literature in several ways. Firstly, the methods used to assess lung density by MRI in these studies have shown to be consistent and reliable: there was a small coefficient of variation for repeated measures of resting lung density; the process involved some qualitative measurement on the part of the researchers, and this process was shown to be reliable

both between and within observers; and finally, the values for lung density achieved in this study match those previously reported. When added to the validation studies of Estilaei et al,[34, 35] the use of MRI as a measure of lung density assessment appears sound. Secondly, these studies clearly demonstrate a lack of change in lung density following sustained exercise both in normoxia and hypoxia, which indicates that pulmonary oedema does not occur as a result of exercise as readily as previously indicated. Diffusion limitation cannot be completely ruled out as a mechanism of EIAH from the results of these studies, as pulmonary oedema and diffusion limitation may occur under different exercise conditions, such as those indicated in the findings by McKenzie et al,[84] and further transient oedema may resolve relatively quickly following exercise making detection post-exercise difficult.

The most comprehensive means of examining the issue of pulmonary oedema following exercise in humans would involve multiple exercise protocols under normoxic and hypoxic conditions followed by MRI assessment of lung density and measures of diffusing capacity. However, MRI is prohibitively expensive when used repeatedly in this way, and there are logistical problems associated with measuring diffusing capacity in a limited time frame between exercise and MRI. Nonetheless, further study of this area is warranted, perhaps combining technologies used in the present study with other indices of pulmonary oedema such as bronchoalveolar lavage, and of right to left shunts such as agitated saline contrast echocardiography in an attempt to expand the knowledge base of pulmonary responses to exercise, and further understand the mechanisms of EIAH.

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APPENDIX A. Individual data from Chapter 3

Subject	Age (y)	Height (cm)	Mass (kg)
1	37	166.2	71.2
2	26	183.7	83.0
3	27	173.1	73.2
4	20	176.5	80.2
5	33	182.6	81.0
6	33	174.5	74.7
7	21	184.7	73.8
8	33	186.2	85.9
9	33	192.9	83.3
10	33	175.5	87.0

Table A.1. Individual descriptive data.

Subject	HR ($b \cdot min^{-1}$)	VE ($L \cdot min^{-1}$)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	Peak Power (W)	Minimum SaO_2 (%)
1	188	119.0	3.7	51.2	347	96.9
2	183	138.8	3.9	46.6	370	96.9
3	187	126.9	3.7	49.4	341	95.4
4	191	174.3	5.2	63.5	407	94.9
5	180	146.3	4.6	56.2	363	96.1
6	189	129.2	4.5	58.8	406	93.8
7	193	181.4	5.3	71.8	430	94.6
8	184	155.3	5.7	65.4	491	94.5
9	174	140.7	5.8	55.8	452	93.0
10	187	151.6	4.7	52.9	400	95.0

Table A.2. Individual maximal exercise data ($F_IO_2 = 21\%$).

Subject	HR ($b \cdot min^{-1}$)	VE ($L \cdot min^{-1}$)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	Peak Power (W)	Minimum SaO_2 (%)
1	185	107.5	3.5	48.2	333	91.2
2	181	143.0	4.4	50.0	362	94.4
3	189	123.7	3.6	48.2	325	92.0
4	189	123.8	3.6	48.2	325	92.0
5	181	144.7	4.4	52.4	363	95.0
6	182	130.6	4.3	55.5	378	88.8
7	196	165.2	4.8	65.7	415	90.3
8	178	164.1	5.2	59.7	452	92.6
9	177	128.8	4.3	50.7	408	90.0
10	182	147.2	4.7	53.3	393	89.3

Table A.3. Individual maximal exercise data (F_IO_2 = 18%).

Subject	HR ($b \cdot min^{-1}$)	VE ($L \cdot min^{-1}$)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	Peak Power (W)	Minimum SaO_2 (%)
1	185	118.9	3.4	48.2	318	84.7
2	184	129.3	3.9	44.6	348	87.1
3	188	107.5	3.3	41.5	291	85.0
4	186	158.9	4.3	53.1	378	84.8
5	187	137.6	4.1	48.3	347	86.0
6	179	138.4	4.2	54.8	355	76.5
7	188	163.1	4.5	59.9	382	79.9
8	178	158.1	4.5	52.8	430	87.8
9	173	129.7	4.0	47.0	413	81.0
10	178	138.9	2.8	31.7	355	80.5

Table A.4. Individual maximal exercise data (F_IO_2 = 15%).

Subject	HR ($b \cdot min^{-1}$)	VE ($L \cdot min^{-1}$)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	Peak Power (W)	Minimum SaO_2 (%)
1	177	114.2	2.7	37.4	273	74.4
2	162	65.7	2.3	26.5	204	72.8
3	131	43.0	2.1	20.8	176	75.5
4	153	59.3	2.4	28.0	153	68.7
5	171	137.1	3.6	43.2	137	73.7
6	128	33.7	1.6	27.8	71	70.8
7	174	79.8	2.8	37.1	243	69.7
8	157	111.1	3.2	36.6	273	74.0
9	169	99.4	3.0	35.5	317	64.9
10	141	53.9	2.1	22.4	168	70.1

Table A.5. Individual maximal exercise data (F_IO_2 = 12%).

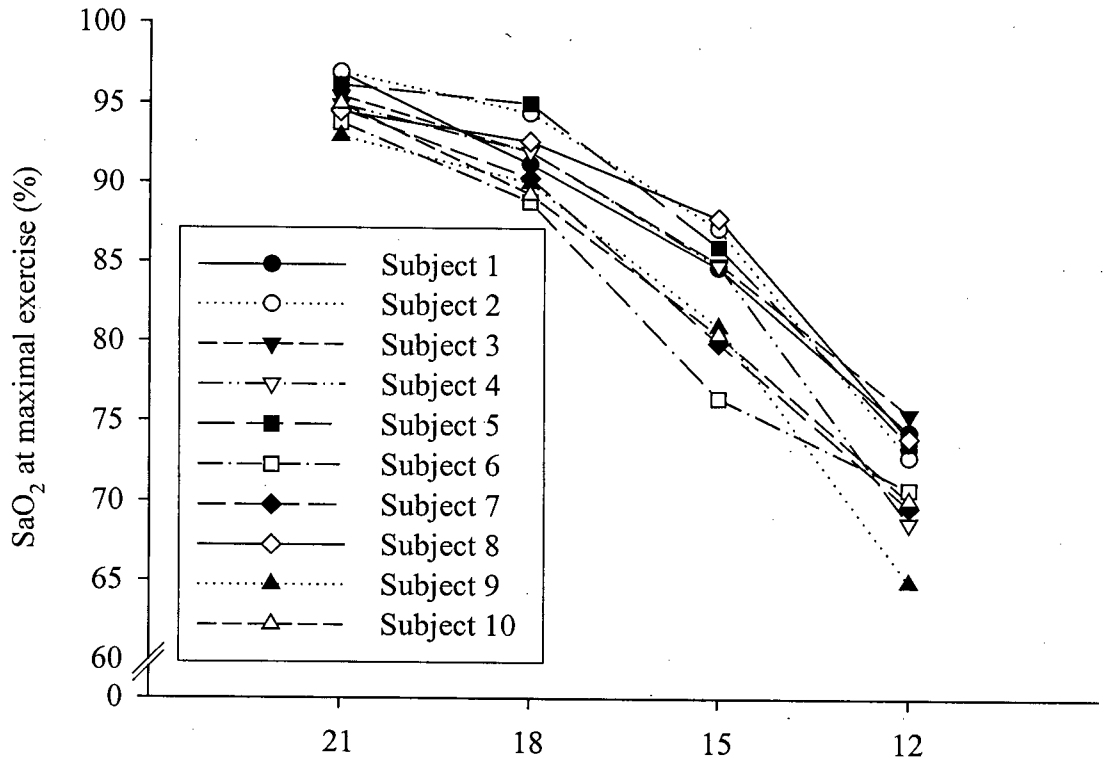

Figure A.1. Individual minimum SaO_2 with varying F_IO_2.

APPENDIX B. Individual exercise data from Chapter 4

Subject	Age (y)	Height (cm)	Mass (kg)	FVC (L)	FEV1 / FVC (%)
1	24	190.0	87.5	7.3	80.7
2	26	177.5	69.5	5.3	81.9
3	23	189.0	89.9	6.8	86.7
4	34	190.0	84.5	6.7	81.1
5	34	184.5	81.6	5.6	80.8
6	23	180.5	67.0	4.4	81.1
7	22	167.5	62.6	5.4	86.2
8	29	192.0	89.4	7.2	86.4
9	21	175.5	80.4	3.9	80.9
10	24	195.0	73.5	6.7	81.1

Table B.1. Individual descriptive and spirometry data.

Subject	HR ($b \cdot min^{-1}$)	VE ($L \cdot min^{-1}$)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	Peak Power (W)	Minimum SaO_2 (%)
1	189	188.0	5.0	56.8	445	94.5
2	176	123.4	4.3	66.4	355	93.8
3	184	177.0	5.5	61.4	450	90.8
4	182	153.1	4.7	56.2	393	95.3
5	183	126.1	4.7	57.1	420	91.6
6	192	144.9	4.7	69.4	460	92.6
7	190	134.1	4.5	72.2	460	92.5
8	191	189.3	5.8	66.5	604	89.5
9	184	157.5	5.1	64.5	480	91.9
10	196	158.8	5.8	79.5	500	90.6

Table B.2. Individual maximal exercise data ($F_IO_2 = 21\%$).

Subject	HR ($b \cdot min^{-1}$)	VE ($L \cdot min^{-1}$)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	Peak Power (W)	Minimum SaO_2 (%)
1	180	168.0	4.3	50.6	386	83.0
2	174	132.5	3.9	58.5	326	83.5
3	180	158.9	4.4	49.0	378	79.0
4	180	134.3	4.5	52.3	355	86.2
5	179	145.0	4.1	50.4	369	77.1
6	184	132.1	4.0	60.0	401	81.6
7	178	147.0	3.6	57.3	393	76.7
8	159	75.2	3.6	39.8	341	72.1
9	184	165.8	4.7	58.8	408	83.4
10	186	152.1	4.4	63.9	415	74.9

Table B.3. Individual maximal exercise data (F_IO_2 = 15%).

Subject	HR ($b \cdot min^{-1}$)	V_E ($L \cdot min^{-1}$)	VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	Power (W)	SaO_2 (%)
1	161.7 ± 3.9	63.9 ± 5.2	37.5 ± 1.9	246.5 ± 2.2	95.3 ± 0.6
2	140.6 ± 6.8	47.0 ± 3.5	37.2 ± 1.5	173.9 ± 1.5	96.3 ± 0.2
3	146.8 ± 7.2	74.4 ± 5.5	38.7 ± 2.3	235.8 ± 7.5	97.2 ± 0.5
4	145.7 ± 5.3	57.9 ± 4.6	33.4 ± 2.2	218.5 ± 5.7	95.8 ± 0.2
5	153.9 ± 4.8	67.7 ± 4.6	41.0 ± 0.9	237.3 ± 1.7	93.9 ± 0.3
6	160.6 ± 13.0	70.1 ± 9.9	46.9 ± 2.9	257.0 ± 28.1	95.3 ± 0.4
7	164.1 ± 9.7	76.3 ± 8.8	51.7 ± 2.9	275.0 ± 10.0	94.6 ± 0.3
8	163.0 ± 4.7	40.5 ± 3.6	28.1 ± 1.7	383.0 ± 6.8	93.2 ± 1.1
9	161.5 ± 6.9	72.9 ± 3.5	43.0 ± 1.3	290.0 ± 0.0	95.4 ± 0.1
10	170.7 ± 5.4	62.7 ± 10.6	40.9 ± 5.3	289.1 ± 27.5	95.4 ± 1.0

Table B.4. Individual exercise intervention data (normoxic condition, averaged over last 50 min; mean ± SD).

Subject	HR ($b \cdot min^{-1}$)	V_E ($L \cdot min^{-1}$)	VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	Power (W)	SaO_2 (%)
1	158.4 ± 4.4	66.9 ± 4.0	32.7 ± 0.9	212.0 ± 0.0	87.5 ± 1.5
2	157.9 ± 3.7	58.0 ± 2.5	39.1 ± 1.1	181.2 ± 1.4	82.0 ± 0.3
3	152.8 ± 4.6	77.4 ± 7.7	33.1 ± 2.4	195.5 ± 3.3	81.7 ± 1.5
4	144.6 ± 5.7	54.0 ± 1.8	27.4 ± 0.6	185.6 ± 8.0	88.0 ± 0.5
5	155.0 ± 4.2	71.7 ± 3.6	36.5 ± 0.9	204.1 ± 4.1	80.8 ± 0.6
6	155.0 ± 10.3	68.4 ± 3.5	38.7 ± 1.2	214.4 ± 14.7	86.1 ± 0.6
7	165.2 ± 6.9	70.1 ± 6.7	44.2 ± 2.3	224.6 ± 2.9	81.4 ± 0.4
8	149.8 ± 7.1	44.7 ± 1.1	25.5 ± 1.1	257.9 ± 13.0	78.8 ± 1.5
9	162.6 ± 4.7	64.7 ± 3.9	32.8 ± 1.9	241.4 ± 3.1	81.1 ± 9.4
10	162.1 ± 4.3	69.9 ± 2.6	45.0 ± 2.1	235.0 ± 0.0	78.2 ± 1.6

Table B.5. Individual exercise intervention data (hypoxic condition, averaged over last 50 min; mean ± SD).

Subject	Total Work in Normoxia (kJ)	Total Work in Hypoxia (kJ)
1	889.5	763.2
2	618.0	647.4
3	840.4	699.6
4	785.2	636.2
5	843.7	712.3
6	884.8	728.3
7	936.9	759.3
8	1366.8	912.6
9	1016.6	854.5
10	1078.0	753.9

Table B.6: Individual exercise intervention data (total work performed).

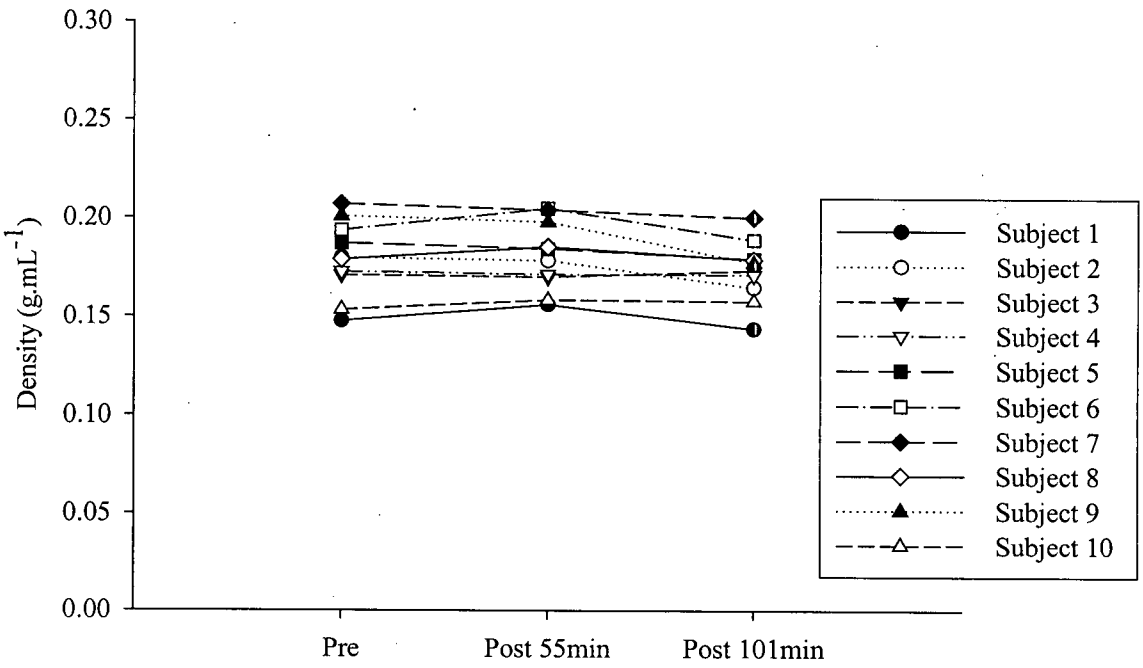

Figure B.1. Individual lung densities following normoxic exercise (density threshold = 0.3 g·mL^{-1}).

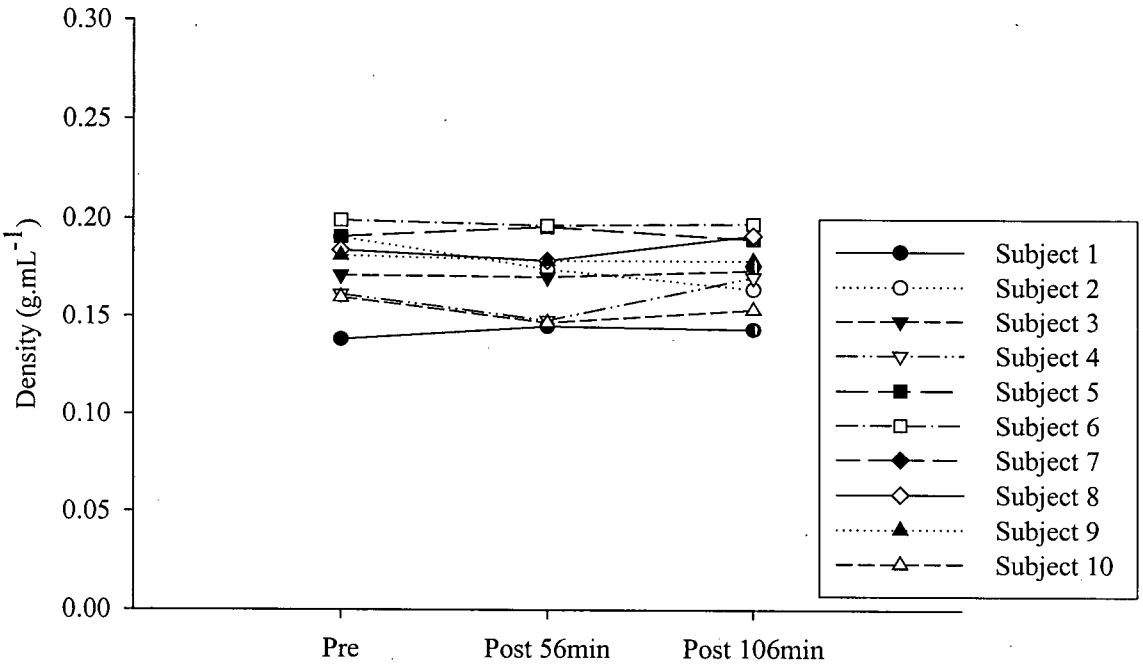

Figure B.2. Individual lung densities following hypoxic exercise (density threshold = 0.3 $g \cdot mL^{-1}$).

APPENDIX C. Individual data: lung densities, gradients, & areas

As described in section 4.2, pixels above a specific density were removed from the lung density images to avoid including vascular tissue in the density calculations. Six different thresholds were used for this process. A threshold of 0.30 $g \cdot mL^{-1}$ was used for all analyses in the manuscript, but lung densities were calculated for all threshold values used (0.25, 0.30, 0.35, 0.40, 0.45, and 1.0 $g \cdot mL^{-1}$). Tables C.1 – C.6 contain these individual lung density data.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	0.141	0.146	0.136	0.132	0.136	0.138
2	0.170	0.166	0.155	0.178	0.158	0.154
3	--	0.154	0.142	0.156	0.157	0.159
4	0.158	0.157	0.157	0.147	0.136	0.154
5	0.170	0.166	0.159	0.170	0.171	0.169
6	0.181	0.173	0.176	0.179	0.176	0.176
7	--	0.188	0.187	0.184	0.183	0.178
8	0.165	0.164	0.167	0.167	0.165	0.172
9	0.181	0.177	0.160	0.168	0.166	0.174
10	0.146	0.148	0.147	0.150	0.135	0.141

Table C.1. Individual lung densities ($g \cdot mL^{-1}$) with a density threshold = 0.25 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	0.148	0.156	0.144	0.138	0.145	0.143
2	0.180	0.179	0.165	0.190	0.174	0.164
3	--	0.167	0.156	0.171	0.170	0.174
4	0.173	0.172	0.172	0.161	0.148	0.170
5	0.187	0.185	0.179	0.191	0.196	0.190
6	0.194	0.205	0.189	0.199	0.197	0.197
7	--	0.209	0.213	0.207	0.204	0.200
8	0.179	0.186	0.179	0.184	0.179	0.192
9	0.201	0.198	0.177	0.181	0.179	0.179
10	0.153	0.159	0.158	0.160	0.147	0.153

Table C.2. Individual lung densities ($g \cdot mL^{-1}$) with a density threshold = 0.30 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	0.152	0.162	0.148	0.142	0.150	0.146
2	0.185	0.188	0.171	0.197	0.185	0.169
3	--	0.173	0.164	0.180	0.178	0.183
4	0.185	0.183	0.183	0.171	0.157	0.183
5	0.198	0.197	0.196	0.205	0.217	0.205
6	0.223	0.199	0.208	0.212	0.209	0.213
7	--	0.224	0.229	0.222	0.220	0.216
8	0.186	0.200	0.187	0.194	0.187	0.206
9	0.214	0.213	0.189	0.188	0.186	0.205
10	0.158	0.165	0.164	0.166	0.154	0.161

Table C.3. Individual lung densities ($g \cdot mL^{-1}$) with a density threshold = 0.35 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	0.155	0.165	0.151	0.145	0.153	0.148
2	0.188	0.193	0.174	0.202	0.190	0.172
3	--	0.178	0.169	0.185	0.183	0.189
4	0.194	0.191	0.192	0.178	0.165	0.193
5	0.203	0.206	0.208	0.214	0.233	0.215
6	0.233	0.206	0.216	0.220	0.217	0.224
7	--	0.233	0.241	0.231	0.230	0.227
8	0.190	0.210	0.191	0.200	0.193	0.216
9	0.222	0.225	0.197	0.192	0.191	0.214
10	0.160	0.170	0.168	0.170	0.160	0.166

Table C.4. Individual lung densities ($g \cdot mL^{-1}$) with a density threshold = 0.40 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	0.156	0.167	0.152	0.147	0.156	0.149
2	0.191	0.196	0.176	0.205	0.193	0.174
3	--	0.181	0.171	0.188	0.187	0.193
4	0.199	0.196	0.197	0.183	0.171	0.200
5	0.207	0.213	0.217	0.220	0.245	0.222
6	0.214	0.240	0.210	0.225	0.222	0.231
7	--	0.240	0.248	0.236	0.236	0.233
8	0.191	0.215	0.194	0.203	0.195	0.222
9	0.227	0.233	0.202	0.195	0.194	0.220
10	0.162	0.173	0.170	0.173	0.164	0.169

Table C.5. Individual lung densities ($g \cdot mL^{-1}$) with a density threshold = 0.45 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	0.160	0.171	0.154	0.155	0.162	0.152
2	0.199	0.201	0.180	0.216	0.201	0.177
3	--	0.186	0.178	0.194	0.195	0.204
4	0.205	0.204	0.204	0.190	0.187	0.216
5	0.213	0.224	0.233	0.229	0.272	0.236
6	0.267	0.233	0.241	0.237	0.239	0.246
7	--	0.255	0.260	0.244	0.247	0.245
8	0.193	0.223	0.196	0.206	0.201	0.231
9	0.238	0.251	0.212	0.199	0.201	0.232
10	0.167	0.180	0.173	0.179	0.171	0.175

Table C.6. Individual lung densities ($g \cdot mL^{-1}$) with a density threshold = 1.0 $g \cdot mL^{-1}$.

The gradient of lung density for every image was calculated as the slope of the best fit line between the average density at each of 3 distances from anterior to posterior lung. The mean data for gradient at a threshold density of 0.30 $g \cdot mL^{-1}$ are discussed in section 4.3. The individual data for each scan at each threshold density are presented in tables C.7 – C.12.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	5.1	4.1	3.3	4.9	4.3	4.8
2	2.4	5.9	6.4	5.9	5.9	5.8
3	--	4.8	7.1	7.1	4.6	5.4
4	5.3	6.2	5.7	5.1	6.8	--
5	2.7	2.5	6.3	4.7	4.1	4.6
6	4.9	6.5	4.9	5.7	5.9	6.7
7	--	3.5	5.0	3.4	4.8	5.9
8	4.6	4.2	4.6	5.2	2.7	3.8
9	--	2.5	5.0	5.2	--	4.8
10	6.3	5.7	6.2	5.3	6.6	5.7

Table C.7. Individual lung density gradients ($mg \cdot mL^{-1} \cdot cm^{-1}$) with a density threshold = 0.25 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	5.8	4.9	3.8	5.4	5.2	5.0
2	3.0	7.1	7.2	6.9	7.4	6.5
3	--	5.4	8.4	8.8	5.6	6.5
4	7.3	8.1	7.0	6.5	8.7	8.9
5	3.0	2.7	8.4	6.1	5.6	6.0
6	6.6	6.8	8.1	7.6	7.8	9.4
7	--	4.9	6.8	4.0	6.7	7.7
8	5.5	5.6	5.8	6.7	3.6	5.3
9	6.7	2.6	6.1	6.4	5.6	5.6
10	7.3	7.7	7.9	6.6	8.4	7.4

Table C.8. Individual lung density gradients ($mg \cdot mL^{-1} \cdot cm^{-1}$) with a density threshold = 0.30 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	6.3	5.5	4.2	5.8	5.7	5.1
2	3.4	7.9	7.7	7.4	8.5	6.9
3	--	5.7	9.1	9.9	6.4	7.2
4	8.9	9.6	8.7	7.5	10.2	10.9
5	3.2	2.5	10.1	7.7	7.3	7.3
6	8.5	9.1	7.8	9.2	9.3	11.8
7	--	6.1	8.8	4.3	8.4	9.0
8	6.0	6.9	6.6	7.9	4.5	6.6
9	7.8	2.8	7.1	6.9	6.3	7.3
10	7.7	8.8	8.9	7.5	9.7	8.1

Table C.9. Individual lung density gradients ($mg \cdot mL^{-1} \cdot cm^{-1}$) with a density threshold = 0.35 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	6.5	5.7	4.4	6.1	6.0	5.1
2	3.5	8.4	7.9	7.7	9.0	7.2
3	--	5.9	9.6	10.4	6.7	7.6
4	10.0	10.6	10.0	8.4	11.4	12.4
5	3.5	2.5	11.5	9.0	8.9	8.4
6	9.6	9.6	8.6	10.2	10.5	13.6
7	--	7.0	10.2	4.9	9.3	10.3
8	6.3	7.6	7.1	8.6	5.1	7.8
9	8.5	2.6	7.7	7.1	6.8	8.0
10	8.0	9.5	9.5	8.3	10.5	8.7

Table C.10. Individual lung density gradients ($mg \cdot mL^{-1} \cdot cm^{-1}$) with a density threshold = 0.40 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	6.6	5.8	4.4	6.3	6.2	5.2
2	3.5	8.7	8.1	7.9	9.3	7.3
3	--	6.0	9.8	10.8	7.0	7.9
4	10.7	11.4	10.6	9.0	12.2	13.8
5	3.8	2.4	12.7	9.8	10.3	9.0
6	8.9	10.6	9.9	10.8	11.0	14.7
7	--	7.5	11.6	5.4	10.2	11.0
8	6.4	7.9	7.4	8.9	5.4	8.6
9	8.8	2.6	8.1	7.2	7.0	8.5
10	8.2	9.9	9.7	8.6	11.1	8.9

Table C.11. Individual lung density gradients ($mg \cdot mL^{-1} \cdot cm^{-1}$) with a density threshold = 0.45 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	6.7	5.8	4.3	6.8	6.5	5.2
2	3.6	8.9	8.2	8.3	9.8	7.4
3	--	6.3	10.1	11.3	7.6	8.5
4	11.2	12.5	11.2	9.6	14.2	16.1
5	4.4	0.6	14.6	11.6	13.4	10.4
6	13.4	11.8	10.4	12.0	12.2	18.8
7	--	8.6	13.2	6.0	10.8	12.2
8	6.5	8.3	7.6	9.1	5.8	9.5
9	9.3	2.7	9.0	7.4	7.3	9.2
10	8.4	10.4	9.9	9.1	11.7	9.0

Table C.12. Individual lung density gradients ($mg \cdot mL^{-1} \cdot cm^{-1}$) with a density threshold = 1.0 $g \cdot mL^{-1}$.

The area of each lung scan slice was measured during the calculation of lung slice density. This area varies depending on subject size, observer slice selection, and the amount of tissue removed above the threshold density. The individual data for each scan at each threshold density are presented in tables C.13 – C. 18.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	21,936	20,923	21,020	21,555	21,355	21,745
2	15,703	14,844	15,895	14,402	13,619	16,064
3	--	13,289	14,445	13,600	13,928	13,708
4	14,569	15,341	14,711	15,483	16,273	14,008
5	12,198	12,202	10,681	10,738	8,634	10,795
6	9,113	11,223	11,467	10,966	10,820	9,806
7	--	7,688	6,238	8,075	7,855	7,653
8	19,255	15,239	17,147	15,833	16,983	14,284
9	12,064	10,859	13,030	13,722	13,756	10,709
10	20,711	18,492	19,313	19,850	19,695	19,550

Table C.13. Individual lung slice areas (mm^2)with a density threshold = 0.25 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	23,169	22,706	22,225	22,469	22,781	22,603
2	17,375	16,919	17,359	16,567	15,919	17,531
3	--	14,800	16,125	15,706	15,698	15,773
4	16,791	17,583	16,761	17,448	17,848	16,181
5	14,616	14,723	12,967	13,497	11,417	13,464
6	13,833	12,313	13,406	13,913	13,750	12,636
7	--	10,200	8,886	10,863	10,256	9,992
8	22,102	18,931	19,481	18,811	19,511	17,711
9	15,344	13,916	15,356	15,773	15,614	15,614
10	22,033	20,181	21,081	21,655	21,438	21,628

Table C.14. Individual lung slice areas (mm^2)with a density threshold = 0.30 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	23,777	23,519	22,842	22,941	23,477	22,975
2	18,056	18,039	18,034	17,470	17,103	18,094
3	--	15,475	16,955	16,727	16,547	16,853
4	18,273	19,006	18,108	18,548	18,827	17,606
5	15,838	16,222	14,647	15,119	13,645	15,222
6	14,513	14,522	15,959	15,588	15,305	14,481
7	--	11,805	10,486	12,508	11,850	11,461
8	23,205	21,227	20,586	20,338	20,716	19,823
9	17,231	15,858	16,747	16,602	16,473	14,659
10	22,586	21,045	21,911	22,484	22,434	22,672

Table C.15. Individual lung slice areas (mm^2)with a density threshold = 0.35 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	24,066	23,913	23,077	23,225	23,822	23,163
2	18,364	18,573	18,347	17,938	17,602	18,341
3	--	15,838	17,356	17,133	16,984	17,423
4	19,153	19,802	19,011	19,244	19,525	18,602
5	16,378	17,067	15,716	16,022	15,266	16,234
6	15,544	15,114	16,705	16,398	16,070	15,477
7	--	12,573	11,417	13,319	12,661	12,297
8	23,670	22,458	21,108	21,031	21,355	21,086
9	18,116	17,064	17,445	16,964	16,906	15,444
10	22,881	21,516	22,325	22,931	23,005	23,252

Table C.16. Individual lung slice areas (mm^2)with a density threshold = 0.40 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	24,202	24,103	23,189	23,400	24,053	23,283
2	18,567	18,805	18,497	18,178	17,878	18,480
3	--	16,019	17,536	17,336	17,227	17,697
4	19,627	20,291	19,458	19,613	20,009	19,217
5	16,648	17,602	16,398	16,470	16,320	16,741
6	15,997	16,173	15,420	16,789	16,456	16,047
7	--	13,009	11,903	13,677	13,048	12,709
8	23,845	23,020	21,359	21,281	21,620	21,794
9	18,542	17,823	17,864	17,152	17,119	15,881
10	23,027	21,806	22,480	23,189	23,381	23,498

Table C.17. Individual lung slice areas (mm^2)with a density threshold = 0.45 $g \cdot mL^{-1}$.

Subject	Normoxic Pre	Normoxic Post1	Normoxic Post2	Hypoxic Pre	Hypoxic Post1	Hypoxic Post2
1	24,430	24,317	23,320	23,841	24,402	23,447
2	19,009	19,086	18,680	18,719	18,273	18,663
3	--	16,277	17,813	17,642	17,634	18,227
4	19,984	20,788	19,842	19,984	20,255	20,145
5	16,975	18,277	17,258	16,978	17,897	17,500
6	17,445	16,350	18,094	17,416	17,263	16,852
7	--	13,669	12,381	14,042	13,514	13,208
8	23,942	23,630	21,503	21,483	21,964	22,448
9	19,191	18,933	18,375	17,375	17,481	16,492
10	23,313	22,188	22,661	23,558	23,823	23,823

Table C.18. Individual lung slice areas (mm^2)with a density threshold = 1.0 $g \cdot mL^{-1}$.

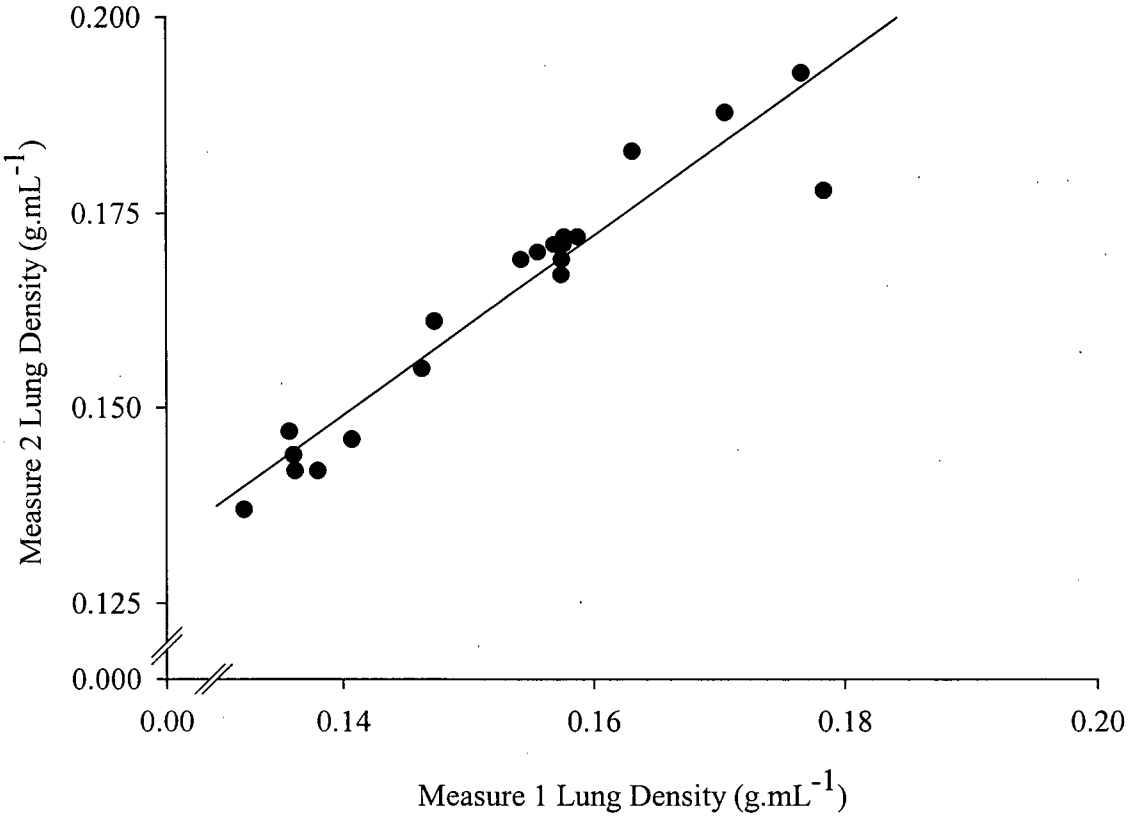

Figure C.1. Intra-observer reliability of lung density, $r = 0.98$, $p < 0.05$, $N = 20$.

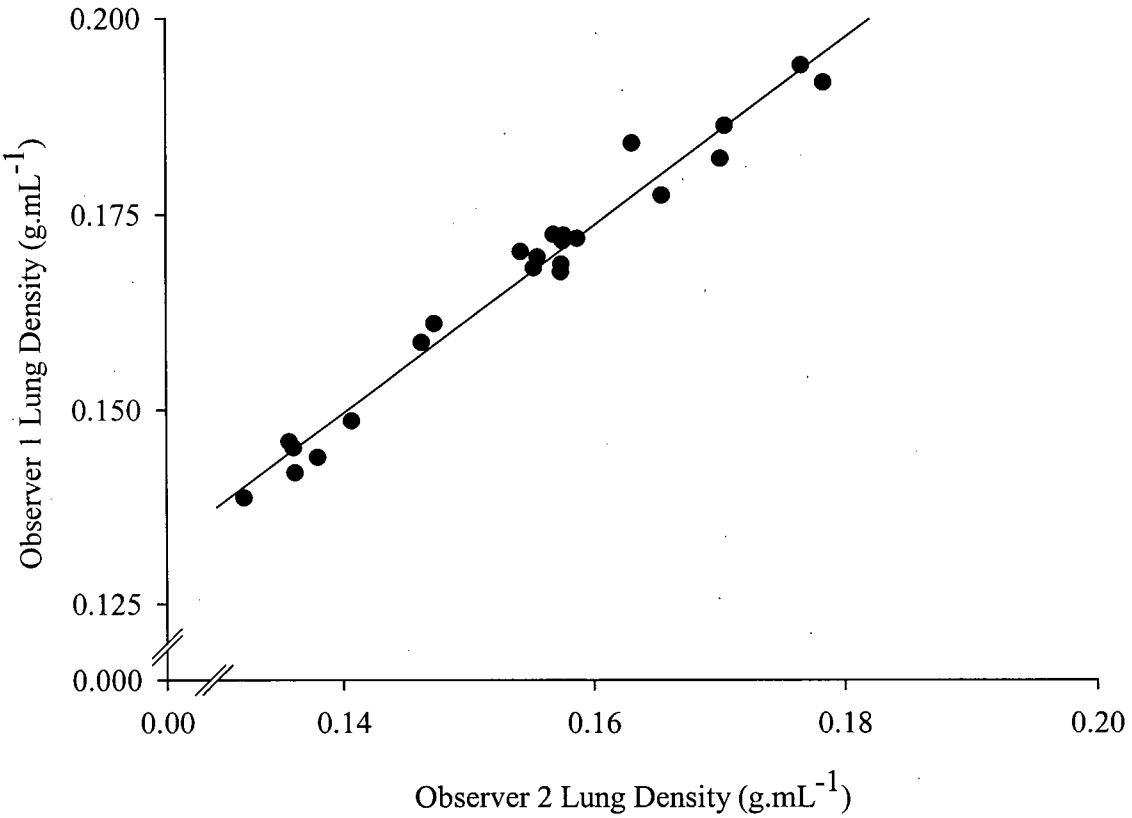

Figure C.2. Inter-observer reliability of lung density r = 0.98, p< 0.05, N = 20.

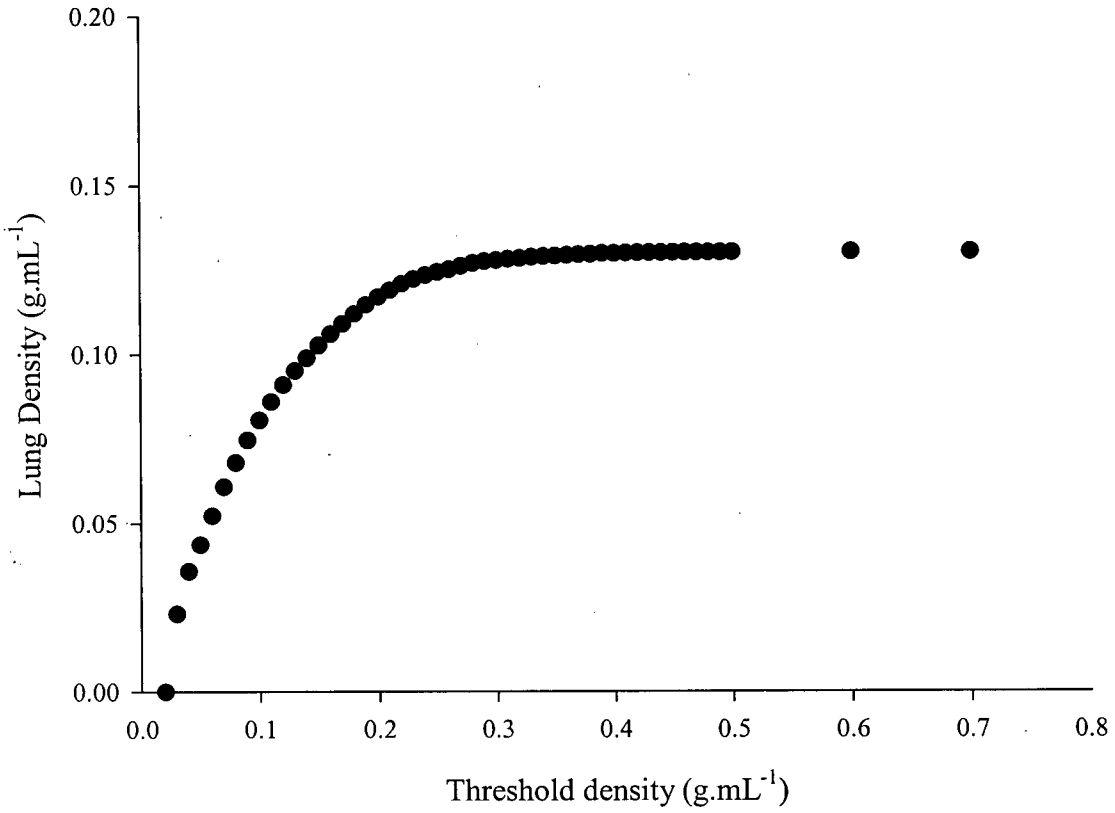

Figure C.3. Lung density vs. lung density threshold.