

**CARDIOVASCULAR AND RESPIRATORY REFLEX  
CONTROL SYSTEMS  
IN THE REGULATION OF PULMONARY BLOOD FLOW  
AND VENTILATION DURING EXERCISE**

by

CHRISTOS LYKIDIS

A thesis submitted to the University of Birmingham for  
the degree of Doctor of Philosophy

School of Sport and Exercise Sciences  
University of Birmingham  
September 2009

UNIVERSITY OF  
BIRMINGHAM

**University of Birmingham Research Archive**

**e-theses repository**

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

# ABSTRACT

The regulation of pulmonary blood flow and ventilation during exercise is mediated by numerous factors, yet the role of certain cardiovascular and pulmonary reflex control systems is unknown. Therefore this thesis investigated the pulmonary vascular response to the activation of the muscle metaboreflex alone, and combined with activation of the muscle mechanoreflex. The ventilatory responses to the activation of the muscle metaboreflex were also studied under a background of activated ventilatory chemoreceptors. Finally the effect of increased metabolism on the ventilatory sensitivity to carbon dioxide was investigated in healthy humans. We found that activation of the muscle metaboreflex induced pulmonary vasoconstriction that was alleviated by muscle mechanoreflex activation. Furthermore a respiratory response to the activation of the muscle metaboreflex was observed in hypercapnia but not when breathing room air. Finally we found that increases in metabolic rate induced elevations in the ventilatory sensitivity to carbon dioxide. Overall these data suggest that the interplay between cardiovascular and respiratory control systems contribute to the regulation of pulmonary haemodynamics and breathing during exercise. Our findings could be implicated in the reduced exercise tolerance seen in chronic heart failure patients.

This thesis is dedicated to my parents  
and to my future wife

# ACKNOWLEDGEMENTS

I would like to thank several people for their contribution in this thesis. Firstly my greatest gratitude belongs to my parents who have supported me by all means throughout my life. My gratitude also goes to my fiancé and future wife, Stella, who has stood by me despite all the difficult times and the distance separating us. I would also like to thank my supervisor Dr George Balanos for his guidance, support and endless patience, especially in times that things were not going under the plan. I am also indebted to my co-supervisor Dr Mike White who has been a constant source of expert advice that has been invaluable throughout my PhD years. Many thanks also belong to Dr Prem Kumar for his input in the submitted manuscripts and poster presentations as well as for his help in surviving the meetings in Oxford and Spain. I would also like to thank my friends those who are in life and those who are not for their support. Special thanks also go to my PhD mentor Dr Rachel Drew, ex-housemate and colleague Dr Laurinho Vianna, Jacqueline Burke, Dr Dave McIntyre and the technical team of the Sportex. Finally I would like to express my gratitude to all these who have contributed in the completion of the thesis and are not included here. They know who they are!

# PUBLICATIONS

## Full papers:

- Lykidis CK, White MJ, Balanos GM (2008). The pulmonary vascular response to the sustained activation of the muscle metaboreflex in man. *Exp Physiol* 93:247-53.
- Lykidis CK, Kumar P, Vaianna, LC, White, MJ, Balanos GM (2010). A respiratory response to the activation of the muscle metaboreflex during concurrent hypercapnia in man. *Exp Physiol*, 95, 194-201.
- Lykidis CK, Kumar P, Balanos GM.(2009). The respiratory responses to the combined activation of the muscle metaboreflex and the ventilatory chemoreflex. *Adv Exp Med Biol* 648:281-287.

## Abstracts

- Lykidis CK, White MJ and Balanos GM (2007). Evidence for increased pulmonary vascular resistance during muscle metaboreflex activation *FASEB J* 21:979.12
- Lykidis CK, Kumar P ,Balanos GM (2008). The role of peripheral and central chemoreceptors in the hypermetabolism-induced increases in the ventilatory chemosensitivity to CO<sub>2</sub> in humans. *Proc Physiol Soc* 12 PC3

# ABBREVIATIONS

Ach = acetylcholine

ADP = adenosine diphosphate

ANS = autonomic nervous system

ANOVA = analysis of variance

ATP = adenosine triphosphate

ATPS = ambient temperature and pressure

CWD = continuous wave Doppler

CHF = chronic heart failure

CPT = cold pressor test

DAP = diastolic arterial pressure

DEGF = dynamic end-tidal gas forcing

ECG = electrocardiography

H<sup>+</sup> = hydrogen ion

HAPO = high altitude pulmonary oedema

HCO<sub>3</sub><sup>-</sup> = bicarbonate ion

HR = heart rate

IHG = isometric handgrip

K<sup>+</sup> = potassium ion

Kcal = calorie

LAP = left atrial pressure

MAP = mean atrial pressure

MR = metabolic rate

MVC = maximum voluntary contraction

NTS = nucleus tractus solitarii

PAP = mean pulmonary artery pressure

P<sub>A</sub>O<sub>2</sub> = partial pressure of arterial oxygen

P<sub>A</sub>CO<sub>2</sub> = partial pressure of arterial carbon dioxide

PP = pulse pressure

PECO = post-exercise circulatory occlusion

$P_{ET}CO_2$  = partial pressure of end-tidal carbon dioxide

$P_{ET}O_2$  = partial pressure of end-tidal oxygen

PVP = mean pulmonary venous pressure

PVR = pulmonary vascular resistance

PWD = pulsed wave Doppler

$\dot{Q}$  = cardiac output

RAP = right atrial pressure

RQ = respiratory quotient

SAP = systolic arterial pressure

SBRS = spontaneous baroreflex sensitivity

SEM = standard error of mean

SNS = sympathetic nervous system

SPAP = systolic pulmonary artery pressure

STP = short-term potentiation

STPD = standard temperature and pressure

SV = stroke volume

$\dot{V}_A$  = alveolar ventilation

$V_E$  = pulmonary ventilation

VHS = video home system

$VO_2$  = oxygen consumption

$VCO_2$  = carbon dioxide production

VTI = velocity-time integral

$\Delta P_{MAX}$  = maximum transtricuspid pressure difference

# CONTENTS

<b>CHAPTER 1: INTRODUCTION</b>	<b>1</b>
<b>RESPIRATORY PHYSIOLOGY AND REGULATION OF PULMONARY BLOOD FLOW AT REST AND EXERCISE</b>	<b>3</b>
<b>1.1: BASIC RESPIRATORY PHYSIOLOGY AND ANATOMY</b>	<b>3</b>
1.1.1: Airways	3
1.1.2: Diffusion	5
<b>1.2: THE PULMONARY CIRCULATION</b>	<b>6</b>
1.2.1: Anatomy and function	6
1.2.2: Blood pressure and resistance to blood flow in the pulmonary circulation	7
1.2.3: Blood flow distribution	8
1.2.4: Control of the pulmonary vasculature; tone and resistance	11
<b>1.3: PULMONARY CIRCULATION AND HAEMODYNAMICS DURING EXERCISE</b>	<b>18</b>
1.3.1: Basic overview	18
1.3.2: Pulmonary haemodynamics	19
1.3.3: Pulmonary haemodynamics during exercise in cardiopulmonary disease	22

<b>REGULATION OF VENTILATION AT REST AND EXERCISE</b>	<b>23</b>
<b>1.4: RESPIRATORY CONTROLLERS: CHARACTERISTICS AND LOCALISATION</b>	<b>23</b>
<b>1.5: RESPIRATORY EFFECTOR MUSCLES</b>	<b>26</b>
<b>1.6: RESPIRATORY RECEPTORS</b>	<b>27</b>
1.6.1: Upper airway receptors	28
1.6.2: Lung, tracheal and bronchial receptors	28
1.6.3: Chemoreceptors	29
1.6.3.1: Central chemoreceptors	29
1.6.3.2: Peripheral chemoreceptors	30
1.6.3.3: Other receptors	31
<b>1.7: THE CAROTID BODY</b>	<b>32</b>
1.7.1: Functions, basic histology and anatomy	32
1.7.2: Main reflexes mediated by the carotid body; hypoxia and hypercapnia	33
<b>1.8: REGULATION OF VENTILATION DURING EXERCISE</b>	<b>34</b>
<b>1.9: MECHANISMS CONTROLLING BREATHING DURING EXERCISE</b>	<b>38</b>
1.9.1: Central command	39

1.9.2: Sensory feedback mechanisms	41
1.9.2.1: Respiratory feedback	41
1.9.2.2: Non-respiratory feedback	44
<b>1.10: REGULATION OF VENTILATION DURING EXERCISE IN CHF</b>	<b>46</b>
<b>MUSCLE AFFERENTS</b>	<b>47</b>
<b>1.11: BASIC ANATOMY AND PHYSIOLOGY</b>	<b>47</b>
<b>1.12: MECHANORECEPTORS</b>	<b>48</b>
<b>1.13: METABORECEPTORS</b>	<b>50</b>
<b>1.14: MUSCLE REFLEXES IN DISEASE</b>	<b>51</b>
<b>AIMS OF THIS PHD; PROPOSED STUDIES</b>	<b>53</b>
<b>CHAPTER 2: METHODS</b>	<b>55</b>
<b>2.1: END-TIDAL GAS FORCING</b>	<b>56</b>
<b>2.2: ECHOCARDIOGRAPHY</b>	<b>64</b>

**CHAPTER 3: THE PULMONARY VASCULAR RESPONSE  
TO THE SUSTAINED ACTIVATION OF THE MUSCLE  
METABOREFLEX IN MAN 77**

3.1: ABSTRACT	78
3.2: INTRODUCTION	79
3.3: METHODS	81
3.4: RESULTS	85
3.5: DISCUSSION	92

**CHAPTER 4: EFFECTS OF INCREASED  
METABOLIC RATE ON THE VENTILATORY  
SENSITIVITY TO INHALED CO<sub>2</sub> 97**

4.1: ABSTRACT	98
4.2: INTRODUCTION	99
4.3: METHODS	101
4.4: RESULTS	108
4.5: DISCUSSION	112

**CHAPTER 5: A RESPIRATORY RESPONSE TO THE ACTIVATION  
OF THE MUSCLE METABOREFLEX  
DURING CONCURRENT HYPERCAPNIA IN MAN 117**

5.1: ABSTRACT	118
5.2: INTRODUCTION	119
5.3: METHODS	121
5.4: RESULTS	126
5.5: DISCUSSION	133

<b>CHAPTER 6: THE PULMONARY VASCULAR RESPONSE TO THE COMBINED ACTIVATION OF THE MUSCLE METABOREFLEX AND MECHANOREFLEX</b>	<b>138</b>
6.1: ABSTRACT	139
6.2: INTRODUCTION	140
6.3: METHODS	141
6.4: RESULTS	146
6.5: DISCUSSION	151
<b>CHAPTER 7: GENERAL CONCLUSIONS</b>	<b>155</b>
<b>REFERENCES</b>	<b>160</b>

# LIST OF FIGURES

## CHAPTER 1: INTRODUCTION

Figure 1.1: Schematic representation of the structure of the bronchial tree.

4

Figure 1.2: The three zones of the lung incorporating the Starling resistor model for each zone inside the outline of the lung up the lung versus blood flow.

9

Figure 1.3: Graph showing how blood flow is reduced as distance from the bottom of the lung increases.

10

Figure 1.4: Mean driving pressure and pulmonary vascular resistance variations with cardiac output during supine and upright exercise.

21

Figure 1.5: Central respiratory controllers.

24

Figure 1.6: Types of respiratory receptors

31

Figure 1.7: The characteristic three phases of the ventilatory response to dynamic exercise.

35

Figure 1.8: Neural control of circulation during exercise.

47

## CHAPTER 2: METHODS

Figure 2.1: Schematic representation of the mouthpiece apparatus used for EDGF.

60

Figure 2.2: Experimental set up of the laboratory for end-tidal forcing studies.

63

Figure 2.3: Graphic representation of the scan lines emitted by a transducer used for cardiac investigations.

66

Figure 2.4: The two-colour spectrum used in Doppler ultrasound to indicate the velocity and direction of a flow.

68

Figure 2.5: A three-beat velocity profile of a tricuspid regurgitation jet measured in CWD.

71

Figure 2.6: apical 4-chamber, 5-chamber and parasternal long-axis view of the heart.

72

Figure 2.7: Tricuspid regurgitation visualised in colour Doppler mode from an apical 4-chamber view.

73

Figure 2.8: The flow of blood exiting the heart through the aortic valve during systole, visualised in colour Doppler mode from an apical 5-chamber view

74

### **CHAPTER 3: THE PULMONARY VASCULAR RESPONSE TO THE SUSTAINED ACTIVATION OF THE MUSCLE METABOREFLEX IN MAN**

Figure 3.1. Schematic representation of the experimental protocol and sequence of echocardiographic measurements of SPAP and cardiac output.

85

Figure 3.2: Percent changes in SPAP and SAP from rest during the isometric handgrip exercise and control protocols.

87

Figure 3.3: Changes in cardiac output, heart rate and stroke volume during the isometric handgrip exercise and control trials.

88

Figure 3.4: Changes in systemic arterial stiffness during the isometric handgrip exercise and control trials.

91

### **CHAPTER 4: EFFECTS OF INCREASED METABOLIC RATE ON THE VENTILATORY SENSITIVITY TO INHALED CO<sub>2</sub>**

Figure 4.1: Pattern of P<sub>ET</sub>CO<sub>2</sub> increases in the hypercapnic challenge.

104

Figure 4.1: A) Example traces from one subject, showing the increase in P<sub>ET</sub>CO<sub>2</sub> and ventilation B) Relationship between changes in ventilation and end-tidal CO<sub>2</sub>. Changes in ventilation are plotted as a function of changes in end-tidal CO<sub>2</sub>.

106-107

Figure 4.3: A) individual values for oxygen consumption during the control and steak trials. B) mean oxygen consumption obtained during the control and steak trials.

110

Figure 4.4: A) individual values for ventilatory sensitivity during the control and steak trials. B) mean values for ventilatory chemosensitivity obtained during the control and steak trials.

111

## **CHAPTER 5: A RESPIRATORY RESPONSE TO THE ACTIVATION OF THE MUSCLE METABOREFLEX DURING CONCURRENT HYPERCAPNIA IN MAN**

Figure 5.1: Schematic representation of the protocol.

123

Figure 5.2: Change in minute ventilation from steady state values during each phase of the hypercapnic and the air breathing trials.

129

Figure 5.3: Change in mean arterial pressure from steady state values during each phase of the hypercapnic and the air breathing trials.

130

Figure 5.4: Change in heart rate from steady state values during each phase of the hypercapnic and the air breathing trials.

131

Figure 5.5: Mean slope values of regression lines calculated from sequence analysis during the ERGO and CHEMO+ERGO trials.

132

Figure 5.6: Mean intercept values of regression calculated from sequence analysis during the ERGO and CHEMO+ERGO trials.

133

## **CHAPTER 6: THE PULMONARY VASCULAR RESPONSE TO THE COMBINED ACTIVATION OF THE MUSCLE METABOREFLEX AND MECHANOREFLEX**

Figure 6.1: Schematic representation of the experimental protocol.

141

Figure 6.2: Mean percentage changes in SPAP from baseline during the isometric exercise and control protocols.

148

Figure 6.3: Mean percentage changes from baseline in cardiac output, heart rate and stroke volume during the isometric exercise and control trials.

149

Figure 6.4: Mean percentage changes from baseline in SAP, DAP, heart rate and MAP during the isometric exercise and control trials.

150

# LIST OF TABLES

## CHAPTER 1: INTRODUCTION

Table 1.1: Humoral receptors in pulmonary vessels. 15

Table 1.2: Autonomic receptors in pulmonary vessels. 17

## CHAPTER 3: THE PULMONARY VASCULAR RESPONSE TO THE SUSTAINED ACTIVATION OF THE MUSCLE METABOREFLEX IN MAN

Table 3.1: Baseline values of the variables studied at the two protocols. 86

Table 3.2: Measurements obtained during the isometric handgrip exercise trial. 90

## CHAPTER 4: EFFECTS OF INCREASED METABOLIC RATE ON THE VENTILATORY SENSITIVITY TO INHALED CO<sub>2</sub>

Table 4.1: Respiratory data obtained in the control and steak trials. 109

## **CHAPTER 5: A RESPIRATORY RESPONSE TO THE ACTIVATION OF THE MUSCLE METABOREFLEX DURING CONCURRENT HYPERCAPNIA IN MAN**

Table 5.1: Mean values for measurements taken before any intervention and after eight minutes of rest under conditions of either room air breathing or euoxic hypercapnia.

127

## **CHAPTER 6: THE PULMONARY VASCULAR RESPONSE TO THE COMBINED ACTIVATION OF THE MUSCLE METABOREFLEX AND MECHANOREFLEX**

Table 6.1: Baseline mean values of the variables studied at the two protocols.

146

# **CHAPTER 1: INTRODUCTION**

In the healthy state, performance of exercise induces a variety of physiological adjustments, which aim to satisfy the increased need for oxygen and metabolic substrates by the musculature, and to maintain an optimal environment for the functioning of other systems in the human body. Broadly these adjustments include increases in ventilation, cardiac output, pulmonary and active muscle blood flow, and reductions in blood flow towards vascular beds that are not actively involved in exercise. Central command and reflex control systems represent the two main pathways, which are involved in the mediation of these adjustments. Of paramount importance are the redundancy and complex relationships that exist between the aforementioned mediators of the exercise responses.

The pulmonary system plays a fundamental part in the adjustments taking place during exercise as it facilitates oxygenation of mixed venous blood and its provision to the left heart while ensuring the maintenance of an optimal acid-base status. However, despite vast amount of research, there are still areas concerning the regulation of pulmonary blood flow and ventilation during exercise that are not clear. Existing gaps in knowledge have presented a burden in the understanding of the reduced exercise capacity of patients with chronic heart disease, which is increasingly prevalent in modern societies. Fortunately, recent technological advances have allowed for an extended scope of measurements to be performed, thereby improving knowledge on physiological functioning.

The first part of this introduction will firstly provide a brief account of the basic aspects of respiratory physiology and anatomy. Particular attention will be given to the regulation of pulmonary blood flow at rest and during exercise in

the healthy and heart failure states. In the second part, the mechanisms mediating the regulation of ventilation at rest and during exercise in the healthy and heart failure states will be outlined. Special mention will be paid to the carotid body as a respiratory controller. The third part of the introduction will briefly portray the role of the muscle reflexes in the cardiovascular control during exercise and in parallel conclude the background needed for the experimental chapters, which will be outlined in the fourth part of this introduction.

## Part 1. RESPIRATORY PHYSIOLOGY AND REGULATION OF PULMONARY BLOOD FLOW AT REST AND EXERCISE

### 1.1: BASIC RESPIRATORY PHYSIOLOGY AND ANATOMY

The lungs constitute an interface between the environment and the cardiovascular system. Their prime function is to allow oxygen from the inspired air to move into venous blood, and expel the carbon dioxide from mixed venous blood into the atmosphere. Given the continuous need of tissues in the human body for oxygen, right ventricular output passes through the lungs during each circulation, ensuring constant re-oxygenation of the venous blood. Importantly, the 'branching tree' design of the main functional components of the lungs, airways and blood vessels, allows fast and efficient gas exchange to occur between blood and air both at rest and during exercise, when systemic oxygen consumption may be very high.

#### *1.1.1: Airways*

Atmospheric air enters from the nose and mouth and flows into the conductive portion of the respiratory system as negative pressure is generated by the expansion of the chest and the contraction of the diaphragm. At this stage inspired air is adjusted to body temperature, filtered and humidified as it passes through the trachea, which represents the first part of the airway 'branching tree' organisation. The inspired air passes into the two branches of the trachea, namely the right and left bronchi, which then split into five branches, known as the lobar bronchi. This dividing process continues down to the terminal bronchioles, which complete the conductive part of the

respiratory system. The collective volume of the conductive part (approximately 150 mL in adults) is known as the anatomical dead space, since no gas exchange takes place in it. Subsequently, the respiratory zone, in which gas exchange occurs, begins with the respiratory bronchioli which branch from the terminal bronchioli. Respiratory bronchioli have occasional alveoli attached on their walls, and give rise to alveolar ducts, which are completely lined up with alveoli (West, 1995).

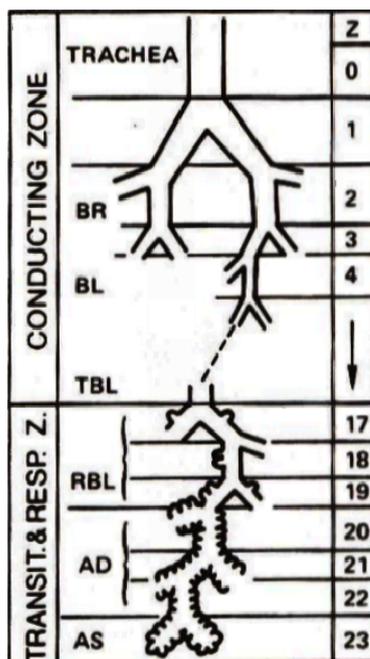


Figure 1.1: Schematic representation of the structure of the bronchial tree. (Adapted from West, 1995).

### 1.1.2: Diffusion

The movement of oxygen and carbon dioxide across the blood/gas barrier constitutes one fundamental process of breathing, known as diffusion. Diffusion refers to the passive movement of molecules across a membrane and along a concentration gradient and is described by Fick's law. Therefore, according to Fick, the rate of diffusion is proportional to the area of the membrane, the gas partial pressure difference between the two sides of the membrane, and a diffusion constant that is directly proportional to the solubility of a gas in the membrane, but inversely proportional to the square root of its molecular weight. The rate of diffusion is inversely proportional to the thickness of the membrane. Fick's law of diffusion is written as:

$$\dot{V}_{\text{gas}} = D \cdot \frac{A}{T} \cdot (P_1 - P_2)$$

where  $V_{\text{gas}}$  is the diffusion rate,  $D$  is the diffusion constant,  $A$  is the area over which a gas diffuses,  $T$  is the thickness of the barrier, and  $(P_1 - P_2)$  is the partial pressure difference of the gas across the barrier.

Diffusion is promoted by the enormous area that the blood/gas barrier covers and also by its thickness, which can be as low as 0.3  $\mu\text{m}$  in some places. The pressure gradient for oxygen and carbon dioxide across the blood/gas barrier (i.e. between alveolar gas and pulmonary arterial blood) is about 60 mmHg (100 alveolar – 40 venous) and 5 mmHg (45 venous – 40 alveolar) respectively. The low-pressure gradient for carbon dioxide is immensely counterbalanced by its superior solubility to oxygen facilitating a diffusion rate 20 times faster compared to that of oxygen.

Diffusion rates for either gas are more than adequate in facilitating rapid gas exchange. Therefore, the alveolar and arterial gas partial pressures between the alveolus and the capillary equilibrate within about 0.25 seconds while a red blood cell takes approximately 0.75 seconds to move through the capillary. This is quite important during exercise when blood flow through the lungs increases dramatically (up to 20-fold) and the red blood cells have substantially less time crossing the alveoli in order for O<sub>2</sub> and CO<sub>2</sub> partial pressures to reach equilibrium (West, 1995).

## **1.2: THE PULMONARY CIRCULATION**

### *1.2.1: Anatomy and function*

The function of the pulmonary circulation is to deliver poorly oxygenated blood at the gas exchange surface and to deliver it to the left heart in order to be distributed throughout the body. Therefore, it commences at the main pulmonary artery, which receives deoxygenated blood from the right heart, and then successively branches into smaller arteries accompanying the pattern of the bronchial tree. This branching pattern ends at the level of the terminal bronchioles where the arteries break up and form an exceptionally dense network of capillaries that lies in the alveolar walls. After the blood becomes oxygenated it is collected by the small pulmonary veins that run into the capillary bed. Small pulmonary veins unite to form the four large veins from which blood drains into the left atrium.

The contraction of the right ventricle drives an amount of blood into the pulmonary circulation (right ventricular output) that is almost equal to that entering the systemic circulation due to left ventricular contraction (left ventricular output). The aorta distributes blood throughout a number of smaller circulations (i.e. renal, intestinal, brain, kidneys and muscles), which can self-regulate blood flow according to the metabolic demands of the organ they perfuse. The pulmonary circulation in turn, which is continuously receiving the entire right ventricular output, presents a higher degree of distensibility than that of the systemic circulation due to the fact that pulmonary arteries have thinner walls and less smooth muscle relative to the systemic ones (Nunn, 2000; West, 1995).

#### *1.2.2: Blood pressure and resistance to blood flow in the pulmonary circulation*

Owing to its high distensibility, the large cumulative cross-sectional area and diffusion barrier, the pulmonary circulation operates under lower pressures as compared with the systemic circulation. Therefore the mean pulmonary artery pressure is about 15 mmHg (25/8 mmHg; systolic vs diastolic) and much lower than its systemic counterpart which is about 100 mmHg (120/80 mmHg; systolic vs diastolic). In addition, the driving pressure (difference between input and output) in the pulmonary circulation is ten times lower compared to that in the systemic circulation.

Pulmonary vascular resistance (PVR) refers to the resistance to flow across lungs and is defined as the ratio between driving pressure across the lungs and flow.

$$PVR = \frac{PAP - PVP}{\dot{Q}}$$

where PAP is mean pulmonary artery pressure, PVP is mean pulmonary venous pressure, and  $\dot{Q}$  is volume (cardiac output). The pulmonary circulation can accommodate large increases in blood flow (i.e. cardiac output) without excessive increases in pulmonary artery pressure, implying that pulmonary vascular resistance decreases with elevations in cardiac output. This property of the pulmonary circulation is attributed to the high distensibility of the vasculature and to the fact that high blood flow force open arteries from previously under-perfused areas of the lungs. The latter functional characteristic is known as recruitment (Naeije, 2000; West, 1995).

### *1.2.3: Blood flow distribution*

Owing to the low resistance in the pulmonary circulation, the distribution of blood in the lungs is greatly affected by gravity. Therefore the lower parts of the lungs are greatly perfused yet blood flow is diminished as the distance from the base of the lung increases. West *et al* (1964) proposed that the lung is divided in three vertical zones based upon the pressure in the alveoli, the arteries and the veins (figure 1.1).

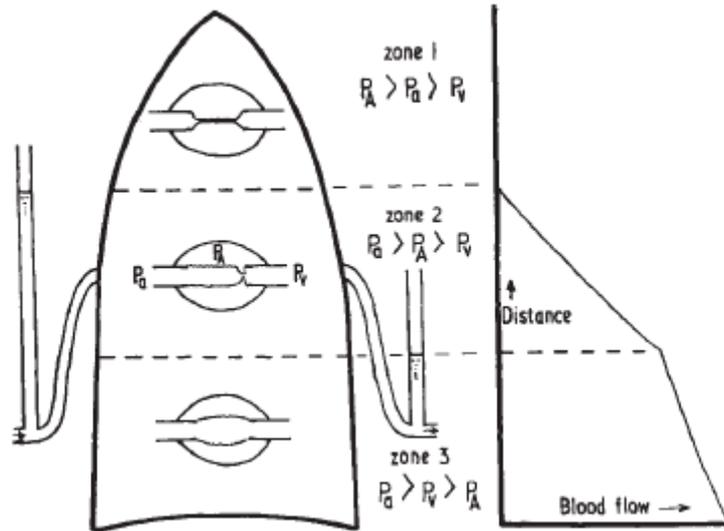


Figure 1.2: *Left*: The three zones of the lung incorporating the Starling resistor model for each zone inside the outline of the lung. *Middle*: relationship of pulmonary artery pressure ( $P_A$ ), pulmonary vein pressure ( $P_V$ ), and alveolar pressure ( $P_a$ ) for each of the three zones. *Right*: graph of distance up the lung versus blood flow (Adapted from West *et al.*, 1964).

Zone 1 resides at the top of the lung (apex) and receives little blood flow. In this zone, alveolar pressure ( $P_A$ ) exceeds both arterial pressure ( $P_a$ ) and venous pressure ( $P_V$ ). As such, the higher pressure in the alveoli collapses the vessels enabling little or no flow through. In Zone 2, which is at the middle section of the lung, alveolar pressure is lower than arterial pressure and higher than venous pressure. Blood flow in zone 2 is mediated by the alveolar-arterial pressure difference as venous pressure is less than alveolar pressure. The greater the arterial pressure, the wider the vessel will open thereby facilitating an enhanced blood flow. Recruitment of previously collapsed vessels also contributes to the increases in blood flow in this zone.

Zone 3 resides at the bottom part of the lung where arterial pressure is greater than venous pressure, and both are greater than alveolar pressure. Given this, the flow in zone 3 is regulated by the arterial-venous pressure difference. Increases in blood flow are mediated mainly by distension of the arteries (West, 1964; 1995). The relationship between distance from the base of the lung and blood flow is diagrammatically shown in figure 1.2. Notably, flow at the very bottom of the lung is reduced compared to the area that resides five centimetres higher. The decreased blood flow through this area, which is sometimes called Zone 4, is thought to be due to the compression of extra-alveolar vessels by hydrostatic pressure (West, 1995).

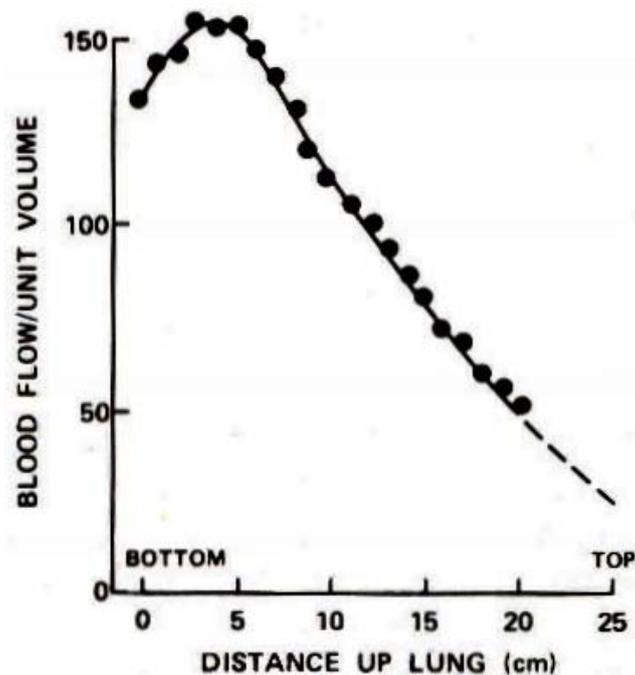


Figure 1.3: Graph showing how blood flow is reduced as distance from the bottom of the lung increases. Also shown is the reduction of blood flow at the very bottom of the lung (zone 4). (Adapted from West, 1995).

#### *1.2.4: Control of the pulmonary vasculature; tone and resistance*

The pulmonary circulation is a high-flow and low-resistance circuit that accommodates the right ventricular output at about 20% of the systemic pressure. The fundamental difference between the pulmonary and the systemic circulations is that the latter is regulated primarily by neural and humoral (active) mechanisms whereas the in the former passive mechanisms predominate (Barnes & Liu 1995).

##### *Passive mechanisms*

As it has been mentioned previously, cardiac output exerts a great influence on PVR and it can be summarised as follows. Elevations in cardiac output lead to increases in pulmonary vascular pressures and in turn in the radius of the pulmonary vessels, given their high distensibility. High blood pressures in the pulmonary circuit will also open and perfuse previously collapsed vessels. Consequently pulmonary vascular pressure will decrease. The opposite effects are seen upon decreases in cardiac output (Dawson *et al.*, 1989; Grover *et al.*, 1985; Hakim *et al.*, 1989; Naeije, 2000). Moreover, fluctuations in left atrial pressure will be transmitted to pulmonary artery pressure thereby influencing resistance. Alternatively, left atrial pressure is a determinant of the downstream pressure in the pulmonary circuit (i.e. PVP), which in turn is a determinant of PVR by definition (Kuramoto & Rodbard, 1962). Systemic arterial pressure can also influence pulmonary haemodynamics via its effects on pulmonary blood flow, left ventricular emptying and left atrial pressure (Reeves *et al.*, 1996)

As it was mentioned previously resistance in the pulmonary circulation is influenced by gravity both in a direct and an indirect fashion since not only it alters the distribution of blood in lungs but can also affect cardiac output (Dawson *et al.*, 1989; Grover *et al.*, 1985; Naeije, 2000). Lung volume represents another passive factor that affects PVR. Therefore at functional residual capacity (i.e. at the end of a passive expiration), resistance is minimal yet above it resistance dramatically increases due to narrowing of alveolar capillaries caused by expansion of alveoli (Culver & Butler, 1980).

#### *Active mechanisms*

Factors that actively regulate the tone and thus resistance in the pulmonary circuit can be classified into three main categories; chemical, humoral and neural.

#### *Chemical control*

It is well known that levels of oxygen and carbon dioxide in the alveoli and in mixed venous blood exert a great influence on pulmonary vascular tone. Therefore vasoconstriction in veins and arteries has been well documented in response to decreased levels of oxygen (hypoxia) in various animal preparations (Brashers *et al.*, 1988; Hillier *et al.*, 1997; Madden *et al.*, 1985) as well as in intact animals (Barer *et al.*, 1970) and humans (Dorrington *et al.*, 1997). In fact, hypoxic pulmonary vasoconstriction represents a physiological response of small arteries (200-600  $\mu\text{m}$  internal diameter) that diverts mixed

venous blood away from hypoxic alveoli. This is known to optimise the matching between ventilation and perfusion thereby preventing arterial hypoxemia (Olschewski & Weir, 2000; Shirai *et al.*, 1986). Hypoxic pulmonary vasoconstriction is known to be intrinsic to the lung and has been suggested to be mediated by an elevation of  $\text{Ca}^{2+}$  in arterial smooth muscle that results in the opening of voltage-gated  $\text{Ca}^{2+}$  channels and myocyte contraction (Aaronson *et al.*, 2006; Olschewski & Weir, 2000). Despite intensive research, the signalling pathways that lead to this cascade of events have yet to be precisely clarified. A number of authors have suggested that  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum occurs secondary to hypoxia-induced increases in cyclic ADP-ribose (Wilson *et al.*, 2001). Others have proposed that hypoxia induces changes in levels of  $\text{H}_2\text{O}_2$  and other reactive oxygen species that lead to an inhibition of voltage-gated  $\text{K}^+$  channels and cell depolarisation (Moudgil *et al.*, 2005). Increased levels of oxygen or hyperoxia are known to produce vasodilation and its degree could be dependent on the pre-existing tone (Fishman, 1990; Fineman *et al.*, 1993).

Blood acidity as well as levels of carbon dioxide and in both inspirate and blood also mediate changes in vascular tone, with hypercapnia (increased levels) and hypocapnia (decreased levels) inducing vasoconstriction and vasodilation, respectively (Kiely *et al.*, 1996). It is generally accepted that hypercapnia and hypocapnia exert their effects via the concomitant changes to pH. Interestingly it is known that both hyperoxia and hypocapnia attenuate the vascular responses to vasoconstrictor stimuli, which are enhanced by both hypoxia and hypercapnia (Archer *et al.*, 1989; Archer & Michelakis, 2002; Barnes & Liu, 1996; Dawson, 1984; Olschewski & Weir, 2000).

### *Humoral control*

Several mediators and hormones can have an effect on pulmonary vascular tone that is mediated via multiple receptors (Table 1.1). Substances that affect tone in the pulmonary vasculature include catecholamines, eicosanoids, amines, peptides, and purine nucleosides, and their effects can vary depending on the species tested, age and the pre-existing tone. Despite intensive research, the physiological role of humoral control has not been clearly elucidated (Barnes & Liu, 1996; Bergofsky, 1980; Dawson, 1984). It has been suggested that maintenance of low pulmonary tone is a result of a balance between vasodilators and vasoconstrictors and that hormones could play a part in this phenomenon (Barnes & Liu, 1996). Coupled to that, it has been postulated that the influence of hormones on pulmonary vascular tone can be greater in course of physiological adaptations such as pregnancy, exercise and cold exposure (Moore, 1989). On more firm grounds is the hormonal involvement in the pulmonary vascular abnormalities seen in various diseases. Therefore cyclo-oxygenase and lipoxygenase products have been known to mediate the pulmonary hypertension seen in adult respiratory distress syndrome (Rounds, 1989). In addition catecholamines have been suggested to play a role in pulmonary hypertension associated to chronic lung disease (Andreas *et al.*, 2005; Salvi, 1999).

Table 1.1: Humoral receptors in pulmonary vessels. (Adapted from Barnes & Liu, 1996).

<b>Receptors</b>	<b>Subtype</b>	<b>Response</b>	<b>Endothelium dependency</b>
Adenosine	A <sub>1</sub>	Contraction	No
	A <sub>2</sub>	Relaxation	No
Angiotensin	AT	Contraction	No
	ANP <sub>A</sub>	Relaxation	No
ANP	ANP <sub>B</sub>	Relaxation	No
	Bradykinin	B <sub>1</sub>	Relaxation
Bradykinin	B <sub>2</sub>	Relaxation	Yes
	Endothelin	ET <sub>A</sub>	Contraction
ET <sub>B</sub>		Relaxation	Yes
Histamine	H <sub>1</sub>	Relaxation	Yes
	H <sub>2</sub>	Relaxation	No
5-HT	5-HT <sub>1</sub>	Contraction	No
	5-HT <sub>1C</sub>	Relaxation	Yes
Thromboxane	TP	Contraction	No
Vasopressin	V <sub>1</sub>	Relaxation	Yes

*Neural control: Basic functional anatomy*

Similarly to that for the hormonal control, the exact physiological role of neural mechanisms on pulmonary vascular tone and resistance has not been adequately defined. The pulmonary circulation receives both sympathetic and parasympathetic afferent and efferent nerve supply that is sparse relative to the systemic vasculature. The density of innervation is greater in the larger elastic arteries, less in the veins and muscular arteries, whereas vessels narrower than 30  $\mu\text{m}$  does not receive nerve supply (Downing & Lee, 1980; Richardson, 1979; Spencer & Leof, 1964). Despite the sparse innervation pulmonary vascular tone is regulated by multiple autonomic receptors (Table 1.2).

### *Sympathetic influence*

Stimulation of  $\beta_2$  adrenergic receptors has been unequivocally shown to mediate vasodilation (Hyman *et al.*, 1981; Murray *et al.*, 1986). On the contrary variable (up to 70%) increases in PVR have been seen in response to stellate ganglia (Daly, 1961; Daly *et al.*, 1970; Duke *et al.*, 1960; Ingram *et al.*, 1970; Kadowitz *et al.*, 1974; Kadowitz & Hyman, 1973; Szidon & Fishman, 1971) or  $\alpha$ -sympathetic stimulation in animal preparations (Barman, 1995; Murray *et al.*, 1986; Piene, 1976). Whereas the degree of vascular responsiveness has been suggested to depend on the strength of sympathetic stimulation (i.e. stimulation frequency), a number of studies have reported increased arterial stiffness (Ingram *et al.*, 1968; Szidon & Fishman, 1971) and/or increased pulmonary vascular impedance (Piene, 1976; Reuben *et al.*, 1971) in response to low-level sympathetic stimulation. Impedance, which can be defined as the ratio of the amplitude of oscillatory pressure to that of oscillatory flow at a given frequency, refers to the opposition to pulsatile flow from the right ventricle into the pulmonary circuit (Dawson *et al.*, 1989; Piene 1986). Thus it incorporates all the factors that oppose ventricular ejection such as the resistive, compliant, inertial and the pulse wave reflection properties of the vascular bed (Piene 1986) and therefore it has been widely suggested as a determinant of right ventricular work (Dawson *et al.*, 1989; Grant & Lieber, 1996; Piene, 1986).

Table 1.2: Autonomic receptors in pulmonary vessels. (Modified from Barnes & Liu, 1996).

<b>Receptors</b>	<b>Subtype</b>	<b>Response</b>
Adrenergic	$\alpha_1$	Contraction
	$\alpha_2$	Contraction
	$\beta_2$	Relaxation
Muscarinic	$M_1$	Relaxation
	$M_3$	relaxation/contraction
Purinerbic	$P_{2X}$	Contraction
	$P_{2Y}$	Relaxation
Tachykinin	$NK_1$	Relaxation
	$NK_2$	Contraction
VIP	n/a	Relaxation
CGRP	n/a	Relaxation

### *Parasympathetic influence*

The cholinergic innervation of the pulmonary circulation is less intense as compared to that of the sympathetic nervous system. Likewise, the effects of parasympathetic nervous system on pulmonary vasculature have not been clearly identified partially due to the confounding role of the cardiovascular changes that accompany alterations in parasympathetic activity. Therefore, vagal withdrawal and activation are associated with cardiac output increases and decreases, respectively that can influence pulmonary vascular tone. In addition, studies on the effects of acetylcholine (ACh) on the pulmonary vascular circuit have produced mixed results, with vasodilation (Cherry & Gillis, 1987; Wilson *et al.*, 1995), vasoconstriction (Chand & Altura, 1980; El-Kashef *et al.*, 1991) and a dual response (Altiere *et al.*, 1986) being reported. A number of studies revealed that the initial vascular tone dictated the pulmonary vascular response to parasympathetic stimulation or inhibition. Therefore infusion of acetylcholine in either isolated vessels, lungs or in the

pulmonary circulation of the intact animal caused vasoconstriction when the initial tone was low and vasodilation when the pre-existing tone was high (Hyman *et al.*, 1988; Hyman & Kadowitz, 1989; Vanhoutte, 1974). Notably all vascular responses to Ach were blocked by the muscarinic inhibitor atropine. In humans, studies on the parasympathetic influence on pulmonary vasculature and haemodynamics have been limited. Acetylcholine failed to affect pulmonary artery pressure at rest (Harris, 1957), whereas the effects of atropine were variable with both unaffected and mildly decreased pulmonary vascular resistance being reported at rest and during exercise (Daly *et al.*, 1963; Williams *et al.*, 1960).

### **1.3: PULMONARY CIRCULATION AND HAEMODYNAMICS DURING EXERCISE**

#### *1.3.1: Basic overview*

During exercise, cardiac output and hence blood flow through the pulmonary circulation increases four-fold above resting levels, allowing for a greater uptake of oxygen and output of carbon dioxide. The increase in blood flow is facilitated through elevations of vascular driving pressure (pulmonary artery pressure minus pulmonary venous pressure) from the pulmonary artery to the left atrium and also due to the fact that pulmonary vascular resistance remains unchanged or decreases (Reeves *et al.*, 1996).

It is imperative that increases in driving pressure are not excessive as this will impose a great stress to the right ventricle and it will also induce severe impairments in gas exchange through fluid leakage into the alveoli. The latter

phenomenon can be observed during intense and/or unaccustomed exercise at high altitude (high altitude pulmonary oedema; HAPO) (Bärtsch *et al.*, 2002).

### *1.3.2: Pulmonary haemodynamics*

The changes seen in vascular driving pressure have been traditionally thought to be largely the result of the increases seen in left atrial pressure (LAP). Elevations in LAP that have been ascribed to the Frank-Starling mechanism, pericardial limitation of ventricular filling and tachycardia-related limitations of diastolic filling time, are transmitted backwards into the pulmonary circulation, thereby increasing pulmonary venous and artery pressures (Reeves *et al.*, 1990; 1996). In addition, it has been suggested that LAP increases account for up to 80% of the variation in the pulmonary arterial pressure (Eldridge *et al.*, 1996; Reeves *et al.*, 1996). However, increases in vascular driving pressure are not always accompanied by increases in LAP, which could remain unchanged or even decrease during exercise (Luepker *et al.*, 1971; Reeves *et al.*, 1996). As such, it has been postulated that pulmonary artery pressure increases during exercise might be also related to other factors such as the degree of vascular distension arising from elevations in pulmonary blood flow (Banchero *et al.*, 1966). Supporting the view that left heart haemodynamics might not be always dictating the pulmonary vascular response to exercise, Bernheim *et al* (2007) showed that the exaggerated increases in systolic pulmonary artery pressure during normoxic exercise seen in HAPO-susceptible individuals compared to controls were not related to Doppler-derived indices of left ventricular filling pressure. Interestingly,

higher levels of sympathetic activity were observed in HAPO-susceptible individuals, and consequently they have been suggested to contribute to the excessive pulmonary vascular pressure response to exercise resembling neurogenic pulmonary oedema (Bärtsch *et al.*, 2005).

Increases in PVR during exercise have been also observed in HAPO-susceptible individuals (Kawashima *et al.*, 1989), although decreases have been also reported (Eldridge *et al.*, 1996). The course of PVR decreases during exercise in normal humans is known to depend on the body posture assumed. Therefore PVR declines gradually as intensity increases during supine exercise whereas, in the upright exercising person, there is a dramatic reduction in PVR during the transition from rest to mild workloads (Figure 1.3). The pronounced decrease in the upright position has been ascribed to recruitment of previously unperfused vessels at zone 1 and 2 yet this suggestion has not been universally acceptable. Vessel distension has been suggested to play a more important role in PVR reductions seen during supine exercise (Reeves *et al.*, 1996). Recently, it has been postulated that active decreases in vascular tone could contribute to PVR response to exercise (Merkus *et al.*, 2008), yet evidence to support this suggestion is inconclusive. In fact, several animal studies have shown that the interaction between vasodilatory and vasoconstrictive influences taking place during exercise has a neutral effect on the pulmonary circulation (Kane *et al.*, 1993; 1994a;b; Koizumi *et al.*, 1996).

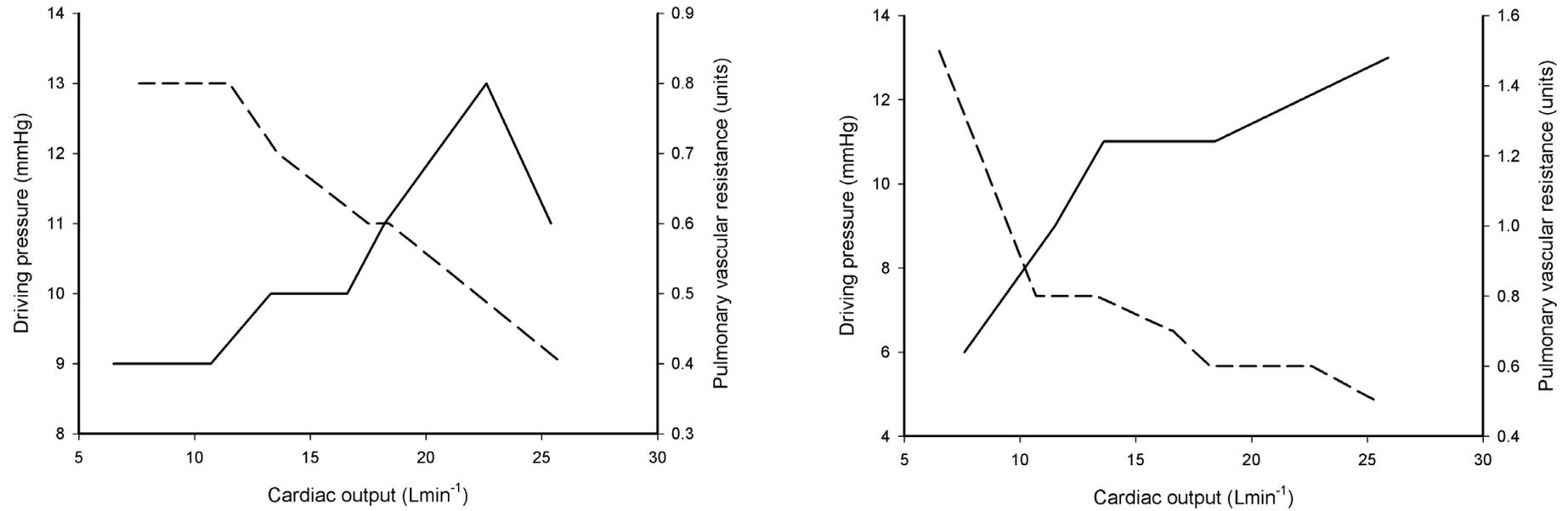


Figure 1.4: Mean driving pressure (solid lines) and pulmonary vascular resistance (dashed lines) variations with cardiac output during supine (left panel) and upright (right panel) exercise. (pooled data from Reeves *et al.*, 1996)

### 1.3.3: Pulmonary haemodynamics during exercise in cardiopulmonary disease

It has been established that pulmonary haemodynamic response to exercise in chronic heart failure (Butler *et al.*, 1999) is characterised by excessive increases in PAP and dampened decreases or even increases in PVR (Butler *et al.*, 1999; Christensen *et al.*, 2004; Janicki *et al.*, 1985). This in turn has been associated to the excessive ventilatory exercise response and limited exercise tolerance in these patients (Reindl *et al.*, 1998). It has been thought that the mechanisms underlying the abnormal haemodynamic responses to exercise are multiple and depend on the underlying pathology (Fink *et al.*, 1986). Increased stiffness of pulmonary vasculature is a common pathological feature in chronic lung and heart disease that could contribute to the increased pulmonary pressures seen during exercise (Berger *et al.*, 2002; Merkus *et al.*, 2008; Milnor *et al.*, 1969; Reuben, 1971; Scrijen *et al.*, 1982). Interestingly, the increased sympathetic activation represents a hallmark of these conditions (Andreas *et al.*, 2005; Cohn *et al.*, 1984) and could be involved in the abnormal pulmonary haemodynamics and inefficient ventilation during exercise through influencing the vasculature.

## Part 2. REGULATION OF VENTILATION AT REST AND EXERCISE

Breathing is a fundamental homeostatic behaviour that aims to oxygenate and remove carbon dioxide from, mixed venous blood while maintaining the arterial  $PO_2$ ,  $PCO_2$  and pH within a narrow range, at levels compatible with effective cell function (Bell, 2006; Ward, 2004). The precise match of respiration to the metabolic needs of the tissues involves integrative functioning of respiratory controllers, receptors and effectors.

### **1.4: RESPIRATORY CONTROLLERS: CHARACTERISTICS AND LOCALISATION**

Breathing is controlled by respiratory neurons located within the lower brainstem that are involved in the creation and preservation of respiratory rhythm, the processing and integration of sensory inputs and the relay of synaptic signals to the respiratory effectors. The respiratory neurones are synaptically-networked and are categorised according to their pattern and time of discharge in relation to phrenic nerve activity into the following neuron groups; pre-inspiratory, early-inspiratory, inspiratory, late-inspiratory, post-inspiratory and expiratory.

Respiratory neurons are also classified according to their location within the lower brainstem into neurons of the pontine, dorsal and ventral respiratory group. Pontine respiratory neurons are involved in the control of inspiratory duration and in modulation of breathing frequency. The dostral respiratory

group is located in the nucleus tractus solitarius, which has connections with pontine respiratory neurons and receives input from baro-, mechano- and metaboreceptors. Finally, the ventral respiratory group, which incorporates the pre-Bötzinger and Bötzinger complex, the para-facial respiratory group and the upper cervical respiratory neurons, is involved in both inspiration and expiration (Cohen, 1979; Mateike & Duffin, 1995; Von Euler, 1997) (Figure 1.5).

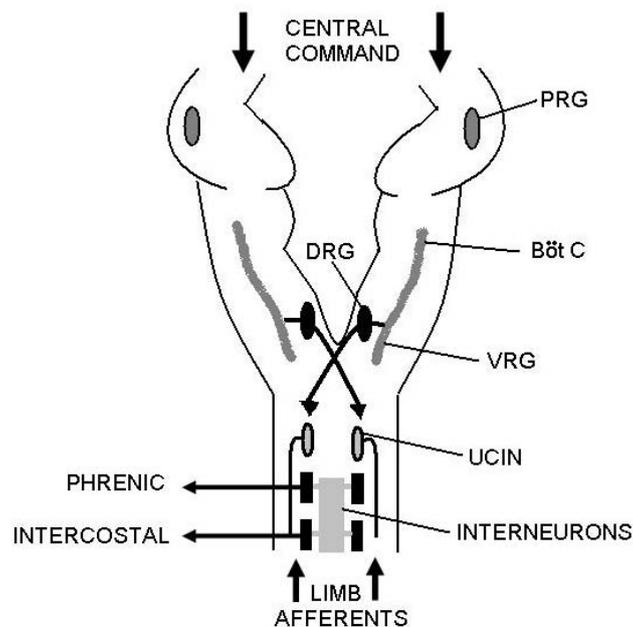


Figure 1.5: Central respiratory controllers. A schematic representation of the location of the main groups of respiratory neurons and associated structures in the feline medulla and spinal cord. PRG; pontine respiratory group, Böt C; Bötzinger complex, DRG; dorsal respiratory group, VRG ventral respiratory group, UCIN upper cervical inspiratory neurons. (Modified from Mateika & Duffin, 1995).

### *Generation of respiratory rhythm and respiratory neural plasticity*

In contrast to the great amount of knowledge that has been accumulated on the control of inspiration and expiration, the neural structures responsible for respiratory rhythmogenesis have been less clearly identified. Therefore a number of theories have been proposed. It has been suggested that the respiratory rhythm is composed of three phases (inspiratory, post-inspiratory and expiratory) that arise from an interaction between respiratory neurons (Onimaru *et al.*, 2000; Paton & Richter, 1995). Others argued for existence of a pacemaker originating from either the pre- Bötzing complex or the para-facial respiratory group (Butera *et al.*, 1999; Onimaru & Homma, 2003). It has been also postulated that respiratory rhythmogenesis could be the result of the combined action of the aforementioned mechanisms (Richter *et al.*, 1986; Ward, 2004).

It has been established that the respiratory response to various challenges such as hypoxia / hypercapnia, exercise and injury is also modulated based on prior experience. This phenomenon, which is known as respiratory plasticity, involves a persistent change in the morphology and function of the neural control system. Two different time domains of respiratory plasticity have been identified; short- and long-term plasticity. The former domain accounts for the elevated ventilation seen after the exercise stimulus is removed. The latter refers to the persistent augmentation of the exercise ventilatory response seen after repeated bouts of exercise are performed with increased external dead space (Mitchell & Babb, 2006; Mitchell & Johnson 2002).

## **1.5: RESPIRATORY EFFECTOR MUSCLES**

The term respiratory effectors encompasses various 'pump' and accessory muscle groups whose periodic contractions collectively results in intrathoracic pressure changes, exchange of air between the environment and the lungs and ultimately ventilation. The respiratory effectors contract in a coordinated manner under the directives of the respiratory controller motor outflow and produce two distinct ventilatory phases; inspiration and expiration.

### *Diaphragm muscle and abdominal wall muscles*

The diaphragm, which plays a fundamental role in inspiration, consists of a large, dome-shaped thin sheet of muscle and fiber that provides a separation of the abdominal and thoracic cavities. Upon contraction, the diaphragm forces the abdominal contents downward and the ribs to be lifted and moved out thereby elongating and enlarging the chest cavity. This results in development of negative intrathoracic pressure, as compared to atmospheric pressure facilitating the inflation of lungs with air. In tidal breathing at rest the level of diaphragm movement is approximately 1 cm whereas with exercise and especially forced breathing the excursion can reach up to 10 cm. Relaxation of the diaphragm at tidal breathing shortens the thoracic cavity and in concert with the lung elastic recoil drives air out of the lungs (West, 1995).

The abdominal wall muscle group, which comprises the rectus abdominus, internal and external oblique and transversus abdominus muscles, plays an important part in the increases in expiratory rates taking place during exercise. As these muscles contract they push the diaphragm and abdominal muscles

upward facilitating an increase in intra-abdominal pressure and assisting in expiration (Campbell & Green, 1955; West, 1995).

#### *Intercostal and accessory muscles*

Intercostal muscles, which are divided into external and internal, are known to be involved in both inspiration and expiration. As such, the external intercostals muscles connect adjacent ribs and they cause the ribs to be pulled upward and forward when they contract, increasing the dimensions of the thorax. The internal intercostals exert an opposite action to that of the external as they pull the ribs downward and inward thereby assisting active expiration. It should be stressed however that the contribution of the intercostal muscles in inspiration and expiration is far more complicated than that described in this brief account (De Troyer *et al.*, 2005).

The accessory muscles comprise the scalenes and the sternomastoids which upon contraction they elevate the first two ribs and the sternum, respectively. These muscles have been known to assist inspiration at rest, during heavy breathing and exercise (De Troyer & Estene, 1984; Hudson *et al.*, 2007; Legrand *et al.*, 2003; Ward, 1995).

### **1.6: RESPIRATORY RECEPTORS**

Various inputs to the respiratory control centres are projected from various receptors including these within the upper airways, the lungs, the trachea, the bronchial tree, the muscles and joints involved in respiration as well as in the brainstem and the carotid bodies. Appropriate levels of respiration and hence maintenance of the arterial blood composition within suitable limits is the end-

result of complex interaction between the effects mediated from activation of respiratory receptors and various other receptors that feed in respiratory control centres or adjacent structures.

#### *1.6.1: Upper airway receptors*

These receptors, which are located in the nasal passages, pharynx, larynx and the upper portion of the trachea, respond to mechanical and chemical stimuli. They serve to protect and defend the lower respiratory tract by initiating reflexes such as sniffing, sneezing and coughing (West, 1995).

#### *1.6.2: Lung, tracheal and bronchial receptors*

Various receptors are spread in the lungs and the tracheobronchial tree initiating regulatory and defence reflexes. Therefore the slowly adapting pulmonary stretch receptors are located in the airway smooth muscle and they provide information on the degree of lung inflation, an inspiratory off-switch and they are also responsible for the Hering-Beuer reflex. The latter refers to the inhibition of inspiration resulting from overinflation-mediated activation of the pulmonary stretch receptors. Signals from these receptors are transmitted to the brain via the vagus nerve at a maintained discharge rate in response to lung inflation (slow adaptation).

The rapidly adapting pulmonary receptors are found between epithelial cells in the airways and they convey impulses to the brain via the vagus nerve. These receptors, which are activated by deleterious gases, dust, cold air and changes in lung compliance, are involved in pulmonary congestion, microembolisms and in asthmatic attacks.

The juxta-capillary receptors are located in the alveolar walls in close proximity to the capillaries and they are stimulated by increases in the volume of interstitial fluid at the alveolar walls inducing rapid, shallow breathing and apnoea.

A number of nerve endings from C-fibers terminate in the walls of the trachea and bronchi and they are activated by pulmonary venous congestion and increases in lung stiffness. They have been proposed to be involved in the cough reflex.

Juxta-capillary receptors or J-receptors, are believed to be located in the alveolar walls in close proximity to the capillaries. They are involved in the dyspnoea sensation associated with heart failure and lung disease (West, 1995).

### *1.6.3: Chemoreceptors*

Receptors sensitive to changes in the blood content of oxygen and carbon dioxide and blood acidity are known to play an important part in the interplay of adjusting breathing according to the metabolic needs of the body while maintaining the levels of oxygen, carbon dioxide and  $H^+$  in the blood within a narrow range. These receptors have been classified to central and peripheral owing to the localisation in the brain and the periphery, respectively.

#### *1.6.3.1: Central chemoreceptors*

Various sites in the brain that have been known to be sensitive to disturbances in the partial pressure of carbon dioxide and acidity in cerebrospinal fluid are capable of modulating changes in ventilation. It has

been established that such receptors exist on the rostral and ventral area of the ventral medullary surface (Bruce & Cherniack, 1987; Lahiri & Forster, 2007). Additional sites of central chemoreception can be found in the dorsal and ventral respiratory groups, in the locus coeruleus (Putnam *et al.*, 2004), the NTS (Nattie & Li, 2002), retrotrapezoid nucleus (Li & Nattie, 1997; Takakura *et al.*, 2006) and in the medullary raphe (Wang *et al.*, 2001).

It has been suggested that the process involved in central chemoreception most likely involves sensing of  $H^+$ , whereas  $CO_2$  acts through its hydration to  $H^+$  and  $HCO_3^-$  (Lahiri & Forster, 2003). As carbon dioxide diffuses across the blood-brain barrier, the acidity of the cerebrospinal fluid bathing the chemosensitive parts of the brain changes according to the disturbances in the partial pressure of carbon dioxide and acidity of arterial blood. The time constant for ventilatory increases due to activation of central chemoreceptors by hypercapnia is known to lie between 65-180 seconds in humans (Tansley *et al.*, 1998). This wide variation could be due to the different employed methodologies and also to the influence of peripheral chemoreception and particular of the carotid body, which is known to play a part in modulating the central chemoresponsiveness to carbon dioxide (Dahan *et al.*, 1990; 2007).

#### *1.6.3.2: Peripheral chemoreceptors*

It is well known that the rapid responses to respiratory stimuli, such as disturbances in  $O_2$  and  $CO_2$ , tension are mediated by peripheral chemoreceptors, which have been identified at multiple extracranial sites including the aortic arch, abdomen and the carotid body. It has been widely

argued that the most important role in peripheral chemoreception is being played by the carotid body (Prabhakar & Peng, 2004).

### 1.6.3.3: Other receptors

Various other control systems, which provide neural input to respiratory centres, are known to influence ventilation. Therefore aortic and carotid sinus baroreceptors, which relay arterial blood pressure changes in the NTS, can either inhibit or enhance ventilation the central and peripheral drive to breathe (Heistad *et al.*, 1974; Somers *et al.*, 1991). Likewise, stimulation of pain (Borgbjerg *et al.*, 1996) and thermal receptors is also known to be capable of inducing changes in ventilation. Finally muscle afferents sensitive to mechanical deformation and/or metabolites have been long thought to contribute to the increases in ventilation seen during exercise. A thorough account of these receptors' involvement in the mediation of exercise hyperpnoea is taken place in the subsequent part of this introduction.

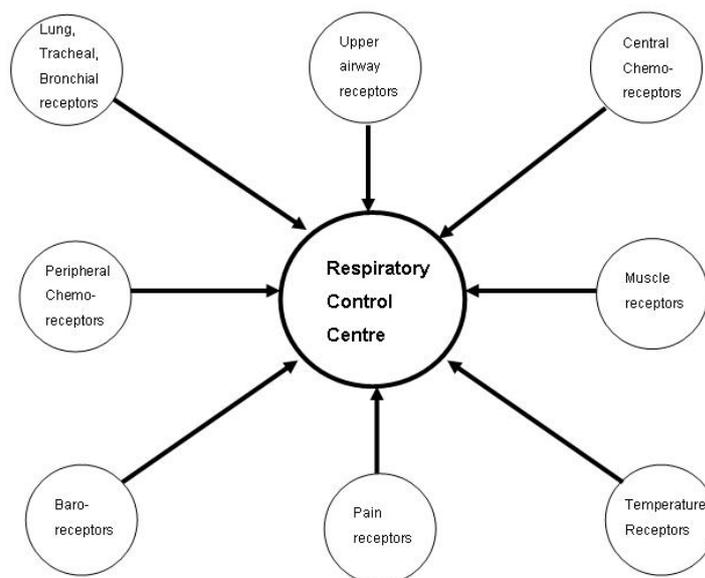


Figure 1.6: Types of respiratory receptors.

## 1.7: THE CAROTID BODY

### 1.7.1: Functions, basic histology and anatomy

The carotid body is a small organ located bilaterally at the bifurcation of the carotid artery that is sensitive to a variety of chemical and physical stimuli. These include perturbations in oxygen, carbon dioxide, pH, blood pressure, temperature,  $K^+$ , osmolarity, glucose, catecholamines and other hormones and substances (Gonzalez *et al.*, 1994; Marshall, 1994). This organ primarily serves to assess the composition of arterial blood before it reaches the brain and initiate reflex ventilatory responses in order to maintain the composition of arterial blood within suitable limits (Nye, 1993). In parallel, it has established that the carotid body plays an important role in neuroendocrine and haemodynamic regulation in response to a variety of stimuli such as hypoxia, hypercapnia and exercise (Kara *et al.*, 2003; Koyama *et al.*, 2001; Marshall, 1994). Recent work on rats has also provided evidence for metabolic rate sensing by the carotid body (Bin Jaliah *et al.*, 2004; 2005) that, in addition, has been heavily implicated in the abnormal cardiovascular and respiratory status of various diseases (Di Vanna *et al.*, 2007; Kara *et al.*, 2003; Stickland *et al.*, 2007).

The carotid body is a high-vascularised organ that receives the greatest rate of perfusion in the body. Being surrounded by an extensive capillary network, Type I cells take almost 80% of the organ's volume and they are the primary site of chemosensation (Gonzalez *et al.*, 1994). Type I cells, which are responsible for catecholamine release, are surrounded by Type II cells. Neural signals from the carotid body are relayed to the NTS via the carotid sinus

nerve (Campanucci & Nurse, 2007; Gonzalez *et al.*, 1994; Kumar & Bin-Jaliah, 2007).

#### *1.7.2: Main reflexes mediated by the carotid body; hypoxia and hypercapnia*

Reductions in  $P_{A}O_2$  or hypoxia constitute the most well known stimulus of the carotid body, which increases its afferent discharge exponentially over the range of 100 to 30 mmHg (Biscoe *et al.*, 1970; Kumar & Bin-Jaliah, 2007; Marshall, 1994). The chemotransduction process involved has yet to be clarified despite extensive research. In fact several theories have been proposed with purines (Lahiri *et al.*, 2007), catecholamines (Gonzalez *et al.*, 1994) and cholines being putative neuromodulators in the process (Nurse, 2005).

Rises in  $P_{A}CO_2$  above a threshold and acidosis (respiratory or metabolic) cause linear increases in the firing discharge of the carotid body that plateau at 100mmHg  $P_{A}CO_2$  (Lorinc *et al.*, 1991; Lahiri & Forster, 2003). The carotid body response has been widely thought to be mediated by changes in the intracellular pH status of type I cells. It has been shown that the time constant of the ventilatory increases due to carotid body stimulation by  $CO_2$  is between 8 and 26 seconds accounting for up to 50% of the total ventilatory response to hypercapnia (Bruce & Cherniack, 1987; Dahan *et al.*, 1990; Tansley *et al.*, 1998; Weil & Swanson, 1991). Interestingly in the physiological range of  $P_{A}CO_2$ , at sea level, chemosensory oscillations are matched by respiratory oscillations. This phenomenon, which was firstly observed by Band *et al.*, (1976) can be explained by the  $P_{A}CO_2$  increase and  $P_{A}O_2$  decrease at expiration and the opposite gas fluctuations at inspiration. Ultimately, the

carotid body provides approximately 20 to 30% of the resting ventilatory drive (Dejours 1962; Mahamed *et al.*, 2001).

### **1.8: REGULATION OF VENTILATION DURING EXERCISE**

Muscular exercise presents a unique, multifaced and potent stress on the ventilatory control system, which has to accommodate the increased need for oxygen and disposal of carbon dioxide by the active musculature while maintaining optimal blood acidity and gases status. Exercise hyperpnoea or the increases in breathing seen during exercise, represents the largest ventilatory adjustment during a normal human life that is inherent even in walking which is the most ordinary of human activities. Despite the immense physiological importance of this phenomenon, and the great attention that it has received by researchers for over a century, the underlying mechanisms are not completely understood (Dempsey *et al.*, 1995; Mitchell & Babb, 2006).

#### *Respiratory response to exercise*

##### *Dynamic exercise*

##### *Constant-load exercise*

In the healthy individual, the ventilatory response to dynamic (involving rhythmic repetitive movements of muscle groups) exercise performed at an

invariable intensity is schematically represented by Figure 1.4. The response is characterised by three distinct phases.

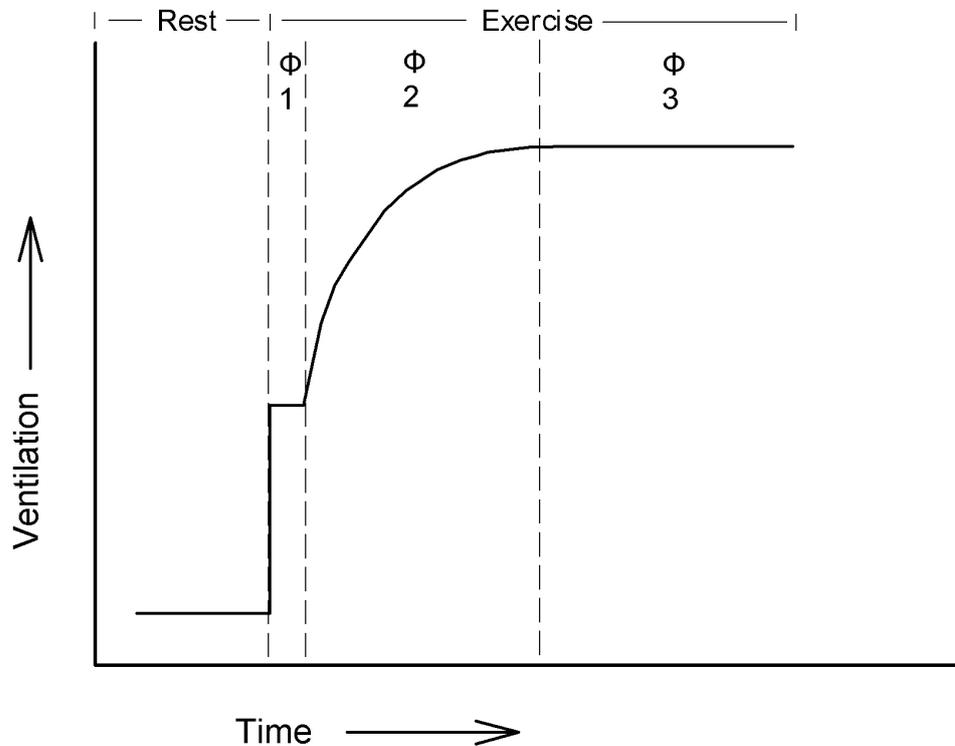


Figure 1.7: The characteristic three phases of the ventilatory response to dynamic exercise.

Phase 1 ( $\Phi_1$ ) takes place immediately at the start of exercise and is characterised by an abrupt rise in ventilation along with an accompanying elevation in the pulmonary gas exchange rates for oxygen and carbon dioxide. Increases in oxygen consumption and carbon dioxide production also occur and they could match closely the gas exchange rates. A mismatch between production and exchange could also occur due to metabolically-produced  $\text{CO}_2$  being taken-up by the muscle and venous blood, and result in a transient

plateau of ventilation for up to 20 seconds (Mateika & Duffin, 1995; Ward, 2000; Whipp, 1991; Whipp & Ward, 1991).

Subsequently, phase 2 ( $\Phi_2$ ) is characterised by an exponential increase in ventilation, oxygen uptake and carbon dioxide elimination. The time constant of the exponential increases of oxygen uptake is smaller than that for ventilation and carbon dioxide elimination, which are almost similar (~10% difference). The shorter  $O_2$  time constant has been thought to depict reasonably faithfully the time course of oxygen uptake by the exercising muscle since body stores for  $O_2$  are limited. In contrast the  $CO_2$  capacity of body stores is large and accounts for the slow kinetics of carbon dioxide output that has been suggested to 'dictate' those of  $V_E$ . As a result of the dissociation between oxygen, carbon dioxide and ventilation kinetics, the partial pressure of oxygen in arterial blood tends to decrease whereas that of the carbon dioxide slightly increases (Mateika & Duffin, 1995; Whipp, 1991; Whipp 2000).

The last portion of the ventilatory response to dynamic exercise of constant, moderate intensity is characterised by a steady-state level of respiration. At this stage there is a match between pulmonary gas exchange and metabolic rate that results in near-resting  $P_ACO_2$  and  $P_AO_2$ . During exercise of constant yet heavy intensity  $\Phi_3$  is characterised by continuous elevations in respiration so that clearance of carbon dioxide is increased at a higher rate than metabolic rate, resulting in reductions in  $P_ACO_2$ . These changes usually occur along with lactic/metabolic acidosis (Mateika & Duffin, 1995; Ward *et al.*, 2000; Whipp, 1991; Whipp & Ward 1998).

### *Incremental exercise*

During the initial stages of exercise incremental intensity with minute increments, respiration increases proportionally with oxygen uptake and/or carbon dioxide clearance, resulting in near-resting  $P_{A}CO_2$  and blood acidity. Upon reaching 40-60% (depending on fitness) of the maximum aerobic capacity, ventilation increases disproportionately to oxygen uptake and carbon dioxide clearance. This point of inflation, which has been known as the anaerobic threshold, is usually accompanied by elevations in arterial lactic acid and decrements in  $P_{A}CO_2$ . As exercise intensity increases to 70-90% of the maximum aerobic capacity, the disproportional increases of ventilation to oxygen uptake and carbon dioxide clearance become greater. In parallel greater elevations and depressions in arterial lactic acid and  $P_{A}CO_2$ , respectively, are seen. This other inflation point has been termed as the second anaerobic threshold (Mateika & Duffin, 1995; Ward, 1983; 2000).

### *Isometric exercise*

Performance of isometric (static muscular contraction) exercise is also associated with increases in ventilation whose magnitude varies according to the level of tension exerted, the duration of effort and the rates of muscular fatigue/pain induced (Duncan *et al.*, 1981; Muza *et al.*, 1983). As such, static muscle contractions exercise below 15% of the maximum voluntary contraction (MVC), which are generally regarded as non-fatiguing, induce small increments (<2 L/min) in breathing that are maintained until the contraction is ceased. Greater increases in ventilation (>5 L/min) are seen after approximately 3 minutes of static exercise performed at intensity above

20% of the MVC or after the onset of muscle fatigue (Fontana *et al.*, 1993; Iellamo *et al.*, 1999; Muza *et al.*, 1983). The magnitude of increases in ventilation has been also related to the muscle mass involved in exercise (Imms & Mehta, 1989; Muza *et al.*, 1983). It has been known that breathing rises due to isometric work are inappropriately high for the increases achieved in carbon dioxide clearance and oxygen uptake, leading to decreases in  $P_{ET}CO_2$  in  $P_ACO_2$  that are pronounced during the latter stages of relatively high-tension exercise (Imms & Mehta, 1989; Poole *et al.*, 1988; Wiley & Lind, 1971).

### **1.9: MECHANISMS CONTROLLING BREATHING DURING EXERCISE**

Uncovering the mechanisms responsible for the increments in respiration in response to exercise has been thought as one of the remaining challenges in the quest to understand the control of human systemic function (Whipp & Ward, 1998). This challenge has long attracted a vast deal of research attention yielding various mechanisms, each of which being accountable for the phenomenon when given in isolation (Dempsey *et al.*, 1995). These mechanisms could be classified to three main categories; a) the non-sensory feed-forward mechanism or central command, b) a non-sensory mechanism intrinsic to brainstem neurons that acts to potentiate the neural respiratory response to stimuli and c) sensory feedback mechanisms (Eldridge, 1994).

### 1.9.1: Central command

The notion that the drive to increase ventilation during exercise, particularly at its onset, originates from various structures in the brain has been long held and it was coupled to the observations that respiration increases abruptly before any locomotor changes take place. Early evidence for the functional importance of these brain areas has been accumulated from employing several techniques in animal preparations that comprise selective electrical stimulation, localised pharmacological stimulation, focal lesioning and antidromic techniques. Regions that have been identified to project into the cardiorespiratory integrative areas of the medulla as well as into the locomotor 'pattern generator' in the spinal cord include the cerebral motor cortex, the hypothalamus, the mesencephalon and the amygdala (Eldridge *et al.*, 1991; Waldrop *et al.*, 1996; Whipp & Ward, 1998). More recently a great deal of well designed studies has been conducted on animal preparations (Ordway *et al.*, 1989), paraplegic and especially non-paraplegic humans with the use of partial neuromuscular blockade (Adams *et al.*, 1984; Brown *et al.*, 1990), servo-assisted positive pressure ventilation (Poon *et al.*, 1987), imaginary exercise (Wuyam *et al.*, 1995), yielding a more comprehensive picture of the central command involvement in exercise hyperpnoea. Therefore it has been accepted that the mediation of hyperpnoea seen during isometric exercise is at least partially accounted for by central command (Asmussen *et al.*, 1965; Duncan *et al.*, 1981; Muza *et al.*, 1983; Poole *et al.*, 1988; Victor *et al.*, 1989; Wiley & Lind, 1971). Furthermore, it has been widely, but not universally, suggested that although central neurogenesis contributes to the rapid increases seen at the onset of dynamic exercise ( $\Phi_1$ ), it is not essential for

mediating the response. Furthermore the contribution of central command in the other phases of exercise hyperpnea remains a possibility although unambiguous experimental confirmation has yet to be achieved (Mateika & Duffin, 1995; Ward *et al.*, 2000; Whipp, 1991; Whipp & Ward 1998).

#### *Non-sensory mechanisms intrinsic to brainstem neurons*

Maintenance and development of the initial increases in ventilation seen at  $\Phi_2$  could be also facilitated by a central neurogenic mechanism, which has slow dynamics and is known as neuronal short-term potentiation (STP). Experiments on animal preparations have shown that when a single respiratory stimulus is given, activation of STP aids in the facilitation of a smooth exponential increase of breathing. It should be noted though that the importance of STP in humans' respiratory control is not well understood and, in addition the mechanism has yet to be anatomically localised (Eldridge, 1994; Mateika & Duffin, 1995; Whipp & Ward, 1998).

Another possible, yet ubiquitous, neuronal influence to respiratory control attests to the plasticity of the exercise responses based on previous experience. This phenomenon, which is known as long-term potentiation of exercise hyperpnea, is possibly mediated through serotonergic pathways, and has been primarily evidenced in animals (Whipp & Ward, 1998). Therefore a persistent and consistent hyperventilation has been observed in goats during the performance of a standard treadmill task after a two-day training period in which they completed the same task repeatedly while breathing through an imposed external dead space (Martin *et al.*, 1993). Similar findings were also reported on human studies (Turner *et al.*, 1997).

### *1.9.2: Sensory feedback mechanisms*

Various feedback mechanisms have been postulated to modulate the increases in ventilation during exercise, and they have been classified into two main categories; a) respiratory feedback through chemoreceptors and afferent input from receptors in respiratory muscles, airways and lungs and b) non-respiratory feedback from receptors in the heart, vessels, lungs and exercising muscle (Eldridge, 1994).

#### *1.9.2.1: Respiratory feedback*

##### *Central chemoreception*

Changes in blood gases and acidity have been long suggested as a potential stimulus for mediating exercise hyperpnoea although exercise up to a moderate intensity is not associated with significant alterations in these parameters. Therefore, it has been thought that central chemoreceptors are unlikely to play an important role given their location, slow response kinetics, and that  $\text{PCO}_2$  of both the arterial and venous blood and in the cerebrovascular fluid remain near normal or decrease during light and moderate exercise (Bellville *et al.*, 1979; Dempsey *et al.*, 1995; Gardner *et al.*, 1980; Weil & Swanson, 1991). Further support to that notion has been provided by data advocating a failure to alter exercise hyperpnoea after manipulation of the acidity of the cerebrovascular fluid (Smith *et al.*, 1988). The dampened oscillations in brain extracellular pH that occur secondary to these in blood could provide a stimulus to central chemoreceptors. However,

the increases in breathing frequency seen during exercise could decrease pH oscillations in blood (Murphy *et al.*, 1987), thus precluding a substantial contribution of extracellular fluid pH oscillations in the dynamics of ventilation during exercise (Dempsey *et al.*, 1995).

### *Peripheral chemoreception*

On the contrary, the peripheral chemoreceptors are known to be responsive to the pH and  $\text{PCO}_2$  oscillations in arterial blood that occur throughout the respiratory cycle (Band *et al.*, 1969; 1980; Cross *et al.*, 1979 a; b; Hornbein *et al.*, 1961). Coupled to that, it was hypothesised that ventilation during exercise could be related to the changes of slope in either the upstroke or the downstroke of the  $\text{P}_{\text{A}}\text{CO}_2$ , which is dependent on the mixed venous pulmonary  $\text{CO}_2$  flux and thus is determined by cardiac output and carbon dioxide production (Band *et al.*, 1980; Cross *et al.*, 1982). It has been also proposed that the rate sensitivity of the peripheral chemoreceptors to these changes in oscillations could account for the precise matching of ventilation and carbon dioxide production at rest and during exercise (Mateika & Duffin, 1995; Saunders 1980). These hypotheses have been challenged on both theoretical and experimental basis (Nye, 1994).

Nonetheless, other studies provided further insight on the potential role of peripheral chemoreception. As such, Phillipson and co-workers (1981) showed a linear relationship between ventilation and  $\text{CO}_2$  production under isocapnic conditions when  $\text{CO}_2$  was added or removed from the peripheral venous blood of awake sheep with intact carotid bodies. Importantly, that relationship became hypercapnic when the carotid bodies were either

denervated or blunted by administering a hyperoxic inspirate (Phillipson *et al.*, 1981). In conjunction to this, data by Bin Jaliah and associates (2005) in rats strongly suggested that the hypermetabolism-hyperpnoea could be due mediated via an increase in the sensitivity of the carotid body to CO<sub>2</sub>. This finding implied a potential link between exercise-induced increases in metabolism and ventilation, and provided the basis on which the effects of metabolism on chemosensitivity were studied as part of this thesis (Chapter 4). Further evidence supporting the role of peripheral chemoreception in the regulation of breathing during exercise has been provided by studies in which blunting of carotid body activation by hyperoxia and sodium bicarbonate resulted in transient decreases in ventilation during exercise along with slowed ventilatory kinetics, which imply impaired coupling between ventilation and carbon dioxide. Coupled to that, an accentuation of ventilatory kinetics during exercise has been noted with enhanced carotid chemosensitivity through metabolic acidosis or hypoxia (Ward *et al.*, 1987; Whipp, 1994; Whipp & Ward, 1998). Additionally the respiratory response to acute metabolic acidaemia during exercise appears to be mediated through the carotid bodies (Whipp & Wasserman, 1980).

On the whole, animal and human data have collectively suggested that peripheral chemosensitivity plays an important role in modulating the tightness with which P<sub>A</sub>CO<sub>2</sub> is regulated during  $\dot{V}_2$  (Whipp, 1994a), contributes ~20% to the ventilatory drive of  $\dot{V}_3$  (Whipp, 1994b; Whipp & Ward, 1998), and in addition it is involved in the hyperpnoea of isometric exercise (Poole *et al.*, 1988). Importantly it has been also established that the carotid body is stimulated by limbs' movement (Biscoe & Purves, 1967) and a variety of

exercise-induced alterations, including increases in  $K^+$  (Paterson, 1992), adenosine (Lahiri *et al.*, 2007), angiotensin (Allen, 1998), osmolarity (Kumar & Bin-Jaliah, 2007), sympathetic activation (Llados & Zapata, 1978; Maskell *et al.*, 2006) and temperature (Landauer *et al.*, 1995).

#### *Other respiratory feedback*

It has been purported that afferent feedback from receptors in airways and respiratory muscles may also play a modulatory role in breathing during exercise. These receptors were suggested to sense the degree of mechanical stress and provide input to higher respiratory centres, which modify ventilation so as to achieve the maximal possible efficiency. Although these inputs can influence ventilation, they can not be considered as a primary drive of exercise hyperpnoea (Dempsey *et al.*, 1995; Eldridge, 1994; Poon, 1987).

#### *1.9.2.2: Non-respiratory feedback*

Feedback not directly related to breathing or blood gases can also exert an influence to the mediation of increases of breathing during exercise. Therefore, type III and IV receptors in the muscle sensitive to mechanical (mechanoreceptors) and metabolic (metaboreceptors) changes (detailed information on the physiological importance of these receptors is given in the next part of the introduction section), respectively, have been suggested to provide a respiratory drive during dynamic exercise by many (Clark *et al.*, 1995; Grieve *et al.*, 1999; Oelberg *et al.*, 1998; Piepoli *et al.*, 1995; 1996; 1999; Scott *et al.*, 2003), but not all (Francis *et al.*, 1999) investigators. Likewise the question whether this drive is obligatory in exercise hyperpnoea

has been debated in the scientific community. Recently, the working group of Haouzi as well as others have strongly supported that intramuscular receptors sensitive to vascular distention / pressure could operate along with the mechano- and metaboreceptors in regulating ventilation and arterial gases during exercise (Haouzi *et al.*, 1993; 1999; 2001; 2003; 2005; 2006; Williamson *et al.*, 1993).

Activation of receptors in the heart and lungs has been also purported to contribute to respiration increases during exercise, according to the long held theory of 'cardiodynamic hyperpnoea'. This theory has arisen by the observation of the respiratory exchange ratio remains close to resting values during the initial 15 seconds of exercise, suggesting proportional increases in alveolar ventilation and cardiac output or pulmonary blood flow (Mateika & Duffin, 1995). In favour of this hypothesis have been studies showing reflex hyperpnoea in response to local mechanical distension of the heart and adjacent vasculature (Jones *et al.*, 1982; Kostreva *et al.*, 1975). However data on heart transplantation patients were not consistent with the 'cardiodynamic' theory as an essentially normal hyperpnoeic response to exercise was demonstrated (Banner *et al.*, 1988). Moreover, ventilation was found not to respond rapidly to an abrupt increase in resting heart rate induced by pacemakers (Jones *et al.*, 1981). It has been recently concluded that the parallel increases in ventilation and cardiac output seen at the start of exercise appear to be elicited by a common central mechanism in the medulla and therefore the operation of a cardiac mechanism in mediating exercise hyperpnoea does not seem to be obligatory in health (Mateika & Duffin, 1995). In contrast, cardiac and particularly pulmonary haemodynamics could be

implicated in the abnormally increases in ventilation seen during exercise in CHF (Guazzi *et al.*, 2007).

#### **1.10: REGULATION OF VENTILATION DURING EXERCISE IN CHF**

The excessive respiratory response to exercise seen in patients with moderate to severe chronic heart failure has been strongly related to symptoms of breathlessness and their limited exercise capacity (Clark *et al.*, 1996; Coats, 2001; Piepoli *et al.*, 2008; Witte & Clark, 2007). Various factors were proposed by earlier studies to underpin exercise hyperventilation in this condition including increased dead space, PVP, PVR and impaired right ventricular function. More recent investigations have strongly supported that an increased drive to breathe due to hyperactive central and peripheral chemoreceptors (Chua *et al.*, 1996; Ponikowski *et al.*, 1997) as well as muscle metaboreceptors (Piepoli *et al.*, 1996; 1999; Scott *et al.*, 2003) may play an important role in the abnormal exercise responses of CHF.

## PART 3: MUSCLE AFFERENTS

### 1.11: BASIC ANATOMY AND PHYSIOLOGY

It is well known that the regulation of the cardiovascular responses to exercise is mediated by the action of and interaction between central and peripheral mechanisms. Therefore neural signals from higher centres of the brain (central command), arterial and carotid baroreceptors and small afferents located in the active skeletal muscles converge in the cardiovascular integrative centres of the brainstem (i.e. ventrolateral medulla, NTS), whose neural projections to vessels, heart and adrenal medulla control heart rate, blood pressure, stroke volume and peripheral resistance by adjusting parasympathetic and sympathetic activation (Figure 1.5) (O'Leary, 2005; Sinoway & Li, 2005; Smith *et al.*, 2005).

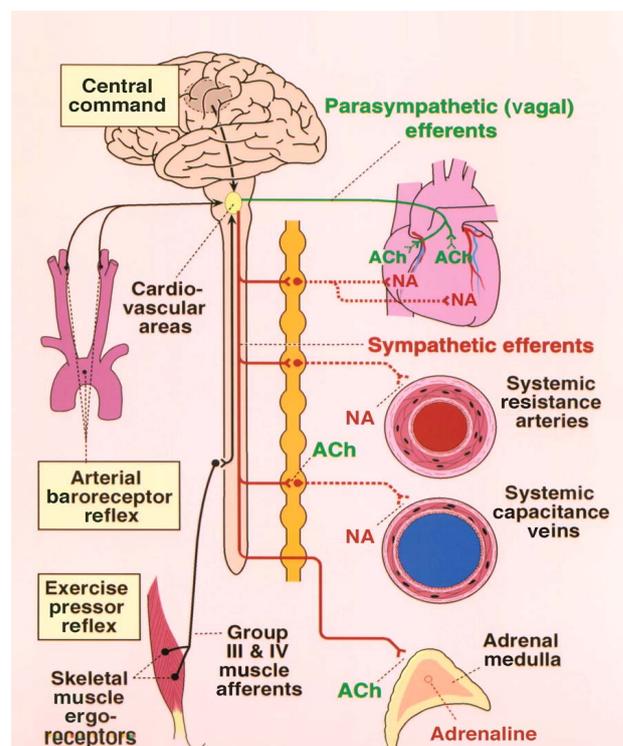


Figure 1.8: Neural control of circulation during exercise (Adapted from Smith *et al.*, 2005)

Muscle afferents are sensory nerve fibres that have free endings located in close proximity to blood vessels, tendon and collagenous tissue in the muscle interstitial space. Two types of polymodal neural fibers have been identified to provide neural signals to the NTS through the dorsal horn of the spinal cord regarding the metabolic, pressure and mechanical changes taking place during muscular work; type III myelinated fibers that are predominantly responsive to mechanical deformation and pressure changes (i.e. mechanoreceptors) and type IV non-myelinated fibres that are mostly sensitive to metabolic changes (i.e. metaboreceptors) (Kaufman & Rybicki, 1987; McCloskey & Mitchell, 1972). Activation of mechanically- and metabolically sensitive receptors induces distinct cardiovascular responses that are encompassed by the terms mechanoreflex and metaboreflex (or ergoreflex), respectively. Collectively, the responses that arise from the activation of the mechano- and metaboreflex are known as the exercise pressor reflex (Rowell & O'Leary, 1990; Smith *et al.*, 2005).

### **1.12: MECHANORECEPTORS**

Activation of receptors sensitive to mechanical deformation by rhythmic / sustained passive stretch or external muscle compression in awake humans and anaesthetised animals has been consistently shown to decrease parasympathetic activation and increase heart rate (Fisher *et al.*, 2005; Gladwell *et al.*, 2002; 2005; Matsukawa & Nakamoto, 2008; Stebbins *et al.*, 1988; Tokizawa *et al.*, 2004 McClain *et al.*, 1994; Williamson *et al.*, 1994). Activation of mechanoreceptors has been also found to increase muscle or cardiac nerve sympathetic activity (Cui *et al.*, 2006; 2008; Murata &

Matsukawa, 2001) and/or blood pressure in some studies (Bell & White, 2005; Drew *et al.*, 2008; Fisher *et al.*, 2005), but not all (Gladwell *et al.*, 2002; 2005). Whereas the above discrepancies have been attributed to methodological differences amongst studies, it has been widely suggested that the tonically active feedback provided by mechanoreceptors to the cardiovascular integrative centres contributes to the heart rate and blood pressure increases as well to the sympathoexcitation seen during dynamic exercise. Elevations in sympathetic outflow during exercise in turn are well known to serve two main important functions. First is redistributing the available cardiac output towards the active skeletal muscle by inducing vaso- and venoconstriction of vascular beds that are not involved in exercise. Second, is to ensure adequate perfusion of the heart and brain in face of the large rises in muscle blood flow (O'Leary, 2006; Sinoway & Li, 2005). In support to the purported role of mechanoreceptors on the above cardiovascular adjustments, evidence suggesting mechanoreceptor-mediated increases in cerebral blood flow (Jorgensen *et al.*, 1992) and vasoconstriction of the renal vascular bed (Middlekauff *et al.*, 1997; Momen *et al.*, 2003; Sinoway & Li 2005; Victor *et al.*, 1989) has been presented. Interestingly enhanced cardiovascular responses to mechanoreceptor activation in presence of sustained metaboreceptor activation were observed in some data (Bell & White, 2005), but not in the available majority thereof (McClain *et al.*, 1994; Fisher *et al.*, 2005; Leshower *et al.*, 2001). Again, while the disparity in the findings could be due to the different methodological approaches employed, it is well known that a high percentage of type III mechanically sensitive afferents are also activated by chemical stimuli (Kaufman & Rybicki, 1987; Rotto & Kaufman, 1988).

### 1.13: METABORECEPTORS

Activation of muscle receptors sensitive to chemical changes has been known to be largely responsible for the blood pressure and sympathetic nervous system activity increases seen during isometric exercise and may also contribute to the aforementioned changes during severe dynamic exercise (Kaufman & Rybicki, 1987; Rowell & O'Leary, 1990). Supporting evidence for this has been provided by experiments in which entrapment of contraction-related metabolites in the active muscle was ensured by occluding the circulation to the limb by either inflation of a cuff around the exercising limb (human experiments; post exercise circulatory occlusion-PECO) or by hindlimb aortic constriction (animal experiments). This ensures that nerve endings from type IV afferents remain sensitised by contraction related metabolic products such as  $H^+$ , lactic acid, bradykinin,  $K^+$ , arachidonic acid, adenosine, analogues of ATP, diprotonated phosphate and prostaglandins (Kaufman & Forster, 1996). Therefore, entrapment of contraction-related metabolites has been shown to maintain the increases in systemic blood pressure and in sympathetic nervous system activity at various vascular beds, including that of the kidneys, skin and inactive muscle, seen during isometric exercise (Momen *et al.*, 2003; Ray *et al.*, 1994; Wallin *et al.*, 1989). In addition, data on animals and humans has suggested that activation of muscle metaboreceptors is involved in the regulation of central haemodynamics and ventricular performance during exercise in healthy animals and humans and in CHF (Crisafulli *et al.*, 2003; 2008; O'Leary & Augustyniak, 1998; Sheriff *et al.*, 1998; Shoemaker *et al.*, 2007).

### **1.14: MUSCLE REFLEXES IN DISEASE**

Given the importance of the metaboreflex and mechanoreflex in cardiovascular regulation during exercise in health, extensive research has been conducted to examine whether these regulatory mechanisms contribute to the impaired exercise tolerance and cardiovascular and respiratory regulation seen in the sympathoexcitatory condition of CHF. Therefore, a number of studies have collectively showed that the contribution of the muscle metaboreflex to the blood pressure, leg vascular resistance and blood flow, and ventilatory responses to dynamic handgrip exercise were significantly higher in CHF patients than in normals (Grieve *et al.*, 1999; Hammond *et al.*, 2000; Notarius *et al.*, 2001; Piepoli *et al.*, 1996; 2006; Scott *et al.*, 2002a; b; 2003). Furthermore, Piepoli and co-workers (1996) found that 6 weeks of forearm physical training reduced the metaboreflex contributions to the aforementioned physiological variables, thereby improving the exercise responses of CHF patients. Moreover, the degree of metaboreflex contribution to ventilation was strongly associated with a worse clinical status, lower exercise tolerance, pronounced exercise hyperventilation, baroreflex impairment and sympathetic activation in CHF patients (Ponikowski *et al.*, 2001; Piepoli *et al.*, 2006).

In contrast, other data obtained on humans and animal models have suggested that the muscle metaboreflex is down-regulated in CHF (Sterns *et al.*, 1991) and that the overactivity of muscle mechanoreceptors plays the pivotal role in the mediation of the abnormal cardiovascular responses to exercise (Middlekauff *et al.*, 2004; Negrao *et al.*, 2001; Smith *et al.*, 2005;). In addition investigations in which changes in renal blood flow during were

estimated with positron emission tomography (Momen *et al.*, 2003) and ultrasound imaging (Middlekauff *et al.*, 2000) provided evidence for mechanoreceptor-mediated renal vasoconstriction during isometric exercise in CHF.

Notably, White and associates have reported decreased systemic arterial pressure values during voluntary and electrically evoked isometric contractions and subsequent circulatory occlusion in CHF patients as compared to their age-matched healthy counterparts. Consequently they argued for a decreased neural input from both the metabo- and mechanoreceptors to the exercise pressor response in this condition (Carrington *et al.*, 2000; Sinoway & Li, 2005).

Despite the lack of consensus amongst studies, that could be related to the differences in the exercise protocol (i.e. modality, duration and intensity) and muscle group used and the severity of heart failure in the patients' cohort (Carrington *et al.*, 2004), it has been accepted that the abnormal cardiovascular and ventilatory responses to exercise in CHF are partially accounted for by increased activation of both the metabo- and the mechanoreflex (Piepoli *et al.*, 2008; Witte & Clark, 2007).

## PART 4. AIMS AND OBJECTIVES OF THIS PHD; PROPOSED STUDIES

The experimental studies included in the present PhD thesis sought to investigate the following subjects;

- Given the role that muscle reflex systems appear to play in cardiovascular regulation during exercise, we aimed to examine whether the muscle metaboreflex is also involved in the regulation of pulmonary blood flow during exercise (Chapter 3). The sustained activation of the muscle metaboreflex in the post-exercise period by circulatory occlusion enabled us to assess the effect of metaboreflex-mediated sympathoexcitation on Doppler-derived estimates of pulmonary artery pressure without the confounding influence of exercise-related increases in cardiac output. Coupled to that, we investigated in a separate study (Chapter 6) the pulmonary haemodynamic response to the combined activation of the muscle metaboreflex and muscle mechanoreflex induced in the post exercise period by circulatory occlusion and passive muscle stretch, respectively.
- In light of the commonly held postulation that the muscle metaboreflex interacts with the ventilatory chemoreflex in the mediation of abnormal breathing during exercise in CHF (Coats, 2001; Schmidt *et al.*, 2005; Tumminello *et al.*, 2007), we aimed to examine whether the combined activation of these reflexes affects ventilation in healthy subjects (Chapter 5). Activation of the metaboreflex and chemoreflex were

accomplished by post-exercise circulatory occlusion and hypercapnia, respectively.

- Recent animal data evidenced an increase in the ventilatory sensitivity to CO<sub>2</sub> after metabolic rate was elevated by insulin infusion, and thus suggested that this phenomenon might be implicated in the mediation of exercise hyperpnoea (Bin Jaliah *et al.*, 2005). We aimed to assess whether small elevations in metabolic induced by food ingestion would affect the ventilatory sensitivity to inhaled carbon dioxide in healthy humans (Chapter 4).

# **CHAPTER 2: METHODS**

## 2.1: END-TIDAL GAS FORCING

Two investigations reported in this thesis required manipulation of the gases that were breathed by subjects in order to induce normoxic hypercapnia. In other words, the inspired gases were controlled in such a way that the partial pressure of oxygen in arterial blood was maintained in normal levels (normoxia or euoxia) whereas the partial pressure of carbon dioxide in arterial blood was increased (hypercapnia).

### *Basic Principles*

The technique used in order to manipulate the inspired gases is called dynamic end-tidal gas forcing (DEGF). The first comprehensive attempt of DEGF took place in 70's by Swanson's group (Swanson & Bellville, 1975). The technique was further developed in the Laboratory of Physiology in Oxford University by Robbins and co-workers (Robbins *et al.*, 1982a; 1982b). One fundamental principle underlying this DEGF is that partial pressures of oxygen and carbon dioxide in the alveolar air of healthy individuals are almost identical to these in the arterial blood that leaves the lungs. Thus the latter can be indirectly assessed by sampling the concentration of oxygen and carbon dioxide in expired gas at the end of expiration. DEGF involves manipulation of the inspired air so as to attain the required end-tidal partial pressure of a gas. The appropriate inspired values of gases required to induce certain end-tidal values at given ventilation are predicted by a cardio-respiratory mathematical model. This model, which relates changes in end-tidal gases to change in ventilation, firstly utilises the mass balance equations for oxygen and carbon dioxide in the lung. For instance the amount of a gas that enters the lungs via

inspiration should be equal to the amount leaving the lungs plus the rate of change of this gas in the alveoli.

$$\dot{V}_L \left( \frac{\Delta P_A}{dt} \right) = \dot{V}_A P_I - \dot{V}_A P_A + \lambda \left( \dot{Q}_B C_V - \dot{Q}_B C_A \right)$$

where  $\dot{V}_A$  is alveolar ventilation,  $P_A$  is the partial pressure of the gas in question in the alveoli,  $P_I$  is the partial pressure of inspired gas,  $\dot{V}_L$  is the lung volume,  $\lambda$  is a coefficient dependent on units,  $\dot{Q}_B$  is pulmonary blood flow,  $C_A$  is the arterial concentration of the gas in the blood, and  $C_V$  is mixed venous concentration of the gas in the blood.

This solved for  $P_I$  would be

$$P_I = \frac{\dot{V}_A P_A + \dot{V}_L \cdot \frac{dP_A}{dt} + \lambda \dot{Q}_B (C_A - C_V)}{\dot{V}_A}$$

A two-compartment model of the respiratory system that incorporate a central and a peripheral component, as well as a multi-compartment model of the circulation are used to solve the above equation (Robbins *et al.*, 1982a; 1982b).

### *Correction*

The correction factor of the scheme compares the actual end-tidal values with the desired ones and adjusts the predicted values of inspired gases in order to minimise the error. The adjustment in the inspired gas partial pressure ( $P_i$ ) is calculated according to the following equation for a given breath  $n$ :

$$P_{I(n)} = P_{I_C(n)} + g_p \cdot (P_{ET_d(n-1)} - P_{ET_m(n-1)}) + g_i \cdot \left( \sum_{j=1}^{n-1} (P_{ET_d(j)} - P_{ET_m(j)}) \right)$$

where  $P_{I_C}$  is the predicted inspired partial pressure,  $P_{ET_d}$  is the desired end-tidal partial pressure,  $P_{ET_m}$  is the measured end-tidal partial pressure,  $g_p$  is the proportional feedback gain, and  $g_i$  is the integral feedback gain. The gains can be adjusted manually, and the value of  $g_p$  is set to be ten times that of  $g_i$ . The proportional gain serves to reduce the effects of breath-to breath-variation, and the integral gain serves to eliminate drift and steady-state error (Robbins *et al.*, 1982a; 1982b).

### *Apparatus for DEGF*

A photographic display of the main parts that comprised the apparatus used for DEGF is shown in Figure 2.2. The subjects were breathing through a mouthpiece that was used along with a nose clip to ensure exclusive mouth breathing. The mouthpiece assembly (Figure 2.1), which integrated a saliva trap, had a port for a gas-sampling tube and was connected to a volume-measuring turbine and to a pneumotach.

The gas-sampling tube was connected to a four-channel mass spectrometer (AirSpec 2000, Case Scientific, London, UK) that sampled expired air at a rate of 80 mL/min and that was tuned to detect O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> on the basis of their mass-to-charge ratio. Output data from the mass spectrometer was updated every 20 ms. Volumes and flows of gases were measured with a combination of a turbine (Cardiokinetics Ltd., UK) and a pneumotach (Pneumo Hans Rudolph pneumotach amplifier 1, Shawnee, USA). The turbine used was a transparent plastic tube which houses an impeller that rotates as air moves past it. The turbine assembly was surrounded by a photodetector pickup device (Durant VMM-400) that collects information on the speed and direction of rotation of the impeller, which is then converted to inspiratory and expiratory volumes (1 revolution = 2 mL). Given that the turbine is not affected by water vapour it is ideal for volume measurements. However, a substantial delay is present due to the inertia of the impeller caused by the change of phase as inspiration progresses expiration and vice versa. Detection of phase change is done by the pneumotach device, which consists of a cylinder (27 mm long by 27 mm diameter) containing a honeycomb of fine tubes. The honeycomb provides a small resistance to flow (approximately 3 mmH<sub>2</sub>O/s/L) resulting in a small pressure drop across the device. An accurate measurement of flow is made since flow through the honeycomb is laminar up to 130 L/min. The information from the mass spectrometer, turbine, and pneumotach was collected by a computer.

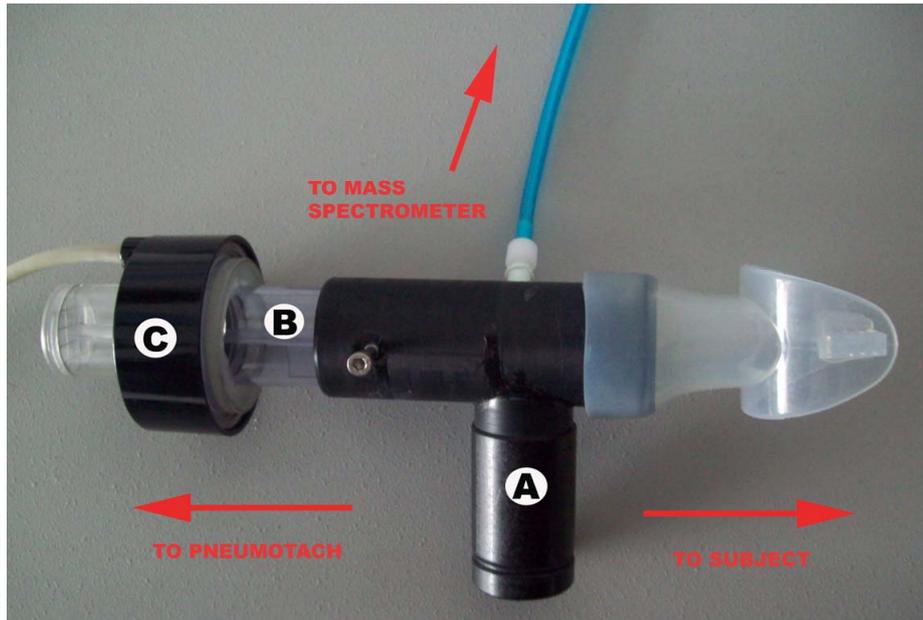


Figure 2.1: Schematic representation of the mouthpiece apparatus used for EDGF.A; saliva trap, B; turbine, C; photodetector device.

End-tidal profiles were generated using a computerized dynamic end-tidal forcing system program (BreatheM, University of Oxford, UK), in which the actual end-tidal gas composition was recorded every breath and compared with desired values by a computer. The computer then passes a signal to a pair of control boxes (MKS Instruments PR 4000 F, Germany), each controlling two of four gas channels: compressed air,  $O_2$ ,  $CO_2$ , and  $N_2$ . The control boxes are designed for use with mass flow controllers (MKS Instruments 1559A, Germany), which accurately measure and control the mass flow rate of gases through the opening of valves for  $O_2$ ,  $CO_2$  and  $N_2$  that allow a calculated duration of flow of each gas at a known rate. Because mass flow is measured, correction for temperature and pressure variations is not necessary. Before the gas mixture reaches the subject it is passed

through a set of humidifiers (Fisher & Paykel Healthcare HC150, Auckland, New Zealand) to make it easier to breathe.

### *Safety*

The safety of the subjects while using the fast gas mixing system and the chamber was assured by monitoring their O<sub>2</sub> saturation in arterial blood and by monitoring the inspired levels of O<sub>2</sub> and CO<sub>2</sub>. Saturation was measured by a pulse oximeter (Datex-Ohmeda 3900, General Electrics). Audiovisual alarms were triggered when preset safety levels were exceeded. Heart rate was also continuously monitored and subjects were instructed to immediately inform the experimenter in case of any signs discomfort (i.e. light-headedness and dizziness) that could arise with hypercapnia. A 2-3 second surge of light-headedness was reported by a number of participants during the transition from normal air breathing to normoxic hypercapnia.

### *Calibration*

Prior to each experiment calibration of the mass spectrometer and of the DEGF system was taken place. For the calibration of the former, gases of known O<sub>2</sub> and CO<sub>2</sub> concentration (15% and 5% respectively) as well as 100% Helium were used. Gases of known O<sub>2</sub> and CO<sub>2</sub> concentrations were also used for calibration of the DEGF whereas volumes were calibrated through repeated evacuations of a 1L pump.

*Brief description of running the DEGF*

A standard procedure was followed prior to each experiment that utilised DEGF. Therefore subjects were initially asked to sit quietly on a chair and breathe through a mouthpiece that was used with a noseclip. Subjects rested for at least 15 minutes while watching television in order to become unaware of their breathing. During the final 5 minutes of that period basal values of  $P_{ET}CO_2$  and  $P_{ET}O_2$  were determined. These values were used to construct the function according to which increases in  $P_{ET}CO_2$  (hypercapnia) in normoxia ( $P_{ET}O_2 = 100$  mmHg) would be forced later during the experiment.

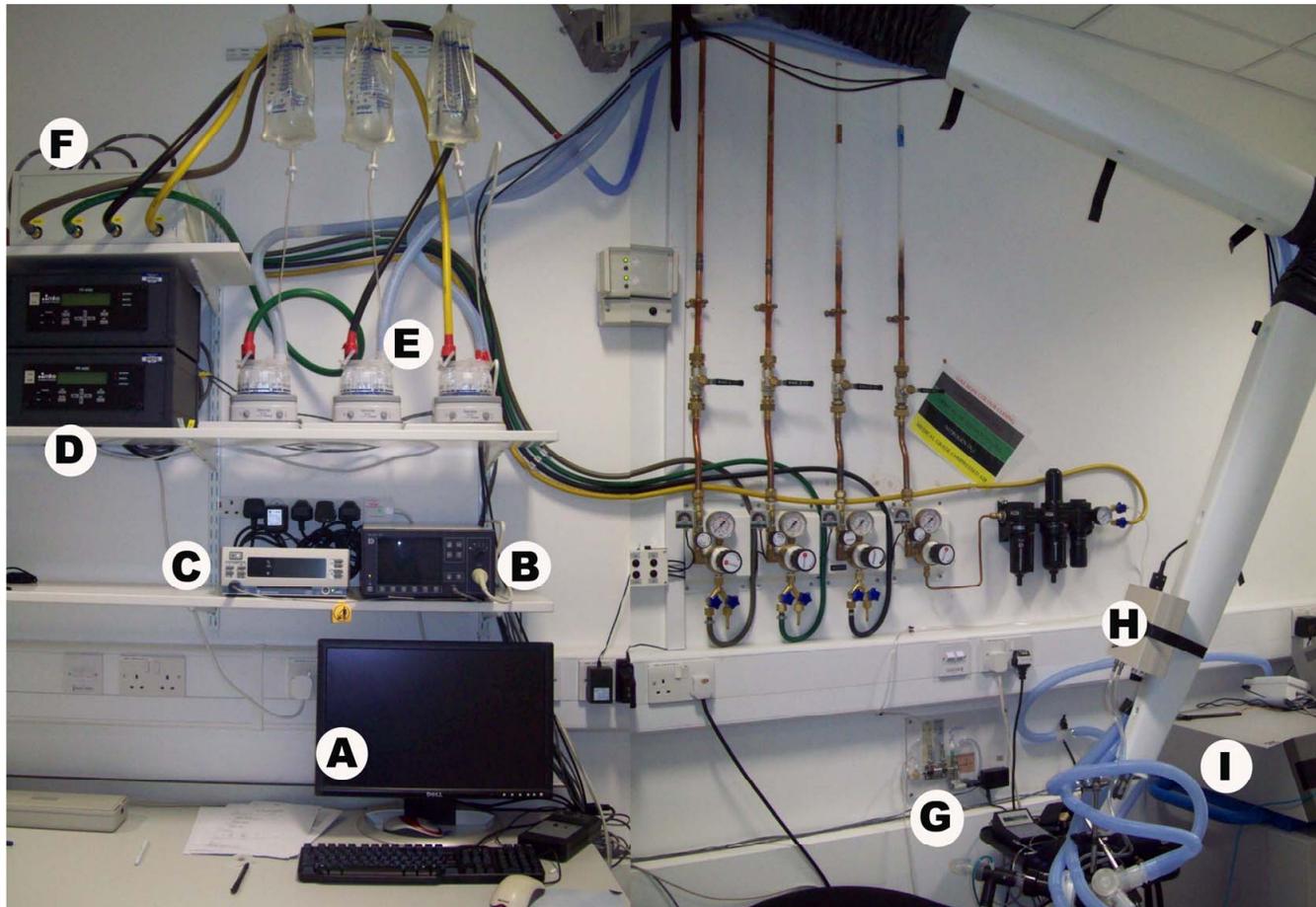


Figure 2.2: Experimental set up of the laboratory for end-tidal forcing studies. A; computer, B; heart rate monitor, C; pulse oximeter, D; control boxes, E; humidifiers, F; mass flow controllers, G; mouthpiece apparatus, H; pneumotach and I; mass spectrometer.

## **2.2: ECHOCARDIOGRAPHY**

Echocardiography, which is one of the earliest applications of medicine-related ultrasonography, has been extensively used in the clinical setting for diagnostic purposes as well as in research owing to its non-invasive nature and its ease of use. Echocardiography employs the principles underlying ultrasound imaging so as to yield multi-dimensional pictures, examine the morphology of the heart, detect blood flow across heart's valves and measure its velocity.

### *Ultrasound imaging*

Ultrasound is generated by a physical principle known as the piezoelectric effect. If an electric voltage is applied across opposite surfaces of a piezoelectric material, the material will physically oscillate producing pressure or sound waves that are inaudible to the human ear (ultrasound). The second important feature of a piezoelectric material is that it will produce an electrical impulse when it is struck by a sound wave. Such a piezoelectric element constitutes the primary component of an ultrasonic transducer which also incorporates electrodes that electrically excite the element and detect the electric impulses.

The transducer transmits a series of very short pulses (batches) of ultrasound which while traveling through the human body, they cross various interfaces. This causes some of the sound waves to be reflected back to the transducer, whereas the remainder is refracted traveling further into the tissue until the next interface. Such interfaces in the heart are usually the junctions formed between blood and the heart valves or the myocardium. The amount and/or strength of the reflected sound waves (echoes) back to the transducer depend on the difference in density of the two materials at the interface, the depth of

ultrasound penetration in the tissue and also the angle at which the sound waves strike the interface.

After the reflected waves are received by the transducer, they are amplified, and processed by means of determining the distance of the reflector given that the wave propagation velocity is known. Ultimately a two-dimensional image of the insonated tissue is built up with returning echoes being shown as variations in brightness. Once all of the echoes of interest have returned, another batch of ultrasound is generated from the transducer. The rate or frequency at which batches are sent can be adjusted by the depth control of an ultrasound machine. A decrease in frequency will enable collection of information from a more distal part at the expense of image quality, and vice versa.

Imaging of the heart presents two main issues that necessitate the use of special transducers; the heart is relatively large structure that is situated behind an arrangement of ribs that are impermeable to ultrasound, and b) the ultrasound signal has to be directed in between the ribs. Therefore for cardiac investigations a special transducer is used that creates a fan of ultrasound (usually consisting of 120 lines) thereby producing a wedge-shaped image (Figure 2.3). Resolution of images with this configuration is high when the insonated tissue lies close to the transducer and vice versa

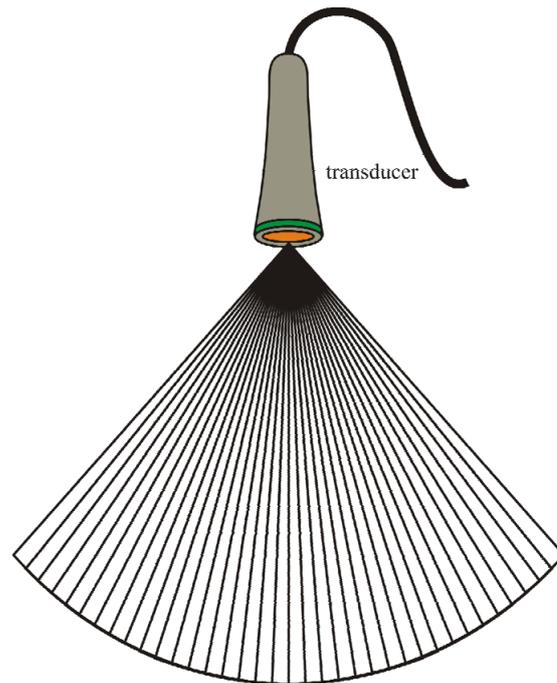


Figure 2.3: Graphic representation of the scan lines emitted by a transducer used for cardiac investigations. Note that as distance from the transducer increases the spreading of scan lines leads to reduction in resolution.

The spatial resolution of an image determines how well the structures that lie close together are distinguished or identified. Spatial resolution is further divided into lateral and axial resolutions which refer to the distinguishing of the structures that are situated side-by-side in a horizontal arrangement and at slightly different depths in a vertical arrangement, respectively. Lateral resolution is affected by the density of scan lines, whereas axial resolution depends on the wavelength or frequency of the ultrasonic beam. Lateral and axial resolution can be optimised by utilising a high frequency transducer at the expense of ultrasound penetration (depth). Temporal resolution, which refers to the ability to accurately locate structures or events at a particular instant of time, is important for studying moving structures such as blood jets. Temporal

resolution is dependent on frame rate, which is improved with minimising depth as well as the density of the scan lines at the expense of lateral resolution.

Importantly, when high accuracy measurements are required, they have to be performed along a scan line rather than across scan lines, especially if the structure to be measured is placed far from the transducer. For instance, if the thickness of a cardiac wall is to be determined, the orientation of the wall boundaries should be adjusted in such a way so that they fall across to a scan line. If the wall's boundaries were adjusted so that they fell along scan lines, then the chance of obtaining an inaccurate measurement would be increased as the boundaries could lie between scan lines. This possibility for inaccurate measurements is further raised when the distance between the structure of interest and the transducer becomes greater (Hatle & Anglesen, 1985).

### *Doppler Ultrasound*

Doppler ultrasound is the modality used in order to examine the flow of blood through organs, vessels and valves. The modality is based on the principle of the Doppler effect, which was named after the person who firstly noticed it, Christian Johann Doppler. Therefore Doppler, who observed that the colour appearance of the stars was different depending on the relative movement between the observer and the light source, argued that stars appearing red move away from the earth whereas those appearing blue move toward it.

The Doppler effect also applies to sound waves, given that they share similar properties to light waves. There are many everyday examples of the Doppler effect; train whistles, police or fire sirens and race car engines produce a sound with a higher pitch when they move towards a standing observer than that produced when they move away from the observer. A sound of higher pitch, or

of higher frequency, is received by the observer due to the denser wave profile arising from the fact that the source of the sound is travelling in the same direction as the sound waves. As the source of the sound passes and moves away from the observer the sound wave profile is not as dense owing to the different direction of travelling that the sound waves and the source of the sound wave.

In the setting of measuring blood flow the modality of Doppler ultrasound is applied as follows. A sound wave pulse of known frequency is transmitted to the blood tissue and it is reflected by the red blood cells back to the transducer. If blood was stationary then the frequency of the reflected sound wave will be the same with that from the initially emitted sound wave. If the blood was moving, the reflected waves will have a different frequency that depends on the direction of the flow. The direction of a flow in Doppler ultrasound is signified by a two-colour spectrum whereas the velocity of the flow by the intensity of the spectrum's colour (Figure 2.4). For instance in figure illustrates an example of such a spectrum in which flow towards the transducer (positive frequency shift) is denoted by a red colour whereas flow away from the transducer is indicated by a blue colour (Hatle & Anglesen, 1985; Houston & Simpson, 1988).

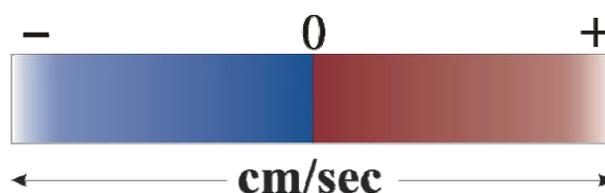


Figure 2.4: The two-colour spectrum used in Doppler ultrasound to indicate the velocity and direction of a flow.

The frequency difference between the emitted and returning waves is given by the following equation.

$$\Delta f = \frac{2vf \cos \theta}{C}$$

Where  $\Delta f$  is the frequency shift,  $v$  is the velocity,  $\theta$  is the angle between the direction of the flow and the sound wave beam (assumed to be  $0^\circ$  degrees Celcius)  $C$  is 1540 m/s, which is the velocity of sound in tissue and  $f$  is the frequency of the emitted sound wave.  $\Delta f$  is measured by the ultrasound machine, whereas velocity of the blood flow can be calculated from equation (Hatle & Anglesen, 1985).

$$v = \frac{\Delta f C}{2f \cos \theta}$$

### *Modes of Doppler Imaging*

Pulsed and continuous wave Doppler represent the two different modalities that are used to investigate low and high velocity flows, respectively. In pulsed wave Doppler (PWD), the emission of a sound wave pulse is followed a pause and the next pulse is not sent out until the reflection returns to the transducer. PWD is also used in order to examine flow at a specific point in the imaging plane. A small *time-gate* or sample volume is positioned at this point along the path of the beam allowing the ultrasound machine to calculate how long it will take for a transmitted sound pulse to travel to and to be reflected back from the red blood cells in the sample volume and travel back to the transducer. A very important technical limitation of PWD that relates to the fact that velocities are being sampled by the pulses is that it can not reliably assess velocities that are higher than 1m/s (Hatle & Anglesen, 1985; Houston & Simpson, 1988).

Continuous wave Doppler (CWD) overcomes this limitation by employing a two-transducer arrangement in which one transducer continuously emits pulses along a focused pathway, and the other continuously receives the backscattered pulses. The receiving transducer is positioned so that its focal zone intersects that of the emitting transducer over a long distance. This instrumentation, which constantly assesses the frequency shifts obtained from the region of the intersection, is used for evaluating high velocity flows in the heart such as these of mitral and tricuspid regurgitation jets (Houston & Simpson, 1988).

### *Spectral analysis*

The returning signals collected from PWD and CWD are undergone directional separation by means of being split up into two components; one of the increased frequencies from structures moving away from the transducer (positive frequency shift) and one of decreased frequencies from structures moving away (negative frequency shifts). The positive and negative shifts are plotted on either side of a horizontal zero line, with the distance from the baseline denoting the frequency shift that is directly related to the velocity of the flow. Time is integrated in the plot along with an ECG signal and a respiratory waveform allowing for the association of flow events to a particular point of the heart and breathing cycles (Figure 2.5).

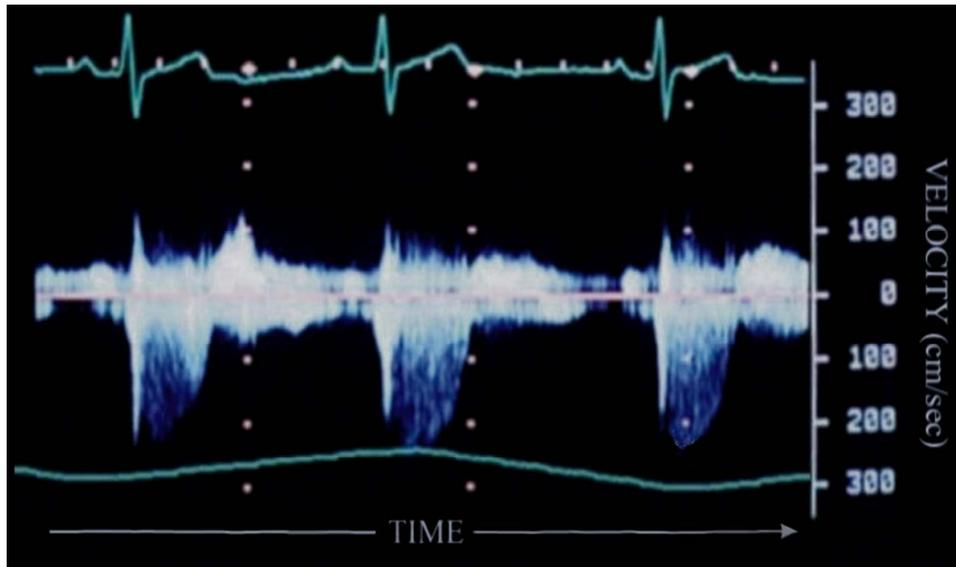


Figure 2.5: A three-beat velocity profile of a tricuspid regurgitation jet measured in CWD. Upper trace: electrocardiogram; middle trace: Doppler signal (related to velocity scale); lower trace: index of respiratory movement

The relationship that exists between the pressure and velocity of an ideal fluid was firstly described by the mathematician Daniel Bernoulli. Bernoulli stated that an increase in the speed of the fluid occurs simultaneously with a decrease in pressure.

The ultrasound machine makes use of a modified version of this equation in order to calculate the pressure drop across an orifice assuming that the following conditions are met: The velocity before the orifice is low, the orifice is short in length and the ultrasound beam is well aligned with the flow.

The modified Bernoulli's equation is

$$\Delta P = 4v^2$$

Where  $\Delta P$  is the pressure drop across the orifice in mmHg, and  $v$  is the flow velocity (m/s). The equation is used for the calculation of both peak and mean pressure drops; the difference being that in the first case only the point furthest away from the baseline of the spectral waveform is measured, while a trace of

the entire profile of a spectral waveform is needed for the mean pressure drop to be calculated (Torbicki & Kurzyna, 2000; Yock & Popp, 1984).

*Echocardiographic measurements obtained for the thesis investigations*

Three echocardiographic views were utilised for the purposes of this thesis; 4-chamber and 5-chamber apical views and a parasternal long axis view (figure 2.6). The two apical views are obtained from the lateral part of the fourth or fifth intercostal space and they are used for the visualisation of the tricuspid valve and of the aortic valve. The parasternal long axis view is obtained from the second or third intercostal space, just lateral to the sternum, and it is used for the visualisation of the aortic valve in a vertical arrangement to accurately measure its diameter.

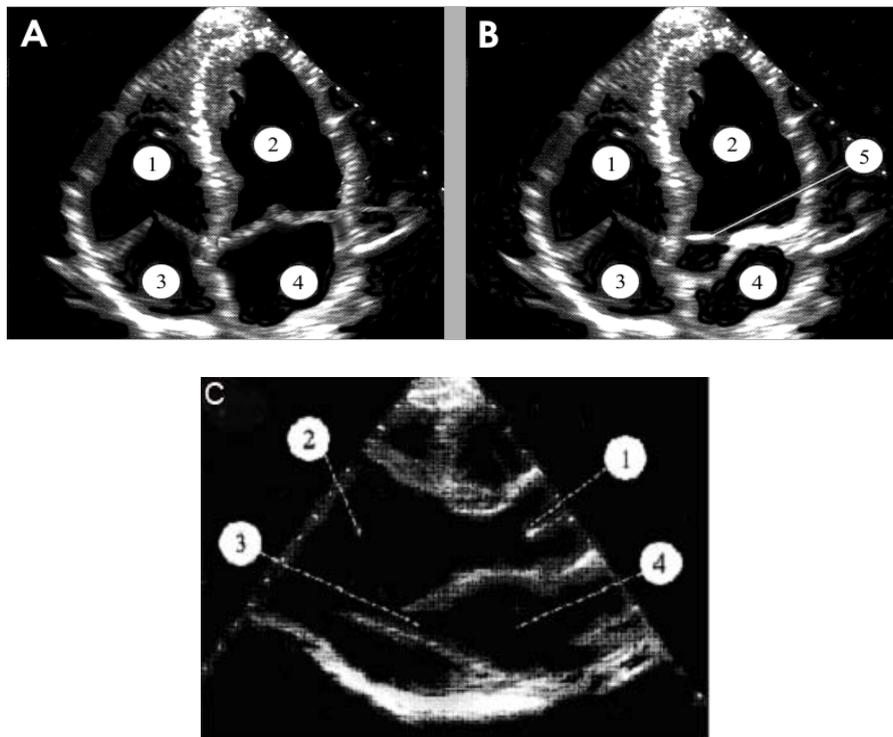


Figure 2.6: A (apical 4-chamber) and B (5-chamber); Right ventricle (1), left ventricle (2), right atrium (3), left atrium (4) and aortic valve (5). C (parasternal long-axis view); Aortic valve (1), left ventricle (2), mitral valve (3) and left atrium (4).

### *Tricuspid regurgitation and estimation of systolic pulmonary artery pressure*

Estimation of SPAP with Doppler ultrasound was initially applied to patients with pathologically elevated tricuspid regurgitation in early 80's (Yock & Popp, 1984). It was later shown that a physiological regurgitation could be detected across the tricuspid valve with Doppler in 80% normal people (Mc Quillan *et al.*, 2001). This non-pathological finding can provide valuable indirect information about changes in pulmonary artery pressure in the absence of invasive techniques. The tricuspid regurgitation is a fast flowing jet travelling at velocities of more than 2 m/s and it is measured in CWD mode from the apical 4-chamber view. To measure the velocity of the regurgitation jet, visualisation of the tricuspid valve is done in two-dimensional black and white mode and then the jet is identified in colour Doppler mode as it flows from the right ventricle into the right atrium during systole (Figure 2.7). Intra- and inter-observer variability for estimation for measurements of a single velocity profile is  $0.9\pm 1.1$  (mean $\pm$ SD) mmHg and  $1.3\pm 1.4$  (mean $\pm$ SD) mmHg, respectively (Balanos *et al.*, 2005).

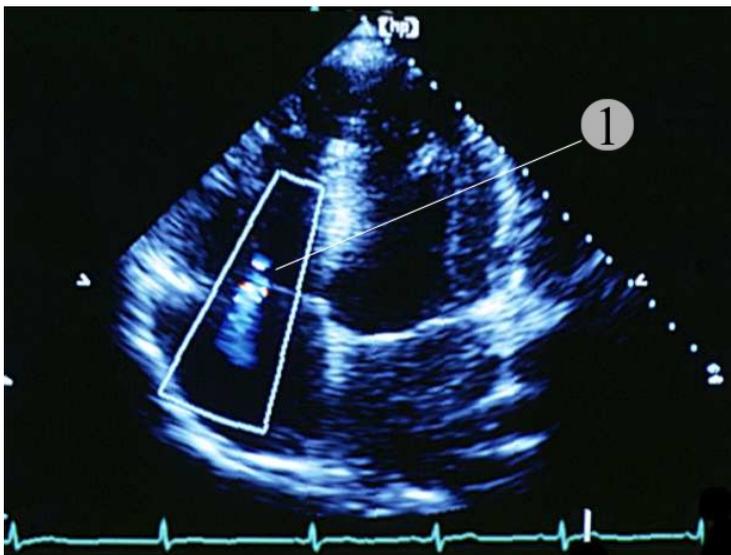


Figure 2.7: Tricuspid regurgitation (1) visualised in colour Doppler mode from an apical 4-chamber view.

Following proper alignment of the Doppler beam with the jet, a spectral profile is obtained with CWD mode and the peak velocity of the regurgitation is measured at the most distal part of the profile. The peak velocity is then automatically converted to peak pressure difference ( $\Delta P_{MAX}$ ) between the right ventricle and the right atrium using Bernoulli's equation.  $\Delta P_{MAX}$  is assumed to change linearly with systolic pulmonary artery pressure in healthy individuals, because right atrial pressure is believed not to vary substantially. In individual subjects, changes in SPAP have been shown to correlate well with changes in pulmonary vascular pressure measured by right heart catheterisation (Dorrington *et al.*, 1997), and as a result SPAP can be used as an index of PVR.

### *Cardiac Output*

Cardiac output can also be measured non-invasively using Doppler ultrasound. Two separate echocardiographic measurements are required for the determination of cardiac output. First the velocity time integral (VTI) is measured from the flow across the aortic valve via a 5-chamber apical view (figure 2.8), and second, the diameter of the location where VTI is obtained is measured via a parasternal long axis view.

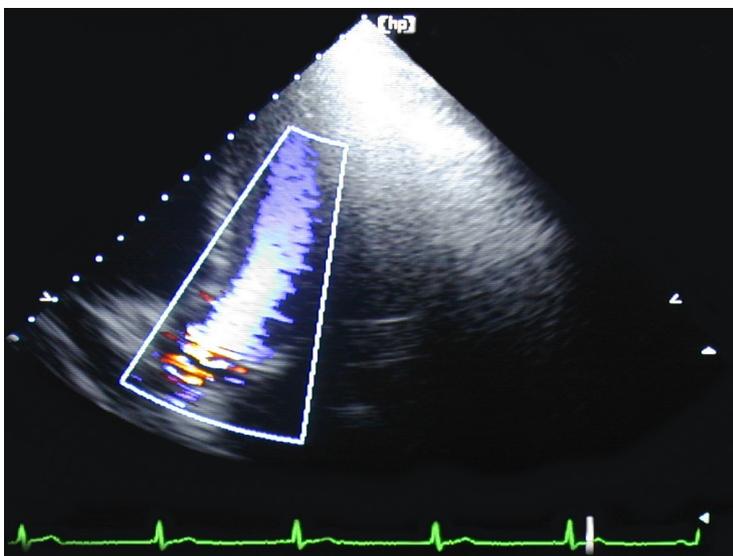


Figure 2.8: The flow of blood exiting the heart through the aortic valve during systole, visualised in colour Doppler mode from an apical 5-chamber view

The area (A) of this site is then calculated from this diameter, assuming that the area consists of a circle. The VTI is measured just below the aortic orifice and it represents the mean distance that is travelled by the blood with each cardiac contraction given in centimetres. The mean velocity of the flow and the duration of systole are taken into account for the result. The exact location where VTI is measured is important because two conditions have to be met; A) the flow must be uniform and B) the location has to be round.

Cardiac output is calculated using the equation below

$$\dot{Q} = VTI \times A \times HR$$

where HR is heart rate.

To perform the VTI measurement the aortic valve is visualised in an apical 5-chamber view and the flow is identified in colour Doppler format. The flow through the aortic valve has a moderate velocity of around 80cm/s and it is analysed using spectral mode to calculate the mean velocity of the jet. The Doppler beam is aligned with the flow and the range of investigation of the PWD is positioned on the ventricular side of the aortic valve. A PWD spectral trace of the flow through the aortic valve is obtained, and its mean velocity is calculated and translated automatically into distance travelled per cardiac cycle. (Lewis *et al.*, 1984). Intra- and inter-observer error for measurement of cardiac output from a single aortic flow profile is  $0.08 \pm 0.05$  (mean $\pm$ SD) L/min and  $0.12 \pm 0.08$  (mean $\pm$ SD) L/min, respectively (Balanos *et al.*, 2005).

#### *Training of investigator*

It has been established that the accuracy in estimating SPAP and  $\dot{Q}$  by Doppler ultrasound is highly investigator-dependent. The main investigator for the

purposes of this study had undergone over 100 hours of training before conducting ultrasound measurements for the first study of this thesis (February-May 2007; Chapter 3). Frequent bouts of ultrasound scanning had been taking place prior the conduction of the second study that involved measurement of SPAP and  $\dot{Q}$  (July-August 2008; Chapter 6).

**CHAPTER 3:  
THE PULMONARY VASCULAR  
RESPONSE TO THE  
SUSTAINED ACTIVATION OF  
THE MUSCLE METABOREFLEX  
IN MAN**

### 3.1: ABSTRACT

The pulmonary circulation is influenced by the autonomic nervous system, yet whether this is physiologically important during exercise in man is not known. The aim of this study was to assess the pulmonary vascular response to sympathoexcitation induced by the maintained activation of the muscle metaboreflex in the post-exercise period. Nine healthy subjects performed a 2-minute bout of intense isometric handgrip exercise followed by circulatory occlusion so as to maintain the muscle metaboreflex activated for 2 minutes (post exercise circulatory occlusion; PECO). A control protocol was also undertaken. Blood pressure measurements and echocardiographically-determined estimates of systolic pulmonary artery pressure (SPAP) and cardiac output, were obtained at various intervals throughout the two protocols. Elevations in SPAP ( $20.06 \pm 2.08\%$ ), cardiac output ( $36.04 \pm 4.97\%$ ) and mean arterial pressure (MAP) ( $25.62 \pm 3.54\%$ ) were noted during isometric exercise as compared to baseline (All  $P < 0.05$ ). Increases in SPAP and MAP persisted during PECO (All  $P < 0.05$ ), whereas cardiac output returned to resting levels. All data are mean  $\pm$  S.E.M. Our findings suggest that the sympathoexcitation induced by isometric exercise affects the pulmonary circulation possibly by inducing vasoconstriction and/or stiffening the large conduit arteries. The exaggerated activation of the sympathetic nervous system that has been evidenced in cardiopulmonary patients could be implicated in their abnormal pulmonary hemodynamic responses to exercise.

### 3.2: INTRODUCTION

Blood flow through the lungs is regulated by active and passive factors according to the contribution of vasomotility to the final outcome. Alternations in blood flow are being brought about independently of changes in vascular tone when passive factors come into play, whereas active factors exert their influence by affecting the contractile and relaxation status of vascular smooth muscle (Barnes & Liu, 1995).

Activation of the sympathetic component of the autonomic nervous system (ANS) in animal preparations is known to influence pulmonary haemodynamics via either vasoconstriction-mediated increases in pulmonary vascular resistance (PVR) (Barman, 1995; Hyman *et al.*, 1981; Kadowitz *et al.*, 1973; 1974; Murray *et al.*, 1986) or decreases in the compliance of large conduit arteries (Ingram *et al.*, 1968; 1970; Szidon *et al.*, 1971). These effects were attributed to the stimulation of  $\alpha_1$  adrenergic receptors by neurogenically-released noradrenaline, while that of  $\beta_2$  receptors was postulated to play an opposing role through inducing vasodilation and / or increases in arterial compliance (Barman, 1995; Hyman *et al.*, 1981; Kadowitz *et al.*, 1973; Murray *et al.*, 1986). Furthermore an interaction between stimulation of adrenergic receptors has been purported to contribute to the existence of basal sympathetic tone in the pulmonary vasculature under normal physiological conditions and in addition it appeared to play a part in PVR regulation during exercise in sheep (Kane *et al.*, 1993; 1994).

Investigations on humans have also been supportive of the view that pulmonary vessels and / or haemodynamics are sensitive to sympathetic influences. PVR elevations were commonly observed during sympathetic activation induced by the cold pressor test (CPT). Yet the fact that this effect was clearly apparent

only when the impact of mechanical factors was prevented or reduced, made the generation of safe inferences concerning the physiological importance of sympathetic influences on the pulmonary circulation not viable (Moruzzi *et al.*, 1988; 1989a; 1989b).

In contrast, the vascular and / or haemodynamic response of the systemic circulation to sympathoexcitation is better understood. Manipulation of the muscle metaboreflex / chemoreflex, in particular, has been of paramount importance for establishing that the activation of type IV neural afferents in the active muscle by various metabolites induces increases in blood pressure via sympathoexcitation-mediated vasoconstriction (Rowell *et al.*, 1990; Smith *et al.*, 2006). Evidence for the latter has been presented at various vascular beds, including the renal (Momen *et al.*, 2003) as well as those of the active and the inactive muscle (Hansen *et al.*, 1994). Moreover, reductions in the compliance of the conduit artery feeding the musculature of the inactive limb, but not of the active, were shown when the muscle chemoreflex was sustained active following isometric exercise (Davies *et al.*, 2007). Data regarding the response of the pulmonary vasculature / haemodynamics has not been available.

The lack of cardiac output alternations with respect to pre-exercise levels that is observed when the muscle metaboreflex is maintained activated in the post-exercise period could provide the optimal environment so as to assess the effect of the ensuing sympathoexcitation on pulmonary haemodynamics (Bastos *et al.*, 2000; Nishiyasu *et al.*, 1994; Sinoway *et al.*, 1989). This not only could potentially verify the existing evidence for an autonomic nervous contribution to lung blood flow regulation in humans, but it might also be of particular value in revealing this aspect of the ANS function under physiological conditions.

Therefore this study sought to examine the pulmonary vascular responses to the maintained activation of the muscle chemoreflex following a bout of intense isometric exercise. An echocardiographically-obtained estimation of systolic pulmonary artery pressure (SPAP) was utilized as an index pulmonary vascular responsiveness (Balanos *et al.*, 2002; Dorrington *et al.*, 1997). Based on the findings of Ingram *et al.* (1968) and Szidon *et al.* (1971) who reported increases in SPAP during sympathetic stimulation in animal preparations, we hypothesized that SPAP would be raised in response to the sympathoexcitation induced in the post-exercise period by the sustained activation of the muscle chemoreflex.

### **3.3: METHODS**

#### *Subjects*

Nine healthy and physically active volunteers (age  $21.8 \pm 6$  years; mean  $\pm$  SD; 5 men, 4 women) gave written consent to take part in this study after they had been given detailed information on the procedures and risks. The study was performed according to the Declaration of Helsinki and was approved by the Local Ethics Committee.

#### *Preliminary session*

A preliminary session was undertaken in order to determine the suitability of subjects and to screen for exclusion criteria, ascertained by questionnaire, which comprised presence of cardiorespiratory disease, recent forearm injury or upper respiratory tract infection. Doppler echocardiographic visualization of tricuspid regurgitation was then undertaken on a specially designed couch

(Ergoselect 1200EL, *Ergoline Gmb*) which allowed for a slight rotation of the body towards the lateral decubitus position. If the acquired ultrasound images were of satisfactory quality, the maximum voluntary contraction (MVC) of the dominant arm was then determined by a handgrip dynamometer (Lafayette Instrument Company) while the participants remained in the same body position. Subjects were instructed to maximally squeeze on the dynamometer and maintain this for at least 3 seconds. The exercising arm was fully extended and was kept close to the body. The mean of three attempts was regarded as the MVC provided that they did not differ more than 10% from each other.

#### *Design of the study*

Participants who completed the preliminary trial were asked to return to the laboratory to carry out a control and an exercise protocol on two separate occasions. The duration of each visit was approximately one hour and the order of the protocols was randomly selected. Subjects were requested to undertake the two protocols at the same time of the day. Additionally they were asked to refrain from caffeine, alcohol and performance of physical activity 12 hours, and eating 3 hours, prior to each visit.

At the beginning of each visit participants were prepared for monitoring of heart rate (ECG), respiratory frequency (pneumograph), and blood pressure (finger plethysmography, Portapres). Finally, a pneumatic cuff was placed around the upper exercising arm for subsequent occlusion of forearm blood flow (E20 Hokanson system). Following their preparation subjects rested on the couch for 15 minutes before measurements commenced. While the first 10 minutes of the resting period were completed in the supine position, the couch was slightly

turned sideways during the final 5 minutes. Subjects were instructed to maintain a self selected but constant respiratory rate throughout both protocols. This was aided by a continuous background sound of a metronome set at a frequency of 60 beats per minute. In addition they were asked to remain quiet and to avoid performing Valsalva maneuvers during measurements.

After the 15 minute rest period, baseline measurements of the  $\Delta P_{MAX}$  and cardiac output were obtained for five minutes. Subjects were then asked to squeeze the handgrip dynamometer to 50% MVC and to sustain this for two minutes. Visual feedback of the target force and output from the dynamometer was given via a monitor placed within the subject's field of view. In the control trial exercise was replaced by two minutes of rest. Two seconds before the cessation of the exercise period (or the equivalent point in the control trial) the pneumatic cuff around the arm was rapidly inflated to 205 mmHg and this was maintained for a further two minutes (PECO period). Upon deflation of the cuff subjects were instructed to remain in the supine resting position for a final two minutes (recovery period). Blood pressure and respiratory waveforms were continuously measured throughout both protocols. Cardiac output and trans-tricuspid pressure difference were measured at the time points indicated in Figure 3.1 during baseline, exercise, PECO and recovery periods. Details of these measurements are given below.

### *Echocardiography*

Echocardiographic measurements were performed using a Philips Sonos 7500 ultrasound machine with a S3 cardiac transducer (1-3 MHz). Heart rate was also recorded on this machine. All measurements were obtained with the subject being slightly rolled towards the lateral decubitus position on the specially designed couch that was used in the preliminary session. Series of consecutive cardiac cycles for each part of the protocol were acquired and stored on VHS tapes. At least three but usually five beats at, or close to, end-expiration were analyzed off line and averaged for each measurement that was made during every stage of the protocol.

### *Estimation of SPAP from measurement of $\Delta P_{MAX}$*

$\Delta P_{MAX}$  was measured for as many cardiac cycles as possible during the correct phase of the respiratory cycle. These measurements were then pooled to give a mean value for each phase of the experiment. Adjustments to the position of the ultrasound beam was required to maintain good quality images and this resulted in some data loss for short periods of the protocol.

### *Cardiac output.*

Cardiac output was measured for approximately 30-second periods during three separate sections of the baseline period, after the initial rapid rise in heart rate had occurred during exercise, at the end of the PECO phase and then again during recovery when heart rate had returned to baseline levels following release of the occluding cuff (figure 3.1).

Given that stroke volume (SV) equals the product of VTI and A, the ratio of SV to pulse pressure (SBP-DBP), which has been widely used as an index of systemic arterial stiffness (De Simone *et al.*, 1999), was also derived.

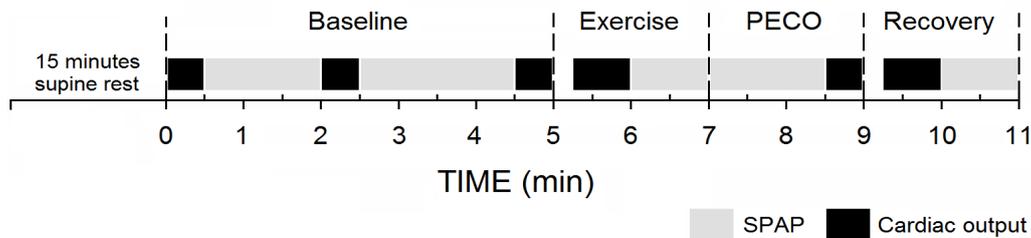


Figure 3.1. Schematic representation of the experimental protocol and sequence of echocardiographic measurements of SPAP and cardiac output. Heart rate and blood pressure were monitored continuously throughout the protocol. PECO, postexercise circulatory occlusion; SPAP, systolic pulmonary artery pressure.

### *Statistical methods*

Statistical analysis of the effects of muscle metaboreflex activation on pulmonary haemodynamics was performed using repeated measures 2-way ANOVA (5x2; time vs condition). If appropriate, *post hoc* analysis was also performed at individual time points by using paired t-tests (two-tailed) that were adjusted for multiple corrections. A power calculation showed that our study had 76.4% power to detect a 10% change in SPAP. The statistical package utilised was SPSS 14.0 (SPSS Inc., Chicago, Illinois, USA).  $P < 0.05$  was considered significant. Data are presented as means  $\pm$  S.E.M.

### **3.4: RESULTS**

Baseline values of the variables obtained at the control and exercise trial are presented at table 3.1. No significant differences were found.

Table 3.1: Baseline values (mean±S.E.M; n=9) of the variables studied at the two protocols. SPAP, systolic pulmonary artery pressure; MAP, mean arterial pressure; SAP, systolic arterial pressure; DAP, diastolic artery pressure; Q, cardiac output; HR, heart rate; SV, stroke volume.

	<b>Control</b>	<b>Exercise</b>
SPAP, mmHg	19.67 ± 0.56	19.61 ± 0.41
Q, L/min	4.10 ± 0.20	4.20 ± 0.20
MAP, mmHg	91 ± 3.26	93 ± 4.01
SAP, mmHg	136 ± 5.77	136 ± 5.78
DAP, mmHg	69 ± 2.95	71 ± 3.73
SV, ml	70.55 ± 2.51	74.29 ± 4.66
HR, bpm	58 ± 1.73	57 ± 1.71

#### *Systolic pulmonary artery pressure*

No significant changes from baseline were noted at any stage during the control trial. In contrast, at the exercise trial SPAP was increased by 20.06±2.08% during isometric handgrip exercise and remained elevated during the first (+16.19±1.45%) and the second (+16.03±1.36%) minute of PECO as compared to the resting values (All P<0.05). SPAP gradually returned towards baseline levels during the recovery period (Figure 3.2 and Table 3.2).

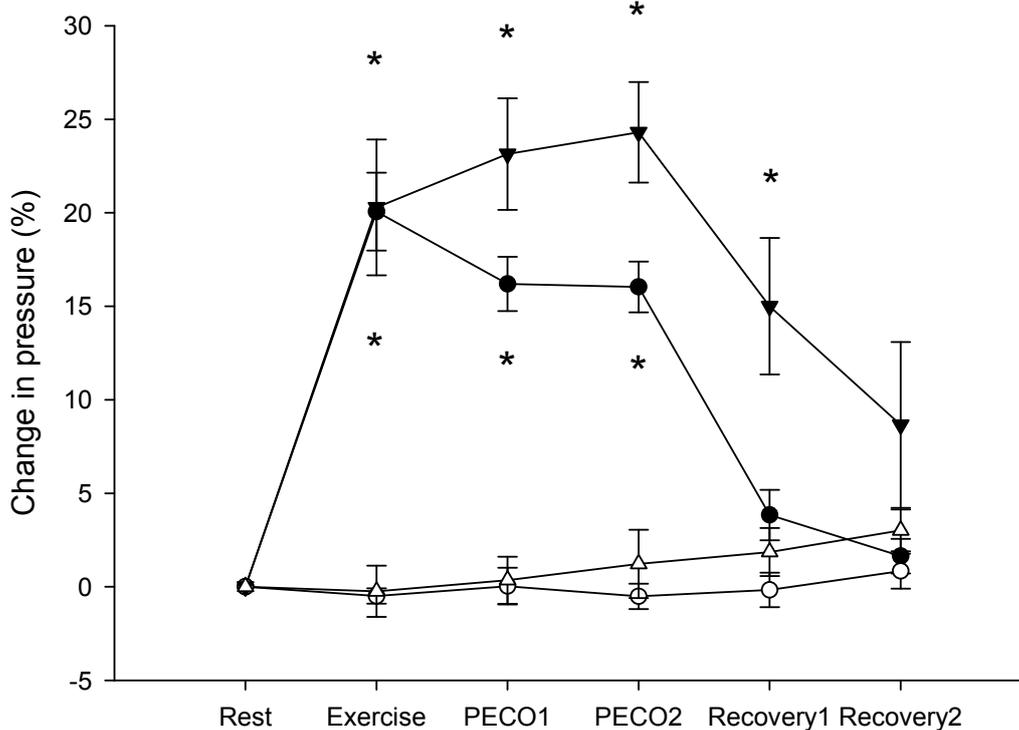


Figure 3.2: Percent changes in SPAP (circles) and SAP (triangles) from rest during the isometric handgrip exercise (closed symbols) and control (open symbols) protocols. “\*” signifies  $P < 0.05$  compared to rest. Data are means  $\pm$  S.E.M;  $N = 9$ .

#### *Cardiac output, heart rate and stroke volume*

Cardiac output, heart rate and stroke volume were not different from baseline at any time during the control trial (Figure 3.3). In the exercise trial, a  $36.04 \pm 4.97\%$  ( $P < 0.05$ ) increase in cardiac output was observed during IHG. This was due to a rise in heart rate ( $P < 0.05$ ), since stroke volume was not significantly elevated with respect to the initial values. During the second minute into PECO cardiac output fell at resting levels ( $P > 0.05$  compared to baseline). Similarly stroke volume increased during the recovery period causing a non-significant  $8.05 \pm 4.40\%$  elevation in cardiac output ( $P > 0.05$  compared to baseline). Heart rate did not change.

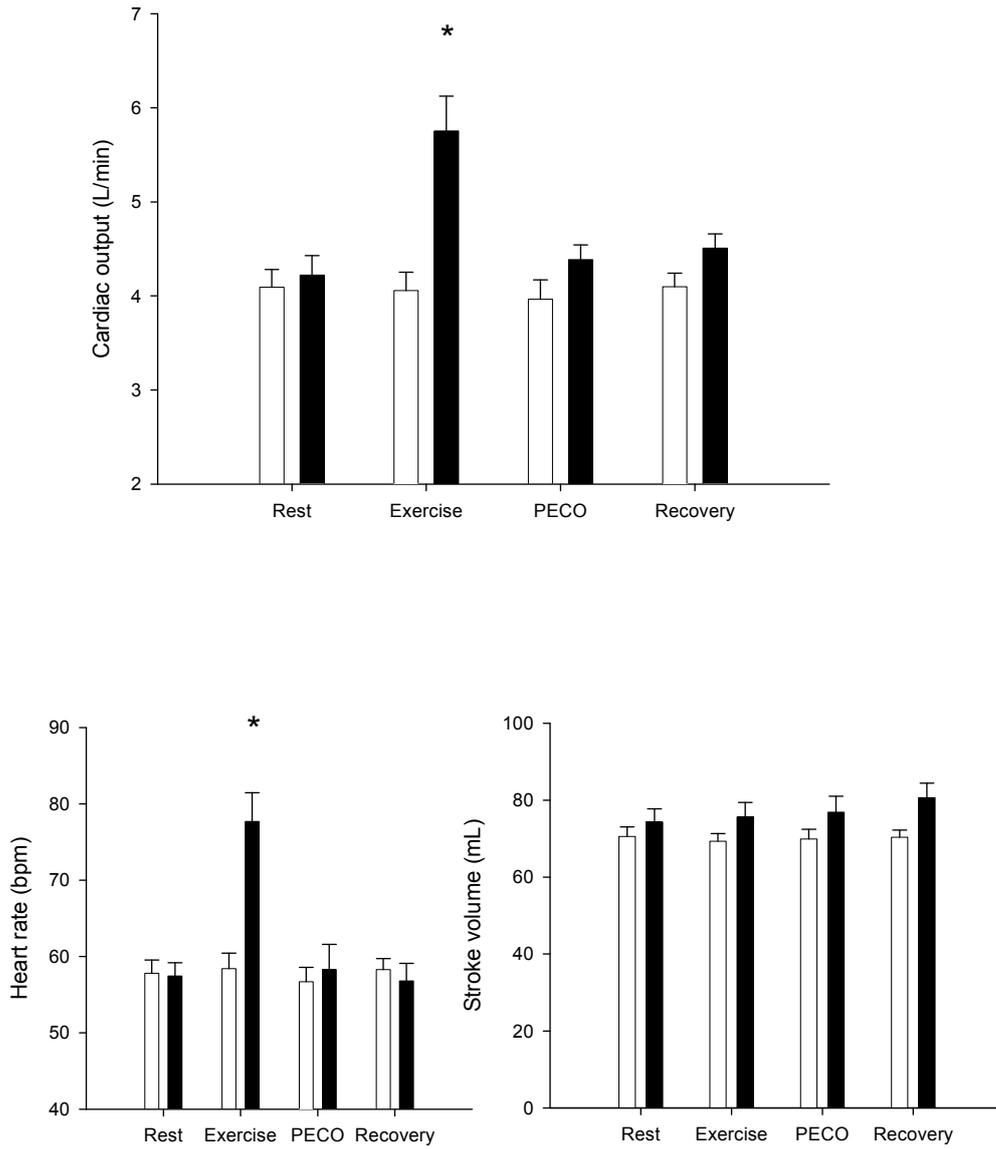


Figure 3.3: Changes in cardiac output (top panel), heart rate (bottom left panel) and stroke volume (bottom right panel) during the isometric handgrip exercise (black bars) and control (white bars) trials. "\*" signifies  $P < 0.05$  compared to rest. Data are means  $\pm$  S.E.M; N=9.

*Systemic blood pressure*

The response of mean blood pressure during the exercise trial is illustrated in figure 3.2. Mean, systolic and diastolic arterial pressures significantly increased during exercise (all  $P < 0.05$  vs rest). Subsequently they all remained elevated above resting levels during the first (all  $P < 0.05$ ) and the second (all  $P < 0.05$ ) minute into PECO. During the first minute of the recovery stage systemic arterial pressures decreased yet they were significantly greater as compared to baseline (all  $P < 0.05$ ). The second minute was characterised by further pressure decreases.

Table 3.2: Measurements obtained during the isometric handgrip exercise trial. For abbreviations see table 1 (means $\pm$ SEM; n=9; “\*” signifies P < 0.05 compared to rest).

	Rest	Exercise	PECO1	PECO2	Recovery1	Recovery2
SPAP, mmHg	19.61 $\pm$ 0.41	23.36 $\pm$ 0.68*	22.80 $\pm$ 0.66*	22.76 $\pm$ 0.59*	20.37 $\pm$ 0.58	19.93 $\pm$ 0.45
Q̇, L/min	4.22 $\pm$ 0.21	5.75 $\pm$ 0.37*		4.38 $\pm$ 0.16	4.51 $\pm$ 0.15	
MAP, mmHg	93.18 $\pm$ 4.01	116.86 $\pm$ 5.64*	116.46 $\pm$ 6.83*	118.97 $\pm$ 5.95*	105.41 $\pm$ 5.67	99.98 $\pm$ 5.38
SAP, mmHg	136.43 $\pm$ 5.03	163.69 $\pm$ 6.72*	168.01 $\pm$ 7.46*	169.52 $\pm$ 7.00*	156.76 $\pm$ 7.23	148.14 $\pm$ 7.98
DAP, mmHg	71.59 $\pm$ 3.74	93.48 $\pm$ 5.24*	90.73 $\pm$ 6.69*	93.73 $\pm$ 5.75*	79.77 $\pm$ 5.39	75.94 $\pm$ 4.69
SV, ml	74.44 $\pm$ 4.67	75.63 $\pm$ 3.77		76.85 $\pm$ 4.15	80.66 $\pm$ 3.74	
HR, bpm	57.66 $\pm$ 2.34	77.69 $\pm$ 3.77*		58.30 $\pm$ 3.28	56.80 $\pm$ 2.32	

### *Systemic arterial stiffness*

The ratio of stroke volume to pulse pressure was significantly reduced during exercise and it remained lower than resting levels during PECO (all  $P < 0.05$ ). In contrast systemic arterial stiffness did not significantly change during the control trial (Figure 3.4).

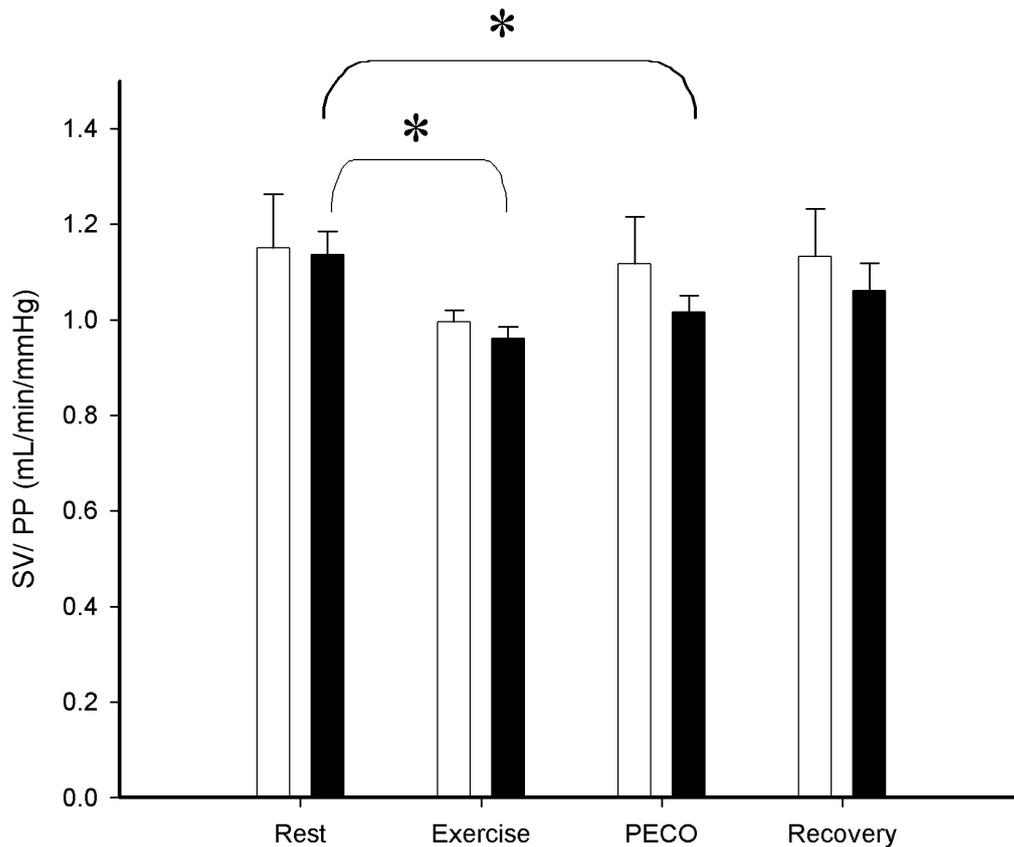


Figure 3.4: Changes in systemic arterial stiffness during the isometric handgrip exercise (black bars) and control (white bars) trials. “ \* ” denotes  $P < 0.05$  compared to rest. Data are means  $\pm$  S.E.M;  $N = 9$ .

### *Respiratory frequency*

Respiratory frequency was not different between rest, PECO and recovery in the control and exercise trials. During exercise respiratory frequency could not be accurately determined because of inadvertent performances of the Valsalva manoeuvre and irregular breathing especially towards the end of the exercise bout.

### 3.5: DISCUSSION

A ~20% increase in SPAP was observed along with an elevation in cardiac output during the performance of intense isometric handgrip exercise. This increase in SPAP was sustained during PECO, despite cardiac output returning to resting values. Therefore these findings suggest that the pulmonary vasculature is responsive to muscle metaboreflex-mediated sympathoexcitation. Studies on sheep have shown the existence of a high degree of  $\alpha$  and  $\beta$  adrenergic-mediated tone in the pulmonary circulation during treadmill exercise, yet it was concluded that the net vasoactive effect of this adrenergic influence was neutral (Kane *et al.*, 1993; 1994). On the contrary, in man concurrent elevations of plasma noradrenaline and pulmonary artery pressure observed during exercise suggest a possible SNS contribution in pulmonary hemodynamics (Richards *et al.*, 1986). In the present study, using SPAP as an index of pulmonary vascular reactivity (Balanos *et al.*, 2005; Grünig *et al.*, 2000) the observed increases in SPAP during PECO identify, for the first time, a role for the muscle metaboreflex in the regulation of the pulmonary circulation.

The notion that the pulmonary circulation is affected by sympathoexcitatory stimuli is supported by studies that show PVR increases of approximately 50% (Moruzzi *et al.*, 1988, 1989a) and 20% (Moruzzi *et al.*, 1989b) above baseline during cold pressor testing. Importantly, performance of the cold pressor test did not alter cardiac output. In fact the latter findings were obtained under conditions of constant but reduced cardiac output throughout the protocol, brought about by inflation of a balloon in the vena cava. When blood flow was unimpeded, non-significant (Moruzzi *et al.*, 1989a, 1989b) or only small (~10%) (Moruzzi *et al.*, 1988) elevations in PVR were shown during the CPT. It was argued that the observed increases in PVR were due to pulmonary venous

and/or arteriolar constriction, which was potentiated by the restriction of cardiac output (Moruzzi *et al.*, 1988).

The maintenance of heightened SPAP along with the return of cardiac output to baseline levels noted during the PECO period in the present experiment implies that vascular constriction-mediated increases in PVR took place. Although the location of constriction at the pulmonary vascular tree could not be determined by our measurements, it is tempting to speculate that vessels of small diameter played an important role in the observed augmentation in resistance. This suggestion has been shared by a number of authors (Daly *et al.*, 1970; Kadowitz *et al.*, 1974), on the basis that arteries and/or veins of small diameter are equipped with abundant smooth muscle and consequently have a greater capacity to constrict as compared to large vessels (Spencer & Leof, 1964).

This is in line with observations on steadily-perfused canine preparations in which responses to sympathetic stimuli were characterised by SPAP elevations and evidence for raised arterial stiffness of large pulmonary conduit arteries (Ingram *et al.*, 1968; Szidon & Fishman, 1971). Studies on pulsatile hemodynamics have concluded that SNS stimulation-associated decrements in the compliance of large proximal arteries represent an additional opposition to right ventricular outflow by means of hindering the ability of the vessels to accommodate the output of the right ventricle without necessitating large rises in pressure (Piene, 1976; 1986). Interestingly, it has been experimentally shown that this negative influence is ameliorated when increases in pulmonary blood flow cause distention of the vessels (Piene, 1976). We observed that the ratio of stroke volume to pulse pressure was higher during PECO as compared to rest in agreement with results from previous reports (Davies *et al.*, 2007; Edwards *et al.*, 2008). This information, along with our SPAP data and studies showing

increases in the resistance to flow in the renal (Momen *et al.*, 2003) and the inactive muscle (Hansen *et al.*, 1994) circulation during PECO collectively suggest that activation of type IV neural afferents in the muscle results in widespread arterial stiffening and/or constriction.

Arterial stiffening in the pulmonary circulation induced by SNS activation might serve to protect the vessels during the usually accompanying elevations in cardiac output by setting a limit for distension (Dowing & Lee, 1980). This could maintain pulmonary blood flow within narrow limits during exercise and in conjunction with constriction of smaller arteries might be involved in the control of blood distribution within the lungs (Elkins & Milnor, 1971; Ingram *et al.*, 1968). Additionally, the generation of high-pressure pulses towards the capillaries that is favoured from SNS-induced changes in compliance and small artery vasomotion coupled to the elevation in right ventricular stroke volume, has been postulated to account for the increased red cell velocity and the drop in transit time seen during exercise (Grover *et al.*, 1983).

On the contrary, the exaggerated resting and exercise SPAP (Himelman *et al.*, 1989) and PVR (Barberà *et al.*, 2003) that characterise pulmonary hypertension could be partly related to the stiffer proximal pulmonary arteries that have been documented in these patients as compared to normotensives (Haneda *et al.*, 1983; Laskey *et al.*, 1993; Reuben, 1971). Along the same lines, the development of high pressures in the pulmonary circuit could be favoured by the muscularisation of small arteries that is seen in pulmonary hypertension (Wagenvoort & Wagenvoort, 1983). Haneda *et al.* (1983), who found that oxygen administration in the pulmonary hypertensive patients caused increases in large artery compliance and falls in plasma noradrenaline levels, suggested that the effects of hyperoxia may be attributed to low sympathetic tone. As a

result, increased attention has been recently given to the contribution of neurohumoral activation to the systemic manifestations of chronic lung disease, which is characterised by increased SNS activity (Andreas *et al.*, 2005; Salvi *et al.*, 1999; Velez-Roa *et al.*, 2004). Likewise, implications might also concern other high-SNS activity / pulmonary hypertensive conditions such as chronic heart failure and high altitude pulmonary oedema (HAPO) (Cohn *et al.*, 1984; Duplain *et al.*, 1999). Markedly, a high correlation ( $r=0.83$ ) was noted between muscle sympathetic nerve activity and SPAP in HAPE susceptible individuals (Duplain *et al.*, 1999).

In estimating SPAP we assumed a value for RAP based on the literature (Grünig *et al.*, 2000) and that this would be unchanged throughout the protocol. Since isometric handgrip exercise has been associated with a small (~1-2mmHg) elevation in RAP (Freyschuss, 1970) in some subjects this assumption may lead to a slight underestimation of SPAP in those who respond in this manner. In favour of the latter postulation have been animal data in which muscle metaboreflex activation during exercise was shown to elicit increases in RAP (Sheriff *et al.*, 1998). However, a possible underestimation of SPAP during PECO in the present study would further strengthen our conclusions rather than negatively affect them.

Tidal volume was not measured in this study and its influence on the observed pulmonary haemodynamic changes could not be excluded during exercise (Jones *et al.*, 2004). However, subjects were continuously monitored to avoid Valsalva manoeuvres and comparable breathing patterns and respiratory frequencies were achieved throughout the duration of the two protocols. In addition, the SPAP responses to isometric exercise observed in this study are similar to previously published data that were obtained invasively (Raynolds *et*

*al.*, 1995). Anyhow the novel finding of this investigation, which is the maintenance of elevated SPAP in response to the activation of the muscle metaboreflex in the post-exercise period, is unlikely to be associated to respiratory changes given that ventilation during PECO is known to return to pre-exercise values (Houssiere *et al.*, 2006; Iellamo *et al.*, 1999; Lykidis *et al.*, 2009; Wiley & Lind, 1971).

In summary, isometric exercise-related elevations in SPAP were sustained during PECO indicating that muscle metaboreflex induced sympathoexcitation caused pulmonary vasoconstriction. This finding might be implicated in the exaggerated pulmonary haemodynamic responses to exercise seen in chronic lung and heart sympathoexcitatory conditions.

**CHAPTER 4:  
EFFECTS OF INCREASED  
METABOLIC RATE ON THE  
VENTILATORY SENSITIVITY TO  
INHALED CO<sub>2</sub>**

#### 4.1: ABSTRACT

The role of hypermetabolism in the mediation of exercise hyperpnoea is contentious. Animal data evidenced that the carotid body could be directly involved in establishing the hyperpnoea via a metabolic rate (MR)-induced elevation in the ventilatory sensitivity to CO<sub>2</sub>. An increase in the chemosensitivity to CO<sub>2</sub> in response to raised MR has been also evidenced yet this finding might be limited to the responses of central chemoreceptors. Therefore we aimed to assess the effect of hypermetabolism on chemosensitivity as it modulated by both central and peripheral receptors.

Eight healthy subjects undertook two randomly assigned trials; in one trial the ventilatory response to a five-minute hypercapnic ramp (P<sub>ET</sub>CO<sub>2</sub> = 0 to 8 mmHg above normal; P<sub>ET</sub>O<sub>2</sub> = 100mmHg) protocol was measured prior and 210 minutes after the ingestion of a fixed amount of beef steak fillet. In the other trial, the ventilatory response to the hypercapnic ramp was assessed at 0 and 210 minutes with subjects ingesting small amounts of food at regular intervals. MR was measured as oxygen consumption prior to each challenge of hypercapnia during both trials.

At 210 minutes MR was significantly increased by meat ingestion (0.23±0.02 to 0.30±0.02 L/min) whereas it did not change during the control trial (0.23±0.02 to 0.25±0.02 L/min). The ventilatory sensitivity to CO<sub>2</sub> was increased significantly in the former (1.13±0.21 to 1.89±0.18 L/min/mmHg; P<0.05), but not in the latter trial (1.22±0.20 to 1.52±0.18 L/min/mmHg; P>0.05).

Our results suggest that the hypermetabolism-induced increases in the ventilatory sensitivity to CO<sub>2</sub> are mediated primarily through elevations in the peripheral chemoreceptor gain. Enhancement of this gain appeared to be

facilitated even by mild increments of MR. Our findings could be involved in the mechanisms responsible for exercise hyperpnoea.

#### **4.2: INTRODUCTION**

Exercise hyperpnoea or the tight coupling that occurs between systemic metabolism and ventilation during exercise is considered to be one of the major remaining challenges to understand the control of human systemic function. Extensive research for over a century has indicated that the mechanisms controlling exercise hyperpnoea involve a number of feedforward (central command), proportional feedback (muscle reflex and carotid chemosensory) as well as learned elements. However, none of these mechanisms has appeared to fully account for the proportional rise of ventilation and metabolism, suggesting a degree of redundancy in this important physiological process (Bell, 2006; Mateika & Duffin, 1995; Mitchell & Babb, 2006; Ward, 2007; Whipp & Ward, 1998)

Although the increases in  $\text{VO}_2$  (i.e hypermetabolism) represent an inherent adjustment to exercise, their role in the mediation of exercise hyperpnoea has not been elucidated. It has been long argued that exercise-induced increases in metabolism could be associated with increased chemosensitivity of peripheral and/or central chemoreceptors. This implies a higher stimulus to breathe for a given partial pressure of arterial carbon dioxide ( $P_{\text{A}}\text{CO}_2$ ), which in fact remains largely unchanged during exercise of intensity lower than that eliciting the anaerobic threshold. Dynamic exercise per se has been shown to increase the ventilatory chemosensitivity to inhaled carbon dioxide in many occasions (Bannister *et al.*, 1954; Clark *et al.*, 1980, Hulsbosch *et al.*, 1981,1982; McConnel & Semple, 1996; Pianosi & Khoo, 1995; Pandit & Robbins, 1992;

Poon & Greene, 1985; Weil *et al.*, 1972), but not always (Casey *et al.*, 1987; Clark & Godfrey, 1969; Duffin *et al.*, 1980; Kelley *et al.*, 1982; Martin *et al.*, 1978). Whereas it has been thought that this lack of consensus is due to the different methodologies used (Poon & Greene, 1985), interestingly, all studies that showed negative results assessed the ventilatory responses to hypercapnia under a background of hyperoxia. Hyperoxia has been known to decrease the carotid body discharge response to carbon dioxide in animals (Figerald & Parks 1971; Lahiri & Delaney, 1975) and also to lessen the contribution of peripheral chemoreceptors to the ventilatory increases in response to hypercapnia at rest (Dahan *et al.*, 1990; Gelfand & Lambertsen, 1973; Lai & Bruce, 1997) and during exercise in humans (Forster *et al.*, 1993). Hyperoxia has also been shown to decrease ventilation during exercise (Hesse *et al.*, 1981). Studies that reported a failure of exercise to affect the ventilatory sensitivity to ramp increases of CO<sub>2</sub> against a background of hyperoxia rather suggest that central chemoreceptors are not involved in exercise hyperpnea. Phillipson and co-workers (1981) provided further support to this notion by reporting significant differences in the ventilatory responses to metabolic CO<sub>2</sub> loading between carotid body intact and carotid body-resected sheep when animals were breathing a normoxic inspirate, but the responses were similar under hyperoxic conditions. It is well known that peripheral chemoreceptors are equipped to respond to quick changes in P<sub>A</sub>CO<sub>2</sub> (Dahan *et al.*, 1990; Nye, 1990) and also that they contribute significantly more to ventilation when hypercapnic stimuli are dynamic rather than steady (O'Regan & Majcherczyk, 1982). However it should be kept in mind that the ventilatory response to a hypercapnic stimulus involves a response that arises from the ventrolateral medulla, in which signals

from both peripheral and central chemoreceptors are integrated (Poon & Greene, 1985).

The same limitations could be also applied for studies that used hyperoxic hypercapnia and found that hypermetabolism increases chemosensitivity. Therefore Zwillich *et al* (1979) showed that elevations in metabolism induced by protein ingestion resulted in enhancements in the ventilatory sensitivity to carbon dioxide assessed by the Read's re-breathing method. Recently Bin-Jaliah *et al* (2005) also evidenced in rats that the ventilatory sensitivity to CO<sub>2</sub> was increased in response to hypermetabolism. Nevertheless both investigations utilised hypercapnia under a background of hyperoxia, which could have partially silenced the peripheral chemoreceptors and consequently prohibiting an integrated response of the chemoreception system to evolve. Therefore in this study we aimed to assess whether hypermetabolism affects the ventilatory chemosensitivity to normoxic hypercapnia in awake humans. Metabolism was raised by protein ingestion and thus in absence of the other changes associated with exercise. Based on the findings by Zwillich *et al* (1979) and Bin-Jaliah *et al* (2005), we hypothesised that metabolic rate increments would result in enhancements in chemoreceptor sensitivity.

### **4.3: METHODS**

#### *Subjects*

Eight subjects (25.63 ±3.02 years; 5 men; 3 females) aged took part in the study, after they had been informed on the aims of this investigation and had given written consent. The experiment was approved by the local ethics committee. All procedures associated with the investigation were undertaken in

the Laboratory of Respiratory Function located in the School of Sport and Exercise Sciences at the University of Birmingham. Temperature in the Laboratory was kept at 20 C° and the light levels were constantly low.

Participants were free from cardiorespiratory disease, non-smokers and their fitness status ranged from untrained to moderately-trained, as it was ascertained from questionnaires. They were recruited from the general population of the University of Birmingham and from personal contacts. Female subjects were asked to participate in the first 14 days of the menstrual cycle, or at any time if they were taking the contraceptive pill. Subjects were acquainted to the procedures involved in this study during a familiarisation trial.

### *Trials*

After they completed the familiarization session, subjects were asked to report to the laboratory twice, at the same time of the day (morning to early afternoon hours). Visits, which were undertaken in a random order, were separated from each other by at least 48 hours. Participants were requested to refrain from food and caffeine consumption for 3 and 12 hours, respectively, prior to each visit. The restriction for consumption of alcohol and performance of exercise was applied for the 12-hour period prior to each visit.

### *Control trials*

At start of the control trial subjects were asked to sit quietly on a chair and breathe through a mouthpiece that was used with a noseclip to ensure exclusive mouth breathing. Subjects rested for at least 15 minutes to watch television so that they become unaware of their breathing. During the final 5 minutes of that period the basal values of  $P_{ET}CO_2$  and  $P_{ET}O_2$ .  $P_{ET}CO_2$  and

$P_{ET}O_2$  were determined. These values were used to construct the function according to which increases in  $P_{ET}CO_2$  (hypercapnia) in euoxia ( $P_{ET}O_2 = 100$  mmHg) would be forced later during the experiment. An initial metabolic rate reading was taken at zero minutes while subjects were watching television using a Douglas bag, dry-gas meter, vacuum pump and oxygen and carbon dioxide analysers. Immediately after the initial metabolic rate reading the first  $P_{ET}CO_2$  forcing procedure was imposed by the DEF. In the forcing procedure of the ramp experiment  $P_{ET}CO_2$  was initially maintained for 80 seconds at 2 mmHg above normal as pilot data indicated that this minimised ventilatory fluctuations thereafter. Then  $P_{ET}CO_2$  was increased at a rate of 0.83 mmHg/min so that at the end of the ramp  $P_{ET}CO_2$  reached 8 mmHg above baseline.  $P_{ET}O_2$  was maintained at 100 mmHg at all times during the ramp protocol. A schematic representation of the  $P_{ET}CO_2$  during the protocol is shown in figure 4.1. The metabolic rate readings and hypercapnic bouts were repeated at 210 minutes after. Subjects were administered one and a half cream crackers each hour so as to maintain metabolic rate at constant levels. During each of the hypercapnic exposures, heart rate and blood saturation were monitored to ensure the safety of participants. In addition during each of these exposures and metabolic rate measurements subjects were watching television.

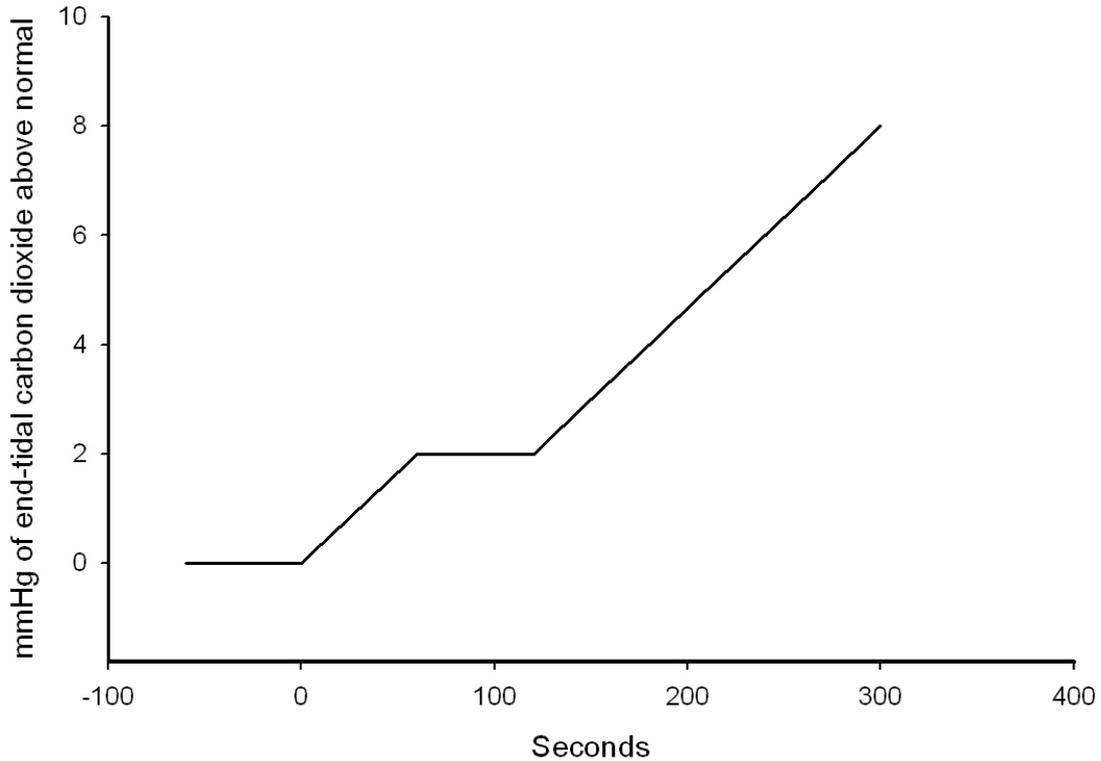


Figure 4.1: Pattern of  $P_{ET}CO_2$  increases in the hypercapnic challenge.

### *Steak trial*

The steak trial had an identical design to the control, with the only difference being that subjects ingested a lean and well-cooked beef lean steak fillet (mean raw weight of  $545 \pm 50$  grams; mean calorific value 980 kcal) immediately after the initial baseline metabolic rate measurement and hypercapnic exposure took place. Subjects were instructed to consume the food within 15 minutes. No crackers were administered. All other measurements were identical to the control protocol.

### *Metabolic rate measurements*

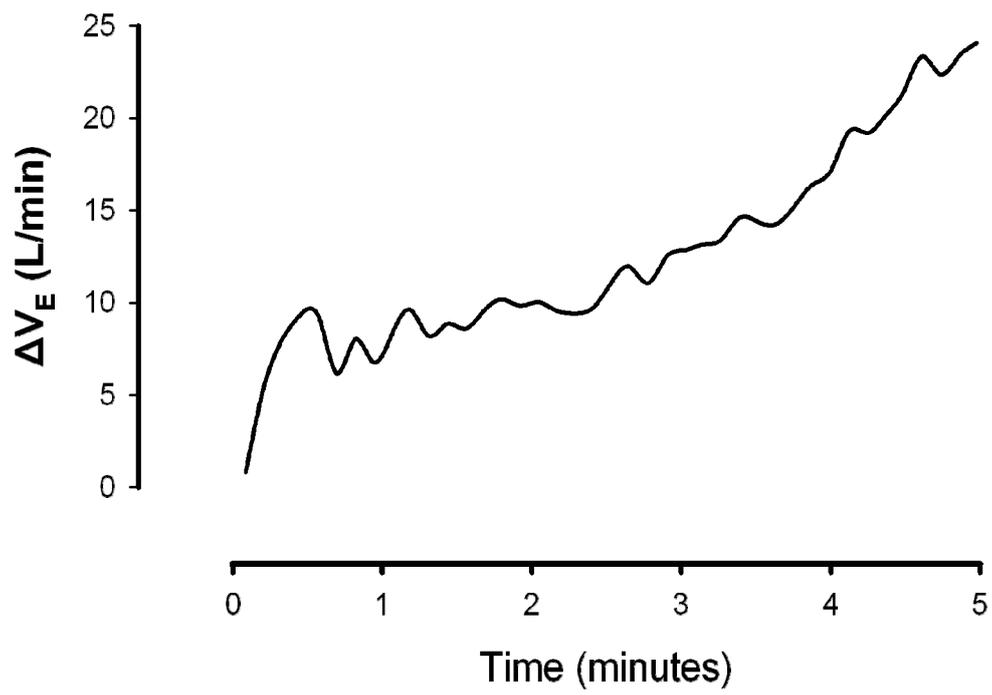
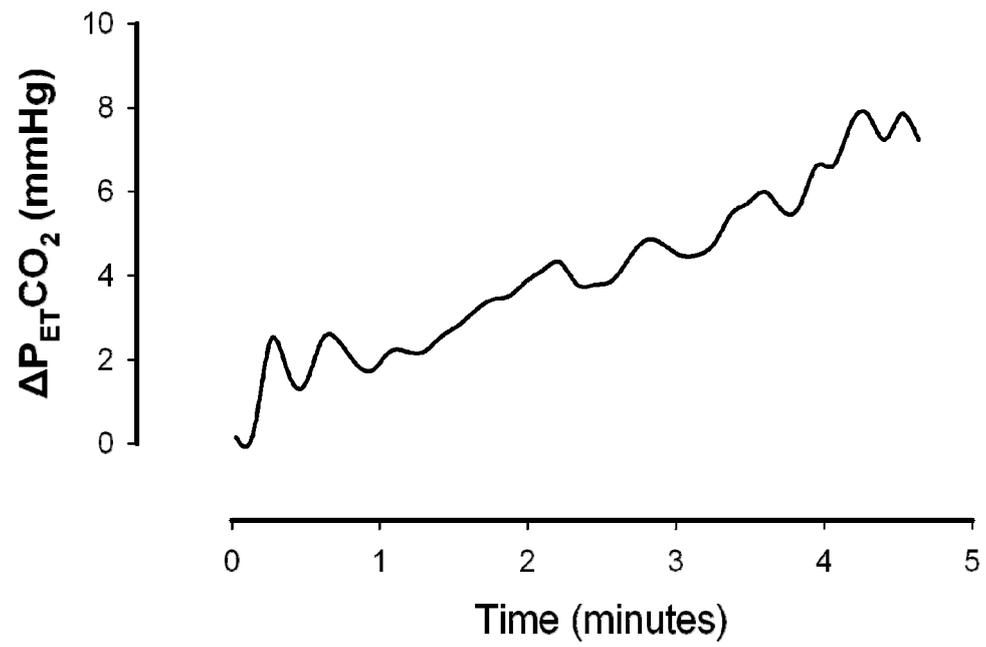
Metabolic rate measurements were conducted using standard indirect open-circuit techniques (indirect calorimetry). Prior each trial the gas analyser was calibrated against gases of known concentrations. A mouthpiece and a noseclip were used to collect expired air in a Douglas bag over a period of 3 minutes. Collection of gases was initiated and finished at end-expiration. After the sample was collected the bag was agitated to ensure consistency of gas concentration throughout, and was analysed for volume, temperature and for fractions of O<sub>2</sub> and CO<sub>2</sub>. The volume taken in acquiring the percentage concentrations, calculated by: flow rate (20ml/sec) x sample time (30 seconds), was also added to the end volume. These volumes were then converted from ambient temperature, pressure and saturated water vapour (ATPS) to standard temperature, pressure and dry (STPD). Subsequently O<sub>2</sub> consumption (VO<sub>2</sub>) CO<sub>2</sub> production, minute ventilation and non-protein Respiratory Quotient (RQ) were calculated using the Haldane transformation of the Fick equation.

### *Ventilatory measurements*

Given that in healthy population and at resting conditions P<sub>ET</sub>CO<sub>2</sub> approximates P<sub>A</sub>CO<sub>2</sub>, the ventilatory responses were related to P<sub>A</sub>CO<sub>2</sub> by the equation  $V_E = S(P_ACO_2 - B)$ , where S is the slope of the response line and B is the extrapolated intercept on the 'x' axis.

Slopes and intercepts of V<sub>E</sub> vs P<sub>ET</sub>CO<sub>2</sub> for the ramp protocol were calculated taking in account P<sub>ET</sub>CO<sub>2</sub> values (and their corresponding V<sub>E</sub> values) that ranged between 3 and 8 mmHg above normal (Figure 4.1a and 4.1b).

A



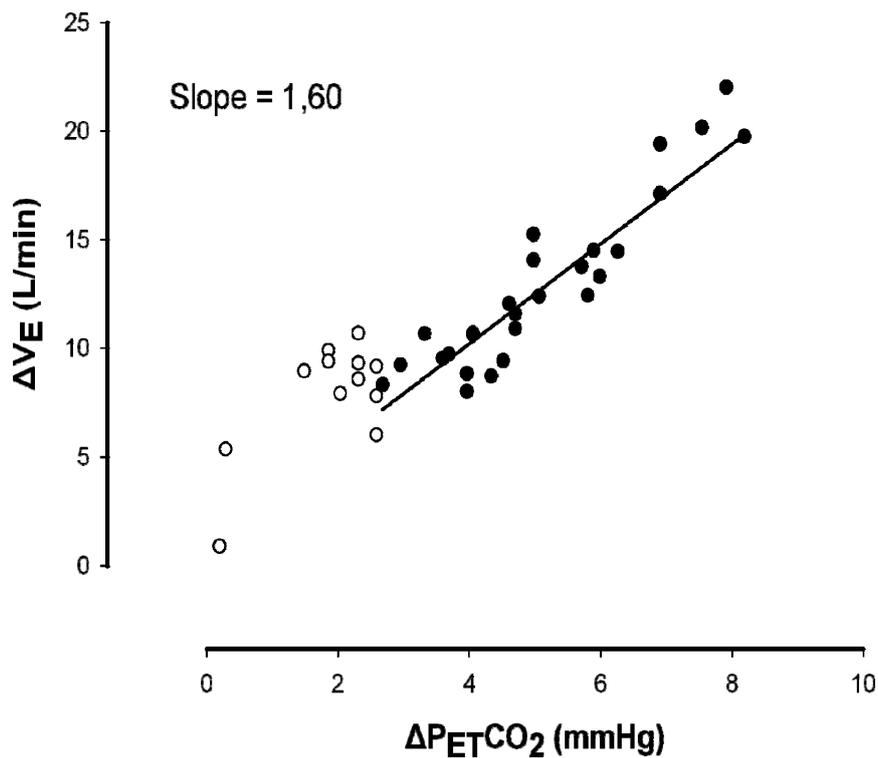
**B**

Figure 4.1: A) Example traces from one subject, showing the increase in  $P_{ET}CO_2$  (at top) and ventilation (bottom). B) Relationship between changes in ventilation and end-tidal  $CO_2$ . Changes in ventilation are plotted as a function of changes in end-tidal  $CO_2$ . A regression line has been fitted to the data.

### *Statistical methods*

The effect of increased metabolic rate on the ventilatory chemosensitivity to  $CO_2$  was analysed by means of repeated measures 2-way ANOVA (2x2; time vs condition). If appropriate this was followed by paired t tests that were adjusted for multiple comparisons (SPSS 16.0). Data are expressed as means  $\pm$  S.E.M. Significance was taken at  $P < 0.05$ .

#### 4.4: RESULTS

Ingestion of the beef steak brought about increases in mean  $\dot{V}O_2$  ( $0.23 \pm 0.02$  to  $0.30 \pm 0.02$  L/min;  $P < 0.0002$ ) (Figures 4.3, 4.3b)  $\dot{V}CO_2$  and RQ (Table 1). In contrast no significant changes in oxygen consumption were noted during the control trial (Figures 4.3, 4.3b). Pre-ramp mean  $P_{ET}CO_2$ , which was obtained while subjects were breathing medical grade air, was not different compared to baseline between trials (Table 4.1;  $P > 0.05$ ). Steak ingestion induced increases in the slope ( $1.12 \pm 0.21$  to  $1.89 \pm 0.18$  L/min/mmHg;  $P < 0.05$ ) but not in the intercept of the ventilation- $P_{ET}CO_2$  relationship ( $36.82 \pm 1.02$  to  $37.17 \pm 0.97$  mmHg;  $P > 0.05$ ; Figure 4.4). Cracker ingestion had no effect on either slope ( $1.24 \pm 0.20$  to  $1.52 \pm 0.18$  L/min/mmHg) or intercept ( $36.44 \pm 0.92$  to  $36.72 \pm 0.97$  mmHg; all  $P > 0.05$ ; Figure 4.4).

Table 4.1: Respiratory data obtained in the control and steak trials. \* indicates  $P < 0.05$  versus baseline (mean  $\pm$  S.E.M; N=8).

		Control trial		Steak trial	
		0 min	210 min	0 min	210 min
<b>P<sub>ET</sub>CO<sub>2</sub> (mmHg)</b>	<i>Mean</i>	37.05	37.32	37.65	37.74
	<i>SEM</i>	1.32	0.61	1.29	1.18
<b>VCO<sub>2</sub> (L/min)</b>	<i>Mean</i>	0.18	0.20	0.18	0.23*
	<i>SEM</i>	0.01	0.01	0.01	0.01
<b>RQ</b>	<i>Mean</i>	0.79	0.84	0.79	0.75*
	<i>SEM</i>	0.01	0.03	0.02	0.02

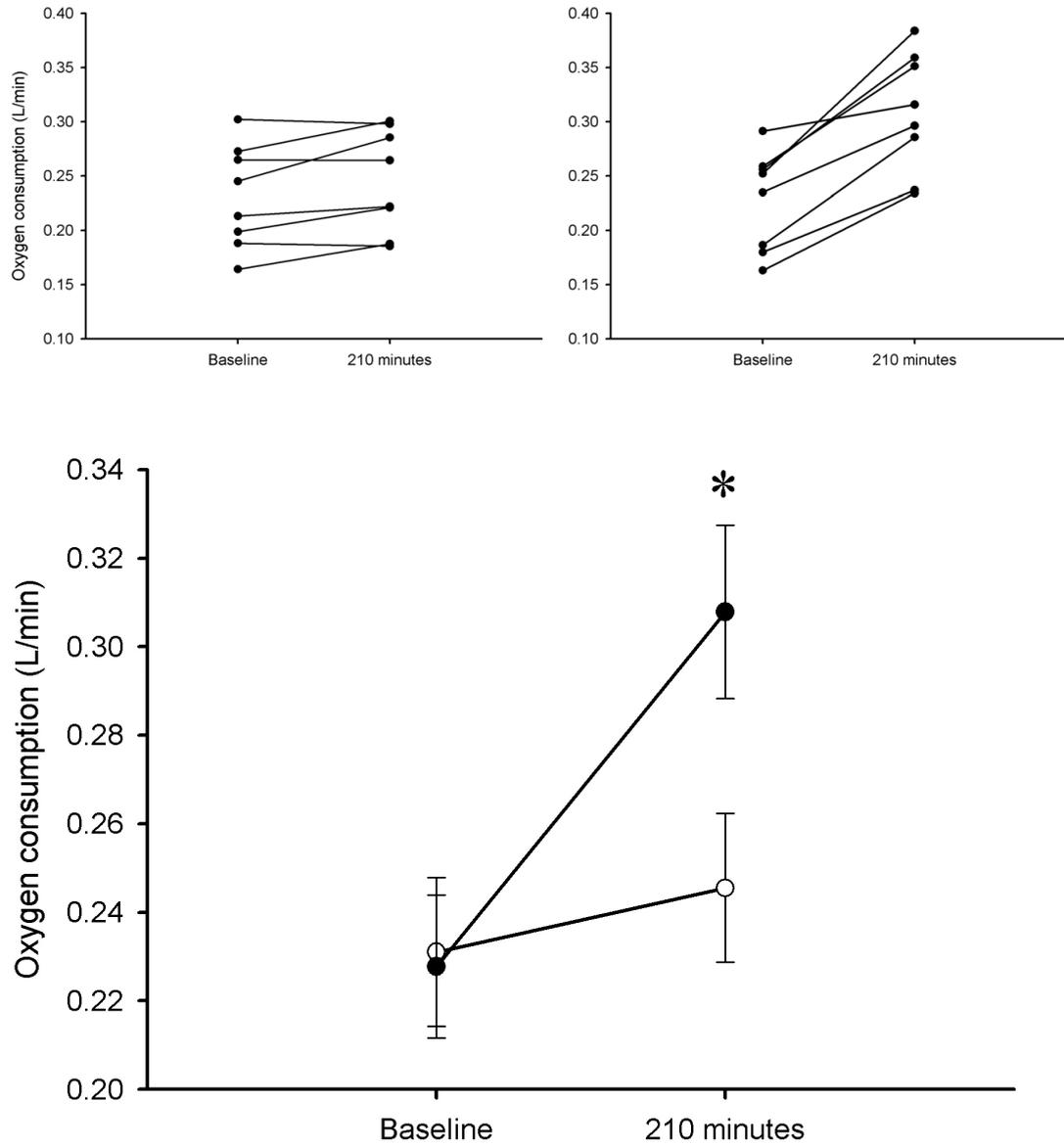


Figure 4.3: A (top panels); individual values for oxygen consumption during the control (left) and steak (right) trials. B (lower panel); mean ( $\pm$ S.E.M) oxygen consumption obtained during the control (open symbols) and steak (closed symbols) trials (N=8).

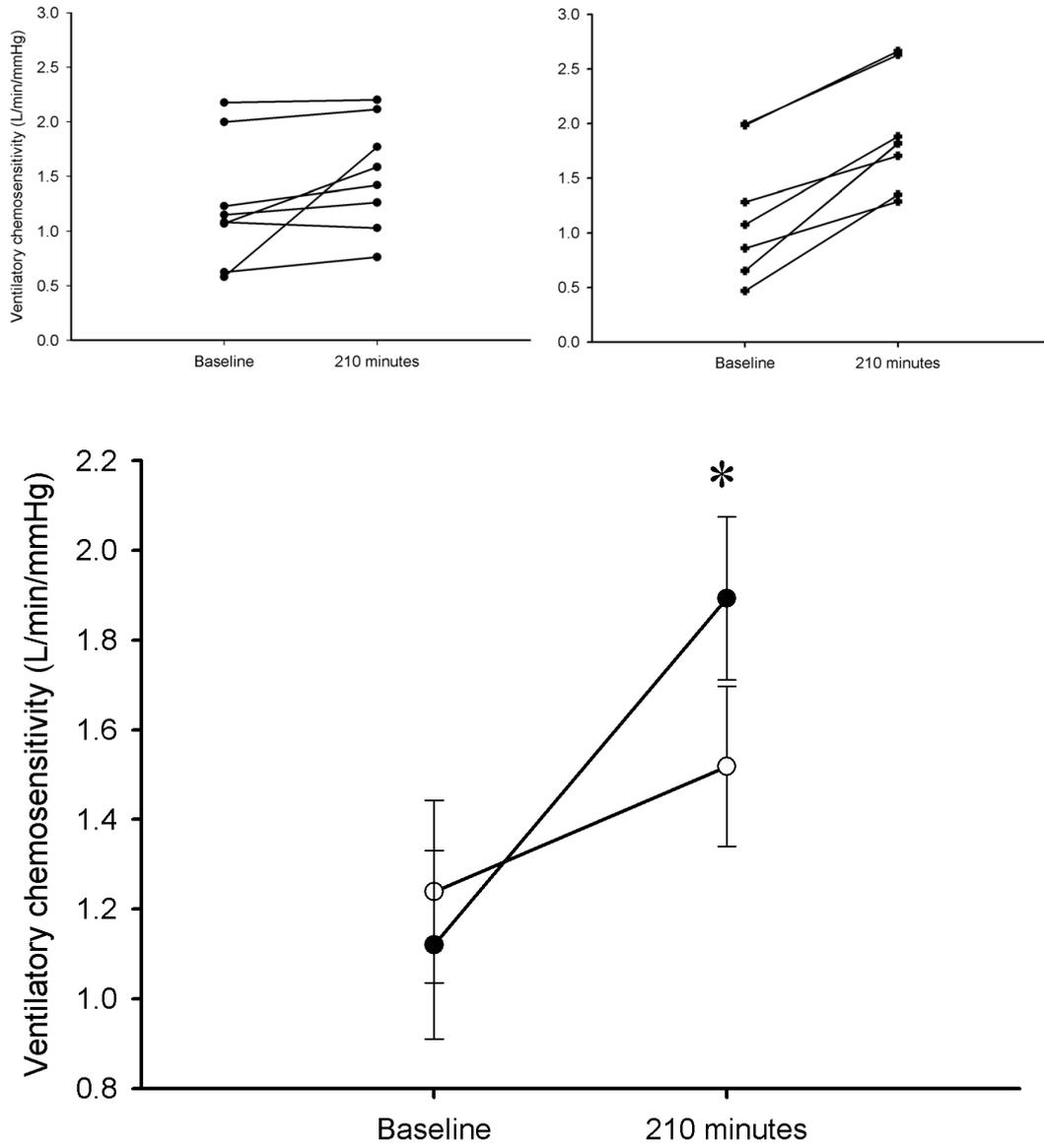


Figure 4.4: A (top panels); individual values for ventilatory sensitivity during the control (left) and steak (right) trials. B (lower panel); mean ( $\pm$ S.E.M) values for ventilatory chemosensitivity obtained during the control (open symbols) and steak (closed symbols) trials (N=9).

#### 4.5: DISCUSSION

The main finding of this investigation was that ventilatory chemosensitivity to normoxic hypercapnia was enhanced by mild increases in metabolic rate induced by ingestion.

Our findings are in agreement with Zwillich *et al* (1979), who showed that metabolic rate increases brought about by ingestion of protein oral bolus induced elevations in the ventilatory chemosensitivity to hyperoxic hypercapnia. A high protein diet administered by intravenous infusion was also found to increase the ventilatory sensitivity to hypercapnia in patients with nutritional depletion compared to a low protein diet (Askanazi *et al.*, 1984). This effect of protein could be associated with its ability to induce a higher thermal effect as compared with the other nutrients (Dagenais *et al.*, 1966; Nair *et al.*, 1983; Raben *et al.*, 2003; Robinson *et al.*, 1990). In close relation to these results it has been evidenced that ventilatory sensitivity is elevated in hypermetabolic states (Engel & Ritchie, 1971; Zwillich *et al.*, 1978) and decreased in hypometabolic states (Zwillich *et al.*, 1975). Specifically in hyperthyroidism, the increased respiratory response to hypercapnia was attributed to the increased adrenergic activity and increased plasma catecholamine levels seen in this condition (Engel & Ritchie, 1971). It has been also long known that either catecholamines (Cunningham *et al.*, 1958; Maskell *et al.*, 2006) or increased sympathetic nervous system activity (Acker & O'Regan, 1981; O'Regan, 1981) can enhance the sensitivity of the carotid body to CO<sub>2</sub>.

It is established that ingestion of protein is followed by sympathoexcitation that is evident several hours in the post-prandial state and serves the

maintenance of cardiac output in face of mesenteric vasodilation. The degree of postprandial sympathoexcitation has been shown to be directly related to the calorific value of the meal (Diamond and LeBlanc, 1987; Soucy & LeBlanc 1999; Van Baak, 2008). Although there are no available data to support that protein ingestion elicited sympathoexcitation in our experiment, we are confident that ingestion of a 580 g steak with a calorific value of approximately 980 kcal was enough to induce postprandial sympathetic activation. Against the notion that ingestion-induced sympathoexcitation results in elevated chemosensitivity, Zwillich *et al* (1979) observed unchanged cardiovascular variables yet enhanced ventilatory responses to hypercapnia following administration of a protein bolus. This lack of cardiovascular change in that study might be related to the reduced palatability of the protein bolus (Le Blanc & Brondel, 1985). Bin-Jaliah *et al* (2005) showed increases in chemosensitivity after metabolic rate was raised by insulin infusion, which has been known to elevate SNS activity (Fagius *et al.*, 1986). Bin-Jaliah *et al* (2005) noted this increase only in carotid body-intact animals but not in the carotid-resected ones, and consequently they suggested that the carotid body was involved in the mediation of elevated chemosensitivity. A role of the carotid body in sensing metabolism and adjusting ventilation has been also apparent in an earlier study, which reported that the ventilatory response to venous VCO<sub>2</sub> loading in carotid body-denervated sheep was markedly reduced as compared to the carotid body intact animals (Phillipson *et al.*, 1981). Therefore it is likely that the increases in metabolism brought about by protein ingestion might have increased the sensitivity of the carotid body to CO<sub>2</sub>. On a theoretical basis, our postulation is also in line with the widely

supported 'oscillations hypothesis', which proposes that the rate sensitivity of the carotid body to changes in metabolic rate could account for the precise matching of ventilation and carbon dioxide production at rest and during exercise (Mateika & Duffin, 1995; Saunders 1980).

The ventilatory chemosensitivity increases we observed could also be accounted for by increases in circulating potassium concentrations usually seen after protein ingestion (Kontessis *et al.*, 1990; Zwillich *et al.*, 1979). Increased blood  $K^+$  has been known to stimulate the carotid body (Band *et al.*, 1985) and has attracted considerable attention as a candidate for exercise hyperpnea (Paterson, 1992). Against the role of potassium in hypermetabolism-mediated increases in  $CO_2$  chemosensitivity have been the results by Bin-Jaliah *et al* (2005), who noted decreases in circulating potassium along with increased chemosensitivity following increases in ventilation. Whereas insulin infusion has been known to decrease plasma potassium via an  $\alpha$ -adrenoreceptor mechanism (Fisher *et al.*, 1991; Strakosch *et al.*, 1976) it is established that during exercise there is a catecholamine-driven suppression of insulin secretion (Galbo *et al.*, 1977) and an increase in potassium blood levels (Paterson, 1992).

Alternatively, the ventilatory sensitivity to  $CO_2$  we observed post-protein ingestion could also be accounted for by increases in body temperature (Vejby-Christensen and Petersen, 1973) that are commonly (Brundin & Wahren, 1994; Burton & Murlin, 1935; Johnston *et al.*, 2002), but not always (Zwillich *et al.*, 1979), observed post-feeding. Nevertheless the increases elicited by digestion are usually small as compared to that needed in order to induce a significant ventilatory increment at rest ( $>1^\circ C$ ) (White, 2006). Finally plasma concentrations of growth hormone, which

have been seen to increase after protein ingestion (Sukkar *et al.*, 1968), could have contributed to the elevation we observed in ventilatory chemosensitivity (Grunstein *et al.*, 1994).

It has been shown that ventilatory chemosensitivity increases at the transition of rest to exercise, or early during exercise, and then it plateaus (Weil *et al.*, 1972) or even decreases (Clark *et al.*, 1980) as the maximal capacity is approached. Increases of 253% and 130% in metabolic rate in these experiments elicited elevations of 30% (Clark *et al.*, 1980) and 55% (Weil *et al.*, 1972) in  $V_E/P_{A\text{CO}_2}$  slope, respectively. The ~20% increase in metabolic rate after protein ingestion in our subjects raised chemosensitivity from 1.12 to 1.89 L/min/mmHg (approximately 70%). A comparison between slopes obtained from exercise slopes and our data may not be valid since we raised metabolic rate independently of limb movement (Biscoe & Purves, 1967) and exercise-related mental factors (Shea, 1996) that could influence ventilation and/or chemosensitivity. Our results are comparable with these from Zwillich *et al* (1979) who showed that a ~10% elevation in metabolism induced an increase in chemosensitivity from 2.8 to 3.6 L/min/mmHg (about 30%). The appreciably lower slope values we reported could be due to the measures that we took in order to minimise the anxiety associated with CO<sub>2</sub> inhalation. Therefore we had familiarised our subjects to the procedures prior to the experimental day and in addition we asked them to watch television during measurements of chemosensitivity. A failure to address the anxiety factor could elevate the ventilatory response to carbon dioxide and yield greater slopes (Singh, 1984). Anyhow, the slopes reported in the

current investigation lie within previously reported ranges (Rebuck & Read, 1971; Saunders *et al.*, 1978).

Our methodological approach did not fully take in account the central chemoreceptor constant time or the time required for ventilation to rise from zero to the 63% of its steady state value due to central chemoreceptor stimulation by CO<sub>2</sub>. Central constant time has been reported to range from 89 to 118 seconds (Berger *et al.*, 1973; Gelfand & Lambertsen, 1973) whereas 80 seconds were allowed in the current investigation before the ramp increases in CO<sub>2</sub> were forced by the DEF system. However, the interpretation of our results could not be confounded by this limitation since it was applied to all trials of this investigation. The increases in the ventilatory sensitivity to CO<sub>2</sub> we observed after metabolic rate was elevated by food ingestion could be implicated in the mechanisms mediating exercise hyperpnoea. Inclusion of cardiovascular data, body temperature and blood analyses for catecholamines, K<sup>+</sup> and acidity in this investigation would undoubtedly provide an insight on the mechanisms mediating the effect of increased metabolic rate.

In summary, we observed increases in the ventilatory chemosensitivity to carbon dioxide in response to mild elevations in metabolic rate induced by protein ingestion. The effects of hypermetabolism on chemosensitivity are likely to be mediated by the carotid body. Our findings could be implicated in the mechanisms responsible for the precise matching of ventilation to metabolic rate at rest and during exercise.

**CHAPTER 5:  
A RESPIRATORY RESPONSE  
TO THE ACTIVATION OF THE  
MUSCLE METABOREFLEX  
DURING CONCURRENT  
HYPERCAPNIA IN MAN**

**5.1: ABSTRACT**

In this study we aimed to assess the ventilatory and cardiovascular responses to the combined activation of the muscle metaboreflex and the ventilatory chemoreflex, achieved by PECO and euoxic hypercapnia (end-tidal  $\text{PCO}_2 = 7$  mmHg above normal), respectively. Eleven healthy subjects (4 women, 7 men;  $29 \pm 4.4$  yrs; mean  $\pm$  SD) undertook four trials, in random order; 2 minutes of isometric handgrip exercise followed by 2 minutes of PECO with hypercapnia, 2 minutes of isometric handgrip exercise followed by 2 minutes of PECO while breathing room air, 4 minutes of rest with hypercapnia and 4 minutes of rest while breathing room air. Ventilation ( $V_E$ ) was significantly increased during exercise in both the hypercapnic ( $+3.17 \pm 0.82$  L/min) and the room air breathing trial ( $+2.90 \pm 0.26$  L/min; all  $P < 0.05$ ). During PECO  $V_E$  returned to pre-exercise levels when breathing room air ( $+0.52 \pm 0.37$  L/min;  $P > 0.05$ ) but it remained elevated in hypercapnia ( $+3.77 \pm 0.23$  L/min;  $P < 0.05$ ). The results indicate that the muscle metaboreflex stimulates ventilation with concurrent chemoreflex activation. These findings have implications for disease states where effort intolerance and breathlessness are linked.

## 5.2: INTRODUCTION

The involvement of muscle reflexes in the cardiovascular control during exercise has been established (Rowell and O'Leary, 1990). Similarly, the role of chemoreceptors in the regulation of ventilation at rest and during exercise has been also extensively appraised in the literature (Dejours, 1962; Whipp, 1994). In contrast, the interaction between these two mechanisms has received circumstantial attention (Makeham *et al.*, 2004) although there could be situations in which the muscle reflexes and the chemoreceptors are operating in concert.

Such a situation could be presented by CHF, in which the importance of the impaired reflex cardio-respiratory control system in disease progression has been recently emphasized (Coats, 2001). Particular consideration has been paid to the muscle metaboreflex, which denotes the reflex activation of sympathetic activity following stimulation of metabolically-sensitive receptors in exercising muscle, and the CO<sub>2</sub> ventilatory chemoreflex (Schmidt *et al.*, 2005). Both reflexes are overactive in CHF and the degree of their overactivation is an independent prognostic marker of exercise ventilatory abnormalities and disease status (Chua *et al.*, 1996; Ponikowski *et al.*, 2001). Overactivation of the muscle metaboreflex in CHF has been attributed to alterations in muscle structure and biochemistry (Piepoli *et al.*, 1999), an impaired baroreflex function (Kim *et al.*, 2005) and also the incapacity to enhance cardiac performance and stroke volume during exercise (Crisafulli *et al.*, 2008). In contrast, the mechanisms underlying the overactivity of the ventilatory chemoreflex remain largely obscure (Chua *et al.*, 1996)

Ponikowski *et al.*, (2001), who evidenced that muscle metaboreflex activation was a strong predictor of ventilatory sensitivity to hypercapnia in CHF patients, argued for the existence of an interaction between the two reflexes. It was suggested that signals arising from metaboreceptors might interact in the central nervous system with input from chemoreceptors. This in turn would lead to an enhanced ventilatory chemosensitivity implying greater ventilation for a given partial pressure of  $P_{A}CO_2$ . This suggestion, which has been widely adopted in the attempt to explain the exaggerated exercise ventilation in CHF, has not been experimentally verified (Chua *et al.*, 1996; Coats, 2001; Piepoli *et al.*, 1999; Schmidt *et al.*, 2005; Tumminello *et al.*, 2007). Such information would be of importance in delineating the role of reflex cardio-respiratory control systems in the abnormal ventilatory responses to exercise in CHF, given that both the metabo- and the chemoreflex are known to be hyperactive in these patients. Therefore the aim of this study was to assess the respiratory and cardiovascular responses to the combined activation of the ventilatory chemoreflex and the muscle metaboreflex, achieved by hypercapnia and PECO, respectively, in healthy humans. Circulatory occlusion following isometric exercise has been known to maintain the elevated sympathetic activation seen during exertion without having an effect on ventilation, which returns to pre-exercise levels (Houssiere *et al.*, 2006; Iellamo *et al.*, 1999). Likewise, it has been shown that the sympathoexcitatory stimuli of lower body negative pressure and head-up tilt have no effect on ventilation under eucapnic conditions, but they significantly enhance ventilation when imposed against a hypercapnic background (Howden *et al.*, 2004; Jordan *et al.*, 2000). Based on the latter findings, we hypothesised that ventilation during PECO under hypercapnic conditions would be greater to that seen with eucapnia, implying a

stimulatory effect of the combined activation of the muscle metaboreflex and the ventilatory chemoreflex on ventilation.

### **5.3: METHODS**

#### *Subjects*

Eleven healthy participants (4 women, 7 men;  $29 \pm 4.4$  yrs; mean  $\pm$  SD) took part in this study. All participants had received verbal and written instructions on the experimental procedures before they gave informed written consent. The investigation was performed according to the Declaration of Helsinki and was approved by the Local Ethics Committee. Selection of hypercapnic and exercise intensity levels that were utilised in the procedures, had been made during pilot work based on reports of minimum discomfort by participants. Participants were not taking any medication, were non-smokers and underwent a preliminary trial during which they became familiarised to the procedures of the study. Female participants were asked to participate during the follicular phase of their menstrual cycle. All experimental procedures took place in our laboratory at which temperature was kept at 21°C.

On the experimental day, participants reported to the laboratory having abstained from consumption of food and caffeine-containing beverages for 4 hours. Restriction for performance of strenuous physical activity and consumption of alcohol was also applied for 12 hours prior to the visit. Upon reporting to the laboratory, participants assumed a seated position and were asked to breathe through a mouthpiece that was used with a nose clip. The MVC force of the forearm muscles at the dominant arm was then measured in triplicate by a handgrip dynamometer (Lafayette Hand Dynamometer; model

70718; Lafayette Instrument Company). Subjects were instructed to maximally squeeze the dynamometer and maintain this force for 3 seconds with their dominant hand. The mean of the three maximal contractions was regarded as the MVC, provided that they were not different by more than 10%. Participants continued to rest quietly in the seated position for 15 minutes in order for the normal  $P_{ET}O_2$  and  $P_{ET}CO_2$  to be determined. During this 15 minute period subjects were watching television so that they became unaware of their breathing. Respired gas was sampled continuously via a catheter attached to the mouthpiece and analysed for  $PCO_2$  and  $PO_2$  by a mass spectrometer (AirSpec 2000, Case Scientific, London, UK). Partial pressures of  $O_2$  and  $CO_2$  were sampled by a computer and breath-by-breath end-tidal gases were identified and recorded with the use of dedicated software.

### *Trials*

Participants then undertook four trials in a random order. The trials were separated by 30 minutes of rest. A schematic representation of the four trials is shown in Figure 5.1. Before each trial and whilst subjects were breathing room air, one minute of resting ventilatory and cardiovascular data was recorded for baseline purposes. The four trials which then followed were: i) CHEMO, during which the ventilatory chemoreflex was investigated by exposing the participants to euoxic hypercapnia ( $P_{ET}O_2=100$  mmHg,  $P_{ET}CO_2=7$  mmHg above normal) whilst they rested for 12 minutes, ii) ERGO, during which participants breathed room air whilst they performed 2 minutes of isometric handgrip exercise at 40% of the MVC followed by a further 2 minutes of PECO, iii) CHEMO+ERGO, during which participants were exposed to euoxic hypercapnia ( $P_{ET}O_2=100$  mmHg,  $P_{ET}CO_2=7$  mmHg above normal) whilst they performed 2 minutes of

isometric handgrip exercise followed by a further 2 minutes of PECO, and iv) CONTROL, during which participants breathed room air whilst they rested for 12 minutes. All trial periods were followed by 2 minutes of recovery data collection before the rest period began.

To ensure that steady state had been achieved in the ventilatory response to euoxic hypercapnia in the CHEMO and CHEMO+ERGO trials, a period of 8 minutes was allowed before any further intervention was performed. An 8-minute period was also applied to ERGO and CONTROL trials for consistency. Subjects were instructed to breathe normally without performing abnormal respiratory manoeuvres at all times. Subjects were watching television at all times except during exercise.

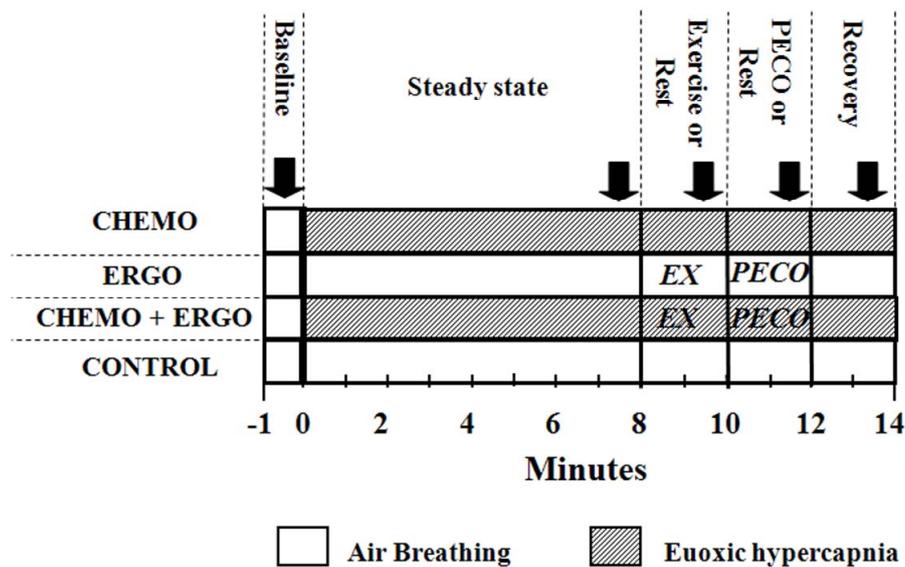


Figure 5.1: Schematic representation of the protocol. 'EX' signifies period of isometric forearm exercise. Arrows indicate 1-minute periods over which measurements of ventilation, blood pressure and heart rate were averaged.

### *Isometric exercise and PECO*

During handgrip exercise participants had visual feedback from a monitor in order to maintain force output as close to the predetermined 40% of their MVC as possible. This relative tension was chosen because it was associated with minimal discomfort when PECO was superimposed to hypercapnia in preliminary trials, while facilitating the ischaemic conditions required for activation of the muscle metaboreflex (Houssiere *et al.*, 2006; Zwartz *et al.*, 2004) Participants were asked not to tense any other muscles apart from those of the forearm, and in addition not to stop squeezing the handgrip dynamometer at any time during exercise to prevent metabolite release into the systemic circulation and a diminished activation of the muscle metaboreflex during the subsequent PECO. PECO was induced 5 seconds prior to the cessation of exercise by rapid inflation to 200mmHg of a pneumatic cuff around the upper arm (E20 Hokanson System, Hokanson, Bellvue, USA).

### *Cardiovascular measurements*

Mean arterial pressure (MAP) and HR were measured continuously using finger photoplethysmography (Portapress, Finapress Medical Systems, the Netherlands) and a three-lead ECG (Micromon 7141 monitor, Kontron Medical, France), respectively. The middle finger of the non-exercising arm, which was rested at heart level, on the armrest of the chair, was used for measurement of blood pressure. The blood pressure and ECG signals were sampled by an analog-to-digital converter (Cambridge Electronic Design 1401 plus, Cambridge, UK). Data were recorded and displayed using Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

Spontaneous baroreflex sensitivity (SBRS) at the operating point of the baroreflex function curve was assessed offline using the sequence technique, which involved detecting sequences of three or more successive beats where systolic blood pressure and RR interval were either both increasing or both decreasing. Regression equations from these sequences of systolic blood pressure (x-axis) and RR interval (y-axis) provided slope values representative of SBRS and provided intercept values (Iellamo *et al.*, 1999). Only those regressions with  $r^2 > 0.95$  were accepted. The minimum cut-off number of sequences for inclusion in analysis was 3 per time interval.

### *Measurements*

Ventilation, blood pressure (Portapress, Finapress Medical Systems, The Netherlands) and HR were continuously recorded throughout the duration of the protocol. Minute averages were calculated for baseline, steady state, second minute into exercise, second minute of PECO and second minute of recovery.

### *Statistical methods*

Differences between baseline values across trials were tested by means of one-way ANOVA for repeated measures. Local weighted scatter plot smoothing was applied in the mean ventilation curves from baseline until the end of steady state for the CHEMO and CHEMO+ERGO trials. This revealed the same distinct ventilatory pattern in both trials; one steep increase followed by a plateau, which was maintained until the end of the exposure, verified that steady state was achieved within approximately 5 minutes. This is in accordance with the results from Berkenbosch *et al* (1989) who, similarly to the

present investigation, used end-tidal forcing to induce hypercapnia. Two-way ANOVA for repeated measures were used to examine differences between trials. When appropriate, multiple-comparison adjusted post hoc tests were then applied to reveal differences. Data are expressed as means  $\pm$  S.E.M. and significance ( $P < 0.05$ ) was tested by means. All statistical analyses were conducted by using a standard statistical package (SPSS, Chicago, IL).

#### **5.4: RESULTS**

##### *Changes taking place from baseline to steady state*

Mean respiration, HR and MAP values at baseline and steady state are presented in table 5.1. When breathing room air, no significant differences in ventilation were observed between baseline and steady state values in the ERGO and CONTROL trials, and HR and MAP values during steady state were also unchanged from those at baseline ( $P > 0.05$ ). However, exposure to euoxic hypercapnia caused minute ventilation, to almost double from baseline levels, at steady state, in both the CHEMO and CHEMO+ERGO trials. The slight increases in mean HR and MAP noted during steady state compared to baseline in these latter two trials were not significant (MAP;  $P = 0.073$ , HR;  $P = 0.17$ ).

Table 5.1: Mean values for measurements taken before any intervention (baseline) and after eight minutes of rest (steady state) under conditions of either room air breathing (CONTROL and ERGO trials) or euoxic hypercapnia (CHEMO and CHEMO+ERGO trials). # signifies significant difference from baseline levels;  $P < 0.001$ ,  $N = 11$ .

		<b>CONTROL</b>	<b>CHEMO</b>	<b>ERGO</b>	<b>CHEMO+ERGO</b>
$V_E$ (L/min)	<i>Baseline</i>	8.13 ± 0.59	9.10 ± 0.66	8.23 ± 0.63	9.17 ± 0.58
	<i>Steady state</i>	8.26 ± 0.54	19.53 ± 1.00#	7.94 ± 0.57	19.22 ± 1.04#
MAP (mmHg)	<i>Baseline</i>	93 ± 3.8	96 ± 2.5	96 ± 2.8	92 ± 4.8
	<i>Steady state</i>	96 ± 4.0	103 ± 3.4	97 ± 2.7	98 ± 4.9
HR (bpm)	<i>Baseline</i>	65 ± 2.5	66 ± 3.3	63 ± 2.9	68 ± 3.8
	<i>Steady state</i>	66 ± 2.3	70 ± 3.5	64 ± 2.9	71 ± 4.3

*Changes with respect to steady state*

Any further changes from the steady state values that were induced in ventilation during each of the trial conditions are shown in Figure 5.2. Exercise while breathing room air (ERGO trial) induced a significant increase of 2.90 L.min<sup>-1</sup> in ventilation compared to steady state. Exercise under hypercapnic conditions (CHEMO+ERGO trial) also brought about a similar, significant, increase in ventilation (~3 L/min) compared to steady state. However, during PECO, in the ERGO trial  $V_E$  fell back to steady state levels ( $P>0.05$  vs steady state) but in the CHEMO+ERGO trial,  $V_E$  remained elevated at the levels seen during exercise throughout this phase of the experiment ( $P<0.05$  vs steady state) (see Figure 5.2). During the Recovery phase of the ERGO and CHEMO+ERGO trials,  $V_E$  was not significantly different from steady state values ( $P>0.05$ ). In the CHEMO and CONTROL trials ventilation was not further altered from steady state values, during any phase of the trials.

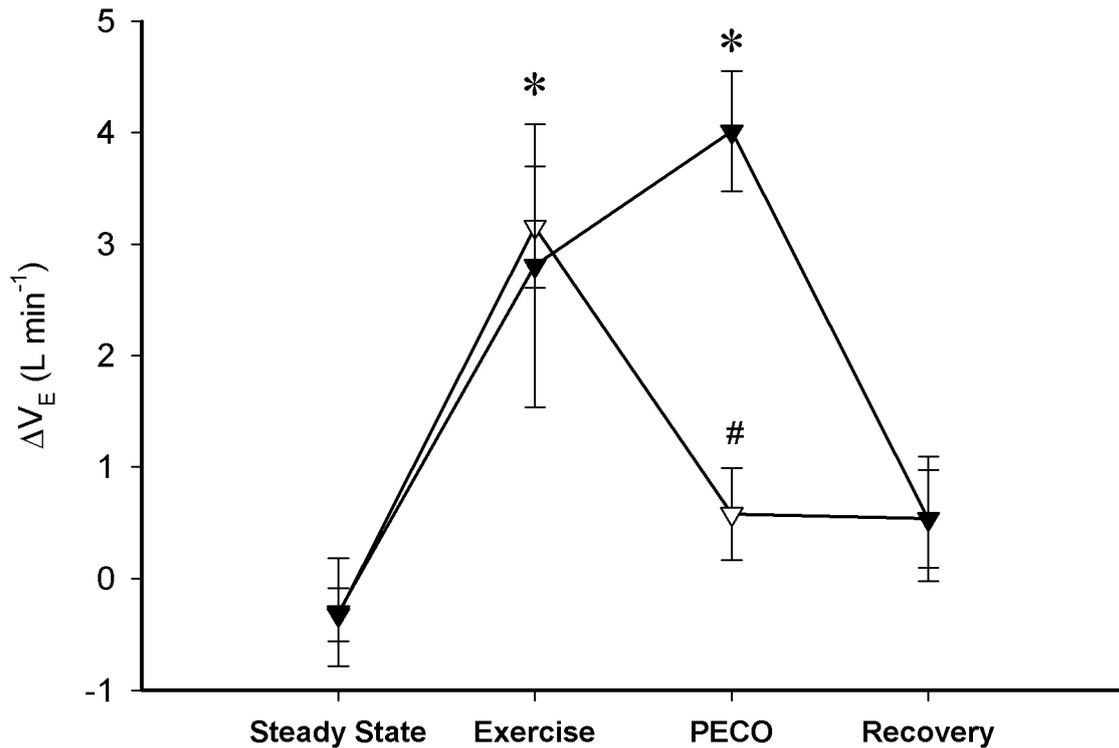


Figure 5.2: Change in minute ventilation from steady state values during each phase of the hypercapnic, CHEMO+ERGO (closed circles) and CHEMO trials (closed triangles) and the air breathing, ERGO (open circles) and CONTROL (open triangles) trials. Mean  $\pm$  S.E.M, N=11. \* denotes significant difference from steady state value, # denotes significant difference between CHEMO+ERGO vs ERGO trials; P<0.05.

Changes from steady state values, in MAP and HR which were seen during Exercise, PECO and Recovery in the four trial conditions are also shown in Figures 5.3 and 5.4, respectively. Exercise induced similar significant increases in MAP from steady state values in both the ERGO and CHEMO+ERGO trials. During PECO, MAP showed the expected fall from end Exercise levels but remained significantly elevated above steady state values by 16-19 mmHg in both trials. Importantly, there was no significant difference between the blood pressure responses to PECO in the two trials (see figure 5.3). HR was also elevated during Exercise in both the ERGO and CHEMO+ERGO trials by approximately 10bpm but it promptly returned to steady state levels during

PECO in both trials (see Figure 5.4). During Recovery in both the ERGO and CHEMO+ERGO trials MAP and HR were not different compared to steady state (all  $P > 0.05$ ). MAP and HR in the CHEMO and CONTROL trials were not significantly altered compared to steady state at any point and there were no significant differences between these hypercapnic and air breathing trials.

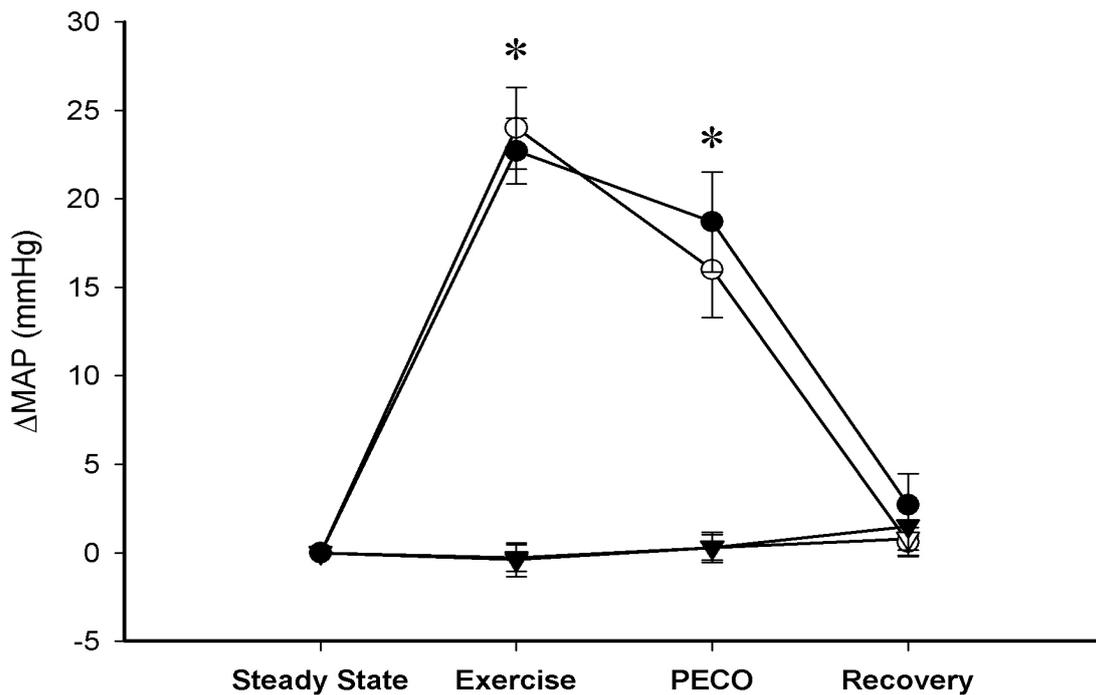


Figure 5.3: Change in mean arterial pressure from steady state values during each phase of the hypercapnic, CHEMO+ERGO (closed circles) and CHEMO trials (closed triangles) and the air breathing, ERGO (open circles) and CONTROL (open triangles) trials. Mean ( $\pm$ S.E.M), N=11. \* denotes significant difference from steady state value;  $P < 0.05$ .

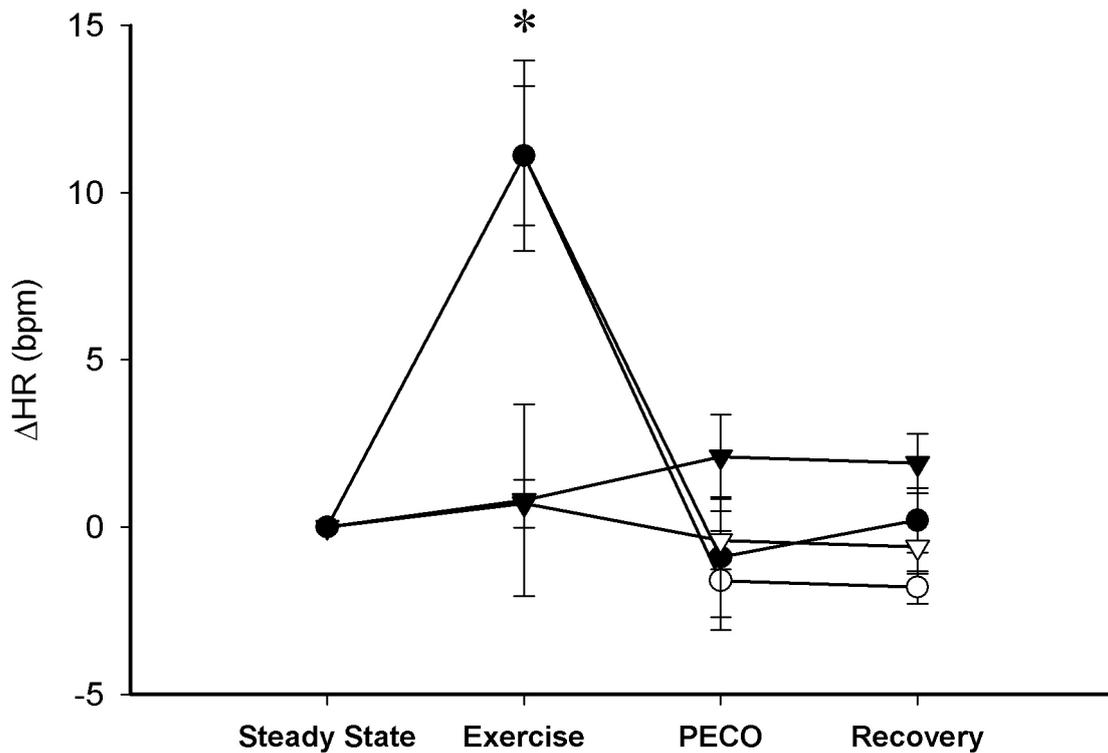


Figure 5.4: Change in heart rate from steady state values during each phase of the hypercapnic CHEMO+ERGO (closed circles) and CHEMO trials (closed triangles) and the air breathing, ERGO (open circles) and CONTROL (open triangles) trials. Mean ( $\pm$ S.E.M), N=11. \* denotes significant difference from steady state value;  $P < 0.05$ .

Our criteria for SBRS analysis were met in only four subjects. The mean slope of the SBP versus RR interval regression was reduced during exercise under either hypercapnic or room air breathing conditions, although this was not significant (Figure 5.5). During the circulatory occlusion period the slope remained decreased in the hypercapnic trial whereas it was elevated above steady state levels while breathing room air (both  $P > 0.05$ ). The mean intercept of the regression was decreased during exercise and then it returned at steady state levels during PECO in the CHEMO+ERGO trial (all  $P > 0.05$ ) (Figure 5.6). In the ERGO trial the mean intercept was not affected by exercise but it was

increased beyond steady state levels during PECO and recovery, although significance was not attained (all  $P > 0.05$ ) (Figure 5.6).

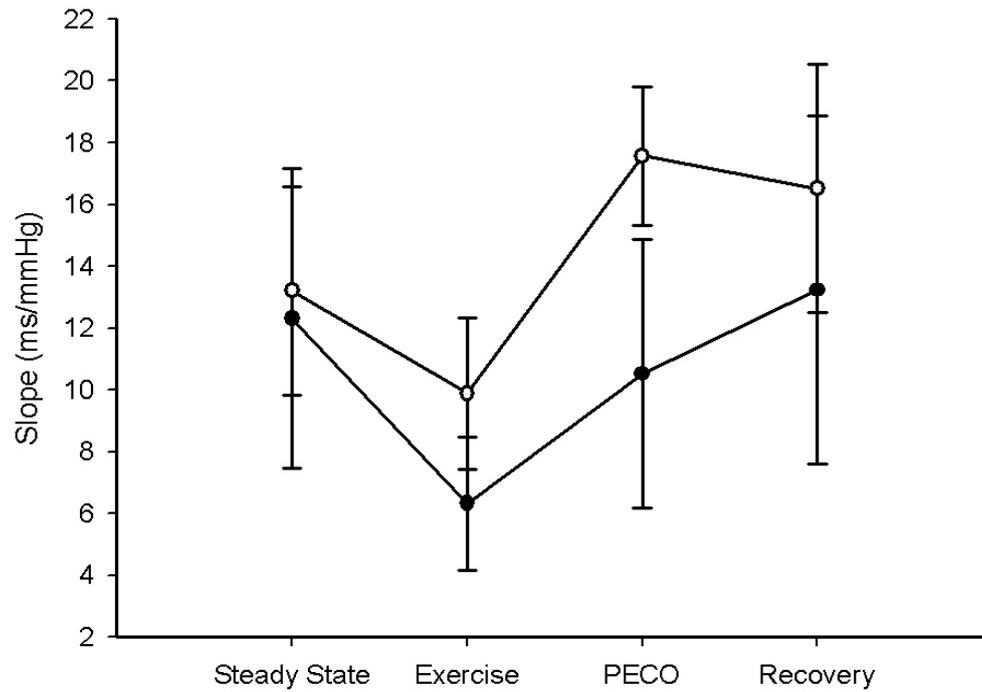


Figure 5.5: Mean slope ( $\pm$ S.E.M) values of regression lines calculated from sequence analysis during the ERGO (open circles) and CHEMO+ERGO (filled circles) trials (N=4).

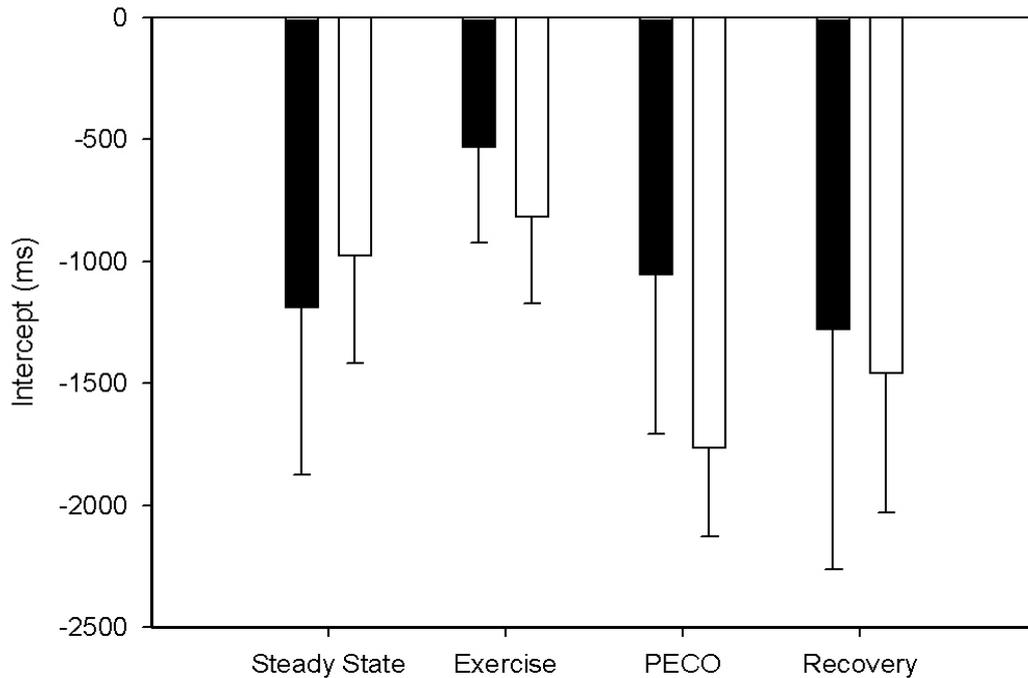


Figure 5.6: Mean intercept ( $\pm$ S.E.M) values of regression calculated from sequence analysis during the ERGO (open bars) and CHEMO+ERGO (closed bars) trials (N=4).

## 5.5: DISCUSSION

The main finding of the present investigation is that under conditions of hypercapnia, the sustained activation of the muscle metaboreflex by post-exercise circulatory occlusion maintained the increases in ventilation at levels seen during exercise, whereas the same procedure in air did not maintain the ventilatory elevation.

We reported that activation of the muscle metaboreflex by circulatory occlusion following a bout of isometric exercise did not significantly affect respiration under eucapnic conditions. This is in accordance to previous published data (Houssiere *et al.*, 2006; Iellamo *et al.*, 1999; Wiley & Lind, 1971). The fact that blood pressure and heart rate were not different between PECO in air and

hypercapnia, precludes the possibility that there was an enhanced muscle afferent feedback during PECO in hypercapnia which could explain the enhanced ventilation. Jordan *et al* (2000) also found that the sympathoexcitatory maneuver of head-up tilt was associated with increases in ventilation only during hypercapnia, but not during eucapnia. The enhancement in ventilation in the previous study was accompanied by attenuations in the hypercapnia-induced elevations in cerebral blood flow. Markedly, when the effect of head-up tilt and hypercapnia was studied with complete ganglionic blockade, diminished elevations in ventilation and enhancements in cerebral blood flow were observed (Jordan *et al.*, 2000).

These authors postulated that the observed sympathoexcitation-induced ventilatory increments were possibly mediated via cerebral vasoconstriction and/or increased peripheral chemosensitivity. It has been widely suggested that cerebral vasoreactivity and / or cerebral blood flow is of paramount importance in modulating the ventilatory response to carbon dioxide, possibly via influencing the gain of central chemoreceptors (Xie *et al.*, 2005; 2006). As such, reduction of brain blood flow in unanesthetized goats provoked increases in the respiratory responses to CO<sub>2</sub> (Chapman *et al.*, 1979). Whether the ventilatory enhancement observed during superimposition of metaboreflex activation on that of the chemoreflex was due to a PECO-induced diminution in brain blood flow is unknown but possible. Against this postulation, have been data showing a failure of PECO to affect cerebral blood flow under conditions euoxic / eucapnic conditions (Jørgensen *et al.*, 1992; Pott *et al.*, 1997; Vianna *et al.*, 2009).

On the other hand, sympathoexcitation and vasoconstriction arising from PECO has been evidenced at various extracranial arterial beds, including that of the

inactive muscle (Seals, 1989), kidney (Momen *et al.*, 2003) and lung (Lykidis *et al.*, 2008). This raises the possibility for increased carotid chemoresponsiveness to contribute to the increases in ventilation we observed during PECO in hypercapnia, given that sympathetic activation has been shown to increase the carotid body gain in response to carbon dioxide (Joels & White, 1968; Maskell *et al.*, 2006). In addition, PECO has been found to induce increases in circulating renin and noradrenaline (Nishiyasu *et al.*, 1998), the latter of which was shown to enhance the respiratory response to CO<sub>2</sub> (Cunningham *et al.*, 1958). Renin, in turn, is converted to angiotensin II which was reported to increase carotid chemoreceptor afferent activity in rats (Allen, 1998) and to contribute to the hypercapnia-mediated ventilatory augmentations seen in unanesthetised dogs (Walker & Jennings, 1995). Nevertheless, our blood pressure data does not support an increased activation of the renin-angiotensin system during PECO in hypercapnia, since no difference was noted compared to PECO while breathing air.

The increases in ventilation seen during PECO against a hypercapnic background in the present study could be also accounted for by a central interaction between the muscle metaboreflex and the ventilatory chemoreflex. Therefore neural signals from metaboreceptors that converge into the ventrolateral medulla could cause augmentation of chemoreceptor input and increase central and/or peripheral chemoreceptor sensitivity to CO<sub>2</sub> (Clark *et al.*, 2000; Schmidt *et al.*, 2005). Although to date no experimental data has been available to directly support this postulation, it has been shown that activation of the cardiac sympathetic afferent reflex in healthy and CHF rats caused an augmentation of the chemoreflex, with the ventrolateral medulla and the NTS being identified as the site of interaction (Gao *et al.*, 2007; Wang *et al.*, 2008). It

has been established that the NTS is involved in the mediation of the muscle metaboreflex and the cardiac sympathetic afferent reflex which have been known to have common activation stimuli including ischaemia, adenosine and capsaicin (Costa *et al.*, 2001; Kaufman *et al.*, 1982; Wang & Ma, 2000; Zahner *et al.*, 2003). Along these lines, activation of the muscle metaboreflex might have also enhanced the carotid body chemoresponsiveness to CO<sub>2</sub>, underlying the ventilatory stimulation seen during PECO in hypercapnia in the present experiment. In addition, the lower mean baroreflex sensitivity during the hypercapnic PECO compared to that during room air breathing, although not being significant, depicts a partial removal of the inhibitory effect that the baroreflex is known to have on the ventilatory responses to chemoreflex activation (Somers *et al.*, 1989; 1991).

The ventilation enhancements we observed when the ventilatory chemoreflex and the muscle metaboreflex were concurrently activated could be implicated in the abnormal respiratory increases that characterise exercise in CHF, given that both reflexes are hyperactive in these patients. Along the same lines, the excessive muscle metaboreflex activation in this condition could increase the gain of central/peripheral chemoreceptors or cause them to overshoot, resulting in increased ventilation for a given P<sub>A</sub>CO<sub>2</sub> or VCO<sub>2</sub> (Coats, 2001; Schmidt *et al.*, 2005). As a corollary to these postulations, exercise training, which has been known to attenuate muscle metaboreflex activation (Piepoli *et al.*, 1996), was shown to diminish both exercise hyperventilation and the activity of the ventilatory chemoreflex in CHF patients (Tomita *et al.*, 2003). In addition, the improvements in exercise ventilation observed after acute and chronic administration of sildenafil to these patients were strongly associated with a lessened metaboreflex activity. Favourable effects of sildenafil have been also

demonstrated for arterial function, breathlessness during exercise and pulmonary haemodynamics (Guazzi *et al.*, 2007; 2008). Pulmonary haemodynamics, in turn, which were closely related to markers of exercise hyperventilation in CHF (Lewis *et al.*, 2008; Reindl *et al.*, 1998), appeared to be affected by the activation of the muscle metaboreflex in healthy humans (Lykidis *et al.*, 2008). In total, the emerging importance of the overactive ergoreflex in the pathogenesis of abnormally high ventilation during exercise in CHF is further strengthened by the results of the present investigation. Nevertheless, the fact that we utilised healthy subjects precludes extrapolation of our findings to CHF and thus necessitates further experiments aiming at assessing the ventilatory effect of the two reflexes' concurrent activation in CHF patients.

In conclusion, we showed that activation of muscle metaboreflex activation against a background of hypercapnia (i.e. ventilatory chemoreflex activation) maintained ventilation at levels seen during exercise. We suggest that our findings could be implicated in the exaggerated ventilation seen during exercise in CHF given that both reflexes are hyperactive in this condition.

**CHAPTER 6:  
THE PULMONARY VASCULAR  
RESPONSE TO THE  
COMBINED ACTIVATION OF  
THE MUSCLE METABOREFLEX  
AND MECHANOREFLEX**

## 6.1: ABSTRACT

The interplay between the muscle metabo- and mechanoreflex is well known to participate in the mediation of the cardiovascular responses to exercise. Whether interplay between these reflexes is operative in the control of the pulmonary vascular response to exercise is unknown. The aim of this study was to assess the pulmonary vascular response to the combined activation of the two muscle reflexes. Nine healthy subjects performed a bout of isometric calf plantarflexion exercise. During exercise and for 9 minutes post-exercise, circulatory occlusion was applied. At 5 minutes into PECO the calf muscle was stretched for 180 s. A control (no exercise) protocol was also undertaken. Blood pressure measurements and echocardiographically-determined estimates of SPAP and cardiac output  $\dot{Q}$ , were obtained at various intervals throughout the two protocols. Elevations in SPAP ( $22.51 \pm 2.61\%$ ),  $\dot{Q}$  ( $26.92 \pm 2.99\%$ ) and mean MAP ( $15.38 \pm 2.29\%$ ) were noted during isometric exercise as compared to baseline (All  $P < 0.05$ ). Increases in SPAP and MAP persisted during PECO (All  $P < 0.05$ ), whereas  $\dot{Q}$  returned to resting levels. Increases in MAP and SPAP were also maintained during stretch which significantly elevated  $\dot{Q}$  (All  $P < 0.05$ ). All data are mean  $\pm$  S.E.M. These data suggest that activation of the muscle mechanoreflex attenuated the vasoconstriction or increases in pulmonary vascular stiffness mediated by activation of the metaboreflex. Our findings could be implicated in the regulation of pulmonary haemodynamics during exercise.

## 6.2: INTRODUCTION

It has been long believed that the regulation of pulmonary haemodynamics and blood flow during exercise is mediated in a passive fashion through the exercise-associated increases in cardiac output ( $\dot{Q}$ ), with the ANS exerting little, if at all any, influence (Mercus *et al.*, 2008; Reeves & Taylor 1996). However, both sympathetic (Chand & Altura, 1980; Szidon & Fishman, 1971; Vanhoutte, 1974) and parasympathetic (Norel *et al.*, 1996; Toga *et al.*, 1996; Wilson *et al.*, 1995) activation have been long known to be capable of influencing pulmonary vascular tone in isolated vessels of animals. Similar evidence has been generated from in various animal preparations (Barman, 1995; Duke *et al.*, 1960; Hyman & Kadowitz, 1988; Hyman *et al.*, 1981; Ingram *et al.*, 1968; 1970; Kadowitz & Hyman, 1973; Nandiwada *et al.*, 1983; Piene, 1976; Porcelli & Bergofsky, 1973; Sada *et al.*, 1987; Szidon & Fishman, 1971) and also in intact animals at rest (Kadowitz *et al.*, 1974; Murray *et al.*, 1986) and during exercise (Kane *et al.*, 1993; 1994; Koizumi *et al.*, 1996; Strubenitsky *et al.*, 1998). Investigations on resting humans have also been supportive of the view that pulmonary vessels and / or haemodynamics are sensitive to neural influences (Moruzzi *et al.*, 1988; 1989a; 1989b) however the exact function of the ANS in pulmonary vascular regulation exercise human studies has been far from clear. Recently, the pulmonary vascular response to the activation of the muscle metaboreflex was appraised in healthy humans by our working group (Chapter 1; Lykidis *et al.*, 2008). We observed that the sustained activation of the muscle metaboreflex in the post-exercise period maintained the exercise-induced increases in systolic pulmonary artery pressure under conditions of resting cardiac output, implying increases in pulmonary vascular resistance (Lykidis *et al.*, 2008). It is established that the sympathoexcitation induced by muscle

metaboreflex activation operates in concert with the vagal inhibition associated with muscle mechanoreflex activation in mediating the cardiovascular adjustments to exercise (Rowell & O'Leary, 1990). However the effect of their combined activation on pulmonary haemodynamics has not been studied in the literature. Such information would be useful in delineating the role of muscle reflex control systems in the regulation of pulmonary blood flow. In addition it would have implications in the exaggerated pulmonary haemodynamic response in CHF given that regulation of muscle metaboreflex and mechanoreflex are abnormal in these patients (Piepoli *et al.*, 2008). Therefore we sought to assess the effect of muscle mechanoreceptor activation by passive stretch of the calf muscle with and without concurrent metaboreflex activation by PECO. Previous studies have evidenced that vagal inhibition by atropine caused pulmonary vasodilation when the pre-existing vascular tone was elevated sympathetic/parasympathetic stimulation (Vanhoutte, 1974; Wilson *et al.*, 1995). Based on these findings we hypothesised that stimulation of the muscle mechanoreflex would offset the increases in SPAP seen during muscle metaboreflex activation.

### **6.3: METHODS**

#### *Subjects*

Nine healthy and physically active volunteers (age  $27 \pm 3.60$  years; mean  $\pm$  SD; 4 men, 5 females) gave written consent to take part in this study after they had been given detailed information on the procedures and risks. The study was performed according to the Declaration of Helsinki and was approved by the Local Ethics Committee. Female participants were asked to take part in the

procedures during the follicular phase of their menstrual cycle. All procedures associated with the investigation were undertaken in the Cardiovascular Laboratory located in the School of Sport and Exercise Sciences at the University of Birmingham.

#### *Preliminary session*

A preliminary session was undertaken in order to determine the suitability of subjects and to screen for exclusion criteria, ascertained by questionnaire, which comprised presence of cardiorespiratory disease, recent leg injury or upper respiratory tract infection. Doppler echocardiographic visualization of tricuspid regurgitation was then undertaken with subjects lying on the couch of a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA). Cushions were placed at the right side of the back support of the Biodex system so that subjects' bodies were slight rotated towards the lateral decubitus position. If the acquired ultrasound images were of satisfactory quality, subjects were asserted as eligible to take part in the study and they were familiarized to the procedures involved in the experimental day.

#### *Experimental day*

Participants who completed the preliminary trial were asked to return to the laboratory to carry out a control and an exercise protocol on the same day. Protocols were separated by each other by 30 minutes of rest. The duration of the visit was approximately one hour and the order of the protocols was randomly selected. Subjects were asked to refrain from caffeine, alcohol and performance of physical activity 12 hours, and eating 3 hours, prior to the visit.

At the beginning of the visit participants were prepared for monitoring of heart rate (ECG) and blood pressure (finger plethysmography, Finapres). Finally, a pneumatic cuff was placed around the lower femur for subsequent occlusion of leg blood flow (E20 Hokanson system). Following this subjects were asked to rest on the Biodex couch. Their right foot was strapped to the footplate so that the lower leg was horizontal to the floor and that the angle between the femur and the shin was 150°. Velcro straps were used to fix the foot and minimise heel lift during voluntary plantarflexor exercise and passive stretch. Passive range of dorsiflexion of the ankle joint was then established by manually moving the footplate as far as was comfortable. This information was programmed into the machine so that the Biodex could perform the subsequent stretching movement automatically. The MVC force of the calf plantarflexors was subsequently assessed by recording maximal efforts. The median force output was regarded as the MVC.

#### *Experimental protocol*

After the MVC was determined subjects continued resting on the couch for 15 minutes before measurements commenced. Subjects were instructed to maintain a self selected but constant respiratory rate throughout the protocols. This was aided by a continuous background sound of a metronome set at a frequency of 60 beats per minute. In addition they were asked to remain quiet and try to avoid performing Valsalva maneuvers during measurements.

After the 15-minute rest period, baseline measurements of the  $\Delta P_{MAX}$  and cardiac output were obtained for five minutes. At the end of this period the cuff

placed around the right thigh was inflated to 200mmHg by a rapid cuff inflator (E20, Hokanson, Bellevue, WA, USA) and this remained inflated for a further 9 minutes. At the time of cuff inflation, subjects were instructed to either rest for a further 1.5 minutes (control trial), or perform ischemic isometric plantarflexion exercise using their right calf muscles in order to produce a force that matched the pre-determined exercise intensity of 50% MVC (exercise protocol) for 1.5 minutes. Visual feedback of the target force and output from the dynamometer was given via a monitor placed within the subject's field of view. After a further 3.5 minutes of circulatory occlusion, the foot was passively dorsiflexed by the Biodex to the predetermined maximum pain-free angle at a velocity of 30 deg/s, and held there for the next 3 minutes with continued occlusion. After this stretch period, the foot was returned to its starting position and circulatory occlusion continued for a further minute. The thigh cuff was then deflated and subjects recovered for a further 2 minutes.

Blood pressure and heart rate were continuously measured throughout both protocols. Cardiac output and trans-tricuspid pressure difference were measured at the time points indicated in Figure 6.1 during baseline, exercise, PECO and recovery periods. Details of these measurements are given below.

#### *Estimation of SPAP from measurement of $\Delta P_{MAX}$*

$\Delta P_{MAX}$  was measured for as many cardiac cycles as possible at, or close to, end-expiration. These measurements were then pooled to give a mean value for each phase of the experiment. Adjustments to the position of the ultrasound beam were required to maintain good quality images and this resulted in some data loss for short periods of the protocol.

### Cardiac output.

Cardiac output was measured for approximately 30-second periods during three separate sections of the baseline period, after the initial rapid rise in heart rate had occurred during exercise, at the end of the PECO and stretch phases and then again during the second minute into recovery (Figure 6.1).

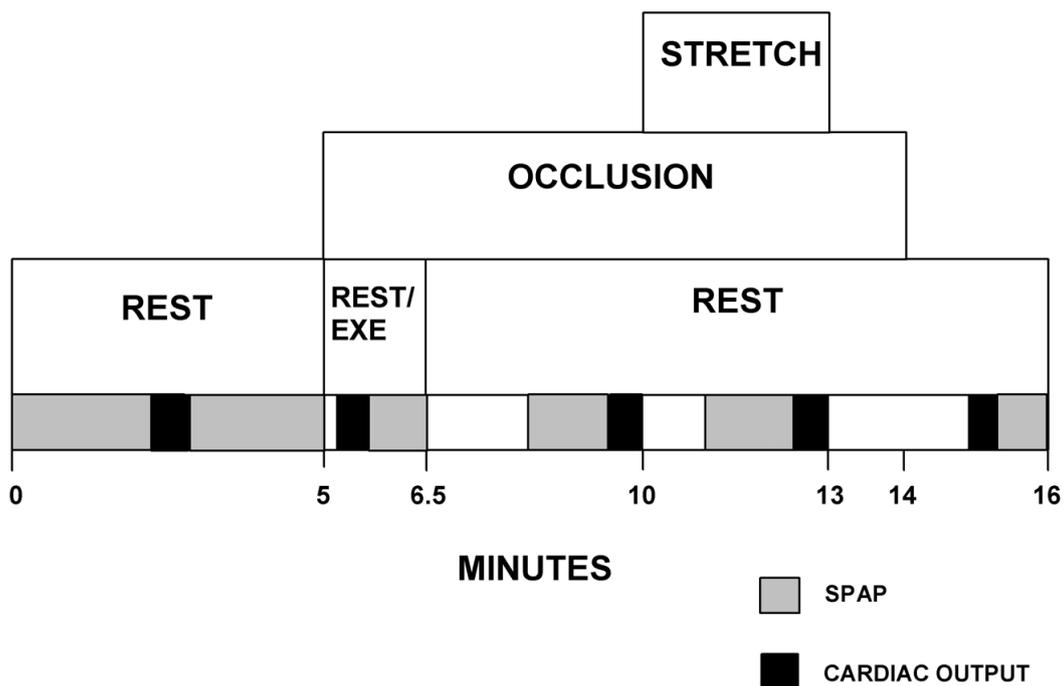


Figure 6.1: Schematic representation of the experimental protocol. EXE; Exercise.

### Statistical methods

Statistical analysis was performed using repeated measures ANOVA (5x2; time vs condition) and *post hoc* analysis using paired t-tests that were adjusted for multiple comparisons, when required. A power calculation showed that our study had 76.4% power to detect a 10% change in SPAP. The statistical package utilised was SPSS 14.0 (SPSS Inc., Chicago, Illinois, USA).  $P < 0.05$  was considered as significant. Data are presented as means  $\pm$  S.E.M.

## 6.4: RESULTS

Baseline values of the variables obtained at the control and exercise trial are presented in Table 6.1. No significant differences were found.

Table 6.1: Baseline mean values ( $\pm$ S.E.M) of the variables studied at the two protocols. SPAP, systolic pulmonary artery pressure; MAP, mean arterial pressure; SAP, systolic arterial pressure; DAP, diastolic artery pressure; Q, cardiac output; HR, heart rate; SV, stroke volume (N=9).

	<b>Control</b>	<b>Exercise</b>
SPAP, mmHg	14.62 $\pm$ 0.43	14.90 $\pm$ 0.47
Q L/min	2.96 $\pm$ 0.12	3.04 $\pm$ 0.13
MAP, mmHg	78 $\pm$ 2.97	80 $\pm$ 3.47
SAP, mmHg	116 $\pm$ 3.46	121 $\pm$ 5.04
DAP, mmHg	59 $\pm$ 3.04	60 $\pm$ 2.83
SV, ml	70.55 $\pm$ 2.51	74.29 $\pm$ 4.66
HR, bpm	59 $\pm$ 2.08	61 $\pm$ 2.13

### *Systolic pulmonary artery pressure*

No significant changes from baseline were noted at any stage during the control trial. In contrast, at the exercise trial SPAP was elevated by  $22.51 \pm 2.61\%$  during isometric exercise and remained increased during (+15.26  $\pm$  3.03%) PECO and (+13.44  $\pm$  2.28%) Stretch compared to the resting values (All  $P < 0.05$ ). SPAP gradually returned towards baseline levels during the recovery period (Figure 6.2 and Table 6.2).

### *Cardiac output, heart rate and stroke volume*

Cardiac output, heart rate and stroke volume were not different from baseline at any time during the control trial (Figure 6.3). In the exercise trial, a  $26.92 \pm 2.99\%$  ( $P < 0.05$ ) increase in Q was observed during exercise. This was due to a rise in

heart rate ( $22.88 \pm 2.65\%$ ;  $P < 0.05$ ) since changes in stroke volume ( $+3.31 \pm 1.17\%$ ) failed to reach significance. During PECO,  $\dot{Q}$  fell at resting levels ( $+6.95 \pm 1.87\%$ ;  $P > 0.05$  vs baseline) and subsequently increased during stretch ( $+19.82 \pm 4.36\%$ ;  $P < 0.05$  vs baseline). This was due to a rise in both heart rate ( $10.93 \pm 2.14\%$ ;  $P < 0.05$ ) and stroke volume ( $7.87 \pm 2.64\%$ ;  $P = 0.08$ ). Heart rate, stroke volume and cardiac output all returned to baseline values during the recovery.

#### *Systemic blood pressure*

Systolic, diastolic and mean blood pressure values during the two trials are illustrated in Figure 6.4. No significant changes from baseline were noted at any stage during the control trial. Mean, systolic and diastolic arterial pressures significantly increased during exercise (all  $P < 0.05$  vs baseline). Subsequently they all remained significantly elevated above resting levels during PECO and Stretch (all  $P < 0.05$  vs baseline) with the exception of DAP ( $P = 0.08$ ). During recovery, systemic arterial pressures decreased towards baseline values (all  $P > 0.05$ ).

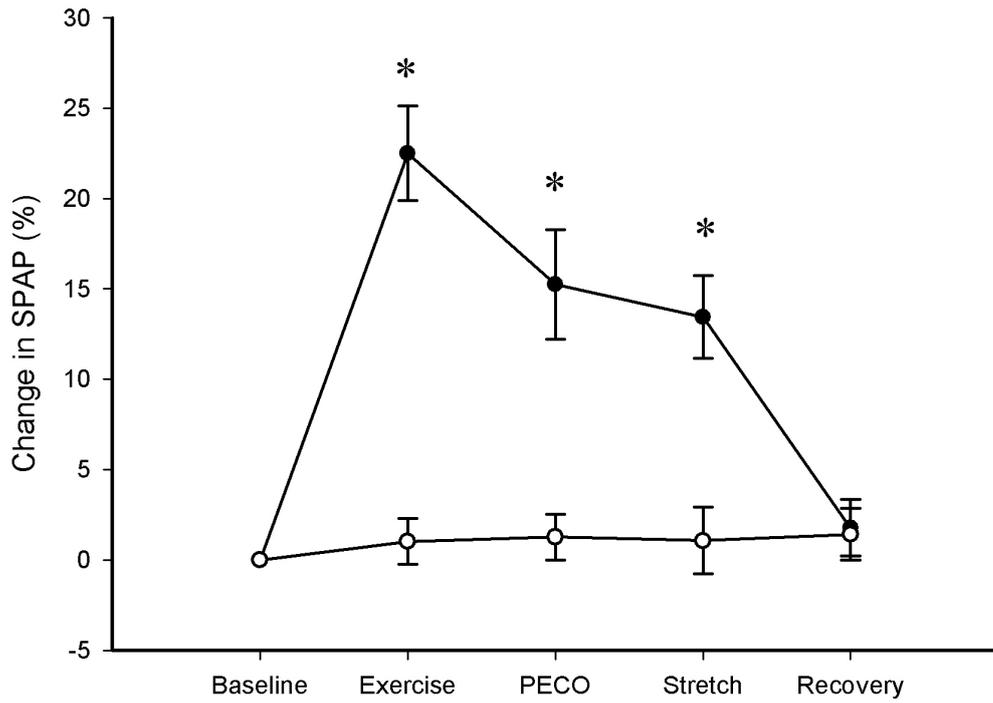


Figure 6.2: Mean percentage changes ( $\pm$ S.E.M) in SPAP from baseline during the isometric exercise (closed symbols) and control (open symbols) protocols (\* indicates  $P < 0.05$  compared to rest;  $N=9$ ).

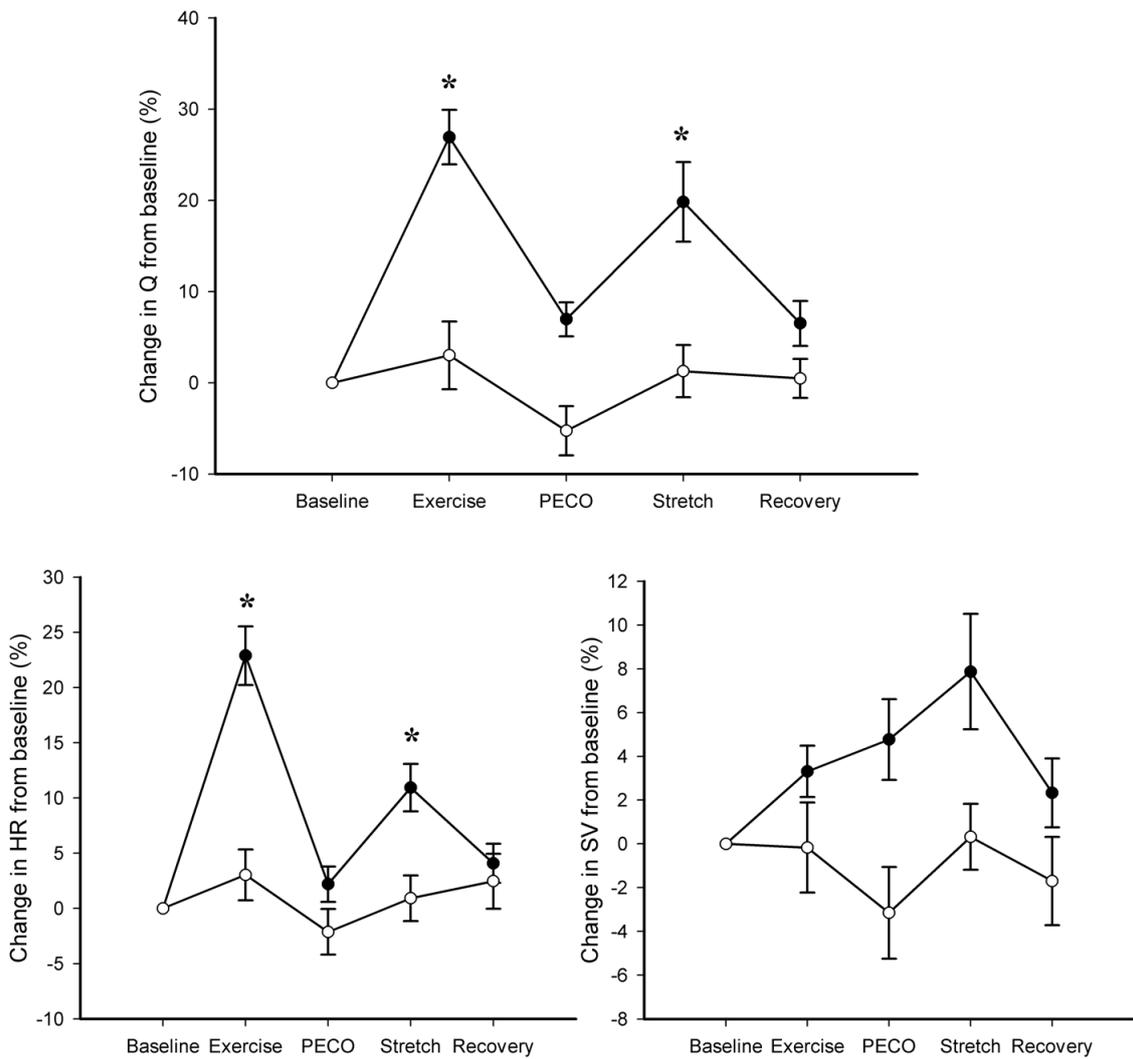


Figure 6.3: Mean ( $\pm$ S.E.M) percentage changes from baseline in cardiac output (top panel), heart rate (bottom left panel) and stroke volume (bottom right panel) during the isometric exercise (black bars) and control (white bars) trials (\* indicates  $P < 0.05$  from baseline;  $N=9$ ).

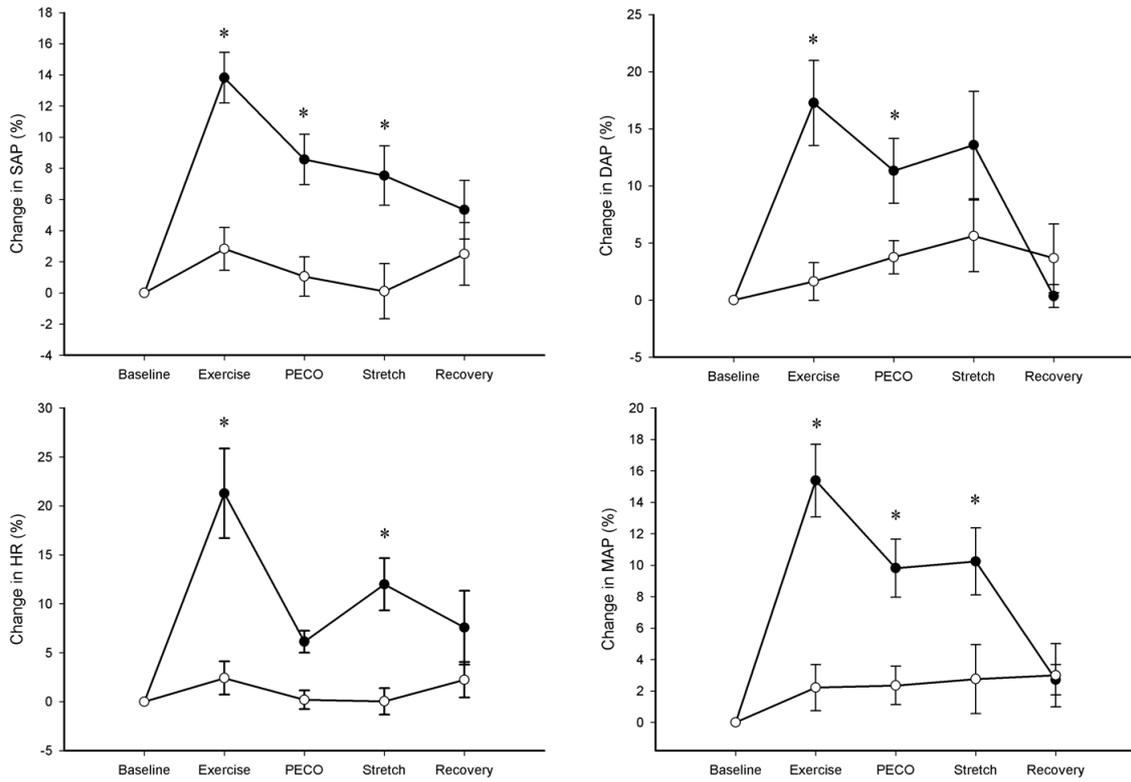


Figure 6.4: Mean ( $\pm$ S.E.M) percentage changes from baseline in SAP (top left panel), DAP (top right panel), heart rate (bottom left panel) and MAP (bottom right panel) during the isometric exercise (closed symbols) and control (open symbols) trials (\* indicates  $P < 0.05$  from baseline;  $N=9$ ).

## 6.5: DISCUSSION

A ~20% elevation in SPAP was observed along with an increase in cardiac output during the performance of isometric plantarflexor exercise. This increase in SPAP was sustained during PECO, despite  $\dot{Q}$  returning to resting values. Induction of calf muscle stretch parallel to PECO resulted in rises in  $\dot{Q}$  while maintaining the elevations in SPAP. Our findings suggest that activation of the muscle mechanoreceptors attenuates the elevated pulmonary vascular tone and/or stiffness mediated by muscle metaboreceptor activation.

Given that activation of muscle mechanoreceptors is known to result primarily in vagal withdrawal, our findings resemble those obtained from various in vitro and in situ animal preparations, which collectively evidenced that parasympathetic inhibition by atropine reversed pre-existing pulmonary vasoconstriction (Altiere *et al.*, 1986; Chand *et al.*, 1980; El-Kashef *et al.*, 1991; Hyman *et al.*, 1988; Vanhoutte, 1974; Wilson *et al.*, 1995). Although a restoration of SPAP towards resting levels did not take place when the mechanoreflex was activated against a background of activated metaboreflex, SPAP was not elevated as it would have been expected due to an increase in  $\dot{Q}$  (Lodato *et al.*, 1985) indicating pulmonary vasodilation. Alternatively, the ability of the pulmonary vasculature to accommodate the increases in cardiac output without increases in pressure could have been due to an increase in arterial compliance. We observed a lack of SPAP change when muscle mechanoreceptors were activated under conditions of basal pulmonary vascular tone in the control trial. The reason underpinning the latter observations might be related to the suggestion that at low (resting) tone the ability to dilate the pulmonary vasculature is very limited (Fishman, 1990).

The effects of atropine on the pulmonary vasculature were shown to depend on cardiac output and hence pulmonary blood flow (Chen *et al.*, 1992; Murray *et al.*, 1986). As such, Chen and co-workers (1992) observed in conscious dogs that the pulmonary pressure to flow ratio was not affected by atropine under conditions of low cardiac output but it was reduced under high cardiac output conditions. In support to our results have been studies showing that administration of atropine in humans induced increases in cardiac output that were accompanied by unchanged or mildly decreased pulmonary artery pressures (Daly *et al.*, 1963; Williams *et al.*, 1960). The effects of atropine on the pulmonary vasculature and haemodynamics have been also attributed to the inhibition of the M<sub>1</sub> and/or M<sub>2</sub> muscarinic receptors (El-Kashef *et al.*, 1991; Wilson *et al.*, 1992), which have been known to mediate the parasympathetic control of the pulmonary circulation in animals (Barnes & Liu, 1996) and humans (Norel *et al.*, 1996; Walch *et al.*, 2000).

The SPAP response (~20% above baseline) that we have observed in response to isometric exercise of the calf isometric muscle was similar to that evidenced during handgrip exercise despite the existing differences in the duration and intensity and muscle group used among the exercise bouts (Chapter 1; Lykidis *et al.*, 2008). These differences could underpin the larger increases in  $\dot{Q}$  and blood pressure we demonstrated during handgrip exercise as compared to these evidenced during calf exercise. The larger  $\dot{Q}$  response to exercise is due to the greater heart rate response seen during handgrip. The stroke volume response to exercise was similar amongst experiments.

During circulatory occlusion following either handgrip or calf isometric exercise SPAP was maintained at the same levels (~15% above baseline). Similarly, the HR, SV and  $\dot{Q}$  responses to PECO were identical between experiments. Blood

pressure during PECO was maintained closely at levels seen during handgrip exercise whereas it was slightly reduced as compared to the values seen during calf exercise. The reasons underlying these differences could be related to sensitivity differences between muscle groups (Scott *et al.*, 2002). The response of SV to PECO was similar amongst experiments.

The heart rate and blood pressure responses that we observed in response to the activation of the muscle mechanoreflex by passive isometric stretch of the calf muscle, with and without concurrent metaboreflex activation, are comparable to these obtained by previous studies (Drew *et al.*, 2008; 2009). We reported no changes in SV in response to the activation of muscle mechanoreceptors by passive isometric stretch. While to our knowledge the current investigation was the first to assess the SV response to static muscle stretching, studies that activated the muscle mechanoreflex in a dynamic fashion through passive cycling have produced mixed results with both unchanged (Nurhayati & Butcher, 1998) and slightly increased SV values (Vorluni & Volianitis, 2008) being reported.

The interplay between the muscle metaboreflex and mechanoreflex has been widely accepted to play a large part in the mediation of cardiovascular control during exercise. Our findings suggest that interplay between the two reflexes could be also operative in controlling pulmonary haemodynamics and vascular resistance during exercise. Therefore the increases in pulmonary vascular pressure and resistance induced by the muscle metaboreflex could be offset by activation of mechanoreceptors during exercise resulting in the mediation of low pulmonary vascular resistance during exercise. This might contribute to the mediation of low PVR seen during dynamic exercise, since activation of the muscle mechanoreceptors by repetitive deformation of the mechanically

sensitive structures could represent a greater stimulus as compared to static stretching (Adreani *et al.*, 1997; Pickar *et al.*, 1994).

On the contrary the mechanoreflex' involvement in the mediation of abnormal pulmonary haemodynamics in CHF seems improbable, based on our present findings. The reason underpinning this postulation is that CHF is characterised by overactive mechanoreceptors, which would mediate pulmonary vasodilation and/or a reduction in PVR. However our findings could not be safely extrapolated to CHF patients given the health status of the subject cohort we used.

In summary, the present data imply that the superimposition of muscle mechanoreflex activation on that of the muscle metaboreflex induced pulmonary vasodilation and/or increases in pulmonary arterial compliance. Our findings might be implicated in the regulation of pulmonary haemodynamics during exercise.

# **CHAPTER 7: GENERAL CONCLUSIONS**

The control of pulmonary haemodynamics and ventilation during exercise is regulated by numerous factors. The interplay between these factors in the healthy state facilitates increases in breathing that are in accordance with the metabolic needs of the body, parallel to elevations in pulmonary blood flow under conditions of low PVR. The role of certain cardio-pulmonary reflex systems and/or their interaction in the mediation of these changes has not been clarified. Such information would be particularly beneficial in the understanding of the reduced exercise tolerance of patients with CHF, given that these reflex systems are deranged in this condition.

The first study in this thesis assessed the pulmonary vascular response to the sustained activation of the muscle metaboreflex, induced by post-exercise circulatory occlusion in the active limb. It was demonstrated that the increases in SPAP induced by exercise were sustained during PECO. This, coupled to the drop of  $\dot{Q}$  towards resting levels seen during PECO suggests that the sympathoexcitation associated with the activation of the muscle metaboreflex induced pulmonary vasoconstriction and/or increases in PVR and arterial stiffness. Our findings uncovered a previously unknown aspect of pulmonary haemodynamics' regulation and in fact could, but they could be also implicated in the reduced exercise capacity of CHF patients, given that the muscle metaboreflex is hyperactive in this condition. Moreover recent data evidenced reductions in metaboreflex activity and breathlessness during exercise along with improvements in pulmonary haemodynamics and in exercise ventilation after administration of sildenafil, which is known to improve systemic arterial function and blunt adrenergic stimulation (Guazzi *et al.*, 2007; 2008). An extrapolation of our results in the findings of these investigations could suggest that the improvements seen in pulmonary vascular pressures and

breathlessness after sildenafil administration could have been at least partially accounted for by decreases in muscle metaboreflex activity.

The second study of this thesis assessed the effect of hypermetabolism on the ventilatory chemosensitivity to inhaled CO<sub>2</sub>. We showed that mild increases in metabolic rate brought about by protein ingestion resulted in elevations in the ventilatory response to a hypercapnic ramp protocol. Although our methodological approach did not allow a comprehensive insight into the mechanisms responsible for our observations, careful consideration of the physiological responses associated to protein ingestion suggests that the effects of increased metabolism on ventilatory chemosensitivity could be at least partly attributed to an increased stimulation of the carotid body. Our findings could be implicated in the mechanisms responsible for the characteristic tight coupling of metabolic rate and ventilation seen at rest and during exercise.

The third study of this thesis examined the ventilatory and cardiovascular responses to the combined activation of the muscle metaboreflex and the ventilatory chemoreflex, achieved by PECO and euoxic hypercapnia, respectively. We observed increases in respiration during PECO in hypercapnia but not when breathing room air. The possible mediating mechanisms comprise decreases in cerebral blood flow and increases in carotid body stimulation due to PECO-associated sympathoexcitation. In addition, an interaction between the neural signals from muscle metaboreflex and the ventilatory chemoreflex at the level of the NTS might have augmented the medullary chemoreceptor input thereby increasing the chemosensitivity to CO<sub>2</sub>. The activation of the muscle metaboreflex accomplished against a background of activated chemoreceptors in this experiment could potentially resemble the interaction that takes place

between these reflexes during exercise in CHF. Therefore, the exercise-induced overactivation of the muscle metaboreflex in these patients could interact with their overactivated chemoreceptors resulting in abnormally greater ventilation related to the amount of CO<sub>2</sub> that is produced metabolically.

The fourth study of this thesis aimed to assess the pulmonary vascular response to the combined activation of the muscle metaboreflex and the muscle mechanoreflex, by PECO and static passive stretch of the calf muscle. It was shown that the elevation in SPAP due to calf isometric exercise was maintained during PECO, in agreement with the findings of first investigation in this thesis. During the subsequent stretch of the calf muscle, increases in SPAP were sustained while an increase in cardiac output was also observed. These data suggest that activation of the muscle mechanoreceptors under conditions of elevated pulmonary vascular tone/stiffness by muscle metaboreceptor activation mediates pulmonary vasodilation and/or increase in arterial compliance. Our findings provide information regarding a previously unknown aspect of pulmonary vascular regulation during exercise.

The work in this thesis enhanced current knowledge on the regulation of pulmonary blood flow and ventilation during exercise. Activation of the muscle metaboreflex alone appeared to induce pulmonary vasoconstriction without having an influence on ventilation. Interestingly, the vasoconstrictive influence of the muscle metaboreflex was shown to be attenuated by the concurrent activation of the muscle mechanoreflex. In addition, when the muscle metaboreflex was activated against a background of activated ventilatory chemoreflex, a respiratory response was observed. In turn, the ventilatory response to the activation of the chemoreflex was enhanced under conditions of elevated metabolic rate. On the whole, the above findings further strengthen the

notion that the physiological control during exercise is the outcome of complex interaction between several systems and influences, whose isolated study could draw deficient, if not erroneous, conclusions.

Findings from the first (Chapter 3), third (Chapter 5) and fourth (Chapter 6) studies in this thesis have potential implications in the reduced exercise tolerance of patients with CHF. However due to the fact that we used healthy young subjects whose physiological functioning is fundamentally different from that in CHF patients, our findings could not be safely extrapolated to that condition. Therefore the ventilatory, systemic and pulmonary haemodynamic, autonomic nervous system response to the activation of the muscle metaboreflex, with and without combined muscle mechanoreflex activation, could be studied in CHF patients and age-matched healthy controls under conditions of hypercapnia and during air breathing. These investigations could be conducted before and after a period of exercise training, given that the latter is known to induce beneficial changes in the ventilatory and cardiovascular control in CHF. Moreover, future studies could compare the contribution of the muscle metaboreflex and mechanoreflex activation to the pulmonary haemodynamic and ventilatory changes taking place during dynamic and isometric exercise. This can be also done under conditions of hypercapnia / air breathing in order to assess whether there is interplay between the associated reflexes in the mediation of the exercise response. In addition animal studies could provide insight on the central integration of neural signals from the muscle metaboreflex, the mechanoreflex and the ventilatory chemoreflex.

An insight into the mechanisms responsible for the hypermetabolism-induced increases in the ventilatory chemosensitivity could be provided by incorporating cardiovascular data, body temperature, blood analyses for growth hormone,

potassium and acidity, and indices of ANS activation into the methodology. Neural blockade could be also employed so as to assess the influence of the autonomic nervous system in the effects of hypermetabolism on chemosensitivity. The above investigations could be also conducted on CHF patients, who are known to have increased levels of sympathetic nervous system activation, and their age-matched healthy controls.

In summary, cardiovascular and respiratory reflex control systems appear to be involved in the control of the pulmonary blood flow and ventilation during exercise. Future studies are needed to examine the contribution of these systems in the reduced exercise tolerance in CHF patients.

## REFERENCES

Aaronson, P.I., Robertson, T.P., Knock, G.A., Becker, S., Lewis, T.H., Snetkov, V and Ward, J.P.T. (2006). Hypoxic pulmonary vasoconstriction: mechanisms and controversies. *J Physiol*, 570, 53-58.

Acker, H.& O'Regan, R.G. (1981).The effects of stimulation of autonomic nerves on carotid body blood flow in the cat. *J Physiol*, 315, 99-110.

Adams, L., Frankel, H., Garlick, J., Guz, A., Murphy, K., Semple, S.J. (1984). The role of spinal cord transmission in the ventilatory response to exercise in man. *J Physiol*, 355, 85-97.

Adreani, C.M. Hill, J.M, & Kaufman, M.P. (1997). Responses of group III and IV muscle afferents to dynamic exercise. *J Appl Physiol*, 82, 1811-1817.

Agusti, A.G.N, Cotes, J. & Wagner, P.D. (1997). Responses to exercise in lung diseases. *Eur Respir Mon*, 6, 32-50.

Allen, A.M. (1998). Angiotensin AT1 receptor-mediated excitation of rat carotid body chemoreceptor afferent activity. *J Physiol*, 510, 773-781.

Altieri, R.J., Kiritsy-Roy, J.A. & Catravas, J.D. (1986). Acetylcholine-induced contractions in isolated rabbit pulmonary arteries: role of thromboxane A<sub>2</sub>. *J Pharmacol Exp Ther*, 236, 535-41.

Andreas, S., Anker, S.D., Scanlon, P.D. & Somers, V.K. (2005). Neurohumoral activation as a link to systemic manifestations of chronic lung disease. *Chest*, 128, 3618-3624.

Archer, S. & Michelakis, E. (2002). The mechanism(s) of hypoxic pulmonary vasoconstriction: potassium channels, redox O(2) sensors, and controversies. *News Physiol Sci*, 17, 131-7.

Archer, S.L., Weir, K.E. & McMurthy, I.F. Mechanisms of acute hypoxic and hyperoxic changes in pulmonary reactivity. Weir, E.K. & Reeves, J.T. (1989). *Pulmonary Physiology and Pathophysiology*, New York, Marcel & Dekker, 51-105.

Askanazi, J., Weissman, C., LaSala, P.A., Milic-Emili, J. & Kinney, J.M. (1984). Effect of protein intake on ventilatory drive. *Anesthesiology*, 60, 106-110.

Asmussen, E., Johansen, S.H., Jorgensen M. & Nielsen, M. (1965). on the nervous factors controlling respiration and circulation during exercise. experiments with curarization. *Acta Physiol Scand*, 63, 343-350.

Balanos, G.M., Dorrington, K.L., Robbins, P.A. (2002). Desferrioxamine elevates pulmonary vascular resistance in humans: potential for involvement of HIF-1. *J Appl Physiol*, 92, 2501-2507.

Balanos, G.M., Talbot, N.P., Robbins, P.A. & Dorrington, K.L. (2005). Separating the direct effect of hypoxia from the indirect effect of changes in cardiac output on the maximum pressure difference across the tricuspid valve in healthy humans. *Pflügers Archiv*, 450, 372-380.

Banchero, N., Sime, F., Penaloza, D., Cruz, J., Gamboa, R & Marticorena, E. (1966). Pulmonary pressure, cardiac output, and arterial oxygen saturation during exercise at high altitude and at sea level. *Circulation*, XXXIII, 249-262.

Band, D.M., Cameron, I.R. & Semple, S.J. (1969). Oscillations in arterial pH with breathing in the cat. *J Appl Physiol*, 26, 261-267.

Band, D.M., McClelland, M., Phillips, D.L. & Wolff, C.B. (1978). Sensitivity of the carotid body to within-breath changes in arterial  $P_{CO_2}$ . *J Appl Physiol* 45, 768-777.

Band, D.M., Wolff, C.B., Ward, J. Cochrane, G.M. & Prior, J. (1980). Respiratory oscillations in arterial carbon dioxide tension as a control signal in exercise. *Nature*, 283, 84-85.

Banister, P, Kay R.H. & Coxon R.V. (1954). Continuous gas analysis in the control of oxygen therapy for infants. *Lancet*, 267, 777-780.

Banner, N., Guz, A., Heaton, R., Innes, J.A., Murphy, K., Yacoub, M. (1988). Ventilatory and circulatory responses at the onset of exercise in man following heart or heart-lung transplantation. *J Physiol*, 399, 437-449.

Barberà, J.A., Peinado V.I. & Santos, S. (2003). Pulmonary hypertension in chronic obstructive pulmonary disease. *Eur Respir J*, 21, 892-905.

Barer, R., Howard, P. and Shaw, J.W. (1970). Stimulus-response curves for the pulmonary vascular bed to hypoxia and hypercapnia. *J Physiol*, 211, 139-155.

Barman S.A. (1995). Effect of catecholamines on pulmonary circulation at elevated vascular tone. *J Appl Physiol*, 78, 1452-1458.

Barnes, P.J.& Liu, S.F. (1995). Regulation of pulmonary vascular tone. *Pharmacol Rev*, 47, 87-131.

Bärtsch, P., Mairbaurl, H., Maggiorini, M. & Swenson, E.R. (2005). Physiological aspects of high-altitude pulmonary edema. *J Appl Physiol*, 98, 1101-1110.

Bastos, B.G., Williamson, J.W., Harrelson, T. & Da Nóbrega, A.C.L. (2000). Left ventricular volumes and hemodynamic responses to postexercise ischemia in healthy humans. *Med Sci Sport Exerc*, 32, 1114-1118.

Bell, H.J. (2006). Respiratory control at exercise onset: an integrated system perspective. *Respir Physiol Neurobiol*, 152, 1-15.

Bell, M.P., White, M.J. (2005). Cardiovascular responses to external compression of human calf muscle vary during graded metaboreflex stimulation. *Exp Physiol*, 90, 383-391.

Bellville, J.W., Whipp, B.J., Kaufman, R.D., Swanson, G.D., Aqleh, K.A., Wiberg, D.M. (1979). Central and peripheral chemoreflex loop gain in normal and carotid body-resected subjects. *J Appl Physiol*, 46, 843-53.

Berger, M., Hecht, S.R., Van Tosh, A. & Lingam, U. (1989). Pulsed and continuous wave Doppler echocardiographic assessment of valvular regurgitation in normal subjects. *J Am Coll Cardiol*, 13, 1540-1545.

Berkenbosch, A., Bovill, J.G., Dahan, A., DeGoede, J. & Olievier, I.C. (1989). The ventilatory CO<sub>2</sub> sensitivities from Read's rebreathing method and the steady-state method are not equal in man. *J Physiol*. 411, 367-377.

Bernardi, L., Gabutti, A., Porta, C. & Spicuzza, L. (2001). Slow breathing reduces chemoreflex response to hypoxia and hypercapnia, and increases baroreflex sensitivity. *J Hypertens*, 19, 2221-2228.

Bernheim, A.M., Kiencke, S., Fischler, M., Dorschner, L., Debrunner, J., Mairböurl, H., Maggiorini, M. & Brunner-La Rocca, H.P. (2007). Acute changes in pulmonary artery pressures due to exercise and exposure to high altitude do not cause left ventricular diastolic dysfunction. *Chest*, 132, 380-387.

Bin-Jaliah, I., Maskell, P.D. & Kumar, P. (2004). Indirect sensing of insulin-induced hypoglycaemia by the carotid body in the rat. *J Physiol*, 556, 255-266.

Bin-Jaliah, I., Maskell, P.D. & Kumar, P. (2005). Carbon dioxide sensitivity during hypoglycaemia-induced, elevated metabolism in the anaesthetized rat. *J Physiol*, 563, 883-93.

Biscoe, T.J. & Purves MJ. (1967). Factors affecting the cat carotid chemoreceptor and cervical sympathetic activity with special reference to passive hind-limb movements. *J Physiol*, 190, 425-441.

Biscoe, T.J. Bradley, G.W. & Sampson, S.R. (1970). The frequency of nerve impulses in single carotid body chemoreceptor afferent fibers recorded in vivo with intact circulation. *J Physiol*, 208, 121-131.

Borgbjerg, F.M, Nielsen, K. & Franks, K. (1996). Experimental pain stimulates respiration and attenuates morphine-induced respiratory depression: a controlled study in human volunteers, *Pain*, 64, 123-128.

Brashers, V.L., Peach, M.J. and Rose, C.R. (1988). Augmentation of hypoxic pulmonary vasoconstriction in the isolated perfused rat lung by vitro antagonists of endothelium-dependent relaxation. *J Clin Invest*, 82, 1495-1502.

Brown, D.R., Forster, H.V., Pan, L.G., Brice, A.G., Murphy, C.L., Lowry, T.F., Gutting. S.M., Funahashi, A., Hoffman, M., Powers, S. (1990). Ventilatory response of spinal cord-lesioned subjects to electrically induced sympathetic nerve traffic. *J Appl Physiol*, 77, 2612-2617.

Bruce, E.N. & Cherniack, N.S. (1987). Central chemoreceptors. *J Appl Physiol*, 62, 389-402.

Brundin, T. & Wahren, J. (1994). Influence of protein ingestion on human splanchnic and whole-body oxygen consumption, blood flow, and blood temperature. *Metabolism*, 43, 626-32.

Butera, R.J., Rinzel, J. and Smith J.C. Models of respiratory rhythm generation in the pre-Botzinger complex. I Bursting pacemaker neurons. *J Neurophysiol*, 82, 382-397.

Butler, J., Chomsky, D.B., Wilson, J.R. (1999). Pulmonary hypertension and exercise intolerance in patients with heart failure. *J Am Coll Cardiol*, 34, 1802-6.

Campanucci, V.A. & Nurse, C.A. (2007). Autonomic innervation of the carotid body: Role in efferent inhibition. *Respir Physiol Neurobiol*, 157, 83-92.

Campbell, E.J.M. and Green, J.H. (1955). The behaviour of the abdominal muscles and the intrabdominal pressure during quiet breathing and increased pulmonary ventilation. Study in man. *J Physiol*, 28, 423-426.

Carrington, C.A., Fisher, J.P., Davies, M.K., White, M.J. (2001). Muscle afferent and central command contributions to the cardiovascular response to isometric exercise of postural muscle in patients with mild chronic heart failure. *Clin Sci*, 100, 643-651.

Carrington, C.A., Fisher, J.P., Davies, M.K., White, M.J. (2004). Muscle afferent inputs to cardiovascular control during isometric exercise vary with muscle group in patients with chronic heart failure. *Clin Sci*, 107, 197-204

Casey, K., Duffin, J. & McAvoy, G.V. (1987). The effect of exercise on the central-chemoreceptor threshold in man. *J Physiol*, 383, 9-18.

Chand, N. & Altura, B.M. (1980). Reactivity and contractility of rat main pulmonary artery to vasoactive agents. *J Appl Physiol*, 49, 1016-1021.

Chapman, R.W. Santsiogo, T.V & Edelman, N.H. (1979). Effects of graded reduction of brain blood flow on chemical control of breathing. *J Appl Physiol*, 47, 1287-1294

Chen, B.B., Nyhan, D.P., Fehr, D.M. & Murray, P.A. (1992). Halothane anesthesia abolishes pulmonary vascular responses to neural antagonists. *Am J Physiol: Heart Circ Physiol*, 262, H117-122.

Cherry, P.D., Gillis, C.N. (1987). Evidence for the role of endothelium-derived relaxing factor in acetylcholine-induced vasodilatation in the intact lung. *J Pharmacol Exp Ther*, 241, 516-520.

Chua, T.P., Clark, A.I., Amadi, A.A. & Coats, A.J.S. (1996). Relation between chemosensitivity and the ventilatory response to exercise in chronic heart failure. *J Am Coll Cardiol*, 27, 650-657.

Clark T.J. & Godfrey S. (1969). The effect of CO<sub>2</sub> on ventilation and breath-holding during exercise and while breathing through an added resistance. *J Physiol*, 201, 551-66.

Clark, A.L. & Cleland, J.G.F. (2000). The control of adrenergic function in heart failure: therapeutic intervention. *Heart Fail Rev*, 5, 101-114.

Clark, A.L., Piepoli, M., Coats, A.J. (1995). Skeletal muscle and the control of ventilation on exercise: evidence for metabolic receptors. *Eur J Clin Invest*, 25, 299-305.

Clark, A.L., Poole-Wilson, P.A. & Coats, A.J.S. (1996). Exercise limitation in chronic heart failure: Central role of the periphery. *J Am Coll Cardiol*, 28, 1092-1102.

Clark, J.M., Sinclair, R.D., Lenox, J.B. (1980). Chemical and nonchemical components of ventilation during hypercapnic exercise in man. *J Appl Physiol*, 48, 1065-76.

Coats, A.J.S. (2001). What causes the symptoms of heart failure? *Heart*, 86, 574-578.

Cohen, M.I. (1979). Neurogenesis of respiratory rhythm in the mammal. *Physiol Rev*, 59, 1105-1173.

Cohn, J.N., Levine, T.B., Olivari, M.T., Garberg, V., Lura, D., Francis, G.S., Simon, A.B. & Rector, T. (1984). Plasma norepinephrine as a guide to

prognosis in patients with chronic congestive heart failure. *N Engl J Med*, 311, 819–823.

Costa, F., Diedrich, A., Johnson, B., Sulur, P., Farley, G. & Biaggioni, I. (2001). Adenosine, a metabolic trigger of the exercise pressor reflex in humans. *Hypertension*, 37, 917-922.

Crisafulli, A Salis, E., Tocco, F, Melis, F., Milia, R., Pittau, G., Caria, M.A., Solinas, R., Meloni, L., Pagliaro, P. & Concu, A. (2008). Impaired central hemodynamic response and exaggerated vasoconstriction during muscle metaboreflex activation in heart failure patients. *Am J Physiol: Heart Circ Physiol*, 292, H2988-H2996.

Crisafulli, A. Scott, A.C., Wensel, R., Davos, C.H., Francis, D.P., Pagliaro, P., Coats, A.J.S, Concu, A. & Piepoli, M.F. (2003). Muscle metaboreflex-induced increases in stroke volume. *Med Sci Sport Exerc*, 35, 221-228.

Cross, B.A., Davey, A., Guz, A., Katona, P.G., MacLean, M., Murphy, K., Semple, S.J., Stidwill, R. (1982). The pH oscillations in arterial blood during exercise; a potential signal for the ventilatory response in the dog. *J Physiol* 329, 57-73.

Cross, B.A., Grant, B.J., Guz, A., Jones, P.W., Semple, S.J., Stidwill, R.P. (1979). Dependence of phrenic motoneurone output on the oscillatory component of arterial blood gas composition. *J Physiol*, 290, 163-84.

Cross, B.A., Hiramoto, T., Mitchell, G.S., Scheid, P. (1979). The effects of intrapulmonary carbon dioxide and airway pressure on phrenic activity in the dog. *J Physiol*, 292:13P-14P.

Cui, J., Mascarenhas, V., Moradkhan, R., Blaha, C., Sinoway, L.I. (2008). Effects of muscle metabolites on responses of muscle sympathetic nerve activity to mechanoreceptor(s) stimulation in healthy humans. *Am J Physiol*, 294: R458–R466.

Cui, J.M., Moradkhan, R., Mascarenhas, V., Momen, A. & Sinoway, L.L. (2008). Cyclooxygenase inhibition attenuates sympathetic responses to muscle stretch in humans. *Am J Physiol: Heart Circ Physiol*, 294, H2693-2700.

Culver, B.H. & Butler, J. (1980). Mechanical influences on the pulmonary microcirculation. *Ann Rev Physiol*, 42, 187-198.

Cunningham, D.J.C., Hey, E.N. & Lloyd, B.B. (1958). The effect of intravenous infusion of noradrenaline on the respiratory response to carbon dioxide in man. *Q J Exp Physiol Cogn Med Sci*, 43, 394-399.

Dahan, A., DeGoede, J., Berkenbosch, A. & Olievier, I.C. (1990). The influence of oxygen on the ventilatory response to carbon dioxide in man. *J Physiol*, 428, 485-499.

Dahan, A. Nieuwenhuijs, D., Teppema, L. (2007). Plasticity of central chemoreceptors: effect of bilateral carotid resection on central CO<sub>2</sub> sensitivity. *PLoS Med*, e239.

Daly, W.J., Ross, J.C. & Behnke, R.H. (1963). The effect of changes in the pulmonary vascular bed produced by atropine, pulmonary engorgement, and positive-pressure breathing on diffusing and mechanical properties of the lung. *J Clin Invest*, 42, 1083-1094.

Davies, T.S., Frenneaux, M.P., Campbell, R.I., White, M.J. (2007). Human arterial responses to isometric exercise: the role of the muscle metaboreflex. *Clin Sci*, 112, 441-447.

Dawson, C.A., Linehan, J.H. & Bronikowski, T.A. Pressure and flow in the pulmonary vascular bed. In Weir, E.K. & Reeves, J.T. (1989). *Pulmonary Physiology and Pathophysiology*, New York, Marcel & Dekker, 51-105.

Dawson, C.A. (1989). Role of pulmonary vasomotion in physiology of the lung, *Physiol Rev*, 64, 544-620.

De Burgh Daly, I., Ramsay, D.J. & Waaler, B.A. (1970). The site of action of nerves in the pulmonary vascular bed in the dog. *J Physiol*, 209, 317-339.

de Daly, I.B. (1961). The nervous control of the pulmonary circulation. *Naunyn Schmiedebergs Arch Exp Pathol Pharmacol*, 240, 431-45.

de Simone, G., Roman, M.J., Koren, M.J., Mensah, G.A., Ganau, A., Devereux, R.B. (1999). Stroke volume/pulse pressure ratio and cardiovascular risk in arterial hypertension. *Hypertension*, 33, 800-805.

Dejours, P. (1962). Chemoreflexes in breathing. *Physiol Rev*, 42, 335-358.

Dempsey, J.A., Forster, H.V. & Ainsworth, D.M. Regulation of hyperpnea, hyperventilation, and respiratory muscle recruitment during exercise. In Dempsey, J.A. & Pack, A.L. (1995). *Regulation of Breathing*. New York, Marcel Dekker, 473-540.

De Troyer, A. and Estene, M. (1984). Coordination between rib cage muscles and diaphragm during quiet breathing in humans. *J Appl Physiol*, 57, 899-906.

De Troyer A. Kirkwood, P.A. & Wilson, T.A. (2005). Respiratory action of the intercostal muscles. *Physiol Rev*, 86, 717-756.

Di Vanna, A., Braga, A.M., Laterza, M.C., Ueno, L.M., Rondon, M.U., Barretto, A.C., Middlekauff, H.R., Negrão, C.E. (2007). Blunted muscle vasodilatation during chemoreceptor stimulation in patients with heart failure. *American J Physiol: Heart Circ Physiol*, 293, H846-52.

Diamond, P. & LeBlanc, J. (1987). Role of autonomic nervous system in postprandial thermogenesis in dogs. *Am J Physiol*, 252, E719-726.

Dorrington, K.L., Clar, C., Young, J.D., Jonas, M., Tansley, J.G., Robbins, P.A.. (1997). Time course of the human pulmonary vascular response to 8 hours of isocapnic hypoxia. *Am J Physiol*, 273, H1126-3411.

Downing, S.E., Lee, J.C. (1980). Nervous control of the pulmonary circulation. *Ann Rev Physiol*, 42, 199-210.

Drew, R.C., Bell, M.P. & White, M.J. (2009). Modulation of spontaneous baroreflex control of heart rate and indexes of vagal tone by passive calf muscle stretch during graded metaboreflex activation in humans. *J Appl Physiol*, 104, 716-723.

Drew, R.C., McIntyre, D.B., Ring, C. & White, M.J. (2008). Local metabolite accumulation augments passive muscle stretch-induced modulation of carotid-cardiac but not carotid-vasomotor baroreflex sensitivity in man. *Exp Physiol*, 93, 1044-1057.

Duffin, J., Bechbache, R.R., Goode, R.C. & Chung, S.A. (1980). The ventilatory response to carbon dioxide in hyperoxic exercise. *Respir Physiol*, 40, 93-105.

Duke, H.N. & Stedeford, R.D. (1960). Pulmonary vasomotor responses to epinephrine and norepinephrine in the cat. Influence of the sympathetic nervous system. *Circ Res*, 8:640-648.

Duncan, G., Johnson, R.H. & Lambie, D.G. (1981). Role of sensory nerves in the cardiovascular and respiratory changes with isometric forearm exercise in man. *Clin Sci*, 60, 145-155.

Duplain, H., Vollenweider, L., Delabays, A., Nicod, P., Bärtsh, P. & Scherrer, U. (1999). Augmented sympathetic activation during short-term hypoxia and high-altitude exposure in subjects susceptible to high-altitude pulmonary edema. *Circulation*, 99, 1713-1718.

Eldridge, F.L. (1994). Central integration of mechanisms in exercise hyperpnea. *Med Sci Sport Exerc*, 26, 319-327.

Eldridge, M.W., Podolsky, A. Richardson, R.S., Johnson, D.H., Knight, D.R., Johnson, E.C., Hopkins, S.R., Michimata, H., Grassi, B., Feiner, J., Kurdak, S.S., Bickler, P.E., Wanger, P.D. and Severinghaus, J.W. (1996). Pulmonary

hemodynamic response to exercise in subjects with prior high-altitude pulmonary edema. *J Appl Physiol*. 81, 911-921.

El-Kashef, H.A., Hofman, W.F., Ehrhart, I.C. & Catravas, J.D. (1991). Multiple muscarinic receptor subtypes in the canine pulmonary circulation. *J Appl Physiol*, 71, 2032-2043.

Elkins, R.C. & Milnor, W.R. (1971). Pulmonary vascular response to exercise in the dog. *Circ Res*, 29, 591-599.

Engel, L.A. & Ritchie, B. (1971). Ventilatory response to inhaled carbon dioxide in hyperthyroidism. *J Appl Physiol*, 30, 173-177.

Fagius, J., Niklasson, F. & Berne, C. (1986). Sympathetic outflow in human muscle nerves increases during hypoglycemia. *Diabetes*, 35, 1124-1129.

Fatemian, M., Nieuwenhuijs, D.J., Teppema, L.J, Meinesz, S., van der Mey, A.G., Dahan, A. & Robbins, P.A. (2003). The respiratory response to carbon dioxide in humans with unilateral and bilateral resections of the carotid bodies. *J Physiol*, 549, 965-73.

Fineman, J.R., Wong, J. and Soifer, S.J. (1993). Hyperoxia and alkalosis produce pulmonary vasodilation independent of endothelium-derived nitric oxide in newborn lambs. *Pediatr Res*, 33, 341-346.

Fink, G.R., Adams, L., Watson, J.D., Innes, J.A., Wuyam, B., Kobayashi, I., Corfield, D.R., Murphy, K., Jones, T., Frackowiak, R.S. (1995). Hyperpnoea during and immediately after exercise in man: evidence of motor cortical involvement. *J Physiol*, 489, 663-675

Fisher, B.M., Thompson, I., Hepburn, D.A., & Frier, B.M. (1991) Effect of adrenergic blockade on serum potassium changes in response to acute insulin-induced hypoglycaemia in non-diabetic humans. *Diabetes Care*, 14, 548-552.

Fisher, J.P., Bell, M.P.D. & White, M.J. (2005). Cardiovascular responses to human calf muscle stretch during varying levels of muscle metaboreflex activation. *Exp Physiol*, 90, 773-781.

Fishman, A.P. (1990). *The pulmonary circulation: Normal and abnormal*. Philadelphia, PA: University of Philadelphia Press.

Fitzgerald, R.S. & Parks, D.C. (1971). Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in cats. *Respir Physiol*, 12, 218-229.

Fontana, G.A., Pantaleo, T., Bongiani, F., Cresci, F., Manconi, R. & Panuccio, P. (1993). Respiratory and cardiovascular responses to static handgrip exercise in humans. *J Appl Physiol*, 75, 2789-2796.

Forster HV, Pan LG. (1994). The role of the carotid chemoreceptors in the control of breathing during exercise. *Medicine and Science in Sports and Exercise*, 26, 328-336.

Forster, H.V., Dunning, M.B., Lowry, T.F., Erickson, B.K., Forster, M.A., Pan, L.G., Brice, A.G. & Effros, R.M. (1993). Effect of asthma and ventilatory loading on arterial PCO<sub>2</sub> of humans during submaximal exercise. *J Appl Physiol*, 75, 1385-1394.

Francis, N., Cohen-Solal, A. & Logeart, D. (1999). Peripheral muscle ergoreceptors and ventilatory response during exercise recovery in heart failure. *Am J Physiol; Heart Circ Physiol*, 245, H913-917.

Freyschuss, U. (1970). *Cardiovascular adjustments to somatomotor activation*. *Acta Physiol Scand, Suppl 342*, 1-60.

Galbo, H., Christensen, N.J. & Holst, J.J. (1977). Catecholamines and pancreatic hormones during autonomic blockade in exercising man. *Acta Physiol Scand*, 101, 428-437.

Gao, L., Pan, Y.-X., Wang, W.-Z, Li, Y., Schultz, H.D., Zucker, I.H. & Wang, W. (2007). Cardiac sympathetic afferent stimulation augments the arterial chemoreceptor reflex in anesthetised rats. *J Appl Physiol*, 102, 37-43.

Gardner, W.N. (1980). The pattern of breathing following step changes of alveolar partial pressures of carbon dioxide and oxygen in man. *J Physiol*, 300, 55-73.

Gelfand, R. & Lambertsen, C.J. (1973). Dynamic respiratory response to abrupt change of inspired CO<sub>2</sub> at normal and high PO<sub>2</sub>. *J Appl Physiol*, 35, 903-913.

Gladwell, V. F. & Coote, J. H. (2002). Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. *J Physiol*, 540, 1095- 1102.

Gonzalez, C., Almaraz, L., Obeso, A. and Rigual, R. (1994). Carotid body chemoreceptors: from natural stimuli to sensory discharges. *Physiol Rev*, 74, 829-98.

Grieve, D.A.A., Clark, A.L., McCann, G.P. & Hillis, W.S. (1999). The ergoreflex in patients with chronic stable heart failure. *Int J Cardiol*, 68, 157-164.

Grover, R.F., Wagner, W.W., McMurthy, I.F. & Reeves, J.T. (1983). Pulmonary Circulation. In: *Handbook of Physiology, Section 2, The Cardiovascular System*, vol. III, *Peripheral Circulation and Organ Blood Flow*. ed. Shepherd JT, Abboud FM, part 2, chapt. 14, pp 103-136. American Physiological Society, Bethesda, MD, USA.

Grünig, E., Mereles, D., Hildebrandt, W., Swenson, E.R., Kübler, W., Kuecherer, H. & Bärtzsch, P. (2000). Stress Doppler echocardiography for identification of high altitude pulmonary edema. *J Am Coll Cardiol*, 35, 980-987.

Guazzi, M., Casali, M., Berti, F., Rossoni, G., Colonna, V.D. & Guazzi, M.D. (2008). Endothelium-mediated modulation of ergoreflex and improvement in

exercise ventilation by acute sildenafil in heart failure patients. *Clin Pharmacol Ther*, 83, 336-341.

Guazzi, M., Samaja, M., Arena, R., Vicenzi, M. & Guazzi, M.D. (2007). Long-term use of sildenafil in the therapeutic management of heart failure. *J Am Coll Cardiol*, 50, 2136-2144.

Guazzi, M.D., De Cesare, N., Fiorentini, C., Galli, C., Montorsi, P., Pepi, M., Tamborini, G. (1986). Pulmonary vascular supersensitivity to catecholamines in systemic high blood pressure. *J Am Coll Cardiol*, 8, 1137-44.

Hakim, T.S., Lisbona, R. & Dean, G.W. (1989). Effect of cardiac output on gravity-dependent and nondependent inequality in pulmonary blood flow. *J Appl Physiol*, 66, 1570-1578.

Hammond, R.L., Augustyniak, R.A., Rossi, N.F., Churchill, P.C., Lapanowski, K., O'Leary, D.S. (2000). Heart failure alters the strength and mechanisms of the muscle metaboreflex. *Am J Physiol: Heart Circ Physiol*, 278, H818-28.

Haneda, T., Nakajima, T., Shirato, K., Onodera, S. & Takishima, T. (1983). Effects of oxygen breathing on vascular input impedance in patients with pulmonary hypertension. *Chest*, 83, 520-527.

Hansen, J., Thomas, G.D., Jacobsen, T.N. & Victor, R.G. (1994). Muscle metaboreflex triggers parallel sympathetic activation in exercising and resting human skeletal muscle. *Am J Physiol: Heart Circ Physiol*, 266, H2508-2514.

Haouzi, P. (2006). Theories on the nature of the coupling between ventilation and gas exchange during exercise. *Respir Physiol Neurobiol*, 151, 267-279.

Haouzi, P., Chenuel, B. & Chalon, B. (2001). Control of breathing and muscle perfusion in humans. *Exp Physiol*, 86, 759-768.

Haouzi, P., Chenuel, B. & Huszczuk, A. (2004). Sensing vascular distension in skeletal muscle by slow conducting afferent fibers: neurophysiological basis and implication for respiratory control. *J Appl Physiol*, 96, 407-418.

Haouzi, P., Hill, J.M., Lewis, B.K. & Kaufman, M.P. (1999). Responses of group III and IV muscle afferents to distension of the peripheral vascular bed. *J Appl Physiol*, 87, 545-553.

Haouzi, P., Huszczuk, A., Porszasz, J., Chalon, B., Wasserman, K. & Whipp, B.J. (1993). Femoral vascular occlusion and ventilation during recovery from heavy exercise. *Respir Physiol*, 94, 137-150.

Hatle, L. and Anglesen, B. (1985). *Doppler ultrasound in cardiology: physical principles and clinical applications*. Philadelphia: Lea & Febiger.

Harris, P. (1957). Influence of acetylcholine on the pulmonary arterial pressure. *Heart*, 19, 272-278.

Hesse, B., Kanstrup, I.L., Christensen, N.J., Ingemann-Hansen, T., Hansen, J.F., Halkjaer-Kristensen, J. & Petersen, F.B. (1981). Reduced norepinephrine response to dynamic exercise in human subjects during O<sub>2</sub> breathing. *J Appl Physiol*, 51, 176-178.

Hillier, S.C. Graham, J.A. Hanger, C.C., Godbey, P.S., Glenny, R.W. & Wagner, W.W. (1997). Hypoxic vasoconstriction in pulmonary arterioles and venules. *J Appl Physiol*, 82, 1084-1090.

Himelman, R.B., Syulbarg, M., Kircher, B., Lee, E., Lee, K., Dean, N.C., Wolfe, C.L. & Schiller, N.B. (1989). Noninvasive evaluation of pulmonary artery pressure during exercise by saline-enhanced Doppler echocardiography in chronic pulmonary disease. *Circulation*, 79, 863-871.

Houssière A, Najem B, Cuylits N, Cuypers S, Naeije R, van de Borne P. (2006). Hyperoxia enhances metaboreflex sensitivity during static exercise in humans. *Am J Physiol: Heart Circ Physiol*, 291, H210-215.

Houssiere, A., Najem, B., Pathak, A., Xhaët, O., Naeije, R. & Van De Borne, P. (2006). Chemoreflex and metaboreflex responses to static hypoxic exercise in aging humans. *Med Sci Sport Exerc*, 38, 305-312.

Houston, A.B. and Simpson, I.A. (1988). *Cardiac Doppler ultrasound*. Wright.

Howden, R., Lightfoot, J.T., Brown, S.J. & Swaine, I.L. (2004). The effects of breathing 5% CO<sub>2</sub> on human cardiovascular responses and tolerance to orthostatic stress. *Exp Physiol*, 89, 465-471.

Hudson, A.L. Gandevia, S.C. Butler, J.E. (2007). The effect of lung volume on the co-ordinated recruitment of the scalene and sternomastoid muscles in humans, *J Physiol*, 584, 261-270.

Hulsbosch, M.A., Binkhorst, R.A. & Folgering, H.T. (1981). Effects of positive and negative exercise on ventilatory CO<sub>2</sub> sensitivity. *Eur J Appl Physiol Occ Physiol*, 47, 73-81.

Hyman, A.L. & Kadowitz, P.J. (1988). Tone-dependent responses to acetylcholine in the feline pulmonary vascular bed. *J Appl Physiol*, 64, 2002-2009.

Hyman, A.L., Nandiwada, P., Knight, D.S., Kadowitz, P.J. (1981). Pulmonary vasodilator responses to catecholamines and sympathetic nerve stimulation in the cat. Evidence that vascular beta-2 adrenoreceptors are innervated. *Circ Res*, 48, 407-415.

Iellamo, F., Massaro, M., Raimondi, G., Peruzzi, G., Legramante, J.M. Role of muscular factors in cardiorespiratory responses to static exercise: contribution of reflex mechanisms. *J Appl Physiol*, 86, 174-180.

Imms, .F.J. & Mehta, D. (1989). Respiratory responses to sustained isometric muscle contractions in man: The effect of muscle mass. *J Physiol*, 419, 1-14.

Ingram, R.H. Jr, Szidon, J.P., Fishman, A.P. (1970). Response of the main pulmonary artery of dogs to neuronally released versus blood-borne norepinephrine. *Circ Res*, 26, 249-262.

Ingram, R.H., Szidon, J.P., Skalak, R. & Fishman, A.P. (1968). Effects of sympathetic nerve stimulation on the pulmonary arterial tree of the isolated lobe perfused in situ. *Circ Res*, 22, 801-815.

Janicki, J.S., Weber, K.T., Likoff, M.J. & Fishman, A.P. (1985). The pressure-flow response of the pulmonary circulation in patients with heart failure and pulmonary vascular disease. *Circulation*, 72, 1270-1278.

Joels, N. & White, H. (1968). The contribution of the arterial chemoreceptors to the stimulation of respiration by adrenaline and noradrenaline in the cat. *J Physiol*, 197, 1-23.

Johnston, C.S., Day, C.S. & Swan, P.D. (2002). Postprandial thermogenesis is increased 100% on a high-protein, low-fat diet versus a high-carbohydrate, low-fat diet in healthy, young women. *J Am Coll Nutr*, 21, 55-61.

Jones, D., Egi, M., Baldwin, I. & Bellomo, R. (2004). A pilot study of the effect of altering airway pressure on systolic pulse pressure variation in the systemic and pulmonary arterial circulations. *Crit Care Res*, 6, 167-174.

Jones, P.W., French, W., Weissman, M.L., Wasserman, K. (1981). Ventilatory responses to cardiac output changes in patients with pacemakers. *J Appl Physiol*, 51, 1103-1107.

Jordan, J. Shannon, J.R. Diedrich, A Black, B., Costa, F., Robertson, D. & Biaggioni, I. (2000). Interaction of carbon dioxide and sympathetic nervous system activity in the regulation of cerebral perfusion in humans. *Hypertension*, 36, 383-388.

Jørgensen, L.G, Perko, M., Hanel, B., Schroeder, T.V. & Secher, N.H. (1992) Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. *J Appl Physiol*, 72, 1123-1132.

Kadowitz, P.J. & Hyman, A.L. (1973). Effect of sympathetic nerve stimulation on pulmonary vascular resistance in the dog. *Circ Res*, 32, 221-227.

Kadowitz, P.J., Joiner, P.D. & Hyman, A.L. (1974). Effect of sympathetic nerve stimulation on pulmonary vascular resistance in the intact breathing dog. *Proc Soc Exp Biol Med*, 147, 68-71.

Kane, D.W., Tesauro, T. & Newman, J.H. (1993). Adrenergic modulation of the pulmonary circulation during strenuous exercise in sheep. *Am Rev Respir Dis*, 147, 1233-1238.

Kane, D.W., Tesauro, T., Koizumi, T., Gupta, R. & Newman, J.H. (1994). Exercise-induced pulmonary vasoconstriction during combined blockade of nitric oxide synthase and beta adrenergic receptors. *J Clin Invest*, 93, 677-683.

Kara, T., Narkiewicz, K. & Somers, V.K. (2003). Chemoreflexes – physiology and clinical implications. *Acta Physiol Scand*, 177, 377-384.

Kaufman, M.P. & Rybicki, K.J. (1987). Discharge Properties of Group III and IV Muscle Afferents: Their Responses to Mechanical and Metabolic Stimuli. *Circ Res*, 61 suppl, I-60 – I-65

Kaufman, M.P., Iwamoto, G.A., Longhurst, J.C. & Mitchell, J.H. (1982). Effects of capsaicin and bradykinin on afferent fibers with endings in skeletal muscle. *Circ Res*, 50, 133–139.

Kawashima, A., Kubo, K., Kobayashi, T. & Sekiguchi, M. (1989). Hemodynamic responses to acute hypoxia, hypobaria, and exercise in subjects susceptible to high-altitude pulmonary edema. *J Appl Physiol*, 67, 1982-1989.

Kelley, M.A., Owens, G.R. & Fishman, A.P. (1982). Hypercapnic ventilation during exercise: effects of exercise methods and inhalation techniques. *Respir Physiol*, 50, 75-85.

Kiely, D.G., Cargill, R.I. & Lipworth, B.J. (1996). Effects of hypercapnia on hemodynamic, inotropic, lusitropic, and electrophysiologic indices in humans. *Chest*, 109, 1215-1221.

Kim, J., Sala-Mercado, J.A., Hammond, R.L. Rodriguez, J., Scislo, T.J. & O'Leary, D.S. (2005). Attenuated arterial baroreflex buffering of muscle metaboreflex in heart failure. *Am J Physiol: Heart Circ Physio*, 289, H2416-H2423.

Koizumi, T., Hermo, C.I., Bjertnaes, L.J., Banerjee, M., Newman, J.H., Kubo, K. (1996). Nitric oxide and nitroglycerin reversal of pulmonary vasoconstriction induced by alpha-activation during exercise. *Am J Physiol*, 270, H875-880.

Kontessis, P., Jones, S., Dodds, R., Trevisan, R., Nosadini, R., Fioretto, P., Borsato, M., Sacerdoti, D. & Viberti, G. (1990). Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins. *Kidney Int*, 38, 136-144.

Kostreva, D.R., Zuperku, E.J., Purtock, R.V., Coon, R.L., Kampine, J.P. (1975). Sympathetic afferent nerve activity of right heart origin. *Am J Physiol*, 229, 911-5.

Koyama, Y., Coker, R.H., Denny, J.C., Lacy, D.B., Jabbour, K., Williams, P.E. & Wasserman, D.H. (2001). Role of carotid bodies in control of the neuroendocrine response to exercise. *Am J Physiol: Endocrinol Metab*, 281, 742-748.

Kumar, P. & Bin-Jaliah, I. (2007). Adequate stimuli of the carotid body: More than an oxygen sensor? *Respir Physiol Neurobiol*, 157, 12-21.

Kuramoto, K., Rodbard, S. (1962). Effects of blood flow and left atrial pressure on pulmonary venous resistance. *Circ Res*, 11, 240-246.

Lahiri, S. & DeLaney, R.G. (1975). Stimulus interaction in the responses of carotid body chemoreceptor single afferent fibers. *Respir Physiol*, 24, 249-266.

Lahiri, S., Mitchell, C.H., Reigada, D., Roy, A. & Cherniack, N.S. (2007). Purines, the carotid body and respiration. *Respir Physiol Neurobiol*, 157, 123-129.

Lahiri, S. & Forster, R.E. (2003). CO<sub>2</sub>/H(+) sensing: peripheral and central chemoreception. *Int J Biochem Cell Biol*, 35, 1413-1435.

Lai, J. & Bruce, E.N. (1997). Ventilatory stability to transient CO<sub>2</sub> disturbances in hyperoxia and normoxia in awake humans. *J Appl Physiol*, 83, 466-476

Landauer, R.C. Pepper, D.R., Kumar, P. (1995). Interaction of temperature and CO<sub>2</sub> in the adult rat carotid body. *J Physiol*, 489P, 162P-163P.

Laskey, D.K., Ferrari, V.A., Palevsky, H.I. & Kussmaul, W.G. (1993). Pulmonary artery hemodynamics in primary pulmonary hypertension. *J Am Coll Cardiol*, 21, 406-412.

LeBlanc, J. & Brondel, L. (1985). Role of palatability on meal-induced thermogenesis in human subjects. *Am J Physiol*, 248, E333-6.

Legrand, A. Schneider, E. Gevenois, P-A, & DeTroyer, A. (2003). Respiratory effects of the scalene and sternomastoid muscles in humans. *J Appl Physiol*, 94, 1467-1472.

Leshower, B.G., Potts, J.T., Garry, M.G. & Mitchell, J.H. (2001). Reflex cardiovascular responses evoked by selective activation of skeletal muscle ergoreceptors. *J Appl Physiol*, 90, 308-316.

Levitzky, M.G. (2006). Teaching the effects of gravity and intravascular and alveolar pressures on the distribution of pulmonary blood flow using a classic paper by West et al. *Adv Physiol Ed*, 30, 5-8.

Lewis, G.D., Shah, R.V., Pappagianopoulos, P.P. & Semigran, M.J. (2008). Determinants of ventilatory efficiency in heart failure: the role of right ventricular performance and pulmonary vascular tone. *Circulation: Heart Failure*, 1, 227-233.

Lewis, J.F., Kno, L.C., Nelson, J.G., Limacher, M.C. and Quinones, M.A. (1984). Pulsed Doppler echocardiographic determination of stroke volume and cardiac output: clinical validation of two new methods using the apical window. *Circulation*, 70, 425-431.

Li, A.H. & Nattie, E.E. (1997). Focal central chemoreceptor sensitivity in the RTN studies with a CO<sub>2</sub> diffusion pipette in vivo. *J Appl Physiol*, 83, 420-428.

Llados, F. & Zapata, P. (1978). Effects of adrenoceptor stimulating and blocking agents on carotid body chemosensory inhibition. *J Physiol*, 274, 501-509.

Lodato, R.F. Michael, J.R. Murray, P.A. (1985). Multipoint pulmonary vascular pressure-cardiac output plots in conscious dogs. *Am J Physiol*, 249, H351-H357.

Lorinc, Z., Derr, J., Snider, M., Lydic, R. (1991). Defining origin of positive slope in hypercapnic ventilatory response curve. *Am J Physiol*, 261, R747-51.

Lykidis, C.K. White, M.J. & Balanos, G.M. (2008). The pulmonary vascular response to the sustained activation of the muscle metaboreflex in man. *Exp Physiol*, 93, 247-253.

Madden, J.A., Dawson, C.A. and Harder, D.R. (1985). Hypoxia-induced activation in small pulmonary arteries from the cat. *J Appl Physiol*, 59; 113-118.

Mahamed, S., Ali, A.F., Ho, D., Wang, B., Duffin, J. (2001). The contribution of

chemoreflex drives to resting breathing in man. *Exp Physiol*, 86, 109-16.

Makeham, J.M., Goodchild, A.K., Costin, N.S. & Pilowski, P.M. (2004). Hypercapnia selectively attenuates the somato-sympathetic reflex. *Respir Physiol Neurobiol*, 140, 133-143.

Mark, A.L., Victor, R.G., Nerhed, C. & Wallin, B.G. (1985). Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circ Res*, 57, 461-469.

Marshall, J.M. (1994). Peripheral chemoreceptors and cardiovascular regulation. *Physiol Rev*, 74, 543-594.

Martin, B.J., Weil, J.V., Sparks, K.E., McCullough, R.E. & Grover, R.F. (1978). Exercise ventilation correlates positively with ventilatory chemoresponsiveness. *J Appl Physiol*, 45, 557-564.

Martin, P.A., Mitchell, G.S. (1993). Long-term modulation of the exercise ventilatory response in goats. *J Physiol*, 470, 601-17.

Maskell, P.D., Rusius, C.J. Whitehead, K.J. & Kumar, P. (2006) Adrenaline increases carotid body CO<sub>2</sub> sensitivity. *Adv Exp Med Biol*, 580, 245-250.

Mateika, J.H. & Duffin, J. (1995). A review of the control of breathing during exercise. *Eur J Appl Physiol*, 71, 1-27.

McClain, J., Hardy, J. C., & Sinoway, L. I. (1994). Forearm compression during exercise increases sympathetic nerve traffic. *J Appl Physiol*, 77, 2612-2617.

McCloskey, D. I. & Mitchell, J. H. (1972). Reflex cardiovascular and respiratory responses originating in exercising muscle. *J Physiol*, 224, 173-186.

McConnell, A.K. & Semple, E.S. (1996). Ventilatory sensitivity to carbon dioxide: the influence of exercise and athleticism. *Med Sci Sport Exerc*, 28, 685-691.

McQuillan, B.M., Picard, M.H., Leavitt, M., Weyman, A.E. (2001). Clinical correlates and reference intervals for pulmonary artery systolic pressure among echocardiographically normal subjects. *Circulation*, 104, 2797-2802.

Merkus, D., de Beer, V.J., Houweling, B., Duncker, D.J. (2008). Control of pulmonary vascular tone during exercise in health and pulmonary hypertension. *Pharmacol Ther*, 119, 242-63.

Middlekauff, H.R., Chiu, J., Hamilton, M.A., Fonarow, G.C., MacLellan, W.R., Hage, A., Moriguchi, J. & Patel, J. (2004). Muscle mechanoreceptor sensitivity in heart failure. *Am J Physiology: Heart Circ Physiol*, 287, 1937-1943.

Middlekauff, H.R., Nitzsche, E.U., Hoh, C.K., Hamilton, M.A., Fonarow, G.C., Hage, A., Moriguchi, J.D. (2000). Exaggerated renal vasoconstriction during exercise in heart failure patients. *Circulation*, 101, 784-9.

Middlekauff, H.R., Nitzsche, E.U., Nguyen, A.H., Hoh, C.K., Gibbs, G.G. (1997). Modulation of renal cortical blood flow during static exercise in humans. *Circ Res*, 80, 62-8.

Milnor WR, Conti CR, Lewis KB, O'Rourke MF. (1969). Pulmonary arterial pulse wave velocity and impedance in man. *Circ Res*, 25, 637-49.

Mitchell G.S., Johnson S.M. (2003). Neuroplasticity in respiratory motor control. *J Appl Physiol*, 94, 358-74.

Mitchell, G.S. & Babb, T.G. (2006). Layers of exercise hyperpnea: Modulation and plasticity. *Respir Physiol Neurobiol*, 151, 251-266.

Momen, A., Leuenberger, U.A., Ray, C.A., Cha, S., Handly, B., & Sinoway, L.I. (2003). Renal vascular responses to static handgrip: role of muscle mechanoreflex. *Am J Physiol: Heart Circ Physiol*, 285, H1247-H1253

Moruzzi, P., Sganzerla, P. & Guazzi, M.D. (1988). Pulmonary vasomotor responses to neural activation in man. *Cardiovasc Res*, 22, 25-30.

Moruzzi, P., Sganzerla, P. & Guazzi, M.D. (1989a). Neural influences on the pulmonary vascular bed in man. *Clin Sci*, 76, 363-368.

Moruzzi, P., Sganzerla, P. & Guazzi, M.D. (1989b). Pulmonary vasoconstriction overreactivity in borderline systemic hypertension. *Cardiovasc Res*, 23, 666-673.

Moudgil, R., Michelakis, E.D. & Archer, S.L. (2005). Hypoxic pulmonary vasoconstriction. *J Appl Physiol*, 98, 390-403.

Murata, J. & Matsukawa, K. (2001). Cardiac vagal and sympathetic efferent discharges are differentially modified by stretch of skeletal muscle. *Am J Physiol: Heart Circ Physiol*, 280, H237-H245.

Murphy, K., Stidwill, R.P., Cross, B.A., Leaver, K.D., Anastassiades, E., Phillips, M., Guz, A., Semple, S.J. (1987). Is hypercapnia necessary for the ventilatory response to exercise in man? *Clin Sci*, 73, 617-25.

Murray, P.A., Lodato, R.F. & Michael, J.R. (1986) Neural antagonists modulate pulmonary vascular pressure-flow plots in conscious dogs. *J Appl Physiol*, 60, 1900-1907.

Muza, S.R., Lee, Y., Wiley, R.L., McDonald, S. & Zechman, F.W. (1983). Ventilatory responses to static handgrip exercise. *J Appl Physiol*, 54, 1457-1462.

Naeije, R. Pulmonary vascular function. In Nunn's Applied Respiratory Physiology. Lumb, A.B. (2000). Butterworth-Heineman, 3-13.

Nair, K.S., Halliday, D. & Garrow, J.S. (1983). Thermic response to isoenergetic protein, carbohydrate or fat meals in lean and obese subjects. *Clin Sci*, 65, 307-312.

Nandiwada, P.A., Hyman, A.L. & Kadowitz, P.J. (1983). Pulmonary vasodilator responses to vagal stimulation and acetylcholine in the cat. *Circulation Research*, 53, 86-95

Nattie, E.E. & Li, A.H. (2002). CO<sub>2</sub> dialysis in nucleus tractus solitarius region of rat increases ventilation in sleep and wakefulness. *J Appl Physiol*, 92, 2119-2130.

Negrão, C.E., Rondon, M.U., Tinucci, T., Alves, M.J., Roveda, F., Braga, A.M., Reis, S.F., Nastari, L., Barretto, A.C., Krieger, E.M., Middlekauff, H.R. (2001). Abnormal neurovascular control during exercise is linked to heart failure severity. *J Appl Physiol: Heart Circ Physiol*, 280, H1286-92.

Nishiyasu, T., Tan, N., Morimoto, K., Sone, R. & Murakami, N. (1998). Cardiovascular and humoral responses to sustained muscle metaboreflex activation in humans. *J Appl Physiol*, 84, 116-122.

Norel, X., Walch, L., Costantino, M., Labat, C., Gorenne, I., Dulmet, E., Rossi, F. & Brink, C. (1996). M<sub>1</sub> and M<sub>3</sub> muscarinic receptors in human pulmonary arteries. *Br J Pharmacol*, 119, 149-157.

Notarius, C.F., Atchison, D.J., Rongen, G.A., Floras, J.S. (2001). Effect of adenosine receptor blockade with caffeine on sympathetic response to handgrip exercise in heart failure. *Am J Physiol: Heart Circ Physiol*, 281, H1312-8.

Nurhayati Y, Boutcher SH. (1998). Cardiovascular response to passive cycle exercise. *Med Sci Sport Exerc*, 30, 234-238.

Nurse, C.A. (2005). Neurotransmission and neuromodulation in the chemosensory carotid body. *Auton Neurosci*, 120, 1-9.

Nye, P.C. (1994). Identification of peripheral chemoreceptor stimuli. *Med Sci Sport Exerc*, 26, 311-318.

O'Leary, D.S. (2005). Altered reflex cardiovascular control during exercise in heart failure: animal studies. *Exp Physiol*, 91, 73-77.

Oelberg, D.A., Evans, A.B., Hrovat, M.I., Pappagianopoulos, P.P., Patz, S. & Systrom, D.M. (1998). Skeletal muscle chemoreflex and pHi in exercise ventilatory control. *J Appl Physiol*, 84, 676-82.

O'Leary, D.S., Augustyniak, R.A. (1998). Muscle metaboreflex increases ventricular performance in conscious dogs. *Am J Physiol: Heart Circ Physiol*, 275, H220-4.

Olschewski, A. & Weir, E.K. Hypoxic pulmonary vasoconstriction and hypertension. In *Nunn's Applied Respiratory Physiology*. Lumb, A.B. (2000). Butterworth-Heinemann, 31-44.

Onimaru, H. and Homma, I. (2003). A novel functional neuron group for respiratory rhythm generation in the ventral medulla. *J Neurosci*, 23, 1478-1486.

Onimaru, H. Arata, I. and Homma, I. (2000). Inhibitory synaptic inputs to the respiratory rhythm generator in the medulla isolated from newborn rats. *Pflugers Arch*. 417, 425-423

Ordway, G.A., Waldrop, T.G., Iwamoto, G.A., Gentile, B.J. (1989). Hypothalamic influences on cardiovascular response of beagles to dynamic exercise. *Am J Physiol: Heart Circ Physiol*, 257, H1247-53.

O'Regan, R.G. & Majcherczyk, S. (1982). Role of peripheral chemoreceptors and central chemosensitivity in the regulation of respiration and circulation. *J Exp Biol*, 100, 23-40.

O'Regan, R.G. (1981). Responses of carotid body chemosensory activity and blood flow to stimulation of sympathetic nerves in the cat. *J Physiol*, 315, 81-98

Pandit, J.J. & Robbins, P.A. (1992). Ventilation and gas exchange during sustained exercise at normal and raised CO<sub>2</sub> in man. *Respir Physiol*, 88, 101-112.

Paterson, D.J. (1992). Potassium and ventilation in exercise. *J Appl Physiol*, 72, 811-20.

Paton, J.F. and Richter, D.W. (1995) Role of fast inhibitory synaptic mechanisms in respiratory rhythm generation in the maturing mouse. *J Physiol*, 484, 502-512.

Peacock, A.J., Challenor, V. & Sutherland, G. (1990). Estimation of pulmonary artery pressure by Doppler echocardiography in normal subjects made hypoxic. *Respir Med*, 84, 335-337.

Phillipson, E.A., Bowes, G., Townsend, E.R., Duffin, J. & Cooper, J.D. (1981). Carotid chemoreceptors in ventilatory responses to changes in venous CO<sub>2</sub> load. *J Appl Physiol*, 51, 1398-1403.

Pianosi, P. & Khoo, M.C. (1995). Change in the peripheral CO<sub>2</sub> chemoreflex from rest to exercise. *Eur J Appl Physiol Occup Physiol*, 70, 360-366.

Pickar, J. G., Hill, J. M. & Kaufman, M. P. (1994). Dynamic exercise stimulates group III muscle afferents. *J Neurophysiol*, 71, 753-760.

Piense, H. (1976). The influence of pulmonary blood flow rate on vascular input impedance and hydraulic power in the sympathetically and noradrenaline stimulated cat lung. *Acta Physiol Scand*, 98, 44-53.

Piense, H. (1986). Pulmonary arterial impedance and right ventricular function. *Physiol Rev*, 66, 606-652.

Piepoli, M., Clark, A.L. & Coats, A.J.S. (1995). Muscle metaboreceptors in hemodynamic, autonomic, and ventilatory responses to exercise in men. *Am J Physiol*, 38, H1428-1436.

Piepoli, M., Clark, A.L., Volteranni, M., Adamopoulos, S., Sleight, P. & Coats, A.J.S. (1996). Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: Effects of physical training. *Circulation*, 93, 940-952.

Piepoli, M., Ponikowski, P., Clark, A.L., Banasiak, W., Capucci, A. & Coats, A.J.S. (1999). A neural link to explain the 'muscle hypothesis' of exercise intolerance in chronic heart failure. *Am Heart J*, 137, 1050-1056.

Piepoli, M., Ponikowski, P., Clark, A.L., Banasiak, W., Capucci, A. & Coats, A.J.S. (1999). A neural link to explain the "muscle hypothesis" of exercise intolerance in chronic heart failure. *Am Heart J*, 137, 1050-1056.

Piepoli, M.F., Dimopoulos, K., Concu, A., & Crisafulli, A. (2008). Cardiovascular and ventilatory control during exercise in chronic heart failure: role of muscle reflexes. *Int J Cardiol*, 130, 3-10.

Piepoli, M.F., Kaczmarek, A., Francis, D.P., Davies, L.C., Rauchhaus, M., Jankowska, E.A., Anker, S.D., Capucci, A., Banasiak, W., Ponikowski, P. (2006). Reduced peripheral skeletal muscle mass and abnormal reflex physiology in chronic heart failure. *Circulation*, 114, 126-34.

Ponikowski, P. P., Chua, T.P., Francis, D.P., Capucci, A., Coats, A.J.S. & Piepoli, M.F. (2001). Muscle ergoreceptor overactivity reflects deterioration in clinical status and cardiorespiratory reflex control in chronic heart failure. *Circulation*, 104, 2324-2330.

Ponikowski, P., Chua, T.P., Piepoli, M., Ondusova, D., Webb-Peploe, K., Harrington, D., Anker, S.D., Volterrani, M., Colombo, R., Mazzuero, G., Giordano, A., Coats, A.J. (1997). Augmented peripheral chemosensitivity as a potential input to baroreflex impairment and autonomic imbalance in chronic heart failure. *Circulation*, 96, 2586-94.

Poole, D.C., Ward, S.A. & Whipp, B.J. (1988). Control of blood-gas and acid-base status during isometric exercise in humans. *J Physiol*, 396, 365-377.

Poon, C.S., Greene, J.G. (1985). Control of exercise hyperpnea during hypercapnia in humans. *J Appl Physiol*, 59, 792-797.

Poon, C.S., Ward, S.A., Whipp, B.J. (1987). Influence of inspiratory assistance on ventilatory control during moderate exercise. *J Appl Physiol*, 62, 551-60.

Porcelli, R.J. & Bergofsky, E.H. (1973). Adrenergic receptors in pulmonary vasoconstrictor responses to gaseous and humoral agents. *J Appl Physiol*, 34, 483-438.

Pott, F., Ray, C.A., Olesen, H.L., Ide, K & Secher, N.H. (1997). Middle cerebral artery blood velocity, arterial diameter and muscle sympathetic nerve activity during post-exercise muscle ischaemia. *Acta Physiol Scand*, 160, 43-47.

Prabhakar, N.R. & Peng, Y-J. (2004). Peripheral chemoreceptors in health and disease. *J Appl Physiol*, 96, 359-366.

Putnam, R.W. Filosa, J.A. & Ritucci, N.A. (2004). Cellular mechanisms involved in CO<sub>2</sub> and acid signalling in chemosensitive neurons. *Am J Physiol*, 287, C1493-1526.

Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J.J. & Astrup, A. (2003). Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. *Am J Clin Nutr*, 77, 91-100.

Ray, C.A., Secher, N.H., Mark, A.L.(1994). Modulation of sympathetic nerve activity during posthandgrip muscle ischemia in humans. *Am J Physiol: Heart Circ Physiol*, 266, H79-83.

Rebuck, A.S. & Read, J. (1971). Patterns of ventilatory response to carbon dioxide during recovery from severe asthma. *Clin Sci*, 41, 13-21.

Reeves, J.T. & Taylor, A.E. Pulmonary hemodynamics and fluid exchange in the lungs during exercise. In *Handbook of Physiology: Section 12, Exercise: Regulation and Integration of Multiple Systems*. Rowell, L.B. & Sheppard, J.T. (1996). Oxford University Press, New York, 585-613.

Reeves, J.T., Groves, B.M., Cymerman, A., Sutton, J.R., Wagner, P.D., Turkevich, D. & Houston, C.S. (1990). Operation Everest II: cardiac filling pressures during cycle exercise at sea level. *Respiration Physiology*, 80, 147-154.

Reindl, I., Wernecke, K-D., Opitz, C., Wensel, R., König, D., Dengler, T., Schimke, I. & Kleber, F.X. (1998). Impaired ventilatory efficiency in chronic heart failure: possible role of pulmonary vasoconstriction. *American Heart Journal*, 136, 778-785.

Reuben, S.R. (1971). Compliance of the human pulmonary arterial system in disease. *Circulation Research*, 29, 40-50.

Richards, A.M., Ikram, H., Nicholls, M.G., Espiner, E.A., Hamilton, E.J. & Richards, R.D. (1986). Ambulatory pulmonary arterial pressures in humans: relationship to arterial pressure and hormones. *American Journal of Physiology: Heart and Circulatory Physiology*, 251, H101-H108.

Richardson, J.R. (1979). Nerve supply to the lungs. *American Review of Respiratory Diseases*, 119, 785-802.

Richter, D.W., Ballantyne, D. & Remmers, J.E. (1986). How the respiratory rhythm is generated? A model. *News in Physiological Sciences*, 1, 109-112.

Robbins, P.A., Swanson, G.D. & Howson, M.G. (1982a). A prediction-correction scheme for forcing alveolar gases along certain time courses. *Journal of Applied Physiology*, 52, 1353-1357.

Robbins, P.A., Swanson, G.D., Micco, A.J. & Schubert, W.P. (1982b). A fast gas-mixing system for breath-to-breath respiratory control studies. *Journal of Applied Physiology*, 52, 1358-1362.

Robinson, S.M., Jaccard, C., Persaud, C., Jackson, A.A., Jequier, E. & Schutz, Y. (1990). Protein turnover and thermogenesis in response to high-protein and high-carbohydrate feeding in men. *American journal of Clinical Nutrition*, 52, 72-80.

Rotto, D. M. & Kaufman, M. P. (1988). Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *Journal of Applied Physiology*, 64, 2306-2313.

Rowell, L.B. & O'Leary, D.S. (1990). Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. *Journal of Applied Physiology*, 69, 407-418.

Sada, K., Shirai, M. & Ninomiya, I. (1987). Vagally and acetylcholine-mediated constriction in small pulmonary vessels of rabbits. *Journal of Applied Physiology*, 63, 1601-1609.

Salvi, S.S. (1999).  $\alpha_1$  – adrenergic hypothesis for pulmonary hypertension. *Chest*, 115, 1708-1719.

Schmidt, H., Francis, D.P., Rauchhaus, M., Werdan, K. & Piepoli, M.F. (2005). Chemo- and ergoreflexes in health, disease and ageing. *International Journal of Cardiology*, 98, 369-378.

Scott, A.C., Davies, L.C., Coats, A.J. & Piepoli, M. (2002). Relationship of skeletal muscle metaboreceptors in the upper and lower limbs with the respiratory control in patients with heart failure. *Clinical Science*, 102, 23-30.

Scott, A.C., Wensel, R., Davos, C.H., Georgiadou, P., Kemp, M., Hooper, J., Coats, A.J., Piepoli, M.F. (2003). Skeletal muscle reflex in heart failure patients: role of hydrogen. *Circulation*, 107, 300-306.

Scott, A.C., Wensel, R., Davos, C.H., Kemp, M., Kaczmarek, A., Hooper, J., Coats, A.J., Piepoli, M.F. (2002). Chemical mediators of the muscle ergoreflex in chronic heart failure: a putative role for prostaglandins in reflex ventilatory control. *Circulation*, 106, 214-20.

Seals, D.R. (1989) Sympathetic neural discharge and vascular resistance during exercise in humans. *Journal of Applied Physiology*, 66, 2472-2478.

Shea, S.A. (1996). Behavioural and arousal-related influences on breathing in humans. *Experimental Physiology*, 81, 1-26.

Sheriff, D.D., Augustyniak, R.A. & O'Leary, D.S. (1998). Muscle chemoreflex-induced increases in right atrial pressure. *American Journal of Physiology:Heart and Circulatory Physiology*, 275, H767-775.

Shirai, M., Sada, K., Ninomiya, I. (1986). Effects of regional alveolar hypoxia and hypercapnia on small pulmonary vessels in cats. *J Appl Physiol*, 61, 440-448.

Singh, B.S. (1984). Ventilatory response to CO<sub>2</sub>. I. A psychobiologic marker of the respiratory system. *Psychosomatic Medicine*, 46, 333-45.

Sinoway, L. (1989). Muscle acidosis during static exercise is associated with calf vasoconstriction. *Journal of Applied Physiology*, 66, 429-436.

Sinoway, L.I. & Li, J. (2005). A perspective on the muscle reflex: implications for the congestive heart failure. *Journal of Applied Physiology*, 99, 5-22.

Smith, C.A., Jameson, L.C., Dempsey, J.A. (1988). Effects of altered CSF [H<sup>+</sup>] on ventilatory responses to exercise in the awake goat. *Journal of Applied Physiology*, 65, 921-7.

Smith, S.A., Mitchel, J.H., Naseem, R.H. & Garry, M.G. (2005). Mechanoreflex mediates the exaggerated exercise pressor reflex in heart failure. *Circulation*, 112, 2293-2300.

Smith, S.A., Mitchell, J.H. & Garry, M.G. (2006). The mammalian exercise pressor reflex in health and disease. *Experimental Physiology*, 91, 89-102.

Somers, V.K., Mark, A.L., Zavala, D.C. & Abboud, F.M. (1989). Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. *Journal of Applied Physiology*, 67, 2101-2106.

Somers, V.K., Mark, A.L. & Abboud, F.M. (1991). Interaction of baroreceptor and chemoreceptor reflex control of sympathetic nerve activity in normal humans. *Journal of Clinical Investigation*, 87, 1953-1957.

Soucy, J. & Leblanc, J. (1999). Protein meals and postprandial thermogenesis. *Physiology & Behaviour*, 65, 705-709.

Spencer, H. & Leof, D. (1964). The innervation of the human lung. *Journal of Anatomy*, 98, 599-609.

Stebbin, C.L., Brown, B., Levin, D. & Longhurst, J.C. (1988). Reflex effect of skeletal muscle mechanoreceptor stimulation on the cardiovascular system. *Journal of Applied Physiology*, 65, 1539-1547.

Sterns, D. A., Ettinger, S. M., Gray, K. S., Whisler, S. K., Mosher, T. J., Smith, M. B., & Sinoway, L.I. (1991). Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation*, 84, 2034-2039.

Stickland, M.K., Miller, J.D., Smith, C.A., Dempsey, J.A. (2007). Carotid chemoreceptor modulation of regional blood flow distribution during exercise in health and chronic heart failure. *Circulation Research*, 100, 1371-8.

Strakosch, C.R., Stiel, J.N. & Györy, A.Z. (1976). Hypokalaemia occurring during insulin-induced hypoglycaemia. *Australian and New Zealand Journal of Medicine*, 6, 314-316.

Stubenitsky, R., Verdouw, P.D. & Duncker, D.J. (1998). Autonomic control of cardiovascular performance and whole body O<sub>2</sub> delivery and utilization in swine during treadmill exercise. *Cardiovascular Research*, 39, 459-474.

Swanson, G.D., Bellville, J.W. (1975). Step changes in end-tidal CO<sub>2</sub>: methods and implications. *Journal of Applied Physiology*, 39, 377-85.

Szidon, J.P. & Fishman, A.P. (1971). Participation of pulmonary circulation in the defense response. *American Journal of Physiology*, 220, 364-370.

Takakura, A.C.T. Moreira, T.S., Colombari, E, West, G.H. Stornetta, R.L. and Guyenet, P.G. (2006). Peripheral chemoreceptor inputs to retrotrapezoid nucleus (RTN) CO<sub>2</sub>-sensitive neurons in rats. *J Physiol*, 572, 503-523.

Tansley, J.G., Pedersen, M.E., Clar, C. & Robbins, P.A. (1998). Human ventilatory response to 8 h of euoxic hypercapnia. *J Appl Physiol*, 84, 431-4.

Toga, H., Bansal, V. & Raj, J.U. (1996). Differential responses of ovine intrapulmonary arteries and veins to acetylcholine. *Respir Physiol*, 104, 197-204.

Tomita, T., Tokaki, H., Hara, Y., Sokamaki, F., Satoh, T., Takagi, S., Yasumura, Y., Aihara, H., Goto, Y. & Sunagawa, K. (2003). Attenuation of hypercapnic carbon dioxide chemosensitivity after postinfraction exercise training: possible contribution to the improvement in exercise hyperventilation. *Heart*, 89, 404-410.

Torbicki, A. & Kurzyna, M. Echocardiography. In *Nunn's Applied Respiratory Physiology*. Lumb, A.B. (2000). Butterworth-Heineman, 89-99.

Tumminello, G., Guazzi, M., Lancellotti, P. & Piérard, L.A. (2007). Exercise ventilation insufficiency in heart failure: pathophysiological and clinical significance. *Eur Heart J*, 28, 673-678.

Turner, D.L., Bach, K.B., Martin, P.A., Olsen, E.B., Brownfield, M., Foley, K.T. & Mitchell, G.S. (1997). Modulation of respiratory control during exercise. *Respir Physiol*, 110, 277-285.

Van Baak, M.A. (2008). Meal-induced activation of the sympathetic nervous system and its cardiovascular and thermogenic effects in man. *Physiol Behav*, 94, 178-186.

Vanhoutte, P.M. (1974). Inhibition by acetylcholine of adrenergic neurotransmission in vascular smooth muscle. *Circ Res*, 34, 317-26.

Vejby-Christensen, H. & Petersen, E.S. (1973). Effect of body temperature on ventilatory transients at start and end of exercise in man. *Respir Physiol*, 17, 315-324.

Velez-Roa, S., Ciarka, A., Najem, B., Vachier, J., Naeije, R. & Van De Borne, P. (2004). Increased sympathetic nerve activity in pulmonary artery hypertension. *Circulation*, 110, 1308-1313.

Vianna, L.C., Araujo, C.G., Fisher, J.P. (2009). Influence of central command and muscle afferent activation on anterior cerebral artery blood velocity responses to calf exercise in humans. *J Appl Physiol*. [Epub ahead of print]

Victor, R.G., Rotto, D.M, Pryor, S.L., Kaufman, M.P. (1989). Stimulation of renal sympathetic activity by static contraction: evidence for mechanoreceptor-induced reflexes from skeletal muscle. *Circ Res*, 64, 592–599.

Von Euler, C. Neural organisation and rhythm generation. In *The Lung: Scientific Foundations*. Crystal, R.G., West, J.B., Barnes, P.J. & Weiber, E.R. (1997). Lippincott-Raven, Philadelphia, 1711-1724.

Vorluni, L., Volianitis, S. (2008). Baroreflex control of sinus node during dynamic exercise in humans: effect of muscle mechanoreflex. *Acta Physiol (Oxf)*, 192, 351-357.

Wagenvoort, C.A. & Wagenvoort, N. (1982). Smooth muscle content of pulmonary arterial media in pulmonary venous hypertension compared with other forms of hypertension. *Chest*, 81, 581-585.

Wagenvoort, C.A. & Wagenvoort, N. (1983). Pulmonary veins in high-altitude residents: a morphometric study. *Thorax*, 37, 931-935.

Walch, L., Gascard, J.P., Dulmet, E., Brink, C. & Norel, X. (2000). Evidence for a M(1) muscarinic receptor on the endothelium of human pulmonary veins. *Br J Pharmacol*, 130, 73-78.

Waldrop, T.G., Eldridge, F.L. Iwamoto, G. & Mitchell, J.H. Ventral neural control of respiration and circulation during exercise. In *Handbook of Physiology: Section 12, Exercise: Regulation and Integration of Multiple Systems*. Rowell, L.B. & Sheppard, J.T. (1996). Oxford University Press, New York, 333-380.

Walker, J.K.L & Jennings, D.B. (1995). During acute hypercapnia vasopressin inhibits an angiotensin drive to ventilation in conscious dogs. *J Appl Physiol*, 79, 786-794.

Wang, W, Tiwari, J.K., Bradley, S.R., Zaykin, R.V. & Richerson, G.B. (2001). Acidosis-stimulated neurons of the medullary rahe are serotonergic. *J Neurophysiol*, 85, 2224-2235.

Wang, W. & Ma, R. (2000). Cardiac sympathetic afferent reflexes in heart failure. *Heart Fail Rev*, 5, 57-71.

Wang, W.-Z., Gao, H.-J., Zucker, I.H. & Wang, W. (2008). Interaction between cardiac sympathetic afferent reflex and chemoreflex is mediated by the NTS AT1 receptors in heart failure. *Am J Physiol: Heart Circ Physiol*, 295, H1216-H1226.

Ward, S.A. (2000). Control of the exercise hyperpnoea in humans: a modelling perspective. *Respir Physiol*, 122, 149-166.

Ward, S.A. (2004). Physiology of breathing. *Surgery*, 22, 1-7.

Ward, S.A. (2007). Ventilatory control in humans: constraints and limitations. *Exp Physiol*, 92, 357-366.

Ward, S.A., Blesovsky, L., Russak, S., Ashjian, A., Whipp, B.J. (1987). Chemoreflex modulation of ventilatory dynamics during exercise in humans. *J Appl Physiol*, 63, 2001-7.

Weil, J.V. & Swanson, G.D. Peripheral chemoreceptors and the control of breathing. In Whipp, B.J. & Wasserman, K. (1991). *Exercise, Pulmonary physiology and Pathophysiology*. New York, Marcel Dekker

Weil, J.V., Byrne-Quinn, E., Sodal, I.E., Kline, J.S., McCullough, R.E., Filley, G.F. (1972). Augmentation of chemosensitivity during mild exercise in normal man. *J Appl Physiol*, 33, 813-819.

West, J.B. (1995). *Respiratory Physiology: The Essentials*. Lippincott Williams & Wilkins, Philadelphia.

West, J.B., Dollery, C.T. & Naimark, A. (1964). Distribution of blood flow in isolated lung; relation to vascular and alveolar pressures. *J Appl Physiol*, 19, 713-724.

Whipp B.J., Wasserman K. (1980). Carotid bodies and ventilatory control dynamics in man. *Fed Proc*, 39, 2668-73.

Whipp, B.J. & Ward, S.A. (1998). Determinants and control of breathing during muscular exercise. *Br J Sport Med*, 92, 199-211.

Whipp, B.J. & Ward, S.A. Coupling of ventilation to pulmonary gas exchange during exercise. In Whipp, B.J. & Wasserman, K. (1991). *Exercise Pulmonary Physiology & Pathophysiology*, New York, Marcel & Dekker, 371-403.

Whipp, B.J. (1983). Ventilatory control during exercise in humans. *Ann Rev Physiol*, 45, 393-413.

Whipp, B.J. (1994a). Peripheral chemoreceptor control of exercise hyperpnea in humans. *Med Sci Sports Exerc*, 26, 337-347

Whipp, B.J. (1994b). Carotid bodies and breathing in humans. *Thorax*, 49, 1081-1084.

Wiley, R.L. & Lind, A.R. (1971). Respiratory responses to sustained static muscular contractions in humans. *Clin Sci*, 40, 221-234.

Williams, M.H., Zohman, L.R., Bertrand, C.A. (1960). Effect of atropine on the pulmonary circulation during rest and exercise in patients with chronic airway obstruction. *Chest*, 37, 597-601.

Williamson, J. W., Mitchell, J. H., Olesen, H. L., Raven, P. B., & Secher, N. H. (1994). Reflex increase in blood pressure induced by leg compression in man. *J Physiol*, 475, 351-357.

Williamson, J.W., Raven, P.B., Foresman, B.H. & Whipp, B.J. (1993). Evidence for an intramuscular ventilatory stimulus during dynamic exercise in man. *Respir Physiol*, 94, 121-135.

Wilson, P.S., Khimenko, P.L., Barnard, J.W., Moore, T.M. & Taylor, A.E. (1995). Muscarinic agonists and antagonists cause vasodilation in isolated rat lung. *J Appl Physiol*, 78, 1404-1411.

Wilson, H.L., Dipp, M., Thoms, J.M., Lad, C., Galione, A & Evans, A.M. (2001). ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase act as a redox sensor. A primary role for cyclic ADP-ribose in hypoxic pulmonary vasoconstriction. *J Biol Chem*, 276, 11180-11188.

Witte, K.K. & Clark, A.L. (2007). Why does chronic heart failure cause breathlessness and fatigue? *Prog Cardiovasc Dis*, 49, 366-84.

Wuyam, B., Moosavi, S.H., Decety, J., Adams, L., Lansing, R.W. & Guz, A. (1995). Imagination of dynamic exercise produced ventilatory responses which were more apparent in competitive sportsmen. *J Physiol*, 482, 713-724.

Xie, A., Skatrud, J.B., Khayat, R., Dempsey, J.A., Morgan, B. & Russel, D. (2005). Cerebrovascular response to carbon dioxide in patients with congestive heart failure. *Am J Respir Crit Care Med*, 172, 371-378.

Xie, A., Skatrud, J.B., Morgan, B. Chenuel, B., Khayat, R., Reichmuth, K., Lin, J. & Dempsey, J.A.. (2006). Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. *J Physiol*, 577, 319-329.

Yock, P.G., Popp, R.L.. (1984). Noninvasive estimation of right ventricular systolic pressure by Doppler ultrasound in patients with tricuspid regurgitation. *Circulation*. 70, 657-662.

Zahner, M.R., Li, D.P., Chen, S.R. & Pan, H.L. (2001). Cardiac vanilloid receptor 1-expressing afferent nerves and their role in the cardiogenic sympathetic reflex in rats. *J Physiol*, 551, 515–523.

Zahner, M.R., Li, D.P., Chen, S.R. & Pan, H.L. (2003). Cardiac vanilloid receptor 1-expressing afferent nerves and their role in the cardiogenic sympathetic reflex in rats. *J Physiol*. 551, 515-23.

Zwartz, M.J. and Arendt-Nielsen, L. (2004). The influence of force and circulation on average muscle fibre conduction velocity during local muscle fatigue. *Eur J Appl Physiol Occup*, 58, 278-283.

Zwillich, C.W., Matthay, M., Potts, D.E, Adler, R., Hofeldt, F. & Weil, J.V. (1978).Thyrotoxicosis: comparison of effects of thyroid ablation and beta-adrenergic blockade on metabolic rate and ventilatory control. *N Engl J Med*, 292, 662-665.

Zwillich, C.W., Pierson, D.J., Hofeldt, F.D., Lufkin, E.G.& Weil, J.V. (1975). Ventilatory control in myxedema and hypothyroidism. *N Engl J Med*, 292, 662-665.

Zwillich, C.W., Sahn, S.A.& Weil, J.V. (1979). Effects of hypermetabolism on ventilation and chemosensitivity. *J Clin Invest*, 60, 900-906.