THE ROLE OF THIN FIBRE MUSCLE AFFERENT FEEDBACK, AND ITS MODIFICATION BY EXERCISE TRAINING, IN HUMAN VENTILATORY CONTROL.

By

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A thesis submitted to

The University of Birmingham

For the degree of

DOCTOR OF PHILOSOPHY

School of Sport, Exercise &Rehabilitation Sciences,

Collage of Life and Environmental Sciences,

University of Birmingham,

September 2018

ABSTRACT

Historically it was believed that activation of the muscle metaboreflex alone by post exercise circulatory occlusion (PECO) does not result in hyperpnea. Recently, studies have shown that activation of the muscle metaboreflex combined with stimulation of the ventilatory chemoreflex (by concurrent hypercapnia) resulted in an increased ventilatory response, suggesting a potential interaction between these neural inputs. This thesis investigated the nature of this interaction by manipulating one of these neural inputs at a time. Firstly, it was observed that the magnitude of the additional ventilatory response to a standardised level of muscle metaboreflex activation (PECO) rose progressively with the level of concurrent hypercapnia, indicating a linear relationship [$\dot{V}_E$ ($l.min^{-1}$) = 0.85 x $P_{ET}CO_2$ (mmHg) + 0.80 ($l.min^{-1}$)] (R=0.78 range, 0.39-0.99). Secondly, the ventilatory chemoreflex was constantly stimulated (constant level of hypercapnia), but inputs from muscle metaboreceptive afferents were altered by local muscle training. Exercise training of one leg for 6 weeks attenuated the muscle metaboreflex and resulted in decreases in ventilatory ($\dot{V}_E$: from 17.5 ± 2 to 9.8 ± 2.1 $L.min^{-1}$, $p \leq 0.05$) and cardiovascular (MAP: from 16.1± 2.3 to 14.1 ± 2.1 mmHg, $p \leq 0.05$) responses to PECO under the hypercapnia condition but only in the trained leg. Overall these findings support the concept of a synergistic interaction between the neural inputs, and the findings from the training study provide evidence that has promising clinical implication for patients with exercise intolerance and dyspnoea.

DEDICATION

To my lovely wife *DANA*

&

My angels *AMAL* & *EISSA*

ACKNOWLEDGMENTS

To start with, I would like to thank my supervisor, Dr. Michael White, for all his support, help, patience and encouragement during my PhD, which made it enjoyable. His guidance and support, helped me to complete this thesis. I would also like to thank my second supervisor, Dr. George Balanos, for his contribution throughout this work and always being near to me whenever I needed help and advice. I would like to express my gratitude to all members of staff and students, with whom I have worked during the past four years, at the School of Sport, Exercise and Rehabilitation Sciences.

Finally, I feel immense gratitude for my family, for supporting me during the four years of PhD study in particular, my father, mother, sisters and brothers. Their continuous love, enthusiasm, support and motivation, helped me to complete this wonderful journey.

PUBLICATIONS

Full papers:

- GHAITH, J. A., BALANOS, G. M., EVES, F. F. & WHITE, M. J. (2018). Sensitivity of the human ventilatory response to muscle metaboreflex activation during concurrent mild hypercapnia. *Experimental physiology*.

Abstracts:

- White, M., Alghaith, J. and Balanos, G. (2016). CO_2 sensitivity of ventilatory responses to muscle metaboreflex stimulation in humans. *The FASEB Journal*, 30, 1261.14-1261.14.

- Alghaith, J. and With, M. (2018). The ventilatory response to muscle metaboreflex activation during concurrent hypercapnia is attenuated by local muscle training. *The FASEB Journal*, 32, 853.1-853.1.

Awards:

The American Physiology Society Enviromental and Exercise Physiology Section, "*CONTROL Environmental Systems Predoctoral Research Award".* At the Experimental Biology 2018 Confrance, in San Diego.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ATP	adenosine triphosphate
ATPS	ambient temperature, pressure and saturated water vapour
BL	baseline period
BTPS	body temperature, pressure and saturated water vapour
CHF	chronic heart failure
C.O	circulatory occlusion trial
CO_2	carbon dioxide
CON	control trial
COPD	chronic obstructive pulmonary disease
CSF	cerebrospinal fluid
DEF	dynamic end-tidal forcing
DRG	dorsal respiratory group
ECG	electrocardiogram
ET	endurance exercise
Ex	exercise
f	breathing frequency
$GABA_A$	gamma-aminobutyric acid

H^+ hydrogen ion

HR heart rate

K^+ potassium ions

Lt. Left

LTM long term modulation

MAP mean arterial blood pressure

Ms milliseconds

MSNA muscle sympathetic nerve activity

MVC maximal voluntary contraction

NTS nucleus tractus solitarii

O_2 oxygen

P_ACO_2 alveolar partial pressure of carbon dioxide

P_aCO_2 partial pressure of arterial carbon dioxide

PAG periaqueductal gray matter

P_AO_2 alveolar partial pressure of oxygen

P_aO_2 partial pressure of arterial oxygen

PECO post exercise circulatory occlusion

PET positron emission tomography

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

$P_{ET}O_2$	end-tidal partial pressure of oxygen
P_ICO_2	partial pressure of inspired carbon dioxide
P_IO_2	partial pressure of inspired oxygen
PR	pressor reflex
P_vCO2	partial pressure of venous carbon dioxide
rCBF	regional cerebral blood flow
RT	resistance training
Rt.	Right
RTN	retrotraezoid nucleus
SD	stander deviation
S.E.M	standard error of the mean
$\dot{V}co_2$	carbon dioxide production
$\dot{V}_E$	minute ventilation
$\dot{V}o_2$	oxygen consumption
VRG	ventral respiratory group
$\dot{V}_T$	tidal volume

CHAPTER 1: LITERATURE REVIEW

1.1 Neural mechanisms controlling ventilation.

Breathing is essential for supplying oxygen to and removing carbon dioxide from pulmonary capillaries so as to meet the metabolic demand of tissues.

The respiratory neurons located in the brainstem, mainly the medulla and the pons, are considered to be the main controllers of ventilation (Figure 1.1). These neurons are responsible for generating and maintaining the respiratory rhythm. Moreover, they receive and integrate sensory inputs from various receptors. Following the integration of sensory information, respiratory neurons initiate an appropriate output to activate the respiratory muscles. Respiratory neurons are classified based on their location in the brainstem (the medulla and pons), with each group of neurons involved in the different mechanisms of breathing (West, 2012). First, there are two identifiable regions in the medulla responsible for generating ventilation. The dorsal respiratory group neurons, within the nucleus tractus solitarii (NTS), contain inspiratory neurons responsible for the generation of inspiration. The NTS receives sensory inputs from arterial baroreceptors, and muscle mechano- and metaboreceptors, which are then integrated and processed. The other region of the medulla contains ventral respiratory group neurons. This neural group contains inspiratory and expiratory neurons, the main responsibility of which is regulating expiration. However, these neurons occasionally also take part in regulating inspiration, secondarily to the dorsal respiratory group neurons (Boron and Boulpaep, 2016). The pons is the other main location of respiratory neurons and the role of these is to modulate respiratory output. These neurons, comprising the apneustic centre (lower pons) and the pneumotaxic centre (upper pons), are involved in regulating inspiratory depth by controlling the transition between inspiration and expiration. Moreover, pontine respiratory neurons are also responsible for modulating respiratory frequency.

The mechanisms underlying generation of respiratory rhythm remain to be fully understood. Several lines of evidence point to two important regions within the rostral ventrolateral medulla that contain neurons with pacemaker capacity. These regions work separately to regulate inspiration and expiration rhythm. The pre-Bötzinger complex is considered to be an essential and sufficient region for generating inspiratory rhythm (Smith et al., 1991, Feldman et al., 2013), while the retrotrapezoid nucleus/parafacial respiratory group is another key region involved in generating active expiration rhythm (Feldman et al., 2013).

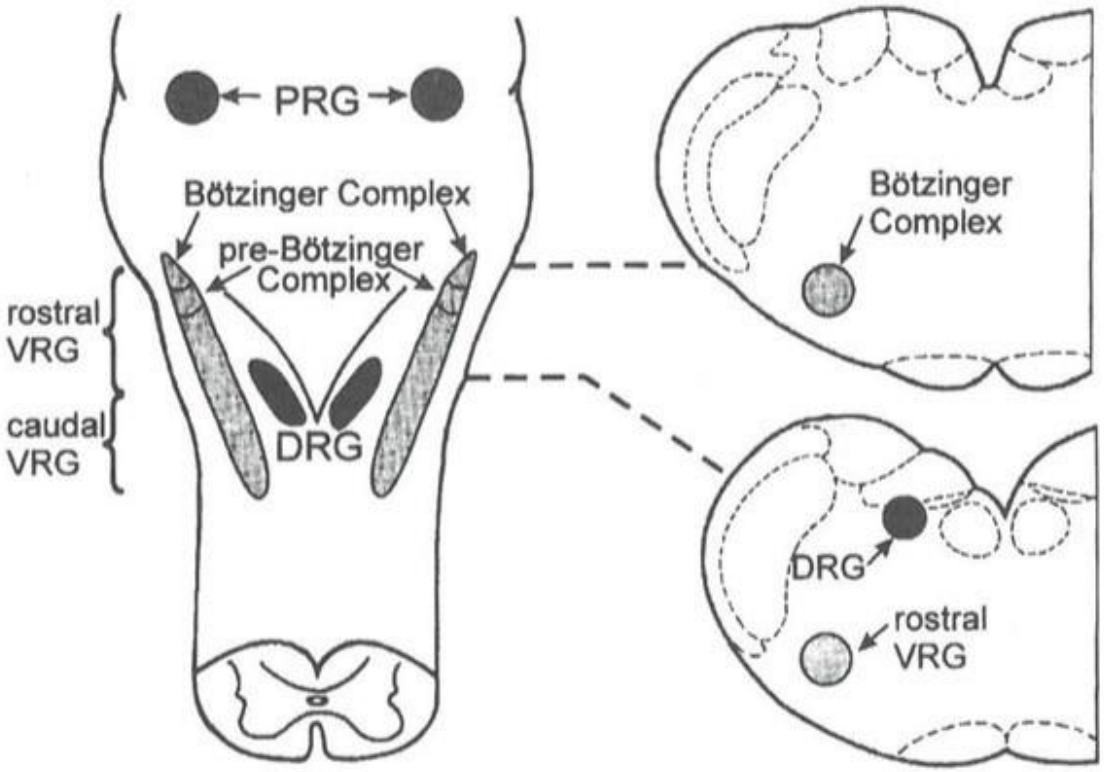

Figure 1.1: Respiratory control areas in the brainstem. A schema representing the respiratory neurons in the medulla and the pons. The left side depicts the dorsal view of the pons and medulla. The right side shows two transverse sections of the medulla, representing the locations of respiratory-related neurons. PRG, pontine respiratory group, DRG, dorsal respiratory group, VRG, ventral respiratory group (Hlastala and Berger, 2001).

1.2 Respiratory receptors

The respiratory control areas in the brainstem receive sensory inputs from different receptors and integrate these input signals to maintain arterial blood gas homeostasis across a range of physical activities (Figure 1.2). These receptors project from within the upper airway, the trachea, the bronchial tree, the lungs, the muscles and joints, the brainstem and the carotid bodies, and alongside arterial partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) respond into a range of stimuli, including pain and temperature, to adjust respiratory function based on activity (West, 2012).

1.2.1 Upper airway receptors

The upper airway receptors are located in the nasal passage, pharynx, larynx and trachea. These receptors respond to mechanical and chemical stimulation and are considered to be the first line of defence in the respiratory system; they initiate different reflexes, such as sneezing, sniffing, coughing and bronchoconstriction, to protect the lower respiratory tract (West, 2012).

1.2.2 Tracheal, lung and bronchial receptors

The respiratory centre in the brainstem receives and integrates various inputs from receptors in the airways and lung to alter the respiratory output.

A) Pulmonary stretch receptors

Pulmonary stretch receptors are mechanoreceptors located within the airway smooth muscle layer that are considered to be slowly adapting receptors. These receptors provide information about lung distension and act as an inspiratory off-switch – the Hering-Breuer

reflex. This reflex works to inhibit inspiration to prevent "lung overinflation" and is considered to be unimportant in adults due to its inactivity while resting. However, this reflex becomes increasingly active during exercise when the tidal volume increases to more than 1 litre (Guz et al., 1964, Guz et al., 1966a, Guz et al., 1966b, West, 2012).

B) Irritant receptors

Irritant receptors are a type of rapidly adapting pulmonary receptor, found between the airway epithelial cells. These receptors are sensitive to chemical stimulants, such as inhaled dust, cold air, noxious gas and cigarette smoke. As a consequence of exposure to these stimulants, the receptors initiate reflex responses, namely bronchoconstriction and hyperventilation (West, 2012). Other rapidly adapting pulmonary receptors respond primarily to stretch (RARs) causing sigh responses during alveolar collapse. In addition, some respond to chemical stimuli such as a cigarette smoke triggering cough (Canning and Spina, 2009).

C) Juxtacapillary receptors

The juxtacapillary receptors (J-receptors) are a type of group IV neurons (C fibres) located in the alveolar wall adjacent to the capillaries and are supplied by the pulmonary circulation. These receptors are also stimulated by chemical stimulants, which trigger a reflex response of rapid shallow breathing (West, 2012).

D) Bronchial C fibres

Contrary to juxtacapillary receptors, these receptors are supplied by the bronchial circulation. They are sensitive toward chemical stimulants and are usually followed by reflexes including bronchoconstriction, rapid shallow breathing and increased mucous secretion into the airways (West, 2012).

1.2.3 Arterial baroreceptors

The arterial baroreceptors are mechanoreceptors found in the aortic arch and carotid sinus. These receptors detect stretching of the arterial wall and function to maintain homeostatic blood pressure. Briefly, arterial baroreceptors detect changes in the distending pressure and relay this information to the NTS region in the brainstem through the glossopharyngeal and vagus nerves (Paton, 1999). Consequently, a baroreflex is initiated to produce changes in the heart rate and cardiac output, as well as vascular resistance, thereby maintaining blood pressure. In addition to controlling cardiovascular responses, studies in animal and human models have shown strong evidence that arterial baroreceptors also play a role in altering ventilation (Heistad et al., 1975, Stewart et al., 2011, West, 2012). These studies concluded that changes in the arterial blood pressure have an inverse relationship with ventilation. Therefore, this 'ventilatory baroreflex' causes a reduction in ventilation as arterial blood pressure rises, while, conversely, a decrease in arterial blood pressure increases ventilation.

1.2.4 Chemoreceptors

Chemoreceptors play an important role in ventilation by closely regulating arterial blood gas parameters (Ballantyne and Scheid, 2001). These receptors can detect changes in oxygen (O_2) and carbon dioxide (CO_2) levels in arterial blood, as well as its acidity. They then adjust breathing based on the metabolic demand of the body to maintain O_2, CO_2 and hydrogen ion (H^+) levels in arterial blood within the optimal narrow range (Pocock et al., 2013). Chemoreceptors are classified into two categories, according on their location: the central chemoreceptors (brain) and peripheral chemoreceptors (periphery).

A) Central chemoreceptors

The central chemoreceptors respond to small changes in the chemical composition of blood and interstitial fluid to adjust the breathing pattern. These receptors are localised to the ventral surface of the medulla (about 200 to 400 μm below ventral surface) (West, 2012) and are surrounded by brain extracellular fluid. The concentration of CO_2 in brain blood flow is a major driver of the pH in this fluid known as cerebrospinal fluid (CSF).

Central chemoreceptors are sensitive to changes in H^+ concentration; an increase in H^+ blood concentration stimulates ventilation. This occurs when the $PaCO_2$ increases; CO_2 diffuses easily (unlike H^+) from blood vessels into the CSF through the blood brain barrier. This acidifies the CSF through formation of carbonic acid, which in turn dissociates to liberate H+ within the CSF. Intraneuronally, H^+ ions, in turn, stimulate central chemoreceptors to increase ventilation and maintain the $PaCO_2$ (West, 2012, Boron and Boulpaep, 2016).

In addition to the ventral surface of the medulla, there are several other regions in the brain that may contain chemoreceptors; namely, the NTS (Nattie and Li, 2002), the retrotrapezoid nucleus (Richerson, 2005), the dorsal and ventral area of the respiratory group within the locus coeruleus (Putnam, 2010), the medullary raphe (Wang et al., 2001), the periaqueductal gray (Lopes et al., 2012) and the pre-Bötziger complex (Feldman et al., 2003).

B) Peripheral chemoreceptors

The peripheral chemoreceptors also play a vital role in ventilation through regulation of arterial blood parameters. In contrast to the central chemoreceptors, the peripheral chemoreceptors function to detect changes in the aforementioned arterial blood gas parameters before the blood reaches the brain. These receptors respond to changes in the PaO_2, pH levels, and increased blood $PaCO_2$ by initiating a rapid reflex ventilatory response to maintain arterial blood gas homeostasis. There are two identifiable regions containing

peripheral chemoreceptors: the carotid bodies (located at the bifurcation of the common carotid arteries) and the aortic bodies (located above and below the aortic arch) (Boron and Boulpaep, 2016).

The exact mechanisms governing the function of the carotid bodies are not completely understood (West, 2012). The primary site of chemoreceptors are type I glomus cells (Gonzalez et al., 1994), which are innervated by the carotid sinus nerve (a branch of the glossopharyngeal nerve) and, in turn, relay neural signals to the NTS (Donoghue et al., 1984, Gonzalez et al., 1994).

The carotid bodies are sensitive to different types of chemical and physical stimulation, such as changes in arterial blood gas parameters, blood pressure, glucose, potassium ions (K^+), catecholamines, osmolarity and temperature (Gonzalez et al., 1994). Moreover, it is well known that carotid bodies play an important role in regulating neuroendocrine and haemodynamic responses to different types of stimulation; for instance, exercise, hypercapnia and hypoxia (Koyama et al., 2001, Kara et al., 2003).

1.2.5 Inputs from central command

There are different areas in the brain, where feedforward or "central command" (see page: 22) signals originate and induce parallel activation of the locomotor and the respiratory control areas of the brain. One of these areas is the cerebellum. It is well established from anatomical studies that there are common neural pathways between the cerebellum and brainstem including NTS and vestibular nucleus (Dormer et al., 1982, Andrezik et al., 1984, Hernandez et al., 2004). Animal studies have shown that electrical stimulation of the anterior lobe of the cerebellum results in inhibition of ventilation (Decima and Euler, 1969). However, ablation the anterior lobe of the cerebellum (by suction) results in stimulation of

ventilation in the anesthetized animal. Moreover, Chen et al. (2005) reported that children with neuropathology of the cerebellum developed breathing disorders and hypoventilation.

The cerebral cortex is another area in the brain involved in the neurocircurity of central command. Zhang and Oppenheimer (1997) suggested that the insular cortex within the cerebral cortex is a site of cardiovascular control. This was based on observations of changes in the regional cerebral blood flow (rCBF) within the insular cortex during exercise resulting in an increase in blood pressure. As central command involvement in both cardiovascular and respiratory responses to exercise is well supported (Turner, 1991, Kaufman and Forster, 1996, Waldrop et al., 1996), it could be suggested that the cerebral cortex plays a prominent role in exercise hyperpnoea. Fink et al. (1995) reported an increase in rCBT of the primary motor cortex during exercise and no activation following exercise. They concluded that the primary motor cortex, known to influence voluntary breathing, is involved in ventilatory control.

Other areas in the brain considered to be involved in central command neurocircurity and have significant influence on ventilation are reviewed in section 1.5 (page: 25).

1.2.6 Other receptors

There are other receptors that provide the respiratory centre with different sensory inputs to regulate ventilation such as thermoreceptors. These receptors are sensitive to changes in body temperature and induce a reflex response that increases ventilation under hyperthermia or reduces it under hypothermia (Fadic et al., 1991, Zapata et al., 1993). Stimulation of pain receptors (nociceptors) by mechanical or chemical stimulants notably can also alter ventilation (Borgbjerg et al., 1996). It is postulated that, in addition, joint receptors can stimulate ventilation in the early stage of exercise (West, 2012). Moreover, muscle afferents

(containing mechanoreceptors and metaboreceptors) are increasingly implicated in the regulation of ventilation during exercise (see section 1.6.2.2, page: 33).

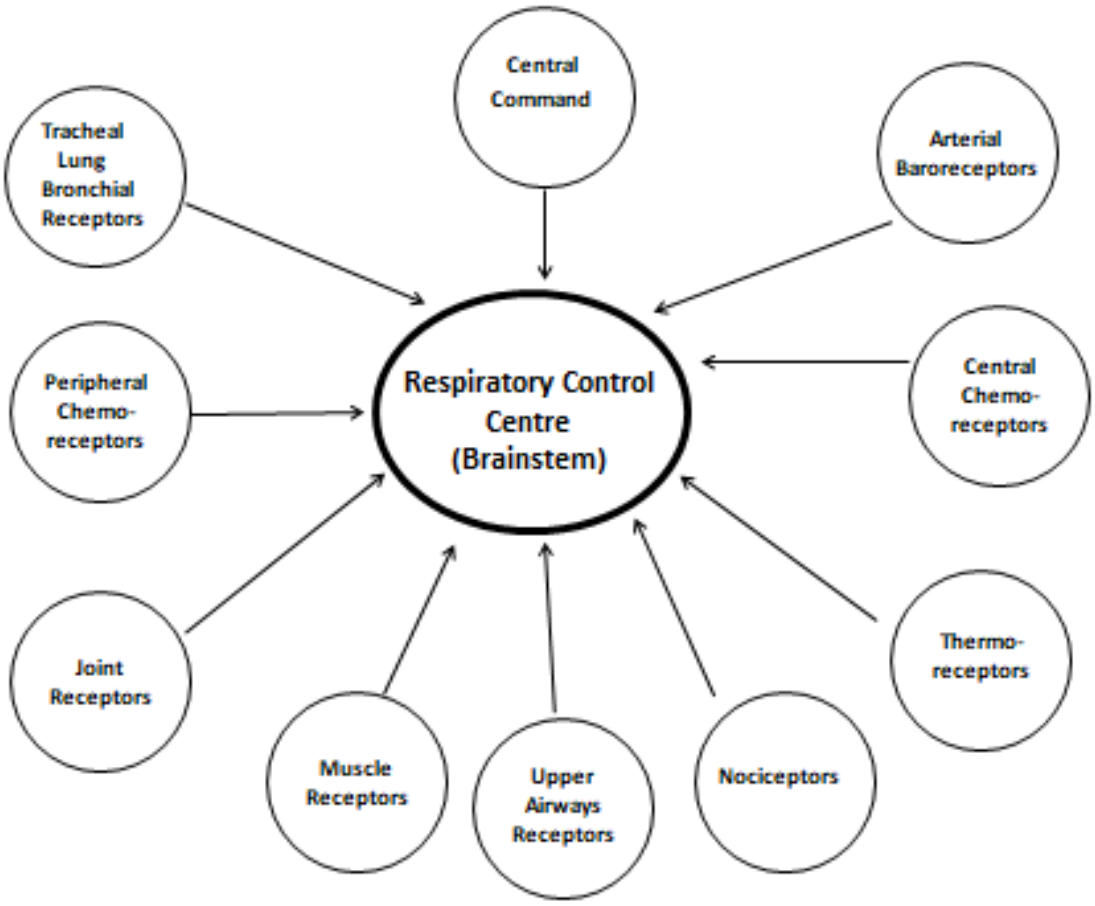

Figure 1.2: Respiratory receptors types.

1.3 The cardiovascular and ventilatory responses to exercise

Exercise exerts significant physiological stress on the cardiovascular and respiratory systems, resulting in acute and long-term effects. Performing muscular exercise induces an increase in blood pressure, heart rate and ventilation (Goodwin et al., 1972). These changes help provide the exercising muscle with more O_2 and remove CO_2 to the meet tissue metabolic demand under different exercising conditions (dynamic or static exercise). The autonomic nervous system mediates and regulates these changes in cardiovascular and respiratory responses in both types of exercise.

1.3.1 Cardiovascular response to exercise

The neural control mechanisms regulating the cardiovascular responses to both dynamic and static exercises are well documented. They include central command (a feedforward mechanism) and the arterial baroreflex and sensory reflexes arising from exercising muscle (mediated by muscle ergoreflex, mechano- and metaboreceptors) (Rowell, 1993, Murphy et al., 2011). These mechanisms provide sensory inputs to the cardiovascular centre in the medulla (e.g. NTS and ventrolateral medulla). In the medulla, this sensory information is integrated and then relayed to the blood vessels, heart and adrenal medulla to effect appropriate cardiovascular responses, according to exercise level (Figure 1.3) (Paton, 1999, Smith et al., 2006).

The cardiovascular response to dynamic exercise depends mainly on the exercise intensity, characterised by increased in heart rate, stroke volume and cardiac output, and reduced total peripheral resistance. These cardiovascular changes occur during dynamic exercise and together lead to a moderate increase in arterial blood pressure. As a consequence, more blood flow is supplied to the exercising muscle to meet the increased oxygen and nutrient demand

(Jordan and Marshall, 1995, Gallagher et al., 1999). However, there is a limit set by the maximum cardiac output of an individual and during heavy exercise involving large muscle masses vasoconstriction must prevail to restrict maximal muscle blood flow and to protect arterial blood pressure (Saltin et al., 1998).

It has been reported that the magnitude of intramuscular pressure has a marked impact on the cardiovascular response to static exercise (Murphy et al., 2011). Isometric contraction can cause a large increase in intramuscular pressure (depending on the muscle mass), which in turn results in restriction of blood flow to the exercising muscle. Therefore, some argue that in an attempt to maintain adequate perfusion to the exercising muscle, the increase in heart rate and resulting cardiac output – along with minor or no change in total peripheral resistance – causes a marked increase in the arterial blood pressure required to sustain perfusion (Rowell, 1993, Smith et al., 2006, Murphy et al., 2011). Notably, whilst this may work for small muscles or during low intensity isometric exercise of large muscles, the cardiovascular system cannot physiologically generate pressures sufficient to overcome the resistance to blow flow found in strongly contracting large muscles. In this case, the pressor response to isometric exercise can be seen more as a failure of regulation, largely driven by muscle afferent feedback activated during sustained high-force ischaemic contraction (Joyner, 1992).

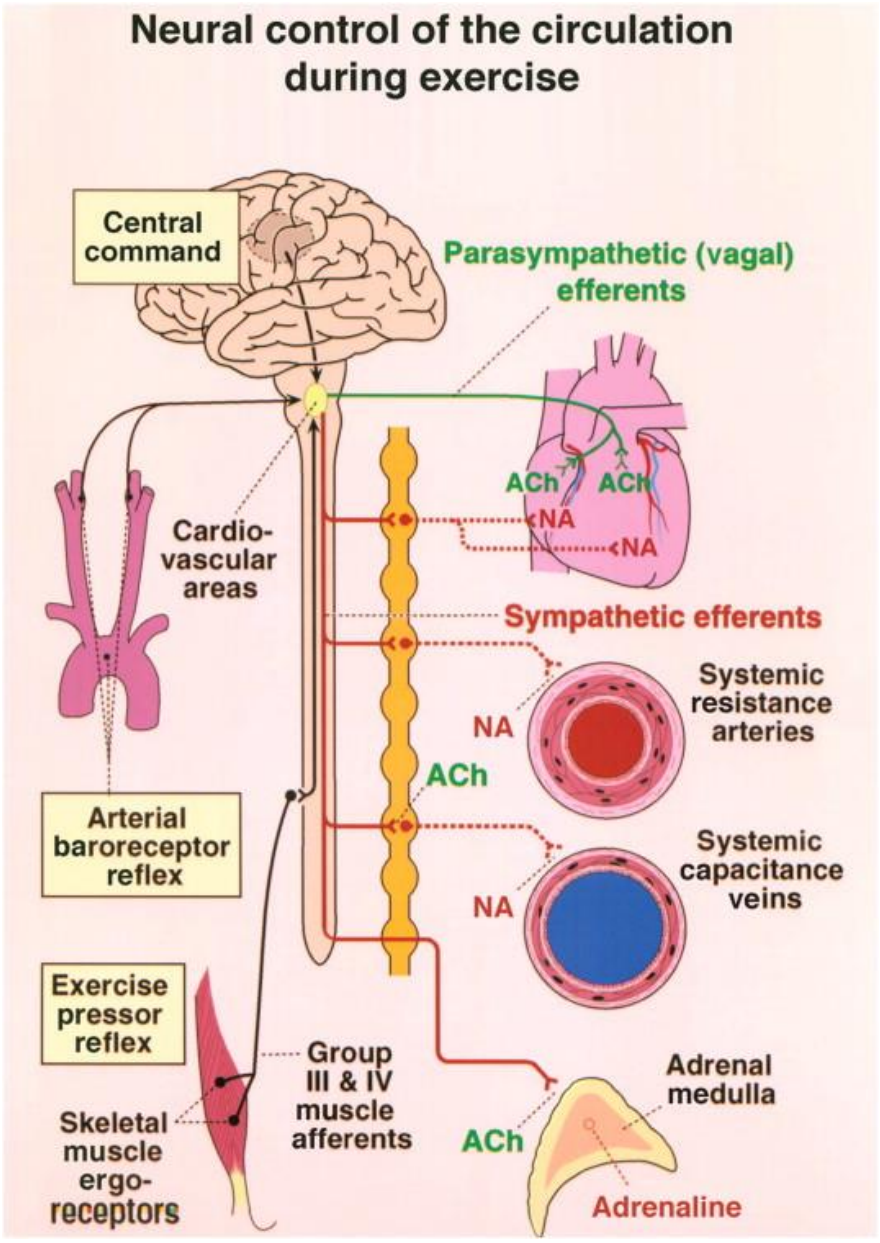

Figure 1.3: Neural mechanisms controlling cardiovascular responses during exercise. During exercise, several neural signals arising from the brain (central command), the carotid artery and aorta (arterial baroreflex) and skeletal muscle afferents (ergoreflex) control sympathetic and parasympathetic nerve activity (Smith et al., 2006).

1.3.2 Ventilatory response to exercise

The neural control mechanisms mediating the ventilatory response to exercise remain to be established and are under intense research. The magnitude of the ventilatory response to dynamic exercise depends on the exercise intensity (Whipp et al., 1982, Wasserman and Whipp, 1983, McArdle et al., 2010). During light to moderate exercise, Phase I comprises of the first 20 seconds of exercise and is characterised by an immediate increase in ventilation and rate of pulmonary gas exchange. During phase II, ventilation continues to increase progressively until it reaches a steady state (usually within two to three minutes), following which phase III begins. During high-intensity exercise, however, steady state ventilation is not achieved as ventilation continues to increase until reaching exhaustion (Casaburi et al., 1989).

During phase II of moderate exercise, the increase in ventilation has a similar pattern to that of oxygen uptake ($\dot{V}_{O2}$) and carbon dioxide removal ($\dot{V}_{CO2}$). However, $\dot{V}_{O2}$ kinetics become faster during late phase II (Wasserman and Whipp, 1983). On the other hand, like ventilation, both the $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ do not reach steady state during high-intensity exercise (Casaburi et al., 1989). Notably, in the case of exercise hyperpnea, the intensity of the ventilation response during the steady state of light to moderate exercise is almost equivalent to $\dot{V}_{O2}$ and $\dot{V}_{CO2}$. During high-intensity exercise however, which is characterised by the production of lactic acid, the ventilation response exceeds $\dot{V}_{O2}$ and $\dot{V}_{CO2}$. This is because the lactic acid that is released from the exercising muscle stimulates the peripheral chemoreceptor, which in turn provides an additional drive to breathe.

With regards to static exercise, the magnitude of the ventilatory response depends on several factors, the most important of which include the degree of muscle tension, muscle mass and level of fatigue. Several studies have shown that the degree of muscle tension (i.e. the % of maximal voluntary contraction; % MVC) has a significant influence on the magnitude of the

ventilatory response (Imms and Mehta, 1989, Fontana et al., 1993, Iellamo et al., 1999b). In other words, the increases in % MVC causes a proportional increase in the magnitude of the ventilatory response. Moreover, muscle mass (small muscle groups vs. large muscle groups) also significantly affects the magnitude of the ventilatory response. Iellamo et al. (1999b) found that during static exercise, the ventilatory response was significantly greater in a leg extension trial compared to a handgrip trial, at the same % MVC. Moreover, the increase in ventilation during high-intensity static exercise is considered to be higher than that required by metabolism. As a result, the end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) is significantly reduced (Imms and Mehta, 1989).

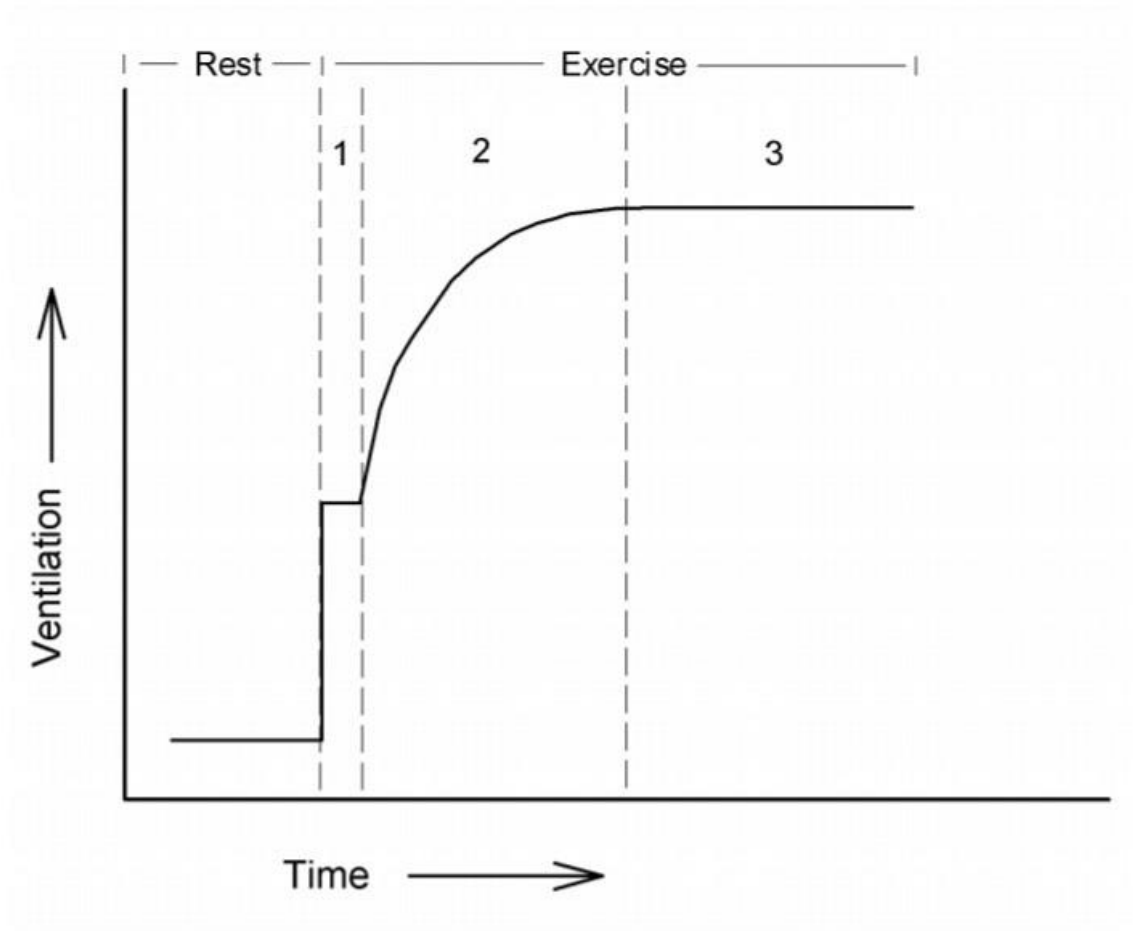

Figure 1.4: Scheme of the three distinct phases (I, II and III) of the ventilatory responses to dynamic exercise.

1.4 Exercise hyperpnea control mechanisms: theory

Exercise causes an increase in ventilation (exercise hyperpnea), which is proportional to the increase in metabolic rate. This increase, in turn, prevents a reduction in PaO_2 and an increase in $PaCO_2$. There are currently different proposed theories as to the regulatory mechanisms underlying exercise hyperpnea. Although, following experimentation, there are already some data in support of the proposed control mechanisms, there are also studies that contradict these; therefore further intensive studies are required to address this controversial topic. This section of the literature review will only address the main theories of control mechanisms underlying exercise hyperpnea.

The first theory, that $PaCO_2$ mediates exercise hyperpnea, was established in the early twentieth century, when Haldane and Priestley (1905) noticed that the increase in ventilation during moderate intensity dynamic exercise was exactly proportional to metabolic rate (they referred to it as CO_2 production). They suggested that an increase in $PaCO_2$ level could stimulate chemoreceptors, which in turn would mediate exercise hyperpnea. However, there have been numerous studies since then suggesting that $PaCO_2$ had no influence on exercise hyperpnea. Douglas and Haldane (1909) found that an increase in ventilation level during light and moderate exercise was exactly equal to the increase in metabolic rate, with no marked change in $PaCO_2$ level. Indeed, generally, at the onset of light to moderate exercise intensity, there is a small transient hypocapnia (about 1 to 3 mmHg of $PaCO_2$ level) from resting levels (Forster et al., 1989). Other studies investigating high-intensity exercise showed that the increase in ventilation had no association to changes in $PaCO_2$ level. However, $PaCO_2$ levels were shown to be decreased due to an excessive clearance rate of CO_2 that exceeded the metabolic rate (Forster et al., 1986, Stringer et al., 1992, Sun et al., 2001).

The second theory proposes that an increase in CO_2 sensitivity during exercise might have an effect on exercise hyperpnea. Krogh and Lindhard (1913) postulated that the immediate and

rapid increase in ventilation at the onset of exercise is due to increased sensitivity of the respiratory centre to H^+, induced by cortical irradiation (now known as central command). However, findings from Duffin et al. (1980) and Duffin and McAvoy (1988) did not support Krogh's and Lindhard's ideas.

In their first study, Duffin et al. (1980) examined the sensitivity of the ventilatory response to CO_2 mediated by the central chemoreceptors during light intensity exercise (25 W) and compared it with rest. This was achieved by examining the ventilatory response to CO_2 using hyperoxia to eliminate peripheral chemoreflex input. Using a rebreathing method under both conditions (resting and light intensity exercise), their results showed no difference in the sensitivity of the ventilatory response to CO_2. In a later study, Duffin and McAvoy (1988) examined the threshold of the ventilatory response to CO_2 that is driven by the peripheral chemoreceptors during resting and exercise onset. This was achieved by stimulating the peripheral chemoreflex using hypoxia with a modified version of the rebreathing technique used in the earlier study. Mild hypoxic gas inhalation combined with prior hyperventilation then created conditions of hypoxia and hypocapnia. They found no changes in either the threshold or the sensitivity of the ventilatory response to CO_2 between rest and onset of exercise under mild hypoxic conditions.

Collectively these two studies indicate that changes in CO_2 sensitivity do not mechanistically contribute to exercise hyperpnea. However, inspiration of CO_2 only does slightly affect ventilation with no associated changes in CO_2 sensitivity (Asmussen and Nielsen, 1957, Clark et al., 1980, Poon, 1987).

A third theory proposes that exercise hyperpnea is elicited as a result of an 'error signal' sent from hypothetical mixed venous chemoreceptors in response to increased venous CO_2 (P_vCO_2). This is because there are no changes in $PaCO_2$ level to provide an error signal. This theory could be seen as highly plausible, whereby the mechanism of exercise hyperpnea would match the ventilation and metabolic rates. However, data from several studies has contradicted this theory. Cropp and Comroe (1961) infused hypercapnic blood into the right ventricle of anesthetized cats and dogs and un-anesthetized dogs. They found that it was only when there was an increase in $PaCO_2$ above the normal level, causing stimulation of arterial chemoreceptors, that ventilation increased. Moreover, in a similar study where Sylvester et al. (1973) infused hypercapnic acid into the superior vena cava of un-anesthetized dogs, they found that ventilatory responses occurred when the hypercapnic blood stimulated arterial chemoreceptors. In a study in human subjects, Stanley et al. (1985) reported a significant lag time between the inflections [1] of $P_{ET}CO_2$ and minute ventilation during reperfusion of exercised muscle following a period of occlusion. Together, these studies provide strong evidence against the concept of mixed venous chemoreceptors.

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[1] Inflections of $P_{ET}CO_2$ or $\dot{V}_E$ defined as the first of three consecutive breaths following the release of circulatory occlusion of exercising muscle, in which the value of each breath is greater than the mean of all breaths values from last minute of preceding circulatory occlusion.

Other proposed mechanisms of regulating exercise hyperpnea relate to the chemical changes in arterial or venous blood, which stimulate peripheral chemoreceptors (carotid bodies) and thus trigger ventilation during exercise. One hypothesis is that carotid body activity is increased immediately during exercise, resulting in elevated minute ventilation (Biscoe and Purves, 1967). Another hypothesis suggests that the concentration of K^+ in arterial plasma released from exercising muscle (during high-intensity exercise) could mediate exercise hyperpnea (Paterson et al., 1990). Several other hypotheses suggest that an increase in the slope/amplitude of the breath-to-breath oscillations in arterial blood gases acts as a trigger of exercise hyperpnea (Yamamoto and Edwards, 1960, Band et al., 1969, Goodman et al., 1974). However, in each case, conflicting studies have raised questions as to the accuracy of the original data supporting the above proposed mechanisms and the rationale behind them. For example, Eisele et al. (1967) used lidocaine to block carotid body sympathetic innervation and this had no effect on the ventilatory response to submaximal exercise. In agreement, Donaldson and Newstead (1988) demonstrated that reducing K^+ levels in the plasma of chronically hyperkalemic patients did not induce any changes in minute ventilation. Furthermore, in an animal model, Warner and Mitchell (1990) found that administration of K^+ into plasma at levels comparable to those during moderate exercise intensity caused no changes in minute ventilation, concluding that hyperkalemia is not a contributing factor to exercise hyperapnea. With regards to breath oscillation in arterial blood gases, Ward and Whipp (1993) reported that these oscillations exerted only a small stimulating effect on exercise hyperapnea. Thus, according to the aforementioned contradicting findings, the proposed mechanistic hypotheses of exercise hyperapnea remain controversial.

Some researchers suggest that the mechanism governing immediate and fast increases in ventilation observed at the onset of exercise (Phase I) cannot be chemically or hormonally

mediated rather, neural regulation has been proposed. There are two primary neural mechanisms that contribute significantly to exercise hyperpnea. One of these is central command. Central command plays an important role in controlling cardiovascular and ventilatory responses to exercise, with abundant evidence supporting this role (to be addressed in next section of this literature review). The other neural mechanism involves mechanically and metabolically sensitive, thin fibre muscle afferents arising from exercising muscle. The role of muscle afferent feedback in controlling the cardiovascular response to exercise is well established (Coote et al., 1971, McCloskey and Mitchell, 1972, Bull et al., 1989, Bell et al., 2005). On the other hand, the role of muscle afferent feedback in controlling ventilation during exercise is considered a controversial subject. Preliminary studies had shown that muscle afferent feedback has no role in controlling exercise hyperpnea. However, recently, their contribution in controlling exercise hyperpnea has been re-examined, and evidence supporting their role has been found (Amann et al., 2010, Lykidis et al., 2010, Gagnon et al., 2012, Bruce and White, 2012, 2015, 2016).

1.5 The role of central command in controlling cardiovascular and ventilatory responses to exercise

Central command is a feed-forward neural mechanism that is considered to be one of two primary mechanisms regulating cardiovascular and ventilatory responses to exercise. Central command involves signals originating from higher brain centres which trigger parallel activation of the locomotor regions to recruit motor neuron units during exercise and activate the cardiovascular and respiratory centres in the brainstem to meet the metabolic demand of exercising muscle. This stimulation activates the autonomic nervous system to in turn regulate cardiovascular and ventilatory outputs during exercise. Studies have shown convincing evidence in favour of central command and its role in controlling cardiovascular and ventilatory responses during exercise. In addition, other neural areas and circuitry likely involved in central command have been identified.

In 1913, Krogh and Lindhard published their first detailed study on this central neural mechanism. They reported that during moderate intensity bicycle exercise in humans, both heart rate and ventilation displayed a sudden and rapid increase. The authors suggested that this rapid change in ventilation was not associated with some form of chemical control, as metabolites released from the exercising muscle would take longer to reach central vascular receptors compared to the fast ventilatory changes (depending on exercise level), indicating that a neural mechanism may be the only likely explanation. They referred to this central neural mechanism as ‘cortical irradiation’. Additionally, Krogh and Lindhard (1917) studied participants performing voluntary or electrically induced leg exercises. They found a delay in heart rate elevations in electrically induced leg exercises (central command removed) and suggested that this was indicative of central mechanisms mediating increased heart rate.

Later on, different methods were used to investigate the role of central command in controlling ventilatory and cardiovascular responses to exercise. One of these methods was

based on blocking neuromuscular transmission by injecting participants with a low dose of d-tubocurarine to cause temporarily muscle weakness, thereby increasing the effort (and presumably central command) required to perform a given task. Asmussen et al. (1965) recruited participants to perform dynamic exercise with and without blockade of neuromuscular transmission. They noted that after reaching the steady state of exercising on a bicycle ergometer (at 380 or 713 kpm/min for 20 to 30 min), responses of blood pressure, heart rate and ventilation were greater during curarisation, compared to control, in both exercise conditions. The findings from Asmussen et al. suggest that increases in cardiovascular and ventilatory responses during exercise with tubocurarine were due to the increase in central command activity, explained by greater motor output required to compensate for muscle weakness and maintain the same exercise intinsity for each exercise condition.

Another investigatory method was used by Asmussen and Nielsen (1964), to examine the role of central command in cardiovascular and ventilatory responses to exercise. In their study, they used pneumatic cuffs placed around participants' upper thighs to block circulation, whilst the participants performed bicycle exercises at different intensities. They found that the rapid increase in ventilatory response at exercise onset did not change during exercise with occlusion. However, during steady state, the ventilatory response was greater in the occluded leg exercise compared to normal exercise (control). Moreover, they reported similar recovery of ventilation to resting levels at cession of exercise in both exercise conditions. They concluded that the increase in ventilation during occluded leg exercise was not due to activation of the muscle metaboreflex, as ventilation returned back to normal levels at cession of exercise while occlusion continued. However, the increase in ventilation was suggested to be attributed to an increase in central neural drive, activating the fatiguing ischemic muscles and maintaining the workload.

In another human study, Innes et al. (1992) recruited patients presenting with painless unilateral weakness (due to neurological or orthopaedic disorders) to perform dynamic leg exercises. Their results showed increased ventilation, heart rate and blood pressure when patients exercised their weakened leg compared to their unaffected leg. The authors concluded that the increases in the ventilatory and cardiovascular responses during exercise of the weakened leg were caused by the magnitude of effort, central command, required to maintain the same workload.

Using a neurophysiological approach, Goodwin et al. (1972) used simple non-invasive methods on healthy participants aiming to determine changes in cardiovascular and ventilatory responses to different degrees of central command. They used a tendon vibration technique to stimulate the primary afferents of muscle spindles during contraction of human biceps and triceps muscles. Vibration is a strong stimulus for muscle spindle endings, which can produce a reflex tension within the muscle without volitional influence (De Gail et al., 1966). The principle of using vibration was to manipulate the voluntary effort required to contract the muscle and, hence, the level of central command. By applying vibration to the contracting muscle, the required effort and central command to achieve target muscle tension was reduced. In contrast, applying the vibration to the antagonist muscle elicited reflex inhibition of the agonist muscle and increased the effort required to achieve target muscle tension. It was also noted that during sustained isometric muscle contraction, increases in heart rate, blood pressure and ventilation were smaller with reduced effort and greater with increased effort. Given that muscle tension did not change during exercise with or without vibration, it can be concluded that changes in cardiorespiratory responses are proportionate to the magnitude of the involved central command.

Overall, these studies provide strong but circumstantial evidence for the existence of central command; however, the anatomical structures and neural circuitry that generate it are still debated.

1.5.1 The neural structures and circuitry involved in central command

The suprapontine brain is considered to be the main area where the feed-forward signals to cardiovascular and respiratory control centres originate (Forster et al., 2012). At the onset of locomotion, neural signals from the suprapontine areas descend to spinal locomotor neurons and to cardiorespiratory control areas resulting in cardiovascular and ventilatory responses.

Eldridge et al. (1981) found that in unanaesthetised decorticate cats, increases in blood pressure and ventilation during actual locomotion on a treadmill were similar to the responses induced by electrical stimulation of the hypothalamus' locomotor region. Although they suggested that these changes were generated with the influence of central command, the authors acknowledged that neural feedback from exercising muscle may also contribute, at least in part, to these responses.

However, further support of the above findings regarding central command was from observations that similar cardiovascular and ventilatory responses resulted from fictive locomotion within paralysed cats, where no muscle afferent feedback was possible. Furthermore, pharmacological stimulation of neural cell bodies in the subthalamic locomotor region using picrotoxin injection (an inhibitor for the neurotransmitter GABA) showed the same responses to those during actual and fictive locomotion (Eldridge et al., 1985).

Other studies done on animals have shown that electrical stimulation of several suprapontine areas – for example the thalamus, hypothalamus, mesencephalic locomotor region and basal

ganglia resulted in the development of locomotion and elevations in blood pressure, heart rate and ventilation. These changes appear similar to those response seeing during exercise (Smith Oa, 1960, Ordway et al., 1989, DiMarco et al., 1983, Bedford et al., 1992, Ángyán, 1994).

More recently, evidence was obtained suggesting that the neurons within the retrotrapezoid nucleus (RTN) that work as a feed-forward neural mechanism, may contribute to ventilation. A study done on anesthetised rats by Fortuna et al. (2009) showed an increase in blood pressure, phrenic nerve activity and RTN discharge resulted from perifornical hypothalamic locomotor region stimulation via administration of gamma-aminobutyric acid ($GABA_A$). It was concluded that the RTN facilitates feed-forward-controlled breathing and locomotion.

In contrast, until very recently, few studies have been conducted on awake humans using methods to record the associated activity or examine the effect of stimulation of different brain areas on cardiorespiratory responses. Most of these studies were conducted on patients undergoing neurosurgery, where electrodes were implanted in distinct brain regions for treatment purpose. In a seminal study, Thornton et al. (2002) recruited patients with movement disorders or chronic pain and found that electrical stimulation of the thalamus, subthalamic nucleus or substantia nigra resulted in significant increases in arterial blood pressure and heart rate, associated with facilitation of movement in awake patients. Moreover, Green et al. (2005) then reported that in awake patients, electrical stimulation of the ventral periventricular/periaqueductal gray matter resulted in a reduction in systolic blood pressure, while stimulation of the dorsal periventricular/periaqueductal gray matter increased systolic blood pressure. These studies in awake patients are suggestive of possible involvement of suprapontine areas in central command neurocircuitry. In 2007, Green et al. provided clear evidence of the importance of periaqueductal gray matter (PAG) as the neurocircuitry involved in central command. The authors reported an increase in neural activity in the PAG during anticipation of exercise, with further increased activity during

normal exercise (low intensity; 15 W) in awake patients. These changes in PAG neural activity were accompanied by a rise in blood pressure, heart rate and ventilation. Therefore, they concluded that the PAG is an important area in the integration and regulation of the cardiorespiratory response to exercise.

The above studies on animals and in patients provide evidence that the suprapontine areas could indeed be involved in the central command neurocircuitry; in that, stimulating these areas induces locomotion and alters cardiovascular and ventilatory responses. Moreover, the studies ruled out the possible involvement of other neural mechanisms, particularly neural reflexes from exercising muscle. Although there was evidence that electrically or chemically stimulating suprapontine areas develops locomotion and elicits changes in cardiovascular and ventilatory responses, other studies showed contradictory results. A direct test on the role of the hypothalamus by performing a lesion on the hypothalamic (subthalamic) locomotor region showed no difference in cardiovascular and ventilatory responses to exercise between pre- and post-lesioning in animals (Hobbs, 1982, Waldrop, 1986). This suggested potential contribution of other neural mechanisms (e.g. neural feedback from exercising muscle) in controlling cardiovascular and ventilatory responses. Moreover, another factor that should be taken into consideration when interpreting findings relating to central command in exercise is that using electrical or chemical stimulation on suprapontine areas clearly does result in alteration of cardiovascular and ventilatory responses. A main concern is whether this stimulation of suprapontine areas activates the same pathways that are commonly used during normal voluntary exercise.

Other methods have been used to identify the active brain regions participating in normal or imagined exercise. Iwamoto et al. (1996) used immunocytochemical labelling of c-fos to identify active brain regions in exercising rats. They noticed that after 45 minutes of treadmill exercise, c-fos labelling in the hypothalamic (posterior and lateral) and subthalmic regions

(NTS and PAG) was upregulated. These findings in awake animals support the aforementioned studies using electrical and chemical stimulation in decorticate animals.

In a human study, Fink et al. (1995) used positron emission tomography (PET) to assess the changes in rCBF in order to determine the active brain regions in healthy participants performing normal exercise. The results showed rCBF increases in the left and right superomedial and superolateral primary motor cortices. In addition to voluntary exercise, Thornton et al. (2001) measured the changes in rCBF using PET during imagined exercise in patients put under hypnosis. They found that imagined exercise resulted in increase heart rate and minute ventilation. These increases were associated with significant activation of the dorso-lateral prefrontal cortex, the primary and supplementary motor areas, sensorimotor areas, superolateral region, thalamus and cerebellum. It is known that these brain areas are involved in generating volitional breathing (Colebatch et al., 1991, Ramsay et al., 1993). As imagined exercise does not involve actual physical exercise, and therefore input from peripheral feedback from the muscle, these changes in the ventilatory and cardiovascular responses to imagined exercise are thought to be mediated by activation of areas of the brain likely involved in the neurocircuitry of the central command mechanism.

However, some researchers suggested that these changes in cardiovascular and ventilatory responses during imagined exercise may not be related to central command; rather, they may represent previous learned experience stored in the memory centres (Guz, 1997). Somjen (1992) stated that "*the central nervous system anticipates present and future needs on the basis of past experiences*". Therefore, changes in these responses during exercise, especially ventilation, could be as a result of previous learned information and practising exercise previously stored in the memory. The primary and supplementary motor areas are considered to be the memory banks of the brain and activation of these areas by central command during

exercise could indeed generate locomotion and changes in cardiovascular and ventilatory responses (Somjen, 1992).

There are several studies that have been conducted on the contribution of memory to the control of breathing, also known as long term modulation (LTM) in humans. An example of the role of LTM in breathing was provided by Wood et al. (2003), who demonstrated that participants that repeatedly exercised under increased external dead space displayed augmentation in exercise hyperpnea when exercising under normal condition. Moreover, Turner and Stewart (2004) found that participants who exercised repeatedly with increased inspiratory resistance showed increased tidal volume response under normal exercise conditions. These findings support a role for memory contribution in exercise hyperpnea.

Thornton et al. (2001) suggested that concurrent activation of the central motor cortex is not necessary for central command responses, as in their studies only the effort was able to drive it. Williamsons et al. (2001) tested this idea and found that those participants who performed imaginary cycling exercise under hypnosis had a significant increase in the heart rate. Together, the findings of Thornton et al. and Williamsons et al. suggested that parallel activation from central command to the locomotor system is not required to control the cardiorespiratory systems. However, central command might activate the locomotor system and cardiorespiratory system separately during exercise.

1.5.2 Conclusions about central command (feed-forward neural mechanism)

The concept of central command as a neural feed-forward mechanism is well developed and several possible locations for the neural structures and circuitry involved in generating central command have been identified in suprapontine areas. Animal studies have shown strong direct evidence of the contribution of central command on cardiovascular and ventilatory

responses. However, a definitive conclusion cannot yet be made as these animal studies do not represent actual voluntary exercise and/or real central command. Similarly, evidence from human studies was indirect and limited, until recently. Based on the available evidence, and considering study limitations, it seems that central command may exist and the PAG may be the most plausible area involved in the neurocircuitry of central command (Iwamoto et al., 1996, Green et al., 2005, Green et al., 2007). Moreover, central command appears to play a significant role in regulating and mediating both cardiovascular responses and exercise hyperpnea.

In contrast, the importance of central command in controlling exercise hyperpnea alone is still a controversial and debated matter (Haouzi, 2006). It is well known that central command initiates an immediate parallel activation of the locomotor and respiratory control areas of the brain during exercise. This action from central command must by definition precede any metabolic or humoral signals arising from exercise and so potentially can be separated temporally by using sinusoidal oscillation in the exercise intensity. In a study in human subjects, Casaburi et al. (1977) examined the ventilatory and pulmonary gas exchange ($\dot{V}_{CO2}$ and $\dot{V}_{O2}$) responses to sinusoidal exercise. Participants were asked to exercise on a cycle ergometer for 30 minutes at a work rate changing sinusoidally between 25W and 80% of the anaerobic threshold of each participant. The period of sinusoidal changes in the work rate was tested at 0.7, 1, 2, 4, 6 and 10 minutes. The authors found that shorting the period of sinusoidal changes in the work rate from 10 to 2 minutes resulted in no change in amplitude (peak to mean) of motor activation. However, it resulted in a decrease in the amplitude of minute ventilation, $\dot{V}_{CO2}$ and $\dot{V}_{O2}$, combining with an increasing in the lag time between work rate changes and ventilatory response. In addition, Haouzi et al. (2004) supported Casaburi et al. findings in a study on sheep. They examined the idea that the system controlling locomotion and ventilation during walking is matched to central command. This was

achieved by examining the locomotor and the ventilatory and pulmonary gas exchange responses in sheep walking normally on a treadmill, where the speed of treadmill changed sinusoidally. Their results showed an increase in the walking frequency with the sinusoidal change in treadmill speed combined with no change in the amplitude of locomotion activation. Moreover, they noticed that shorting the period of sinusoidal changes in the treadmill speed (from 10 to 1 minute) resulted in no lag time between the changes in treadmill speed and walking frequency. On the other hand, they found a significant decrease in the amplitude of ventilatory and pulmonary gas exchange combined with an increase in the lag time between the changes in treadmill speed and ventilation, when the period of sinusoidal changes in the treadmill speed decreased.

These findings from Casaburi et al. and Haouzi et al. studies regarding the lag time between the exercise intensity changes and ventilatory responses are taken as evidence that exercise is unlikely to cause a simple parallel activation of central command and ventilation. These finding suggests another neural mechanism could be involved in mediating exercise hyperpnea, which is more likely dissociated from the central command and more related to metabolic rate.

The neural feedback from exercising muscle (metabolically and mechanically sensitive receptors) is another primary neural mechanism that has displayed a significant role in controlling cardiovascular response to exercise during complete absence of central command influence. Neural feedback has been proposed as another mechanism that mediates exercise hyperpnea, which may provide an error signal to match ventilation with the metabolic rate. Evidence regarding the muscle afferent feedback mechanism on contributing ventilatory and cardiovascular response will be discussed below.

1.6 The role of skeletal muscle afferent feedback in controlling cardiovascular and ventilatory responses to exercise

1.6.1 Types of muscle afferents and their discharge characteristics

There are 5 types of sensory (afferent) nerves innervating skeletal muscle. These afferent nerve fibres are labelled I (subtypes Ia and Ib) to IV. The major difference between these muscle afferents is their size and degree of myelination, which determines their conduction velocity. Group I and II muscle afferents are classified as thick myelinated nerves with conduction velocity ranging between 72 and 120 m/s and 31 and 71 m/s, respectively. Group Ia and II muscle afferents innervate muscle spindles and both are activated by muscle stretching. However, while Ia muscle afferents provide signals about the rate of muscle stretch rate, group II muscle afferents do not. Group Ib muscle afferents innervate Golgi tendon organs and provide signals about the rate of muscle stretch and contraction during activation. These groups of muscle afferents have no effect on cardiovascular responses (Coote et al., 1971, McCloskey and Mitchell, 1972) and their role in ventilation seems to be negligible (McCloskey and Mitchell, 1972)

Group III muscle afferents are primarily mechanoreceptors. They are characterized by a thin myelinated sheet covering their fibre and they have a conduction velocity of 2.5 to 30 m/s. Group III muscle afferents are mainly positioned close to myotendinous junctions. They discharge rapidly during muscle contraction and fire during passive muscle-tendon stretch (Kaufman et al., 1983). There are other pure mechanical stimuli that produce increases in group III firing, including probing, squeezing and external pressure on the muscle (Bell and White, 2005, Hayes et al., 2005, McCord and Kaufman, 2010). However, some of these muscle afferents are considered to be polymodal as they can also be activated by chemical stimulation (e.g. metabolite accumulation) (Kaufman andForster, 1996).

Group IV muscle afferents (C-fibres) are mainly metaboreceptors. These types of muscle afferent are unmyelinated, and their conduction velocity is the lowest of the 5 muscle afferents (less than 2.5 m/s). They are commonly positioned in the connective tissue and venous and lymph vessels. Chemical stimulation can activate these muscle afferents; Kaufman et al. (1983) demonstrated in cats that group IV muscle afferents responded to the build-up of metabolites, caused by electrically evoked muscle contraction. Moreover, local circulatory occlusion of the exercising muscle caused trapping of the metabolites within the muscle, which in turn resulted in continued stimulation of muscle metaboreceptors (Kaufman et al., 1984). Metaboreceptors also can be stimulated by injecting chemical substances into the muscle, such as lactic acid, H^+, adenosine triphosphate (ATP), bradykinin or K^+, which results in increased discharge of these muscle afferents (McCloskey and Mitchell, 1972, Mense and Meyer, 1988, Rotto and Kaufman, 1988, Hanna et al., 2002).

Both group III and IV muscle afferents are considered to be the main afferents transmitting information about metabolic and mechanical conditions of muscle to the medulla (NTS), where these sensory inputs are integrated to regulate cardiorespiratory responses during exercise (Kaufman and Forster, 1996, Paton et al., 2001).

1.6.2 Skeletal muscle afferents: cardiorespiratory control in animal studies

1.6.2.1 Cardiovascular control in animal studies

There have been several neurophysiological studies conducted in animal models showing that feedback from these muscle afferents (group III and IV) contribute to controlling cardiovascular and ventilatory responses to exercise in the complete absence of central command. Electrical stimulation was one of the methods used in early studies to induce muscle contractions without the presence of central command. Two prominent studies

(McDowall, 1936, Euler and Liljestrand, 1946) reported that direct electrical stimulation of hind-limb muscles resulted in contradictory findings to those reported in humans; in fact, they found that arterial blood pressure occasionally fell during contraction of hind-limb muscles in anaesthetised animals.

These above studies did not use appropriate methodology to examine the role of muscle afferent feedback. Coote et al. (1971) suggested that applying direct electrical stimulation to the hind-limb muscles would stimulate many muscle afferents fibres and subsequently block them, thereby masking the real effect of muscle afferent feedback from the contracting muscles. Coote et al. (1971) therefore used an improved method of examining the effect of muscle afferent feedback on cardiorespiratory responses in their study. The authors found that hind-limb muscle contraction induced by electrical stimulation of the ventral roots (L6-S1; efferent nerves) of anaesthetised cats resulted in an increase in blood pressure and smaller increases in heart rate and ventilation. These cardiorespiratory responses were abolished when the dorsal roots (L6-S1; afferent nerves) were cut or muscle contraction was blocked using the non-depolarising neuromuscular blocker gallamine. It was concluded that the cardiorespiratory responses may have been attributed to accumulation of metabolites within the muscle, resulting in activation of group III and IV muscle afferents. The above findings supported previous evidence that electrical stimulation of group I and II muscle afferents in anaesthetised cats had no effect on sympathetic output, whereas electrical stimulation of group III and IV increased it (Coote and Perez-Gonzalez, 1970).

Similarly, McCloskey and Mitchell (1972) investigated the contribution of muscle afferent feedback on the cardiovascular and ventilatory responses to electrically induced muscle contraction. The researchers blocked group I and II muscle afferents (at the dorsal root) in anaesthetised cats, using the anodal block technique, and found that cardiovascular and ventilatory responses were not abolished during electrically stimulated hind-limb muscle

contraction. However, when they blocked group III and IV muscle afferents using local anaesthetic, all of these responses were abolished during electrical stimulation of hind-limb muscles. Additionally, they found that circulatory occlusion of the muscle after contraction resulted in sustained increases in blood pressure, but not in heart rate and ventilation levels.

1.6.2.2 Ventilatory control in animal studies

There are several studies suggesting that muscle afferents play an important role in controlling ventilation, as well as cardiovascular responses. In 1943, Comroe and Schmidt reported an increase in ventilation during muscle contraction induced by electrical stimulation of the ventral roots of anaesthetised dogs. However, this response was abolished by spinal cord transection. These findings support the concept of muscle afferent feedback as a mediator of exercise hyperpnea. Several years later, Kao (1963) examined the effect of muscle afferent feedback on ventilation in isolation from central command influence and hormonal stimuli using a cross circulation technique on anaesthetised dogs. Electrical stimulation of the ventral roots was used to induce muscle contraction of the hind-limb of one dog (neural dog), while the muscle of a second dog (hormonal dog) was continually perfused by blood from the neural dog. Interestingly, the authors noted an immediate increase in ventilatory response (about 6 $l.min^{-1}$) during onset of muscle contraction in the neural dog, but not in the hormonal dog. Furthermore, they noticed that transection of the spinal cord resulted in abolition of the ventilatory response in the neural dog. They concluded that a peripheral reflex is one of the mechanisms contributing to exercise hyperpnea. Furthermore, Tibes (1977) provided clear evidence of the role of muscle afferent feedback in ventilation by cold-blocking group III and group IV muscle afferents. His results showed the elimination of ventilatory responses during electrical stimulation of dynamic hind-limb contraction in dogs. Bennett (1984) also reported an insufficient increase in ventilatory response to maintain

*P*aCO_2 homeostasis during electrically evoked exercise in anesthetised dogs whose muscle afferents were cut. Thus, it was concluded that, when intact, muscle afferents play an important role in regulating ventilation.

Other methods have also been used to investigate the role of muscle afferent feedback in induction of exercise hyperpnea. These methods selectively activated group III (mechanoreceptors) and group IV (metaboreceptores) muscle afferents using mechanical and metabolic stimuli, respectively. Activation of the muscle metaboreceptors alone by injecting animal hind-limb arteries with lactic acid, bradykinin and ATP caused a ventilatory response; this response was abolished when the nerve supply to the muscle was sectioned (Tallarida et al., 1979, Rotto et al., 1989).

Activation of muscle mechanoreceptors alone in animals by passive muscle stretching of the triceps surae resulted in an increase in ventilation. However, this ventilatory response was abolished by arterial injection of gadolinium, which blocks mechanosensitive ion channels (Rotto et al., 1989, Wilson et al., 1994, Hayes and Kaufman, 2001).

Conversely, some animal studies have shown different results, which has led researchers to question the contribution of muscle afferent feedback to exercise hyperpnea. Comroe and Schmidt (1943) reported that when the spinal cord of anaesthetised dogs was transected, the ventilatory response to electrically induced muscle contraction was abolished. However, this was not seen in anaesthetised cats. Lamb (1968) also reported that increases in metabolic rate and ventilatory responses (maintaining blood gas homeostasis) to electrically evoked muscle contractions were not altered after transection of the lumber spinal cord in both cats and dogs. Other investigators have supported these findings; Cross et al. (1982) compared the ventilatory and metabolic responses before and after spinal cord transection. Their results showed no differences in these responses to electrically induced muscle contraction in

anesthetised dogs. Levine (1979), on the other hand, reported somewhat surprising findings. They showed that after transection of the spinal cord, and with electrically induced muscle contraction, ventilatory response and metabolic rate were increased by around 170 % in anesthetised dogs. The authors, therefore, concluded that exercise hyperpnea is stimulated by humoral factors.

Although electrically stimulated muscle contraction studies show that muscle afferent feedback does play a regulatory role in the cardiovascular response to exercise, its contribution to hyperpnea has not been fully established in anesthetised animals. This may be due to two factors: the effects of the aesthetic regime; and, some argue, the small changes in ventilation and metabolic rate obtained from these studies that may have not translated into the responses seen in high-intensity exercise of a large muscle mass.

1.6.3 Skeletal muscle afferents: cardiorespiratory control in human studies

1.6.3.1 Cardiovascular control in humans

Reflexes arising from exercising muscle that provide sensory information about its mechanical and metabolic conditions play an important role in driving the cardiovascular responses to exercise (known as the exercise pressor reflex). A brief explanation about the function of exercise presser reflex is that during exercise the reflex works on removing the metabolic error signal. This is achieved by assisting adequate blood flow to the exercising muscle to wash out metabolites and prevent early muscle fatigue (Rowell and O'Leary, 1990, Rowell, 1993).

Evidence for the role of muscle afferent feedback in regulating cardiovascular responses to exercise independent of central command is well documented in humans. Classically, Alam

and Smirk (1937) were the first investigators to give a full experimental demonstration of the role of muscle afferents in controlling the blood pressure response to dynamic leg exercise in awake humans. They recruited participants to perform dynamic leg exercise with circulatory occlusion by placing a cuff around that upper thighs and inflating it to supra-systolic pressure. The researchers noted that after cession of exercise, and with circulatory occlusion, the blood pressure dropped slightly from the end exercise level but remained significantly higher than resting level. However, the blood pressure then returned to its resting level when the cuff was deflated. Performing circulatory occlusion prevents blood flow through exercising muscle and traps metabolites in the muscle. The authors concluded that the maintained elevation of arterial blood pressure during post-exercise circulatory occlusion (PECO) could only result from activation of reflexes from the exercising muscle and not from circulating metabolites and hormones, which would be unaltered by PECO or central command (as this is absent during PECO).

Blocking afferent feedback from working muscle using lumber epidural anaesthesia is another method used to examine the contribution of muscle afferent feedback in cardiovascular responses. Studies have found that blocking muscle afferent reflexes using bupivacaine, lidocaine or fentanyl results in attenuation of cardiovascular responses to voluntary exercise (Hornbein et al., 1969, Fernandes et al., 1990, Amann et al., 2010), depending on the level of block and, hence, reduction in muscle afferent feedback.

Bull et al. (1989) used another technique to examine (non-invasively) the role of muscle afferent feedback in the cardiovascular response to isometric exercise. They examined the cardiovascular responses to voluntary and electrically evoked muscle contraction followed by PECO. They found that electrically evoked muscle contractions resulted in increases in blood pressure and heart rate, which were similar to those observed during voluntary exercise. Moreover, on cessation of muscle contraction, but with circulatory occlusion, the blood

pressure remained significantly above baseline, while the heart rate returned back to resting level during voluntary and electrically evoked muscle contraction. They suggested that elevation of blood pressure above baseline during PECO period was due to accumulation of trapped metabolites within the exercised muscle, which in turn activate a reflex (metaboreflex). While, returning the heart rate to baseline during PECO period was due to losing the influence of other receptors that are more likely sensitive to mechanical stimulation (mechanoreflex). This suggestion was supported by the similar increase in the heart rate response observed during both voluntary and electrical evoked muscle contractions which was performed at the same exercise intensity. These findings from Bull et al. (1989) study provided clear evidence that muscle afferents play an important role in regulating cardiovascular responses to exercise, especially during electrically evoked muscle contraction followed with PECO, where the central command is completely eliminated. Other studies have used different techniques to attempt to examine the contribution of each muscle afferent alone. This was done by selectively stimulation of muscle mechanoreceptors (mechanoreflex) or muscle metaboreceptors (metaboreflex) and assessing the cardiovascular response induced.

A) Mechanoreflex

The exercise pressor reflex can be activated either by mechanical or metabolic stimuli. The muscle mechanoreflex functions to provide feedback about muscle contraction force and this sensory feedback plays an important role in adjusting the cardiovascular response according to the exercise level (Kaufman and Forster, 1996). Passive muscle stretching and muscle compression are considered to be effective non-invasive techniques that can selectively stimulate the mechanoreceptors, with complete isolation from central command influence, and determine their contribution to the cardiovascular response (Hayes et al., 2005, McCord and Kaufman, 2010). Studies conducted in animals have shown increases in blood pressure,

heart rate and cardiac sympathetic tone combined with a reduction in cardiac vagal tone during passive tendon stretching (Stebbins et al., 1988, Murata and Matsukawa, 2001, Kaufman, 2012). Moreover, they reported that the degree of muscle tension evoked by muscle stretching has an effect on the magnitude of the responses.

Hollander and Bouman (1975) were the first investigators to suggest that mechanoreceptors contribute to the human heart rate response to exercise and coined the term 'the muscle heart reflex'. They reported that during the onset of electrically induced isometric elbow flexion exercises, the heart rate increased rapidly due to withdrawal of vagal activity determined using atropine blockade. Moreover, regarding the stretching technique, Gladwell and Coote (2002) and Gladwell et al. (2005) provided evidence that sustained passive stretching of calf muscle resulted in a significant increase in heart rate only (no change in blood pressure), which was vagally mediated since the response was attenuated by an anticholinergic glycopyrrolate blockade (Gladwell et al., 2005).

Fisher et al. (2005) examined cardiovascular responses to muscle metaboreflex activation (by PECO), and concurrent activation of muscle mechanoreflex by standardized passive calf muscle stretching in human. The authors reported that applying standardised sustained passive calf muscle stretching for 1 minute during circulatory occlusion at rest alone resulted in increases in blood pressure and heart rate. Moreover, they found that during PECO period following graded increases in isometric exercise (at 30, 50, and 70% of MVC), blood pressure was maintained significantly above baseline, and the magnitude of this increase in blood pressure was higher with a higher level of exercise intensity. However, their main finding was that applying standardised sustained passive calf muscle stretching during PECO resulted in a significant further increase in the blood pressure and heart rate above that reported during sustained passive calf muscle stretching alone and PECO alone. In addition, Drew et al. (2008) supported Fisher et al. (2005) findings. They reported that applying a

standardised sustained passive calf muscle stretch for 3 minutes during PECO period, resulted in a significant increase in blood pressure and a higher heart rate compared to sustained passive calf muscle stretching alone and during PECO alone. These findings from Fisher et al. (2005) and Drew et al. (2008) suggest that activation of the muscle mechanoreflex by sustained passive muscle stretching could increase sympathetic vasomotor activity (seen by further increase in blood pressure), as well as the increase in the heart rate due to withdrawal of cardiac vagal tone. In addition to their contribution on controlling blood pressure, Cui et al. (2008) provided supporting evidence that the muscle mechanoreflex also plays a small role in mediating increased muscle sympathetic nerve activity response (MSNA) to exercise. They examine MSNA and blood pressure responses to muscle mechanoreflex activation by sustained passive muscle stretching (wrist dorsiflexion) under 3 conditions; A) under free muscle perfusion, B) under circulatory occlusion of the upper arm during resting, alone and C) under PECO, following 30% MVC dynamic handgrip exercise. The authors' results showed that MSNA and blood pressure responses to sustained passive muscle stretching during PECO were greater than other responses from the other three conditions alone.

These findings from Fisher et al. (2005), Drew et al. (2008) and Cui et al. (2008) support the view that some muscle mechanoreceptors have polymodal characteristics that are sensitive to muscle metabolites and force (see Kaufman and Forster, 1996 for review).

External muscle compression also has the capability to activate the muscle mechanoreflex. Studies on animals (Stebbins et al., 1988) and humans (Williamson et al., 1994) using this technique have shown that external muscle compression caused an increase in the muscle interstitial pressure, resulting in elevated arterial blood pressure, but not heart rate. The possible explanation of this increase in blood pressure might be the activation of muscle metaboreflex. This is because external compression causes local circulatory occlusion, which

results in trapping and accumulation of metabolites within the muscle, stimulating of metaboreceptors. However, Bell and White (2005) provided another explanation for this; in their study, they evaluated the cardiovascular response to mechanoreflex activation (by external muscle compression) under different levels of concurrent muscle metaboreflex stimulation. They applied external compression to the muscle during a PECO period after different levels of isometric calf exercise. They found that a standard level of external compression of the muscle resulted in a graded increase in the blood pressure response to compression; moreover, the magnitude of this increase depended on the intensity of the preceding exercise. These findings were in support of the evidence for polymodality of a population of muscle mechanoreceptors that are sensitive to muscle metabolites.

In conclusion, the above evidence shows that activation of muscle mechanoreflex by passive muscle stretching causes inhibition of cardiac vagal activity and results in increased heart rate. Moreover, some muscle mechanoreceptors have polymodal characteristics and activating them by passive muscle stretching or external muscle compression, in combination with metabolite accumulation (e.g. during PECO) results in increased blood pressure and MSNA.

B) Metaboreflex

During exercise, it has been suggested that the muscle metaboreflex works to provide the cardiovascular centre in the brainstem with metabolic information representing the mismatch between the blood supply and metabolic demand of the exercising muscle (Kaufman and Foster 1996). The contribution of the muscle metaboreflex to the cardiovascular response during exercise has been studied intensively and different techniques have been used to activate these muscle afferents. The most common technique used is PECO, as it is non-invasive and considered to be useful for evaluating the contribution of muscle metaboreflex

activation on the exercise pressor reflex in complete absence of central command and muscle mechanoreflex. PECO works by preventing blood flow to the exercising muscle, which leads to accumulation of metabolites in exercised muscle and results in stimulation of muscle metaboreceptors. This, in turn, evokes a reflex that raises sympathetic activity and peripheral vasoconstriction, ultimately resulting in elevation of blood pressure.

Many studies have demonstrated that isolated activation of muscle metaboreceptors via PECO results in a slight reduction of blood pressure from end exercise levels**,** although this level is maintained significantly above resting level throughout the PECO period, muscle sympathetic nerve activity (MSNA) remains elevated at end exercise levels while heart rate returns to resting levels (Alam and Smirk, 1937, Rowell et al., 1976, Mark et al., 1985, Victor et al., 1987, Bull et al., 1989). Preliminary findings from these studies suggest that muscle metaboreflex may influence only blood pressure and MSNA, but not the heart rate. Therefore, another explanation for the reduction in the heart rate during PECO was proposed. This was the reactivation of cardiac parasympathetic tone in response to a baroreflex mechanism (resetting of baroreceptors), or loss of central command or muscle mechanoreflex at the end of exercise, masking the effect of the muscle metaboreflex mediating the increase in heart rate response (O'Leary, 1993, Nishiyasu et al., 1994, Iellamo et al., 1999a).

Evidence from an animal study showed that abolishing cardiac parasympathetic tone by atropine injection resulted in increased heart rate during PECO, mediated by the now un-masked cardiac sympathetic tone (O'Leary, 1993). Similarly, in a human study, Fisher et al. (2010) re-examined the contribution of muscle metaboreflex to autonomic control of heart rate using drugs that selectively inhibit either the sympathetic tone or cardiac parasympathetic tone. They reported a small increase in heart rate above baseline (although not significant) during PECO following moderate intensity isometric handgrip exercise (25 % MVC). This increase in the heart rate was abolished after administration of β-adrenergic blockade

(propranolol or metoprolol) and was augmented with administration of parasympathetic blockade (using glycopyrrolate).

Although it was proposed that reactivation of cardiac parasympathetic tone is normally responsible for returning the heart rate to resting level during PECO, findings from Fisher et al. (2010), using high intensity exercise, revealed contradictory results, which question the influence of the muscle metaboreflex on heart rate. Their results showed that the increase in heart rate level during high intensity isometric handgrip exercise (40 % MVC) was sustained significantly above baseline during PECO period and this differed from the moderate intensity response (25 % MVC) of recovery to baseline. Moreover, the authors reported that this increase in heart rate during PECO following 40% MVC exercise was attenuated after administration of β-adrenergic blockade but was not changed with cardiac parasympathetic blockade. Their conclusion was that the muscle metaboreflex mediated the increase in cardiac sympathetic tone during PECO in the 40 % MVC condition. Thus, it seems that in order to maintain a heart rate above resting levels during PECO, strong activation of the muscle metaboreflex is required to overcome reactivation of cardiac parasympathetic tone once exercise inhibition of the vagus nerve has ended.

These recent findings from the Fisher et al. (2010) study support previous findings from Iellamo et al. (1999a), where a slight increase in heart rate during PECO was seen and this increase was dependent on the size of the exercising muscle (handgrip vs. knee extension) as well as the level of exercise intensity (15 % vs. 30 % MVC). Collectively, this evidence leads to the conclusion that the muscle metaboreflex can drive blood pressure and MSNA responses. Moreover, high levels of muscle metaboreflex activation during PECO, following high intensity exercise, can also affect heart rate response. This occurs by offsetting the reactivation of cardiac parasympathetic tone at end exercise, when its inhibition by central command and the muscle mechanoreflex is withdrawn.

As described earlier (page 33), other studies have activated the muscle metaboreflex by injecting chemical substances into the muscle. Intraarterial administration of diprotonated phosphate, ATP, lactic acid, H^+ and bradykinin into hind-limb muscles resulted in a pressor response, which was abolished upon sectioning of the nerve supply to the muscle (Stebbins and Longhurst, 1985, Rotto et al., 1989, Sinoway et al., 1994, Li and Sinoway, 2002, Hanna et al., 2002).

Victor et al. (1988) and Sinoway et al. (1989) used ^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMC) to determine the intracellular mediators of the pressor reflex in humans. These studies found that the intercellular concentration of H^+ during handgrip exercise correlated with the time course and the magnitude of the exercise pressor response. Ettinger et al. (1991) also evaluated MSNA responses to isometric handgrip exercise and PECO before and after intravenous administration of dichloroacetate. Dichloroacetate works by inhibiting lactic acid production and increasing pyruvate dehydrogenase levels. The results of the study showed attenuation of the increase of MSNA and venous lactate levels in the dichloroacetate group compared to control.

In patient studies, Pryor et al. (1990) examined the effect of lactic acid on stimulating the exercise pressor reflex on McArdle's disease patient and healthy participants. Due to their myophosphorylase deficiency, McArdle's patients are known to produce limited amounts of lactic acid in their muscles. The authors' results showed that the exercise pressor and MSNA responses to isometric handgrip exercise were significantly lower compared to healthy participants. Similar findings were reported by Fadel et al. (2003), who found that in comparison to the wide range of healthy control responses, there was an attenuation in the MSNA response to moderate intensity isometric handgrip exercise in McArdle's disease patients.

1.6.3.2 Ventilatory control in humans

Exercise causes an increase in ventilation (known as exercise hyperpnea) to meet the metabolic demands of the body (more O_2 uptake and rapid removal of CO_2) and to maintain the blood PaO_2 , $PaCO_2$ and pH levels within resting levels (Dempsey et al., 1995).

The neural mechanisms underlying the initiation and regulation of exercise hyperpnea have been subject to intensive investigation over the last century. Several mechanisms have been proposed as the main regulators of exercise hyperpnea; the most widely agreed mechanism for primary control of exercise hyperpnea is central command (feed-forward mechanisms) (Krogh & Lindhard, 1913, Eldridge et al., 1981, Green et al., 2007). However, the contribution of muscle afferents as neural mechanisms of regulating exercise hyperpnea remain a subject of debate in exercise physiology. Evidence from animal studies using different methods of investigation (described above: section 1.6.2.2) has shown differing contributions of muscle afferent feedback to exercise hyperpnea in complete absence of central command. On the other hand, several studies have been conducted in humans (reviewed below), aiming to establish the role of neural mechanisms in exercise hyperpnea.

A) Suppression of muscle afferent feedback

Various methods have been used to investigate the contribution of muscle afferents in controlling cardiovascular and ventilatory responses to exercise; one of these was blocking the muscle afferent feedback from working muscles using epidural anaesthesia. Fernandes et al. (1990) assessed cardiovascular and ventilatory responses to cycling exercise. They used spinal anaesthesia, injecting bupivacaine into the L3–L4 vertebral space, to block sensory feedback from the exercising muscles. Their results showed a decrease in blood pressure only in the epidural anaesthetic group; heart rate and minute ventilation levels did not differ

significantly between this group and the control group. Therefore, they concluded that muscle afferent feedback is not an important mechanism for mediating exercise hyperpnea. It should be noted that using epidural anaesthesia in this study did not only cause attenuation in muscle afferent feedback, but may also have affected efferent motor activity, as demonstrated by Dempsey et al. (2014), who found a reduction in lower limb strength by 20 – 40 % following epidural anaesthesia. In order to maintain the constant workload for decreased afferent feedback, central command influence was shown to be increased to recruit and repetitively stimulate motor neurons to overcome the local anaesthesia. Consequently, there was no change in minute ventilation during exercise with local aesthesia, which corroborated earlier findings by Fernandes's (1990).

Amann et al. (2010) examined the role of muscle afferent feedback in cardiovascular and ventilatory responses to dynamic exercise under spinal anaesthesia. Unlike Fernandes's (1990) study, Amann et al. (2010) used fentanyl (a μ-opioid receptor agonist) to selectively block afferent feedback in their participants at the L3 − L4 vertebral space. Their findings showed a significant reduction in mean arterial blood pressure, heart rate, minute ventilation and breathing frequency within the fentanyl group, compared to the placebo group, during dynamic exercise (50 – 325 Watts). In addition to Amann's (2010) work, other studies have reported that blocking muscle afferent feedback by intrathecal injection of fentanyl resulted in decreased ventilation during dynamic exercise in chronic obstructive pulmonary disease (COPD) (Gagnon et al., 2012) and chronic heart failure (CHF) patients (Olson et al., 2014).

It is well known that fentanyl inhibits feedback from group III and IV muscle afferents only, without affecting muscle strength (Pomeroy et al., 1986, Hill and Kaufman, 1990, Amann et al., 2009). Hence, Amann et al. (2010), Gagon et al. (2012) and Olson et al. (2014) clearly demonstrated the contribution of muscle afferent feedback in controlling exercise hyperpnea without any change in central command activity.

B) Integration between central command and muscle afferent feedback on controlling ventilation

Several studies had examined the contribution of muscle afferent feedback in controlling the abrupt changes of ventilation at the onset of exercise. Instead of inhibiting the muscle afferent feedback, these studies activate them alone (by passive movement) or with central command (by active movement) to examine their contribution to ventilation in humans. A passive exercise, where the limb is passively moved either manually or using a special apparatus, is considered to be one way for minimizing muscle activation and thereby, has minimal involvement of central command. Therefore, any changes occurring in ventilatory responses to passive movement will be taken to account for the activation of muscle afferent from the exercising limb.

Bell et al. (2003) examined the ventilatory response to two types of passive leg exercise (A: cycling movements performed on a tandem bicycle, B: passive leg extension performed on a chair apparatus). Minute ventilation was examined in two periods, firstly from rest to onset of the passive exercise, and then from onset of passive exercise to the end of passive exercise. The authors reported increases in the minute ventilation in the two types of passive movement exercises during two conditions of assessments. However, they noticed that the magnitude of the increase in minute ventilation during passive exercise on a tandem bicycle was significantly greater than during leg extension on a chair apparatus. The possible explanation for this difference is due to the number of muscles involved in both types of passive exercise. Passive cycling on a tandem bicycle involved more muscles than leg extension, which in turn increased muscle afferent feedback and as a result increased ventilation. Alternatively, it could be due to the involvement of central command during passive cycling on a tandem. This is because, several participants tried to maintain balanced during passive cycling, which in turn caused activation of other muscles (e.g. trunk muscle)

leading to activation of central command. Thereby, increasing in the magnitude of minute ventilation response. In a later study, Bell and Duffin (2006) examined the contribution of muscle afferent feedback in controlling the rapid increase in ventilation alone and with the a central command influence. A tandem chair was used to perform the passive and active leg extension exercise. Minute ventilation was examined in a transition manner; from rest to passive leg extension, passive to active leg extension, and active leg extension to rest. Also, it was examined from rest to active leg extension. Their results showed immediate increases in minute ventilation when participants starting performing passive leg extension from rest. Moreover, there was a further increase in minute ventilation when the subject started the voluntary control of the leg extension. However, the main finding of this study was that the sum of minute ventilation response observed from rest to passive leg extension and passive to active leg extension was equal to that obtained from rest to active leg extension alone.

These findings from the Bell et al. (2003) and Bell and Duffin (2006) studies, suggest that activation of muscle afferent feedback alone (by passive movement) contribute in controlling the rapid increase of ventilation at the onset of exercise. Also, these findings suggests a possible integration between central command and muscle afferent feedback during active movement, which plays an essential role in controlling exercise hyperpnea.

Other studies had examined the contribution of each muscle afferent alone. This is achieved by selectively activating the muscle metaboreflex (by PECO) or muscle mechanoreflex (by passive stretching) and examining the ventilatory response induced (below).

C) Activation of muscle afferents (mechanoreflex and metaboreflex)

In order to accurately investigate the contribution of muscle afferents to exercise hyperpnea regulation, muscle afferents should be activated alone, in complete isolation from central command. Local circulatory occlusion for the exercising limb during or after exercise is considered a good non-invasive technique for stimulating muscle metaboreceptors. This is because, during exercise, muscles produce metabolic compounds (e.g. lactic acid, bradykinin and ATP), to which muscle metaboreceptors are sensitive. Using PECO to trap these metabolic substances in exercising muscle and prevent them from being washed out via systemic circulation causes activation of the muscle metaboreflex and, on returning to rest, the influence of central command is removed.

It is well documented that activation of the muscle metaboreflex leads to an increase in sympathetic efferent activity (Smith et al., 2006), which in turn causes vasoconstriction and a resultant increase in blood pressure. However, the effect of activation of the muscle metaboreflex (by PECO) on the ventilatory response is still a subject of controversy.

Studies on static exercise have shown different results regarding the contribution of muscle afferent feedback to exercise hyperpnea. One such study, by Wiley and Lind (1971), assessed the ventilatory response during handgrip and leg exercise at different levels (30 %, 40 % and 50 % of MVC). They reported an increase in minute ventilation during the exercise period. However, this increase was not sustained during the PECO period and instead dropped back to baseline, suggesting that muscle afferents (or at least the muscle metaboreflex in this case) played no role in controlling exercise hyperpnea.

More such studies have been conducted on dynamic exercise than on static exercise. Rowell et al. (1976) examined the cardiovascular and ventilatory responses to total circulatory occlusion for the both legs (upper thighs) immediately after cycling exercise performed at 50

- 250 Watts, and at 10, 20, 30 seconds before the end of exercise performed at 100 - 150 Watts. They found that, during PECO (lasting for 3 minutes) in both exercise conditions, minute ventilation was not sustained at the exercise level but dropped back to baseline level, whereas blood pressure was maintained significantly above baseline until the end of the PECO period. They, therefore, suggested that activation of the muscle metaboreflex does not elicit a ventilatory response during high-intensity dynamic exercise.

Haouzi et al. (1993) also evaluated the ventilatory and cardiovascular responses to high-intensity cycling exercise (130 - 150 Watts) in five healthy participants. Their results supported those of Rowell et al. (1976); they reported increased minute ventilation during exercise, which was not sustained during the PECO period (2 minutes) but dropped back to baseline, suggesting that activation of the muscle metaboreflex had no significant role in controlling exercise hyperpnea. Haouzi et al. (2001) conducted a further study to determine the ventilatory response to the activation of muscle metaboreceptors during a brief (12 seconds) high-intensity cycling exercise (400 Watts) in 6 healthy participants. They found that, after the cessation of exercise and during two PECO conditions (total leg occlusion and partial leg occlusion for 90 seconds), minute ventilation dropped back to baseline. Scott et al. (2000) similarly reported a decrease in the ventilation level during PECO (3 minutes) after a cycling exercise of moderate intensity (30 - 70 Watts) for 6 minutes in 12 healthy participants.

In addition to the previous studies on dynamic exercise, Fukuba et al. (2007) investigated the effect of leg circulatory occlusion on the ventilatory response. Seven healthy participants took part in this study, which required them to perform an upright cycling exercise at either sub-anaerobic or supra-anaerobic threshold intensity. Their results showed a rapid recovery of minute ventilation during the PECO periods (2 minutes). These findings supported those of Haouzi et al. (1993), Scott et al. (2000) and Haouzi et al. (2001), suggesting that activation of

the muscle metaboreflex alone by local circulatory occlusion after the cessation of exercise has no significant effect on exercise hyperpnea. All these studies (Wiley and Lind, 1971, Rowell et al., 1976, Haouzi et al., 1993, Scott et al., 2000, Haouzi et al., 2001, Fukuba et al., 2007) suggest that the muscle metaboreflex does not play an important role in exercise hyperpnea.

These studies also suggested that, during moderate or heavy-intensity dynamic exercise, activation of the muscle metaboreflex (by PECO) did not prevent ventilation from dropping back to baseline. It is worth mentioning, however, that some aspects of the used methodology should be taken into consideration before drawing any conclusions about the influence of muscle metaboreflex on exercise hyperpnea.

One such aspect is the PECO duration, which may have been too short to allow sufficient metabolites accumulation to drive a ventilatory response. Although the exercise intensity performed in these studies was high enough to produce significant levels intramuscular metabolite these will have been removed in part by the blood flow of dynamic exercise. During 2 to 3 minutes of PECO, metabolites would then increase but perhaps not to a level sufficient to activate ventilatory drive (Adreani et al., 1997, Light et al., 2008, Jankowski et al., 2013, Pollak et al., 2014). Allowing PECO to continue for a longer period of time may have revealed the true effect of muscle metaboreflex on ventilation. This is because the degree of muscle metaboreflex response increases as the local circulatory occlusion period increases (Scott et al., 2000).

The other limiting factor may be abolition of the discharge of some muscle afferents (group III and IV) that are sensitive to vascular distension during PECO (Haouzi et al., 1999). Although Haouzi et al. (2001) reported reductions in ventilatory responses during partial circulatory occlusion (venous occlusion) and total circulatory occlusion of exercising muscle,

they noted that during partial circulatory occlusion, the decrease in ventilatory response was slower compared to total circulatory occlusion. Moreover, both Eiken (1987) and Oelberg et al. (1998) reported small increases in minute ventilation as the circulatory occlusion of the exercised limb was partially occluded (below systolic blood pressure). This entertains the possibility that these specific muscle afferents may have exerted an effect on ventilation, which was in turn abolished by PECO.

A further point of discussion is whether the drop in ventilation to baseline during the PECO period an effect of the ventilatory baroreflex (Stewart et al., 2011). During the PECO period, when the muscle metaboreflex is activated and any central command influence is absent, blood pressure is sustained above baseline due to baroreflex resetting (see Raven et al., 2006 for review). This results in stimulation of the baroreceptors and activation of the ventilatory baroreflex, which may mask the effect of the muscle metaboreflex in mediating hyperpnea – thus, resulting in reduction in ventilation during PECO (Stewart et al., 2011).

In effect, the above studies either selectively blocked muscle afferents using fentanyl while central command was active (e.g. Amann et al., 2010, Gagnon et al., 2012, Olson et al., 2014), or used the PECO technique to activate the muscle metaboreflex in the absence of central command, indicating that muscle afferents have a limited effect on driving exercise hyperpnea on their own. However, it is possible that when combined with other interactions or synergistic inputs from either the central command or chemoreflex to the central respiratory neuronal pool, they may generate a much greater ventilatory response.

The main aim of the above studies (Wiley and Lind, 1971, Rowell et al., 1976, Haouzi et al., 1993, Scott et al., 2000, Haouzi et al., 2001, Fukuba et al., 2007) was to examine the contribution of the muscle afferent feedback in controlling exercise hyperpnea without a central command influence. Their findings showed that the muscle afferent (metaboreflex)

has a limited ability to drive ventilation on its own unless it is combined with another input into the respiratory control centre. Therefore, recent studies have used an improved method of investigation to effectively unmask the contribution of muscle afferent feedback on ventilation by enhancing the respiratory control centre for the input from muscle afferents. This is achieved by stimulating the ventilatory chemoreflex (as a substitute for the central command effect) to examine the contribution of muscle afferent on ventilation, and find out if there is a synergistic interaction between these inputs.

A study conducted by Lykidis et al. (2010) aimed to assess the cardiovascular and ventilatory responses to combined activation of the muscle metaboreflex (by PECO) and stimulation of the ventilatory chemoreflex (by inhaling a mild hypercapnic gas mixture). In their study, a mild hypercapnic gas mixture was induced using a dynamic end-tidal forcing system (EDF). Eleven healthy participants performed 2 minutes (40 % MVC) of an isometric handgrip exercise, followed by PECO. They found that, during inspiration of mild concurrent hypercapnia ($P_{ET}CO_2$ set at *7* mmHg, above resting values) combined with activation of the muscle metaboreflex (by PECO), the minute ventilation level was maintained significantly above baseline and this was observed only in the hypercapnic condition (not when breathing room air).

In another study, Bruce and White (2012) examined the cardiovascular and ventilatory responses to muscle metaboreflex (by PECO) and mechanoreflex (by sustained passive calf muscle stretching) activations combined with ventilatory chemoreflex stimulation (by inhaling a mild hypercapnic gas mixture). In this study, a mild hypercapnic gas mixture was induced by breathing from a Douglas bag (5% CO_2 gas mixture). Thirteen healthy participants were recruited to perform 1.5 minutes of isometric calf muscle exercise (at 50% MVC) with circulatory occlusion of the right leg. Following isometric exercise for 3.5 minutes and under a continuance circulatory occlusion, sustained passive calf muscle

stretching was applied for 3 minutes, and then rest. This protocol was conducted under both conditions; normal room air and hypercapnia ($P_{ET}CO_2$ set at 10 mmHg, above resting). Their results showed that during PECO period and only under hypercapnic conditions, minute ventilation was sustained above hypercapnic baseline. Moreover, under hypercapnic conditions only, the authors found that applying sustained passive calf muscle stretching during PECO period resulted in a further increase in minute ventilation above what was observed during PECO alone.

Both of these studies (Lykidis et al., 2010, Bruce and White, 2012) have successfully shown that activation of muscle afferents in combination with the ventilatory chemoreflex (by inhaling mild hypercapnic gas mixture) elevated ventilation, despite an appreciable pressor response during the PECO period. This implies an overpowering of the ventilatory baroreflex to reveal this contribution of muscle afferent on ventilation.

It is also worth mentioning here that the magnitude of minute ventilation elevation during circulatory occlusion in the Bruce and White study was higher (~ 7 $l.min^{-1}$) than in the Lykidis et al. study, in which it was ~ 3.5 $l.min^{-1}$. This may be attributed to two reasons: first, there may have been differences in the magnitude of minute ventilation increases due to differences in muscle mass recruitment in the Bruce and White (calf muscle) and the Lykidis et al. (forearm) studies. Second, the results may have been caused by a difference in the exercise intensity used in the Lykidis et al. (40 % MVC) and Bruce and White (50 % MVC) studies, which may have played a significant role in triggering muscle afferent discharge (Imms and Mehta, 1989, Fisher and White, 2004).

It is well documented that COPD patients and CHF patients have severe exercise intolerance and dyspnoea in response to low exercise intensity; this is due to early muscle acidosis during exercise, which results in abnormal early activation of muscle afferents (Mancini et al., 1992,

Serres et al., 1998, Grieve et al., 1999). Bruce et al. (2016) recently investigated the effect of muscle metaboreflex activation on the ventilatory response in COPD patients. They found that during PECO following rhythmic isometric handgrip exercise, ventilation dropped slightly but was maintained significantly above baseline in the patient group but not in the healthy control group. They concluded that muscle afferents play an important role in controlling ventilation in COPD patients. Moreover, Machado et al. (2017) assessed the ventilatory response to combined activation of muscle metaboreflex and peripheral chemoreflex in CHF patients. Their results showed that during activation of muscle metaboreflex (by PECO) under hypoxic conditions, ventilation was significantly increased compared to normoxia. The authors concluded that activation of the muscle metaboreflex during PECO enhances the peripheral chemoreflex to regulate ventilation.

Silva et al. (2017) recently assessed, in healthy volunteers, the ventilatory response to combined activation of muscle mechanoreflex and peripheral chemoreflex. They reported that during passive knee flexion and extension of the non-dominant leg under hypoxia, the ventilatory response was increased significantly compared to resting. Conversely, the ventilatory response during passive movement under hyperoxia was decreased and this decrease was similar to resting. It was, therefore, suggested that interaction between activation of the muscle mechanoreflex and peripheral chemoreflex was what resulted in regulation of ventilation.

These recent studies clearly demonstrate that muscle afferent feedback is able to drive exercise hyperpnea in the presence of other inputs to ventilatory control. They also suggest that potential synergy or interaction between inputs from muscle afferent feedback and the ventilatory chemoreflex (central and/or peripheral chemoreceptors) into the central respiratory neural pool may drive ventilation during exercise.

Bruce and White (2015) extended these findings in an attempt to explain the nature of the interaction between the muscle afferents and ventilatory chemoreflex. It is well demonstrated from the previous study (Lykidis et al. 2010, Bruce and White, 2012) that activation of muscle metaboreflex (by PECO) and mechanoreflex (by passive muscle stretching) in combination with ventilatory chemoreflex stimulation (by inhaling mild hypercapnic gas mixture), resulted in sustained in minute ventilation above hypercapnic baseline. Bruce and White (2015) aimed to investigate, whether the ventilatory responses response seeing during PECO was due to sensitization of respiratory control centre caused by acute hypercapnia to enhance inputs from muscle afferent, or whether it was due to muscle acidosis caused by exposure to acute hypercapnia and exacerbates exercise. Ten healthy participants were recruited in this study to performed 2 trials (local trial and systemic trials). In the local trial, participants were exposed to systemic acute hypercapnia (for 5 minutes) followed by circulatory occlusion of the calf muscle, and then exposed to room air. While, in the systemic trial, local circulatory occlusion for the calf muscle was applied under room air condition firstly, and then participants were exposed to systemic hypercapnia. The authors' results showed that during local trials and following 50 % MVC isometric calf muscle exercise, activation of muscle metaboreflex by PECO did not prevent minute ventilation to drop back to baseline. However, in the systematic trial, activation of muscle metaboreflex by PECO resulted in a sustained increase in minute ventilation above hypercapnic baseline. These finding suggests central interaction between muscle afferent feedback and ventilatory chemoreflex feedback (central and/or peripheral chemoreceptors). A further investigation was carried out by the authors to determine the main driver for this interaction (central or peripheral chemoreceptors?). The authors reported that brief exposure to hyperoxic (95 % O_2) and hypercapnic (5% CO_2) condition, in order to block carotid chemoreceptors, resulted in no change in minute ventilation response during PECO period. This follow up finding suggest

that peripheral chemoreceptors had little to no involvement, while central chemoreflex is the main driver for this synergistic interaction.

The findings form Bruce and White's (2015) study further strengthened the view that sustained minute ventilation and cardiovascular response during PECO may be caused by excessive acidosis of the exercising muscle after exposure to acute hypercapnia. This is because early animal studies have reported that increases in ventilation (Rotto et al., 1989) and cardiovascular responses (Rotto and Kaufman 1988, Rotto et al., 1989) to exercise were linked to muscle acidosis. However, local hypercapnia of exercised muscle did not prevent ventilation from recovery to baseline during PECO in Bruce and White's study.

To summarise, the contribution of muscle afferent feedback in controlling ventilatory response to exercise was a controversial subjects. Recently, Amman and colleagues (2010) eliminate this doubt by selectively blocking these afferents with Fentanyl, which showed significant attenuation in the ventilatory and cardiovascular responses to dynamic exercise. Findings from Amman et al. study revived interest in re-investigating the role of muscle afferent feedback in controlling exercise hyperpnea. The preliminary evidence in this area (Wiley and Lind, 1971, Rowell et al., 1976, Innes et al., 1992, Haouzi et al., 1993, Haouzi et al., 2001, Fukuba et al., 2007) goes against muscle afferent feedback, as activation of muscle afferents (e.g. by PECO) appear to make no significant contribution to controlling exercise hyperpnea, at least under breathing room air. However, more recent investigations revealed the opposite, whereby hypercapnic or hypoxic gas mixture was used to stimulate the ventilatory chemoreflex in combination with muscle afferent activation (Lykidis et al., 2010, Bruce and White, 2012, Bruce and White, 2015, Machado et al., 2017, Silva et al., 2017). This thesis will continue the work previously carried out in this laboratory on cardiovascular and ventilatory responses to combined activations of muscle metaboreflex (by PECO) and ventilatory chemoreflex (acute hypercapnia), with an aim to provide more comprehensive

answers to the questions posed by recent studies (Lykidis et al., 2010, Bruce and White, 2012, 2015): i.e. what is the relationship between the level of hypercapnia and the magnitude of the additional ventilation produced in response to a standardised level of muscle afferent activation? (Chapter 3).

1.7 Effect of exercise training on muscle afferent feedback

Muscle afferent feedback is an important mechanism controlling cardiovascular, and now it appears, ventilatory responses during dynamic or static exercise in the presence of other synergic interaction input (e.g. ventialtory chemoreflex) (Bull et al., 1989, Gladwell et al., 2005, Fisher et al., 2005, Lykidis et al., 2010, Bruce and White, 2012).

Exercise muscle training has been shown to be a useful method for altering muscle afferent activity (Mostoufi-Moab et al., 1998); specific forms of training can induce changes in skeletal muscle by improving oxidative capacity and reducing muscle acidosis during exercise, which, in turn, attenuates activation of the muscle metaboreflex and results in reduction of increased exercise pressor reflex and, also likely, the ventilatory response to exercise (Bruce & White, 2016).

Several studies conducted on whole body training reported that exercise had little influence on the autonomic nervous system during exercise or rest. One of these studies, by Seals (1991), reported no notable difference in MSNA and cardiovascular responses to three types of mild physical stress between untrained participants and highly trained endurance athletes. Moreover, Saito et al. (1993) found no significant differences in MSNA and cardiovascular responses to static and dynamic exercises between the dominant and non-dominant forearms of racket sports players. Saito's (1993) findings are somewhat surprising, as one would expect that the metabolic profile (Saltin et al., 1976), motor unit recruitment and discharge pattern (Sale, 1987) of the non-dominant and dominant limbs would affect or delay metabolite accumulation. This, in turn, would attenuate activation of the muscle metaboreflex in the dominant limb. However, it is possible that occluding intramuscular pressure differences in the large trained and smaller untrained forearms may have masked the training-induced differences between the arms.

Longitudinal studies, on the other hand, have demonstrated that local muscle training-induced alterations in muscle afferent activity were associated with changes in MSNA and cardiovascular response to exercise. Sinoway et al. (1989a) reported decreases in the MSNA response to exercise after training the forearm and attributed this to attenuation of muscle metaboreflex activation. Similarly, Somers et al. (1992) demonstrated that 6 weeks of unilateral endurance training of the handgrip in healthy humans resulted in an attenuated MSNA response during PECO. They concluded that this attenuation of MSNA response was due to a decrease in muscle metaboreflex stimulation. Moreover, Mostoufi-Moab et al. (1998) reported that 4 weeks of non-dominant handgrip training resulted in significant decreases in mean arterial blood pressure, venous lactate and pH responses during ischaemic dynamic handgrip exercise, compared to pre-training values. The authors suggested that the reduction in these metabolic parameters resulted from attenuation of muscle metaboreflex activation.

Carrington et al. (1999) also found a significant attenuation of the blood pressure response to electrically evoked isometric calf muscle exercise in sprinters specialising in 400 m runs, compared to 100 and 200 m sprinters. Carrington and colleagues suggested that long-term exposure to muscle acidosis during sprinting over distances longer than 400 m could cause desensitisation in muscle afferent feedback, resulting in attenuated pressor response.

Moreover, in their longitudinal study, Fisher and White (1999) assessed the cardiovascular responses to voluntary and electrically evoked isometric exercise prior to and following 6 weeks of local muscle training of the dominant leg (calf raise). Their results showed that following exercise there was a reduction in the magnitude of diastolic blood pressure increases as a response to voluntary and electrically evoked isometric exercise at 30 % MVC (drop by a mean of 28 % and 27 %, respectively) and during PECO periods in the trained leg. In the contralateral untrained leg, and following exercise training of the trained leg, there was

reduction in the increase of diastolic blood pressure (by 24 %) during voluntary isometric exercise, but no change during electrically evoked exercise. Moreover, there were no changes in the diastolic blood pressure response of the untrained leg during PECO periods in both exercise conditions. The authors concluded that local muscle training induced attenuation of muscle afferent feedback, evidenced by a reduction in magnitude of the increased diastolic blood pressure response during PECO periods under both exercise condition, in the trained leg only. Moreover, they suggested a cross-over effect of exercise training from the trained to the contralateral untrained leg, revealed by a decrease in the pressor response to voluntary muscle contraction in the untrained limb. This indicates adaptation of central command.

Fisher and White's study demonstrated the effect of local muscle exercise training on altering muscle afferent feedback, which resulted in attenuation of the cardiovascular response to isometric exercise. Based on the above literature, it may therefore be expected that such training would also attenuate the ventilatory response to activation of the muscle metaboreflex, in combination with chemoreflex activation, by exposure to mild hypercapnia. This will be investigated in Chapter 5 of this thesis.

1.8 Proposed studies in human participants

The following studies, and their aims, are proposed for this thesis.

To assess:

1. CO_2 sensitivity of the ventilatory response to muscle metaboreflex stimulation in humans.
   - Aims:

     Examine the relationship between the level of systemic hypercapnia and the magnitude of the additional hyperpnea produced in response to a standardised level of muscle afferent activation.

2. The ventilatory response to muscle metaboreflex activation during concurrent hypercapnia after 6 weeks of dynamic training of the calf muscles.
   - Aims:

     A) Investigate the effect of attenuation in muscle afferent feedback, following an exercise training programme, on the ventilatory and cardiovascular responses to muscle metaboreflex activation (by PECO) combined with stimulation of ventilatory chemoreflex (by inhaling mild hypercapnic gas).

     B) Evaluate the cross-over effect of exercise training on the contralateral untrained leg during leg isometric exercise.

     C) If the cross-over is present, determine the level of the cross-over effect of exercise training, if occurring at the segmental (spinal cord) or higher central level.

CHAPTER 2: GENERAL METHODS

2.1 Dynamic end-tidal forcing system

The DEF system was developed by Robbins and colleagues (1982) at the University Laboratory of Physiology at the University of Oxford. This system operates based upon the principle that, in healthy young individuals, the alveolar partial pressure of O_2 (P_AO_2) and CO_2 (P_ACO_2) is almost the same as that of the arterial partial pressure of these gases. Sampling of P_AO_2 and P_ACO_2 with gas anaylsis of the end-tidal air therefore provides a good index of arterial partial pressure in the population studies in this thesis.

The DEF system was used in all studies here to manipulate the partial pressure of inspired O_2 and CO_2 (P_IO_2 and P_ICO_2, respectively) on a breath-by-breath basis, so as to induce hypercapnia. This was achieved by maintaining the $P_{ET}O_2$ at normal levels (normoxia), while increasing $P_{ET}CO_2$ levels to hypercapnia.

2.1.1 The control of end-tidal forcing gases.

Hypercapnia was induced using an open circuit system via a DEF system (Figure 2.1) to control the $P_{ET}O_2$ and $P_{ET}CO_2$ by manipulating the inspired gases. Participants sat in the isometric dynamometer, or chair, breathing through a mouthpiece while their nose was occluded to ensure breathing only by mouth.

The DEF system contains apparatus required to record the $P_{ET}O_2$ and $P_{ET}CO_2$, and deliverer the desired P_IO_2 and P_ICO_2. A personal laptop computer was used to control the DEF system using dedicated software (BreatheDP; University of Oxford, UK) (Figure 2.2). In order to induce hypercapnia a desired level of $P_{ET}O_2$ and $P_{ET}CO_2$ was set in the software. Then the controlling computer calculated the required P_IO_2 and P_ICO_2 to achieve the desired end tidal partial pressure of gases. The inspired gas mixture was delivered by a fast gas mixing system,

where the O_2, CO_2, N_2 and air were mixed and delivered to the participant for inspiration. All the respiratory variables were sampled by the controlling computer every 10ms.

Participants were connected to the apparatus via a mouthpiece including a saliva trap with their nose occluded. The mouthpiece was attached to a calibrated bidirectional turbine (Cardiokinetics Ltd, UK), measuring the volume and the direction of respiratory flow (inspiration and expiration) (calibration details of the turbine: section 2.1.3, page: 72). The turbine is a plastic tube containing an impeller, which rotates when the air moves through it during breathing and, thereby, detects flow direction and speed. The information collected by the impeller is read by a photodetector device (VMM-400, Interface Associates, Irvine, CA, USA) surrounding the turbine. A photodetector converts this information to inspiratory and expiratory volumes, measuring each revolution of the impeller as 2 mL and presents them in BreathDP software, with response time less than 30ms.

From a port between mouthpiece and turbine, tidal gases were sampled continuously via a capillary tube connection to the O_2 and CO_2 gas analysers (MOXAR Respiratory System, AEI Technologies, Pittsburgh, PA, USA) (Figure 2.3). The response time for the O_2 gas analyser is less than 100ms, while for the CO_2 gas analyser it less than 25ms. The output readings from the O_2 and CO_2 gas analysers are continuously updated and displayed on a computer using the BreatheDP software. The software continuously measures the $P_{ET}O_2$ and $P_{ET}CO_2$ values on a breath-by-breath basis and compares them with the desired value of $P_{ET}O_2$ and $P_{ET}CO_2$. In cases where $P_{ET}O_2$ and $P_{ET}CO_2$ deviated from the desired value, the P_IO_2 and P_ICO_2 were manipulated to correct this deviation. This was achieved using an integral proportional feedback control scheme, where inspired partial pressure of the gases was adjusted according to the deviation of the actual value from the desired value.

The mouthpiece assembly (figure 2.4) was connected via a T piece arrangement with the outlet arm allowing exhaust to room air and the other inlet arm connected to the mixing chamber and inflow of the inspired gas mixture, without forced pressure from the mixing chamber. The participants breathed on demand (Figure 2.5).

In order to deliver the required inspired gas to the participant, the controlling computer sends a signal to two control boxes (MKS Instruments PR 4000 F, Germany). Each of these two control boxes is responsible for controlling two of four gas channels: O_2, CO_2, N_2 and compressed medical grade air. These control boxes used mass flow controllers (MKS Instruments 1559A, Germany) to precisely measure and control the mass flow rate of the gases that delivered. Before, the inspired gas mixture is supplied to the participant, it passes through a set of humidifiers (Humidity control sets at 2: normal), (Fisher and Paykel Healthcare HC150, Auckland, New Zealand) in order to make it suitable for breathing.

In this thesis, hypercapnia was induced in all studies by using the DEF system. During hypercapnia condition, the $P_{ET}O_2$ was maintained at 100 mmHg (normoxia), while the $P_{ET}CO_2$ was increased by raising the partial pressure of inspired CO_2 to achieve the desired level of hypercapnia (e.g. $P_{ET}CO_2$ clamped at +10 mmHg, above resting value). Moreover, DEF system was used during trails that conducted under room air condition. During room air condition, the system supplies the participant with compressed medical-grade air. In the control trial, the $P_{ET}O_2$ and $P_{ET}CO_2$ were measured continuously on a breath-by-breath basis throughout the trial, without any manipulation of the inspired gases.

Figure 2.1: Dynamic end-tidal forcing system: A) Computer, B) Control boxes, C) Mass flow controllers, D) Humidifiers, E) Pulse oximeter, F) ECG machine, G) Gas analysers, I) Mouthpiece apparatus.

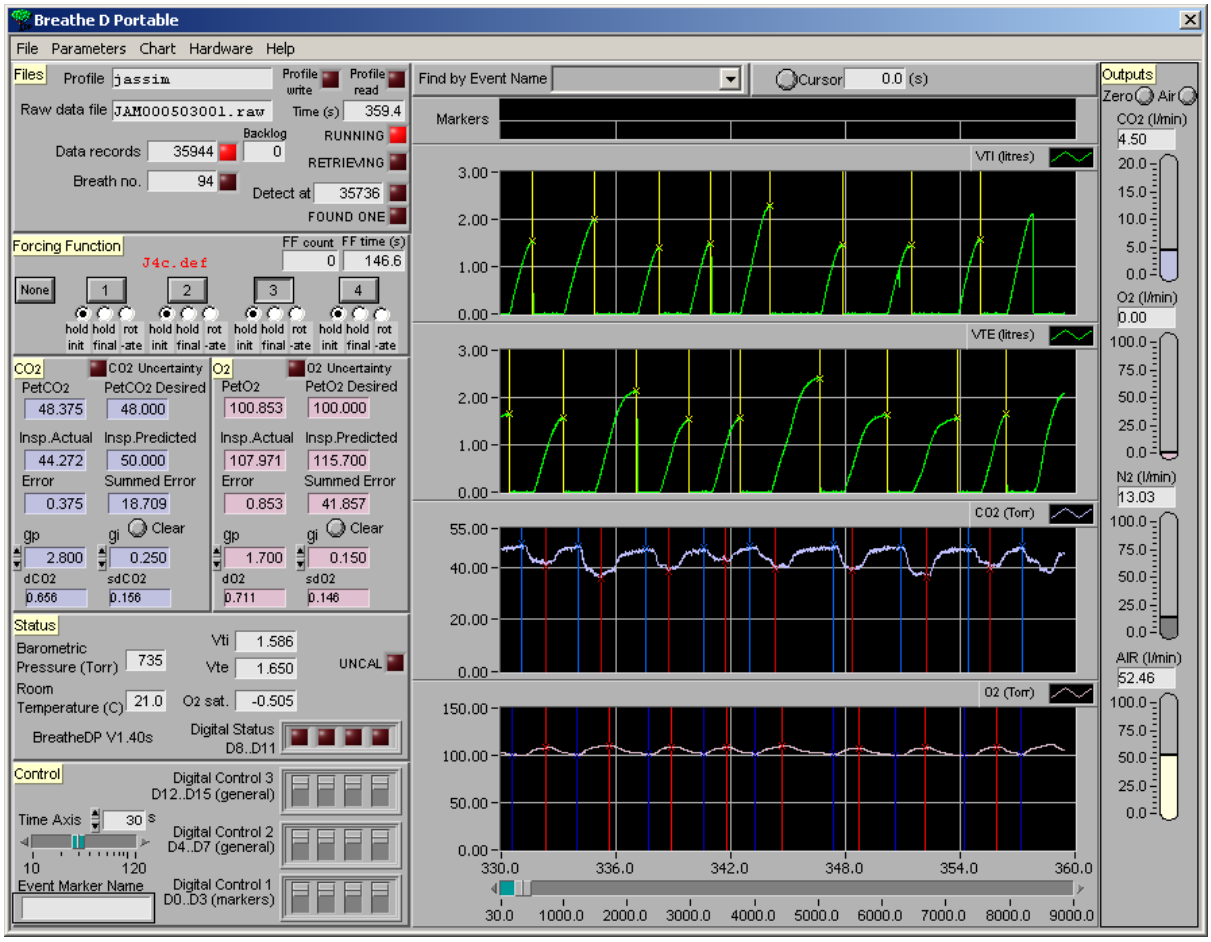


Figure 2.2: BreatheDP software; original recording of the ventilatory response during a leg trial under hypercapnic condition.

Figure 2.3: Gas analysers; A) Oxygen analyser, B) Carbon dioxide analyser.

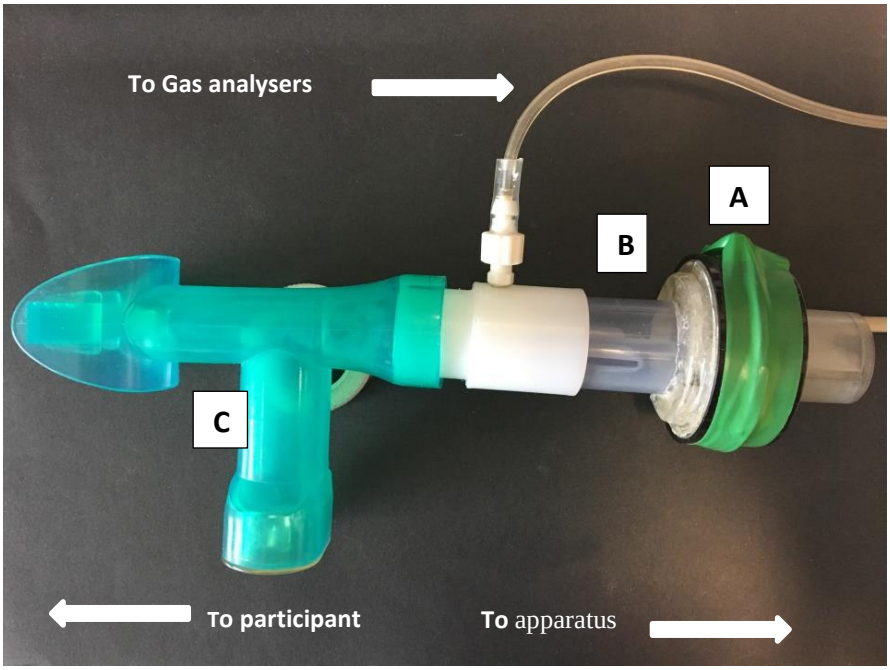


Figure 2.4 Mouthpiece assembly: A) Photodetector device, B) Turbine, C) Saliva trap.

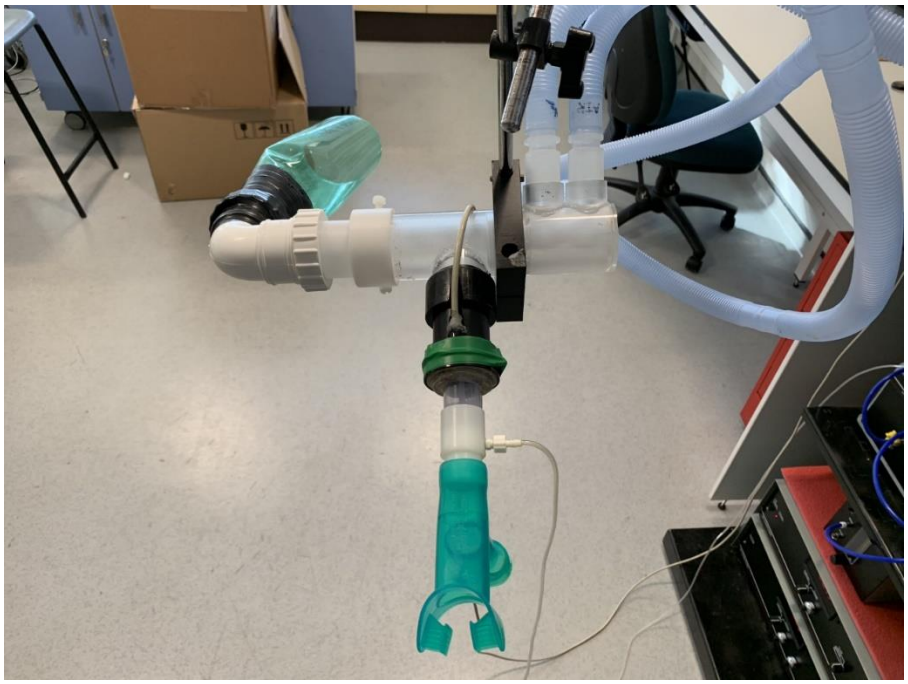


Figure 2.5: T piece arrangement.

2.1.2 Safety management.

Prior to starting the experiment, each participant was connected to electrocardiogram (ECG) leads to monitor their heart rate continuously throughout the experiment. Parallel with the ECG, the O_2 saturation in the arterial blood was monitored non-invasively, using a pulse oximeter (Datex-Ohmeda 3900; General Electrics). Moreover, every participant was asked to immediately report to the investigator any sign of discomfort during exposure to hypercapnia (e.g. dizziness and light headache).

2.1.3 Calibration of the DEF system.

A calibration of the DEF system was performed on each testing day. The calibration of the system is divided into 3 stages. Firstly, an environmental calibration for the system was performed by collecting the barometric pressure and temperature of the laboratory from a Fortin Barometer (Russell Scientific Instruments, Dereham, UK).

Following the environmental calibration, calibration of the turbine was performed. The daily calibration of the turbine was by connecting it to an automated 1L calibration syringe (Series 5540, Hans Rudoph,Inc, Kansas, US). The automated calibration syringe pumped 10 times through the turbine, and the turbine recorded the input volume and displayed readings on the BreathePD software. The output volumes from the turbine were compared against the known volume of the calibration syringe, to confirm the calibration.

Previously, the validity of the turbine measurement was examined across a range of volumes by using a larger graduated 3L calibration syringe (Series 5530, Hans Rudoph, Inc, Kansas, US). The turbine was connected to the 3L calibration syringe, and tested for 3 volumes (1, 2, and 3L, respectively). Each volume was tested 3 times, and during each time 10 pumps were performed. Following testing, an average of the turbine measurement during 3 times for each

volume was calculated and presented in graph. Figure 2.6 showed there is linear relationship between the volumes of the calibration syringe inputs and turbine measurement outputs.

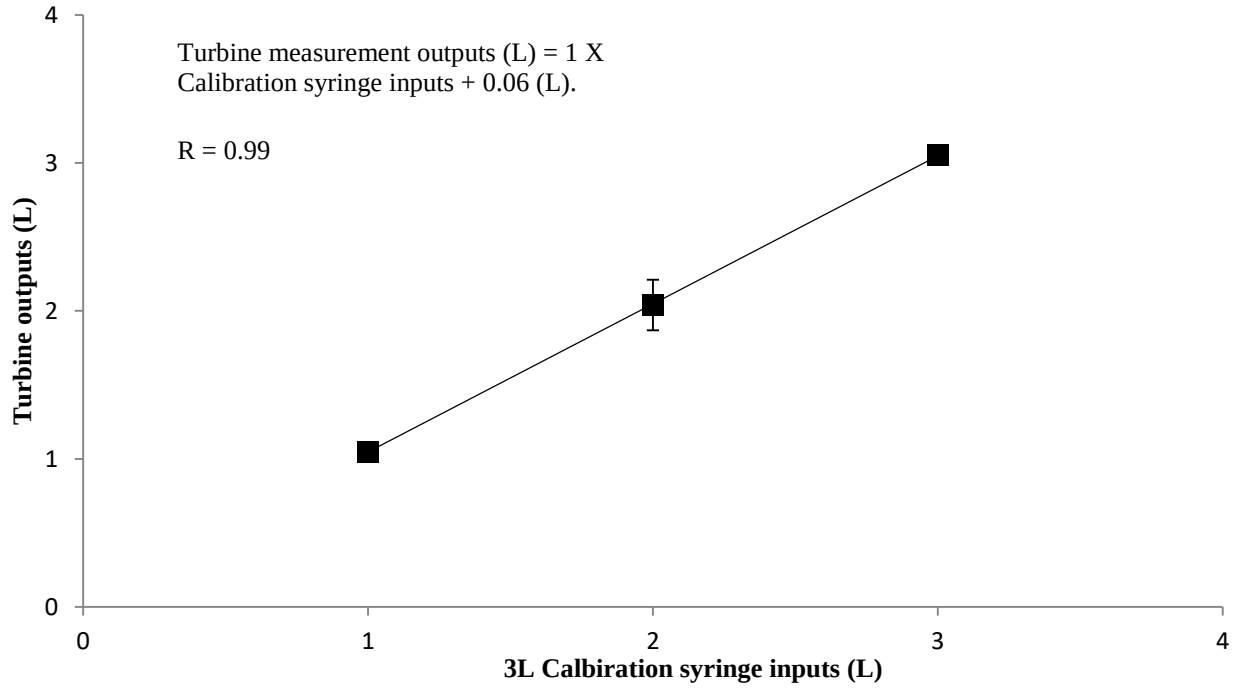


Figure 2.6: Relationship between the volume of 3L calibrated syringe inputs and turbine measurement outputs (means ± SD).

The third stage of calibrating the DEF system is calibrating the CO_2 and O_2 gas analysers. The daily calibration of the gas analysers were performed by using room (CO_2:0.03% and O_2: 20.93%) and certificated gases (CO_2: 5%; O2 15.05% in air; BOC gases, Guildford, UK). Calibration of the gas analysers was also checked for linearity and baseline drift after every hypercapnic trial.

Previously the CO_2 and O_2 gas analysers were examined across their operating ranges separately to test their validity. The CO_2 gas analyser was examined against 5 levels of CO_2 concentration (at Air: 0.03%, 2.01%, 4.06%, 5.06% and 7.04%). This sequence of calibration gases was delivered to the analyser 5 times. The reading was taken directly from the CO_2 gas analyser screen after 3 seconds of exposure to each gas. The average of 5 readings was

calculated for each level of CO_2, and are presented in figure 2.7. Figure 2.7 reveals there is a linear relationship between concentration levels of CO_2 (inputs) and the CO_2 gas analyser measurement (outputs).

With regards to the O_2 gas analyser, 3 levels of O_2 concentration (in gas mixture: 15.04%, 17.99% and in room air: 20.93%) were used to determine its validity. Each level of O_2 concentration was fed in sequence into the O_2 gas analyser 5 times. Readings were taken from the O_2 gas analyser screen, after 3 seconds from starting the gas. Then the average of 5 readings was calculated. Figure 2.8 shows there is a linear relationship between concentration levels of O_2 (inputs) and the O_2 gas analyser measurement (outputs).

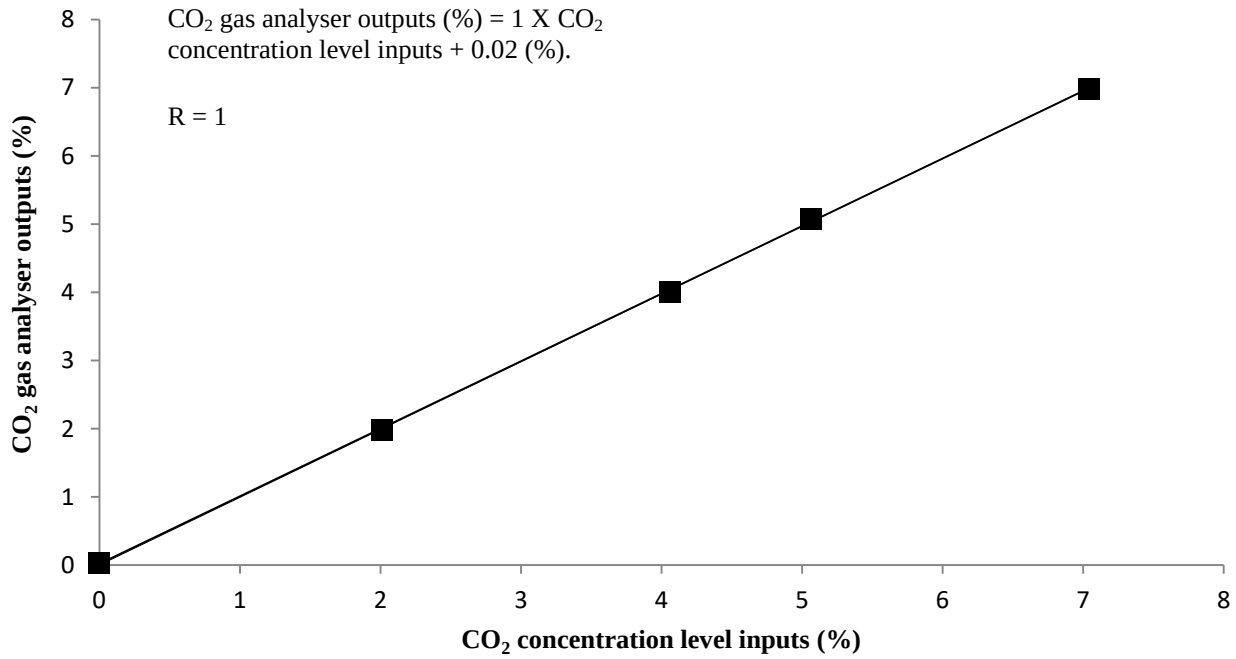


Figure 2.7: Relationship between the CO_2 concentration levels inputs and the CO_2 gas analyser measurement outputs (means ± SD).

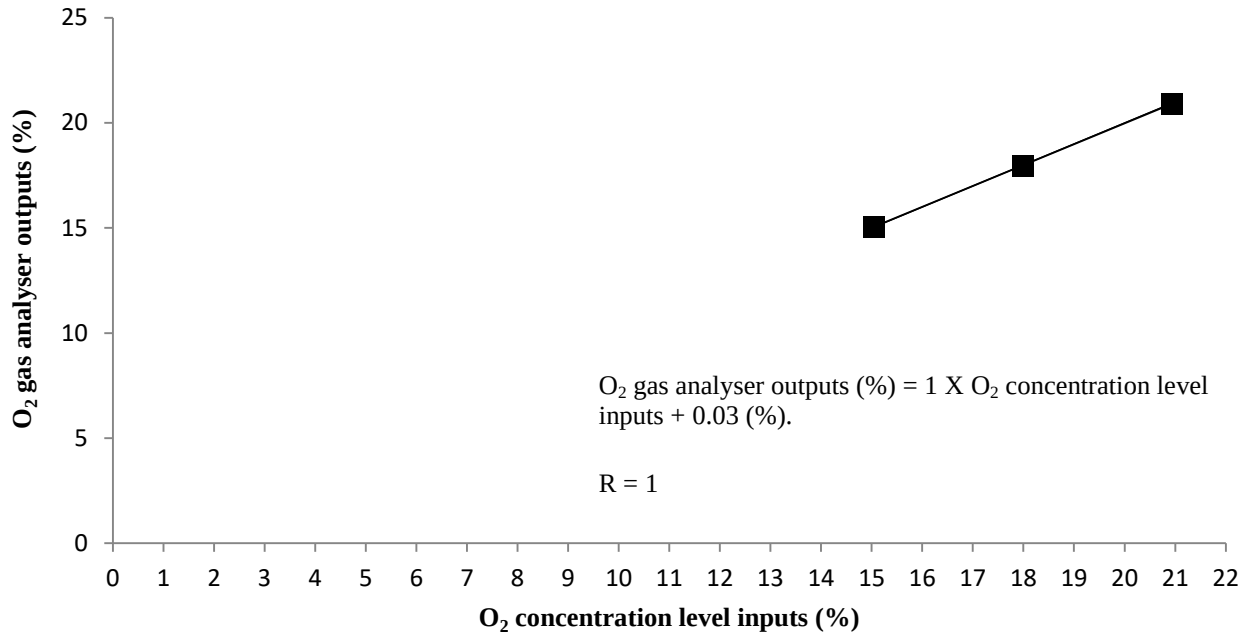


Figure 2.8: Relationship between the O_2 concentration levels inputs and the O_2 gas analyser measurement outputs (means ± SD).

2.1.4 Measuring normal $P_{ET}O_2$ and $P_{ET}CO_2$ using the DEF system.

On the day of testing, a normal level of $P_{ET}CO_2$ was measured for each participant. This was performed by asking the participant to rest, sitting down, for 15 minutes and breathe through the mouthpiece only, while wearing a nose-clip. In order to distract participants from their breathing during this period, a television was positioned at eye level playing documentary programme (Planet earth, BBC, UK). Normal levels of $P_{ET}O_2$ and $P_{ET}CO_2$ were determined in the last 5 minutes of this period, provided that a stable profile of gases was achieved. These normal levels were used later on as a baseline from which to induce hypercapnia at the desired level.

2.2 Experimental procedures

2.2.1 Leg isometric dynamometer

The leg isometric dynamometer was used in Chapters 3, 4 and 5 (only in the leg protocol). Participants were seated in the calibrated isometric dynamometer, designed to measure ankle plantar flexor force (Davies et al., 1982). The leg was fixed with the thigh horizontal and the knee and ankle set at 85 degrees, using a goniometer. Adjustments were made using wooden footplates (Figure 2.10). A shaped plate was then tightened onto the leg and this connected to a steel bar, which was instrumented with strain gauges to transduce force.

MVC of the ankle plantar flexors was recorded before each trial. The participants were asked to perform ankle plantar flexion by pushing against the force transducer as hard as possible, to then hold that force for 3 seconds and finally relax for 1 minute, before starting again. 5 MVC attempts were performed and accepted for experimentation, provided that they did not differ from one another by more than 10 %. The highest measure from these attempts was recorded as the MVC. Then, for the exercise phase of each experimental trial, 50 % of the MVC was calculated and used for all leg protocols. The rationale for this is to achieve the greatest possible activation of muscle metaboreflex during the PECO period.

In each trial, a second computer screen was placed at participant eye level, which displayed the target force (50 % MVC) to be achieved during the exercise period.

Calibration of leg dynamometer

The leg dynamometer was calibrated on the day of the testing. The calibration was conducted after 30 minutes of switching on the amplifier in order to allow the amplifier to warm up and to prevent drift. The calibration of the leg dynamometer was performed by using 10:1 lever system attached above the transducer of the leg dynamometer. Known weights (5, 10, 15 and 20 Kg) were used for calibration. These weights were hung onto the 10:1 lever system giving

weights of 50, 100,150 and 200Kg. Figure 2.9 shows that there is a linear relationship between the known weight input and the leg dynamometer measurement outputs.

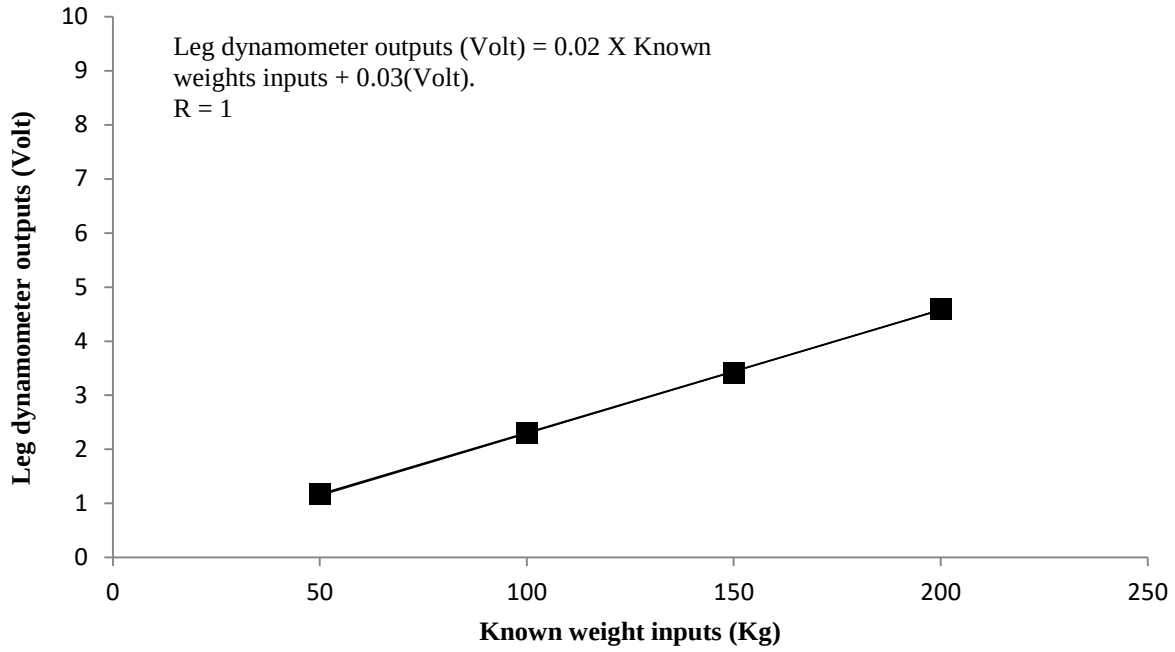


Figure 2.9: Relationship between the known weight inputs and the leg dynamometer measurement outputs (means ± SD).

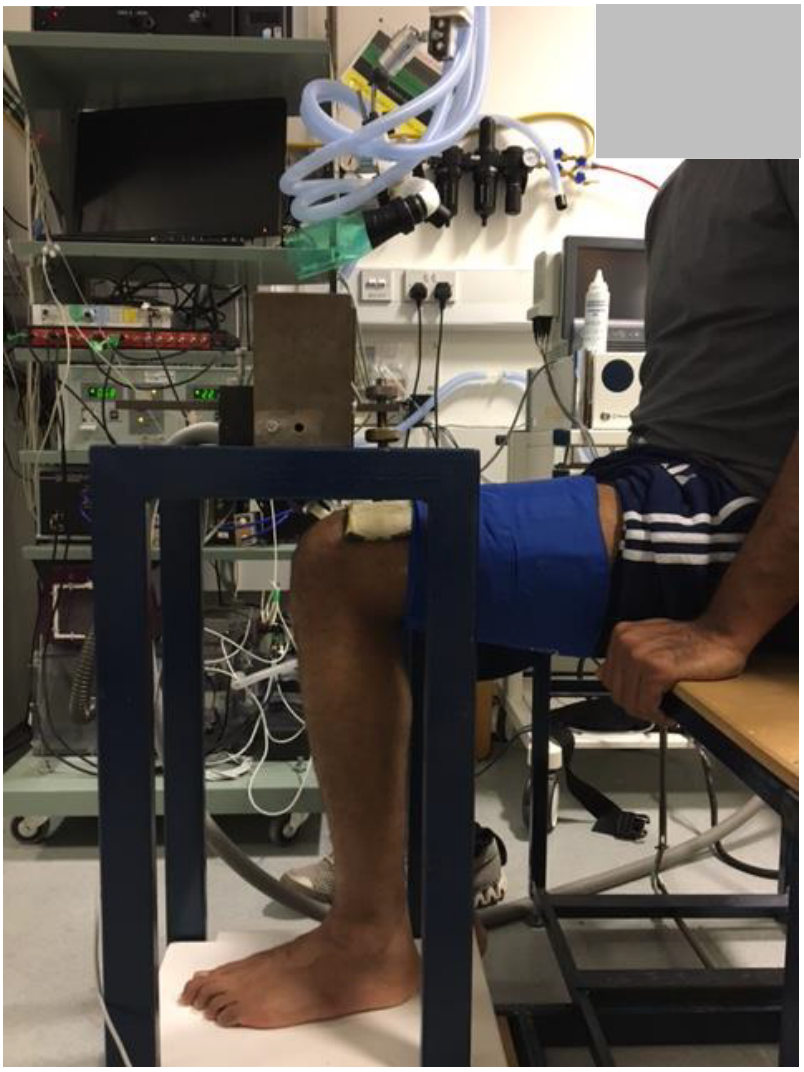


Figure 2.10: Participant in the leg isometric dynamometer.

2.2.2 Handgrip dynamometer.

A calibrated custom-made handgrip dynamometer was used in Chapter 5 (hand protocols) (Figure 2.12). The dynamometer was clamped onto the table surface to prevent it from moving during the isometric handgrip exercise. Participants were seated in an upright position and the dynamometer was held, horizontally, by the exercising arm. MVC was measured before each hand trial. Participants were asked to hold the handgrip dynamometer and squeeze as hard as possible for 3 seconds, then relax for 1 minute before starting again. 5 MVC attempts were carried out and, as mentioned, a 10 % margin of error was accepted. The

highest measured value was the recorded MVC. In Chapter 5, isometric handgrip exercise was performed in two different levels (30 % and 50 % MVC), in separate trials and on separate occasions. For this reason, the desired % of MVC was calculated from the highest pre-trial MVC. During the trial, a second computer screen was placed in front of the participant at eye-level, displaying the desired target force needed for the exercise period.

All studies in this thesis were conducted in the School of Sport, Exercise and Rehabilitation Sciences, laboratory 208, where the ambient temperature was set to 21 °C.

Calibration of hand dynamometer

The hand dynamometer was calibrated on the testing day. Calibration was conducted after 30 minutes of worming up the amplifier. Known weights (5, 10, 15 and 20Kg) were used for calibration. These weights were hung onto the hand dynamometer using the G clamp. Figure 2.11 reveals that there is a linear relationship between known weights inputs and hand dynamometer measurement outputs.

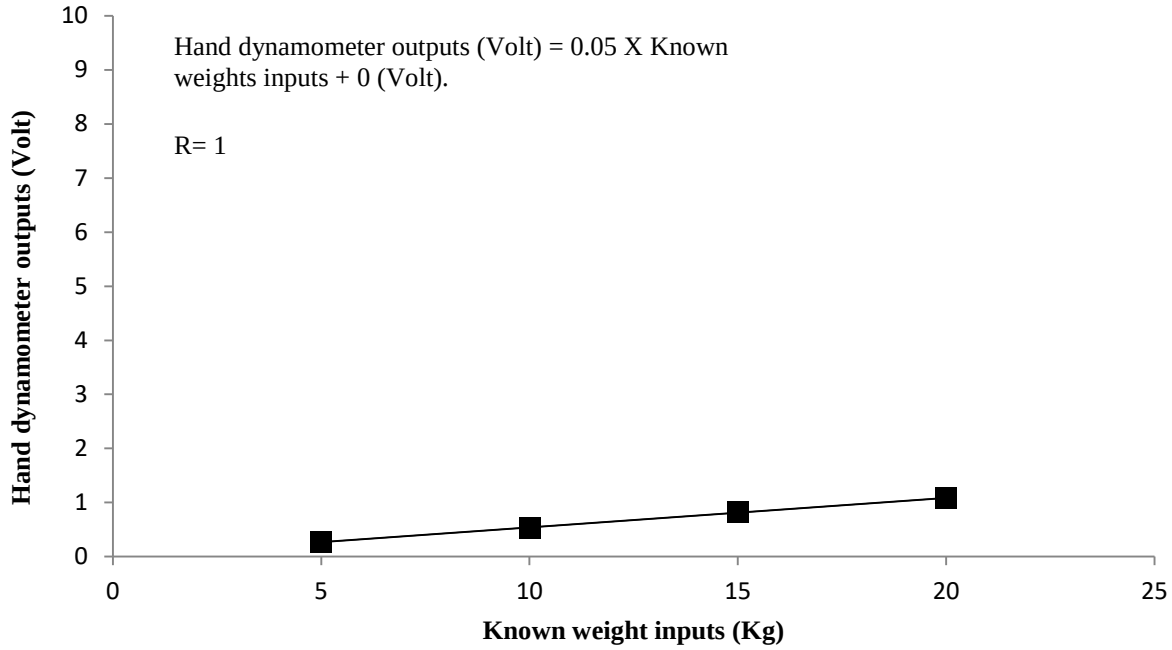


Figure 2.11: Relationship between the known weight inputs and the hand dynamometer measurement outputs (means ± SD).

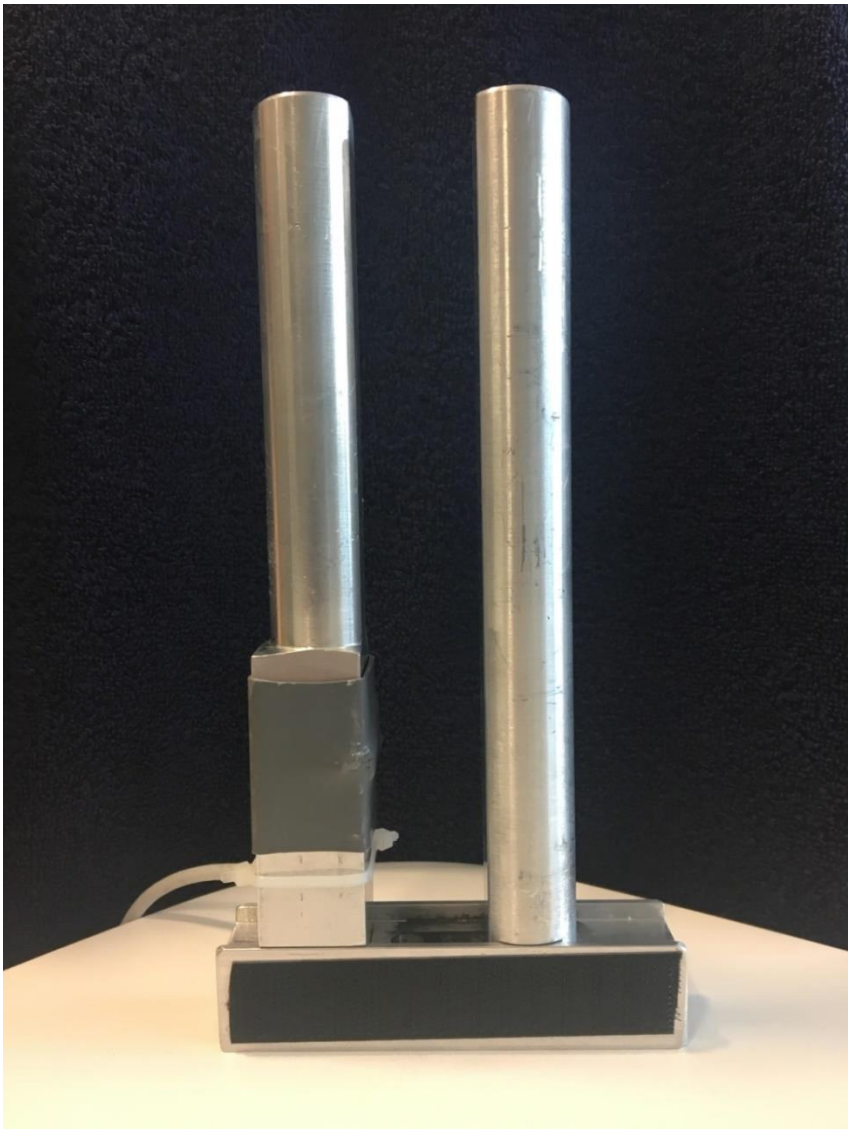


Figure 2.12: Custom-made handgrip dynamometer

2.2.3 Post-exercise circulatory occlusion (PECO)

PECO was performed for all trials in this thesis. PECO was achieved by placing a cuff around the thigh of the exercised leg (leg trials) or around the upper arm of the exercised forearm (hand trials). The cuff was inflated rapidly to 200 mmHg using a rapid cuff inflator system (E20; Hokanson, Bellevue, WA, USA) 15 seconds before cession of the exercise and was released 3 minutes later.

2.2.4 Exercise training programme

In the study described in Chapter 5, all participants underwent an exercise training programme for 6 weeks. Participants were asked to visit the laboratory for supervised exercise training 3 days (Monday, Wednesday and Friday) per week. In these training session with the participants in the standing position, they were asked to perform, with their dominant leg, 4 sets of 30 calf raises using just their body weight at the beginning. The rate of calf raises were 1 lift and 1 lower every 2 seconds, with 1 minute rest between sets. Participants followed a constant rhythm set by a digital metronome at a rate of 60 beats.min^{-1}. When the participants failed to sustain the maximum heel rise (determined by placing a small ball above participants' heads as a target, at their maximum lift height), the exercise was stopped and the number of repetitions and sets were documented.

Once the participants completed 120 repetitions successfully, they exercised by carrying a load inside a rucksack weighing 10 % of their body mass. This load was further increased by 5 % in subsequent training sessions, each time participants successfully completed the 4 sets of 30 heel raises.

2.3 Cardiovascular and ventilatory measures.

A Finapres cuff was used to measure blood pressure non-invasively, by the plethysmographic method of the unloaded arterial wall (Portapress; Finapres Medical Systems, Amsterdam, The Netherlands), whereby a small cuff inflator was wrapped around the middle finger of the non-exercising hand and placed on a support fixed at heart level. Finapres provides accurate estimation of the changes in intra-arterial pressure over short periods during experiments, especially in studies on exercise (Parati et al., 1989, Friedman et al., 1990). Heart rate (HR) was recorded continuously by measuring the R_R interval using a three-lead ECG

(Cardiorator CR7; Cardiac Records Ltd, London, UK) in the leads II position. The three leads were placed over the skin**,** one on the lower left rib and the other two on the left and right collarbones. The force output from the examined muscle was measured by isometric dynamometer. The force was transduced by strain gauges bonded to the steel bar (RS components foil type polyesterencapsulated). These strain gauges shaped half of the Wheatstone bridge circuit, while the other half was combined with the bridge balancing circuit. The output from the bridge was amplified and sampled by the computer after AD conversion. With regards to the hand isometric dynamometer, the force output was measured by squeezing the bars of the dynamometer and the same strain guage transduction process as the leg rig generated a voltage output recorded by the computer after AD conversion.

All cardiovascular response outputs from the Finapres and the ECG as well as force outputs, were converted from analog to digital using a Cambridge Electrical Design 1401 plus converter (Cambridge Electrical Design, Cambridge, UK), at a sampling frequency of 1250 Hz. The outputs readings of the mean blood pressure (MAP), heart rate (HR) and force were displayed and recorded on computer using Spike 2 software (CED, Cambridge, UK). In all studies, cardiovascular responses were measured beat by beat, reporting the average values for each 15 second period from the start of the baseline period to the end of the trial.

The respiratory volumes were recorded continuously every minute from the baseline to the end of the trial using the DEF system. During each trial, participants were connected to the mouthpiece while wearing a nose-clip. Tidal gases and the partial pressure of O_2 and CO_2 were sampled by the O_2 and CO_2 gas analysers at sample flow rate 10-500 $ml.min^{-1}$. The output readings from the O_2 and CO_2 gas analysers were transmitted to the BreathePD software at a sampling frequency of 100Hz. The software works on measuring and recording the $P_{ET}O_2$ and $P_{ET}CO_2$ values on a breath-by-breath basis. The inspiratory and expiratory

volumes were measured by the turbine. This information was collected by the turbine and transmitted to BreathetPD software at a sampling frequency of 100 Hz so that minute ventilation ($\dot{V}_E$), tidal volume (V_T) and breathing frequency (*f*) were measured and recorded on a breath-by-breath basis.

2.4 Data analysis.

The cardiovascular raw data was analysed offline using a customised written script files and Microsoft Excel macros. With regards of the ventilatory raw data, customised written averaging software was used to analysed the data offline. Following that, the ventilatory data were converted from ambient temperature, pressure and saturated water vapor (ATPS) to body temperature, pressure and saturated water vapor (BTPS). All statistical analyses were carried out using a standard statistical package (SPSS, version 22; SPSS, Inc., Chicago, IL, USA). Details of the statistical tests that were used in each study will be described in the methods section of each Chapter in this thesis.

CHAPTER 3: SENSITIVITY OF THE HUMAN VENTILATORY RESPONSE TO MUSCLE METABOREFLEX ACTIVATION DURING CONCURRENT MILD HYPERCAPNIA.

3.1 Introduction

Evidence supporting a link between activation of descending motor pathways and control of both the cardiovascular and respiratory responses to exercise is well developed, with this feedforward mechanism being known as central command (Krogh and Lindhard, 1913, Goodwin et al., 1972, Eldridge et al., 1981, Green et al., 2007). In addition, it is established that thin fibre afferents exert feedback control over the cardiovascular response to exercise (Alam and Smirk, 1937, Coote et al., 1971, McCloskey and Mitchell, 1972); fibres activated by muscle force generated during contraction trigger the muscle mechanoreflex, while those activated by the associated metabolite accumulation engage the muscle metaboreflex. Some polymodal afferents are also shown to respond to both types of stimulation (Kaufman and Forster, 1996). Typically, in humans, the influence of the muscle mechanoreflex can be investigated by passive stretch of the muscle (Gladwell and Coote, 2002, Drew et al., 2008) or by examination of responses evoked during the initial phases of electrically evoked muscle contraction (Bull et al., 1989). The effect of muscle metaboreflex activation is commonly examined by trapping metabolites within the previously exercised muscle, using a period of local occlusion of the circulation immediately following exercise (PECO). This, in the absence of central command and muscle force generation, reveals the influence of the activation of muscle metaboreflex alone.

The role of thin fibre muscle afferent feedback in controlling human exercise hyperpnea has been intensely debated. Historically, some researchers were confident that receptors "*somewhere in the periphery, most probably the muscle*" (Asmussen, 1967) played an important role (Kao, 1963, Dejours, 1967). However, many others postulated that afferent activation was unimportant, pointing to evidence of the, now classic, observation in healthy humans that continued muscle metaboreflex activation after exercise ceased by PECO does not sustain the hyperpnea occurring during exercise (Wiley and Lind, 1971, Rowell et al.,

1976, Innes et al., 1989, Haouzi et al., 2001). In addition, blocking muscle afferent feedback using epidural local anaesthesia was shown to have no effect on the magnitude of exercise hyperpnea. However, more recent experiments employed blockade of the feedback from the legs by intrathecal administration of fentanyl (Amann et al., 2010). Unlike the earlier experiments with local anaesthesia, this opioid agent does not affect the ability of the somatic system to voluntarily activate the leg muscles. Therefore, this method to evaluate the effects of reducing afferent input on ventilation is not confounded by a compensatory increase in central command. Indeed, this approach has consistently revealed a reduced ventilatory response during dynamic cycle exercise in healthy subjects, as well as in COPD and CHF patient groups, (Amann et al., 2010, Gagnon et al., 2012, Olson et al., 2014). The above contradictory findings may be reconciled if one was to accept that thin fibre muscle afferent feedback does have an important influence on exercise hyperpnea, if combined with other inputs to respiratory control (in this case, central command).

Recently, Lykidis et al. (2010) and Bruce and White (2012, 2015, 2016) investigated the synergistic interaction between muscle afferent input and activation of the chemoreflex – another notable input – with respiratory control. These studies showed that a significant increase in ventilation occurs either during muscle mechanoreflex or muscle metaboreflex activation in a resting human muscle (hence, in the absence of central command); however, this is shown to occur only under conditions of concurrent systemic hypercapnia, not during normal air breathing. These experiments were performed on different muscle groups, namely the forearm or the calf muscles, using different exercise modes, for instance; sustained or rhythmic isometric contractions, simple inspiration of hypercapnic gas from a Douglas Bag or more precise automated end-tidal clamping to elevate $P_{ET}CO_2$ levels. While the results of these various approaches were consistent in supporting a ventilatory response to muscle afferent activation during mild hypercapnia, the levels of hypercapnia attained in these

experiments varied between 7 - 10 mmHg above normal resting $P_{ET}CO_2$, while the level of metaboreflex activation clearly would have varied between the muscles groups, exercise modes and durations of muscle contraction. Therefore, a simple but important question remains unanswered; what is the relationship between the level of systemic hypercapnia and the magnitude of the additional hyperpnea produced in response to a standardised level of muscle afferent activation? This relationship could have important implications for comprehensive interpretation of studies where muscle afferent feedback is blocked and followed by a subsequent rise in $P_{ET}CO_2$ (Amann et al., 2010) or in conditions where CO_2 may be retained due to disease (such as COPD) (Richerson and Boron, 2005). In this chapter, the ventilatory response to a standardised high level of activation of the muscle metaboreflex in human calf muscles in the presence of a range of concurrent hypercapnia levels, ranging from 1mmHg to 10mmHg above normal $P_{ET}CO_2$, value was examined.

In view of the limited data available in this field, it is hypothesised that a simple linear relationship exists between the level of hypercapnia and magnitude of the additional hyperpnea produced in response to a standardised level of muscle afferent activation.

3.2 Methods

All participants received written and verbal information regarding the experimental procedures prior to giving informed written consent. They were habituated to the experimental procedures, which conform to the *Declaration of Helsinki* and were approved by the local ethical committee. Participants refrained from consuming food and caffeine 4 hours prior to performing strenuous physical activity or consuming alcohol 12 hours before all trials. Fifteen young healthy subjects aged 22.5 ± 4.1 years old; 175.5 ± 10.1 cm height and 74.2 ± 8.8 kg weight (mean ± SD) performed four trials in random order, on two occasions. On each day, 2 trials were performed with 45 minutes resting periods between them.

3.2.1 General procedure.

Participants were attached to ECG leads via electrodes placed in the lead II position and connected to a monitor. A Finapres cuff was wrapped around the middle finger of the left hand, which was placed on a support fixed at heart level, and a thigh cuff was wrapped around the thigh of the dominant (right) leg. This cuff was connected to a rapid inflator system. In each trial, participants were seated in an isometric dynamometer designed to measure ankle plantar flexor force (Davies et al., 1982) (Figure 2.5). The leg was fixed with the thigh horizontal and knee and ankle set at 85 degrees. A shaped plate was tightened onto the leg and this connected to a steel bar above, which was instrumented with strain gauges to transduce ankle plantar flexor force. Outputs from the dynamometer, ECG monitor and Finapres were converted from analogue to digital and displayed on the PC. The maximal voluntary contraction force of the plantar flexors was determined prior to each trial. This was taken as the highest value attained in 5 maximal efforts separated by 1 minute of rest. 50 % of this maximal value was then calculated and displayed as a target force on a computer screen within view of the subject for subsequent exercise periods. Participants were then connected

to the mouthpiece of the dynamic end-tidal forcing (DEF) system and allowed to rest to establish a baseline cardiovascular and respiratory state.

3.2.2 Experimental protocol

Participants were instrumented and positioned as described above, with measurements taken during a 2 minute control period whereupon they were breathing room air to re-establish a baseline. Pilot trials indicated that following the MVC assessment, two minutes were sufficient to allow the cardiovascular and ventilatory responses to reach the previously established resting levels. Also, given the rigid seat of the leg dynamometer there was a need to minimise the duration of the protocol for participant comfort.

Following the initial 2 minute baseline, participants were exposed to one of 4 levels of hypercapnia (+1, +3, +7 and +10 mmHg above the individual's baseline $P_{ET}CO_2$) for 15 minutes. During this hypercapnic period, 5 minutes of further rest was followed by 2 minutes of sustained isometric calf muscle contraction at 50% of the previously determined maximal voluntary strength. Just prior to cessation of exercise, the thigh cuff was inflated rapidly to 200 mmHg. This remained inflated for 3 more minutes before deflation and the final 5 minutes of exposure to hypercapnia (Figure 3.1).

In all trials, participants were informed to breathe normally through the mouthpiece and avoid any abnormal respiratory manoeuvres. Moreover, they were instructed to notify the investigator for any sings of pain or discomfort caused by circulatory occlusion or hypercapnia, in which the trial would be immediately stopped.

$P_{ET}CO_2$ 1mmHg	BL	Rest	Ex	PECO	Recovery
$P_{ET}CO_2$ 3mmHg	BL	Rest	Ex	PECO	Recovery
$P_{ET}CO_2$ 7mmHg	BL	Rest	Ex	PECO	Recovery
$P_{ET}CO_2$ 10mmHg	BL	Rest	Ex	PECO	Recovery

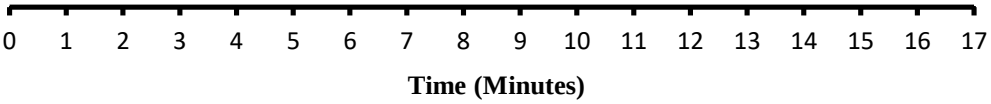


Figure 3.1: Schematic diagram of the protocol. Shaded areas indicate inspiration of hypercapnic gas, which clamped $P_{ET}CO_2$ at 1, 3, 7, or 10 mmHg above resting value (BL, baseline, Ex, exercise, PECO, post-exercise circulatory occlusion).

Minute ventilation (V_E), respiratory frequency (*f*) and tidal volumes (V_T) were continuously recorded throughout the protocol using the DEF system, which clamped $P_{ET}CO_2$ at the chosen values. HR and blood pressure (mean arterial pressure; MAP) were continuously recorded throughout the protocol.

3.2.4 Statistical methods

Throughout all trials, ventilatory and cardiovascular responses were measured as described in Chapter 2 (section 2.3). Then, mean average values for $\dot{V}_E$, *f*, V_T, HR and MAP were calculated for each minute. The cardiovascular and ventilatory responses to each period of the trial and between the different levels of hypercapnia were compared using analysis of variance with repeated measures. When appropriate, Tukey test was used to determine the differences between the 4 levels of hypercapnia.

Data are expressed as means ± S.E.M and statistical significance was taken as $p < 0.05$. Statistical analysis was conducted using a standard statistical package (version 22.0, SPSS, Chicago, IL, USA).

3.3 Results

The 50 % MVC values used during exercise were not significantly different between the 4 trials (*+1 mmHg*: 662.9 ± 41.6 N; *+ 3 mmHg*: 660 ± 45.4 N; *+ 7 mmHg*: 671 ± 45.4 N; *+ 10 mmHg*: 654.7 ± 43.3 N) ($p > 0.05$). The baseline cardiovascular and respiratory variables measured at rest for the 15 subjects prior to each of the four trials are shown in table 3.1. There were no significant differences between the trials for resting $\dot{V}_E$, f, V_T, MAP and HR. During the baseline period, the mean $P_{ET}CO_2$was 38.6 ± 0.8 mmHg, and did not significantly differ across the 4 trials or from the previously determined values recorded during the initial assessment period.

Table 3.2 shows the cardiovascular and ventilatory values recorded during the last 2 minutes of the hypercapnic resting period. There were no significant differences between all trials for hypercapnic resting HR and MAP. However, $\dot{V}_E$ and V_T values in +7 and +10 trials were significantly higher than those in +1 and +3 trials ($p < 0.05$).

Table 3.1: Values recorded during the 2 minute baseline period of each trial. No significant differences between trials (mean ± S.E.M).

Trial	HR (beats.min^{-1})	MAP (mmHg)	$\dot{V}_E$ (l.min^{-1})	V_T (L)	*f* (breaths.min^{-1})
$P_{ET}CO_2$ +1 mmHg	81 ± 5.2	96.4 ± 4.8	19.7 ± 1.6	1.20 ± 0.09	17.5 ± 1.5
$P_{ET}CO_2$ +3 mmHg	88 ± 4.6	97 ± 3.4	18.1 ± 1.2	1.23 ± 0.1	16.5 ± 1.6
$P_{ET}CO_2$ +7 mmHg	88 ± 5.3	95.4 ± 7.9	19.6 ± 1.5	1.34 ± 0.15	15.7 ± 1
$P_{ET}CO_2$ +10 mmHg	84 ± 4.9	93.4 ± 2.8	19.2 ± 2.1	1.24 ± 0.1	16.2 ± 1.3

Table 3.2: Values recorded during the last 2 minutes (6 & 7) of the hypercapnic resting period of each trial (mean ± S.E.M).

Trial	HR (beats.min^{-1})	MAP (mmHg)	$\dot{V}_E$ (l.min^{-1})	V_T (L)	*f* (breaths.min^{-1})
$P_{ET}CO_2$ +1 mmHg	82 ± 4.9	98.2 ± 4.6	26.4 ± 2.9	1.49 ± 0.14	17.9 ± 1.2
$P_{ET}CO_2$ +3 mmHg	90 ± 4.6	98.8 ± 3.6	32.7 ± 2.5	1.74 ± 0.15	19.5 ± 1.3
$P_{ET}CO_2$ +7 mmHg	91 ± 5.0	108.3 ± 4.3	49.5 ± 3.4*†	2.46 ± 0.15*†	20.7 ± 1.3
$P_{ET}CO_2$ +10 mmHg	83 ± 3.6	100.7 ± 3.0	58.9 ± 3.3*†	2.82 ± 0.15*†	21.2 ± 1.0

***** Significantly different from +1 mmHg ($p < 0.05$). † Significantly different from +3 mmHg ($p < 0.05$).

Heart rate:

The mean HR did not change significantly from baseline, during rest, at any level of hypercapnia. During exercise, on the other hand, the HR rose significantly above baseline in all trials by 12-15 b.min^{-1} ($p < 0.05$), although there were no significant differences in HR between trials (Figure 3.2). The HR fell back to baseline within the first minute of PECO in the +1, +3 and +7 trials but remained slightly elevated above baseline (~5 beats.min^{-1}) in the +10 condition. On deflation of the thigh cuff, there was a transient elevation in HR in all trials before a return to hypercapnic rest values.

Figure 3.3 shows changes in mean HR values when normalised to the last 2 minutes of hypercapnic rest values (Table 3.2). The results revealed that, during exercise, the HR rose significantly in all trials by 6.5 – 13.5 beats.min^{-1} compared to hypercapnic rest values ($p < 0.05$); however, again, there were no significant differences between any trials. During the PECO period, the HR fell below hypercapnic rest values within the first minute in +1, +3, and +7 trials, but was slightly elevated above hypercapnic rest values (~5 beats.min^{-1}) in the +10 trials.

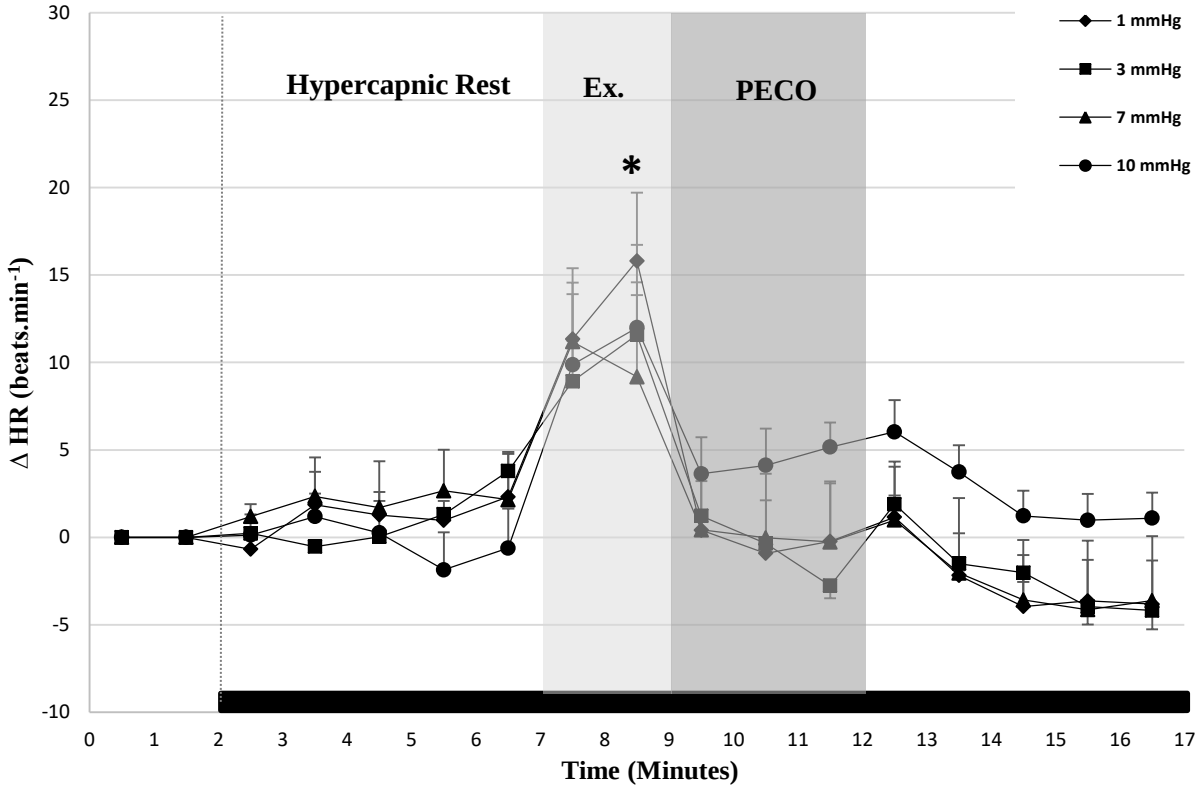


Figure 3.2: Mean changes in HR from baseline during each period of the +1, +3, +7 and +10 mmHg trials (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia**. *** All conditions are significantly different from baseline values ($p < 0.05$).

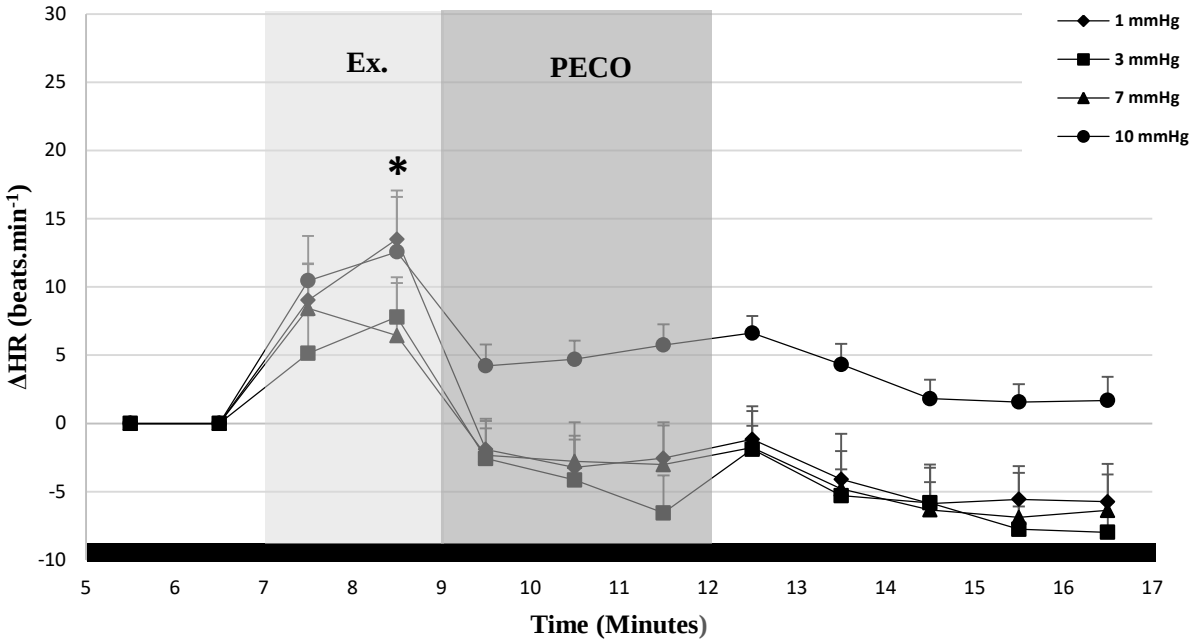


Figure 3.3: Mean changes in HR from the hypercapnic rest period during each period of the +1, +3, +7 and +10 mmHg trials (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia**. *** All conditions are significantly different from hypercapnic rest values ($p < 0.05$).

Blood pressure

Figure 3.4 shows that, over the hypercapnic rest period, the MAP gradually increased from baseline values and this increase reached significance by the 5th minute in all trials ($p < 0.05$). The increase at this time point was significantly greater in the +10 trials than the +1 and +3 trials ($p < 0.05$); however, there were no other significant differences in MAP between trials at this time point. During isometric exercise, MAP progressively rose and was significant above the baseline period in all trials ($p < 0.05$). Again, there were no significantly differences between end exercise values in the four trials. As expected, the increases observed during exercise were partially sustained by PECO in all trials, with the MAP

remaining significantly elevated above baseline ($p < 0.05$). On release of the thigh cuff, the MAP fell to hypercapnic baseline values in all trials.

When MAP changes were normalised to mean MAP values in the last 2 minutes of rest during the baseline hypercapnia period, all differences between trials disappeared (Figure 3.5).

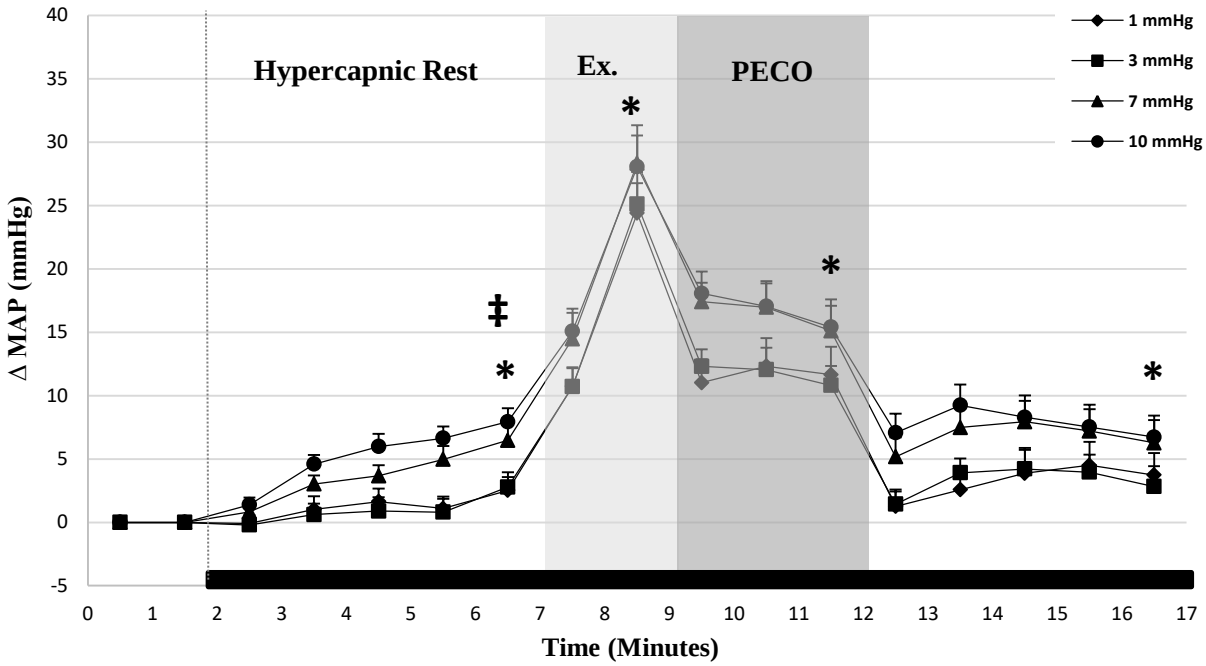


Figure 3.4: Mean changes in MAP from baseline during each period of the +1, +3, +7 and +10 mmHg trials (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-Exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia**. *** All conditions are significantly different from baseline values ($p < 0.05$). ‡ MAP value in the +10 mmHg trial is significantly higher than MAP values in +1 mmHg & +3 mmHg trials ($p < 0.05$).

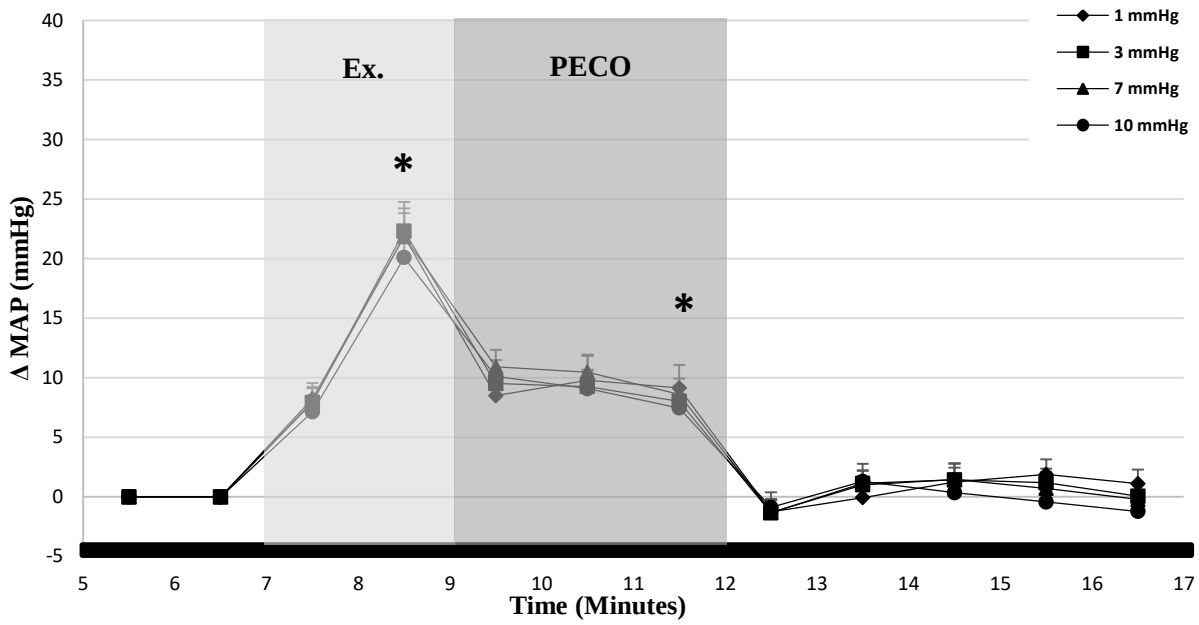


Figure 3.5: Mean changes in the MAP from the hypercapnic rest period, during each period of the +1, +3, +7 and +10 mmHg trials (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia**. *** All conditions are significantly different from hypercapnic rest values in ($p < 0.05$).

Ventilation

From the stable resting values given in Table 3.1, $\dot{V}_E$ increased significantly in all trials by the 5th minute of the baseline period of hypercapnic gas inspiration ($p < 0.05$). This was by virtue of significant increases in both V_T (0.26 ± 0.12, 0.59 ± 0.12, 1.15 ± 0.15 and 1.58 ± 0.13L) (($p < 0.05$) and f (0.9 ± 0.8, 2.7 ± 1.0, 5.3 ± 0.8 and 5.6 ± 0.7 breaths.min^{-1}) ($p < 0.05$) in the +1, +3, +7 and +10 conditions, respectively.

Changes in $\dot{V}_E$, normalised to the respective resting values, are shown for the 4 trials in Figure 3.6. In all trials, $\dot{V}_E$ was significantly greater than the resting values after 5 minutes of exposure to hypercapnia ($p < 0.05$). The responses to +7 mmHg and +10 mmHg above

baseline $P_{ET}CO_2$ were significantly greater than those to +1 and +3 mmHg ($p < 0.05$). There were no other significant differences between trials.

During exercise, $\dot{V}_E$ increased further still in all trials before going on to reach a peak value in the first minute of PECO. It then fell back during the next 2 minutes, to a level which was still significantly above baseline in all trials ($p < 0.05$). The same pattern of statistical significance as was found at the end of the rest period, also was found when examining the differences between trials during both the last minute of the exercise and PECO periods. On release of the occluding thigh cuff, there was a small transient increase in $\dot{V}_E$, followed by a gradual decline towards baseline.

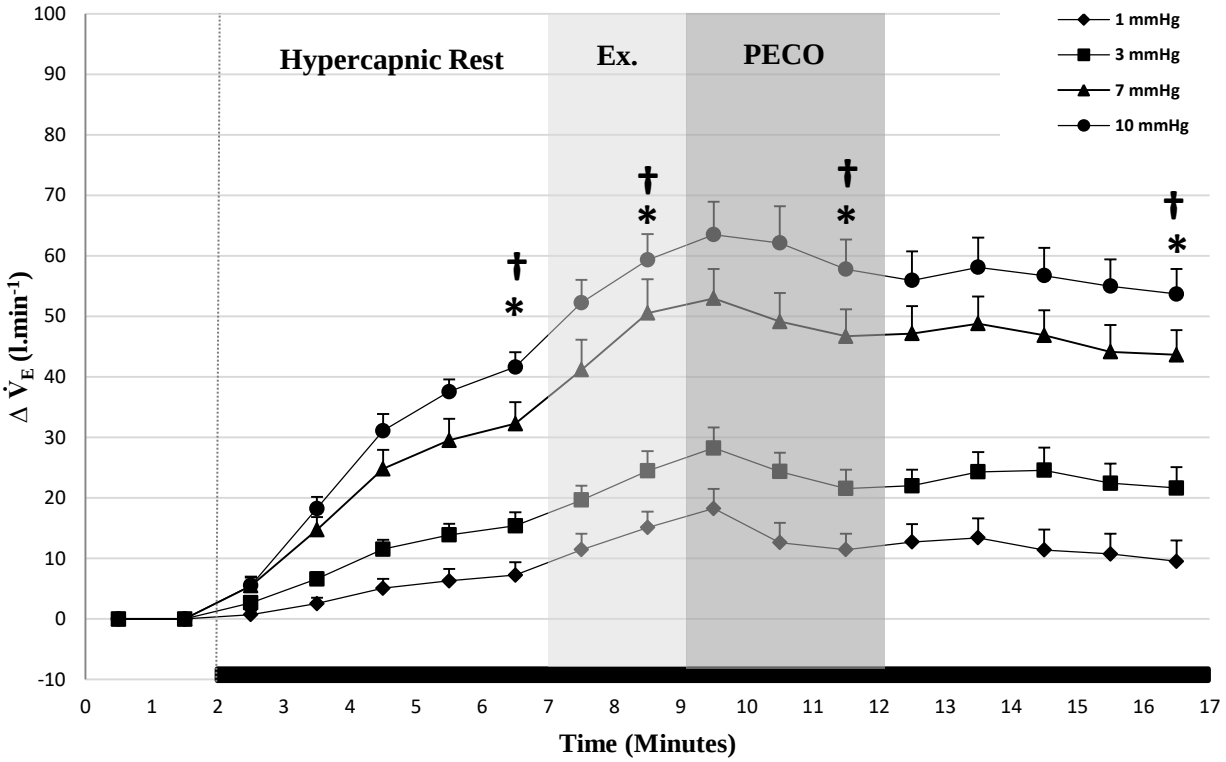


Figure 3.6: Mean changes in $\dot{V}_E$ from baseline period, during each period of the +1, +3, +7 and +10 mmHg trials (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia**. *** All conditions are significantly different from baseline values ($p < 0.05$). † $\dot{V}_E$ values in both +7 mmHg & +10 mmHg trials are significantly higher than +1 mmHg & +3 mmHg trials ($p < 0.05$).

Figure 3.7 shows the changes in $\dot{V}_E$ respective to hypercapnia resting values in table 3.2. At the end of exercise, $\dot{V}_E$ was significantly greater than during the hypercapnia resting period in all trials ($p < 0.05$). This increase in $\dot{V}_E$ dropped slightly in the last 2 minutes of the PECO period to a level that remained above hypercapnia resting values in all trials ($p < 0.05$). When the circulation was restored in the recovery period, there was a small transient increase in $\dot{V}_E$, followed by a progressive decline towards hypercapnic rest values. During the last minute of the recovery period, $\dot{V}_E$ values in all trials were significantly greater than hypercapnic rest

values ($p < 0.05$). In each phase, the increases in $\dot{V}_E$ values in both +7 mmHg and +10 mmHg trials were significantly higher than those in +1 mmHg and +3 mmHg trials (all $p < 0.05$). There were no other significant differences between trials.

Changes in $\dot{V}_E$ in Figure 3.7 were due to changes in V_T and f. V_T was not significantly altered during exercise and PECO periods from hypercapnic resting values in any trials. Meanwhile, f increased significantly ($p < 0.05$) by ~4 breath.min^{-1} during exercise in all 4 trials, and was significantly elevated by ~3-4 breath.min^{-1} ($p < 0.05$) during PECO in the +7 mmHg and + 10 mmHg trials (Figure 3.8).

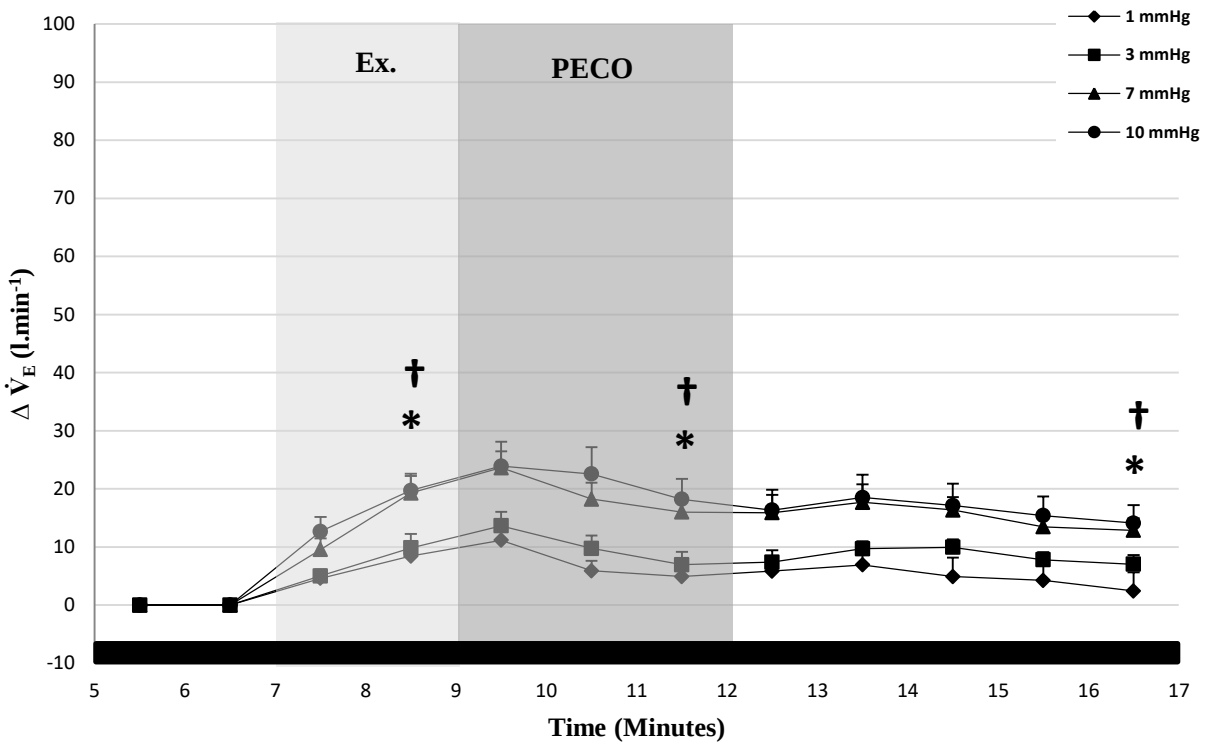


Figure 3.7: Mean changes in $\dot{V}_E$ from the hypercapnic rest period baseline during each period of the +1, +3, +7 and +10 mmHg trials (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) phase and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia. *All conditions are significantly different from hypercapnic rest values ($p < 0.05$). † $\dot{V}_E$ values in both +7 mmHg & +10 mmHg trials are significantly greater than in the +1 mmHg & +3 mmHg trials ($p < 0.05$).

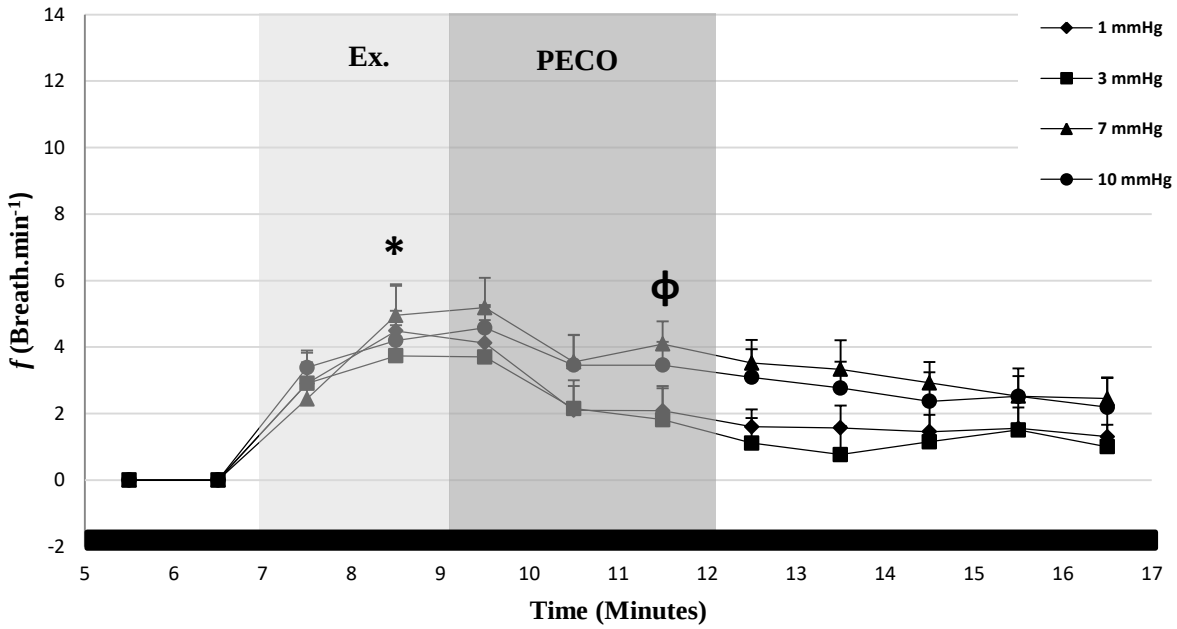


Figure 3.8: Mean changes in *f* from the hypercapnic rest period baseline during each period of the +1, +3, +7 and +10 mmHg trials (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia. *All conditions are significantly different from hypercapnic rest values ($p < 0.05$). ϕ +7 mmHg & +10 mmHg trials are significantly different from hypercapnic rest values ($p < 0.05$).

It is clear from Figures 3.6 and 3.7 that, in all trials, $\dot{V}_E$ did not recover to pre-exercise hypercapnic baseline values by the end of the recovery period (minute 17 of the protocol). It appears that $\dot{V}_E$ had not fully adjusted to the elevated $P_{ET}CO_2$ levels prior to the start of exercise and, as a result, there was a small baseline drift during the exercise, PECO and recovery periods in all trials. To correct for this, linear regression lines were constructed, for each subject in each trial, using $\dot{V}_E$ values at minutes 6, 7, 16 and 17 of the protocol (the final 2 minutes of the hypercapnia resting period and recovery periods) (for details, see Chapter 4, part B). From the slope and intercept of these regression lines, $\dot{V}_E$ values in each trail and for

each subject were adjusted from minutes 6 - 17. This corrected data is shown in Figure 3.9. From this figure, it is clear that despite the exercise intensity being fixed, $\dot{V}_E$ increased more during exercise with exposure to greater levels of hypercapnia. $\dot{V}_E$ levels during the last minute in the +7 mmHg and + 10 mmHg trials were significantly greater than in the +1 mmHg trials ($p < 0.05$). During PECO, ventilation fell from its peak values in all trials to a level which remained significantly above pre-exercise hypercapnic baseline levels, until removal of the thigh cuff. Again, the increases in sustained $\dot{V}_E$ during PECO were progressively greater with higher levels of hypercapnia; the + 10 mmHg values were significantly greater compared to the +1 mmHg and +3 mmHg values ($p < 0.05$). Using values from each subject during the last minute of PECO, linear regression was performed for each subject, plotting change in corrected ventilation from hypercapnic baseline against the 4 end-tidal CO_2 increases. Then, using the values for each subject's slope (15 values) and intercept (15 values), an averaged regression value for all 15 subjects was calculated as follows: $\dot{V}_E$ ($l.min^{-1}$) = 0.85 x $P_{ET}CO_2$ (mmHg) + 0.80 ($l.min^{-1}$) (R=0.78 range, 0.39 - 0.99), as shown in Figure 3.10. The mean R value was calculated by transforming each individual R value to R' from the standard table then calculating the mean R' value, before converting back to R using the standard table.

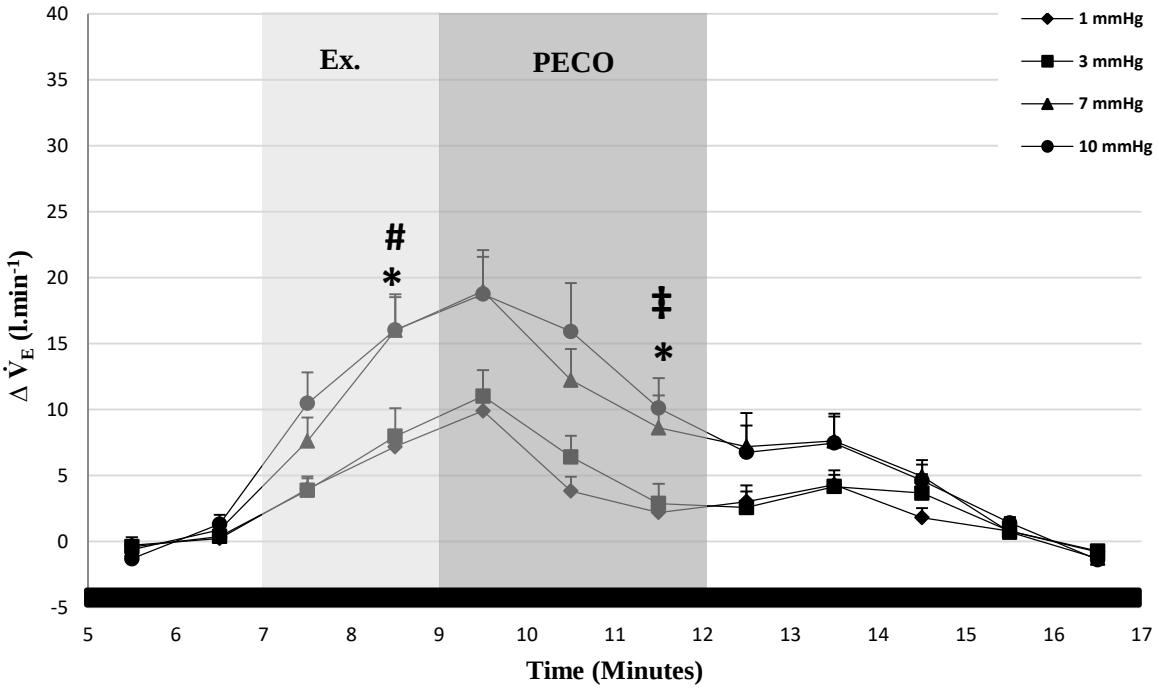


Figure 3.9: Mean changes in $\dot{V}_E$ during each period of the +1, +3, +7 and +10 mmHg trials, corrected for baseline shift between minutes 6, 7 and 16, 17 (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia. *****All conditions are significantly different from baseline values ($p < 0.05$). # both +7 mmHg & +10 mmHg are significantly higher than +1mmHg ($p < 0.05$). ‡ +10 mmHg is significantly higher than +1 mmHg & +3 mmHg ($p < 0.05$).

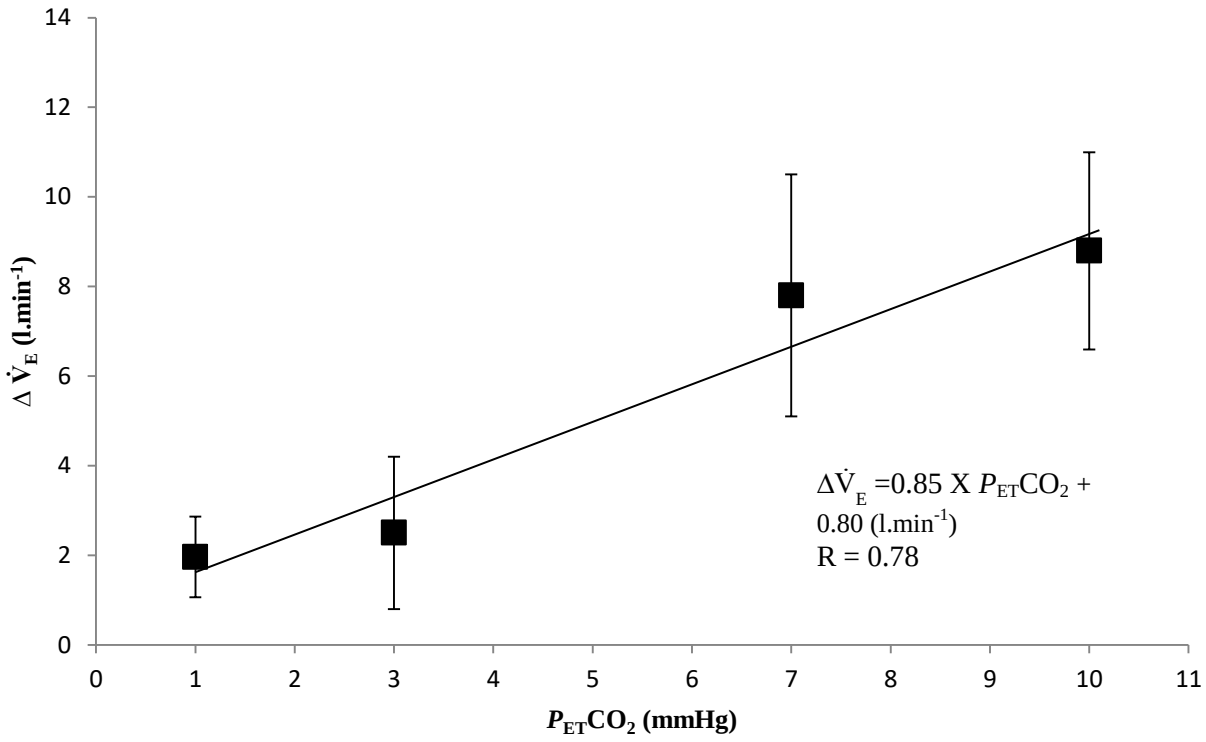


Figure 3.10: Relationship between minute ventilation ($\dot{V}_E$) and end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) (mean ± S.E.M).

3.4 Discussion.

This study found that conducting a standardised isometric exercise protocol, followed by a period of PECO, under 4 different levels of hypercapnia, results in a consistent cardiovascular response that is independent of the immediate baseline shifts caused by hypercapnia (Figure 3.5). The blood pressure response to a period of PECO is well accepted as the prime indicator of the level of activation of the muscle metaboreflex, which drives this pressor reflex (PR) (Alam and Smirk, 1937, Smith et al., 2006).

Thus, in the experiments described in this chapter, the PR gives a clear indication of a consistent and equal activation of the muscle metaboreflex in all trials. During PECO combined with 4 different levels of hypercapnia, ventilation was elevated above levels seen under the same levels of hypercapnia alone; moreover, the magnitude of the increase in ventilation during PECO progressively increased with the level of hypercapnia (Figure 3.10). Given that metaboreflex activation appears to be consistent across the 4 conditions, the progressive increase in the ventilatory response to PECO with increasing levels of hypercapnia supports the idea of synergistic interaction between the muscle metaboreflex and the chemoreflex. Furthermore, it suggests a sensitisation of this interaction with increasing levels of hypercapnia.

The PR to isometric exercise is independent of resting baseline blood pressure (Lind, 1983), and can be sustained until high levels of concurrent dynamic exercise (Lind and McNicol, 1967). This robust effect is also unaffected by concurrent exposure to other powerful activators of the autonomic nervous system, such as the cold pressor test (Peikert and Smolander, 1991, Vianna et al., 2012). It is, therefore, perhaps not surprising that hypercapnia, a powerful activator of the sympathetic nervous system (Somers et al., 1989, Kara et al., 2003) which causes baseline blood pressure elevation (Figure 3.4), does not affect

the PR to a standardised bout of isometric exercise and PECO (Figure 3.5). This indicates that the level of muscle metaboreflex activation during PECO is consistent, with there being no central command or muscle mechanoreflex activation at this time. The pressor responses observed are consistent with previous values (reported by the same laboratory) for this level of isometric exercise of the human calf muscles (Bell and White, 2005). Resting HR did not change significantly during exposure to low or moderate levels of hypercapnia used in the present study. Isometric exercise caused the HR to rise by 10 -15 beats.min^{-1} in all trials; however, unlike blood pressure, there was considerable intra-subject variability apparent in this response, and this rise from baseline only reached statistical significance in the +10 mmHg trial. Furthermore, in contrast to the other 3 trials, in this trial the HR did fall during PECO, although it remained elevated above baseline until release of the thigh cuff. Conversely, in the other trials, the HR promptly fell to baseline or below during PECO and recovery. This is consistent with the idea that cardiac sympathoexcitation due to activation of the muscle metaboreflex, combined with sympathoexcitation caused by this highest level of hypercapnia, provides sufficient opposition to the returning vagal tone, which occurs when muscle mechanoreflex and central command inhibition of the vagus are removed at the end of exercise (Gladwell and Coote, 2002, Brack et al., 2004, Gladwell et al., 2005, Fisher et al., 2010), to result in a slightly elevated HR in comparison to the other trials.

It is possible that exposure to increasing levels of systemic hypercapnia could make the active muscle progressively prone to fatigue due to acidosis and increased levels of metabolites produced during exercise, which then activate the exercise PR. However, the consistent pressor responses seen during PECO argue against this. In addition, in isolated mammalian muscles, the effects of even severe hypercapnia-induced acidosis during intense sustained isometric exercise on muscle contractile characteristics are very small and, indeed, at normal

exercising muscle temperatures, they are likely to be non-existent (Westerblad et al., 1997). Finally, it was noted that participants in the present study were able to sustain the required force levels in all trials, albeit with considerable effort on each occasion.

Previous work from this laboratory showed that the augmented ventilatory response to hypercapnia was not dependent on the muscle being exposed to it; indeed, local occlusion of the circulation to the muscle prior to exposure of the systemic circuit to hypercapnia did not prevent the increase in ventilation seen during muscle metaboreflex activation by PECO. Importantly, brief exposure to systemic hyperoxic (95 % O_2) and hypercapnic (5 % CO_2) conditions, intended (as a form of modified DeJours test) to suppress carotid chemoreceptor activity, had no effect on the ventilatory response suggesting little involvement of the peripheral (carotid) chemoreflex under these conditions and a predominance of central chemoreflex activation. In contrast, exposure of the muscle to systemic hypercapnia followed by local circulatory occlusion, and then a return to room air breathing (i.e. systemic normocapnia), did abolish the ventilatory response during PECO, suggesting a key role for central chemoreception in facilitating generation of this response (Bruce and White, 2015). Edgell and Stickland (2014) reported that hypoxic activation of the carotid chemoreflex combined with PECO does increase ventilation, but that this increase is not greater than the sum of responses to hypoxia and PECO individually. The authors argued that this finding indicates that the metaboreflex does not sensitise the carotid chemoreflex. Therefore, taken together with the MAP response data discussed earlier and previously published experimental data from this laboratory, it seems that in the current work the muscle metaboreflex is equally active during exercise and PECO in all 4 trials. If this drive from the muscle during PECO is constant, then the progressively larger ventilatory responses observed here with increasing

levels of hypercapnia may have been attributed to increasing central chemoreflex sensitivity to this input.

Based on the recommended duration of hypercapnia exposure taken from previous literature (Mateika and Sandhu, 2011), it was expected that ventilation would have reached a new steady state after 5 minutes of exposure to all levels of hypercapnia. However, it is clear in Figures 3.6 and 3.7 that the ventilatory responses to all 4 levels of hypercapnia had not quite reached a true steady state prior to the start of exercise and that, during the recovery periods following PECO, ventilation was stable but at a slightly higher level than that seen at the end of the new baseline period. This drift observed during the exercise and PECO periods appears to be linear (see Chapter 4, part A). The correction of the ventilatory data for this drift was, therefore, carried out from this assumption. The changes in ventilation from these corrected baselines, during standardised activation of the muscle metaboreflex, show a linear increase with increasing $P_{ET}CO_2$ (Figure 3.10). This finding could have important implications for interpretation of studies where the role of the muscle metaboreflex is investigated and where $P_{ET}CO_2$ may be elevated due to experimental intervention (Amann et al., 2010) or by disease (COPD) (Bruce et al., 2016). For example, Amann et al. (2010) found that ventilation during cycling exercise was reduced when muscle afferent feedback from the legs was blocked by intrathecal fentanyl infusion. Quite rightly, they argued that as $P_{ET}CO_2$ became elevated during exercise (as ventilation was reduced during the block), the decrease in ventilation would in fact even greater in the absence of the hypercapnia. They then 'corrected' their exercise ventilatory data for this stimulating effect of hypercapnia based on CO_2 sensitivity data gathered on their resting subjects. The outcome of this was an estimate that muscle afferent activity during cycling exercise could account for as much as 50 % of the ventilatory response. The present data suggests that even this might be an underestimate. Under

conditions of muscle metaboreflex activation, combined with hypercapnia, the ventilatory response is bigger than with hypercapnia alone. This sensitisation effect would apply to metaboreflex feedback from any muscles that are active and, as in the Amann study, still capable of feedback during cycling (e.g. forearm, respiratory and stabilising torso musculature). Further work is required to precisely quantify the magnitude of the effect of this interaction. It is of note that the decline in ventilation during blockade was related to a reduction in *f* in the Amann et al. study, which at first glance may seem at odds with the present observation of an increase in *f* during PECO together with the two increased levels of hypercapnia. However, it is possible that together these observations point to a role for the muscle metaboreflex in controlling breathing frequency, a reduction in feedback by blockade lowering *f* and excitation of the metaboreflex by PECO under hypercapnia inducing its increase.

In conclusion, this study shows a linearly increasing ventilatory response to a standardised level of muscle metaboreflex stimulation, when this reflex is activated in combination with increasing levels of systemic hypercapnia. The data further support the idea of synergistic interaction between central chemoreflex and muscle metaboreflexes.

CHAPTER 4:

1. PART A: CONTROL STUDY

2. PART B: CORRECTION OF BASELINE DRIFITING

4.1 Part A: Control study.

4.1.1 Introduction.

The previous study in this thesis (Chapter 3) revealed that activation of the muscle metaboreflex (by PECO) under different levels of concurrent hypercapnia resulted in sustained elevation of minute ventilation above the hypercapnic baseline level. Moreover, the magnitude of the increase in minute ventilation during the PECO period was progressively increased with the level of hypercapnia. However, ventilation did not fully return to pre-exercise levels after removal of PECO in these trials; therefore, a correction was applied to compensate for this apparent baseline drift. To examine the causes of this drift, further investigation was performed in the following control (pilot) study to evaluate the ventilatory and cardiovascular responses to each component.

4.1.2. Methods

This study was approved by the local ethical committee that conforms to the *Declaration of Helsinki*. 5 healthy male participants (23.4 ± 4.9 years old; 171.5 ± 6.7 cm height; 69.2 ± 7.6 kg weight; mean ± SD) from the University of Birmingham took part in this study. They received verbal and written information about the study, although the specific purpose of the study was not disclosed to participants. After obtaining written consent, participants were asked to visit the laboratory on 3 occasions. During the first visit, participants were habituated to the experimental procedures. Prior to the 2nd and 3rd visits (experimental days), participants were asked to abstain from food and beverages containing caffeine for 4 hours. They were also instructed to avoid consuming alcohol or preforming strenuous physical activity 12 hours before each trial. During the investigation, participants performed 3 trials in

random order. The 3 trials were performed on 2 separate occasions, with 45 minute rest periods between them.

4.1.2.1 Experimental procedures

Participants were seated in the leg isometric dynamometer and instrumented, as described in the general methods section of this thesis (Chapter 2, section 2.2.1), following which they were connected to the mouthpiece of the calibrated DEF system and then performed one of three 17 minute hypercapnic experimental protocols (Figure 4.1.1);

1) Control trial (CON): In this trial, participants rested for 2 minutes, breathing normal air, to establish a baseline measurement. Then, they were exposed to hypercapnia ($P_{ET}CO_2$ set at 10 mmHg, above normal level) for 15 minutes while resting. This level of hypercapnia was chosen based on observations from the previous experimental study (Chapter 3) where, at this level, the activation of muscle metaboreflex by PECO resulted in a significant increase in $\dot{V}_E$.

2) Exercise (Ex): During this trial, participants were sitting in the leg isometric dynamometer (Figure 2.5).

The exercise trial was the same as the control trial, except that at minute 7 of the protocol, participants started to perform 50 % of MVC of their pre-determined calf plantar flexion for 2 minutes, then rested.

3) Circulatory occlusion trial (C.O): This trial was the same as the control trial, except that at minute 9 of the protocol, a thigh cuff was inflated to 200 mmHg using the rapid cuff inflator to achieve local circulatory occlusion. The cuff remained inflated for 3 minutes, before it was deflated and participants continued to rest.

During all trials, participants were asked to breathe normally through the mouthpiece and avoid any abnormal respiratory manoeuvres.

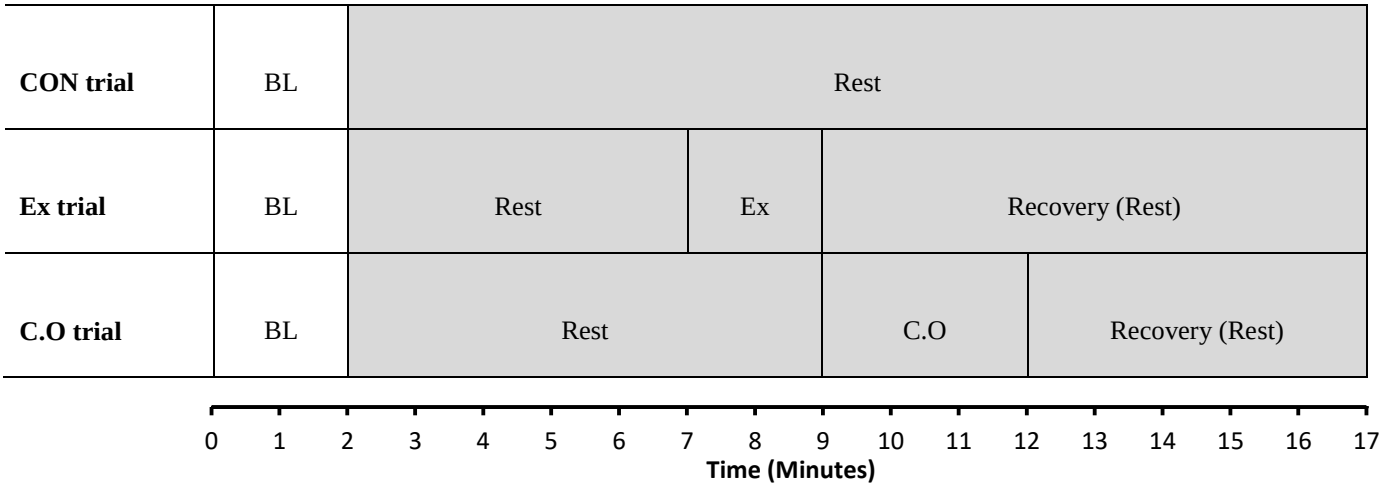


Figure 4.1: Schematic diagram of the control study protocol. Shaded areas indicate inspiration of hypercapnic gas mixture, which clamped $P_{ET}CO_2$ at 10 mmHg above normal levels. CON, control, Ex, exercise, C.O, circulatory occlusion, BL, baseline period.

4.1.2.2 Statistical methods.

A Student's *t* test was used in the CON trial to evaluate the differences in ventilatory and cardiovascular responses between baseline and the last minute of the trial. A repeated analysis of variance and, where appropriate, Tukey test were used to examine the differences in the ventilatory and cardiovascular responses during the last minute of each period of the

Ex and C.O trials (baseline, last minute of intervention and last minute of recovery). These statistical analyses were also used to investigate the differences in the ventilatory and cardiovascular responses between the pre-intervention resting period (average of last 2 minutes of this period), last minute of intervention and last minute of recovery period. The carry-over effect of exercise on the ventilatory response was examined by analysis of variance. This was done by comparing the last minute of pre-exercise resting period value (minute 7) with the first 3 minutes after the exercise period (minute 10, 11 and 12) of the protocol. Data are presented as mean ± S.E.M and statistical significance was shown by $p < 0.05$. Statistical analysis was conducted using a standard statistical package (version 22.0, SPSS, Chicago, IL, USA).

4.1.3. Results

Table 4.1 shows the baseline values recorded for the first 2 minutes of each trial. There were no significant differences between the trials in the resting HR, MAP, $\dot{V}_{E,}$ V_T and *f*. The mean $P_{ET}CO_2$ was 39.6 ± 0.3 mmHg and did not significantly differ across the 3 trials.

Table 4.1: Values recorded during the 2 minute baseline period of each trial. No significant differences between trials (mean ± S.E.M).

Trial	HR (beats.min^{-1})	MAP (mmHg)	$\dot{V}_E$ (l.min^{-1})	V_T (L)	*f* (breaths.min^{-1})
CON trial	69 ± 3	80.3 ± 4.3	17.0 ± 2.2	1.28 ± 0.2	13.6 ± 1.0
Ex. trial	73 ± 6	95.5 ± 6.2	17.9 ± 0.5	1.27 ± 0.1	15.0 ± 1.5
C.O trial	72 ± 5	96.1 ± 7.1	19.5 ± 2.2	1.29 ± 0.2	16.1 ± 2.2

Abbreviation: CON, Control, Ex., Exercise, C.O, Circulatory Occlusion.

Heart rate

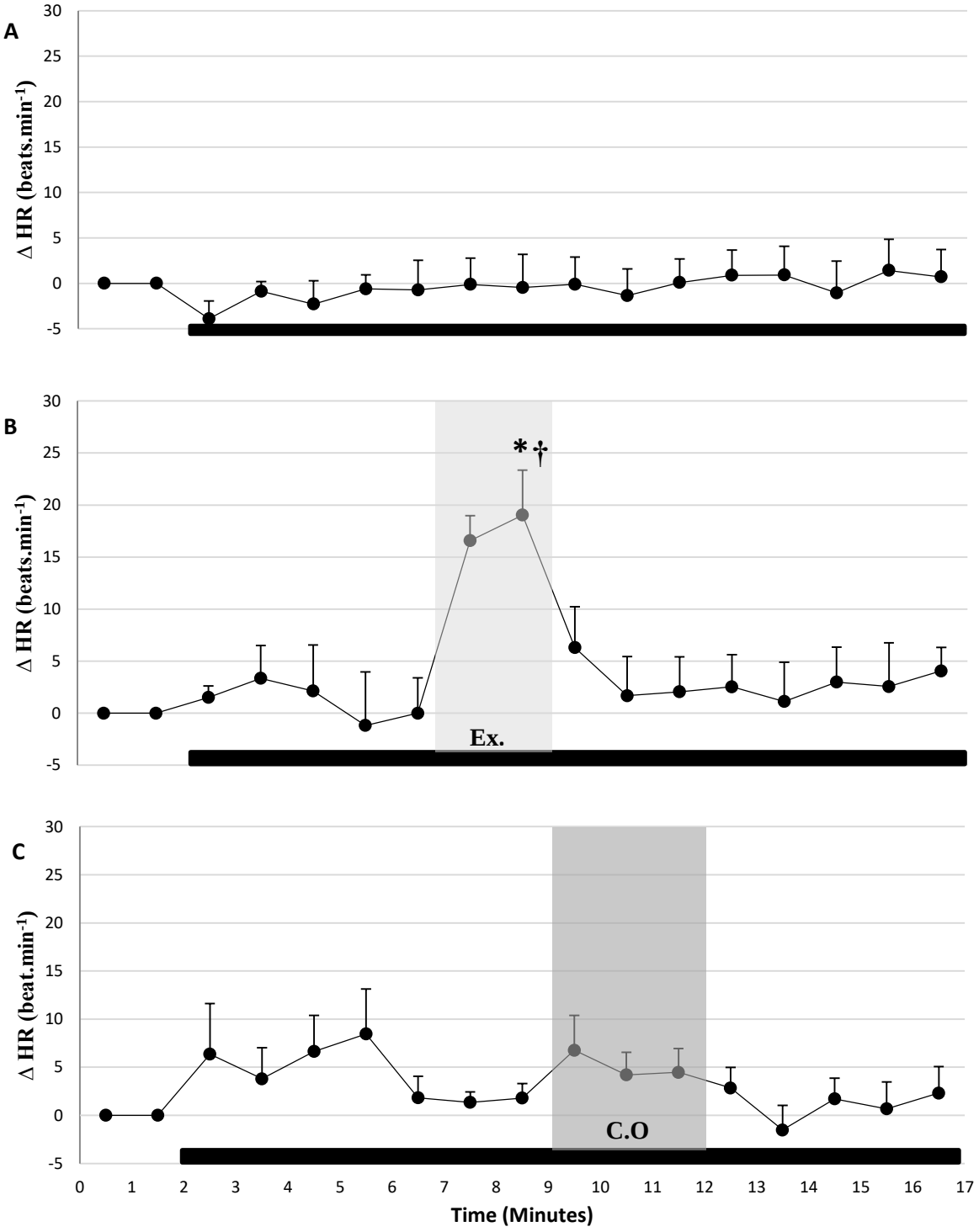


Figure 4.2: Mean changes in the HR from to baseline in the 3 trials (A: CON trial; B: Ex. trial and C: C.O trial) (mean ± S.E.M). The black bar indicates hypercapnia. * Significant difference from baseline values ($p < 0.05$). † Significant difference from the pre-intervention value ($p < 0.05$).

The HR was not notably changed throughout the CON trial (Figure 4.2: A). However, in the Ex. trial (B), isometric calf exercise at 50 % MVC resulted in the expected significant increase in HR by 19 beats.min^{-1} compared to the baseline and, also, compared to the pre-exercise resting periods ($p < 0.05$). Although the HR level during the pre-exercise resting period was observably lower than during the last minute of the recovery period, it did not reach statistical significance. During the C.O trial (C), the HR rate increased by ~ 4 beats.min^{-1} by the end of the C.O period; however, this change did not reach statistical significance when compared to baseline and pre-circulatory occlusion resting periods. There was no difference in the HR value between the last minute of pre-circulatory occlusion resting period and the last minute of the recovery period.

Blood pressure

Figure 4.3 revealed that exposure to hypercapnia resulted in an increase in the MAP from the 2 minute point of the protocol in all trials, reaching a stable level between minutes 5 - 7 of the protocol. During complete rest (CON trial; A), the MAP gradually rose over this time period and remained ~ 7 mmHg above control levels at the end of trial. In the Ex. trial (B), isometric calf exercise induced a significant increase in the MAP (~ 35.3 mmHg compared to baseline value ($p < 0.05$) and ~ 26 mmHg compared to the pre-exercise resting period ($p < 0.05$)). Upon cession of exercise, the MAP began to decline until the end of the recovery period (minute 17), whereupon it remained significantly higher than baseline ($p < 0.05$) but did not differ from the immediate pre-exercise resting level. There was no significant difference in the MAP value between the pre-exercise resting period and the last minute of the recovery period.

Cuff inflation for 3 minutes in the C.O trial (C) resulted in a small further increase in the MAP from the value observed during hypercapnia immediately prior to cuff inflation; however, this ~ 2.7 mmHg rise recovered back to the pre-circulatory occlusion resting level (~ 10.7 mmHg from baseline value, $p < 0.05$) by the end of the trial. There was no significant difference in the MAP between the pre-circulatory occlusion resting period and the last minute of the recovery period.

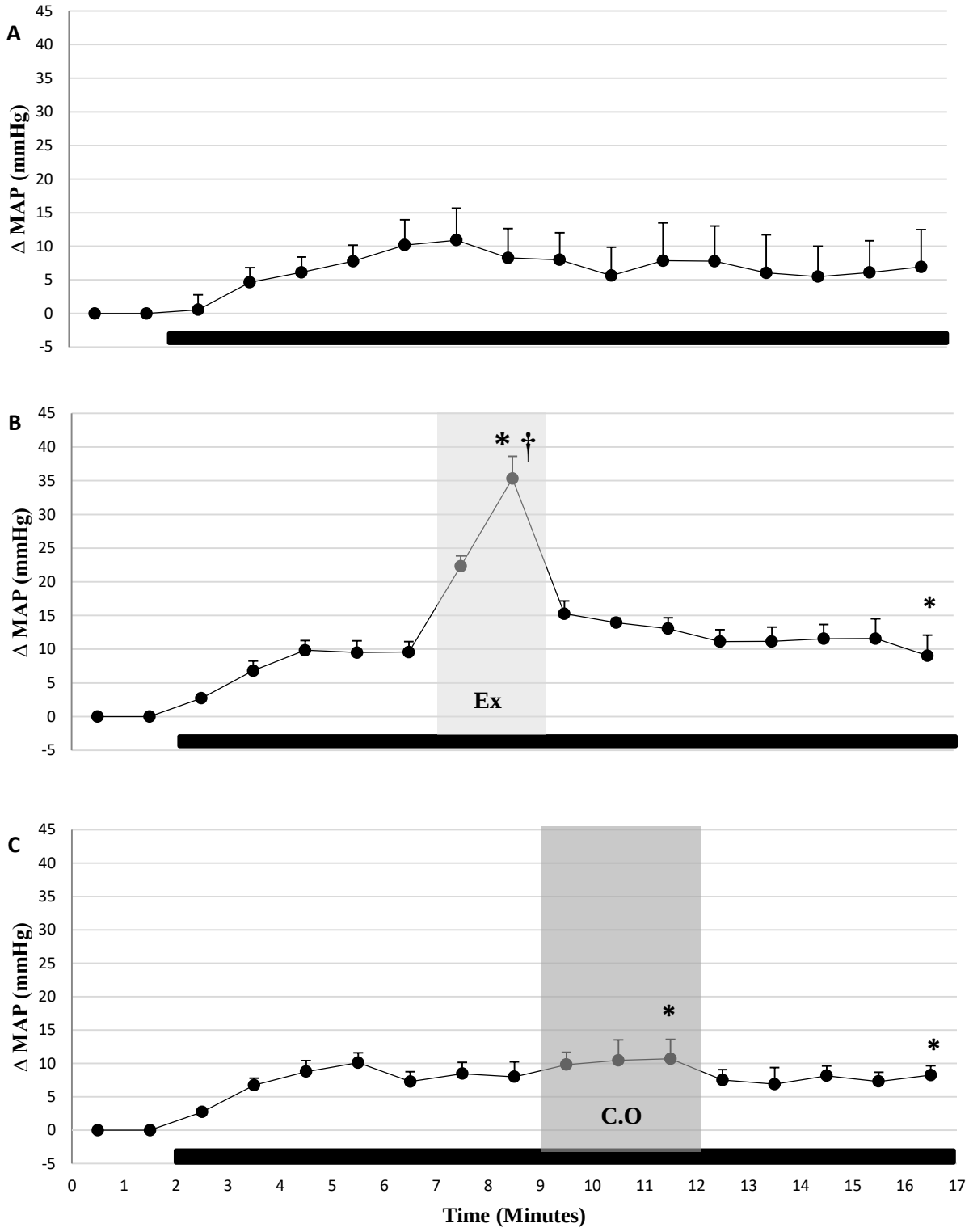


Figure 4.3: Mean changes in MAP from baseline in 3 trials (A: CON trial; B: Ex. trial and C: C.O trial) (mean ± S.E.M). The black bar indicates hypercapnia. * Significant difference from baseline values ($p < 0.05$). † Significant difference from the pre-intervention value ($p < 0.05$).

Minute ventilation

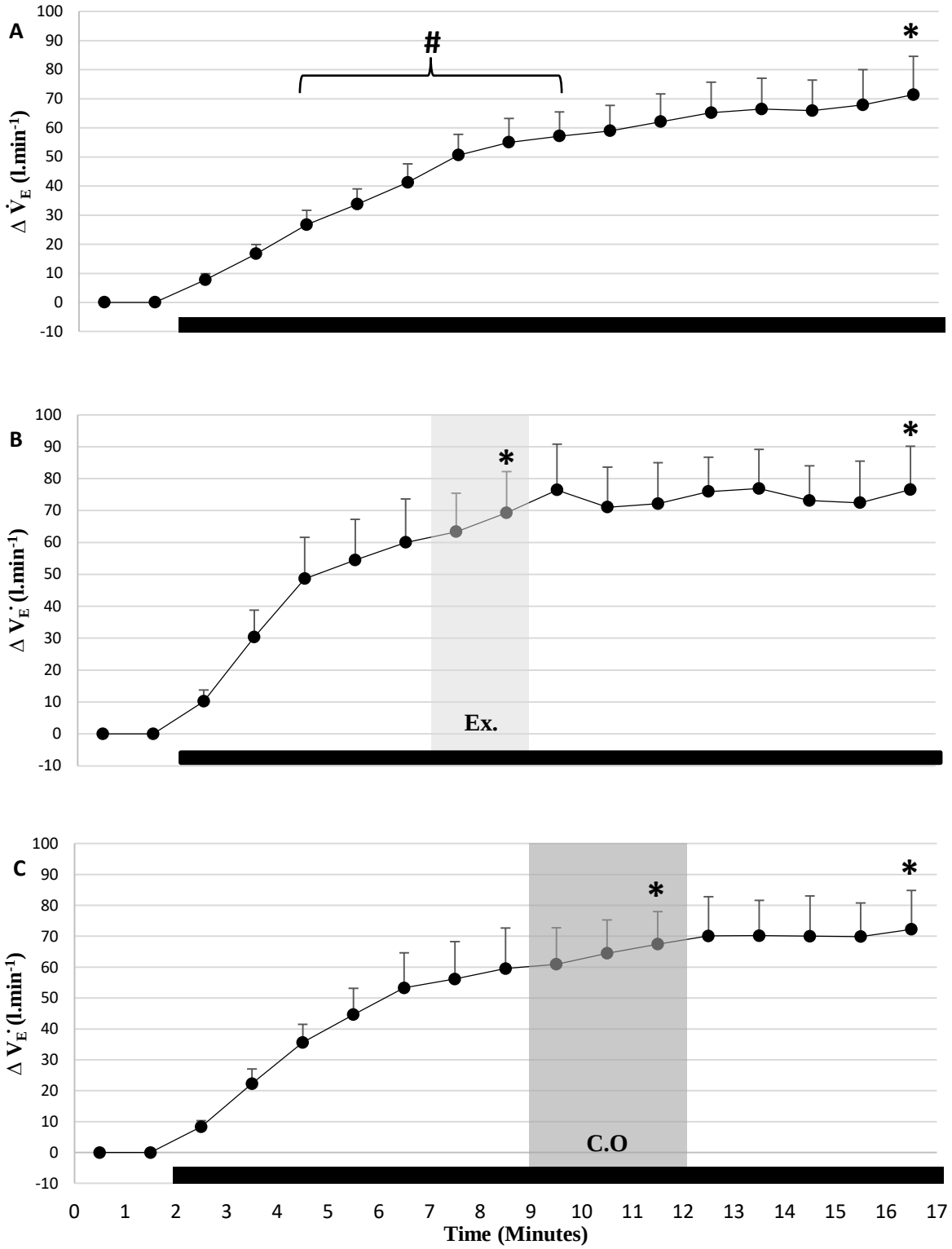


Figure 4.4: Mean changes in $\dot{V}_E$ from baseline in 3 trials (A: CON trial; B: Ex. trial and C: C.O trial) (mean ± S.E.M). The black bar indicates hypercapnia.* Significant difference from baseline values ($p < 0.05$). # Significant difference in $\dot{V}_E$ between minute 5 and 10 ($p < 0.05$).

Unsurprisingly, exposure to hypercapnia resulted in an increase in the $\dot{V}_E$ in all trials. It can be noted from all trials that the ventilatory response to 15 minutes of hypercapnia (+10 mmHg above resting $P_{ET}CO_2$) had a biphasic pattern, whereby in the first 5 minutes of the exposure to hypercapnia the $\dot{V}_E$ increased rapidly, before the rate of rise slowed and became more stable at approx. 70 l.min-1above baseline by the end of the trial. A comparison in the minute ventilation values between minute 5 and minute 10 of the CON trial shows there is a significant difference between these time points ($p < 0.05$). This indicates that minute ventilation did not reach steady state at minute 5. However, it was progressively rising.

Figure 4.4 (B) shows that isometric exercise caused an increase in the rate of $\dot{V}_E$ rise and this increase continued to the first minute after cessation of exercise, before a slight decline in ventilation and returning to stability. When minute 7 from the protocol timeline was compared to minutes 10, 11 and 12, to examine the carry-over effect of exercise on ventilation, $\dot{V}_E$ was not significantly changed between these minutes. During the recovery period, $\dot{V}_E$ become stable and significantly greater than at baseline ($p < 0.05$).

In the C.O trial (Figure 4.4: C), $\dot{V}_E$ was significantly higher compared to baseline at the end of the trial ($p < 0.05$). C.O appeared to have a small effect on the ongoing change in ventilation. After thigh cuff inflation, there was a small transient increase in the rate of increase of $\dot{V}_E$, which disappeared upon cuff deflation. $\dot{V}_E$ values in the last minute of the recovery period were similar to those in the other trials.

Both V_T and the *f* values (Figures 4.5 and 4.6, respectively) showed the same patterns of change and statistical significance as applied to the changes in $\dot{V}_E$.

Tidal volumes

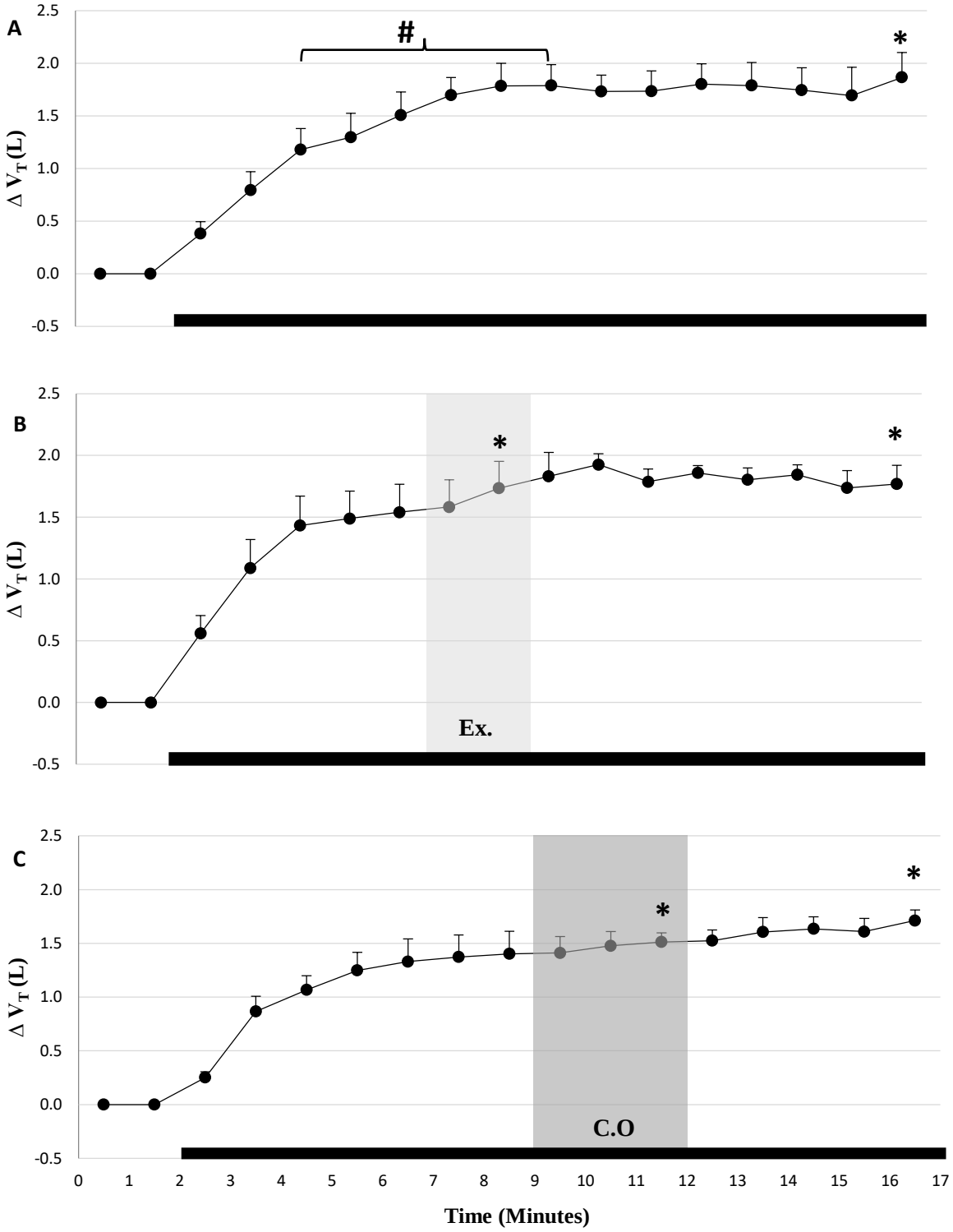


Figure 4.5: Mean changes in V_T from baseline in the 3 trials (A: CON trial; B: Ex. trial and C: C.O trial) (mean ± S.E.M). The black bar indicates hypercapnia. * Significant difference from baseline values ($p < 0.05$). # Significant difference in V_T between minute 5 and 10 ($p < 0.05$).

Breathing frequency

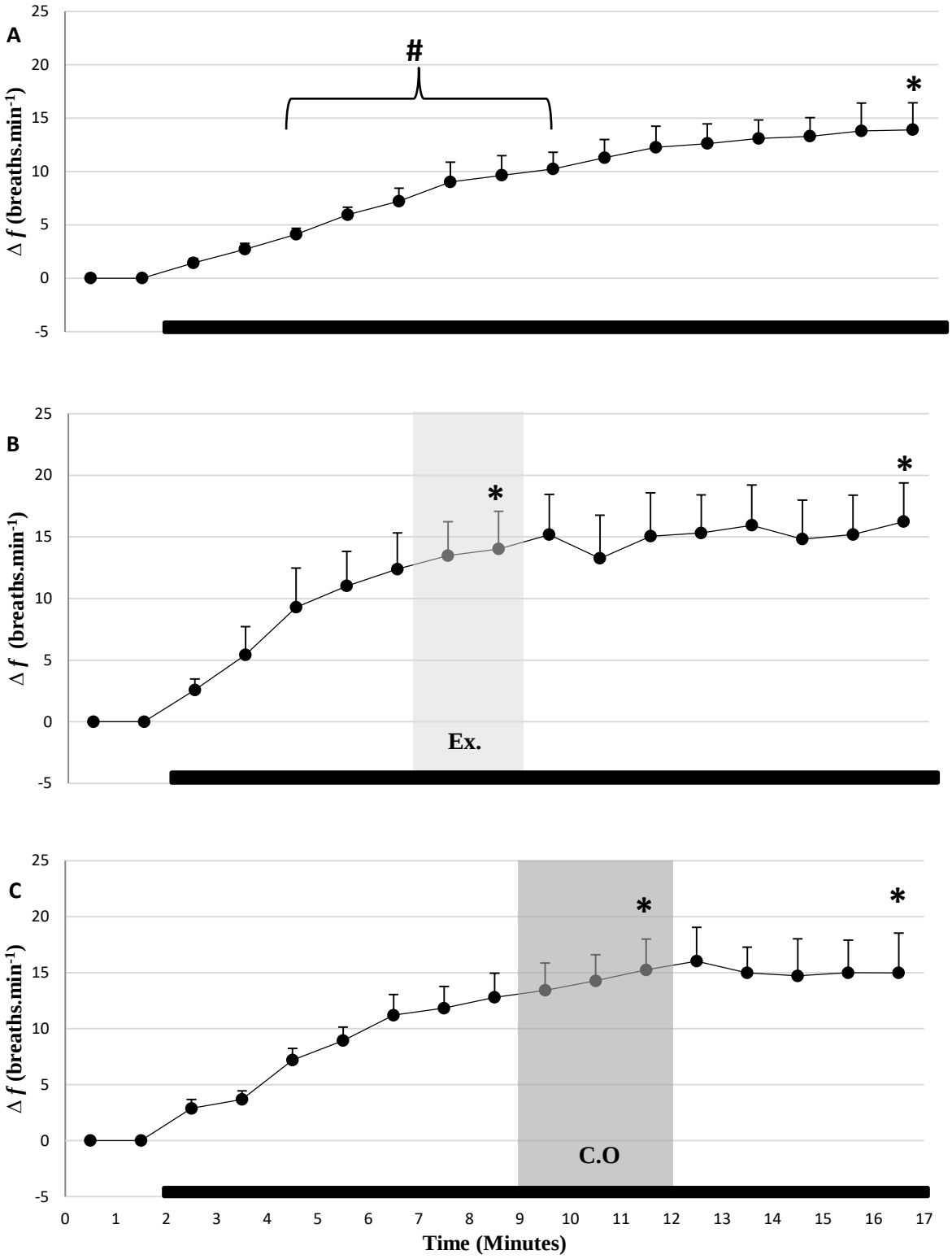


Figure 4.6: Mean changes in *f* from baseline in the 3 trials (A: CON trial; B: Ex. trial and C: C.O trial) (mean ± S.E.M). The black bar indicates hypercapnia. * Significant difference from baseline values ($p < 0.05$). # Significant difference in *f* between minute 5 and 10 ($p < 0.05$).

4.1.4. Discussion

This control study aimed to evaluate the ventilatory and cardiovascular responses to each component of the previous study protocol (Chapter 3) alone. The main findings are that exposure to hypercapnia in all 3 trials resulted in an increase in the cardiovascular response. This confirms earlier observations that breathing hypercapnic gas mixtures resulted in increased arterial blood pressure and HR (Kontos et al., 1968, Somers et al., 1989, Somers et al., 1991). These results could be attributed to chemoreflex activation, which is considered as a powerful activator of the sympathetic nervous system, causing an elevation in blood pressure. Somers et al. (1991) found that exposure to hypercapnia (7 % CO_2) alone for 5 minutes resulted in a significant increase in MSNA, which in turn was associated with a significant increase in mean blood pressure.

It has been consistently reported that performing isometric exercise elevates blood pressure. As expected, performing isometric exercise here (Ex. trial) (Figure 4.3: B) caused a progressive increase in the MAP, reaching ~35 mmHg above baseline during hypercapnia. When this increase in the MAP value during exercise was normalised to the last 2 minute pre-exercise resting values (to remove the effect of hypercapnia on the MAP), the MAP increased by ~ 26 mmHg. This magnitude of increase in blood pressure in the present study was higher than that similar to those previously reported at the same exercise intensity (50 % MVC), which was ~ 12 mmHg (Bruce and White, 2012). This inconsistency can be explained by differences in the experimental methodology; for instance, exercise duration (120 seconds in the present study vs. 90 seconds in previous study).

In the leg circulatory occlusion at rest (C.O) trial, there was an observed increase in the MAP above baseline. However, when examining the difference in MAP between the last 2 minutes of pre-occlusion (to remove the effect of hypercapnia on the MAP), and the last minute of occlusion period, it shows a small increase of about ~2.7 mmHg. This finding corroborates

the data of Rowell et al. (1976) and Bonde-Petersen et al. (1978), who reported small increases in blood pressure during brief leg occlusion at rest. They attributed this increase in blood pressure to a mechanical increase in total peripheral resistance, not due to stimulation of muscle metabosensitive afferents. However, even a small rise in blood pressure implies some permissive response by the baroreflex, which would normally regulate blood pressure around the resting operating point. Muscle afferents are now known to be able to reset the reflex to a higher operating point (see Raven et al.2006 for review) and it is, therefore, possible that some muscle afferent activation does occur in this resting situation. However, to initiate a muscle metaboreflex, there needs to be sufficient accumulation of metabolites which can take from 30 - 40 seconds, even during intense contraction, in animal experiments (see Kufaman and Forster 1996 for review). Thus, it is likely that a considerably longer period of time is required for this to happen in an occluded resting muscle and a progressively rising blood pressure response would be expected as metabolites accumulate. Muscle mechanoreflex activation could be triggered by thigh compression; however, rapid adaptation of this response is likely given the nature of the mechanoreceptor. Again, this does not match the profile of the small blood pressure increase observed.

The other main finding of the present study was that exposure to hypercapnia resulted in a progressive increase in minute ventilation in all 3 trials over a longer period than expected, which appears to be a form of ventilatory long-term facilitation (Reynolds et al., 1972, Gerst III et al., 2010, Griffin et al., 2012). In the CON trial (Figure 4.4: A), minute ventilation progressively increased throughout the protocol. This present finding contradicts Harris et al. (2006), who reported that exposure to hypercapnia ($P_{ET}CO_2$ clamped at +5 mmHg above normal values) for 5 minutes did not change minute ventilation significantly from the initial hypercapnic baseline measurement. This difference between the present findings and those of Harris et al. could be due to the duration of exposure to hypercapnia. In this study,

participants were exposed to hypercapnia for 15 minute, while in the previous study they were only exposed for 5 minutes.

On the other hand, the present findings are in agreement with the findings of Griffin et al. (2012), who reported that exposure to hypercapnia ($P_{ET}CO_2$ set between 4 - 5 mmHg above normal value) for approximately 110 minutes resulted in a gradual rise in minute ventilation, which was significant when compared to hypercapnic baseline measurements. Taken together, these recent findings suggest that sustained hypercapnia for periods longer than 5 minutes does result in a progressive rise in minute ventilation, which was not expected at the outset of the experiments described in this thesis.

Another important observation in the CON trial (Figure 4.4: A) is that minute ventilation became more stable (the rate of rise declined) after 10 minutes of hypercapnia exposure. This is based on the significant difference in minute ventilation values between minute 5 and 10 of the protocol timeline. This finding contradicts the reports of Mateika and Sandhu (2011). The authors suggested that 5 to 7 minutes were required for minute ventilation to reach a steady state during hypercapnia. The finding from the CON trial here shows that minute ventilation was still progressively increasing during this time period (5 - 7 minutes). Therefore, it would seem that at least 10 minutes of hypercapnic exposure should be allowed before minute ventilation can be said to be at, or at least approaching, a steady state, upon which a new hypercapnic baseline measurement can be established.

In this study, isometric exercise of calf muscle under hypercapnic conditions resulted in an increase in the average minute ventilation of about 9.5 $l.min^{-1}$ (relative to the last 2 minutes of pre-exercise resting values) in the Ex. trial. This magnitude of minute ventilation increase was higher than reported by Bruce & White (2012, 2015), where the minute ventilation increase during a similar level of exercise was ~6 & 4.5 $l.min^{-1}$, respectively. These

differences in the magnitude of the minute ventilation increase between the present and previous studies are likely attributed to differences in exercise duration; 120 seconds in the present study vs. 90 seconds in the earlier studies.

Both Reynolds et al. (1972) and Griffin et al. (2012) reported that exposure to hypercapnia during rest resulted in a progressive increase in minute ventilation. This was observed in the present study, where minute ventilation progressively increased and did not fully recover to pre-exercise values after the cession of exercise; instead, it continued rising throughout the trial (Figure 4.4: B). Based on the observation of a similar rise in the control trial where no exercise took place, a form of ventilatory long-term facilitation, rather than a carry-over effect of the exercise, likely explains the continued rise in minute ventilation during the first 3 minutes following cession of exercise. This pattern of ventilatory response was also seen during the C.O trial, as during circulatory occlusion an increase in minute ventilation occurred of approximately 6 $L.min^{-1}$ relative to the last 2 minutes of pre-circulatory occlusion resting values. Again, the increase in baseline appears to be largely due to the influence of the duration of hypercapnia exposure, rather than the thigh compression and local circulatory occlusion per se as indicated by the small 1- 2 L rise in ventilation after cuff deflation.

Taken together, these present ventilatory findings suggest that 15 minutes of exposure to hypercapnia resulted in a brisk increase in ventilation, followed by a progressive gradual increase in minute ventilation, which equates to a drifting baseline (in terms of analysis of the response to an intervention during this period). It is clear that this will affect the measurement of any such response and, therefore, a method of measurement which takes into account this drift was required. This is explained in Part: B, below.

4.2 Part B: Correction of drifting baseline.

It is clear in Figures 4.4 (A, B and C) that the ventilatory responses to exercise and CO are superimposed upon a slight drift upwards in ventilation, with the rapid adjustment in ventilation to hypercapnia having already happened. In order to examine the true effect of the interventions, and correct for the minute ventilation drifting baseline, a linear regression line was created for each subject using the values of minute ventilation ($\dot{V}_{E.\ actual}$) for the last 2 minutes of resting period during hypercapnia (minutes 6 & 7) and the final 2 minutes of the hypercapnia trials (minutes 16 & 17). As an example, data in Figure 4.7 was used to calculate a linear regression line (Figure 4.8). From the slope and intercept of this line, the values of minute ventilation from minutes 6 to 17 were adjusted ($\dot{V}_{E.\ adjusted}$) (Figure 4.9). Then, the differences between the $\dot{V}_{E.\ actual}$ and $\dot{V}_{E.\ adjusted}$ were calculated to reveal the precise change in minute ventilation ($\Delta\ \dot{V}_E$) (Figure 4.10).

$$\Delta\ \dot{\mathbf{V}}_{\mathbf{E}} = \dot{V}_{E.\ actual} - \dot{V}_{E.\ adjusted}$$

This new method of calculation will be utilised in the upcoming study in this work and applied to the previous study (Chapter 3) for $\dot{V}_E$, V_T and *f*.

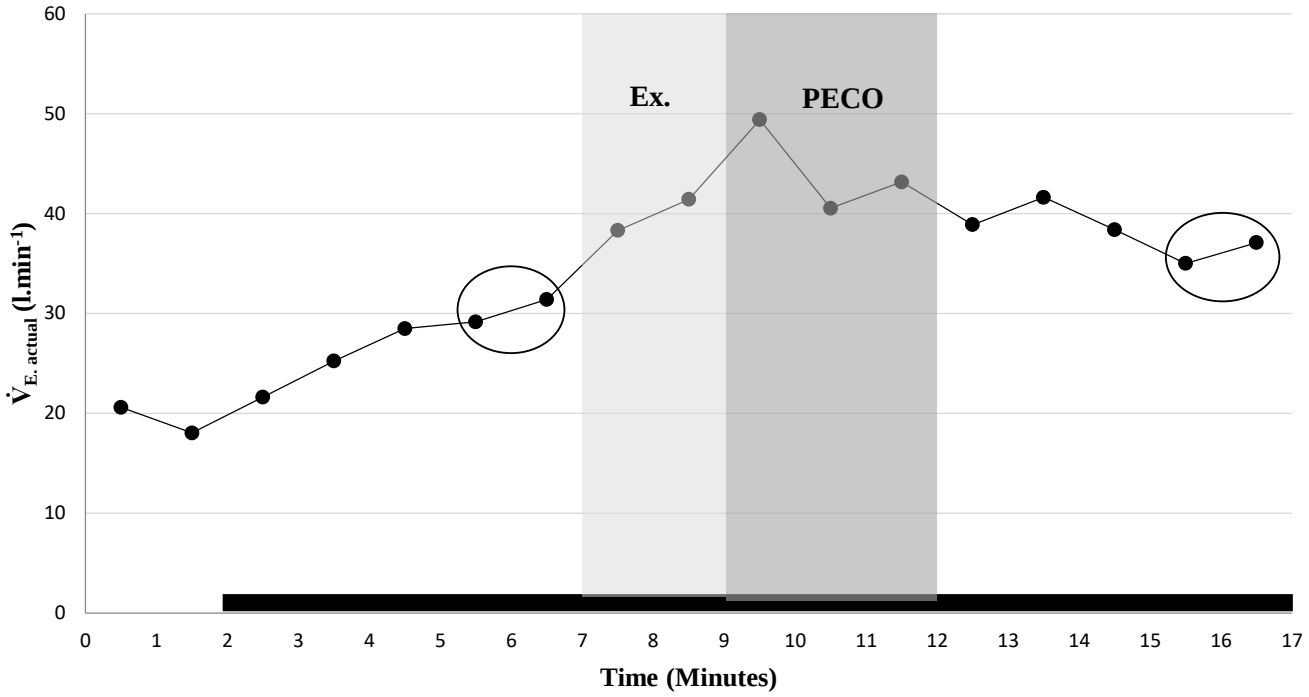


Figure 4.7: An example of the actual minute ventilation ($\dot{V}_{E\ actual}$) ($P_{ET}CO_2$ set at +7 mmHg) taken from 1 participant in previous study (Chapter 3). The light-shaded area indicates the Exercise (Ex.) period and the dark-shaded area indicates post-exercise circulatory occlusion (PECO) period. The circled values represent the actual value of $\dot{V}_E$ for the chosen minute to create the regression line. The black bar indicates hypercapnia.

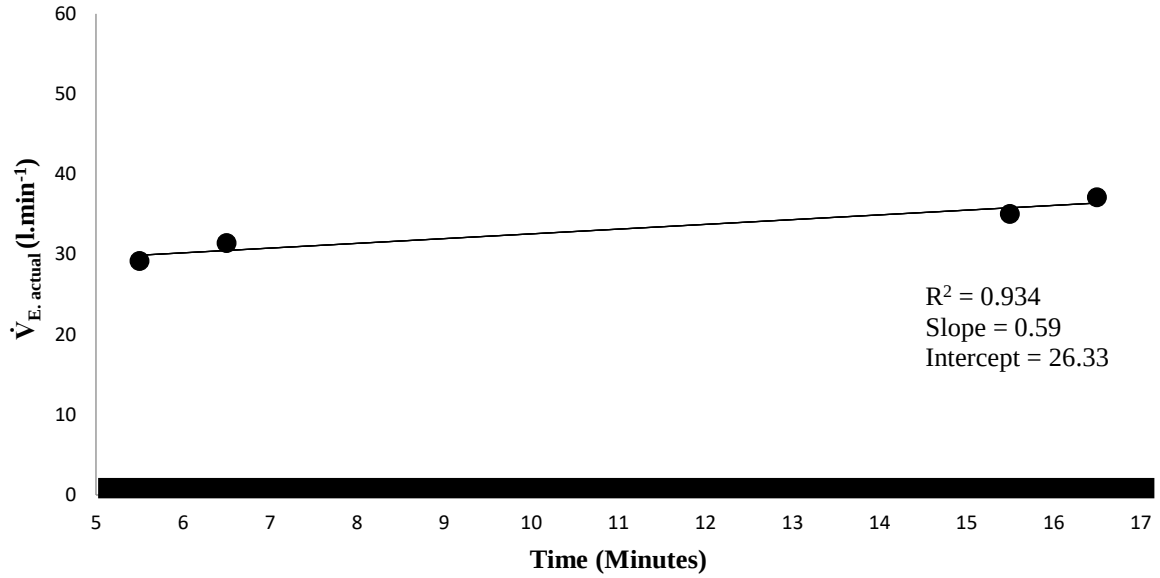


Figure 4.8: The linear regression line generated using the $\dot{V}_{E\ actual}$ values of minutes 6, 7, 16 & 17 and the slope and intercept values of this regression line. The black bar indicates hypercapnia.

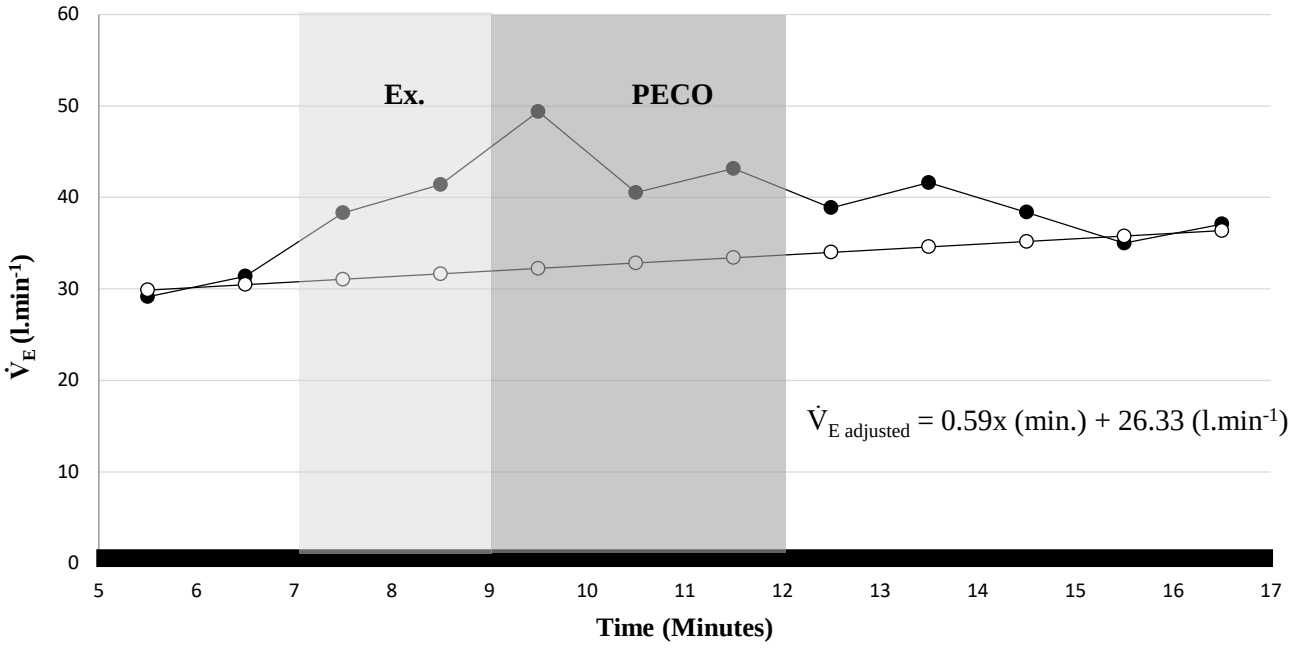


Figure 4.9: The actual values of minute ventilation ($\dot{V}_{E.\ actual}$) (closed circle) and calculated values of expected baseline ventilation in the absence of exercise and PECO ($\dot{V}_{E.\ adjusted}$) (open circle). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory Occlusion (PECO) period. The black bar indicates hypercapnia.

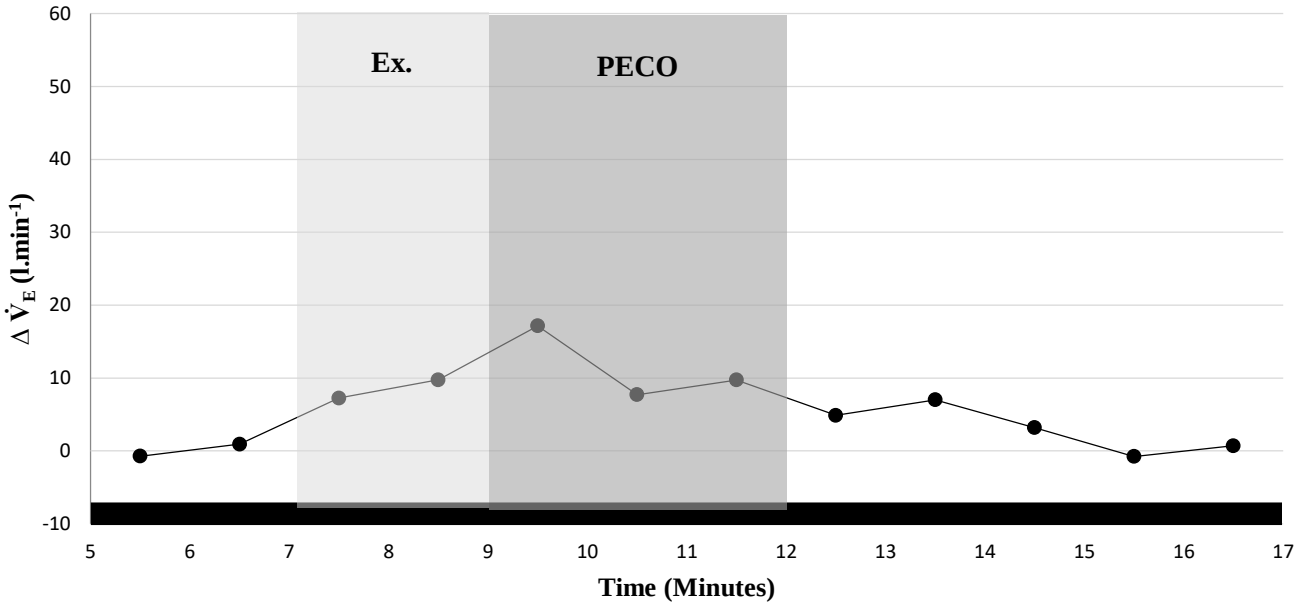


Figure 4.10: Mean changes in $\dot{V}_E$ during each period, corrected for the baseline shift between minutes 6, 7 and 16, 17 (mean ± S.E.M). The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia.

CHAPTER 5: THE VENTILATORY RESPONSE TO MUSCLE METABOREFLEX ACTIVATION DURING CONCURRENT HYPERCAPNIA IS ATTENUATED BY LOCAL MUSCLE TRAINING.

5.1. Introduction

Input from mechanically and metabolically sensitive thin fibre muscle afferents (group III and group IV) arising from exercising muscle contributes to driving exercise hyperpnea, aside from other neural control mechanisms, such as central command and the chemoreflex. For instance, studies discussed in the literature review (Chapter 1, section 1.6.3.2) have shown that complete abolition of muscle afferent feedback, with intrathecal administration of the opioid receptor agonist, fentanyl, resulted in significant attenuation of ventilatory and cardiovascular responses to exercise in healthy participants (Amman et al., 2010) and patients with disease (e.g. COPD and CHF) (Gagnon et al. 2012; Olson et al. 2014). Alternatively, other studies showed that activation of these muscle afferents (by PECO) in combination with stimulation of the ventilatory chemoreflex (by inhaling mild hypercapnic gas mixture) resulted in sustained increases in ventilation above the hypercapnic baseline during PECO (discussed in the literature review) (Lykidis et al., 2010, Bruce and White 2012), and this level of ventilation progressively increased with the level of concurrent hypercapnia exposure (Chapter 3). These latter findings support the idea that muscle afferents cause an increase in ventilation in a resting subject, in the presence of synergistic interactions from other inputs (e.g. the ventilatory chemoreflex) (Bruce and White, 2015). However, the following question is raised: if muscle afferent feedback mediates hyperpnea, would a training-induced attenuation of muscle afferent feedback reduce the ventilatory response to a standardised test protocol? If so, this may have important implications for athletic training and exercise intolerance in patient groups. The oxygen cost of ventilation increases disproportionately as exercise approaches maximal levels, which are often experienced by athletes in training and competition, particularly in women (see Sheel et al., 2016 for review). Furthermore, the increasing competition between respiratory musculature and locomotor muscles for cardiac output as the exercise intensity rises is seen as a major dilemma for the system by integrative

physiologists (Sheel et al., 2018). In addition, perception of effort is partly related to respiratory sensations, increasingly so in old age (Jensen et al., 2011), possibly deterring many from undertaking exercise. Therefore, even a small reduction in ventilation during exercise, whilst maintaining gas exchange might be beneficial across a wide range of the population.

It is well established that local muscle training can alter muscle afferent feedback. For instance, Fisher and White (1999) demonstrated that one-legged training of the calf muscles (calf rises for 6 weeks) resulted in attenuation of the PR of the trained leg during sustained voluntary and electrically evoked isometric muscle contraction (reduced by 28 and 27 %, respectively), and during the subsequent PECO periods. Meanwhile, in the contralateral untrained leg, there were no changes in the PR response to electrically evoked muscle contraction and PECO following exercise training. However, during voluntary contraction of the untrained leg, following the training period, there was attenuation of the PR by 24 % during voluntary contraction, with no effect of training on the PECO period. This attenuation in the rise of the PR during the PECO period in the trained legged in the 2 exercise conditions following exercise training provided evidence that exercise training has the ability to attenuate the muscle metaboreflex in the trained leg.

The data here also supports the idea of adaptation of central command after training, as reduction in the PR during *voluntary* contraction of the untrained leg was not seen in electrically evoked exercise of the limb, where central command was absent. This reduction in the PR in both limbs following training indicated the presence of a cross-over effect of exercise training from the trained leg to the untrained leg. However, whether this effect occurred at a local segmental level as a result of the training, or it represented general alteration in central command, was unclear as no comparison testing of muscle in the upper limb were performed.

The present study followed the same exercise training programme as previously used in Fisher and White (1999) study, which showed attenuation in muscle afferent feedback following exercise training. The first part of the present study aims to investigate the effect of attenuation in muscle afferent feedback in the trained leg on the ventilatory and cardiovascular responses to muscle metaboreflex activation (by PECO), combined with stimulation of the ventilatory chemoreflex (by inhaling mild hypercapnic gas mixture). The study also aims also to evaluate the cross-over effect of exercise training on the contralateral untrained leg during leg isometric exercise. In the second part of the study, a further investigation of the precise level of any ascending or cross-over effect of exercise training (segmental level: spinal cord; or central level: cardiorespiratory control centre) was carried out by examining the ventilatory and cardiovascular responses to exercise and activation of the muscle metaboreflex (by PECO) under mild hypercapnia in the right forearm muscles, by hand grip testing on the trained side. In addition, to extend the previous observations of Fisher and White of a cross-over effect from the trained to the untrained limb, hand grip tests were carried out here on both untrained hands under room air conditions.

5.2. Methods

The inclusion criteria for this study were: male participants aged from 18 to 40 years. Participants with right dominate leg were recruited to take part in this study. On the other hand, participants who had recent surgical intervention (< 3months), or musculoskeletal, neurological, respiratory and/or cardiovascular problems was excluded from this study.

With local ethical committee approval, 11 healthy male participants (Age: 19.5 ± 0.8 yrs; height: 175 ± 7.3 cm; body mass; 70.5 ± 8.2 kg; mean ± SD) from the University of Birmingham student population took part in this study. They received written and verbal information regarding the experimental protocols and procedures, while remaining blind to the exact purpose of the study. All participants completed a general health questionnaire prior to giving informed written consent. They were then habituated to the experimental procedures, according to the *Declaration of Helsinki*. Participants were instructed to refrain from consuming food and caffeinated substances for 4 hours before testing. In addition, they were instructed to avoid consuming alcohol or performing strenuous physical activity for 12 hours before testing.

In this study, participants performed two experimental protocols, with the protocol trials performed in randomized order. The first experimental protocol was a leg protocol, which was conducted on both legs. This was designed to examine the effect of single calf exercise training on the ventilatory and cardiovascular responses to activation of the muscle metaboreflex under room air and hypercapnia of the trained and untrained legs. Moreover, this study aimed to evaluate the potential cross-over effect of exercise training from the trained leg to the contralateral untrained leg. The second protocol (hand protocols) was designed to determine the level of any cross-over effect of exercise training (segmental or central).

5.2.1 Experimental protocol procedures

A) Leg protocol procedures

Participants were seated in the leg isometric dynamometer and instrumented as described in the general methods (Chapter 2, section 2.1.4 and 2.2.1).

Leg trials

In the leg protocol, every participant performed, at random, 2 trials of 22 minutes on each leg, one under room air condition (control) and the other under mild hypercapnia (experimental) before and after a 6 week exercise training programme of the dominant (right) leg (Figure 5.1). These 4 trials were performed in randomized order ,and over two separate visits to the laboratory, where 2 trials (with a 45 minute resting period in between) were conducted per a day.

During the initial 2 minutes of the leg trials, participants rested in the isometric leg dynamometer and breathed room air through the mouthpiece. Then, participants were exposed to a mild hypercapnic gas mixture ($P_{ET}CO_2$ clamped at +10 mmHg, above resting value) while resting for a further 10 minutes (experimental trial), or continued breathing room air, while resting, for another 10 minutes (control trial). $P_{ET}CO_2$ was clamped at +10 mmHg above resting value, based on observations from Chapter 3 (Figure 3.9), which showed a large magnitude of increase in $\dot{V}_E$ during activation of muscle metaboreflex during the PECO period. On minute 12 under both conditions, participants began to perform sustained isometric calf muscle contraction at 50 % MVC (pre-trial measured relative to the day of testing) for 2 minutes. Fifteen seconds before cessation of exercise, a cuff wrapped around the exercising thigh was inflated rapidly to 200 mmHg using the rapid inflator system. The cuff remained inflated for 3 minutes. During last minute of the PECO period (3rd minute), the

pain level was measured by placing a pain scale (see appendix A) in front of the hand of the participant, who then pointed to their pain level. Following these 3 minutes of PECO, the cuff was deflated, to allow restoration of blood flow to the exercised muscle, and participants rested for a further 5 minutes (recovery).

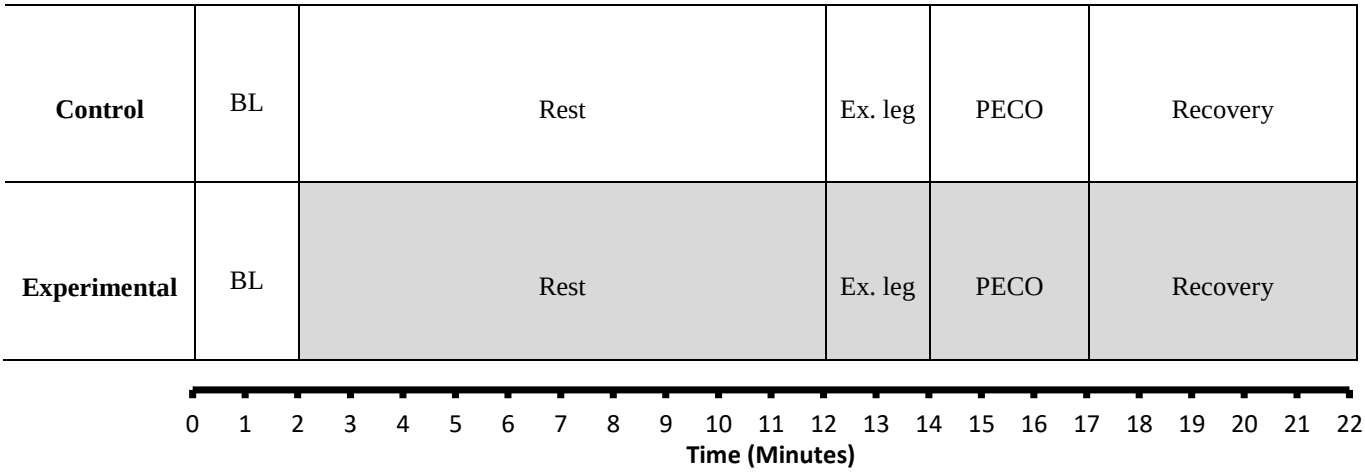


Figure 5.1: Schematic diagram of the leg protocol. The white area indicates inspiration of room air. The shaded area indicates inspiration of mild hypercapnic gas mixture ($P_{ET}CO_2$ clamped at + 10 mmHg, above resting value). BL, Baseline period, Ex, Exercise period (at 50% of MVC), PECO, post-exercise circulatory occlusion period.

B) Hand protocol procedures

A sub-group of 7 participants performed protocols A and B of the hand trials. These two hand protocols were conducted on separate days and carried out as described in Chapter 2 (sections 2.1.4 and 2.2.2).

1) Hand trial (Protocol A)

Protocol A of the hand trials is depicted in Figure 5.2. This protocol was performed on the dominant (right) hand for 22 minutes under room air (control) or hypercapnic (experimental) conditions, before and after exercise training of the dominant (right) leg. The two hand trials of Protocol A were conducting on the same day with 45 minutes apart, the same as the leg trial protocol. During the first 2 minutes of the hand trial, participants were seated and breathed room air via the mouthpiece (nose clamped) to establish a cardiovascular and ventilatory baseline. Following the first 2 minutes, participants were exposed to a mild hypercapnic gas mixture ($P_{ET}CO_2$ clamped at +10 mmHg, above resting value) for 10 minutes while resting (experimental), or continued to breathe room (control) air for another 10 minutes. On minute 12 of the hand protocol (A) timeline, participants started to perform the isometric handgrip exercise for 2 minutes at 50 % MVC (pre-trial measured relative to the day of testing). Fifteen seconds before exercise cessation, a cuff wrapped around the upper arm of the exercising hand was inflated up to 200 mmHg by using the rapid cuff inflator. The cuff remained inflated for 3 minutes, then circulation was restored and participants continued resting for 5 minutes (recovery).

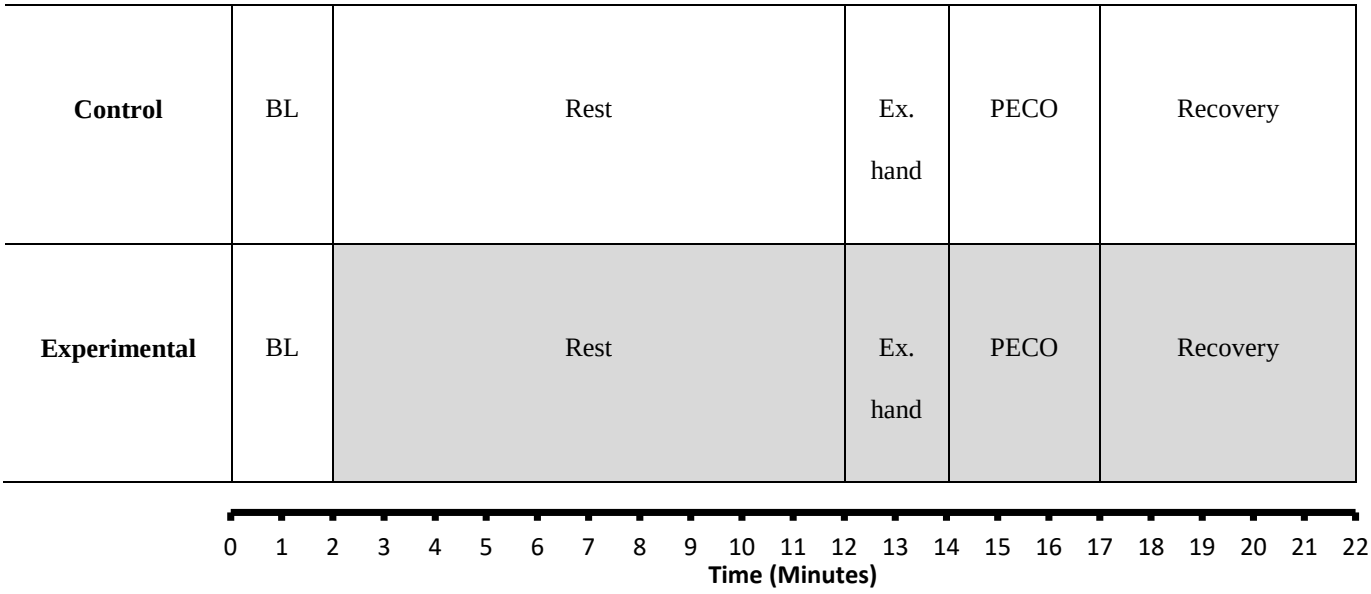


Figure 5.2: Schematic diagram of the right hand trial (Protocol A). The white area indicates inspiration of room air. The shaded area indicates inspiration of mild hypercapnic gas mixture ($P_{ET}CO_2$ clamped at + 10 mmHg, above resting value). BL, Baseline period, Ex, Exercise period (at 50% of MVC), PECO, post-exercise circulatory occlusion period.

2) Hand trial (Protocol B)

The same sub-group of participants also performed Protocol B of the hand trials (Figure 5.3). This protocol was performed on each hand (right and left) for 8 minutes under room air only. Hand protocol B trials were conducted on a different day from the hand Protocol A trials, with a 45 minute resting period between each trial, as previously. Figure 5.3 depicts the experimental hand protocol (B). In the first 2 minutes of the trial, the cardiovascular and ventilatory baselines were measured during rest. Then, participants performed isometric handgrip exercise at 30 % MVC (pre-trial measured relative to the day of testing) for 2 minutes, followed by 2 minutes of upper arm circulatory occlusion. On completion of the 2 minute PECO period, the cuff was deflated and participants rested for 2 minutes (recovery).

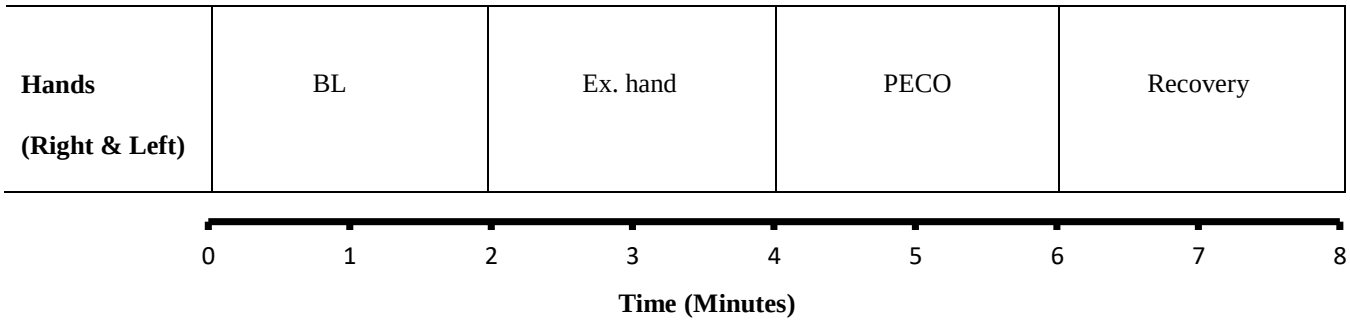


Figure 5.3: Schematic diagram of both hands trial (Protocol B) during inspiration of room air. BL, Baseline period, Ex, Exercise period (at 30% of MVC), PECO, post-exercise circulatory occlusion period.

5.2.3 Measurements

In all leg and hand trials, the ventilatory and cardiovascular responses were measured as described in Chapter 2 (section 2.3). Then, mean average values for $\dot{V}_E$, V_T, *f*, HR and MAP were calculated during each minute

Blood lactate levels were measured at the end of the leg trials (approximately 5 minutes after releasing the thigh cuff) using finger pricking and a Lactate analyser (Biosen C-Line Clinic; EKF Diagnostics for life, Cardiff, UK

5.2.4 Exercise training programme

All participants enrolled in the exercise training programme had completed the initial experimental protocols. The exercise training programme was described in Chapter 2 (section 2.2.4).

5.2.5 Statistical analysis

The Kruskal-Wallis H test was used to determine significant differences in ventilatory and cardiovascular responses in the last minute of each period (where the steady state was achieved) in all experimental protocols. If a statistical significance between these periods was detected, a Wilcoxon's signed-rank test was used to compare the pre-exercise baseline values and the last minute of each period.

A summary measurement was calculated (area under the curve) (Matthews et al., 1990) during the exercise and PECO periods of each trial in all experimental protocols (leg and hand). The before- and after-exercise training values of the ventilatory and cardiovascular responses for the same period in the same trial were compared using Wilcoxon's signed-rank test. Data is expressed as mean ± S.E.M and statistical significance was assumed when $p \leq 0.05$. All statistical analysis was conducted using a standard statistical package (version 22.0, SPSS, Chicago, IL, USA).

5.3. Results

A) Results of the leg protocols

Eleven participants completed all six weeks of the dominant leg (right) exercise training programme successfully. In both conditions, MVCs were measured prior to each leg trial under room air. Table 5.1 shows that there was no significant difference in MVC before and after exercise training in each leg. Moreover, there was no significant difference in MVC between trained (right) and untrained (left) legs and between conditions ($p \geq 0.05$ for all). Measurement of blood lactate revealed that exercise training had no significant effect on lactate levels in each leg trial, between both legs and between conditions ($p \geq 0.05$ for all). The pain level at the end of PECO was scored by participants as 3 to 4 (mild to moderate) in both legs under both conditions, while exercise training had no significant effect on pain level ($p \geq 0.05$ for all).

Table 5.2 shows the average values that were recorded in minutes 11 & 12 of the leg protocol. These 2 minutes were considered as a new baseline (pre-exercise resting baseline), as most of cardiovascular and ventilatory responses in both conditions reach the steady state by that time. There were no significant differences in the mean resting values of cardiovascular (MAP and HR) and ventilatory ($\dot{V}_E$, V_T and f) values before and after training in each leg, and between legs, under both conditions. However, as expected, exposure to concurrent mild hypercapnia ($P_{ET}CO_2$ clamped at +10 mmHg, above resting value) resulted in significant increases in the resting cardiovascular and ventilatory values to above room air conditions ($p \leq 0.05$ for all). The mean $P_{ET}CO_2$ was 38.5 ± 0.2 mmHg and did not significantly change after the training programme.

Table 5.1: Measurement of MVC and blood lactate before and after the training programme (mean ± S.E.M).

	Room Air				**Hypercapnia**			
	Trained (Right)		**Untrained (Left)**		**Trained (Right)**		**Untrained (Left)**	
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***	***Pre***	***Post***
MVC (N)	1351.1	1342.5	1222.8	1232.6	1354.6	1365.9	1285.5	1276.2
Lactate (mmol.l^{-1})	2.13 ± 0.2	2.26 ± 0.2	2.93 ± 0.4	2.89 ± 0.4	2.38 ± 0.3	2.44 ± 0.3	3.33 ± 0.5	3.17 ± 0.6
Pain level (0-10)	3	3	3	3	3	3	4	4

Table 5.2: Values recorded during the last minutes of the pre-exercise resting period (minute 11 & 12) (Pre-exercise baseline) of each trial (mean ± S.E.M).

	Room Air				**Hypercapnia**			
	Trained (Right)		**Untrained (Left)**		**Trained (Right)**		**Untrained (Left)**	
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***	***Pre***	***Post***
MAP (mmHg)	95.4 ± 2.9	95.6 ± 2.5	94.3 ± 3.5	93.0 ± 2.2	103.3 ± 2.8[1]	100.8 ± 1.6[2]	104.7±2.5[1]	100.1±3.2[2]
HR (beats.min^{-1})	78 ± 3.8	76 ± 4.5	78.0 ± 4.3	76 ± 4.0	83 ± 4.4[1]	81 ± 3.9[2]	84 ± 3.8[1]	81 ± 4.5[2]
$\dot{V}_E$ (l.min^{-1})	17.0 ± 5.1	17.0 ± 0.9	18.0 ± 5.4	17.5 ± 0.8	76.9 ± 3.2[1]	76.0 ± 7.1[2]	77.6 ± 3.4[1]	73.0 ± 7.3[2]
$V_{T\,(L)}$	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	2.9 ± 0.9[1]	3.0 ± 0.2[2]	2.9 ± 0.15[1]	2.9 ± 0.2[2]
***f* (breaths.min^{-1})**	15.3 ±1.4	15.8 ±0.9	17.0 ±1.7	16.4 ±1.0	26.9 ±1.9[1]	25.5 ±2.0[2]	27.4± 2.2[1]	25.6 ±2.0[2]

1 $p \leq 0.05$ from pre- (Room Air). 2 $p \leq 0.05$ from post-exercise training (Room Air)

Blood pressure (leg protocol):

Figures 5.4 and 5.5 show the changes in the 15 second average MAP values from pre-exercise resting baseline levels, before and after the exercise training programme, for both the trained and untrained legs. As expected, similar PRs relative to the pre-exercise resting baseline under room air and hypercapnia were generated by sustained isometric exercise in both legs. This was followed by a reduction in MAP to a level remaining above pre-exercise baseline, and which remained stable during PECO in all trials. Following training, the responses in the trained leg were reduced during exercise and PECO, under both conditions, while there appeared to be a small reduction, in the untrained leg, in the MAP response to exercise; particularly, under hypercapnia. However, there was change in the PECO responses. When the changes in MAP were averaged over 1 minute periods for statistical analysis (Figure 5.6), it became clear that isometric exercise results in increased MAP to above pre-exercise resting baseline values by the 2nd minute of exercise, in both legs, before and after training. Values for the trained leg under room air and hypercapnia, before and after the training period, were: 28.8 ± 2.0 & 24.7 ± 1.6 and 24.3 ± 1.7 & 22.1 ± 1.5 mmHg, respectively ($p \leq 0.05$). In the untrained leg, these values were: 23.7 ± 2.4 & 22.1 ± 2.5 and 23.3 ± 2.2 & 20.4 ± 1.9 mmHg, respectively ($p \leq 0.05$). The fall in the MAP during PECO observed to be at a level significantly higher than pre-exercise resting baseline in both legs, under both conditions ($p \leq 0.05$ for all). Releasing the thigh cuff at the end of the PECO period resulted in the MAP returning to the pre-exercise resting baseline in both legs, under both conditions.

Within leg, pre- vs. post-exercise training comparisons of MAP

The changes in the MAP, described above, became much clearer after a summary measurement (area under the curve) was calculated (Figure 5.7). Following the exercise training period, the reduction in the rise of the MAP during exercise in the trained leg, when breathing room air, did not reach statistical significance ($p = 0.065$); however, under hypercapnia, the decrease in MAP was statistically significant (18.1 ± 1.4 vs. 15.6 ± 1.4 mmHg, $p \leq 0.05$). In the contralateral untrained leg, small reductions in the MAP response to exercise under both conditions did not reach statistical significance ($p \geq 0.05$). During the PECO period, the MAP responses were reduced significantly only in the trained leg, under room air and hypercapnia (16 ± 2.6 vs. 14.1 ± 2.2 and 16.1 ± 2.3 vs. 14.1 ± 2.1 mmHg, respectively, $p \leq 0.05$) following exercise training. In both conditions, the MAP values during exercise and PECO periods of the trained leg were greater compared to the untrained leg.

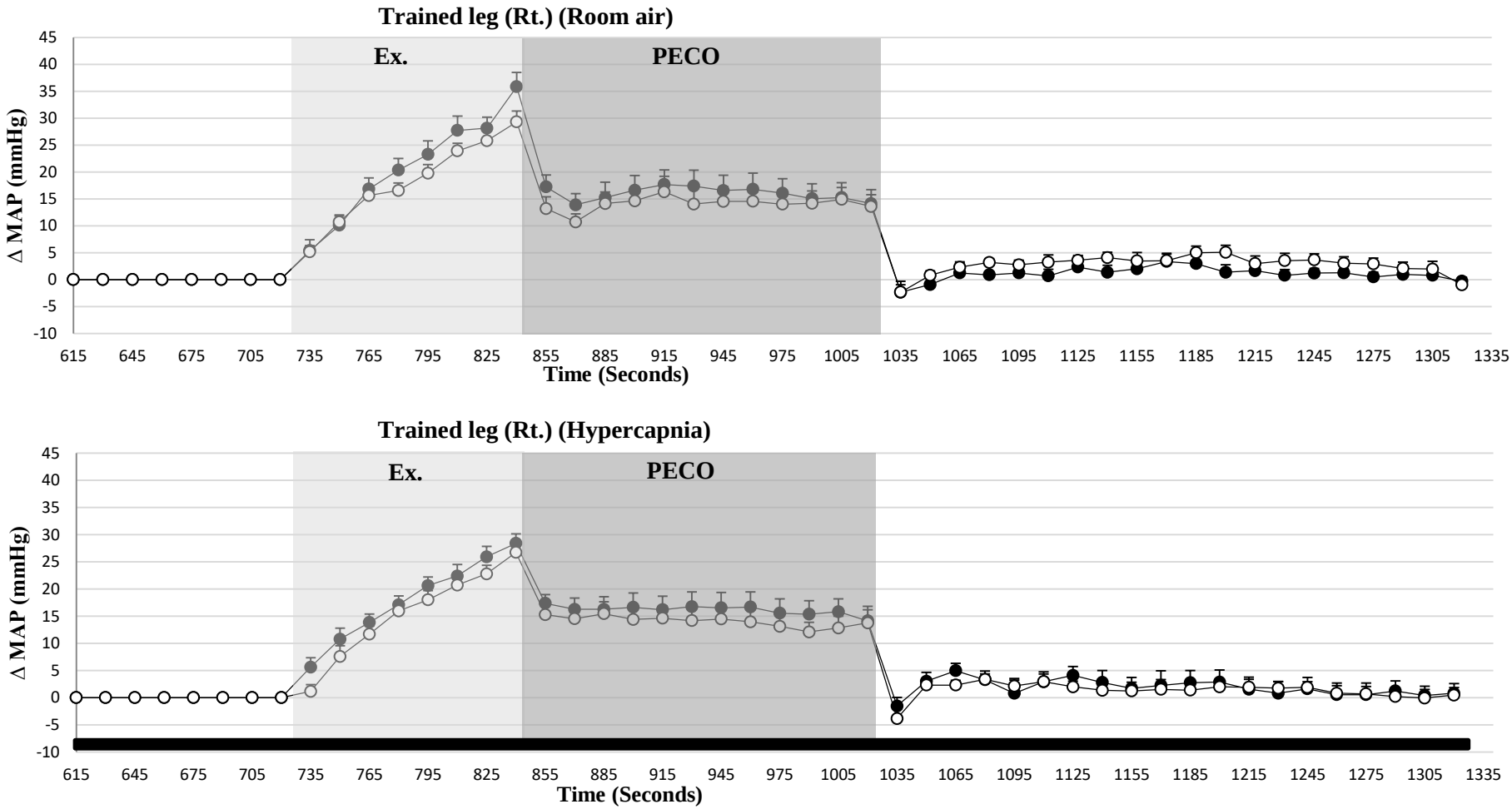


Figure 5.4: Mean changes in the MAP from the pre-exercise resting baseline of the trained leg (Rt.) under room air and hypercapnia (mean ± S.E.M) every 15 seconds. Closed circles indicate before and open circles indicate after the exercise training programme was completed. The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia.

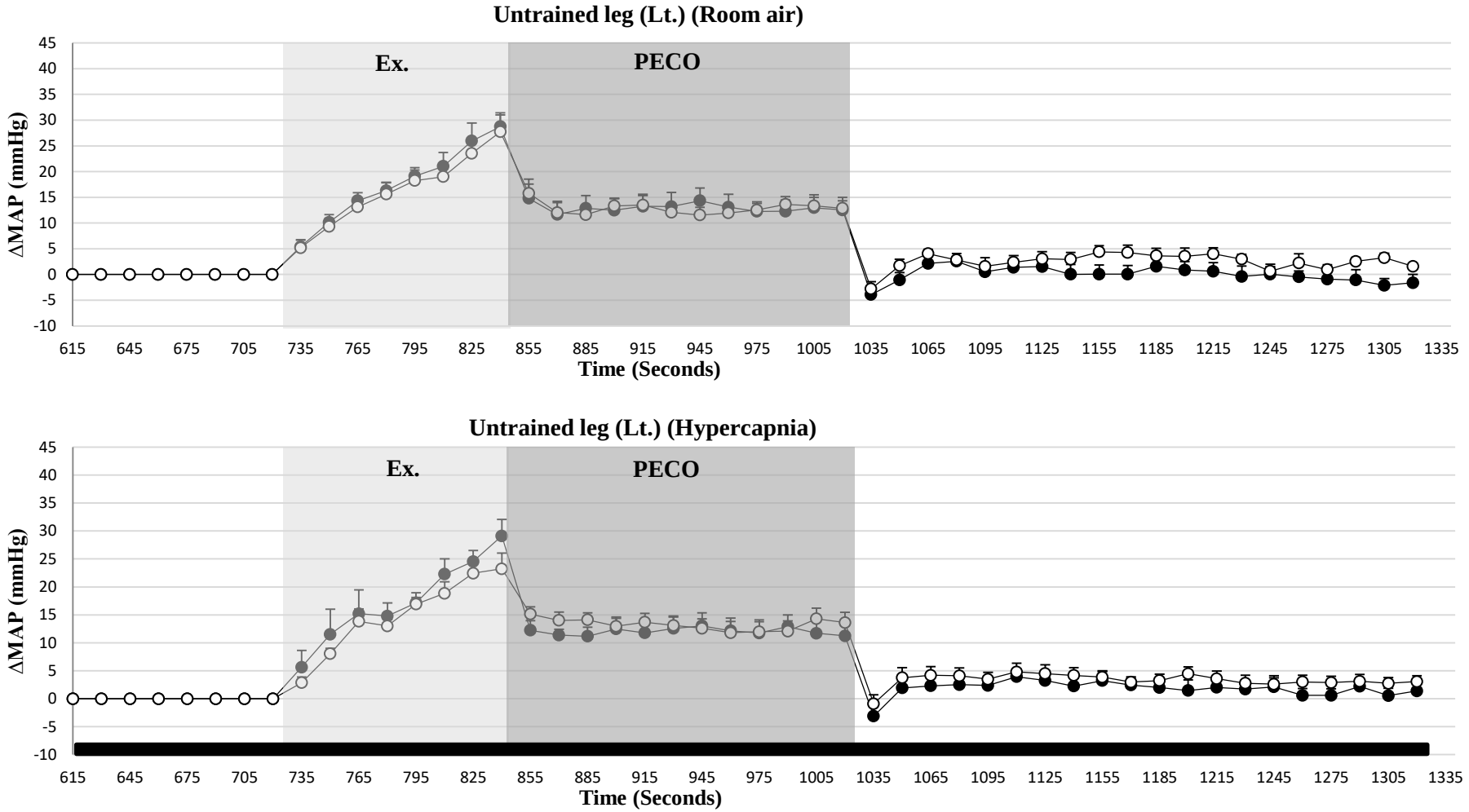


Figure 5.5: Mean changes in the MAP from the pre-exercise resting baseline of the untrained leg (Lt.) under room air and hypercapnia every 15 seconds (mean ± S.E.M). Closed circles indicate before and open circles indicate after the exercise training programme was completed. The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia.

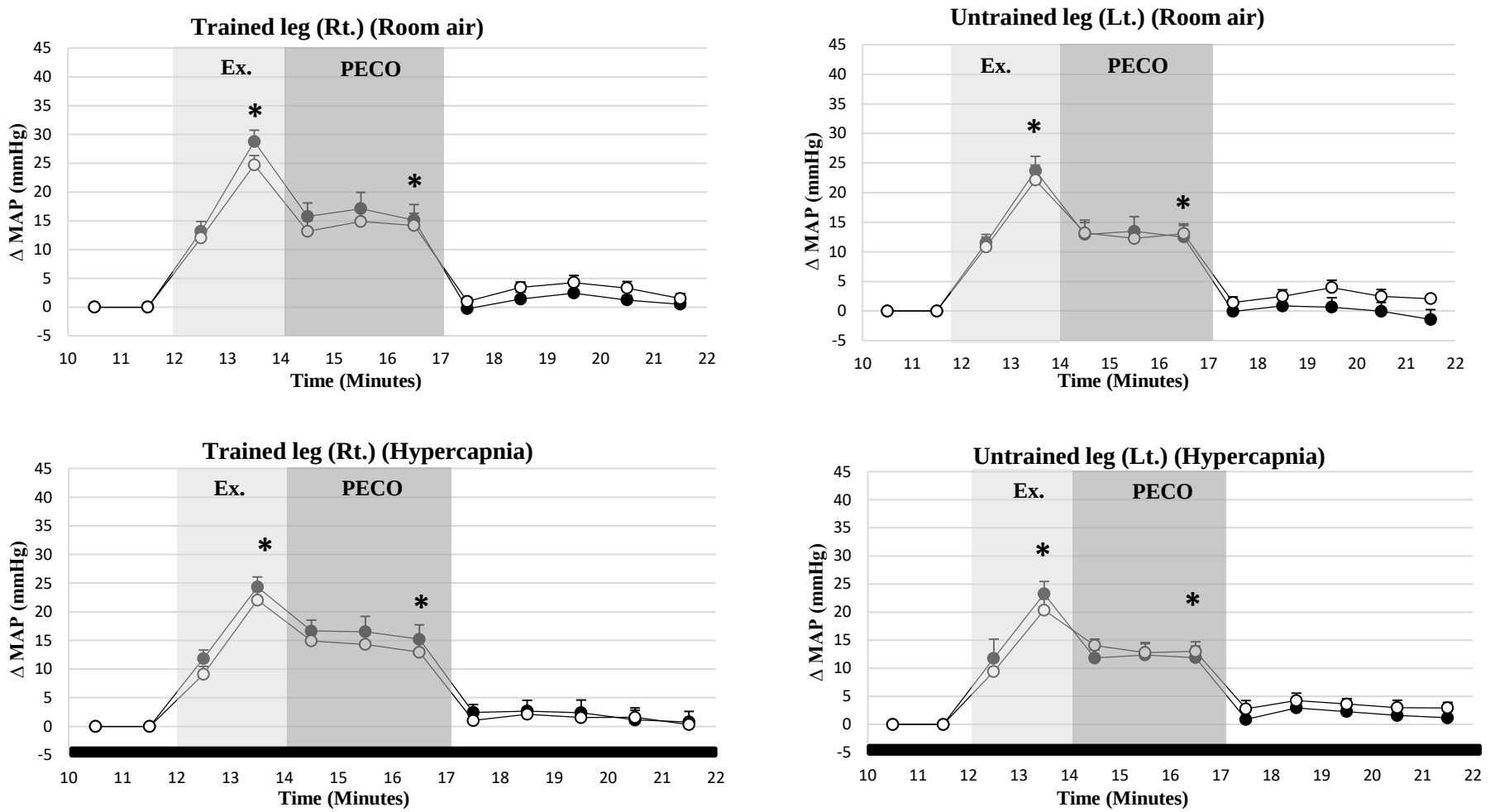


Figure 5.6: Mean changes in the MAP from the pre-exercise resting baseline of trained and untrained legs under room air and hypercapnia every minute (mean ± S.E.M). Closed circles indicate before and open circles indicate after the exercise training programme was completed. The lack bar indicates hypercapnia. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$).

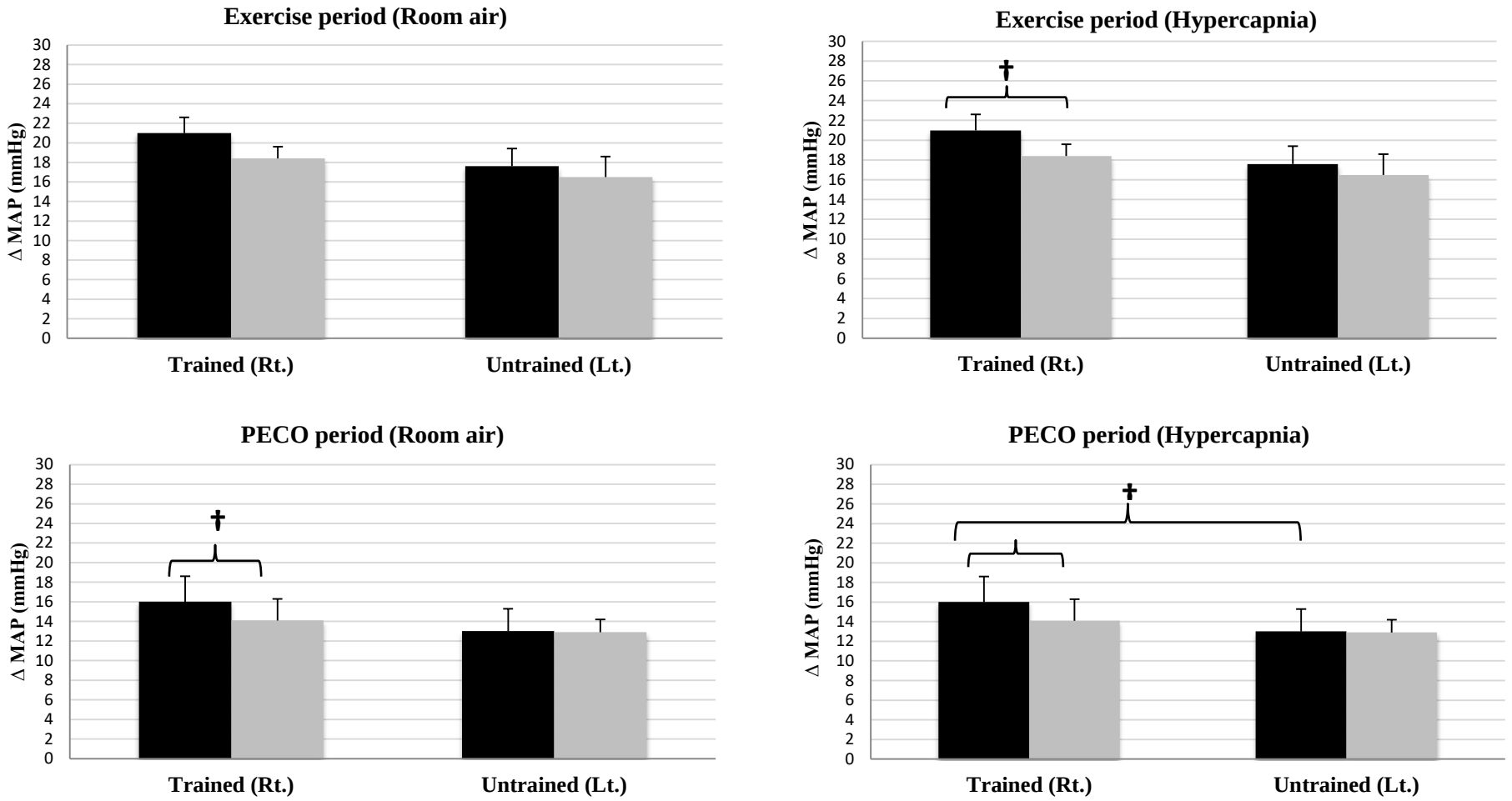


Figure 5.7: Summary measurements of the average changes in the MAP from the pre-exercise resting baseline of the trained and untrained legs over exercise and PECO periods, under room air and hypercapnia (mean ± S.E.M). Before (black bar) and after (grey bar) exercise training values are indicated. † Significant difference between values ($p \leq 0.05$).

Heart rate (Leg protocol):

Figure 5.8 and 5.9 show the changes, in 15 seconds, in the average HR values from pre-exercise resting baseline levels, before and after the exercise training programme, for both the trained and untrained legs. As expected, similar HR responses, relative to the pre-exercise resting baseline conditions under room air and hypercapnia, were generated through sustained isometric exercise in both legs. This was followed by a reduction in the HR almost to pre-exercise resting baseline during the PECO period in all trials. Following training, the HR response in the trained leg was slightly reduced during exercise under both conditions; however, it did not change in the PECO period. In the untrained leg, the HR response was not altered during exercise and PECO. The changes in HR were averaged over 1 minute periods for statistical analysis (Figure 5.10). It was clear that isometric exercise resulted in a significantly increased HR above pre-exercise resting baseline values by the 2nd minute of exercise, in both legs, before and after training ($p \leq 0.05$ for all). There were no significant differences in the HR, under room air and hypercapnia, before and after exercise of the trained leg ($p \geq 0.05$); similarly, in the untrained leg test, the HR did not significantly change before and after exercise, under both conditions ($p \geq 0.05$). It was also observed that the HR responses fell to the pre-exercise resting baseline in both legs, under room air, before and after exercise. However, under hypercapnia, the HR responses were shown to be slightly elevated above pre-exercise resting levels in both legs, before and after exercise ($p \geq 0.05$). Releasing the thigh cuff at the end of the PECO period resulted in a small transient increase in the HR (~ 5 beats.min^{-1}) in both legs, under both conditions, following which it returned to the pre-exercise resting baseline.

Within leg, pre- vs. post-exercise training comparisons of HR

Exercise training had no effect on the HR responses to isometric exercise and PECO in both the trained and untrained legs, under both conditions. Figure 5.11 more accurately depicts the changes in the HR before and after exercise, during exercise and during PECO periods displaying the summary measurements of these periods.

Exercise training had no significant effect on exercise-induced increases in HR in both legs, under both conditions ($p \geq 0.05$ for all); moreover, average changes in the HR over the PECO period showed no significant difference with exercise training in either leg, under both conditions ($p \geq 0.05$ for all).

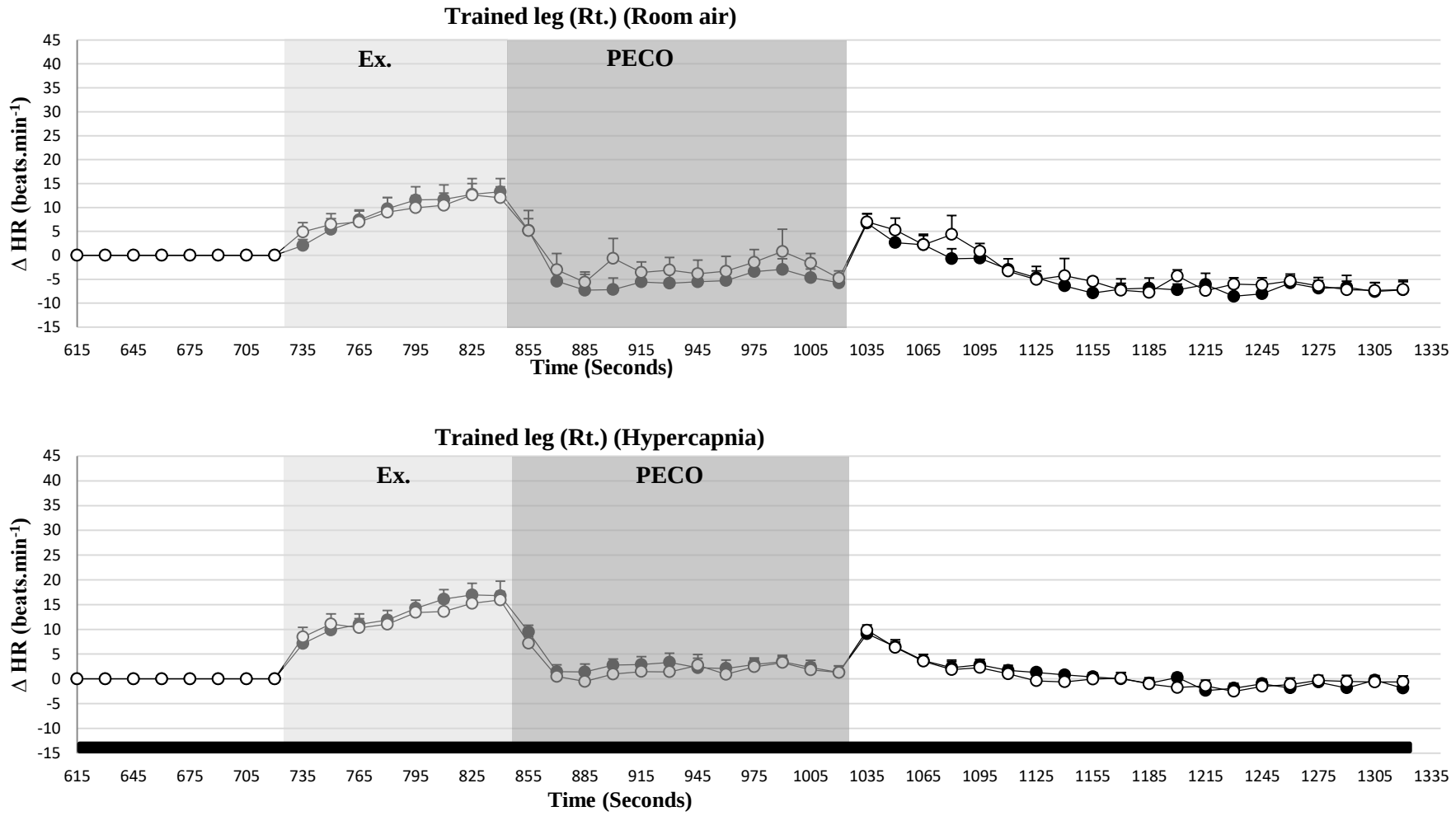


Figure 5.8: Mean changes in the HR from the per-exercise resting baseline of the trained leg (Rt.), under room air and hypercapnia, every 15 seconds (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia.

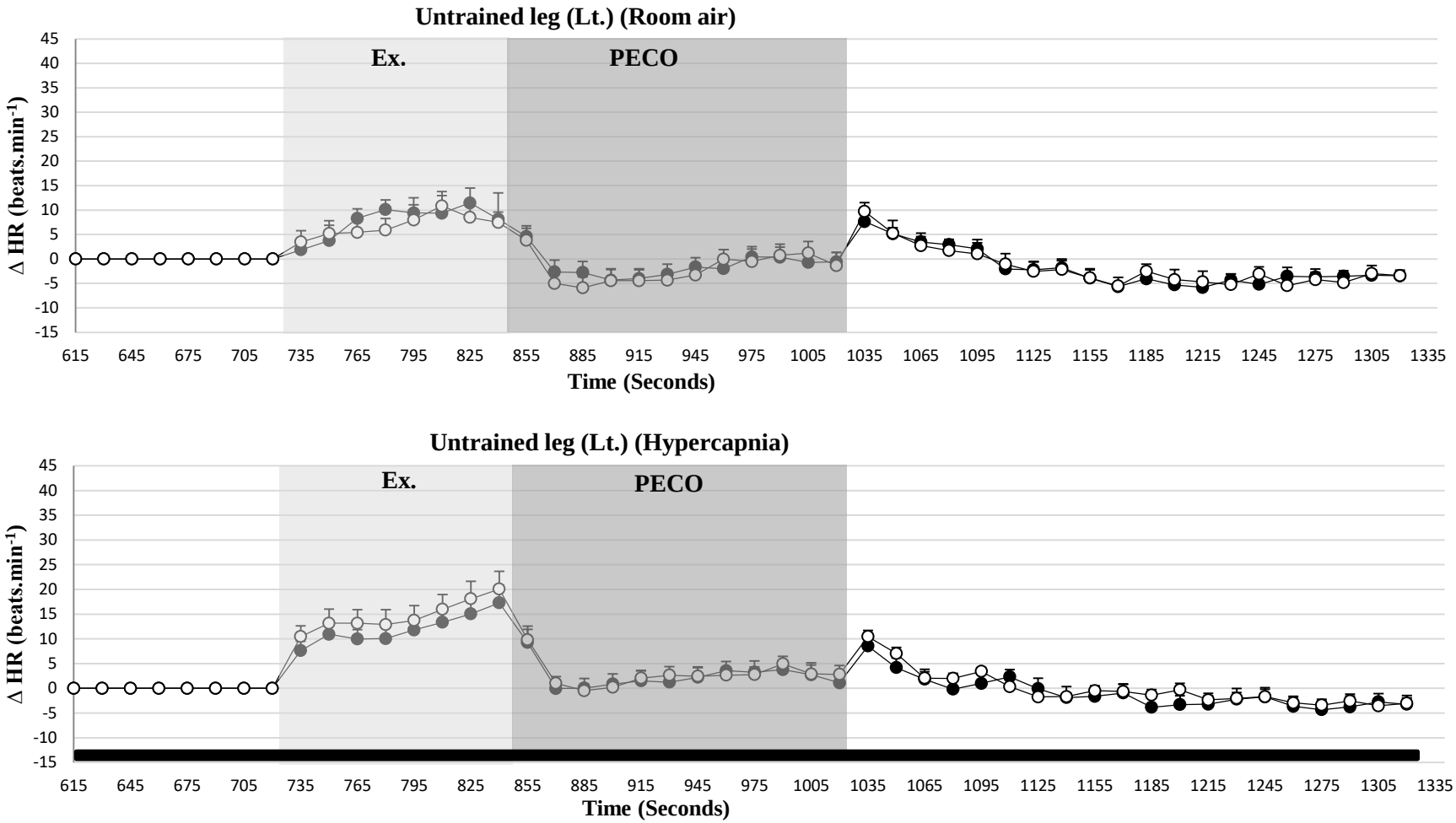


Figure 5.9: Mean changes in the HR from the per-exercise resting baseline of the untrained leg (Lt.), under room air and hypercapnia conditions, every 15 seconds (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia.

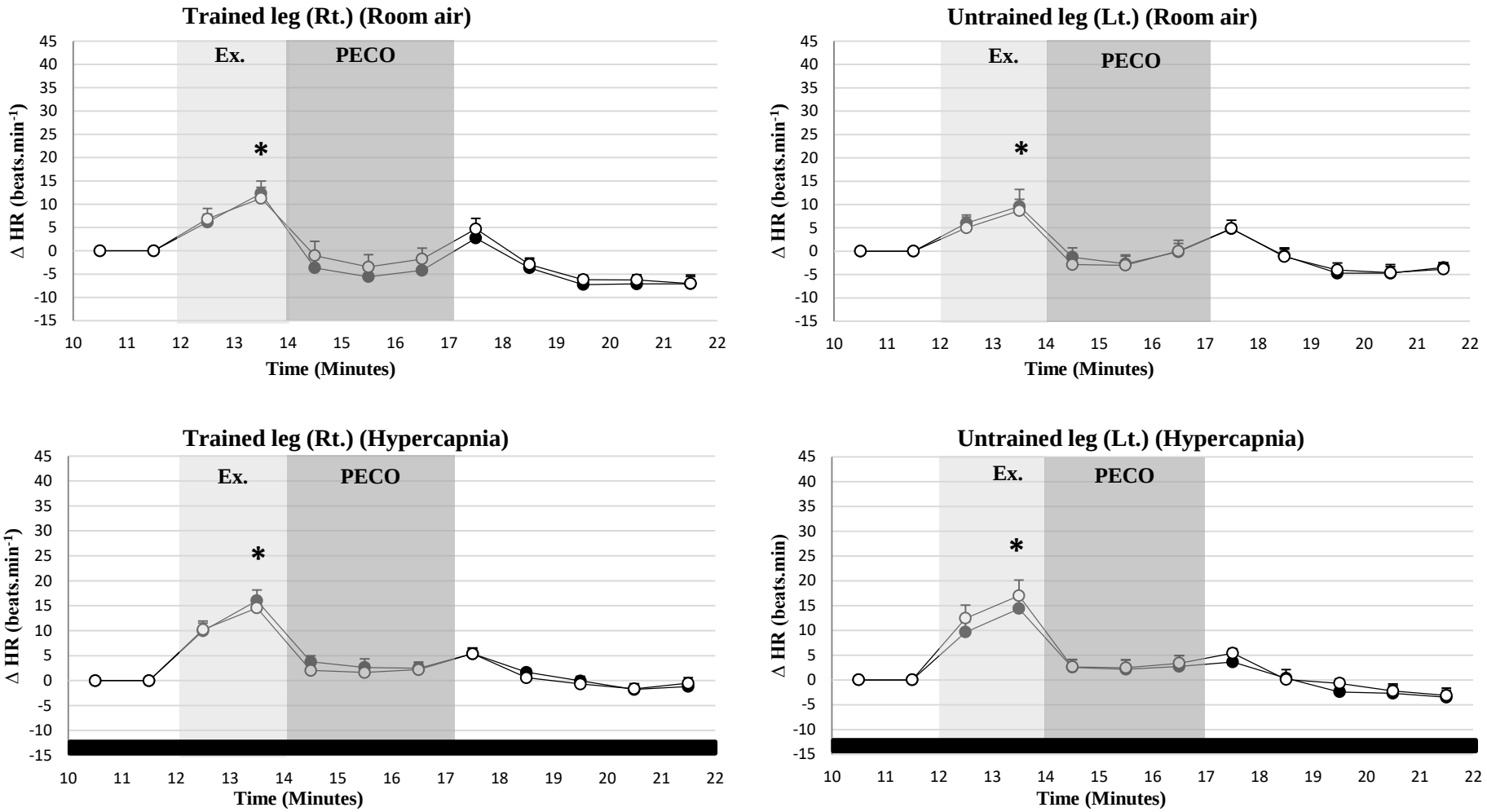


Figure 5.10: Mean changes in the HR from the pre-exercise resting baseline of the trained and untrained legs, under room air and hypercapnia, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The black bar indicates hypercapnia. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$).

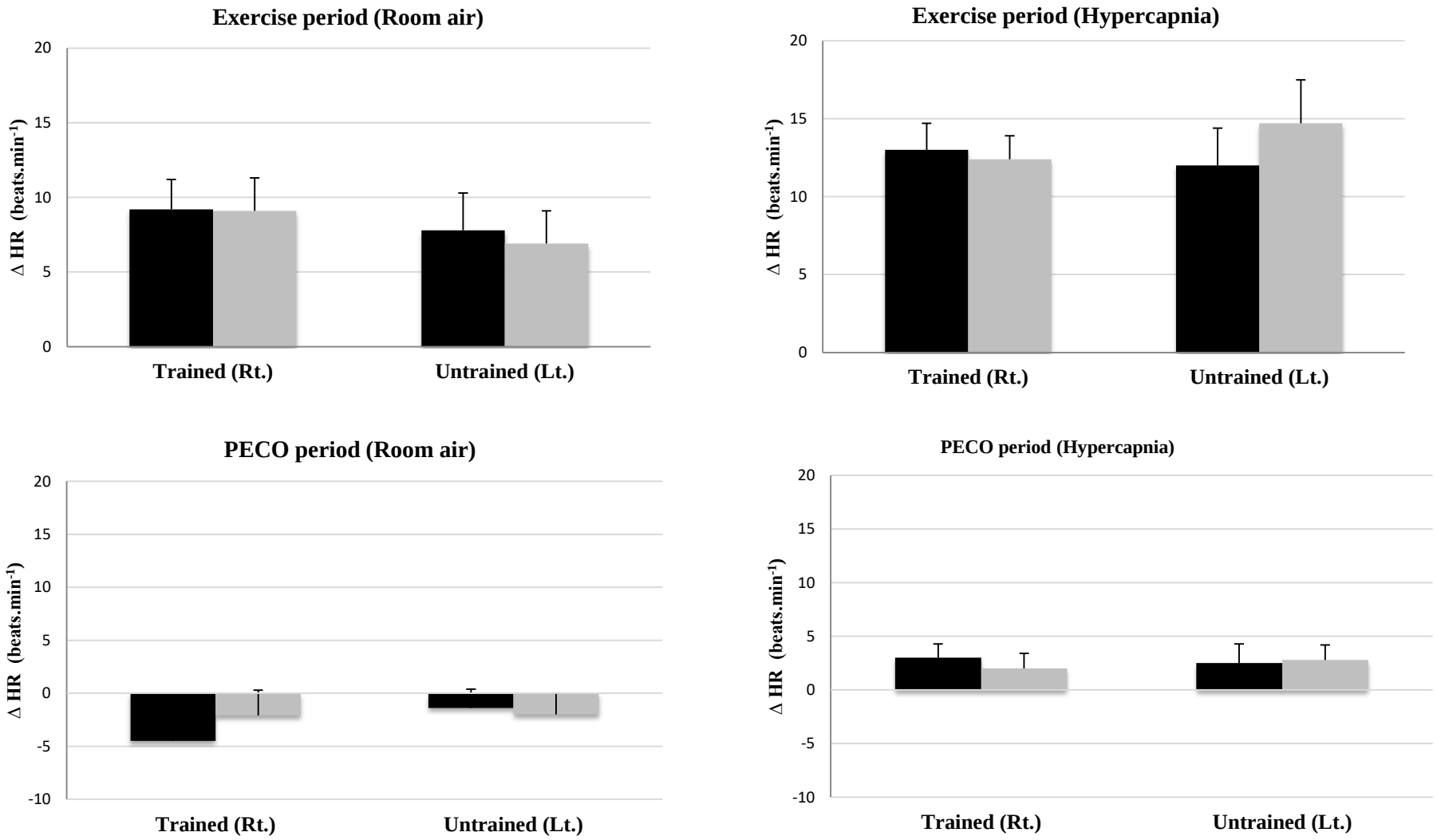


Figure 5.11: Summary measurements of the average changes in the HR from the pre-exercise resting baseline of the trained and untrained legs over exercise and PECO periods, under room air and hypercapnia, (mean ± S.E.M). Before (black bar) and after (grey bar) exercise training values are shown.

Minute ventilation (Leg protocol):

Figure 5.12 shows that, under room air, isometric exercise increased the $\dot{V}_E$ significantly during the last minute of exercise to above the pre-exercise resting level in both trials (before and after exercise training) of both the trained (8.5 ± 2.5 & 6.9 ± 1.1 l.min^{-1}, respectively, $p \leq 0.05$) and untrained (8.8 ± 2.7 & 7.4 ± 1.3 l.min^{-1}, respectively, $p \leq 0.05$) legs. This increase in $\dot{V}_E$ during exercise was further elevated in the first minute of the PECO period, before it dropped back close to pre-exercise resting baseline levels in both leg tests (all, $p \geq 0.05$). When circulation to the leg was restored in the recovery period, a small and transient increase in $\dot{V}_E$ was observed, followed by a fall to pre-exercise resting levels at the end of the trial. Following exercise training, there were small reductions in the $\dot{V}_E$ at the peak of the exercise period in both the trained and untrained legs. However, during PECO, training had no effect on $\dot{V}_E$ in either leg.

Under hypercapnia, the $\dot{V}_E$ observed during the last minute of exercise was significantly increased to above pre-exercise resting levels in both trials (before and after exercise training) in the trained (24.2 ± 3.9 & 16.2 ± 3.0 l.min^{-1}, respectively, $p \leq 0.05$) and untrained (22.2 ± 3.3 & 17.3 ±2.7 l.min^{-1}, respectively, $p \leq 0.05$) legs. Moreover, this increase in $\dot{V}_E$ was continued into the first minute of PECO period. This value then declined over the next 2 minutes of PECO, although, to a level remaining significantly greater than that during pre-exercise resting in both trials of the trained and untrained legs ($p \leq 0.05$ for all). When the thigh cuff was deflated in the recovery period, there was a small transient increase in the $\dot{V}_E$, followed by a progressive decline to pre-exercise resting levels. It is clear from Figure 5.12 that exercise training of the dominant leg resulted in attenuation of the $\dot{V}_E$ increase during exercise in the trained and untrained legs, under both conditions. However, during the PECO period, the $\dot{V}_E$ response was reduced following exercise training only in the trained leg. These

changes in $\dot{V}_E$ became more prominent after calculation of the summary measurements for the exercise and PECO periods.

Within leg, pre- vs. post-exercise training comparisons of $\dot{V}_E$

Figure 5.13 shows changes in the mean $\dot{V}_E$ values during exercise and PECO periods. During exercise, the mean average changes in the $\dot{V}_E$ increases were reduced in both the trained and untrained legs, under room air conditions. Under hypercapnia, the mean average changes in the $\dot{V}_E$ increases were significantly attenuated following exercise training in the trained and untrained legs (17.5 ± 2.8 vs. 12.1 ± 2.6 and 18.4 ± 2.9 vs. 14.4 ± 2.0 $l.min^{-1}$, respectively, $p \leq 0.05$ for all). Moreover, during circulatory occlusion following isometric exercise, the mean average increase in $\dot{V}_E$ was significantly attenuated after exercise training only in the trained leg and only under hypercapnia (17.5 ± 2.0 vs. 9.8 ± 2.1, $p \leq 0.05$) which equates to a 45 % reduction in the ventilatory response to PECO.

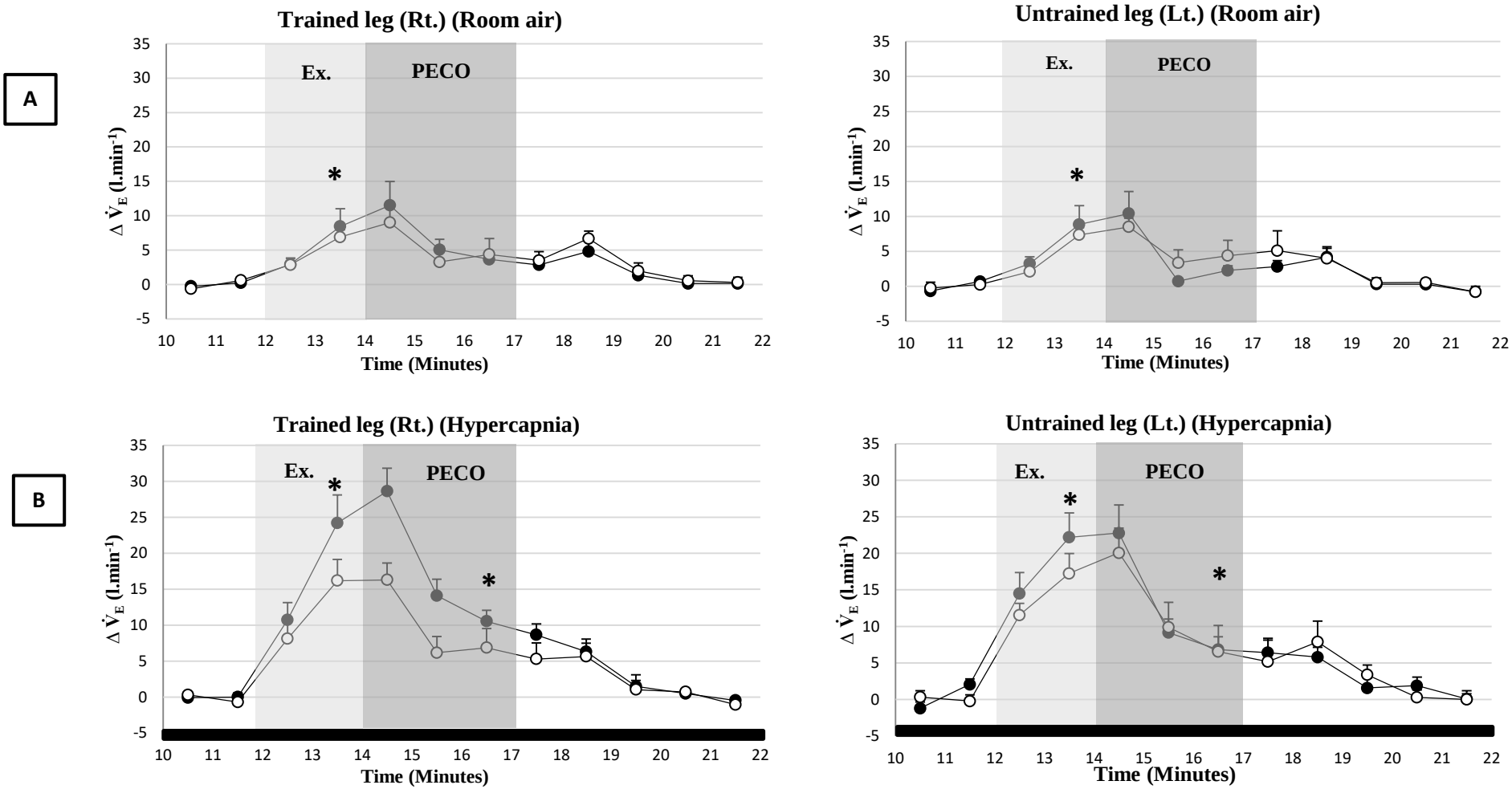


Figure 5.12: A) Mean changes in $\dot{V}_E$ from the pre-exercise resting baseline of the trained and untrained legs under room air. B) Mean change in $\dot{V}_E$ corrected for baseline shift (between minute 11, 12, 21 & 22) under hypercapnia (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The black bar indicates hypercapnia. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$).

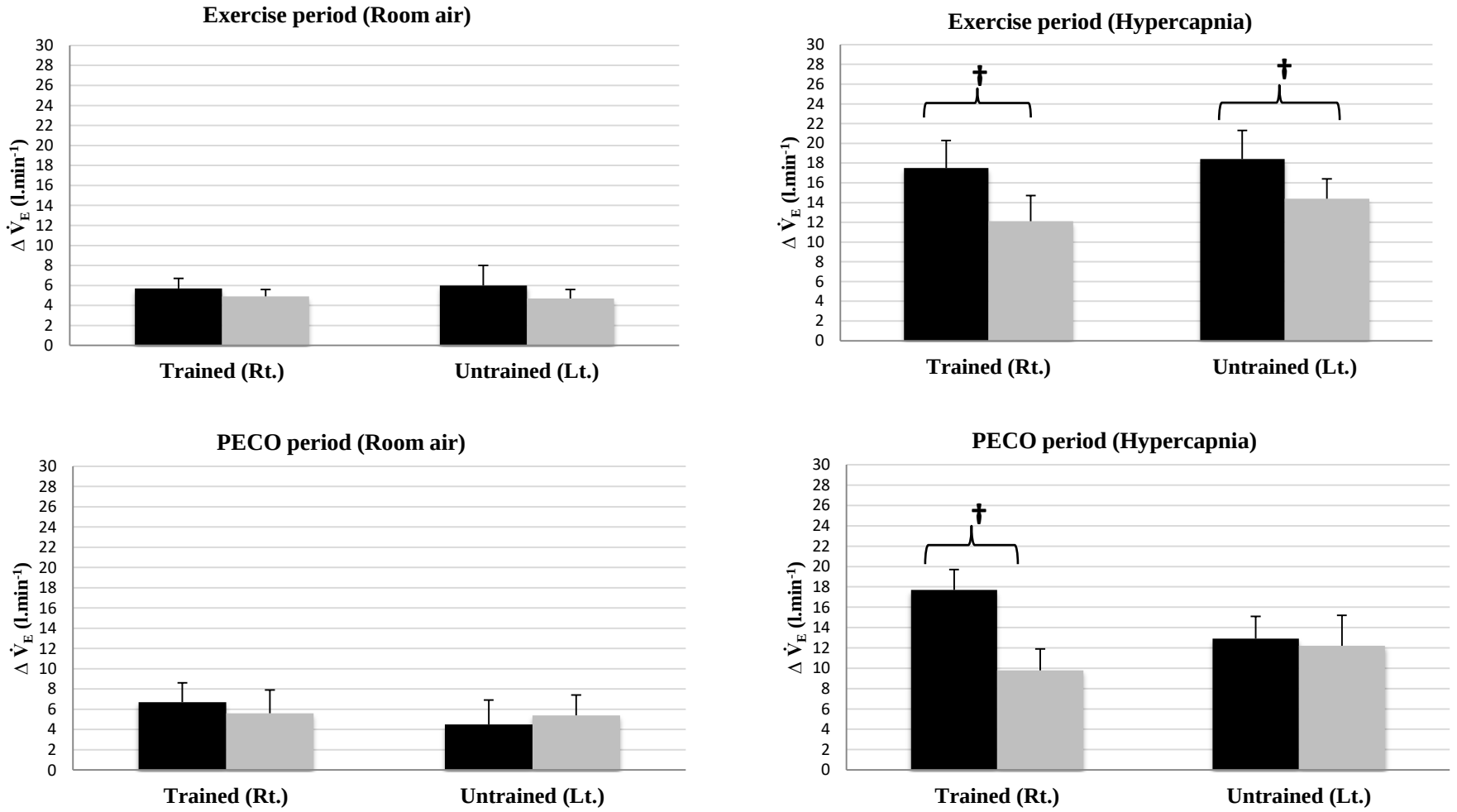


Figure 5.13: Summary measurements of the average changes in $\dot{V}_E$ of the trained and untrained legs, during exercise and PECO periods, under room air and hypercapnia (mean ± S.E.M). Before (black bar) and after (grey bar) exercise training values are shown. † Significant difference between the values ($p \leq 0.05$).

Breathing frequency (Leg protocol):

Changes in $\dot{V}_E$ seen throughout the trials performed on both legs under both conditions were not surprisingly associated with changes in tidal volume and breathing frequency. However, only the changes in *f* reached statistical significance following exercise training. Figure 5.14 shows, that under room air condition the *f* during the last minute of exercise in before- and after-exercise training trials was significantly greater compared to pre-exercise resting levels in trained (6.9 ± 2.1 & 4.3 ± 1.7 breaths.min^{-1}, respectively, $p \leq 0.05$) and untrained (3.8 ± 1.2 & 4.6 ± 0.8 breaths.min^{-1}, respectively, $p \leq 0.05$) legs. During the PECO period, the *f* observably fell from exercise levels to pre-exercise resting levels in both trials (before- and after-exercise training) in both the trained and untrained legs ($p \geq 0.05$ for all).

When hypercapnia was induced, *f* during the last minute of exercise was significantly greater than at pre-exercise rest in both trials (before- and after-exercise training) of the trained (5.7 ± 1.1 & 3.7 ± 0.9 breaths.min^{-1}, respectively, $p \leq 0.05$) and untrained (4.0 ± 0.8 & 4.6 ± 0.7 breaths.min^{-1}, respectively, $p \leq 0.05$) legs. During circulatory occlusion following isometric exercise, *f* reduced but remained significantly above the pre-exercise resting level in both trials of the trained and untrained legs ($p \leq 0.05$ for all).

Within leg, pre- vs. post-exercise training comparisons of f

Figure 5.14 shows that, following exercise training, there was a small reduction in the increases of *f* during exercise and PECO, compared to before-training values in the trained leg under both conditions. Meanwhile, no effect of training on the *f* was observed in the untrained leg. Figure 5.15 represents the average changes in *f* values during exercise and PECO periods. During exercise period, there was no significant reduction of the average changes in *f* increases after exercise training in the trained leg under room air; however, this

reduction reached statistical significance under hypercapnia ($p \leq 0.05$). No significant changes were observed in the untrained leg under both conditions. The average changes in the *f* rise over the PECO period was significantly reduced following exercise training only in the trained leg, under both conditions ($p \leq 0.05$ for all).

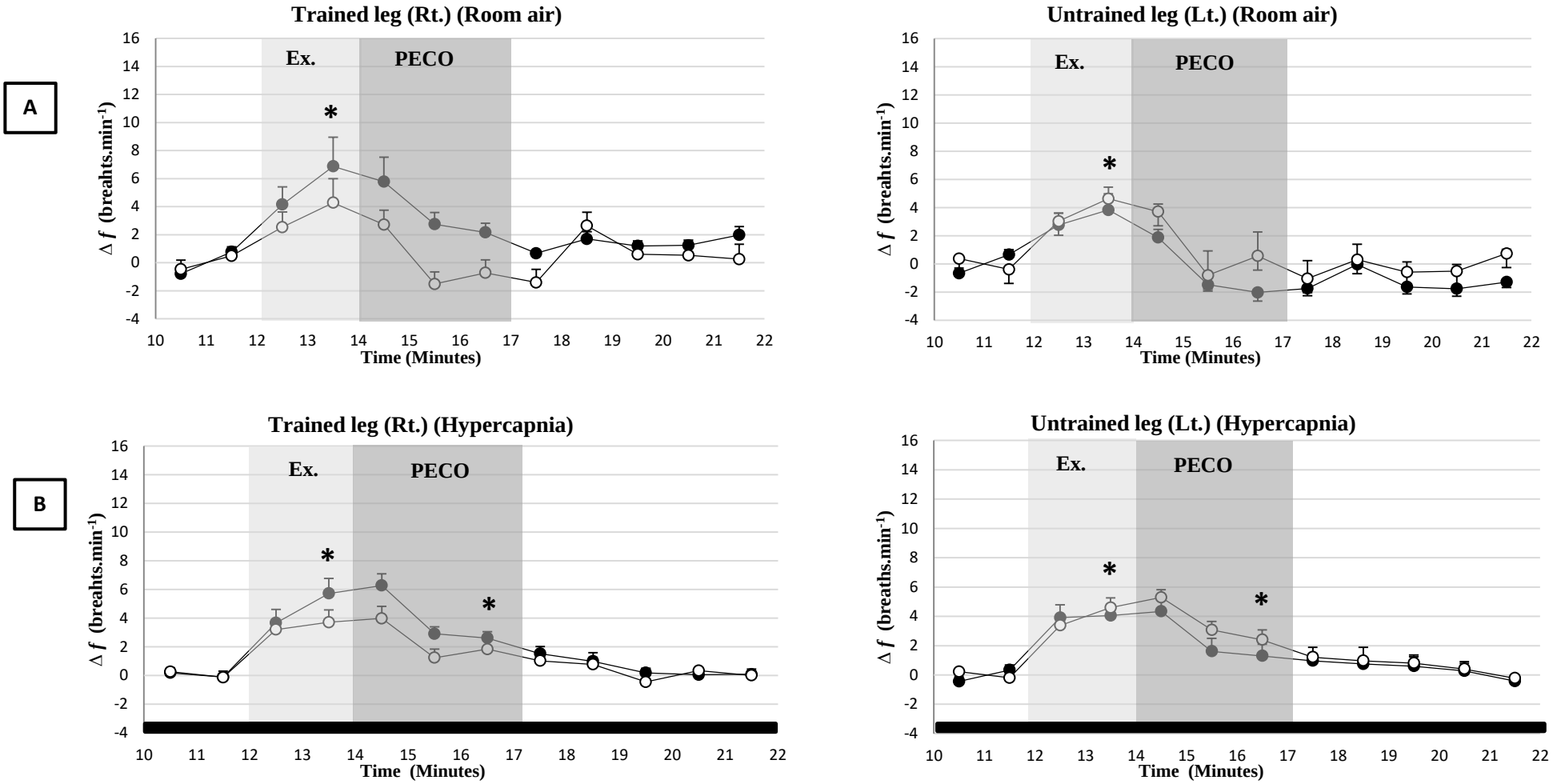


Figure 5.14: A) Mean changes in *f* from the pre-exercise resting baseline of the trained and untrained legs, under room air. B) Mean changes in *f* corrected for baseline shift (between minute 11, 12, 21 & 22) under hypercapnia (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The black bar indicates hypercapnia. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$).

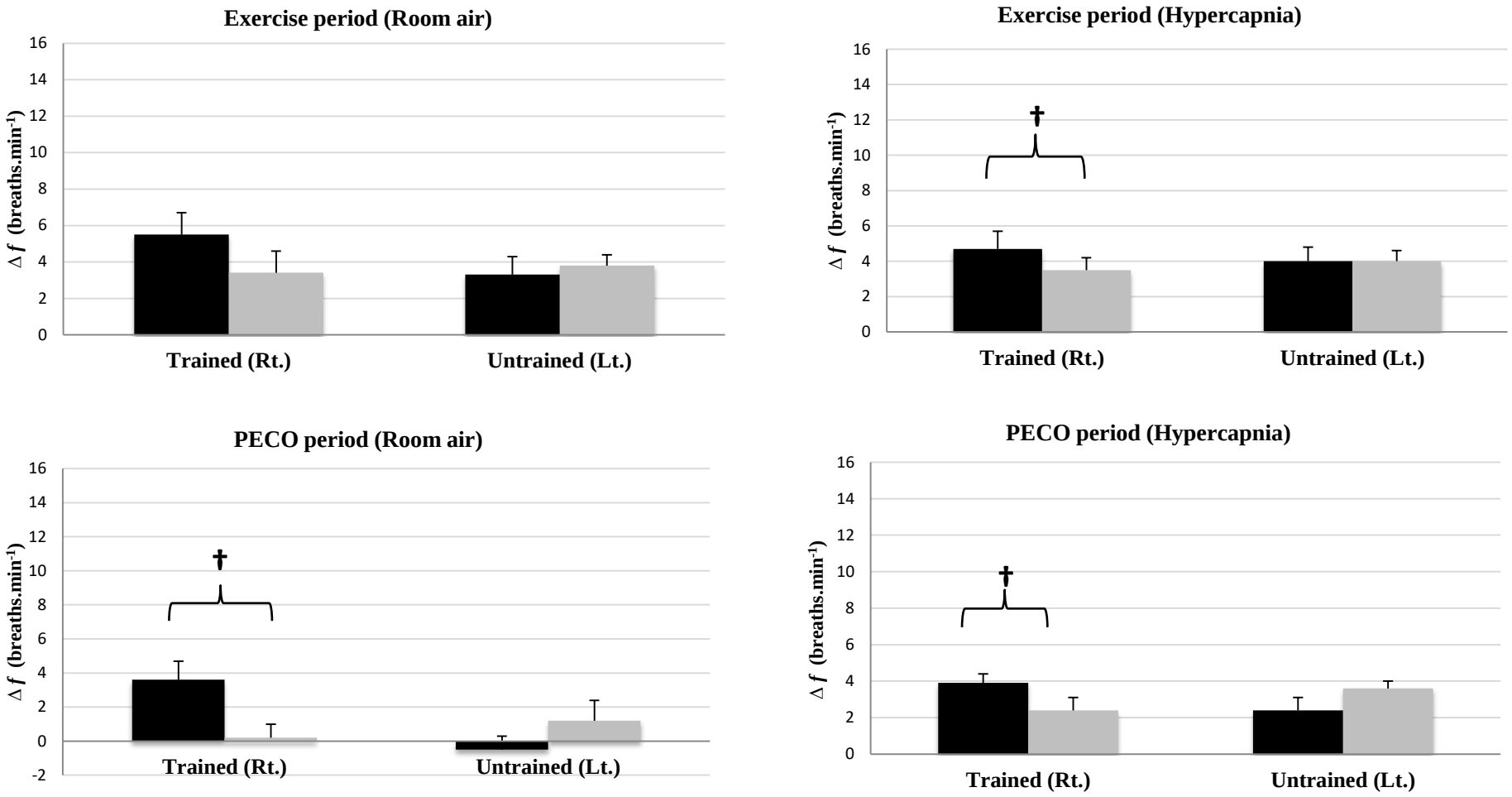


Figure 5.15 Summary measurement of the average change in *f* of the trained and untrained legs during exercise and PECO periods, under room air and hypercapnia (mean ± S.E.M). Before (black bar) and after (grey bar) exercise training values are shown. † Significant difference between the values ($p \leq 0.05$).

B) Results of the hand protocols

1. Hand Protocol A:

A subgroup of 7 participants completed Protocol A of hand testing (right hand only; at 50 % MVC) under room air and hypercapnia, following exercise training of the dominant (right) leg for 6 weeks. Measurement of MVCs before each trial was conducted under room air. There were no significant differences in MVC measurement for the right hand before and after exercise training, within and between conditions (Room air; 395.7N vs. 427.9N and Hypercapnia; 398.4N vs. 424.8N; respectively; $p \geq 0.05$ for all).

Table 5.3 shows that exercise training of the dominant (right) leg had no effect on the resting cardiovascular and ventilatory values, measured over the last 2 minutes of pre-exercise rest in the right hand trials, within both conditions ($p \geq 0.05$ for all). However, as expected, inducing mild hypercapnia ($P_{ET}CO_2$ clamped at +10 mmHg, above resting value) resulted in significant increases in the MAP, $\dot{V}_E$ and f resting values to above room air values ($p \leq 0.05$ for all). The mean $P_{ET}CO_2$ was 38.6 ± 0.3 mmHg and this did not significantly change after the training programme.

Table 5.3: Values recorded during the last minutes of the pre-exercise resting period (minute 11 & 12) (Pre-exercise baseline) in right hand trials (Protocol A) (mean ± S.E.M).

	Room air		**Hypercapnia**	
	Pre	***Post***	***Pre***	***Post***
MAP (mmHg)	88.4 ± 3.3	83.1 ± 4.1	99 ± 4.3[1]	91.6 ±5.3[2]
HR (beats.min^{-1})	81 ± 5.6	77 ± 8.1	90 ± 4.4	87 ± 5.1
$\dot{V}_E$ (l.min^{-1})	17.2 ± 0.8	16.6 ± 0.4	73.4 ± 3.5[1]	71.8 ± 4.8[2]
V_T (L)	1.2 ± 0.1	1.16 ± 0.1	3.0 ± 0.2[1]	2.9 ±0.2[2]
***f* (breaths.min^{-1})**	15.3 ± 1.7	14.4 ± 1.5	25.0 ± 2.0[1]	24.3 ± 1.4[2]

1 $p \leq 0.05$ from pre- (Room Air). 2 $p \leq 0.05$ from post- exercise training (Room Air)

Blood pressure (Hand protocol A)

Figure 5.16 shows the mean changes in the MAP (from pre-exercise resting baseline) every 15 seconds in right hand trials, under room air and hypercapnia (Hand Protocol A). When these values converted into 1 minute averages (Figure 5.17 A), the MAP during the last minute of isometric handgrip exercise was significantly greater compared to the pre-exercise resting baseline in both trials (before- and after-exercise training), under room air and hypercapnia. Circulatory occlusion of the upper arm following isometric handgrip exercise resulted in a slight fall in MAP values in both trials, although it remained significantly above pre-exercise resting baseline values under both conditions ($p \leq 0.05$ for all). Summary measurements of the changes in MAP over exercise and PECO periods (Figure 5.17 B) showed that exercise training of the dominant leg had no significant effect on the MAP in right hand trials, under both conditions ($p \geq 0.05$ for all).

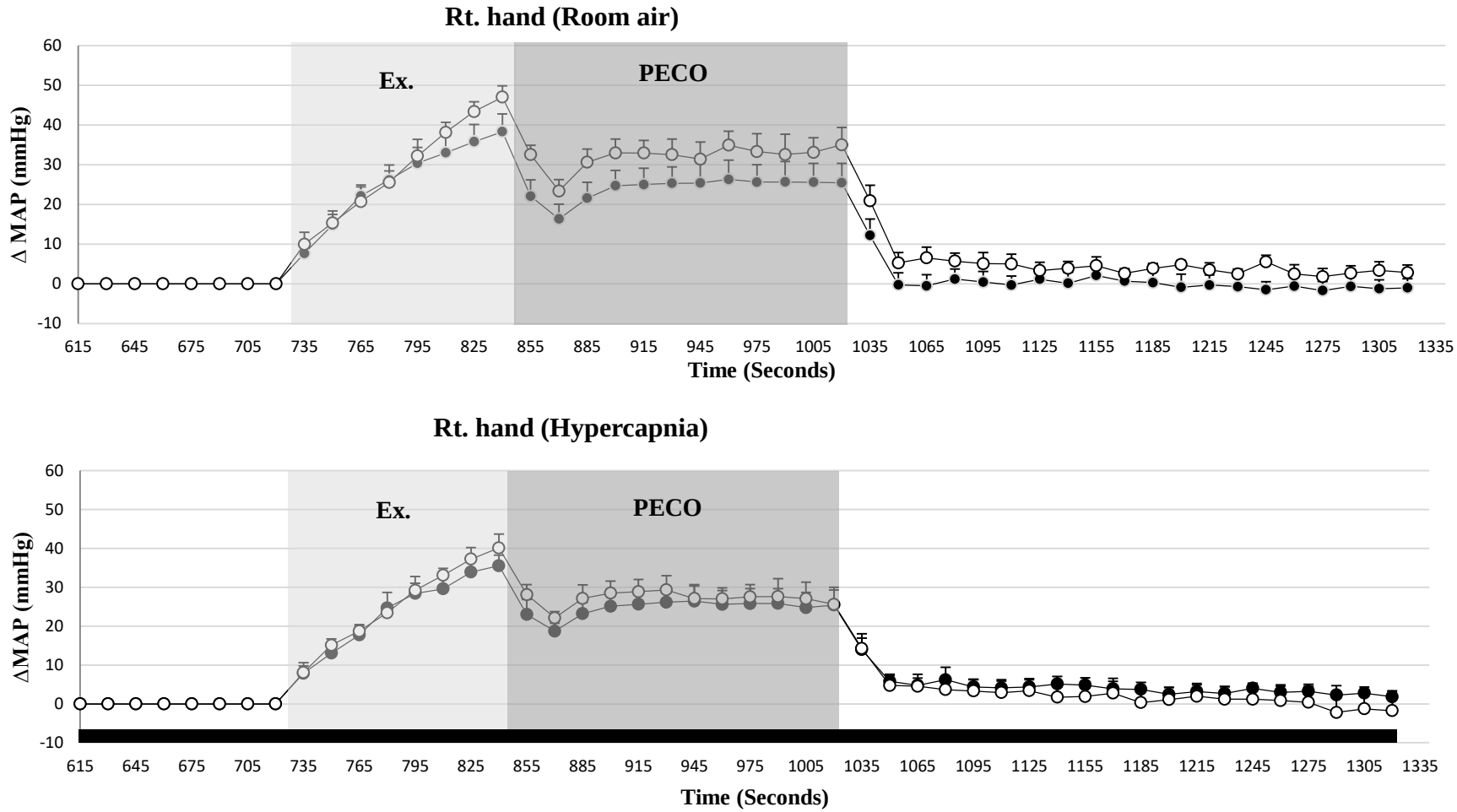
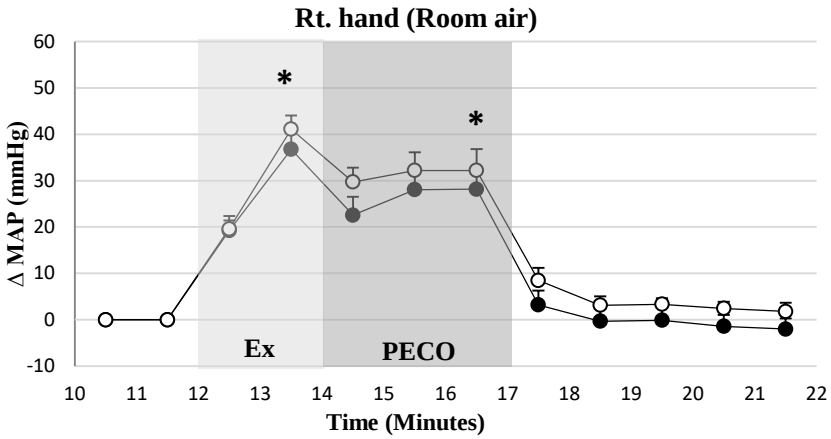
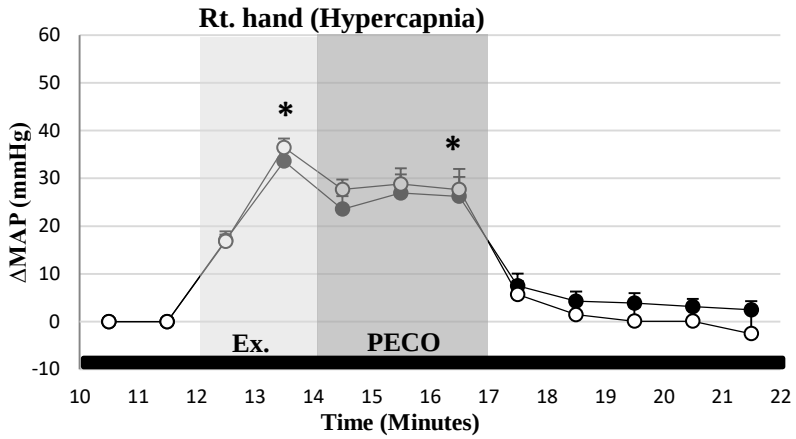


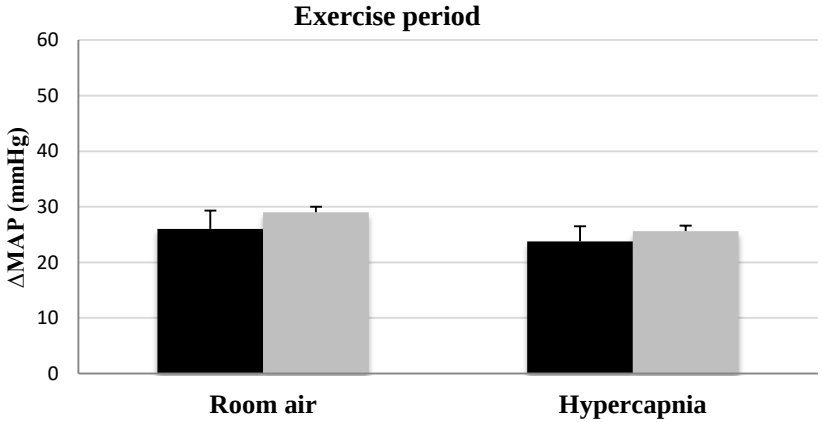
Figure 5.16: Mean changes in the MAP from the pre-exercise resting baseline of the right hand, under room air and hypercapnia, every 15 seconds (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training programme. The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) periods. The black bar indicates hypercapnia.

A





B



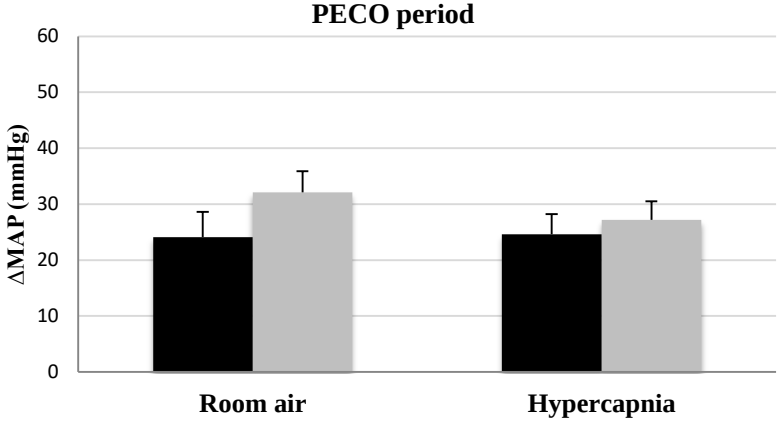


Figure 5.17: A) Mean changes in MAP from the pre-exercise resting baseline of the right hand, under room air and hypercapnia, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The black bar indicates hypercapnia. B) Summary measurements of the average change in MAP from pre-exercise resting baseline of the right hand, during exercise and PECO, under both conditions. Before (black bar) and after (grey bar) training. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$).

Heart rate (Hand protocol A):

Figure 5.18 shows the mean changes in the HR, relative to the pre-exercise resting baseline, in right hand trials under both conditions and every 15 seconds. Averaging these 15 seconds into 1 minute (Figure 5.19 A) revealed that, during the last minute of exercise, the HR increased significantly to above the pre-exercise resting baseline (~ 10 beats.min^{-1}) in both trials under room air ($p \leq 0.05$). There was also a significant increase in the HR in both trials (~ 14 beats.min^{-1}) to above the pre-exercise resting baseline under the hypercapnia ($p \leq 0.05$). During the PECO period, the HR dropped back to the pre-exercise resting baseline under both conditions ($p \geq 0.05$ for all). The area under the curve (Figure 5.19 B) revealed no significant difference in the HR values before and after exercise training, in the dominant leg, during exercise and PECO periods of the hand trials, under both conditions ($p \geq 0.05$ for all).

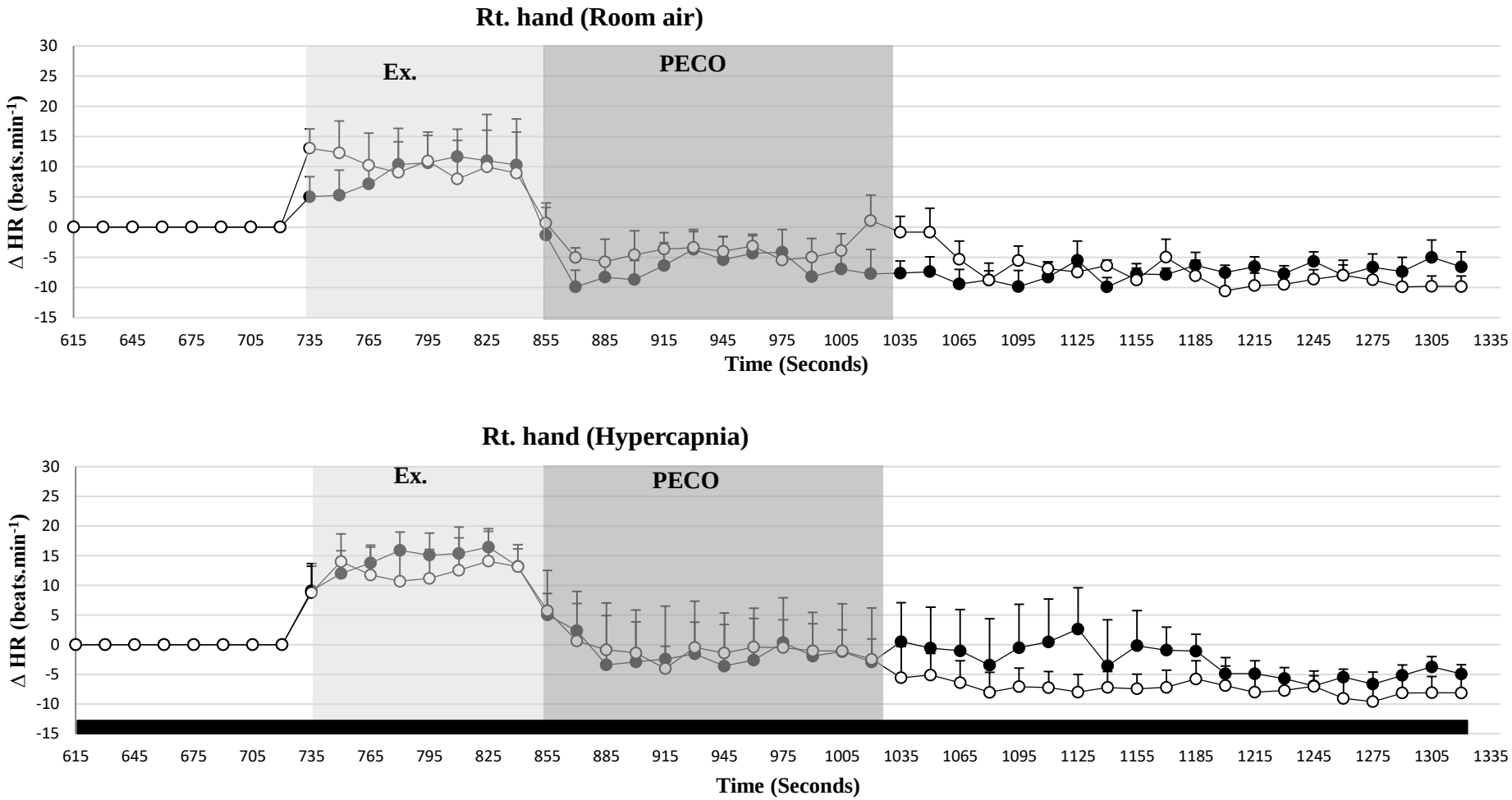


Figure 5.18: Mean changes in the HR from the pre-exercise resting baseline of the right hand under room air and hypercapnia , every 15 seconds (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) period. The black bar indicates hypercapnia.

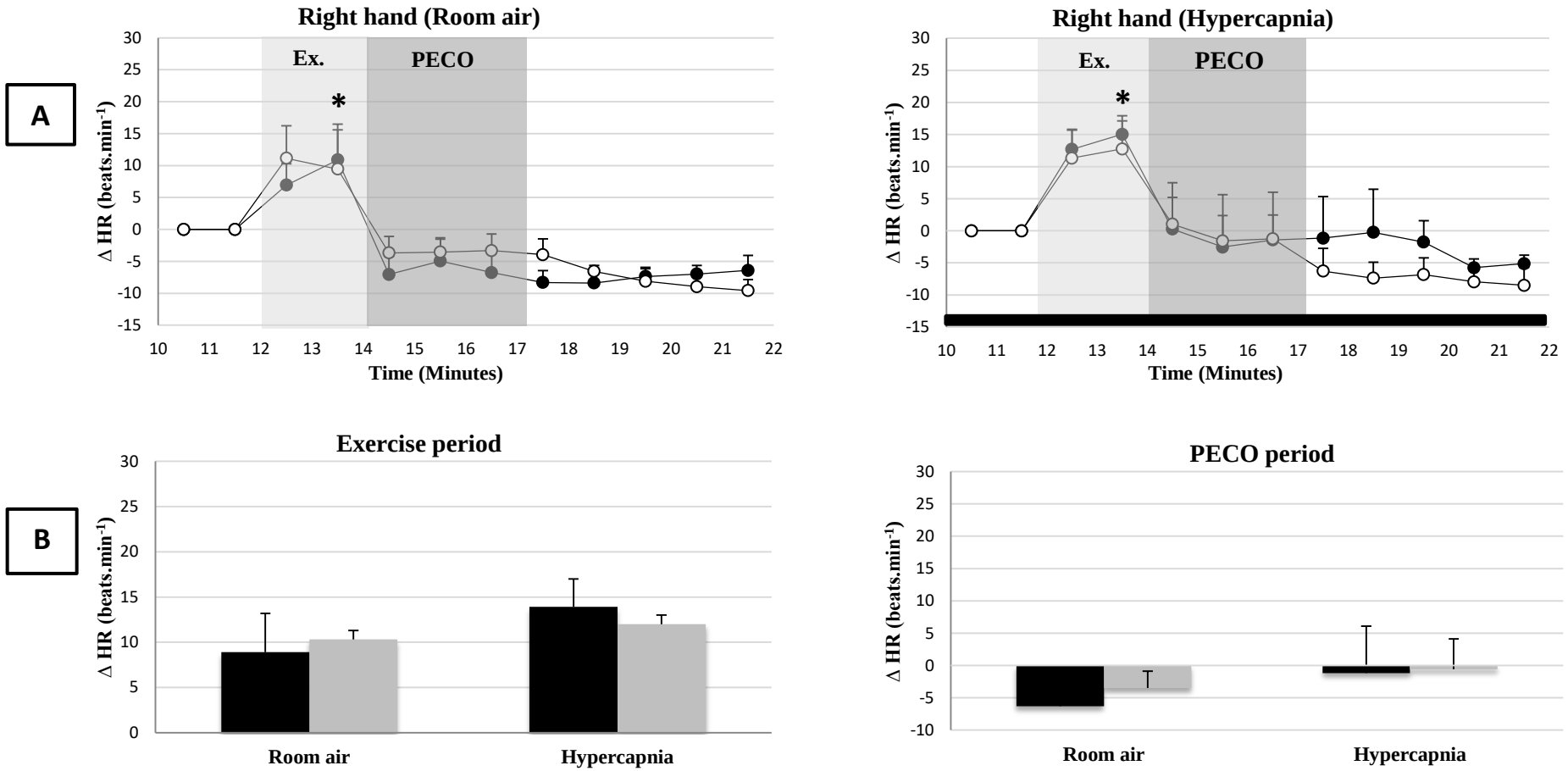


Figure 5.19: A) Mean changes in the HR from the pre-exercise resting baseline of the right hand, under room air and hypercapnia, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The black bar indicates hypercapnia. B) Summary measurements of the average changes in the HR from the pre-exercise resting baseline of the right hand, during exercise and PECO, under both conditions. Before (black bar) and after (grey bar) training. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$).

Minute ventilation (Hand protocol A):

Figure 5.20 (A) showed that isometric handgrip exercise resulted in a significant increase in $\dot{V}_E$ during the last minute of exercise to above pre-exercise resting levels, before and after exercise training trials, under room air (16.4 ± 3.6 & 19.2 ± 3.1 $l.min^{-1}$, respectively, $p \leq 0.05$). However, this increase in the $\dot{V}_E$ seen during exercise declined close to pre-exercise resting baseline levels in the second minute of PECO ($p \geq 0.05$). In contrast, when participants were exposed to hypercapnia, the $\dot{V}_E$ during the last minute of isometric exercise was significantly greater compared to the pre-exercise baseline level, before and after the training period (29.1 ± 5.4 & 28.8 ±6.0 $l.min^{-1}$, respectively, $p \leq 0.05$). During circulatory occlusion following isometric handgrip exercise, the $\dot{V}_E$ slightly dropped from exercise levels but was sustained at significantly greater levels than pre-exercise rest ($p \leq 0.05$). Under both conditions, when the circulation was restored, the $\dot{V}_E$ dropped to pre-exercise resting levels.

The summary measurements of the mean average changes in $\dot{V}_E$ over exercise and PECO periods (Figure 5.20 B) showed that there was no significant difference in $\dot{V}_E$ before and after exercise training, under each condition ($p \geq 0.05$). However, exposure to mild hypercapnia resulted in a significant increase in $\dot{V}_E$ to above the values observed under room air ($p \leq 0.05$).

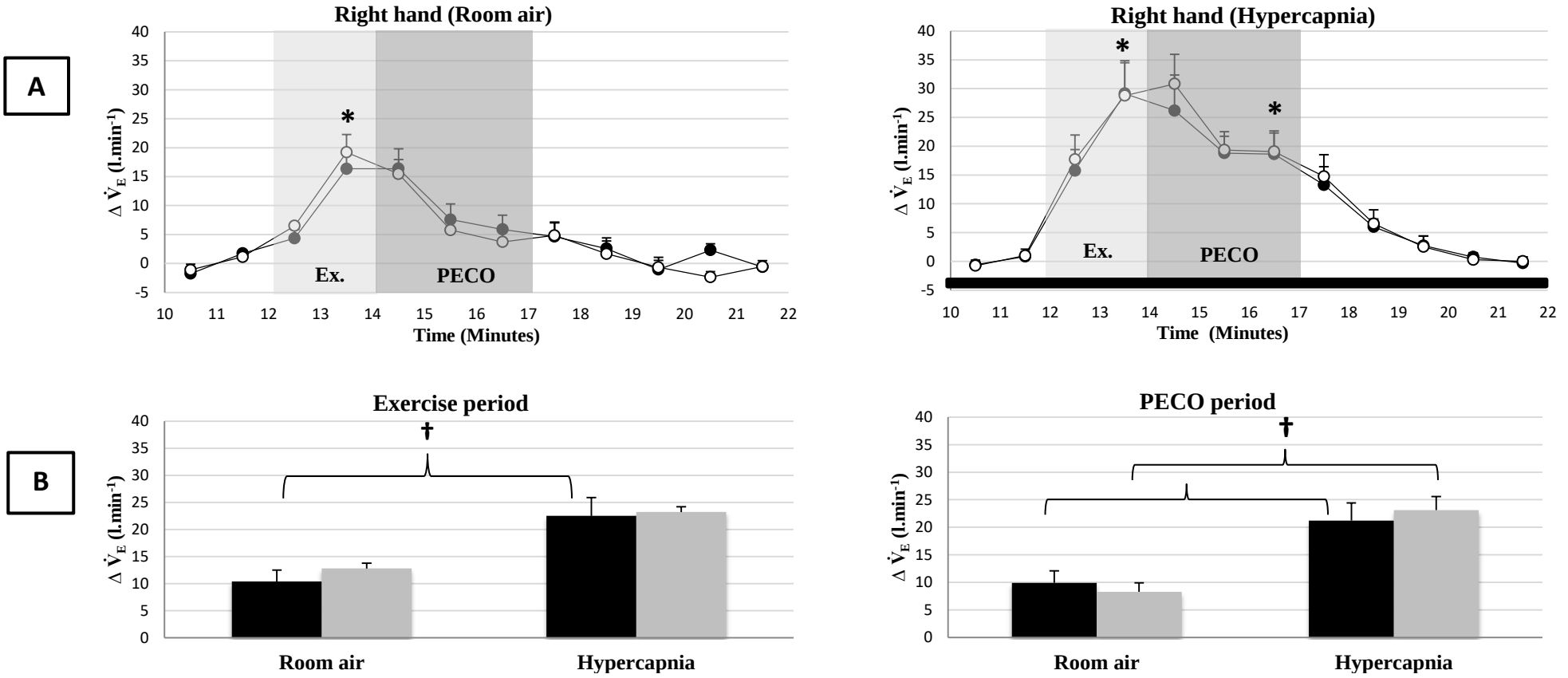


Figure 5.20: A) Mean changes in $\dot{V}_E$ from the pre-exercise resting baseline of the right hand under room air, and mean changes in $\dot{V}_E$ corrected for baseline shit (between minute 11, 12, 21 & 22) under hypercapnia, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The black bar indicates hypercapnia. B) Summary measurements of the average change in $\dot{V}_E$ of the right hand, during exercise and PECO, under both conditions. Before (black bar) and after (grey bar) training values shown. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$). † Significant difference between values ($p \leq 0.05$).

Breathing frequency (Hand Protocol A):

The increase in $\dot{V}_E$ during isometric exercise and PECO periods reflects the changes in the tidal volumes and breathing frequency. However, only the changes in *f* reached statistical significance here. Figure 5.21(A) shows that, during the last minute of exercise period, *f* was significantly greater than pre-exercise resting levels in before- and after-exercise training trials, under room air (6.9 ± 2.7 & 8.8 ± 1.6 breaths.min^{-1}, respectively, $p \leq 0.05$) and hypercapnia (5.5 ± 1.7 & 5.2 ± 1.2 breaths.min^{-1} , respectively, $p \leq 0.05$). This increase in *f* dropped close to pre-exercise resting levels in room air trials ($p \geq 0.05$), but remained significantly elevated to above pre-exercise resting baseline values in hypercapnia trials ($p \leq 0.05$). Figure 5.21 (B) shows the summary measurements of the average changes in *f* during exercise and PECO periods, which revealed no significant effect of the exercise training programme.

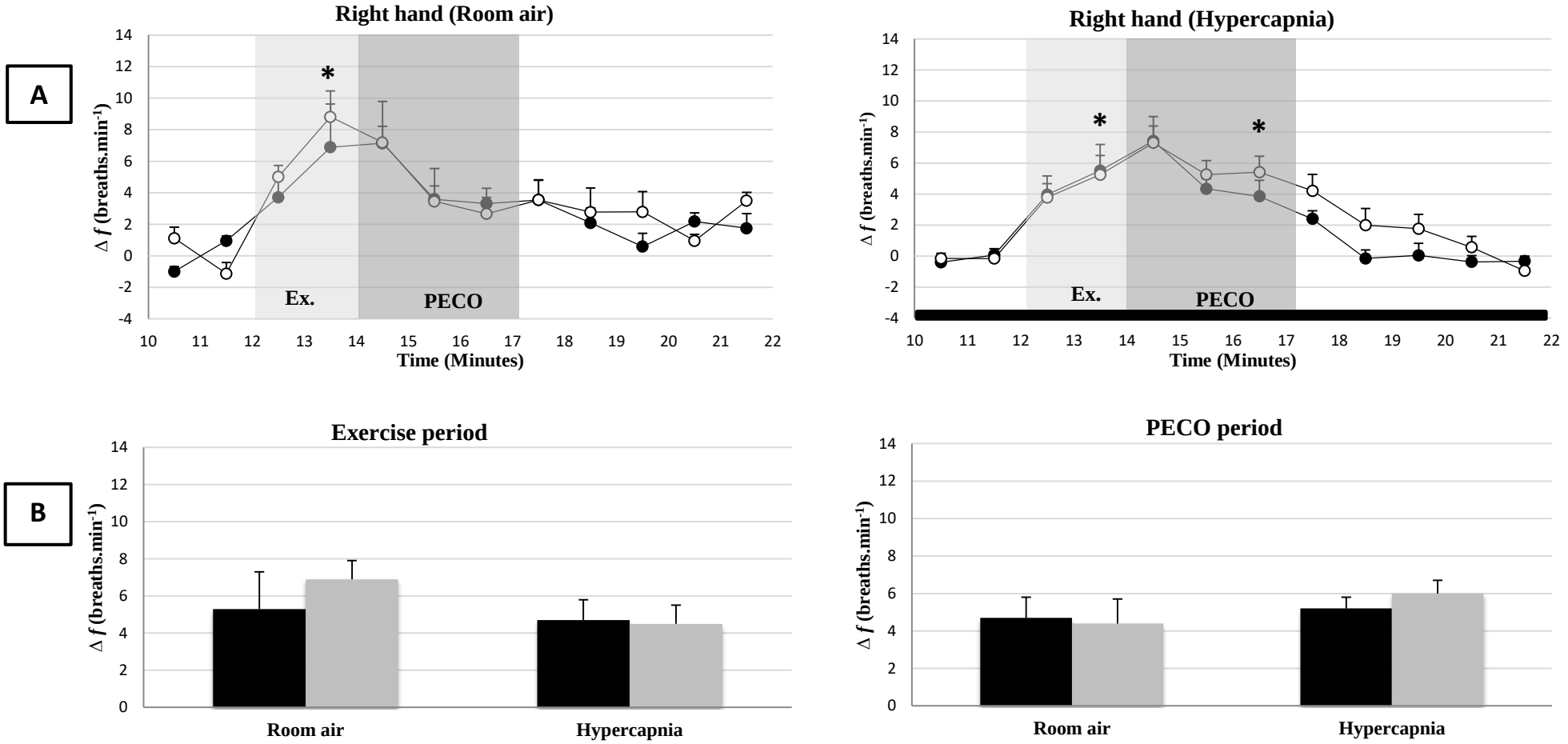


Figure 5.21: A) Mean changes in *f* from the pre-exercise resting baseline of the right hand, under room air condition, and the mean changes in *f* corrected for shift baseline (between minute 11, 12, 21 & 22) under hypercapnia, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. The black bar indicates hypercapnia. B) Summary measurements of the average change in *f* of the right hand, during exercise and PECO, under both conditions. Before (black bar) and after (grey bar) training values shown. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$).

2. Hand Protocol B (both hands):

The same subgroup of 7 participants who completed Protocol A of the hand experiment also completed Protocol B. This protocol was performed on both hands (right and left) at 30 % of MVC, under room air only. Measurements of the MVC revealed no effect of exercise training of the dominant (right) leg on the right and left hands (384.8 N vs. 414 N and 374.6 N vs. 377.2 N, respectively, $p \geq 0.05$ for all). Although the MVC of the right hand naturally tends to be slightly higher compared to the left hand, in the tests here there was no significant difference between them ($p \geq 0.05$).

Table 5.4 shows the cardiovascular and ventilatory resting values recorded during the first two minutes of the baseline period. There were no significant differences in these values before and after exercise training in each hand. Moreover, there was no significant difference in resting values between the hands. The mean $P_{ET}CO_2$ was 39.3 ± 0.4 mmHg and this did not change after exercise.

Table 5.4: Values recorded over 2 minutes of the baseline in each hand trial (Protocol B) (mean ± S.E.M).

	Right Hand		**Left Hand**	
	Pre	***Post***	***Pre***	***Post***
MAP (mmHg)	89.8 ± 3.9	85.5 ± 4.6	87.6 ± 5.5	85.4 ± 3.8
HR (beats.min^{-1})	77 ± 7.4	75 ± 4.8	73 ± 5.0	78 ± 4.5
$\dot{V}_E$ (l.min^{-1})	14.9 ± 0.5	15.5 ± 0.4	15.3 ± 0.5	14.8 ± 0.4
V_T (L)	1.11 ± 0.08	1.09 ± 0.1	1.03 ± 0.04	1.0 ± 0.13
***f* (breaths.min^{-1})**	13.9 ± 0.7	15.3 ± 1.6	15.1 ± 0.6	14.9 ± 1.7

Blood pressure (Hand Protocol B)

Mean changes in the MAP, relative to the baseline, measured every 15 seconds during 8 minutes of right and left hand trials are shown in Figure 5.22. The end point of isometric handgrip exercise in Figure 5.23 (A) shows significant increases in the MAP to above baseline levels before and after exercise training in the right (25.2 ± 1.7 & 28.7 ± 3.4 mmHg, respectively, $p \leq 0.05$) and left (18.9 ± 2.0 & 21.8 ± 3.9 mmHg, respectively, $p \leq 0.05$) hands. This rise in the MAP during the exercise period dropped to a level that remained significantly above baseline during PECO in both hand trials ($p \leq 0.05$ for all). On cession of PECO, the MAP fell to baseline.

Summary measurements of the mean average changes in MAP, during exercise and PECO of the right and left hand (before and after exercise training) are shown in Figure 5.23 (B). Exercise training had no effect on these MAP values in both periods of either hand ($p \geq 0.05$ for all). During exercise, the MAP of the right hand was notably significantly greater compared to the left hand before and after training ($p \leq 0.05$ for all).

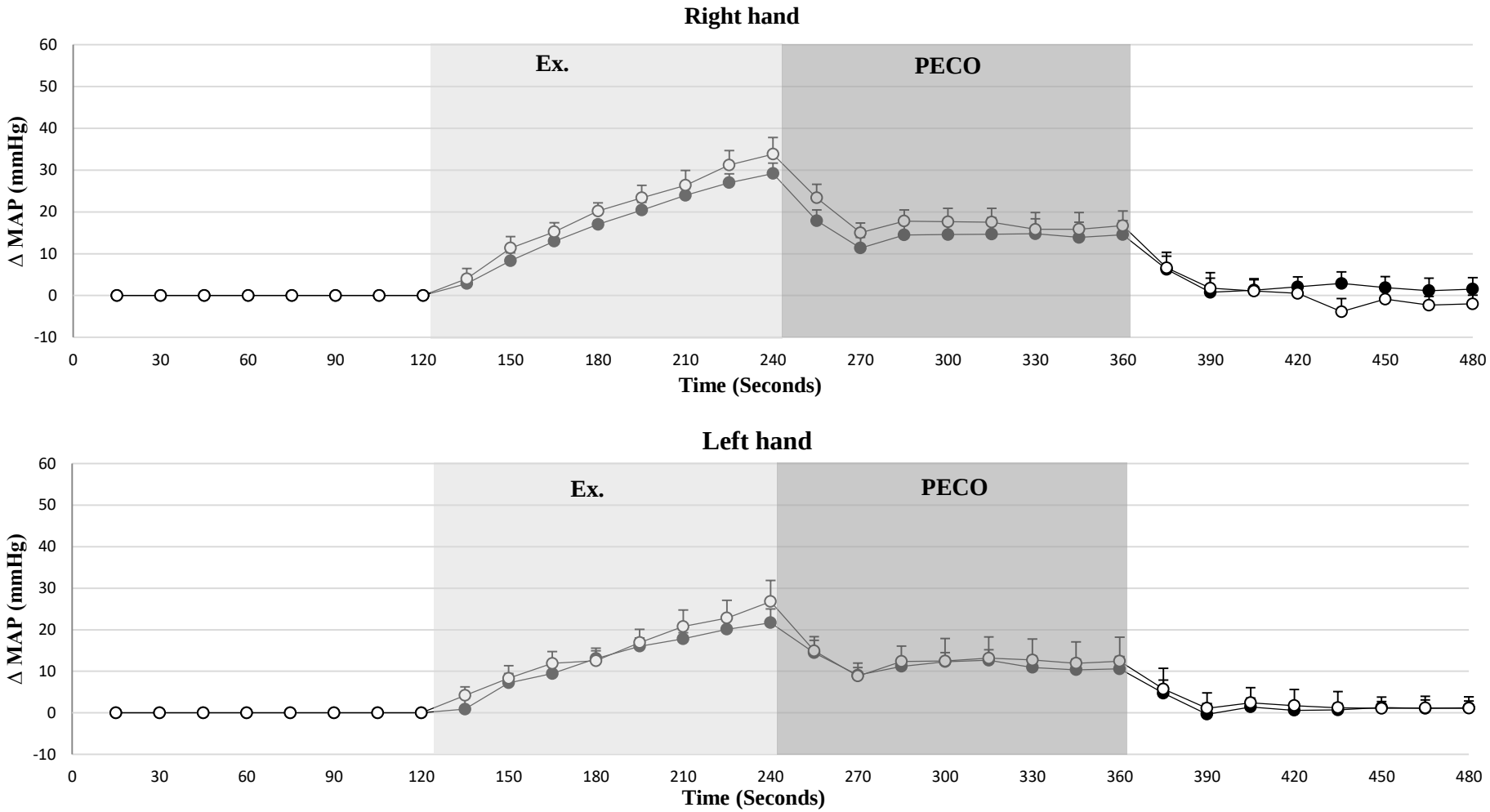


Figure 5.22: Mean changes in the MAP from the baseline of right and left hands, under room air, every 15 seconds (mean ± S.E.M). Before (closed circle) and after (open circle) right leg exercise training values are shown. The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates the post-exercise circulatory occlusion (PECO) periods.

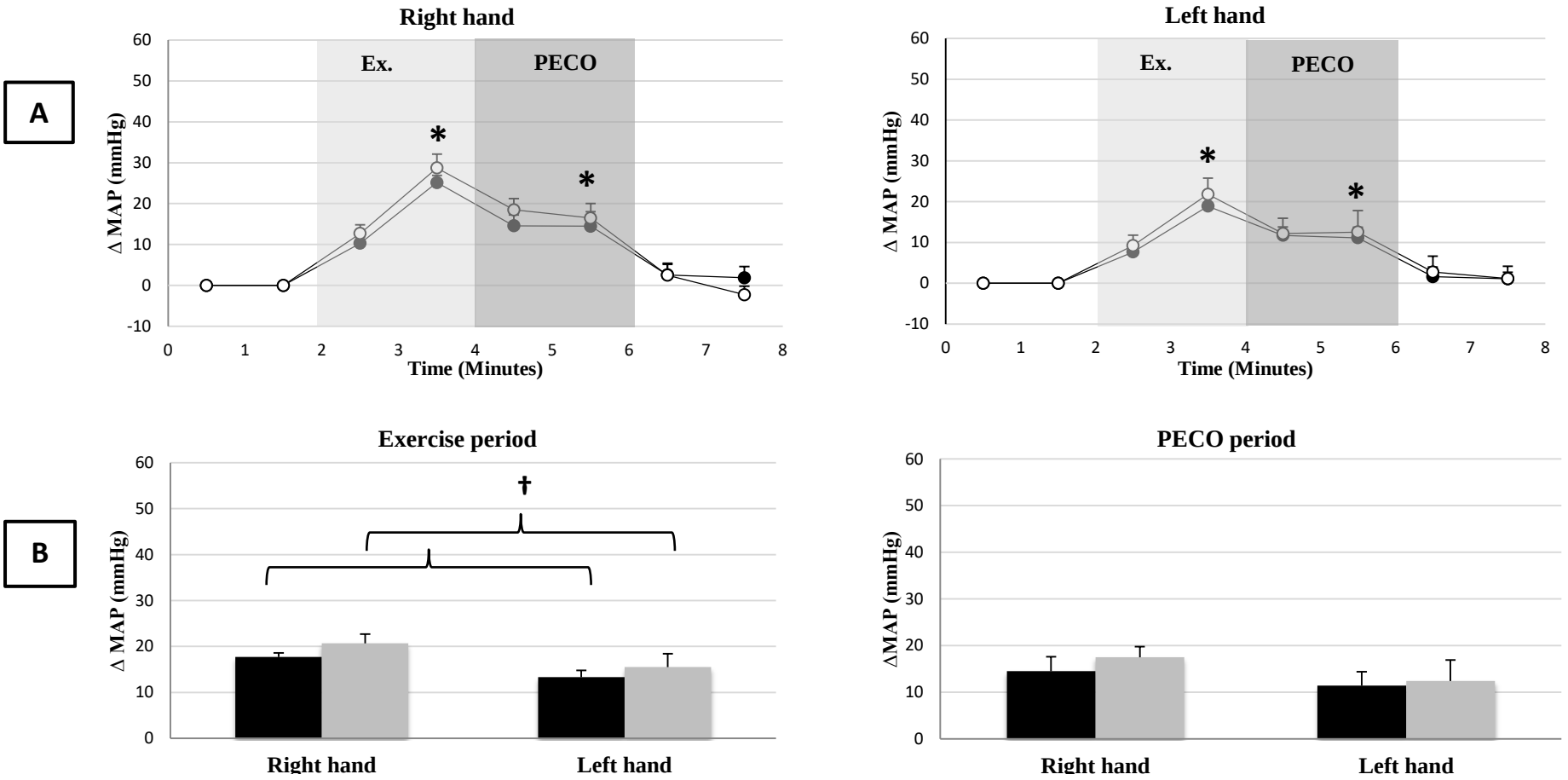


Figure 5.23: A) Mean changes in the MAP from baseline of the right and left hands, under room air, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) right leg exercise training values are shown. B) Summary measurements of the average change in the MAP from baseline of the right and left hands during exercise and PECO. Before (black bar) and after (grey bar) exercise training values shown. * Both values were significantly different from baseline values ($p \leq 0.05$). † Significant difference between values ($p \leq 0.05$).

Heart rate (Hand Protocol B):

Changes in the HR relative to baseline measured every 15 seconds in both right and left hand trials are shown in Figure 5.24. Figure 5.25 (A) shows that at the peak of isometric handgrip exercising, the HR significantly increased to above baseline in the before- and after-exercise training trials of the right (6.9 ± 1.2 & 8.6 ± 2.5 beats.min^{-1}, respectively, $p \leq 0.05$) and left (10.9 ± 2.2 & 10.7 ± 3.3 beats.min^{-1}, respectively, $p \leq 0.05$) hands. On cession of exercise, during the PECO period, the HR returned to baseline levels in both hand trials ($p \geq 0.05$).

Figure 5.25 (B) shows the summary of the mean average changes in HR during the exercise and PECO periods, which revealed no effect of exercise training on the HR in either hand trial ($p \geq 0.05$).

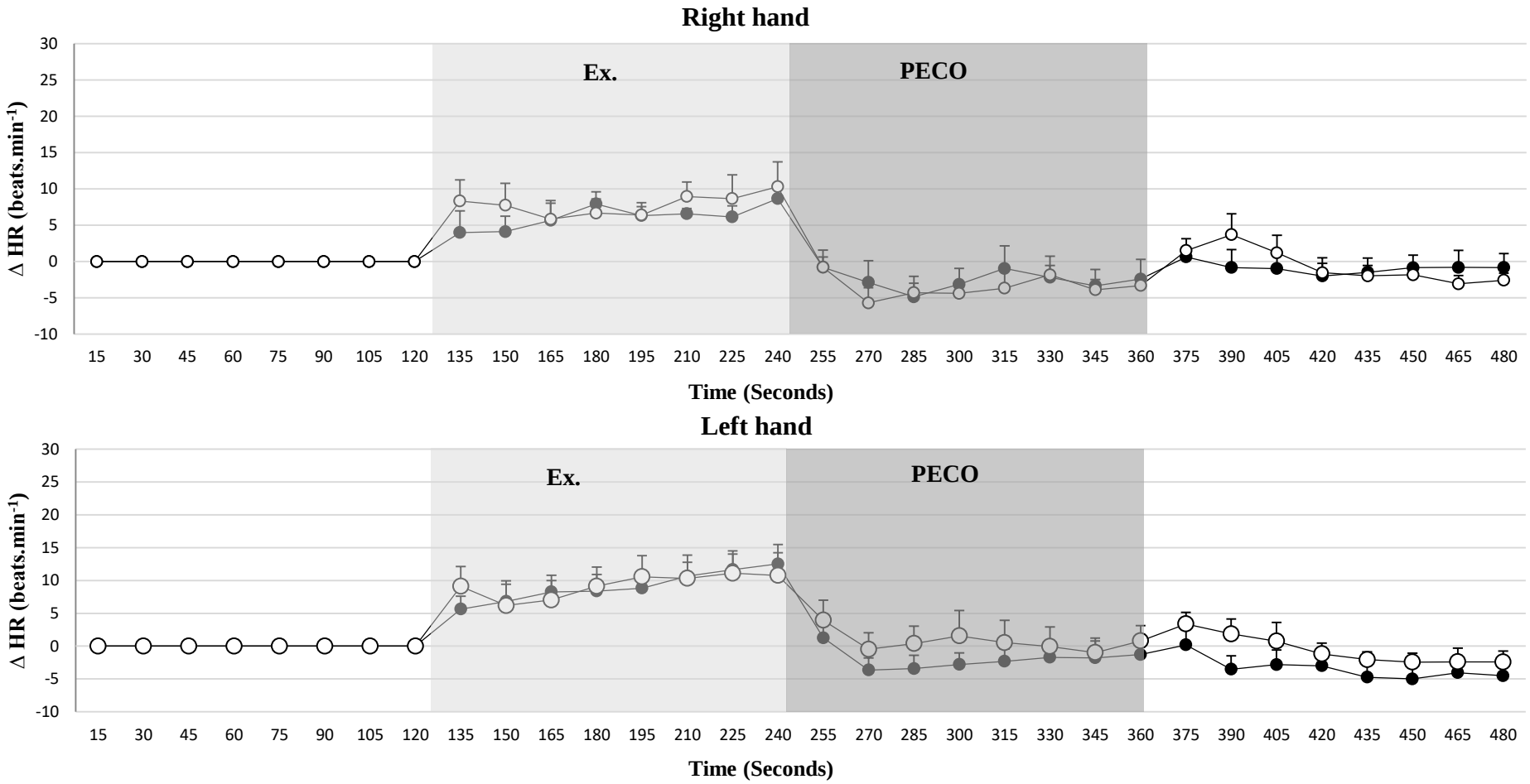


Figure 5.24: Mean changes in the HR from the baseline of the right and left hands, under room air, every 15 seconds (mean ± S.E.M). Before (closed circle) and after (open circle) right leg exercise training values are shown. The light-shaded area indicates the exercise (Ex.) period and the dark-shaded area indicates post-exercise circulatory occlusion (PECO).

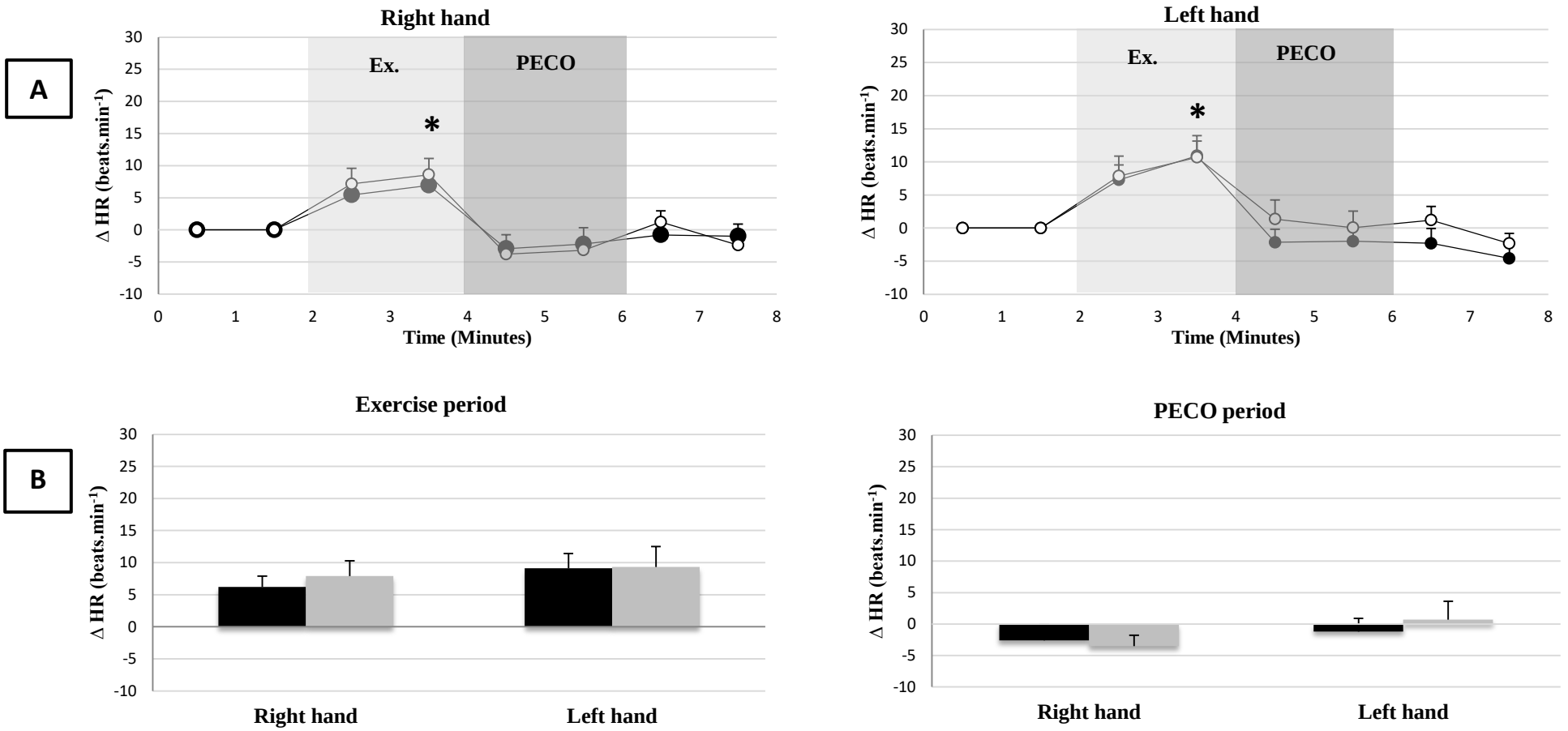


Figure 5.25: A) Mean changes in the HR from baseline of the right and left hands, under room, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) right leg exercise training values are shown. B) Summary measurements of the average changes in the HR from baseline of the right and left hands during exercise and PECO periods. Before (black bar) and after (grey bar) training values shown. * Both values were significantly different from pre-exercise resting baseline values ($p \leq 0.05$).

Minute ventilation (Hand protocol B):

Figure 5.26 (A) shows that the $\dot{V}_E$ during the last minute of isometric handgrip exercise significantly increased to above the baseline in the before- and after-exercise training trials of the right (4.7 ± 0.9 & 6.1 ± 1.3 $l.min^{-1}$, respectively, $p \leq 0.05$) and left (4.6 ± 1.3 & 4.8 ± 1.1 $l.min^{-1}$, respectively, $p \leq 0.05$) hands. During PECO, the $\dot{V}_E$ dropped back to baseline in both hand trials ($p \geq 0.05$ for all). Exercise training of the dominant leg had shown no significant effect on $\dot{V}_E$ values in either hand ($p \geq 0.05$), as shown in Figure 5.26 (B).

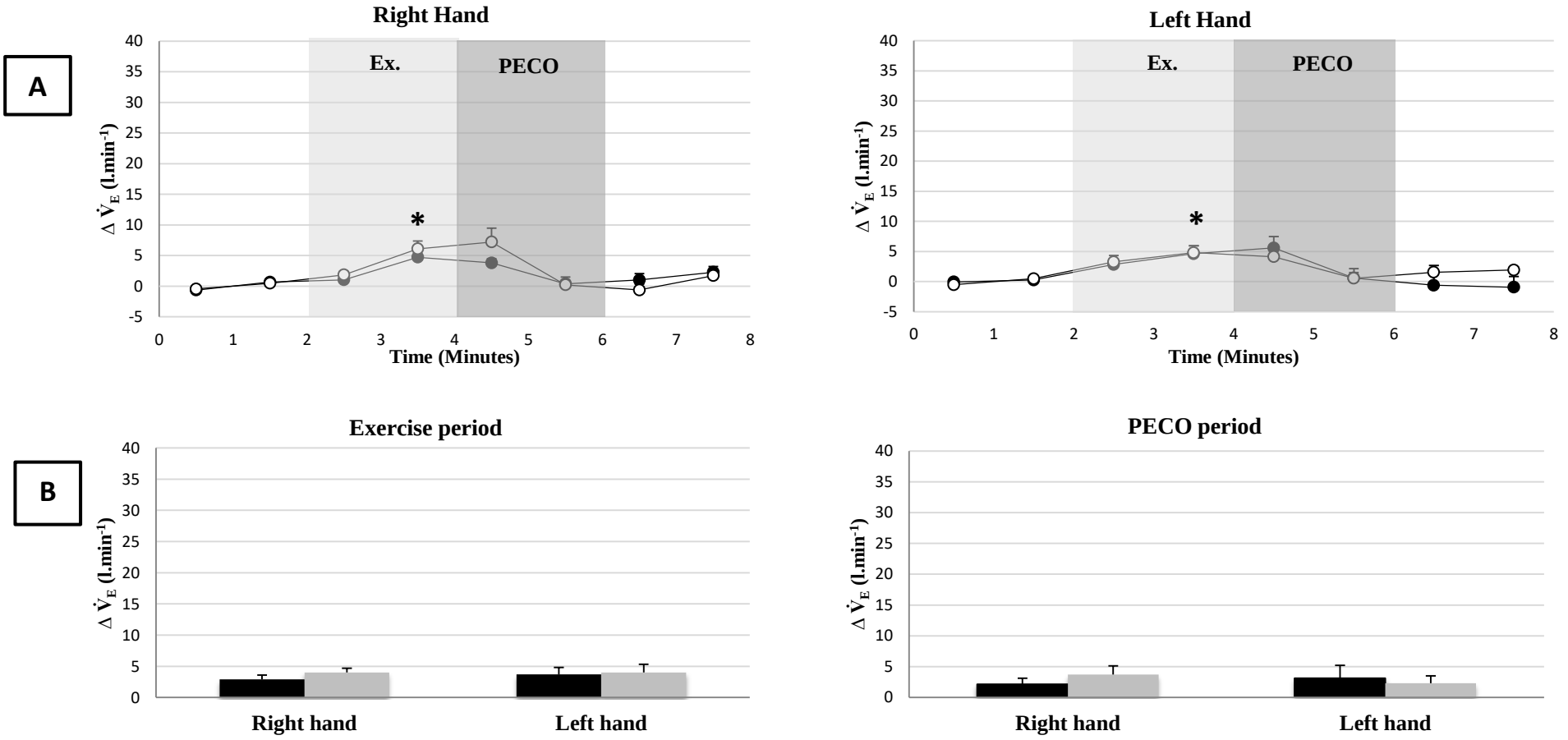


Figure 5.26: A) Mean changes in in the $\dot{V}_E$ from the baseline of the right and left hands, under room air, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) right leg exercise training values are shown. B) Summary measurements of the average changes in $\dot{V}_E$ of the right and left hands during exercise and PECO. Before (black bar) and after (grey bar) training values shown. * Both values were significantly different from baseline values ($p \leq 0.05$).

Breathing frequency (Hand protocol B):

Changes in $\dot{V}_E$ are associated with changes in V_T and *f*. However, only *f* was shown to reach statistical significance. Figure 5.27 (A) shows that the changes in *f* during the last minute of isometric handgrip exercise were significantly greater than baseline in the before- and after-exercise training trials of the right (2.8 ± 0.8 & 3.7 ± 1.0 beats.min^{-1}, respectively, $p \leq 0.05$) and left (4.5 ± 1.1 & 4.5 ± 1.7 beats.min^{-1}, respectively, $p \leq 0.05$) hands. This increase in *f* observed during the exercise period declined to baseline levels during the PECO period in both the right and left hand trials ($p \geq 0.05$).

Exercise training of the dominant leg, had shown no significant effect on *f* values over exercise and PECO periods in either hand ($p \geq 0.05$), as shown in Figure 5.27 (B).

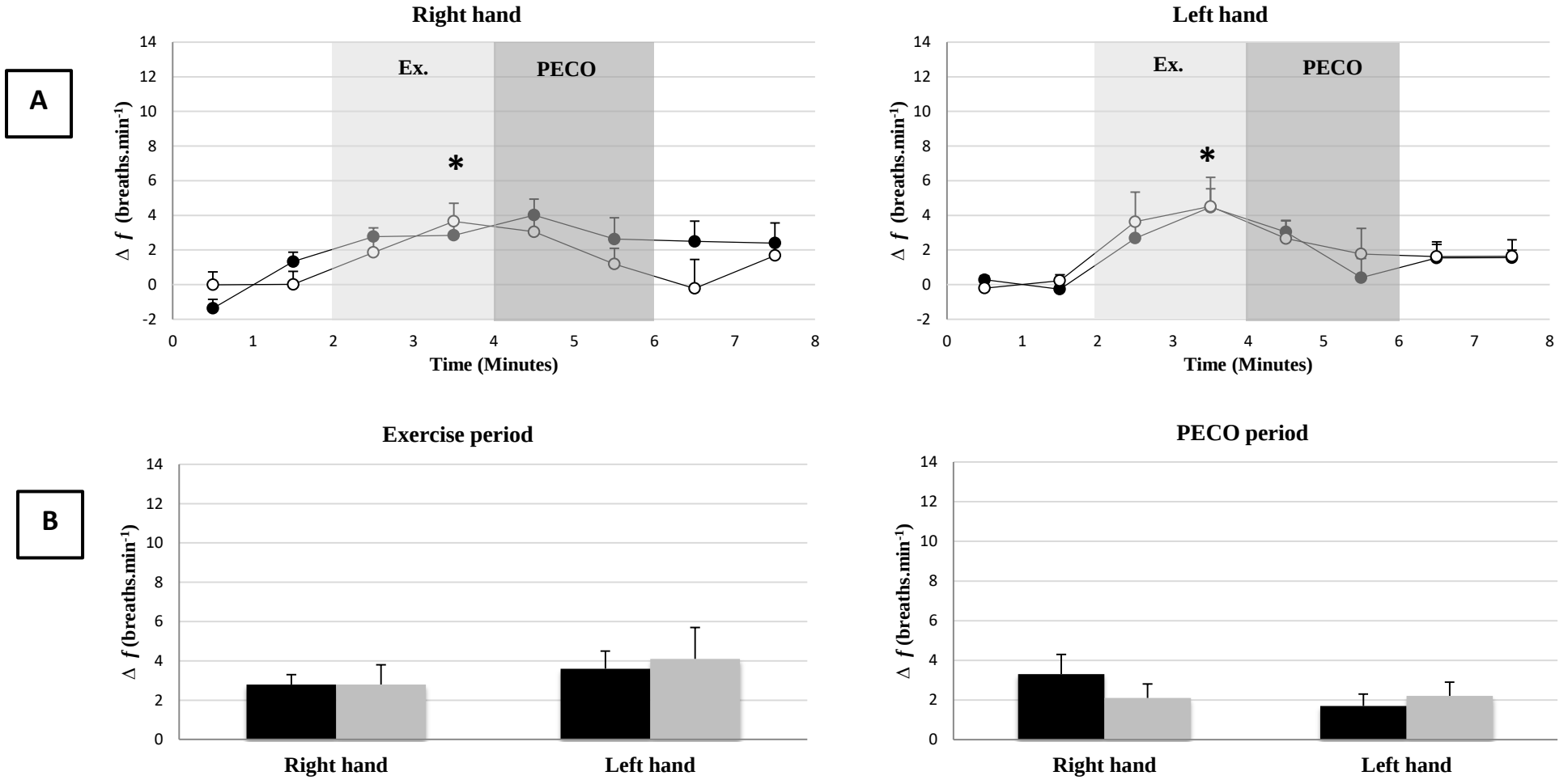


Figure 5.27: A) Mean changes in *f* from the baseline of the right and left hands under room air, every minute (mean ± S.E.M). Before (closed circle) and after (open circle) exercise training values are shown. B) Summary measurements of the average changes in *f* of the right and left hands during exercise and PECO. Before (black bar) and after (grey bar) training values shown. * Both values were significantly different from baseline values ($p \leq 0.05$).

5.4. Discussion

The present study first aimed to assess the effect of high-intensity dynamic exercise training of calf muscles on altering muscle afferent feedback. This was achieved by examining the ventilatory and cardiovascular responses to human muscle metaboreflex activation (by PECO) in both trained and untrained legs, under room air or hypercapnia. Second, the study aimed to investigate the potential cross-over effect of exercise training from the trained leg to the contralateral untrained leg and its level.

The main findings were that 6 weeks of single-leg calf raise training attenuated both the increases in the MAP (under both room air and hypercapnia conditions) and minute ventilation (only under hypercapnia) during the PECO period, only in the trained leg indicating attenuation of the muscle metaboreflex driven increase in ventilation. The study also showed that the above responses were attenuated in both legs by voluntary isometric calf plantarflexion exercise, under both conditions, after training of the dominant leg confirming previous reports of a cross-over effect from the trained to the contralateral untrained leg. This cross-over appears to be at the segmental level, as no changes in responses to handgrip exercise were observed in either limb.

The effect of exercise training on the muscle metaboreflex.

The magnitude of the increased blood pressure response during local circulatory occlusion of exercising muscle is considered to be a reliable and sensitive method of measuring the level of activation of the muscle metaboreflex (Coote et al., 1971, McClosky and Mitchell 1972, Kaufman et al., 1983, Drew et al., 2008). This is because, during this period, central command and muscle mechanoreflex influences are absent while the muscle rests. Prior to exercise training in the present study, the MAP values observed during PECO were similar

under room air and hypercapnia in each leg. Moreover, this pattern was also found after exercise training, where the MAP values were similar for each leg under both conditions albeit with observable training induced adaptations between the legs. These findings indicate a constant level of muscle metaboreflex activation in each leg under both inspired gas conditions

The MAP and HR values reported here during isometric exercise of the human calf muscle, and during the PECO period prior to exercise training, are consistent with Bell and White's (2005) findings. The MAP values observed at the end point of exercise, and during PECO in both legs under both conditions (Figure 5.4 & 5.5), were in agreement with the previous study. The small differences seen in the responses of both legs likely stem from improved activation of the dominant leg and its ability to generate truer MVC. The absolute exercise intensity performed by the non-dominant left leg is, therefore, slightly lower compared to the right leg and, consequently, central command and muscle metabolite accumulation will be proportionately reduced. Nevertheless, this difference was consistently observed throughout the study and should, therefore, not affect limb comparisons.

Isometric calf exercise caused the HR to rise by 10 - 16 beats.min^{-1} in all trials prior to exercise training. However, during PECO and only under hypercapnia, HR values did not recover to the pre-exercise resting baseline but remained slightly elevated until circulation was restored in both legs. Conversely, under room air, the HR recovered to the pre-exercise baseline during PECO in both legs (Figure 5.10). This finding could be attributed to the high level of muscle metaboreflex activation combined with the high level of hypercapnia ($P_{ET}CO_2$ +10 mmHg, above resting value), which may have induced powerful cardiac sympathoexcitation thereby, offsetting the cardiac parasympathetic tone (Somers et al., 1989, Brack et al., 2004, Gladwell et al., 2005, Fisher et al., 2010) (discussed in Chapter 3, page 94).

Following exercise training of the dominant leg, there were changes in the MAP responses in both legs. There was reduction in the increases in MAP during exercise in both legs under both conditions. This was also shown during the PECO period under both conditions, only in the trained leg. These changes in the MAP may reflect central adaptation caused by exercise training. However, the differential change in MAP during PECO can only be explained by attenuation of the muscle afferent feedback (muscle metaboreflex). Plentiful evidence in the literature demonstrates that local muscle training for at least 4 weeks alters the muscle afferent feedback by reducing MSNA. This is shown to be associated with significant decreases in blood pressure during exercise, under several fatigue conditions (Sinoway et al., 1996) or ischaemia (Mostoufi-Moab et al., 1998).

The present study is in agreement with the Fisher and White (1999) findings, who, after training subjects in exactly the same manner as the here, reported attenuation of the muscle afferent feedback only in the trained leg. The authors also reported reduction in the increase in MAP of ~ 30% during the PECO period, following voluntary and electrically evoked muscle contraction, only in the trained leg. This observation provided clear evidence that exercise training attenuated muscle afferent feedback, especially during electrical evoked muscle contraction. This is because, during this trial, any central command influence was completely removed.

Although the present study shows significant attenuation in the increases in MAP during the PECO period of the trained leg, under both conditions, this did not reach the 30 % reduction reported by Fisher and White (in fact, a 15 % reduction was seen here). This is could be due to methodological differences. Despite testing same muscle group (calf) and using the same exercise period (2 minutes), the previous authors set the exercise intensity to 30 % MVC, unlike 50 % MVC set in this study; hence, the increased PRs observed here showed that the participants approached muscle exhaustion more closely at the end of exercise period than in

the previous study. It is likely that there is less scope for reduction in metabolite accumulation in a 50% MVC contraction held for 2 minutes, which is near exhaustion both before and after the training period, as compared to a lower intensity contraction (30% MVC) held for the same duration, which is substantially less than the maximal holding time for that intensity. The present study used the highest exercise intensity tolerable for 2 minutes to achieve the biggest possible muscle metaboreflex response during the PECO period. This was chosen to maximise the ventilatory response, which was shown to give the best possible scope for change after training. Another limitation to consider in the Fisher and White study is that while 30 % MVC can be readily sustained for 2 minutes in a voluntary contraction, it is the limit in an electrically evoked contraction, as higher levels would cause pain and this would alter the observed cardiovascular responses.

This study demonstrated attenuation of the muscle metaboreflex in the trained leg, which in turn had an effect on ventilation. Previous studies consistently reported that activation of muscle metaboreflex by PECO did not prevent ventilation from returning back to baseline levels, suggesting that muscle afferents play no role in ventilation (Rowell et al., 1976, Innes et al., 1989, Haouzi et al., 2001, Fukuba et al, 2007). Indeed, this was also observed here (Figure 5.12), where minute ventilation during PECO returned to levels close to the pre-exercise baseline in both legs under room air. This also shows that exercise training had no effect on minute ventilation under this condition. However, under hypercapnia, activation of the muscle metaboreflex (by PECO) resulted in sustained minute ventilation above pre-exercise resting levels in both legs, which is in agreement with previous reports from this laboratory (Lykidis et al., 2010, Bruce and White 2012) and Chapter 3 of this thesis.

It can be argued that this increase in minute ventilation, observed during the PECO period, is brought about by pain. However Alam and Smirk (1938) refuted that as the HR was not elevated during PECO in their study. This was also observed here under room air conditions.

During hypercapnia, on the other hand, the HR was elevated during PECO; however, as already discussed, this appears to be related to sympathoexcitation via chemoreflex stimulation not pain. Moreover, pain should progressively increase during PECO and this should arguably elicit a parallel progressive increase in HR. This was not observed here; in fact, participants reported only mild pain during the PECO period in both legs.

The present study revealed that exercise training attenuated the muscle metaboreflex, as the attenuation in MAP increases during the PECO period was only observed in the trained leg. Moreover, there was attenuation of the increase of minute ventilation during PECO in the trained leg, only under hypercapnia (~ 45 %) (Figure 5.13). This could be explained by changes in systemic sensitivity to hypercapnia following exercise training. However, as minute ventilation did not change in both legs following exercise training (Table 5.2) and the hypercapnia level did not change across trials from the normal level of $P_{ET}CO_2$ measured on each day of the testing ($P_{ET}CO_2$ set at +10 mmHg, above resting value), this explanation is not likely especially as the day to day variability in $P_{ET}CO_2$ was only 0.33%. The MVC did not change in both legs after training (Table 5.1) (trained (Rt.): + 1% and untrained (Lt.): + 0.05%), and therefore both absolute and relative exercise intensity remained constant during each trial. Since the ventilatory response to PECO with hypercapnia decreased by an average of 45% it is clear that there can be no relationship between changes in MVC and ventilation.

This finding supports evidence gained by Bruce and White (2015) on potential synergistic interaction between the muscle metaboreflex and the chemoreflex. The present findings suggest that desensitisation of one of these interacting neural mechanisms (the muscle metaboreflex) attenuated increases in minute ventilation. Muscle metaboreflex desensitisation is also supported by the observation that blood lactate levels measured after exercise did not change following exercise training here (Table 5.1). The ability of muscle afferents to become desensitised to metabolites was proposed by Sterns et al. (1991), who reported

attenuation in MSNA levels during PECO in patients with CHF, compared to healthy participants. The authors explain that this attenuation was caused by desensitisation of muscle metaboreceptor afferents, especially in CHF patients chronically exposed to acidotic conditions. Furthermore, in healthy participants, Carrington et al. (1999) demonstrated attenuation in the exercise PR to a standard electrically evoked exercise in sprint-trained participants (400 meter sprint), who were chronically exposed to high levels of anaerobic exercise.

The effect of exercise training on central command (cross-over effect).

The present study successfully replicated the cross-over effect of exercise training from the trained leg to the contralateral untrained leg during isometric exercise previously reported by Fisher and White (1999). This was observed in reduction of the increases of MAP and minute ventilation during the exercise period in the untrained contralateral leg, under both conditions, following training (Figure 5.6 and 5.12). This cross-over effect of exercise training is unlikely to be explained by a habituation effect as the exact purpose of the study was not revealed to participants. Moreover, before conducting baseline measurements, participants were carefully habituated to the experimental procedures and more than 6 weeks was allowed before and after exercise training measurements. Therefore, habituation can be excluded as a confounding factor in the present study. However, the idea of a cross-over effect of exercise training from trained to contralateral untrained limbs has been demonstrated in numerous previous studies. For instance, Davies et al. (1985) reported that hand exercise training for 8 weeks resulted in increased strength of the trained hand and 40 % of the this strength increase crossed over into the untrained hand. The authors concluded there was adaptation in the neural drive. Furthermore, Lewis et al. (1984) reported that 50 % of the

strength gained in the quadriceps was transferred to the contralateral untrained leg. Moreover, they found that when the participants performed the exercise at the same relative force (% MVC) as in pre-training, the blood pressure, HR and electromyography (EMG) activity of both legs did not change following exercise training of one leg. However, when the exercise was performed at the same absolute force (as in the before trials), these parameters were reduced compared to pre-training values in both legs. In the present study, exercise training had no effect on the MVC of both legs and all participants exercised at the same relative and absolute intensities during pre- and post-training trials. This indicates that changes in the cardiovascular and ventilatory responses during isometric exercise of both legs under both conditions were not due to difference in exercise intensity.

The attenuation of MAP during exercise seen here in the trained and contralateral untrained legs, under both conditions, corroborates the findings of Fisher and White (1999), who postulate that this represents adaptation in the command. Their argument was based on the observation of cardiovascular responses to voluntary and electrically evoked exercise (where central command influence is absent) of the contralateral untrained leg following exercise. They noted attenuation of the cardiovascular response only during the voluntary exercise, but not in electrically evoked exercise of identical intensity. In the present study, the rise in the MAP in the contralateral untrained leg was attenuated during voluntary isometric exercise under both conditions (Figure 5.7). Furthermore, it was successfully illustrated here that, during voluntary isometric exercise, increases in minute ventilation were attenuated in both legs under room air conditions and significantly so under hypercapnia (Figure 3.13).

The level of cross-over effect of exercise training.

The present study further evaluated the degree of the cross-over effect of exercise training, which may occur at segmental (spinal cord) or central (brainstem: cardiovascular area) level. Findings from the Hand Protocol A showed no changes in the ventilatory and cardiovascular responses to right isometric handgrip exercise (at 50 % MVC) and PECO, under both conditions, following dominant leg exercise training (Figure 5.17 & 5.20). Furthermore, when the participants were tested at 30 % MVC (Hand Protocol B; same as the leg protocol in the Fisher and White (1999) study, in order to provide a wide scope for changes to occur (unlike at 50 % MVC), both hands showed no changes in ventilatory and cardiovascular responses to isometric handgrip exercise and PECO following exercise training of the dominant leg (Figure 5.23 & 5.26). This indicates that the cross-over effect of exercise training should take place somewhere at the segmental level. This finding emphasised previous work by Yue and Cole (1992), who reported that exercise training of the abductor muscle of the fifth hand digit for 4 weeks resulted in increases in the maximum contraction force of this muscle in the trained hand (by 30 %) and untrained hand (by 14 %) . Moreover, there were no changes in the maximum contraction force of the big toe extensors muscle, thereby concluding that exercise training hand no general effect on muscle strength. Furthermore, Hortobágyi et al. (1999) demonstrated that 6 weeks of an ipsilateral training programme resulted in an increase in the quadriceps muscle strength of the trained and untrained legs, but with no changes in handgrip strength. These previous findings, as well as the results of this work, suggest that cross-over effects of exercise training occur at the segmental level (spinal cord).

Conclusion.

The present study confirms that calf muscle metaboreceptor activation by PECO (when central command is absent and muscle mechanoreflex activity is unlikely) has little effect on ventilation during room air breathing. However, during exposure to concurrent hypercapnia, calf muscle metaboreceptor activation does increase ventilation substantially above that caused by hypercapnia alone.

The exercise training programme involving calf raise training for 6 weeks resulted in attenuation of muscle metaboreflex activation in the trained limb muscles, as indicated by significant reductions in the PR during PECO in the trained limb, under both experimental conditions.

This study has shown, for the first time that attenuation of muscle metaboreflex activation, following local muscle training, is associated with a decrease in ventilation, as revealed during exposure to concurrent mild hypercapnia. This apparent reduction in the drive to breathe from the trained muscle may have important clinical implications; training interventions could potentially be employed for treatment of diseases characterised by exaggerated muscle afferent activation (as a possible link to breathlessness) during exercise.

CHAPTER 6: GENERAL CONCLUSIONS

6.1. General conclusions:

It is well established that mechanically and metabolically sensitive thin fibre muscle afferents (group III and IV) play a controlling role in the cardiovascular response during exercise, alongside central command. On the other hand, their roles in mediating exercise hyperpnea is a poorly defined subject that has been debated over a century. Historically, it was postulated that muscle afferents had no influence on ventilation, based on several lines of evidence (see literature review) showing that activation of these muscle afferents (e.g. muscle metaboreflex by PECO) alone did not prevent ventilation form returning to resting baseline levels. However, recently, a surge in new evidence in this field led to the idea that muscle afferents in fact play a role in mediating exercise hyperpnea. These findings were based on observations from blocking muscle afferent feedback using the μ-opioid receptor agonist (fentanyl), which attenuated ventilation during exercise in healthy participants (Amman et al., 2010) and patients with COPD and CHF (Gagnon et al., 2012, Olson et al., 2014). Other methods showed that activation of these muscle afferents (by PECO), in combination with stimulation of the ventilatory chemoreflex (by inhaling a mild hypercapnic gas mixture), resulted an increase in the ventilation to levels above the hypercapnic baseline (Lykidis et al., 2010; Bruce and White, 2012, 2015, 2016). These recent observations all suggest that inputs from muscle afferents can cause an increase in ventilation in resting subjects, combined with other synergistic inputs form central command or from the chemoreflex. This thesis further examined the nature of this synergistic interaction between muscle afferent inputs (the muscle metaboreflex) and the ventilatory chemoreflex using methodology that allows for controlling of one mechanism while experimentally manipulating the other.

The first study in this thesis (Chapter 3) examined the ventilatory and cardiovascular responses to activation of the muscle metaboreflex by PECO under different levels of concurrent hypercapnia. This study aimed to determine the nature of relationship between the

level of hypercapnia and the magnitude of the additional ventilation produced in response to a standardised level of muscle metaboreflex activation. The result showed a consistent cardiovascular response to activation of the muscle metaboreflex during PECO, following standardised isometric exercise, which was independent from hypercapnia levels. Moreover, this shows that the magnitude of the increase of the ventilation response to a standardised level of muscle metaboreflex activation progressively increased along with the level of hypercapnia suggesting a linear relationship. This finding supports previous evidence indicating synergistic interaction between the muscle metaboreflex and the ventilatory chemoreflex. Furthermore, it suggests that the sensitivity of this interaction in turn increases with increasing levels of systemic hypercapnia.

Chapter 4 of this thesis (control study) aimed to examine the causes for the ventilation drifting baseline. This was achieved by evaluating the ventilatory and cardiovascular responses to each component of the 1st study protocol alone. Results revealed that exposure to hypercapnia caused an increase in MAP in the 3 trials (resting, exercise and circulatory occlusion). The other main finding was that exposure to hypercapnia ($P_{ET}CO_2$ set at +10 mmHg, above resting value) resulted in a progressive increase in minute ventilation in all 3 trials over a longer period than expected, which appears to be a form of ventilatory long-term facilitation. It was expected that ventilatory responses to hypercapnia would become more stable within 5 minutes form hypercapnia exposure, as suggested by Mateika and Sandhu (2011). However, observations from this control trial showed that minute ventilation was still progressively increasing during this period and became more stable (the rate of increase decline) after 10 minutes of hypercapnia exposure.

The continuous rise in the minute ventilation was observed following intervention in the exercise and circulatory occlusion trials; this did not fully recover to pre-intervention baseline values, which was attributed to a form of ventilatory long-term facilitation. This

interpretation was based on a similar pattern of minute ventilation increase observed during the control trial, suggesting a drifting baseline which is known to affect measurements of any intervention response. Therefore, a new method had to be developed which would take into consideration the drifting baseline caused by hypercapnia. This method involved plotting a linear regression line using the actual value of the ventilatory response (values in the last 2 minutes of the pre-exercise resting period and final 2 minutes of recovery period) for each participant in each trial. From the slope and intercept of these values, ventilatory responses between theses minutes were adjusted. Then, the difference between the actual values and adjusted values were calculated. This method was used only in the hypercapnic trials in studies 1 and 2 (Chapter 3 & 5, respectively).

The second study in Chapter 5 of this thesis aimed to investigate the effect of local muscle exercise training (calf raises) on altering muscle afferent feedback, which may affect the contribution of these fibres to hyperpnea. This was achieved by examining the ventilatory and cardiovascular responses to a standardised level of muscle metaboreflex activation (by PECO), combined with stimulation of the ventilatory chemoreflex (under hypercapnic conditions) before and after 6 weeks of local muscle training. Findings from this study revealed that exercise training induced attenuation of the muscle metaboreflex, observed by a significant reduction in the increases in MAP (by ~ 15 %) in the trained leg only, during PECO, under room air and hypercapnia. Furthermore, this study displayed, for the first time, attenuation of the increases in minute ventilation (by ~ 45 %) only in trained leg under hypercapnia. Furthermore, this study successfully demonstrated attenuation of the increases of MAP and minute ventilation during isometric calf planterflexion in both legs, under both conditions, which indicates a cross-over effect of exercise training from the trained leg to the contralateral untrained leg. This cross-over effect of exercise training was not detected in hand protocols (Protocols A and B) following exercise training of the right leg. The reason

for this is that cardiovascular and ventilatory responses to handgrip exercise and PECO did not change following exercise training. Therefore, it is suggested that the cross-over effect of exercise training may occur at the segmental level.

In healthy humans, activation of the muscle metaboreflex by PECO under room air is does not prevent minute ventilation from returning to baseline levels. According to this, most of the preliminary evidence discussed here suggests that muscle afferents have no role in mediating ventilation during exercise. However, this finding should be approached with caution, as suppression of ventilation during PECO may be explained by activation of the ventilatory baroreflex. Activation of the muscle metaboreflex by PECO caused an increase in MSNA, which is associated with an increase in blood pressure to maintain blood perfusion to the exercising muscle. This increase in the blood pressure is caused by stimulation of the baroreceptors, which initiate a reflex. Activation of the ventilatory baroreflex can, in turn, mask the contribution of the muscle metaboreflex to ventilation during PECO, resulting in a drop of the ventilation to resting levels (Stewart et al., 2011). Previous studies in this laboratory, and this thesis, used systemic hypercapnia to manipulate the sensitivity of the respiratory control centre to muscle afferent inputs. This technique showed that, only under hypercapnia, activation of the muscle metaboreflex by PECO resulted in a sustained increase of the minute ventilation to above hypercapnic baseline suggesting synergistic interaction between the muscle metaboreflex and central chemoreceptor inputs. Therefore, stimulating the ventilatory chemoreflex can inhibit the ventilatory baroreflex, which in turn un-masks the contribution of the muscle metaboreflex to ventilation during PECO.

Studies 1 and 2 (Chapter 3 and 5, respectively) investigated the idea of synergistic interaction between the inputs further. In study 1, activation of the muscle metaboreflex was constant across 4 trial (as shown in Figure 3.5), while the levels of hypercapnia varied ($P_{ET}CO_2$ clamped at +1, +3, +7 and +10 mmHg, above resting value). The results showed a

progressive increase in the magnitude of minute ventilation with increasing levels of hypercapnia during the PECO period, which, therefore, suggests a linear relationship between the these two parameters.

Conversely, in the second study (Chapter 5), both the hypercapnic level ($P_{ET}CO_2$ clamped at +10 mmHg, above resting value) and the level of muscle metaboreflex activation (50 % MVC) were constant across the leg trials. The difference between the two studies is that in the latter, the level of hypercapnia remained constant across all trials, while feedback from muscle metaboreceptors was manipulated by exercise training (no changes in exercise intensity and PECO duration). As expected, the study showed that exercise training caused attenuation in the muscle metaboreflex. This was reflected by a significant reduction in the increases in MAP (~ 15 %) and minute ventilation (~ 45 %) during PECO of the trained leg, under hypercapnia.

The findings from both studies provided clear evidence for the presence of synergistic interaction between the inputs, especially the muscle afferent feedback (metaboreflex) and central chemoreflex. This is in agreement with previous studies by Amman et al (2010), Gagnon et al. (2012) and Olson et al. (2014), where fentanyl abolished muscle mechano- and metaboreflex inputs by blocking afferent feedback, in the presence of the central command. As a result, there was significant attenuation of the ventilatory response during exercise. Taken together, these findings suggest that muscle afferents have a limited ability to drive ventilation during exercise alone, unless it is combined with other neural inputs such as, central command and/or the central and/or peripheral chemoreflex. An illustration of this synergistic interaction between the inputs shown in Figure 6.1.

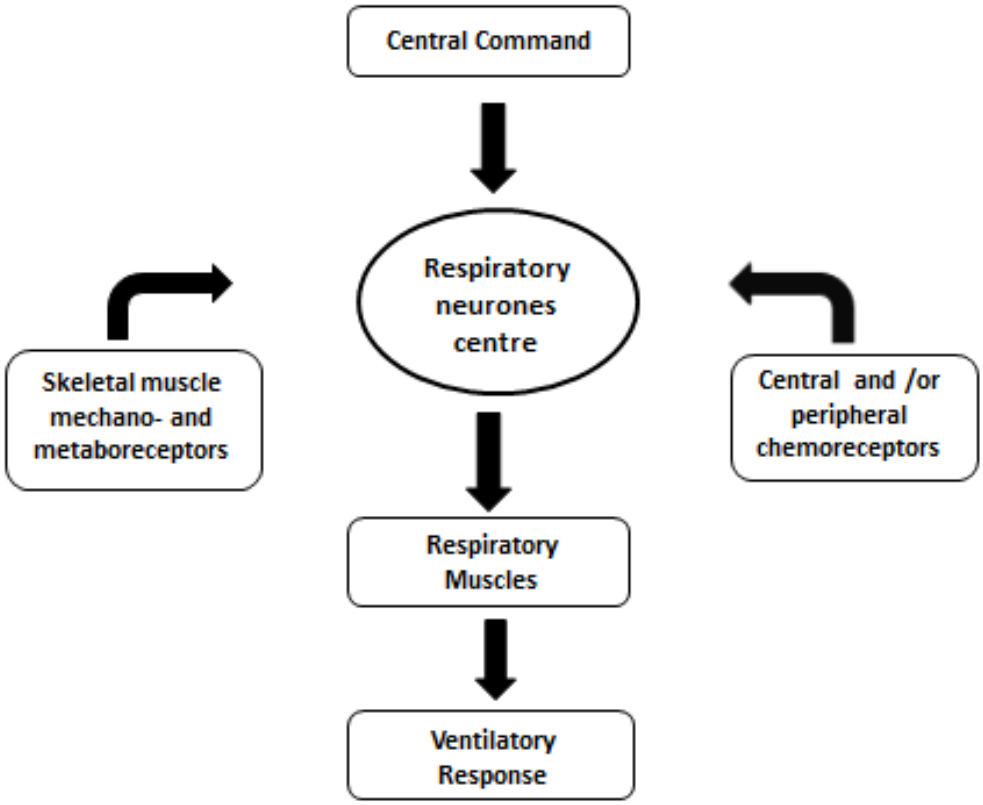


Figure 6.1: Illustration of the synergistic interaction between central command input, skeletal muscle afferent feedback (mechano- and metaboreceptores), neural input central and/or peripheral chemoreceptors at the respiratory neurons centre in the brainstem.

6.1.1. Possible site for the synergistic interaction between neural inputs.

There are two possible sites in the brainstem that may project the aforementioned synergistic interaction between the inputs (Figure 6.2). One of these sites is the NTS. The NTS is a crucial location where these inputs are integrated and processed, as it contains respiratory group neurons that function to regulate respiration (Paton, 1999). It is well established that muscle afferents and peripheral chemoreceptors are projected to the NTS (Kalia et al., 1981, Donoghue et al., 1984, Gonzalez et al., 1994, Li et al., 1997, Paton et al., 2001). Moreover,

both Feldman (2003) and Nattie and Li (2009) suggested that central chemoreceptors may be located in the NTS.

It was recently suggested that the PAG may play a key role in regulating the cardiorespiratory centre during exercise (Paterson, 2014). It is well demonstrated, from human studies, that the PAG is involved in the central command neurocircuitry; Green et al. (2005) noted that stimulation of the ventral PAG caused a decrease in systolic blood pressure, whereas stimulation of the dorsal PAG conversely induced an increase in systolic blood pressure. Furthermore, the authors found an increase in PAG neural activity during exercise anticipation and more so during actual exercise performance (Green et al., 2007). Relating to muscle afferent feedback, Williams et al. (1990) found that removal of the PAG resulted in attenuation of the PR to electrically evoked exercise in cats. Furthermore, in humans, Basnayake et al. (2010) demonstrated an increase in PAG activity during activation of the muscle metaboreflex during PECO. The above electrophysiological evidence suggests that the PAG plays an essential role in integrating neural inputs from skeletal muscle afferents to produce cardiovascular and ventilatory responses. Recent evidence has also suggested that central chemoreceptors that are sensitive to small changes in the concentration of CO_2/H^+ may be found in the PAG. Lopes et al. (2012) showed that chemical lesioning of the PAG in rats resulted in a significant decrease in the ventilatory response to hypercapnia. All of the above findings point to the PAG as a primary location for integrating inputs from central command, skeletal muscle afferents and chemoreceptors relaying these signals to respiratory muscles to initiate the ventilatory response.

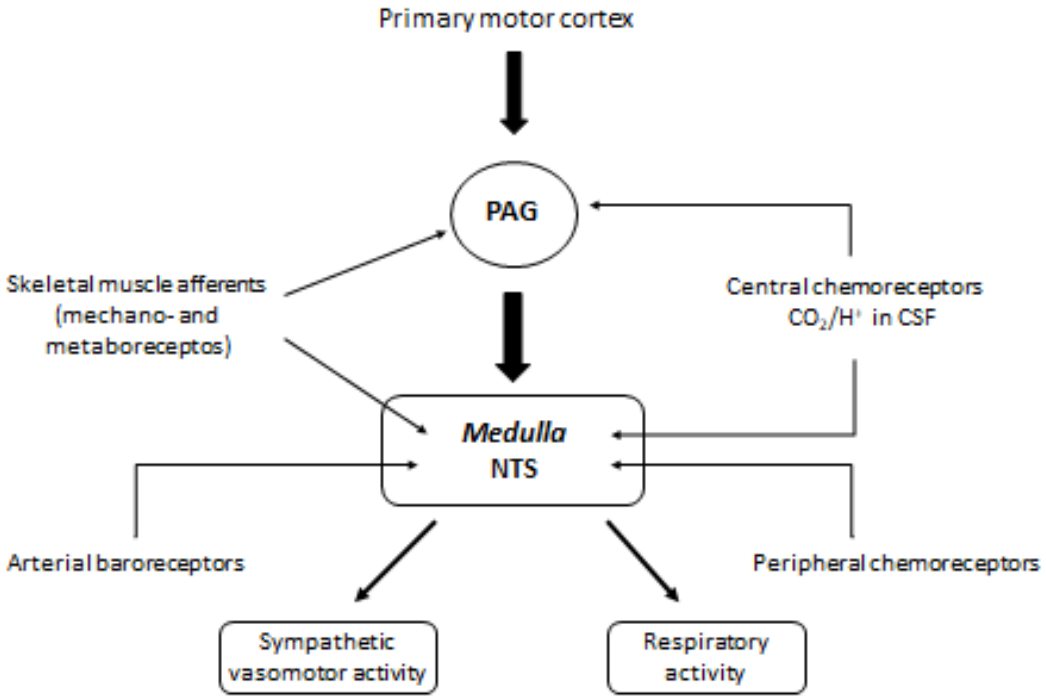


Figure 6.2: Schematic of the potential region of the brainstem where inputs from central command, muscle afferents and central/peripheral chemoreceptors may be integrated. CSF, cerebrospinal fluid, PAG, Periaqueductal gray, NTS, Nucleus tractus solitarius.

6.2. Implications

Studies conducted on patients with CHF and COPD have shown that activation of muscle afferents by PECO alone resulted in sustained minute ventilation above the pre-exercise resting baseline (Piepoli et al., 1996, Bruce et al., 2016). These findings indicate that muscle afferents contribute to increasing ventilatory responses to exercise in such diseases. A further detailed investigation into the involvement of these muscle afferents in hyperpnea was performed using epidural anesthesia in the above patients. Gagnon et al. (2012) used with the μ-opioid agonist, fentanyl, injected into the L3 - L4 vertebral space to block neurotransmission of group III/ IV muscle afferents in lower limb muscle in patients with

COPD. The results of this study revealed a significant reduction in minute ventilation and breathing frequency to constant work rate cycling exercise the fentanyl group, compared to placebo. Moreover, the study shows a significant reduction in dyspnea levels in the fentanyl group. The authors further reported that patients receiving fentanyl showed improvement in exercise tolerance, observed by significantly increased exercise duration from around 7 to 10.5 minutes. Olson et al. (2014) also used fentanyl injections in CHF patients to selectively block muscle afferent feedback. The authors showed significant reduction in minute ventilation in these patients, which was associated with a significant reduction in breathing frequency to constant work rate cycling exercise, compared to control. The findings taken together provide strong evidence that muscle afferent feedback contributes to ventilation during exercise, as well as playing an important role in exercise intolerance and dyspnea.

It is well known that CHF patients experience reduced exercise tolerance as well as increased muscle fatigue and dyspnoea, although this is not directly associated with impaired left ventricular function and central hemodynamics. Findings from surgical interventions (heart transplantation) (Marzo et al., 1992) and pharmacological therapy (Franciosa and Cohn, 1979, Wilson et al., 1984), show improvement in both ventricular function and central haemodynamics but not in exercise tolerance.

The symptoms are however associated with the deconditioning of the muscle, secondary to dyspnoea and associated avoidance of physical activity as well as chronic underperfusion of the muscle during exercise due to augmented sympathetic vasomotor activity (Piepoli et al., 2008, Rehn et al., 2012). Abnormal metabolism and early acidosis during exercise provide a stimulus to initiate an early and exaggerated increase in cardiovascular and ventilatory responses, causing exercise intolerance and dysponea.

Skeletal muscle dysfunction is also characterized by muscle atrophy (Piepoli et al., 2008) and shifting of muscle fibres from type I to II (slow to fast) (Piepoli and Crisafulli, 2014). Another feature is metabolic abnormality, such as increases in acid phosphates (Lipkin et al., 1988), reduction of mitochondrial density and abnormal oxidative metabolism activity (Piepoli and Crisafulli, 2014). These features of skeletal muscle dysfunctions were previously reported in COPD patients (Jobin et al., 1998, Engelen et al., 2000, Gosker et al., 2002, 2007) and, notably, changes in muscle metabolism in CHF and COPD patients could significantly contribute to exercise intolerance and dyspnea. Early acidification of the muscle during the onset of exercise leading to abnormal firing and over-activation of metabolic sensitive thin fibre muscle afferents (metaboreflex), resulting in abnormal cardiovascular and ventilatory responses to exercise (Grieve et al., 1999, Piepoli and Crisafulli, 2014).

Exercise training has been shown to improve the metabolic capacity of skeletal muscle in CHF and COPD patients (Casaburi et al., 1991, Sala et al., 1999, Ventura-Clapier et al., 2007); improved muscle metabolism may, in turn, diminish the level of muscle metaboreceptive stimulation and reduce the ventilatory response to exercise and dyspnea. Indeed, Piepoli et al. (1996) found that 6 weeks of single-hand rhythmic exercise training in CHF patients resulted in reductions in the DBP and ventilatory responses during PECO indicating attenuation of the muscle metaboreflex. Although they did not measure muscle metabolic activity, the authors attributed the attenuation of muscle metaboreflex to this.

An alternative explanation could be desensitisation of muscle metaboreceptive afferents following exercise training. In the training study (Chapter 5), healthy participants with normal physiology were recruited. Findings from this study provided evidence that exercise training caused attenuation of the muscle metaboreflex, which in turn was associated with attenuation of the MAP (under room air and hypercapnia and ventilatory responses (under hypercapnia) to voluntary exercise of the trained leg. Moreover, as there was no significant

difference in lactate acid levels before and after exercise training, it can be suggested that muscle afferents become desensitised following exercise training.

This hypothesis is supported by the findings of Sterns et al. (1991) and Carrington et al. (1999) (discussed on page 180 of this thesis), as well as the aforementioned COPD studies, which showed no significant difference in the increases in blood pressure during PECO periods between patients and healthy participants indicating the same level of muscle metaboreflex activation (Roseguini et al., 2008, Sherman et al., 2011, Bruce et al., 2016). From this finding, it is more likely that desensitisation of muscle metaboreceptive afferents following the exercise training occurs and results in the attenuation in the rises of MAP. Although the effect of exercise training on altering muscle afferent feedback showed attenuation on blood pressure, MSNA and minute ventilation in CHF patients (Piepoli et al., 1996) and healthy participants (Sinoway et al., 1996, Mostoufi-Moab et al., 1998, Fisher and White, 1999), as well as in Chapter 5 of this thesis, there has so far been no controlled study examining the ventilatory and cardiovascular responses to muscle metaboreflex activation following exercise training in COPD patients.

Studies such as the training study in the current thesis, and previous literature, could potentially be highly relevant for patients whereby they could be recommended certain exercise programmes. There may also be a paradigm shift as to the importance of local muscle training, compared to whole body training, as the former e.g. hangrip training is shown to improve exercise tolerance and reduce dyspnea (Piepoli et al., 1996). Recent meta-analysis of training studies on COPD indicates that a combination of resistance training (RT) and endurance training (ET) is more effective than ET alone in increasing leg muscle strength (Iepsen et al., 2015). As the higher forces and increased metabolite levels associated with resistance training are likely the stimuli which cause hypertrophy in COPD patients and are also likely to have caused the adaptation in the training study in this thesis, it is highly likely

that RT would be useful in reducing afferent feedback in COPD patients. The exact combinations of intensity and duration of exercise that are tolerable but required, to adapt afferent feedback in patients groups remains to be discovered but may be less than in healthy people given the historical inactivity patterns of patient groups.

An alternative pharmacological approach that desensitizes muscle metaboreceptive afferents may also be benefit. This, as well as exercise training, could have a great impact on improving exercise tolerance and reducing the level of dyspnoea in patients with CHF and COPD. For instance amiloride (used to treat hypertension or swelling due to heart failure), which blocks acid sensing ion channels (ASICs), resulted in attenuation of the blood pressure response to isometric calf exercise and PECO in decerebrate cats (Hayes et al., 2008, McCord et al., 2009). Cui et al. (2011) used pyridoxine hydrochloride (vitamin B_6), locally infused into the isolated circulation of the human forearm and found a significant attenuation of MSNA and blood pressure during both isometric exercise and PECO periods. Pyridoxine hydrochloride works on selectively blocking P2- receptors, which are known to contribute to stimulation of the muscle metaboreceptors. It is not yet known whether such treatments would safely attenuate ventilatory responses to exercise. Given the additional systemic benefits of exercise it would seem sensible to promote this approach until pharmacological treatment is available or indeed in addition to it.

6.3 Final conclusion

In conclusion, muscle afferent feedback in healthy humans shows a limited ability to drive ventilation in isolation. However, in the presence of synergistic interaction from the systemic chemoreflex, activation of the muscle metaboreflex caused augmentation in ventilation during PECO. The ventilation observed during PECO linearly increased with the level of hypercapnia. Exercise training was able to alter muscle metaboreceptive afferent feedback most likely as results of desensitisation, which in turn lead to attenuation in blood pressure and minute ventilation responses during PECO of the trained leg under hypercapnia. Future studies into the effect of exercise training on altering muscle afferent feedback in patients are essential to provide a comprehensive understanding about the role of these afferents in mediating ventilatory and cardiovascular responses to exercise, as well as their modification by exercise training.

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APPENDIX

Pain scale:

This pain scale was used in the leg trails in study 2 (Chapter 5).