

THE ROLE OF SKELETAL MUSCLE AFFERENT
FEEDBACK IN VENTILATORY AND
CARDIOVASCULAR CONTROL DURING HUMAN
EXERCISE

By

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Abstract

Stimulating muscle metaboreceptive afferents alone via post exercise circulatory occlusion (PECO) typically does not result in hyperpnea in healthy humans. However ventilatory responses have been observed if metabolite accumulation is great enough (e.g. in diseased states) or during a concurrent hypercapnia-induced chemoreflex, suggesting a possible synergistic interaction. This thesis investigated the ventilatory responses to interactions between muscle afferent feedback and potentially synergistic inputs. It was firstly observed that muscle metabo/mechanoreflex activation (via PECO and passive muscle stretch, respectively) increases ventilation but only during acute hypercapnia. Additional investigations suggested that these ventilatory responses were caused by a central interaction, possibly between the medullary input from muscle afferents and central chemoreceptors. Secondly, experimental augmentation of the muscle metaboreflex enhances the ventilatory response during exercise, but not during PECO, suggesting interactions between the metaboreflex and other inputs activated in exercise. Lastly, PECO caused increased ventilation in COPD patients but this was unrelated to chronic hypercapnia. Collectively these findings suggest that in health, muscle metabo/mechanoreflex stimulation induces ventilatory responses, but their effects only appear to be unmasked in combination with secondary synergistic inputs. However, when the metaboreflex is powerful enough, arguably such as in COPD, ventilatory responses to metaboreceptor stimulation alone can be observed.

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- Bruce, R.M. and White M. J. (2012) The ventilatory responses to muscle metaboreflex stimulation during concurrent hypercapnia in humans: roles of central and peripheral chemoreception. *J. Physiol (online) Proc Physiol Soc* 26 C07 & PC07
- Bruce, R.M., Turner, A. and White M. J. (2013) The ventilatory responses to muscle metaboreflex stimulation in patients with COPD. *FASEB J* March 29, 26:702.7

ABBREVIATIONS

ANOVA = analysis of variance

ASIC = acid-sensing ion channel

ATP = adenosine triphosphate

BP = blood pressure

BECF = brains extracellular fluid

BTPS = body temperature and pressure, saturated

CHF = chronic heart failure

CNS = central nervous system

CO = circulatory occlusion

CO₂ = Carbon dioxide

COPD = chronic obstructive pulmonary disease

DBP = diastolic blood pressure

ECG = electrocardiogram

Ex = exercise

Ex L = exercise of the left leg

Ex R = exercise of the right leg

GABA = gamma-aminobutyric acid

GOLD = global initiative for chronic lung disease

H⁺ = hydrogen ion/proton

H_2PO_4^- = diprotonated phosphate

HCO_3^- = Bicarbonate

HR = heart rate

IRTX = iodo-resiniferatoxin

K^+ = potassium ion

Kin₁ = Bradykinin receptor

MAP = mean arterial blood pressure

mmHg = millimetres of mercury

MSNA = muscle sympathetic nerve activity

MVC = maximal voluntary contraction

NTS = nucleus tractus solitarius

P₁ = purinergic 1

P_{2X} = purinergic 2X

P_{2Y} = purinergic 2Y

PaCO_2 = partial pressure of arterial carbon dioxide

PAG = periaqueductal grey area

PaO_2 = partial pressure of arterial oxygen

PECO = post-exercise circulatory occlusion

PECO (L) = post-exercise circulatory occlusion of the left leg

PECO (R & L) = post-exercise circulatory occlusion of the right and left legs

PECO (R) = post-exercise circulatory occlusion of the right leg

P_{ETCO_2} = partial pressure of end-tidal carbon dioxide

PLP = pyridoxal-5-phosphate

PPADS = pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid

RRI = R-R interval

SBP = systolic blood pressure

SD = standard deviation

SEM = standard error of the mean

SNS = sympathetic nervous system

TRPA1 = transient receptor potential A1 channel

TRPV1 = Transient receptor potential vanilloid 1 receptor

$\dot{V}\text{CO}_2$ = carbon dioxide production

\dot{V} = minute ventilation

$\dot{V}\text{O}_2$ = oxygen consumption

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CHAPTER 1: LITERATURE

REVIEW

1.1 Neural structures controlling ventilation at rest

The respiratory neurones within the pons and medulla of the brainstem control breathing by generating and maintaining the respiratory rhythm, integrating sensory inputs and also relaying signals to the respiratory muscles. Respiratory neurones can be categorised based upon their location and each group provide different functions (West, 2008). Within the medulla are two identifiable areas. Dorsal respiratory group neurones (which is composed of cells in the nucleus tractus solitarii; NTS) are thought to be primarily responsible for the generation of inspiration and for receiving and processing sensory inputs from arterial chemoreceptors and baroreceptors. The ventral respiratory group regulates expiration as well as inspiration secondarily to the dorsal respiratory group. Finally, neurones in the pneumotaxic centre (pontine respiratory group) and apneustic centre, of the upper and lower pons respectively, can coordinate the transition between inhalation and exhalation by regulating inspiratory depth. Although there is an incomplete understanding in how respiratory rhythm is generated, two locations in the rostral ventrolateral medulla contain neurones with pacemaker like properties; the pre-Bötzinger complex (Smith *et al.*, 1991) and the parafacial respiratory group (Onimaru & Homma, 2003), and there is evidence that these groups separately regulate inspiration and expiration, respectively (Janczewski & Feldman, 2006). For a review see Feldman *et al.* (2013).

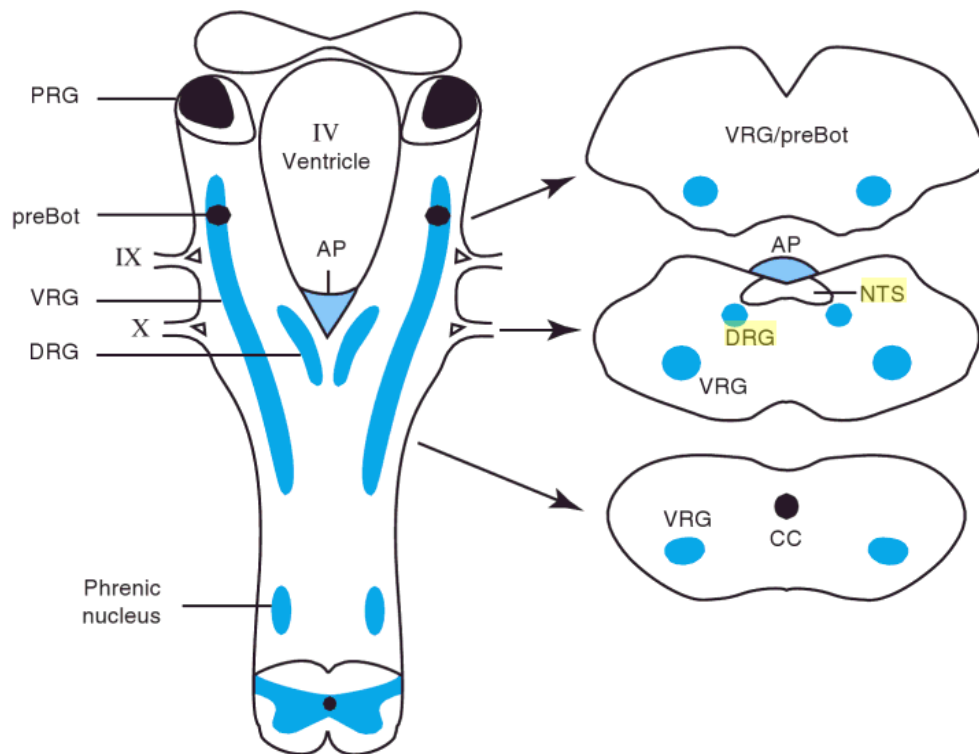


Figure 1.1 Respiratory centres in the brainstem. Left, dorsal view of the medulla and pons with cerebellum removed; Right, transverse sections of the medulla at three different levels. PRG, pontine respiratory group; PreBot, pre-Bötzinger complex; VRG, ventral respiratory group; DRG, dorsal respiratory group; IX cranial nerve (glossopharyngeal); X cranial nerve (vagus); NTS nucleus tractus solitarius; CC, central canal. (Powell Jr, 2003)

Respiratory receptors.

Several receptors project to the respiratory control centres of the brainstem and alter respiratory function (West, 2008). The integration of these separate inputs primarily aims to preserve arterial blood gas tensions and pH, and/or also to protect the health and physical condition of the respiratory airways and lungs.

Upper-airway, tracheal, bronchial and lung receptors

Receptors within the upper airways located in the nasal passage, pharynx, larynx and trachea are stimulated by both chemical and mechanical stimuli and elicit reflexes such as sneezing, coughing and bronchoconstriction to protect the lower portions of the respiratory tract (West, 2008). Similarly, the rapidly adapting pulmonary receptors (or irritant receptors) located between airway epithelial cells are stimulated by dust, cold air, noxious gases and also mechanical stimuli and elicit reflex responses such as bronchoconstriction and increases in ventilation.

The slowly adapting pulmonary stretch receptors are mechanoreceptors found in the smooth muscle of airways and are stimulated by lung inflation. These receptors are responsible for the Hering-Breuer reflex, which is the reflex inhibition of inspiration from lung over-inflation. This reflex is thought to be unimportant in adult humans unless tidal volume increases above resting levels such as in exercise (Richerson & Boron, 2005).

Juxta-capillary receptors or J-receptors are the free nerve endings of non-myelinated C fibres (group IV fibres), which are located in the alveolar walls close to capillaries. These receptors are stimulated by both chemical and mechanical stimulation, resulting in a reflex response of rapid shallow breathing, bronchoconstriction and increased secretion of mucus into the airways (Richerson & Boron, 2005). Similarly bronchial C-fibres respond to chemical stimulation which also results in rapid shallow ventilation, mucus secretion and bronchoconstriction.

Arterial Baroreceptors

The arterial baroreceptors, located in the aortic arch and carotid sinuses, are mechanoreceptors and are primarily responsible for maintaining blood pressure

homeostasis. These receptors detect changes in pressure and signal information via the glossopharyngeal and vagus nerves back to areas of the brainstem such as the NTS (Paton *et al.* 1999), which results in reflex changes in autonomic output to the heart and vasculature and the maintenance of blood pressure (the baroreflex). However, the neural feedback from arterial baroreceptors also alters the drive to ventilation (Heistad *et al.* 1974, 1975; De Burgh Daly, 1997; Stewart *et al.* 2011). For example Stewart *et al.* (2011) demonstrated that when blood pressure is acutely changed, using the modified Oxford technique (bolus administrations of sodium nitroprusside and then phenylephrine) ventilation changes in an inverse relationship with blood pressure. So increases in blood pressure not only reflexly reduce heart rate and total peripheral resistance but also suppresses ventilation.

Peripheral chemoreceptors

Multiple peripheral sites responsible for chemoreception have been identified and include the aortic body (located on the aortic arch) and the carotid body, with the latter widely considered as the most important (Prabhakar & Peng, 2004). The carotid bodies are highly vascularised bilateral organs located at the bifurcation of the carotid artery. The glomus type 1 cells of the organ, which are their primary site of chemoreception, are innervated by the carotid sinus branch of the glossopharyngeal nerve and neural signals are relayed to the NTS (Donoghue *et al.* 1984; Gonzalez *et al.* 1994). The carotid bodies sample the composition of arterial blood before it reaches the brain and are sensitive to changes in PO_2 , PCO_2 and pH. It initiates rapid reflex ventilatory responses in order to maintain homeostasis of arterial blood gas composition. The sensory responses of the carotid bodies are fast allowing the detection of the breath to breath changes and oscillations in arterial blood gas composition.

Central chemoreceptors

Arterial PCO_2 is the most sensitively regulated variable in the control of ventilation and the central chemoreceptors are its primary regulator. These chemoreceptors respond to changes in H^+ concentration in brain extracellular fluid (BECF). H^+ concentration in the BECF increases when arterial PCO_2 increases. Unlike H^+ , CO_2 can freely diffuse across the blood brain barrier, so an increase in arterial PCO_2 results in a proportional increase of the PCO_2 in BECF, cerebral spinal fluid and inside brain cells. This results in the acidosis of these compartments due to the liberation of H^+ (Richerson & Boron, 2005). The following stimulation of the central chemoreceptors causes increases in ventilation and the maintenance of arterial PCO_2 homeostasis. It was thought that these receptors sensitive to CO_2/H^+ are located near the ventral medullary surface (Loeschcke, 1982). It has since been shown that there are several possible individual receptor sites in the brain and include, among others, the retrotrapezoid nucleus, the NTS, the locus coeruleus, the medullary raphè, the periaqueductal grey (PAG) and the pre-Botzinger complex and Li *et al.* 1999; Nattie & Li, 2001, 2002; Feldman *et al.* 2003; Takakura *et al.* 2006; Krause *et al.* 2009; Putnam *et al.* 2010, Lopes *et al.* 2012)

1.2 The cardio-respiratory responses to exercise

Exercise places a complex and powerful stress upon the ventilatory and cardiovascular systems as they attempt to meet the demands for greater oxygen delivery and carbon dioxide disposal from the working muscles. These demands will be met if alveolar ventilation increases proportionately to metabolic rate and if skeletal muscle blood flow is rapidly enhanced in order to deliver the required oxygen and nutrients to the exercising muscle.

It has been well established that the cardiovascular responses to dynamic and static exercise are mediated by the autonomic nervous system, where sympathetic nerve activity increases and parasympathetic activity is withdrawn. Dynamic exercise results in intensity dependent increases in heart rate, stroke volume and cardiac output but only moderate increases in blood pressure as total periphery resistance may reduce. All together the hemodynamic alterations results in the increase of blood flow to the working muscles (Rowell, 1993). During static exercise however muscle blood flow is reduced due to the large sustained increases in intramuscular pressure. Therefore the heart rate mediated increase in cardiac output combined with a small or no change in total peripheral resistance results in a marked increase in blood pressure, which attempts to maintain perfusion of the muscle (Rowell, 1993). The neural control mechanisms behind these responses have been well demonstrated and include central command, the arterial baroreflex and the feedback from skeletal muscle afferents (Kaufman & Forster, 1996; Fisher & White, 2004; Smith *et al.* 2006; Williamson *et al.* 2006, Murphy *et al.* 2011; Basnayake *et al.* 2012), all of which converge and integrate into areas in the brainstem related to cardiovascular control such as the NTS (Paton, 1999). Neural projections from these centres relay to the heart, blood vessels and adrenal medulla so that appropriate cardiovascular responses to exercise are produced (Figure 1.1)

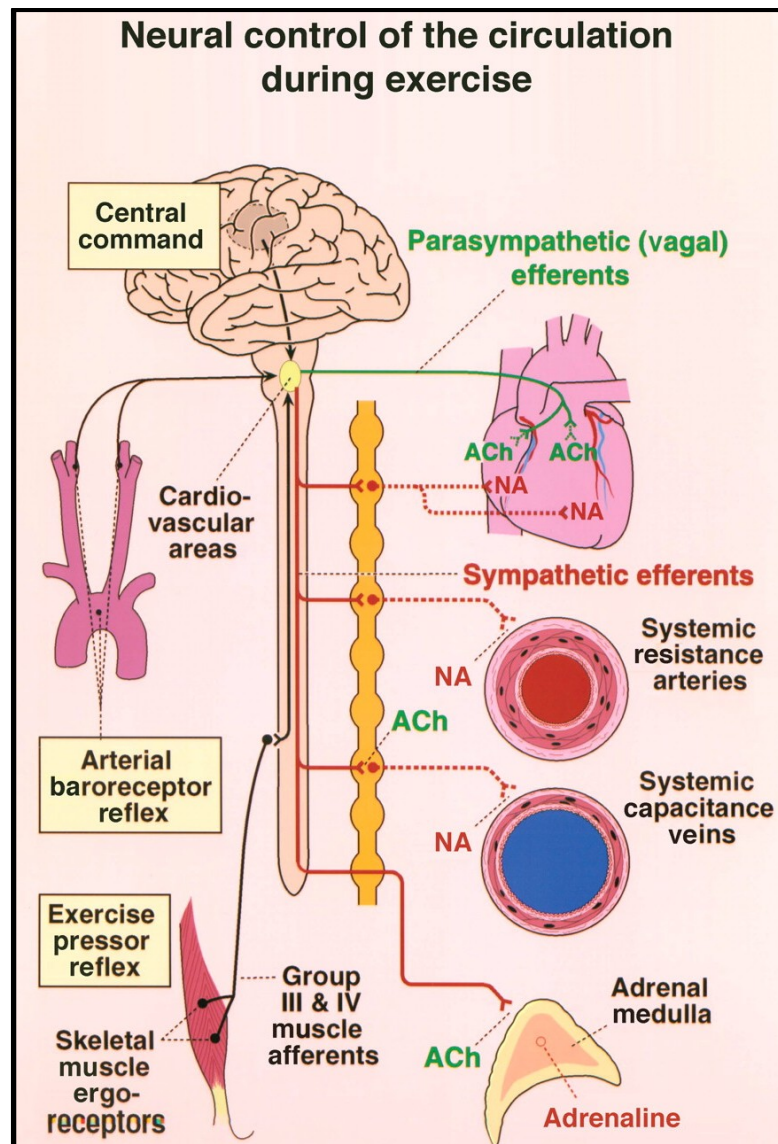


Figure 1.2 Neural cardiovascular control during exercise. Neural signals originating from the brain (central command), the aorta and carotid arteries (arterial baroreflex), and skeletal muscle (exercise pressor reflex) are known to modulate sympathetic and parasympathetic nerve activity during exercise (from Smith *et al.* 2006)

Despite the poor understanding of the control mechanisms behind the exercise hyperpnea, the ventilatory responses to exercise have been well described. In isometric exercise the increase in ventilation is related to the degree of muscle tension, where static contractions performed at higher intensities (% of the maximal voluntary contraction; MVC) results in significantly greater ventilatory responses (Imms & Mehta, 1989; Fontana *et al.* 1993, Iellamo *et al.* 1999). The magnitude of the ventilatory response is also related to the size of the muscle mass involved. The isometric exercise of larger muscle masses (handgrip exercise v.s. knee extensions) at the same relative intensity, but greater absolute intensity, elicits larger increases in ventilation (Iellamo *et al.* 1999). Furthermore fatiguing high intensity isometric exercise is associated with increases in ventilation which are disproportionately higher than metabolic rate and so can result in significant reductions in P_{ETCO_2} (Imms & Mehta, 1989).

The magnitude of the ventilatory response to dynamic exercise is also dependent on exercise intensity and at a constant work rate the response appears to comprise of three distinct phases (Figure 1.2, Bennet *et al.* 1981; Whipp *et al.* 1982; Wasserman and Whipp 1983; Casaburi *et al.* 1989). At the onset of exercise ventilation increases immediately (first phase) and after a transient plateau it continues to rise exponentially (second phase) until a steady state is reached (third phase). This steady state can be achieved within 2 to 3 minutes of light to moderate intensity exercise, but during heavy exercise steady state is not achieved as ventilation continues to elevate until exhaustion (Casaburi *et al.* 1989). At moderate work rates the increases in oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) have a similar pattern to that of ventilation, although $\dot{V}O_2$ kinetics can be faster in phase two (Wasserman & Whipp 1983). Similarly to ventilation however, a steady state in $\dot{V}O_2$ and $\dot{V}CO_2$ is not achieved in phase three of heavy exercise (Casaburi *et al.* 1989). The key characteristic of the hyperpnea to light-moderate exercise is that at

steady state, the increase in ventilation is in proportion with $\dot{V}O_2$ and $\dot{V}CO_2$, whereas in heavy exercise ventilation increases comparatively more.

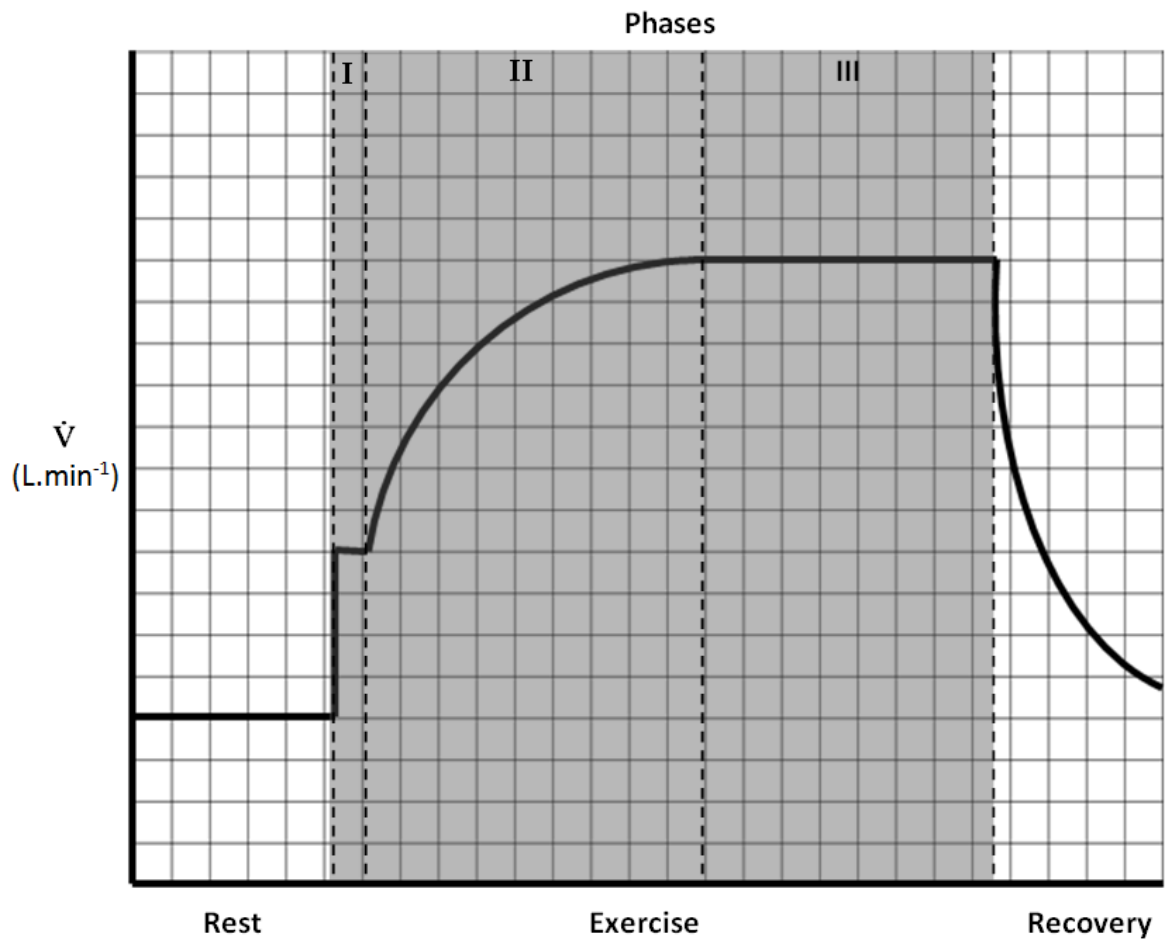


Figure 1.3 Minute ventilation (\dot{V}) during steady-state exercise, at a light to moderate intensity). The period of exercise is shaded in grey. The ventilatory responses to exercise can be separated into three distinct phases (I, II and III).

1.3 Control of the exercise hyperpnea

Haldane & Priestley (1905) were among the first to show that the increase in pulmonary ventilation during moderate intensity exercise was in exact proportion to the increase in metabolic rate (as indexed by $\dot{V}CO_2$). It was suggested that during exercise an increase in arterial PCO_2 (from the increased $\dot{V}CO_2$) would stimulate chemoreceptors and so link ventilation to metabolic rate. However, there is considerable evidence that suggests that the exercise hyperpnea is not driven by alterations in the PCO_2 stimulus.

Firstly the increase in metabolic rate during light and moderate exercise is so precisely matched by ventilation that arterial PCO_2 does not change significantly from resting levels (first shown by Douglas & Haldane, 1909). In general a small transient hypocapnia is often observed at the onset of exercise, and during steady state light-moderate exercise $PaCO_2$ only changes at most by 1-3 mmHg from resting levels (Barr *et al.* 1964; Forster *et al.* 1986). Preventing hypercapnia in spite of the large increase in $\dot{V}CO_2$ characteristic of exercise is an impressive accomplishment of the respiratory system given that its most tightly controlled variable, $PaCO_2/H^+$, does not provide an error signal for the central and/or peripheral chemoreceptors to trigger a reflex increase in ventilation. Furthermore during exercise at a heavy intensity and the concurrent lactic acidosis, phase three (figure 1.2) can be characterised by a slow yet continuous increase in ventilation despite carbon dioxide clearance rate exceeding metabolic rate and the consequential decline in arterial PCO_2 (Forster *et al.* 1986; Stringer *et al.* 1992; Sun *et al.* 2001). The most obvious and widely accepted mechanism for this hyperventilation is the lactic acid/ H^+ , produced from anaerobic metabolism, stimulating carotid chemoreceptors, but the issue is still controversial and several other mechanisms may contribute (see Forster *et al.* 2012 for review).

Secondly, it is possible that the gain of the chemoreceptors increases in exercise so the same stimulus level ($PaCO_2/H^+$) results in greater receptor output. However the weight of evidence suggests that CO_2 inhalation only has an additive effect on the exercise hyperpnea, with no observable changes in CO_2 sensitivity (Asmussen & Nielson, 1957; Clark *et al.* 1980; Duffin *et al.* 1980; Poon *et al.* 1987).

Third, if error signals are not provided by alterations in $PaCO_2$ it is possible that the increases in venous PCO_2 during exercise may provide an error signal for hypothetical mixed venous chemoreceptors. This would be the ideal mechanism to match metabolic rate with ventilation. However the infusion of hypercapnic blood into the right atrium or pulmonary artery of dogs only increases ventilation once the blood has passed through the pulmonary circulation and stimulates arterial chemoreceptors (Cropp & Comroe, 1961; Sylvester *et al.* 1973). Additionally in humans, when the circulation to exercising muscles becomes free flowing after a period of occlusion there is a significant lag time between inflections of $P_{ET}CO_2$ and minute ventilation (Stanley *et al.* 1985), again implying the apparent absence of mixed venous chemoreceptors.

Finally, the increase in ventilation immediately at the onset of exercise occurs too rapidly for it to be caused by the slow development of chemical factors in arterial or even venous blood (Krogh & Lindhard, 1913, 1917). It is therefore likely that the ventilatory response, in phase 1 at least, is triggered by a fast neural mechanism.

So what is/are the mechanism/s responsible for signalling ventilation to increase immediately at the onset of exercise and in proportion to metabolic rate? To date no single postulated mechanism has proven to account for the entire ventilatory response. However, several mechanisms have been proposed which may be capable of stimulating ventilation in exercise (for review see Turner *et al.* 1991; Kaufman & Forster 1996; Waldrop *et al.* 1996; Forster *et al.* 2012). These include other humoral mechanisms where stimulation of

the peripheral chemoreceptors could mediate a hyperpnea: 1) the workload dependent increases in arterial plasma potassium ($[K^+]$) released from exercising muscles (Paterson, 1992) or 2) increases in the amplitude/slope of the breath-to-breath oscillations of $PaCO_2/H^+$, which may provide a signal for an exercise hyperpnea despite the maintained mean $PaCO_2$ (Yamamoto *et al.* 1960, Band *et al.* 1969, 1980). However the evidence for both of these hypotheses and the size of their relative contributions to the exercise hyperpnea remains controversial (Forster *et al.* 1989; Warner & Mitchell, 1990; Nye, 1994; Kaufman & Forster, 1996).

This review and thesis is concerned with examining the role played by neural feedback from metabolically and mechanically sensitive skeletal muscle afferent fibres, which is an attractive mechanism as it could hypothetically provide a stimulus at the onset of exercise and an error signal for steady-state ventilation to match metabolic rate. However the mechanism which currently seems to have the most convincing supportive data is that of central command, the evidence for which is briefly examined below. As such it is widely regarded to have the capacity to drive cardiorespiratory responses in exercise (Waldrop *et al.* 1996).

1.4 Central command – cardio-respiratory control in exercise

The feedforward control mechanism known as central command consists of descending neural signals from higher brain centres, that excite locomotor neurones during exercise, concurrently converging onto cardiovascular and respiratory control areas of the brainstem to meet the anticipated demands of the exercising muscles. Thus there is a simultaneous excitation of those neuronal circuits containing locomotor neurones and also cardiorespiratory medullary neurones leading to the modulation of the sympathetic and parasympathetic output to blood vessels and the heart and to the motor output to respiratory muscles. However it has been a continual challenge for physiologists to definitively examine the concept of central command as a cardiorespiratory control mechanism.

The central command hypothesis was first established from the work of Johansson (1895) and Krogh & Lindhard (1913, 1917). Krogh & Lindhard's (1913) experiments were the first performed on humans and proposed that the concept of “cortical irradiation” (central command) caused the immediate ventilatory and heart rate increases observed at the beginning of exercise, and were responsible for the altered cardio-respiratory responses to changes in perceived load of a cycling exercise. Furthermore Krogh & Lindhard (1917) demonstrated that at the onset of electrically induced leg exercise, where central command is absent, the increase in heart rate is delayed. Although these results clearly demonstrate that other mechanisms must be capable of producing the tachycardia, albeit slightly delayed (e.g. reflexes from the peripheral muscle), they do suggest that mechanisms of a central origin also play a role.

Over many years, a wide variety of techniques have been used to study the role of central command in ventilatory and cardiovascular control. In general these studies fall into two

categories: 1) where the level of central command during exercise is manipulated, and 2) where attempts have been made to identify neural regions that may provide the drive to locomotor and cardiorespiratory control centres in exercise.

Altering the level of central motor drive required to perform an exercise task

Goodwin *et al.* (1972) used the technique of tendon vibration to manipulate the levels of central command required to perform a particular muscular action. During isometric exercise of the biceps or triceps brachii a vibrator was applied to the tendon of either the exercising muscle or its antagonist. Vibration is a stimulus for muscle spindle afferent endings and reflexly elicits tension in the muscle. Vibrating the tendon of the exercising muscle would therefore reduce the level of central command required to produce a given tension within the muscle and vibrating the tendon of the antagonist would increase it. As such the ventilatory and cardiovascular responses to the isometric exercise was greater when the level of central command was increased and these responses increased less when central command was reduced. Crucially the same muscle tension was produced during the exercise with and without vibration, so it appears that the altered responses were due to differences in central command involvement.

Several studies have used the method of partial neuromuscular blockade (induced by curare) to examine the role of central command. Asmussen *et al.* (1965) and Galbo *et al.* (1987) demonstrated in humans that a partial curarization produced a greater response in ventilation, pulse rate and blood pressure during exercise. It is likely that the augmented cardio-respiratory responses to exercise reflect the greater central command activation needed to maintain the constant workload in the weakened muscles. In addition Innes *et al.* (1992) analysed the ventilatory and cardiovascular responses to single legged cycling

exercise in patients suffering from unilateral leg weakness caused by either orthopaedic or neurological disorders. It was shown that exercise with the weak leg produced exaggerated ventilatory and cardiovascular responses to achieve a given workload. These results provide evidence, albeit indirect, for the concept of central command as they demonstrate that the magnitude of the central drive affects cardio-respiratory responses to exercise.

Identifying the neural structures and neurocircuitry involved in central command

Several investigations with animals have electrically stimulated regions of the brain to locate possible areas involved in the parallel activation of locomotion and the cardiorespiratory systems. Using unanaesthetized decorticate cats Eldridge *et al.* (1981) was able to induce locomotion through electrical stimulation of the hypothalamic site called the subthalamic locomotor region, which resulted in respiratory and blood pressure responses similar to that of spontaneous exercise. Crucially, similar responses were generated when “fictive” locomotion was induced in paralysed cats, ruling out the factor of neural feedback from the exercising muscles. These results were subsequently confirmed (Eldridge *et al.* 1985; Waldrop *et al.* 1988) with the pharmacological stimulation of neuronal cell bodies in the subthalamic locomotor regions with picrotoxin (antagonist of the inhibitory neurotransmitter GABA) which produced similar responses even during fictive locomotion.

Other decorticate animal studies have also demonstrated that the electrical stimulation of various areas of the brain, including the thalamus, hypothalamus, the basal ganglia and mesencephalic locomotor region (MLR), can also induce locomotion and also ventilatory and cardiovascular responses similar to spontaneous exercise (Smith *et al.* 1960; DiMarco *et al.* 1983; Ordway *et al.* 1989; Angyan, 1991; Bedford *et al.*, 1992). These results are

consistent with the concept of central command as a locomotor/cardiorespiratory control mechanism during exercise (in animals at least) and provides evidence that these subcortical structures could theoretically be involved in its neurocircuitry. They also imply that the involvement of other mechanisms such as muscle afferent feedback may not be essential for normal cardiorespiratory responses. However their involvement of course cannot be ruled out particularly as hypothalamic lesioning seems to have no effect on the cardiorespiratory responses to exercise in the awake animal (Waldrop *et al.* 1986), suggesting an element of redundancy in the system.

In addition, functional neurosurgery has made it possible to electrically stimulate regions of the brain in awake humans. Those undergoing surgery for movement disorders or chronic pain have deep brain stimulating electrodes implanted stereotactically into the relevant areas of the midbrain. This procedure not only allows the stimulation of these sites but can also provide information regarding their neural activity by recording local field potentials (below). It has been shown that stimulation of the subthalamic nucleus, thalamus and substantia nigra all result in significant increases in heart rate and mean arterial pressure (Thornton *et al.* 2002). Furthermore Green *et al.* (2005) found that the stimulation of ventral and dorsal periventricular/periaqueductal grey matter results in a decrease and increase in blood pressure, respectively. Although respiratory measurements could not be made in these studies, the findings indicate that these subcortical regions of the brain may be involved in the neurocircuitry of central command in humans.

However, one must be cautious with the interpretation of these findings in relation to central command in exercise. Although these structures may form part of a relay system to the respiratory and cardiovascular control centres in the brainstem, “their role has to be viewed in context with the higher centres that are responsible for initiating exercise” (Thornton *et al.*, 2002). So although electrical/chemical stimulation of these areas can

induce cardiorespiratory responses, it is not possible to determine whether their stimulation is analogous to activation of these pathways during normal exercise.

As such various techniques have been used to establish regions of the brain that are active during exercise. For example in rats, areas active during exercise have been identified using the immunocytochemical labelling of c-fos, where its expression was used as a marker for neural activity (Iwamoto *et al.* 1996). After dynamic exercise on a treadmill there was increased activation of hypothalamic and mesencephalic locomotor regions, the periaqueductal gray matter (PAG) as well as the NTS, and the rostral and caudal ventrolateral medulla. These results appear to support the findings of those studies discussed above that used electrical/chemical stimulation techniques.

Extensive work has also been conducted in humans during exercise. Using positron emission tomography to record changes in the regional cerebral blood flow Thornton *et al.* (2001) analysed the areas of increased brain activity during imagined exercise under hypnosis. The advantage of imagined exercise is that any cardiorespiratory responses and/or alterations in regional cerebral activity will not be altered by peripheral feedback from the muscles. When the hypnotised subjects were asked to imagine exercising on a bicycle an increase in ventilation and heart rate was observed, and is consistent with the concept of central command. Furthermore several motor areas of the brain were also shown to be activated during the imagined exercise (e.g. dorsolateral prefrontal cortex, the primary and supplementary motor areas, cerebellum), as they are in voluntary exercise (Fink *et al.* 1995). These areas are known to be involved with motor control including the volitional control of respiratory muscles (Colebatch *et al.*, 1991; Ramsay *et al.* 1993) and as such these structures could be part of a central command mechanism which mediates the exercise hyperpnea.

In addition these findings might support the notion that the central command mechanism for ventilatory control incorporates a learned component. Because the respiratory responses to the imagined exercise might be driven by neurocircuitry involved in locomotion and voluntary respiratory control, and that the “dorsolateral prefrontal cortex is crucial in actions generated on the basis on working memory” (Thornton *et al.* 2001), it could mean that the feed-forward control of ventilation in exercise is based upon previously learned information and practice in producing suitable ventilatory responses to exercise. Indeed it was concluded by Thornton *et al.* that “these areas may encode a respiratory motor programme (central command) that evolves as the motor task is learnt in development, interacting with classical error signals in real exercise”.

The regulation of breathing from memory can also be called long term modulation (LTM). In humans the LTM of breathing has been demonstrated where by the exercise hyperpnea under normal conditions is augmented after subjects repeatedly exercised with increased internal dead space (Wood *et al.*, 2003) or increased respiratory resistance (Turner & Stewart, 2004). These results may provide insight into how a feed-forward mechanism that regulates ventilation could be calibrated by sensory feedback (in this case from repeated increases in chemoreceptor activity), so it can learn to more closely match the respiratory response with metabolic rate.

The results from Thornton *et al.* also imply that a central command response may not necessarily require the concurrent activation of a central motor command but perhaps the sense of effort drives the response. This idea was further demonstrated by Williamson *et al.* (2001, 2002) also using hypnoses techniques so participants imagined performing cycling or handgrip exercise respectively. When performing exercise it was demonstrated that in those with high ‘hypnotisability’, imagining the exercise resulted in significant increases in heart rate and blood pressure. Therefore it is possible that central commands

control of the cardiorespiratory system does not require the parallel activation of locomotor systems, but in fact central command in exercise may involve the concurrent activation of two separate networks; the locomotor system and the cardiorespiratory systems.

More recently Green *et al.* (2007) examined the neural activity of subcortical regions of the brain during exercise by recording local field potentials with deep brain stimulating electrodes. In anticipation to and during a low intensity (15W) pedalling exercise PAG activity increased. It was concluded that this area is possibly an important site in the control of the cardiorespiratory system during exercise and perhaps acts as a relay centre for central command from higher brain centres to cardiorespiratory neurones in the medulla. Furthermore the activity of the PAG increases during skeletal muscle afferent activation (via post-exercise circulatory occlusion, Basnayake *et al.* 2011) signifying that this site may be also be important in integrating the cardiorespiratory responses to muscle afferent activation and central command.

In conclusion, the human studies described in this section have provided indirect evidence for central command and offer several possible cortical and subcortical areas that could be involved in its neurocircuitry, such as the PAG. Animal studies have provided more direct evidence (e.g. Eldridge *et al.* 1981) but it is not possible to tell whether the conditions of the studies are analogous to the activation of central command during normal exercise. Nevertheless, despite no definitive experiment and a lack in the full understanding behind the neurocircuitry involved, based upon the available evidence it is likely that central command does play a role in eliciting the normal ventilatory and cardiovascular responses in exercise.

However, the relative importance of central command (in ventilatory control in particular) is still debated (Haouzi *et al.* 2006). The effects of metabolic rate and motor activity on

ventilation can be separated out by sinusoidal changes in work rate. Ventilation in exercise reduces in response to a decrease in the work rate oscillation period and as such follows the changes in $\dot{V}O_2$ and $\dot{V}CO_2$ despite the constant work rate amplitude. (Casaburi *et al.* 1977, Haouzi *et al.* 2004). Therefore it appears that the ventilatory responses to exercise may be less related to the level of central drive and more to metabolic rate. This must make us consider that other mechanisms unrelated to central drive are involved in the control of the exercise hyperpnea. The neural feedback from metabolically and mechanically sensitive receptors in skeletal muscle, that clearly plays a role in controlling the cardiovascular system in exercise (below), has been proposed as a control mechanism for ventilatory increases in exercise and perhaps provides the error signal for steady-state ventilation to match metabolic rate. Indeed, Eldridge *et al.* (1981) concludes that other factors, such as feedback from the exercising muscle, cannot be ruled out as a mechanism contributing to the ventilatory response and they may be required to track ventilation with metabolic events in exercise.

1.5 Skeletal Muscle afferent feedback

Skeletal muscle is innervated by five types of afferent nerve fibre. Group I (subdivided into Ia and Ib) and group II are thickly myelinated afferents and conduct impulses between 72-120 m/s and 31-71 m/s, respectively. Group Ia and II afferents innervate muscle spindles and group Ib innervate Golgi tendon organs. Group III (A δ) muscle afferents are thinly myelinated and conduct impulses between 2.5-30 m/s. Group IV afferents (C-fibres) are slower still (<2.5 m/s) and are unmyelinated. The free nerve endings of group III muscle afferents are often located in connective tissue and tendon, whereas the free nerve endings of group IV afferents are often found within the vascular network of muscle. Group III and IV afferents synapse in the laminae of the dorsal horn of the spinal cord and project to the medulla including the NTS (which also integrates the inputs from other afferents in the glossopharyngeal and vagus nerves) and can provide the brainstem with information regarding the conditions within the muscle (Kaufman & Forster, 1996).

It has long been established that muscle afferent feedback is an important factor in the control of the cardiovascular system in exercise. Alam & Smirk (1937) demonstrated that after the cessation of a dynamic calf exercise, performed with thigh cuffs inflated to a supra-systolic pressure and thus occluding blood flow to the exercising muscles, blood pressure reduced from the exercising level but was maintained significantly above resting levels. The occlusion of blood flow traps the metabolites produced in exercise within muscles and this blood pressure response to the post-exercise circulatory occlusion (PECO) was maintained for as long as the thigh cuffs were inflated. As no exercise was being performed during PECO it was therefore concluded that a reflex arising from the muscles themselves was maintaining blood pressure. This is known as the exercise pressor reflex. One simple explanation for the function of this reflex is that during exercise at low muscle contractile forces it assists adequate perfusion of the muscles, washing out

metabolites to remove the metabolic error signal and protecting against early muscle fatigue (Fitzpatrick, 2012)

Neurophysiological evidence for the exercise pressor reflex

The reflex component of this pressor response was further examined by Coote *et al.* (1971) by electrically stimulating the ventral roots (L6-S1) in anaesthetised cats to induce hind limb muscle contractions. This allowed the investigators to examine the contribution of muscle afferent feedback to the cardiorespiratory responses during exercise in isolation from the influence of central command. The electrical stimulation produced an increase in blood pressure as well as smaller increases in heart rate and ventilation. Furthermore, this response was abolished if the muscle contraction was blocked by gallamine (a neuromuscular blocking agent) or if dorsal roots (L6-S1) were sectioned. It was suggested by Coote *et al.* (1971) that the pressor response was likely caused by the accumulation of metabolites which stimulated group III and group IV muscle afferents operating as “metabolic receptors” (or metaboreceptors). Indeed Coote & Perez-Gonzalez (1970) determined that the electrical stimulation of group III and IV afferents increases sympathetic nerve output in anaesthetised cats whereas as the stimulation group I and II muscle afferents did not.

McClosky & Mitchell (1972) confirmed this by providing an anodal block to the dorsal roots from the hind limb muscles of anaesthetised cats. While selectively blocking group I and II muscle afferents the electrical stimulation of hind limb muscles resulted in the expected large increase in blood pressure and the smaller heart rate and respiratory responses. But these responses were abolished when the thin myelinated and unmyelinated group III and IV afferents were blocked. Furthermore the increase in blood pressure (but

not heart rate or ventilation) in response to the muscle contractions were augmented by the superimposition of circulatory occlusion during and after the contractions. All together these important studies suggest that feedback from contracting muscles can evoke cardiovascular and respiratory reflexes via group III and group IV muscle afferents.

Discharge properties of group III and IV afferents

Both electrically evoked isometric (Kaufman *et al.* 1983) and rhythmic (Kaufman *et al.* 1984) hind limb muscle contractions increase the discharge of both group III and IV muscle afferents in anaesthetised cats and result in a pressor response. By analysing the discharge patterns it has been possible to speculate that these two afferent groups tend to respond to different stimuli. Group III afferents primarily fire rapidly after the initiation of the contractions and also in synchrony with rhythmic contractions and so appear to be stimulated to a greater extent by mechanical distortion of skeletal muscle (Kaufman *et al.* 1983, 1984). These afferents are also stimulated by the non-noxious and purely mechanical stimuli of squeezing, probing, and external pressure on the muscle (Kaufman *et al.* 1983, 1984; Mense & Stahnke, 1983; Kaufman & Rybicki, 1987; Hayward *et al.* 1991). Furthermore Mense & Stahnke observed that most group III fibres were stimulated by tendon stretching, which was greater during sustained rather than repeated stretches, and concluded that these afferents tend to do be “contraction sensitive units with a mechanical mechanism of activation”.

Conversely group IV afferents tend to respond with a latency consistent with the build up of metabolites produced during electrically evoked contraction contractions (Kaufman *et al.* 1983). Indeed, when these contractions were induced during the occlusion of local blood supply, the resulting metabolite accumulation within the muscle caused a greater

increase in the discharge of group IV afferents compared with group III afferents (Kaufman *et al.* 1984a). This implies that the group IV afferents are stimulated more by metabolic by-products of contraction.

What metabolic by-products of muscle contraction stimulate muscle afferent fibres

McCloskey & Mitchell (1972) demonstrated that the arterial injection of potassium chloride produced similar cardiovascular and ventilatory responses compared with electrically induced hindlimb exercise. It was suggested that substances like potassium, which are released by the exercising muscle, stimulate metabosensitive afferents and cause a reflex increase in blood pressure, heart rate and ventilation. Although potassium injection does stimulate group III and IV afferents (Kaufman & Rybicki 1987), this response adapts quickly while interstitial potassium levels remained elevated making it unlikely that it contributes to the exercise pressor response. However many more chemical substances which, are by-products of muscle contractions, have been identified which stimulate group III and IV muscle afferents in cats and rats.

The injection of bradykinin (Kaufman *et al.* 1983; Mense & Meyer, 1988) and lactic acid/H⁺ (Rotto & Kaufman, 1988) into the arterial supply of anaesthetised cat hindlimbs increases the discharge of both group III and IV afferent fibres. Furthermore prostaglandins also appear to play a role as the injection of arachidonic acid increases the discharge of these afferents and the application of indomethacin, which inhibits the cyclooxygenase enzyme that converts arachidonic acid to prostaglandins, attenuates this increased afferent discharge (Rotto & Kaufman (1988). Finally the intra-arterial injection of ATP stimulates group IV afferent fibres but only some of the slowest conducting (<4 m/s) group III afferents (Hanna & Kaufman, 2004).

Polimodality of group III muscle afferents

After the rapid and transient discharge of group III afferents upon the initiation of the static muscle contractions almost half of them displayed a secondary increase in firing (Kaufman et al 1983). These polymodal discharge properties suggest that a population of group III afferents may be sensitive to both the mechanical effects of the contraction and the metabolic by-products as well. These chemical stimuli may include ATP, lactic acid/H⁺, arachidonic acid metabolites (e.g. prostaglandins) and bradykinin as their intra-arterial injection have all been shown to stimulate some group III muscle afferent fibres (Kaufman *et al.* 1983, Mense & Meyer, 1988; Rotto & Kaufman, 1988, 1990; Sinoway *et al.* 1993; Hanna & Kaufman, 2004).

Furthermore, metabolites have also been found to sensitise group III afferent responses to mechanical stimuli. Adreani et al. (1997, 1998) and Hayes et al. (2006) induced rhythmic muscle contractions using MLR stimulation, a technique which is thought to more closely resembles normal voluntary exercise in terms of motor unit recruitment compared with ventral root stimulation. Contractions resulted in the activation of both group III and IV muscle afferents and during muscle ischemia their discharge was potentiated to a similar extent by the resultant local metabolite accumulation (Adreani & Kaufman, 1998). However, if this muscle ischemia continued after exercise (i.e. PECO) the increased discharge of group IV afferents continued but the discharge of group III afferents returned to baseline (Hayes et al. 2006). Therefore it appears that group III muscle afferents were not being stimulated by the accumulated metabolites per se but were perhaps sensitised by them to the mechanical stimulus of the muscle contractions.

All together the findings above suggest that some group III afferents are not exclusively mechanoreceptive but can either be stimulated or sensitised by chemical stimuli and thus can be considered as polymodal.

1.6 Skeletal muscle afferent feedback – cardiovascular control

Several investigations in humans have clearly demonstrated the importance of muscle afferent feedback in the control of the cardiovascular system in exercise. Electrically evoked muscle contractions, which will stimulate muscle afferents in isolation from central command activation, result in similar blood pressure compared with voluntary exercise (Bull *et al.* 1989) and also induces increases in heart rate, albeit with a delay by 1 cardiac cycle not observed in voluntary exercise (Krogh & Lindhard 1917, Iwamoto *et al.* 1987). Investigations have also inhibited the transmission of muscle afferent feedback from lower limbs by injecting anaesthetic (e.g. lidocaine, bupivacane or fentanyl) into the lumbar epidural space. It has been consistently demonstrated in humans that the cardiovascular responses to voluntary and electrically evoked exercise can be diminished by this reduced sensory feedback (Freund *et al.* 1979, Mitchell *et al.* 1989, Fernandes *et al.* 1990, Kjaer *et al.* 1994; Amann *et al.* 2010). These studies clearly suggest that muscle afferent feedback is an important control mechanism in the normal pressor responses to exercise. However, several techniques have also been used to try and differentiate the contributions of muscle metaboreceptor stimulation (metaboreflex) and mechanoreceptor stimulation (mechanoreflex) in the cardiovascular responses to exercise.

Mechanoreflex

It is thought that the mechanoreflex might provide feedback on the force of contraction so that suitable cardiovascular responses are made to the exercise (Kaufman & Forster, 1996). The techniques of passive muscle/tendon stretch and external muscle compression have been used to selectively stimulate muscle mechanoreceptors in isolation and therefore observe their relative contribution to the control of the cardiovascular system.

In animal models, stretching the tendon increases both blood pressure heart rate with a decrease in cardiac vagal activity and increase in cardiac sympathetic activity (Stebbins *et al.* 1988; Murata & Matsukawa, 2001). These responses could also be graded depending on the tension developed in the muscle by the stretch. In humans, sustained passive stretching of the calf muscle increases in heart rate which is vagally mediated as the response was abolished with glycopyrrolate administration (Gladwell & Coote 2002; Gladwell *et al.* 2005). Fisher *et al.* (2005) and Drew *et al.* (2008) also observed increases in heart rate and progressive increase in blood pressure throughout the stretch period of the calf. These results suggest that activation of the mechanoreflex through stretch increases heart rate through vagal withdrawal but it is also capable of stimulating populations of mechanoreceptive afferents that can signal for an increase in sympathetic vasomotor activity. Sustained passive stretch of the forearm PECO has been shown to cause increases in MSNA, but only when the preceding handgrip exercise was performed to fatigue and not when it was non-fatiguing (Cui *et al.* 2008). This suggests that metabolites, which might include prostaglandins (Middlekauf & Chiu, 2004; Cui *et al.* 2008a), may sensitise muscle mechanoreceptors but only when they accumulate above a certain threshold.

External compressions of the muscle increases interstitial pressure and in both animals (Stebbins *et al.*, 1988) and humans (Williamson *et al.*, 1994; Bell & White, 2005) increases in blood pressure has been observed. However, the local occlusion caused by compression will trap metabolites within muscles and concurrently stimulate muscle metaboreceptors, which might entirely explain the increase in blood pressure. Therefore Bell & White (2005), applied compression to the calf during periods of PECO which followed graded intensities of isometric calf exercise. The compressions caused incrementally larger increases in blood pressure following the greater exercise intensities and thus when there was a greater metabolic stimulus. This suggests that populations of

polymodal muscle mechanoreceptors stimulated by compression can be sensitised by the trapped metabolites within the muscle.

Metaboreflex

It is thought that the metaboreflex may signal for a greater blood supply to be sent to contracting muscles as there is mismatch between blood supply and the metabolic by-products being produced (Kaufman & Forster, 1996). As there is no tension in the muscle PECO is a useful technique to examine the relative contribution of the metaboreflex to the exercise pressor response from other cardiovascular control mechanisms. As described above, metaboreflex activation through PECO causes reflex increases in blood pressure (Alam & Smirk 1937). Furthermore the pressor response observed during PECO increases along with the of the preceding exercise intensity and degree of muscle ischemia, and as such is proportionate to the degree of metabolite production and accumulation (Alam & Smirk 1937, Rowell *et al.* 1976).

Throughout PECO there is a sustained sympathoexcitation and thus vasoconstriction until cuff deflation which accounts for the blood pressure responses first observed by Alam & Smirk (Mark *et al.* 1985; Victor *et al.* 1987; Seals *et al.* 1988). However it has been classically shown that heart rate returns to resting levels during PECO (Rowell *et al.* 1976). It is likely that this recovery of heart rate is either because after exercise the baroreflex masks the response or because the metabolic error signal in the muscle plays little role in controlling the heart. Indeed other mechanisms have shown to provide a control of heart rate, such as the muscle mechanoreflex (Gladwell & Coote, 2002 and Gladwell *et al.* 2005; see below) and central command (Mark *et al.* 1985; Victor *et al.* 1989). For example Victor *et al.* (1989) observed that attempted handgrip contractions,

which yielded almost no force in the muscle due to neuromuscular blockade with tubocurarine, increased heart rate to a similar level recorded before the blockade but the blood pressure and MSNA responses were significantly smaller. Moreover the heart rate responses during attempted handgrip were abolished by atropine. Together these results suggest that central command is an important mechanism in controlling parasympathetic outflow to the heart but plays a much smaller role in the control of sympathetic outflow to skeletal muscle.

More recently however the role of the metaboreflex in human cardiac control has been re-examined by Fisher *et al.* (2010). During a period of circulatory occlusion following a moderate intensity static handgrip exercise (25% MVC) heart rate remained slightly elevated (but not significantly) above resting levels. This heart rate response to PECO was augmented during the pharmacological elimination of cardiac parasympathetic tone (glycopyrrolate), and it could be reduced during adrenergic blockade (propranolol). Similar findings have also been shown in animals (O'Leary, 1993) and collectively these results suggest that the metaboreflex increases cardiac sympathetic nerve activity during PECO, and can elicit a tachycardia, but this response is usually masked by parasympathetic reactivation post exercise. It is thought that this parasympathetic reactivation and resultant bradycardia in PECO is a consequence of the loss of central command and mechanoreflex activity and also the influence of the baroreflex on the heart.

However, Fisher *et al.* also observed that during circulatory occlusion following a higher intensity static handgrip exercise (40% MVC) heart rate remains significantly elevated above baseline. These findings support those made by Iellamo *et al.* (1999) who found small heart rate response to PECO but only if the exercise was at a high enough intensity (15 vs. 30% MVC) and in a large enough muscle mass (handgrip vs. knee extension). Furthermore this incomplete recovery of heart rate was not affected by parasympathetic

blockade but was abolished by β -adrenergic blockade (Fisher *et al.* 2010). This suggests that stronger muscle metaboreflex activation (from a greater local metabolite accumulation) is required for the increase in cardiac SNA to overcome the cardiac parasympathetic reactivation and cause a heart rate response. Overall these results demonstrate that the metaboreflex may be capable of eliciting heart rate responses in normal exercise, but during PECO this affect may be masked by parasympathetic reactivation post exercise.

Several chemicals which are by-products of muscle contraction have been identified which are known to stimulate metabolically sensitive afferents and cause the reflex cardiovascular responses. The injection of bradykinin, lactic acid/ H^+ , ATP, and diprotonated phosphate ($H_2PO_4^-$) into the arterial supply of anaesthetised cat hindlimbs all result in a pressor response (Stebbins & Longhurst, 1985; Rotto *et al.*, 1989; Sinoway *et al.*, 1994; Li & Sinoway, 2002; Hanna *et al.* 2002) which is abolished with the sectioning of the ventral and dorsal roots showing the reflex nature of the response. Arachidonic acid metabolites (such as prostaglandins) are also thought to stimulate the cardiovascular responses to exercise as the injection of the cyclooxygenase inhibitor, indomethacin, reduces the pressor response to electrically induced exercise (Stebbins *et al.* 1986).

By using ^{31}P Nuclear Magnetic Resonance Spectroscopy (^{31}P NMR), it is possible to perform investigations in humans. The time course and magnitude of the exercise pressor response to handgrip exercise was associated with intracellular concentrations of H^+ , suggesting that this is an important metabolic stimulus for the cardiovascular responses to exercise (Victor *et al.* 1988; Sinoway *et al.* 1989). In addition, Dichloroacetate infusion can blunt lactic acid production by stimulating pyruvate dehydrogenase activity. This therefore decreases the relative proportion of pyruvate converted into lactate by shifting the metabolism of pyruvate towards oxidation in the mitochondria. The infusion of

Dichloroacetate into healthy humans has shown to reduce the sympathetic nerve responses to static exercise and PECO (Ettinger *et al.* 1991). As infusion into anaesthetised cats reduces the discharge of group III afferent fibres to electrically induced static contraction (Sinoway *et al.* 1993), these effects are likely caused by a blunted lactic acidosis and a consequential reduction in muscle afferent activation. This again suggests that Lactic acid/H⁺ may be an important metabolite in stimulating the pressor response in humans.

In conclusion, all the chemical substances described above, which are by-products of muscle contraction, do appear to be capable of stimulating metabolically sensitive receptors found on the free nerve endings of muscle afferents and provide a stimulus for the exercise pressor reflex. However, in order to show that a specific metabolite, or mechanical stimulus, can contribute to the exercise pressor reflex, investigations must also demonstrate that blocking those receptor/s that are activated by the stimulus results in an attenuation of the cardiovascular response to: 1) the application or injection of the receptors agonist/ligand metabolite and more importantly to 2) exercise (e.g. electrically induced muscle contractions).

1.7 Receptor subtypes

Since the investigations by Kaufman *et al.* (1983), more recent investigations have determined the sensitivity of group III and IV afferents to particular stimuli is determined by the receptor subtype/s located on the free nerve endings of these fibres. This research has studied the sympathetic, cardiovascular and/or respiratory responses to mechanical and metabolic stimuli during the blocking or stimulation of specific receptor subtypes.

Mechanosensitive receptors

Gadolinium blocks mechanoreceptive channels and its administration in anaesthetised cats reduces group III muscle afferents discharge to passive muscle stretching, electrically induced static muscle contractions (Hayes and Kaufman 2001) and dynamic exercise induced via MLR stimulation (Hayes et al 2009). But gadolinium had no effect on group IV muscle afferent impulse activity. Furthermore, Hayes and Kaufman (2001) demonstrated that these mechanoreceptive channels are capable of evoking cardiorespiratory responses, as these responses to muscle stretching and muscle contractions were also reduced with the administration of gadolinium. When Nakamoto & Matsukawa (2007, 2008) injected gadolinium locally into the myotendinous junction of the triceps surae of anaesthetised rats, the pressor response induced from passive stretching of the muscle and electrically induce static contractions were significantly reduced. It is unlikely that any mechanoreceptors were located in the more distal part of the tendon as the cardiovascular responses to stretching and contraction were similar pre and post sectioning of the Achilles tendon. All together these results suggest that mechanosensitive receptors may be at least partly located in the myotendinous junction of the Achilles tendon and can evoke reflex cardiovascular and respiratory responses.

Metabosensitive afferents

Transient receptor potential vanilloid 1 (TRPV1) receptor

In both cats and rats the stimulation of the TRPV1 receptor (also known as vanilloid receptor 1 or capsaicin receptor) with its agonist capsaicin results in increases in blood pressure and heart rate, similar to that during exercise (Kaufman *et al.* 1982; Smith *et al.* 2005) and is primarily mediated through group IV afferents (Kaufman *et al.* 1983). However, the role of the TRPV1 receptor in eliciting the exercise pressor response is controversial.

Kindig *et al.* (2005) found that blocking this receptor, with the arterial injection iodo-resiniferatoxin (IRTX), abolished the pressor response to capsaicin injection but did not attenuate the exercise pressor response of cats to electrically stimulated static muscle contractions. Recently however, Smith *et al.* (2010) demonstrated in rats that the pressor response to electrically induced muscle contractions and capsaicin injection was significantly attenuated with the application of three antagonists of the receptor (capsazepine, IRTX and ruthenium red). However they had no effect on the pressor response to passive stretch, which may be expected as group IV afferents are more sensitive to capsaicin (Kaufman *et al.* 1983).

Therefore, TRPV1 receptors do appear to contribute to the metabolic component of the exercise pressor response in rats and that these receptors mediate in the activation of group IV afferents. Furthermore Gao *et al.* (2006) has shown in rats that a possible metabolite that stimulates the TRPV1 receptor may be H_2PO_4^- . Unlike monoprotonated phosphate (HPO_4^{2-}), the injection of H_2PO_4^- into cat hindlimbs evokes increases in BP (Sinoway *et al.* 1994), which could be attenuated by ~60% with capsazepine (Gao *et al.* 2006). However,

because lactic acid/ H^+ has no effect on TRPV1 receptors (Li *et al.* 2004) it is unlikely that its stimulation by diprotonated phosphate is mediated by H^+ delivery.

However, due to the apparent species difference between cats and rats it becomes difficult to establish the role of the TRPV1 receptor in the human exercise pressor reflex and until testing can be completed in humans its role will remain unclear.

Transient receptor potential A1 (TRPA1) channel

Similar to the TRPV1 receptor, TRPA1 channels appear also to be preferentially found on group IV muscle afferents (Kobayashi *et al.* 2005; Anand *et al.* 2008) in rats and humans respectively. Recently Koba *et al.* (2010) demonstrated that stimulating TRPA1 channels with its agonist Allyl isothiocyanate (mustard oil) significantly increases renal SNA and blood pressure in a dose dependant manner, and this can be abolished with sciatic nerve section or with the application of the channels antagonist HC-030031. Crucially, the pressor response to electrically induced sustained muscle contraction was reduced with HC-030031 suggesting that the TRPA1 channel is involved in the metabolic component of the exercise pressor reflex. However this response to intermittent contractions (arguably a more mechanical stimulus) was not attenuated by HC-030031, either suggesting that the mechanical stimulus was not great enough to stimulate them or that TRPA1 channels are unresponsive to mechanical stimuli. Furthermore the increase in BP (and RSNA) from the arterial injection of arachidonic acid, bradykinin and diprotonated phosphate were all reduced between ~30 and ~50% with HC-030031, suggesting that these by products of muscle contraction may be stimuli for or mediate in the stimulation of TRPA1 during exercise.

Acid sensitive ion channels (ASIC)

As previously described, the arterial injection of lactic acid/H⁺ and H₂PO₄⁻ results in a pressor response, which seems to be mediated through the stimulation ASICs as the administration of the ASIC antagonist amiloride significantly attenuates the response (Li *et al.* 2004; Gao *et al.* 2006). Furthermore injection the ASIC antagonist A-317567 into cats, reduces the blood pressure response to both static muscle contraction and post contraction circulatory occlusion (Hayes *et al.* 2008; McCord *et al.* 2009), implying its involvement in eliciting the exercise pressor reflex. However because there was no attenuation in the blood pressure response to tendon stretch, it is likely that ASIC play no role in the mechanical component of the pressor reflex (Hayes *et al.* 2008).

Kinin B2 receptor

Using two antagonists (NPC 11731 and HOE 140) Pan *et al.* (1993) demonstrated that blocking the bradykinin (or kinin) B2 receptor in cats significantly reduced the blood pressure (and heart rate) response to arterial injection of bradykinin and to electrically induced static contraction of the hindlimb muscle (by ~50%). This suggests that bradykinin is involved in inducing the cardiovascular responses in exercise and is at least partially mediated through the kinin B2 receptor. Furthermore, the application of indomethacin inhibits the pressor response to bradykinin injection, so perhaps prostaglandins sensitise muscle afferent nerve endings to bradykinin (Stebbins & Longhurst 1985; Pan *et al.* 1993). Indeed, the binding of bradykinin to the kinin B2 receptor causes prostaglandin synthesis, so these receptors may mediate increases in the concentration of prostaglandins in the vicinity of muscle afferent nerve endings and as such the effects of bradykinin on kinin B2 receptors may be enhanced (Pan *et al.* 1993)

Purinergic receptors

The agonist of the purinergic 1 (P1) receptor is adenosine, but its stimulation does not appear to have any cardiovascular effects (Hanna *et al.* 2002). The Purinergic 2 (P2) receptor is stimulated by ATP, the interstitial level of which increases during exercise in humans (Mortenson *et al.* 2009). The P2 receptor is expressed as two subtypes, P2X and P2Y. In cats the arterial injection of α,β -methylene ATP, a selective agonist of the P2X receptor, increases blood pressure (Hanna *et al.* 2002; Li & Sinoway, 2002; Hayes *et al.* 2008a; McCord *et al.* 2010) but the selective stimulation of P2Y receptor has no such effect (Hayes *et al.* 2008a). Furthermore the arterial injection of α,β -methylene ATP in cats primarily stimulates group IV afferents suggesting that these fibres are where P2X receptors are principally located.

However Kindig *et al.* investigated further and showed that both the group III and IV impulse activity responses to static muscle contraction could be significantly reduced with the P2 receptor antagonist pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS; Kindig *et al.* 2006). Hence P2 receptors on both group III and IV afferents may contribute to the exercise pressor response, but because PPADS only reduce the discharge of group IV afferent to the pure metabolic stimulus of circulatory occlusion post-muscle contractions (Kindig *et al.* 2007), it is likely that only P2 receptors on group IV afferents contribute to the metabolic component of the exercise pressor reflex.

P2X receptors may play an even greater role however, as their stimulation may potentiate the pressor responses to mechanical stimuli. This is evidenced by 1) α,β -methylene ATP injection enhances the blood pressure increases to passive muscle stretching (Li & Sinoway, 2002), and 2) PPADS attenuate the impulse activity responses of group III muscle afferents to tendon stretch (Kindig *et al.* 2006). As such it appears that group III

afferents fibres and can be sensitised to mechanical stimuli by ATP present in the muscle interstitium.

As discussed above, there may be a species difference in the relative importance of each receptor that are capable of evoking the exercise pressor response, so experiments in humans are always preferential if possible. Recently Cui *et al.* (2011) locally infused pyridoxine hydrochloride (vitamin B₆) into the forearms of humans via a Bier block. Pyridoxine hydrochloride converts into pyridoxal-5-phosphate which is a P2 receptor antagonist, and during its application MSNA (~50%) and blood pressure (5 mmHg) responses were significantly attenuated to both isometric handgrip exercise and PECO. However, in contrast to the animal data of Li & Sinoway (2002), the block did not affect the increase in MSNA to passive stretch from levels observed in PECO suggesting that P2 receptor stimulation does not potentiate the cardiovascular responses to the mechanical stimuli of stretch in humans. This may signify an underlying species difference or the contrasting results may be due to the stretch being performed during PECO in the study by Cui *et al.* Therefore other metabolites will have accumulated which may sensitise mechanoreceptors independently of P2 receptors e.g. prostaglandins, (Cui *et al.* 2008a). Notwithstanding, Cui *et al.* (2011) have elegantly demonstrated that P2 receptor stimulation are involved in exercise pressor response in humans.

In conclusion these studies collectively suggest that ATP, Lactic acid/H⁺, diprotonated phosphate, bradykinin and also arachidonic acid metabolites may all have a role in the generation of the exercise pressor reflex by stimulating the metabolically sensitive receptors on the free nerve ending of muscle afferent fibres.

1.8 Skeletal muscle afferent feedback- ventilatory control

Due to the clear importance of muscle afferent feedback on cardiac and circulatory control in exercise it is also reasonable to hypothesise that this feedback also plays a role in eliciting the exercise hyperpnea. Indeed neural signals emanating from an exercising muscle may not only be in proportion to metabolic rate but also may account for the rapid increase in ventilation that is classically associated with the onset of exercise (Asmussen, 1973).

Animal studies

Extensive work has been completed in animal preparations which utilise electrically induced muscle contractions to activate the muscle without the co-activation of central command. Early studies performed by Comroe & Schmidt (1943) demonstrated that muscular work induced by electrical stimulation of the ventral roots in anaesthetised dogs results in hyperpnea, which can be abolished through spinal cord transection. Kao *et al.* (1963) improved upon this study design by using a cross-circulation technique in anaesthetised dogs so the effects of muscle afferent feedback on ventilation could also be isolated from humoral factors. The ventral roots of the “neural dog” were electrically stimulated to induce hindlimb muscles contractions, while these muscles were concurrently perfused by the blood of the “humoral dog”. The muscle contractions resulted in the immediate increase of ventilation ($\sim 6 \text{ l.min}^{-1}$) in the neural dog, but not in the humoral dog, and this response was abolished through the transection of the spinal cord. These findings support the concept that nervous signals emanating from the working muscle can mediate in the exercise hyperpnea.

In addition to the studies of Coote *et al.* (1971) and McClosky & Mitchell (1972) described above, Tibes (1977) also examined the reflex component of the exercise hyperpnea. Tibes demonstrated that the ventilatory responses of dogs to electrically stimulated dynamic hindlimb contractions were eliminated by a cold block of muscle afferents, but only when the blocking temperature reached that for group III and IV afferent fibres. All together these findings clearly indicate that neural feedback from the contracting muscles of animals can cause respiratory reflexes via group III and group IV muscle afferents. Comroe & Schmidt (1943) suggested that these respiratory responses could also be caused by the stimulation of receptors located in joints. However Coote *et al.* prevented joint movements by ensuring that the limbs were in a fixed position suggesting that the cardiorespiratory responses observed were caused by afferent feedback from the muscle.

Several investigations have examined the possible metabolic and mechanical stimuli which can activate group III and IV afferent fibres and cause a reflex hyperpnea. Although respiratory data is rarely recorded in these types of studies there is evidence that the injection of bradykinin, lactic acid/H⁺ and ATP into arteries supplying the hind limb of animals causes increases in ventilation (Tallarida *et al.* 1979, Rotto & Hill 1989; Hanna *et al.* 2002). In addition the hyperpnea could be abolished with either the sectioning of the ventral and dorsal roots (Tallarida *et al.* 1979; Rotto & Hill, 1989) or the blockade of ATP sensitive P2 receptors with PPADS (Hanna *et al.* 2002) showing the reflex nature of the response.

In anaesthetised cats the mechanical stimulus of passive muscle stretching of the triceps surae also causes increases in ventilation, the magnitude of which is related to the degree of the stretch (Wilson *et al.* (1994) and which is completely abolished by blocking mechanosensitive channels with the arterial injection of gadolinium (Hayes *et al.* 2001).

Furthermore in addition to the stretch the ventilatory response to electrically induced muscle contractions are also abolished by gadolinium application. As such this provides clear evidence in animals that muscle contraction and passive stretching of muscles can stimulate muscle mechanoreceptors and contribute to ventilation in exercise.

However, the supportive findings for the involvement of muscle afferent feedback in the generation of the exercise hyperpnea described above have not been unanimously demonstrated. Although Comroe & Schmidt (1943) found that spinal cord transection eliminated the ventilatory response to muscle contraction in anaesthetised dogs, it was shown to have no effect on the response in anaesthetised cats. Other investigators have shown similar finding in cats and dogs whereby after spinal cord transection ventilation still increased proportionately to metabolic rate (with maintained arterial blood gas homeostasis) in response to electrical stimulation of hindlimb muscles (Lamb, 1968; Levine, 1979; Weissman *et al.* 1980). Weissman *et al.* concluded that muscle afferent feedback was not required for matching ventilation to the metabolic demands of the exercising muscle. Furthermore because all afferent feedback was abolished and central command was not activated these imply that humoral factors may stimulate the exercise hyperpnea, which is in direct contrast to the findings of Kao *et al.* described above. As such it is not yet possible to conclude in animals whether feedback from muscle afferents stimulated by electrically induced contractions can cause a hyperpnea. The significant variation in results may be related to the highly unphysiological nature of the experiments. Furthermore, the electrically induced muscle contractions in animals only cause very small increases in ventilation which either means that 1) this is an unreliable method to test whether feedback from exercising muscles plays a role in the associated hyperpernea or 2) muscle afferent feedback plays no significant role. Certainly, further examination is required.

Human studies - electrically induced muscle contractions

Adams *et al.* (1984) and Brice *et al.* (1988) demonstrated in awake humans that both voluntary exercise and electrically induced muscle contractions produce similar increases in ventilation which are exactly proportional to metabolic rate. Although it is obviously not possible to examine the ventilatory response to electrically induced muscle contraction pre and post spinal cord transection in humans, these investigators compared the ventilatory responses between healthy individuals and paraplegic humans with complete spinal cord transection. In this population all afferent feedback from skeletal muscles below the level of the lesion will be blocked from reaching respiratory control areas in the brainstem. Adams *et al.* (1984a) and Brice *et al.* (1988a) demonstrated that electrically induced muscle contractions of the quadriceps and hamstring muscles, stimulated ventilation in both healthy and paraplegic individuals. Although the responses of paraplegics were often smaller (e.g. 4.4 vs. 7.6 l.min⁻¹, Adams *et al.* 1984a), critically the increase in ventilation in both groups was in proportion to $\dot{V}CO_2$. These findings therefore suggest that muscle afferent feedback is not necessary to produce an exercise ventilatory response which matches the increased metabolic rate. As such the findings seem to provide support for a humoral mechanism.

However when venous return is inhibited by the inflation of cuffs around the contracting muscle, ventilation decreases in paraplegics but only several seconds after, and in proportion to, a decrease in $P_{ET}CO_2$ (Brown *et al.* 1990). Furthermore the decrease in ventilation was predictable from normal CO_2 responsiveness. This suggests that the change in $PaCO_2$ caused this reduction in the ventilatory response and thus does not support the concept of a humoral mechanism controlling the exercising hyperpnea, as of course during normal mild-moderate exercise in healthy humans $PaCO_2$ remains stable. It is worth noting that further examination of the data shows that ventilation did not return to resting levels

during occlusion as ~50% of the response was maintained (Brown *et al.* 1990). As the contractions were applied in the apparent absence of a central command, muscle afferent and humoral stimulus the mechanism of the response is unknown.

To summarise, the cause of the hyperpnea in paraplegic humans is not clear but feedback from muscle afferents may not be necessary to induce an increase in ventilation that matches metabolic rate. However the finding by Brown *et al.*, and the inconsistency in the animal data (described above), may imply that electrically induced muscle contractions may not be a suitable or reliable method for analysing the role of muscle afferent feedback in controlling the exercise hyperpnea.

Epidural anaesthesia during exercise in humans

By using spinal anaesthesia (e.g. lidocaine or bupivacane injected into the lumbar epidural space) afferent feedback from lower limbs during exercise can be reduced. Seemingly in contrast to cardiovascular responses the hyperpnea to cycling exercise is not attenuated during anaesthetic block in man (Hornbien *et al.* 1969; Fernandes *et al.* 1990), suggesting that muscle afferent feedback is not required to elicit the normal hyperpnea to exercise. However, one concern with the studies is that epidural anaesthesia will also reduce efferent nerve activity to the exercising muscles and thus cause muscle weakness. This will enhance the level of central motor drive required to achieve the same work load and thus increase central command activation, possibly masking any effects from the reduced sensory feedback.

More recently however, Amann *et al.* (2010) injected the μ -opiate agonist fentanyl intrathecally into the L3-L4 vertebral space to inhibit the neurotransmission from group III and IV muscle afferents projecting into the dorsal horn of the spinal cord. Crucially

fentanyl has shown not to alter the force generating capacity of skeletal muscle (Amann *et al.* 2009). Amann *et al.* (2010) demonstrated that the ventilatory and cardiovascular responses to dynamic cycling exercise were significantly attenuated with the use of fentanyl compared to normal exercise. This data is clearly the most compelling evidence to date that muscle afferent feedback does contribute to the mediation of the exercise hyperpnea.

Muscle metaboreflex activation via PECO

PECO stimulates metabolically sensitive afferents in complete isolation of central command, and although it is known to increase sympathetic vasomotor activity most evidence suggests it does not cause increases in ventilation. For example Rowell *et al.* (1976) found that following a bout of exercise on a cycle ergometer (50-250 W) PECO of both legs using thigh cuffs inflated to 300 mmHg did not maintain ventilation at exercising levels but returned to baseline. Conversely blood pressure was maintained at the exercising level until the thigh cuffs were realised. In direct contrast to the findings of Amann *et al.* above, Rowell *et al.* concluded that because the “muscle ischemia (post exercise) did not alter minute ventilation... respiratory control ‘centres’ appear to receive no significant input from ‘metabolic sensors’ in muscle”. This finding has been confirmed in healthy humans by several other investigations employing circulatory occlusion following cycling exercise (Innes *et al.* 1989; Haouzi *et al.* 1993, 2001; Scott *et al.* 2000; Fukuba *et al.* 2007, Olson *et al.* 2010) and rhythmic handgrip exercise (Scott *et al.* 2002). The method of PECO after static exercise has been less extensively tested but similar effects have been found. Wiley & Lind (1971) demonstrated that following static handgrip exercise of varying intensities (30-50% MVC until fatigue), blood pressure remained elevated throughout PECO but ventilation returned to resting levels.

Therefore the technique of PECO has been widely used to test the role of the muscle metaboreflex in cardio-respiratory control. Although it clearly is a powerful activator of the sympathetic nervous system and plays a role in controlling blood pressure, it appears to play no critical role in ventilatory responses for healthy humans. However, the technique of PECO used in the way described above may not be suitable for testing the ventilatory responses to the stimulation of muscle metaboreceptive afferents. This is because of two factors that may suppress ventilation during PECO.

Firstly, some group III and IV afferents appear to respond to vascular distension, perhaps to monitor blood flow to the muscle, as their impulse activity increases in response to vasodilatory agents and venous occlusion (Haouzi *et al.* 1999). Furthermore inflating thigh cuffs to sub-systolic pressures after cycling exercise, in order to occlude blood flow *from* previously exercised legs muscles, results in a slower and attenuated recovery of ventilation compared with complete circulatory occlusion *to* the exercised muscle (Haouzi *et al.* 2001). Therefore during PECO where muscle blood flow is occluded the discharge of these few group III and IV afferents may be abolished and their possibly stimulatory effects on ventilation may be removed. However, it is not clear whether this will have more than a negligible effect as PECO of course takes place at rest when the discharge rates of these afferents appear to be low (Haouzi *et al.* 1999). Therefore the difference in the impulse activity between resting and arterial occlusion conditions may be negligible and so may not suppress any ventilatory responses to muscle metaboreflex activation.

Secondly we must consider the effects of the ventilatory baroreflex. PECO induced metaboreflex activation preferentially causes the elevations in blood pressure to be maintained post exercise, and so the consequential stimulation of the baroreceptors will suppress ventilation via the opposing effects ventilatory baroreflex (Heisted *et al.* 1975; Stewart *et al.* 2011). Therefore during PECO, where the stimulatory effect of central

command is not activated, ventilation may return back to baseline because the metaboreflex alone cannot overcome the suppression from the simultaneous activation of the ventilatory baroreflex. The effects of the mechanoreflex will also be lost during PECO which could additionally contribute to a reduction of ventilation. Currently however, unlike central command, the role of the mechanoreflex on ventilation in humans is still unclear and requires further examination. Data from studies which have utilised rhythmic passive limb movements in humans have hinted that mechanically sensitive muscle afferents can stimulate ventilation (Bell & Duffin, 2006), but one cannot discount the influence of joint receptor activation (Barron & Coote, 1973). Therefore sustained passive stretch, which increases ventilation in animals (Wilson *et al.* 1994), may provide a more selective stimulus (chapter 3).

Nevertheless these observations above may provide a explanation for the apparent disparity in the conclusions drawn from the experiments by Amann *et al.* (2010) and those that applied PECO to stimulate muscle afferents (e.g. Rowell *et al.* 1976). When inhibiting the neural transmission of muscle afferents *during* exercise, when central command is activated, the hyperpnea is attenuated. But stimulating afferents *after* exercise with PECO, where central command is not active, classically has no effect on ventilation in healthy individuals. Therefore it is possible that muscle afferent stimulation alone is ineffective at driving ventilation without the presence of a potentially interactive/synergistic input to the central respiratory neuronal pool such as central command, or perhaps a ventilatory chemoreflex.

Indeed recently Lykidis *et al.* (2010) has demonstrated that muscle metaboreflex activation through PECO maintains ventilation significantly above baseline until cuff deflation, but only under conditions of concurrent hypercapnia ($P_{ET}CO_2$ of 47 mmHg) and not while breathing air. These observations suggest the occurrence of a synergistic interaction, where

in effect the hypercapnia-induced ventilatory chemoreflex seems to unmask the involvement of muscle afferent feedback in ventilatory control. A central interaction is possible as both skeletal muscle and peripheral chemoreceptor afferents project to the NTS (Paton, 2001), a possible site of central chemoreception itself (Nattie & Li, 2009).

Conversely, there is an additional/alternative mechanism that might be accountable for the ventilatory responses observed by Lykidis *et al.* The acute hypercapnia may decrease the pH of the exercised muscle further and consequently augment the afferent feedback. Indeed in animals muscle acidosis is a factor in stimulating the exercise pressor reflex (Rotto & Kaufman, 1988; Rotto *et al.* 1989; Sinoway *et al.* 1992, 1993) and ventilatory response (Rotto *et al.* 1989). Therefore maybe only during concurrent hypercapnia is the metaboreflex powerful enough to maintain ventilation above resting levels during PECO. Whether this possible interaction in the peripheral exercised muscle contributes to the observed ventilatory responses requires further examination (chapter 4).

Nevertheless this concept may account for the atypical findings of Iellemo *et al.* (1999) where ventilation was maintained slightly (yet significantly) above baseline during PECO but only when the exercised muscle mass was great enough (static knee extension vs. static handgrip) and when the preceding exercise was performed at a high enough intensity (30% vs. 15% MVC for 3 minutes). It was argued that there would be greater local metabolite accumulation and as such only when the resultant metaboreflex is powerful enough can ventilatory responses to PECO be observed. Indeed it is possible the metaboreflex must be robust enough for the ventilatory responses overcome the opposing effects of its baroreflex.

To summarise, the role of muscle metaboreflex activation on ventilatory control seems to be unmasked by the sensitising effects of hypercapnia. Currently it is unclear whether this is achieved through a central synergistic interaction or an interaction in the peripheral

muscle. Furthermore the role of the mechanoreflex in human ventilatory control is poorly understood and requires further examination.

Chronic obstructive pulmonary disease (COPD)

The observation of an augmented ventilatory response to metaboreflex activation during acute hypercapnia (Lykidis *et al.* 2010) could have implications for disease states that can result in CO₂ retention and chronic hypercapnia such as that in chronic obstructive pulmonary disease (COPD). COPD is an umbrella term for the occurrence of both chronic bronchitis and emphysema and results in increases in airflow resistance. One of the most common symptoms of COPD is a decreased exercise capacity which is thought to be largely due to the sensations of dyspnea (O'Donnell *et al.* 2009), but also due to abnormalities in skeletal muscle metabolism and function resulting in weakness and early muscle fatigue (Serres *et al.* 1998; Casaburi *et al.* 2001).

One characteristic of some importance is the occurrence of an early and exaggerated muscle acidosis during exercise (Kutsuzawa *et al.* 1992), which may of course increase the stimulation of muscle metaboreceptive afferents. Patients with chronic heart failure (CHF), who appear to have similar abnormalities in skeletal muscle (Franssen *et al.* 2002), have been shown to display augmented metaboreflex activity during PECO producing exaggerated ventilatory responses (Piepoli *et al.* 1996, 1999; Scott *et al.* 2000, 2002; Olson *et al.* 2010). Therefore augmented skeletal muscle afferent feedback may perhaps provide a neural link between skeletal muscle dysfunction and dyspnea during exercise in COPD, as there would be an even greater mismatch between the central drive to increase ventilation and the lungs impaired capacity to do so. However, whether these patients have an augmented metaboreflex and/or a metaboreflex induced ventilatory response is still yet

to be fully examined. Furthermore, based upon the findings of Lykidis *et al.*, it is conceivable that the chronic hypercapnia of some patients may also enhance the ventilatory response to muscle metaboreflex activation and as such could provide another mechanism that causes dyspnea upon exertion in these patients. Indeed it has been shown that in patients with a wide range in COPD severity and who also retain CO₂, the most important stimulus for dyspnea during an incremental cycling exercise appeared to be the increased PaCO₂ (Cloosterman *et al.* 1998).

Summary

The cardiovascular system in exercise is regulated by a combination of central command, the arterial baroreflex and the feedback from group III and IV skeletal muscle afferents responding to mechanical and metabolic stimuli in the exercising muscle. The control mechanisms of the exercise hyperpnea are still not fully understood, yet central command is widely thought to play an integral role. Historically there has been limited and conflicting evidence that neural feedback from exercising muscles can control ventilatory responses in humans. Recently however evidence has suggested that muscle afferent feedback is capable of driving human ventilatory responses as: inhibiting its neurotransmission to the dorsal horn of the spinal cord attenuates the exercise hyperpnea, and the stimulation of muscle afferents in combination with the sensitising effects of hypercapnia causes ventilatory responses. However the latter evidence requires further examination.

1.9 Proposed studies in human participants

Based upon the preceding literature review the following studies are proposed.

1) To examine:

- the ventilatory responses to muscle metaboreflex activation in the calf muscle during concurrent acute hypercapnia.
- The ventilatory responses to muscle mechanoreflex activation during concurrent hypercapnia.
- The combination of metabo and mechanoreflex activation during concurrent hypercapnia

2) To determine:

- If the interaction between muscle afferent activation and hypercapnia occurs centrally (i.e. in the CNS) or peripherally (i.e. in the exercised muscle).
- Additionally to investigate whether the interaction between muscle afferent activation and hypercapnia occurs via a hypercapnia-induced stimulation of the central and/or peripheral chemoreceptors

3) To investigate:

- Whether the experimental augmentation of muscle afferent feedback can increase the exercise hyperpnea.
- Whether muscle mass influences the ventilatory responses to muscle metaboreflex activation

4) To determine whether patients with COPD display augmented ventilatory responses to muscle afferent stimulation (PECO) and if so is this related to chronic hypercapnia from CO₂ retention.

CHAPTER 2: GENERAL METHODS

2.1 Experimental procedures

Isokinetic dynamometer – calf plantarflexion exercise

For the experiments in chapters 3 and 4 all participants were seated in a semi-recumbent position in a Biodex system 3 Pro isokinetic dynamometer (Biodex medical systems, Shirley, NY, USA). Their right foot was firmly strapped to the footplate using Velcro straps, with the centre of rotation of their ankle aligned with that of the machine with their lower leg parallel to the floor and knee flexed at 30° (Figure 2.1). The maximal voluntary contraction (MVC) of the calf plantarflexors was assessed by instructing participants to perform maximal efforts against the footplate and accepting three that were within 10% of each other. Each effort was separated by 1 minute and the highest of the three was taken as the MVC. During the experimental protocol participants maintained the required percentage of their MVC (50%) by matching their torque output to a target torque displayed on a computer screen positioned in front of them at eye level.

Isokinetic dynamometer – passive calf muscle stretch

In chapter 3 where passive stretch of the calf was also applied, the passive range of dorsiflexion around the ankle joint was determined before each trial by manually altering the angle of the footplate to a position as far as was comfortable for the participant. The information was programmed into the Biodex, so that the following passive stretching movement was automatically performed by the machine. Figure 2.2 shows an original record of the typical torques generated during a sustained passive stretch.

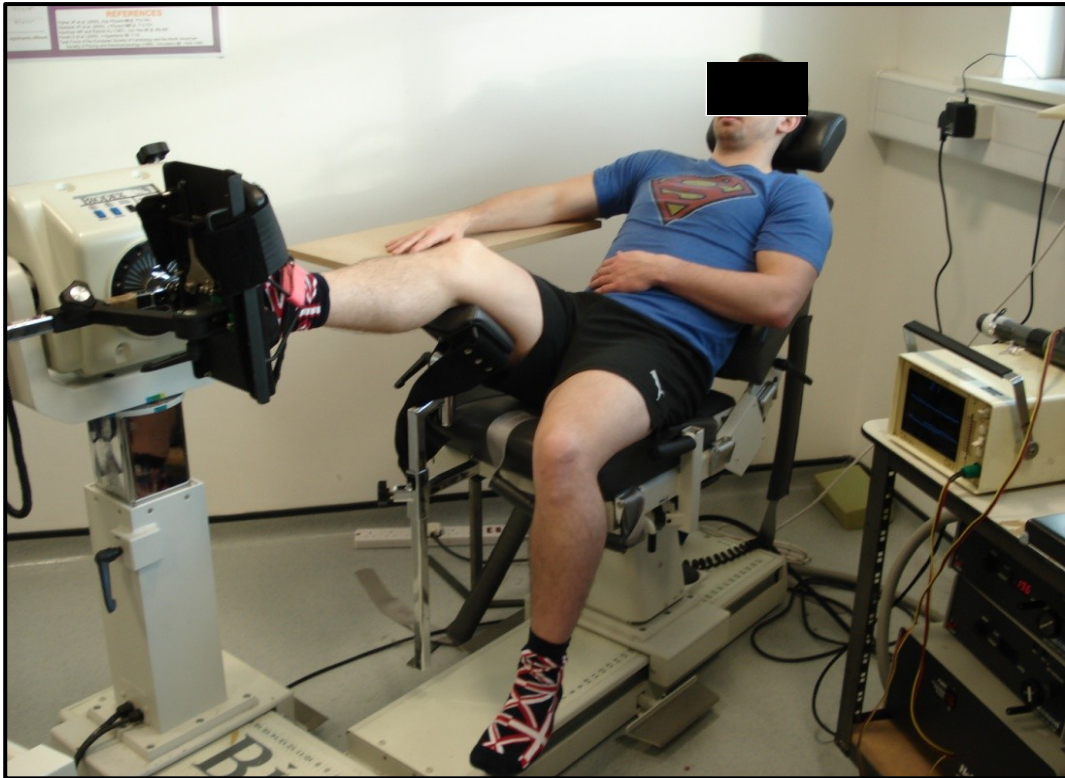


Figure 2.1 Participant seated in the isokinetic dynamometer

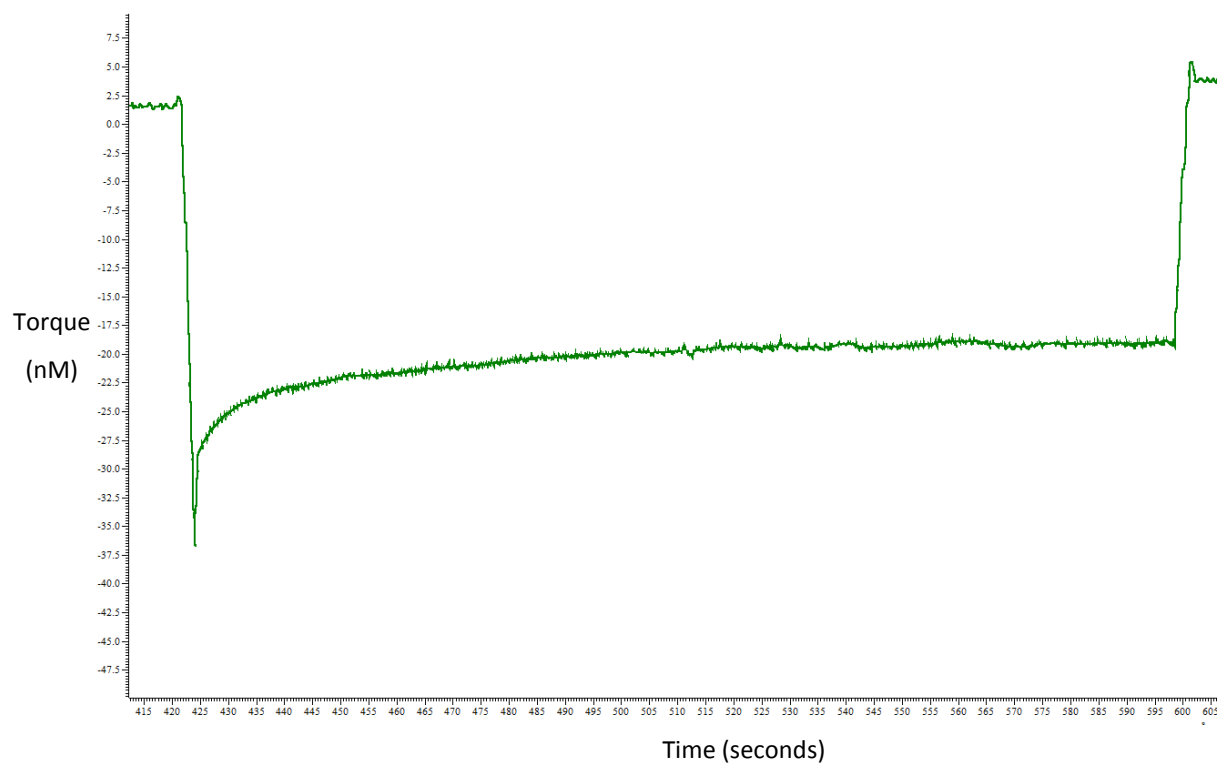


Figure 2.2 Original recording of a typical torque trace during three minutes of muscle stretch

Cycle ergometer exercise

In chapter 5, participants were seated in a semi-recumbent position in a customised electrically braked cycle ergometer (Angio, Lode, Groenigen, The Netherlands; Figure 2.3). The seat of the cycle ergometer was adjusted for each individual, so that the participants knee was almost completely extended when their foot went through the bottom of the pedalling stroke. The participant's right foot was strapped to the right pedal with Velcro straps by the experimenter. The exercise intensity performed by the participants was 50 watts (at 60 RPM).



Figure 2.3 Customised electrically braked cycle ergometer

Handgrip dynamometer exercise

In chapter 6, participants were seated in an upright position with their right hand holding a custom-made handgrip dynamometer (Figure 2.4). The device was clamped to a surface top which also supported the participants right arm. Maximal voluntary contractions (MVC) were determined by instructing participants to perform maximal handgrip efforts and accepting three that were within 10% of each other. Each effort was separated by 1 minute and the highest of the three was taken as the MVC. During the experimental protocol participants maintained the required percentage of their MVC (40%) by matching their force output to a target force displayed on a computer screen positioned in front of them at eye level. The rhythmic handgrip exercise task was performed at a duty cycle of 1-second contraction to 1-second relaxation

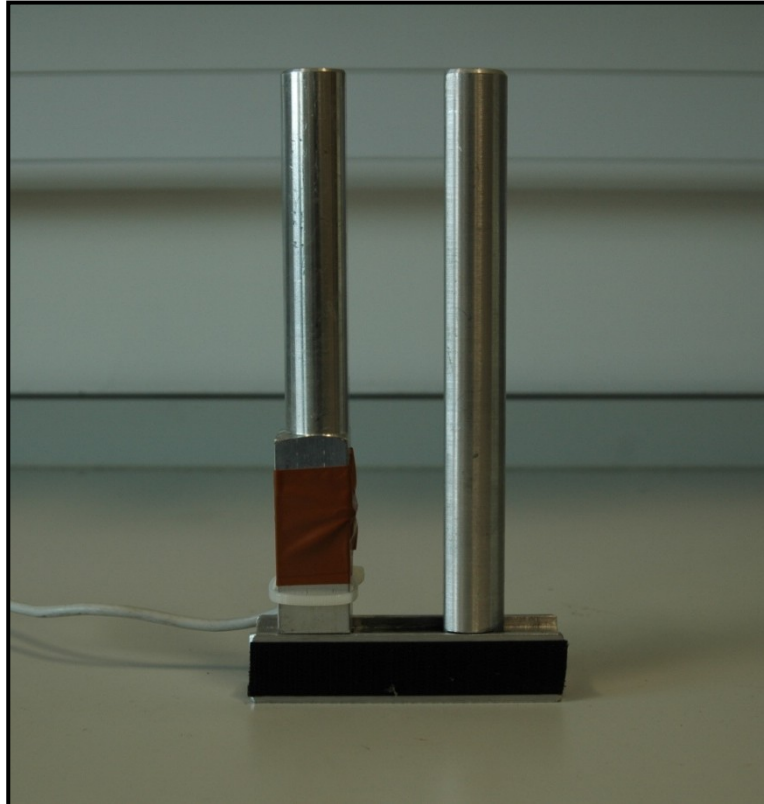


Figure 2.4 Custom-made handgrip dynamometer

Circulatory occlusion

Circulatory occlusion was achieved through the inflation of a thigh/arm cuff to a suprasystolic pressure (200 mmHg) using a rapid cuff inflator (E20, Hokanson Bellevue, WA, USA). In all trials in the thesis the circulatory occlusion of previously exercised muscles (PECO) maintained blood pressure above baseline until circulation was restored via cuff deflation (e.g. Figure 2.5). The deflation of the cuff also resulted in a transient hyperpnea. These observations are consistent with the trapping of exercised generated metabolites during cuff inflation and the consequential stimulation of the metaboreflex throughout PECO.

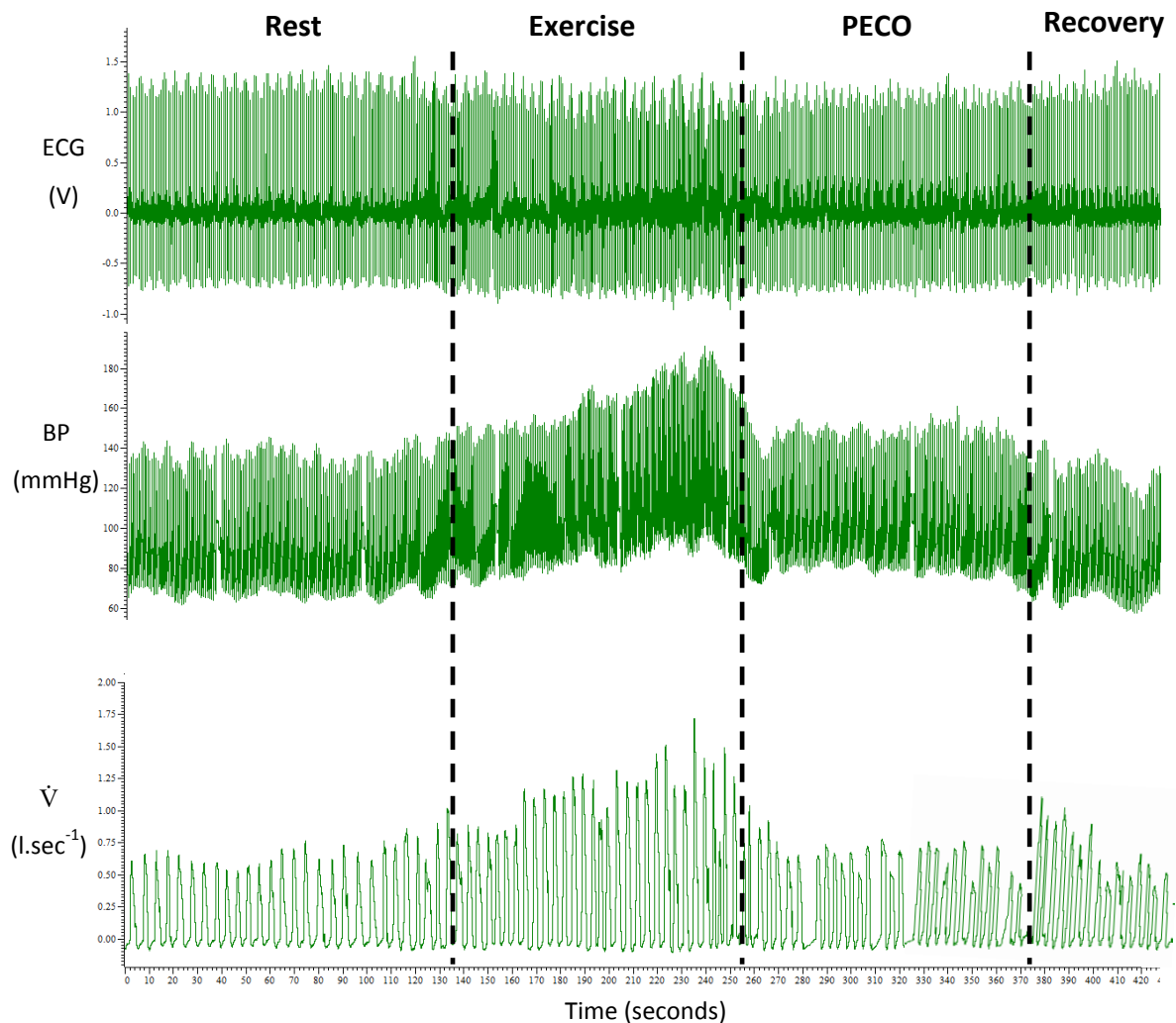


Figure 2.5 Original recording of ECG, BP and ventilation (\dot{V}) during rest, a rhythmic handgrip exercise task (40% MVC, 1 second duty cycle), PECO and recovery upon cuff deflation

2.2 Respiratory and Cardiovascular measures

In all experiments ventilation was continuously monitored with a pneumotachograph (Flowmetrics, BRDL, fr-41 s, ultrasonic pneumotachograph, Chatsworth CA, USA) attached to the inspiratory side of a breathing valve (T-Valve) with a mouthpiece (Figure 2.6). From this the inspiratory airflows (in litres per second) were recorded and minute ventilation (\dot{V}) was calculated offline using custom-written script files. All volumes recorded were converted to BTPS. In chapters 3 and 4, where participants inhaled hypercapnic gas mixtures, corrugated tubing leading to a Douglas bag (filled with the hypercapnic gas mixture) was attached to the other end of the pneumotachograph. When participants inhaled air in these trials the corrugated tubing lead to open air. The pneumotachograph was calibrated with a three litre syringe to give a linear output over the range of 0.1 litres to 3 litres .

End-tidal partial pressure of CO₂ ($P_{ET}CO_2$) was recorded throughout the protocol with a rapid gas analyser (Servomex, 1440, Sussex, UK) sampling the end tidal gases in the breathing valve. The analyser was calibrated using gases with a known concentration of CO₂ (between 0% and 10%; BOC gases, Surrey, UK).

R-R interval was measured from a 3-lead electrocardiogram (ECG; Cardiorator CR7, Cardiac Records Ltd, London, UK) in the lead II position. From this HR was continuously measured. Blood pressure was measured using finger photoplethysmography (Portapress, Finapress Medical Systems, Amsterdam, The Netherlands) with the cuff placed on the middle finger, and the hand supported at heart level on an adjustable table.

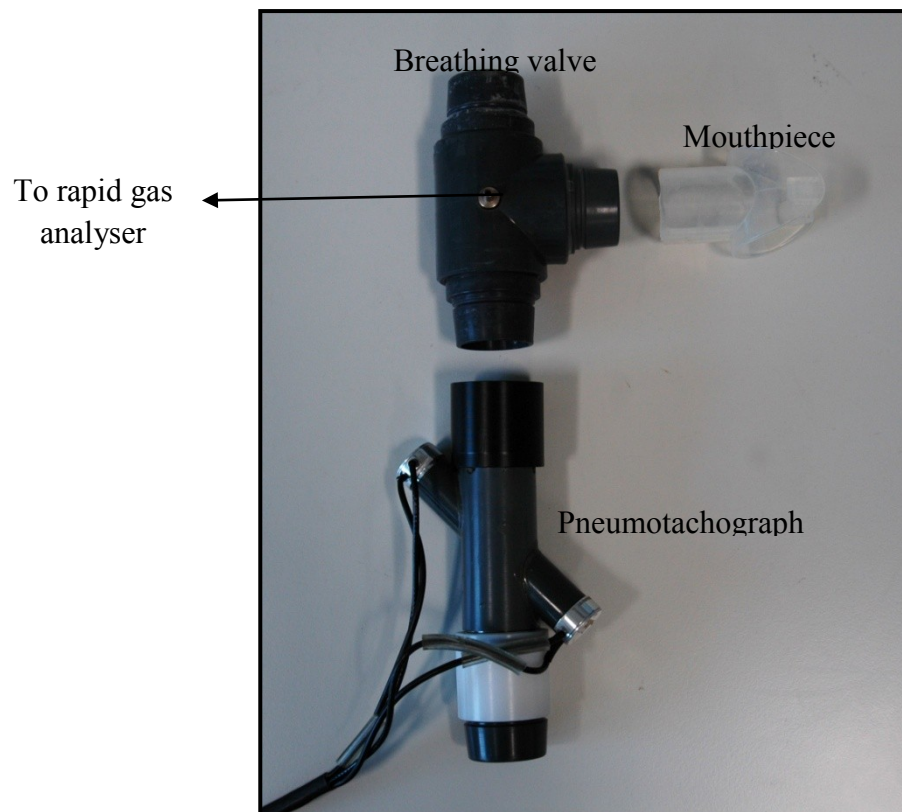


Figure 2.6 Pneumotachograph attached to the inspiratory side of a breathing valve (T-Valve) with a mouthpiece.

2.3 Data analysis

In all the experiments, all signals were sampled by an analogue-to-digital converter (Cambridge Electronic Design 1401 plus, CED, Cambridge, UK) at 1250 Hz, except the torque signal from the Biodex and handgrip dynamometer which was sampled at 625 Hz. Data was recorded and displayed using Spike 2 software (CED, Cambridge, UK). Raw data files were analysed offline using Custom Spike 2 script files which determined breath-to-breath values for minute ventilation and $P_{ET}CO_2$ as well as beat-to-beat values for heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure. All statistical analysis was conducted using a standard statistical package (SPSS, Chicago, IL, USA). The statistical tests used in each experiment are detailed in the method sections in each chapter.

**CHAPTER 3: THE VENTILATORY
AND CARDIOVASCULAR
RESPONSES TO MUSCLE
METABOREFLEX AND
MECHANOREFLEX ACTIVATION
DURING CONCURRENT
HYPERCAPNIA**

3.1 Introduction

It has been well established that the cardiovascular responses to exercise are in part controlled by the thin fibre (groups III and IV) afferent feedback arising from the exercising skeletal muscle (Alam & Smirk 1937; Coote *et al.* 1971, McCloskey & Mitchell 1972). Traditionally, group IV afferents are primarily classified as chemically sensitive whereas group III afferents are predominantly mechanically sensitive, (Mense & Stahnke, 1983; Kaufman *et al.* 1983, 1987), although the latter does display polymodal characteristics (Adreani & Kaufman 1998; Hayes *et al.* 2006). The role of muscle afferent feedback in the control of ventilation in exercise is more controversial. Several pieces of evidence from decerebrate animal preparations, where the modulating influences of higher brain areas are removed, have demonstrated that both metabolic and mechanical muscle afferent stimulation can result in hyperpnea (Coote *et al.* 1971; McCloskey & Mitchell 1972, Tibes, 1977, Wilson *et al.* 1994, Hayes & Kaufman, 2001).

In humans however the role of muscle afferent feedback was considered unimportant as evidenced by the classic finding in healthy humans that the activation of metabolically sensitive skeletal muscle afferents using PECO does not prevent the recovery of ventilation to resting levels (Rowell *et al.* 1976; Innes *et al.* 1989; Scott *et al.* 2000; Haouzi *et al.* 2001, Fukuba *et al.* 2007). Recently however Lykidis *et al.* (2010) found that muscle metaboreflex activation through PECO can produce ventilatory responses but only during a concurrent hypercapnia. The nature of this interaction between the metaboreflex and hypercapnia is not certain as of yet, but given that the muscle mechanoreflex may play a significant role in generating the respiratory response to exercise (Wilson *et al.* 1994; Hayes & Kaufman 2001) this study aims to examine the effects of muscle mechanoreflex activation during hypercapnia. In addition the study will examine the combined effects of muscle mechano and metaboreflex activation on human ventilatory control in combination

with hypercapnia. A simple method of evaluating the effectiveness of human muscle mechanoreceptive afferents is the use of a sustained passive muscle stretch. This has the advantages of avoiding the involvement of both central command and muscle metaboreflex activation.

Respiratory and cardiovascular responses to combinations of passive calf muscle stretch and PECO during inhalation of a hypercapnic gas mixture were examined in a series of trials. These controlled for the effects of 1) the sensitising effects on muscle afferents of metabolites produced during exercise and 2) hypercapnia induced elevated central respiratory drive, in the absence of central command. It was hypothesised that during concurrent hypercapnia, activation of the muscle metaboreflex would maintain ventilation at exercise levels and activation of the muscle mechanoreflex would further increase ventilation above levels recorded in PECO. During normocapnia it was hypothesised that mechano and metaboreflex activation would have no effect on ventilation.

3.2 Methods

13 healthy male participants (20.8 ± 1.2 years old; 78.2 ± 11.1 kg; 179 ± 6.7 cm; mean \pm SD) from the University of Birmingham student population took part in this study. All participants received written and verbal information regarding the experimental procedures prior to giving informed written consent. All subjects were habituated to all the experimental procedures, which conform to the Declaration of Helsinki and were approved by the local ethics committee. Participants visited the laboratory on two separate days following habituation. Before each day participants refrained from consuming food and caffeine in the 4 hours before the trial and from performing strenuous physical activity or consuming alcohol in the 12 hours before the trial. This study followed a within subject design with all subjects participating in all four trials. The order of each trial was randomised with a 30 minutes rest period in between.

Experimental procedures

Subjects were seated in the isokinetic dynamometer (Figure 2.1) and were prepared for the experiment as described in chapter 2. The experimental protocol is shown in figure 3.1. Prior to the start of the 13 minute protocol, participants inhaled either air or a normoxic, hypercapnic gas mix (5% CO₂ in air; BOC gases, Surrey, UK) for 5 minutes (depending on the trial). This was done in order to establish a steady state in the ventilatory response to the hypercapnic gas mix before any further intervention. Pilot testing demonstrated that 5 minutes was a long enough period to achieve this. Participants inhaled the 5% CO₂ gas mix from a Douglas bag connected to the inspiratory side of a breathing valve and mouthpiece via a 2 metre length of corrugated tubing. Participants inhaled room air

through the corrugated tubing which was attached to a filled Douglas bag, but with a tap turned to room air.

Following this 5 minute preparation period, data gathering commenced (time zero in figure 3.1). Baseline measurements were taken for 2 minutes while the participants continued to rest. At 1: 55 minutes of this period a cuff around the participant's right thigh was inflated to 200 mmHg to achieve circulatory occlusion. The 4 trials which then followed were:

- 1) Control trial (CON), where after a further 5 minutes in the protocol, (minute 7 in figure 3.1) passive stretch of the right calf was applied for 3 minutes.
- 2) Exercise trial (EX), during which participants performed 90 seconds of isometric plantarflexion exercise (50% MVC) with their right leg (between 2 min and 3 min 30 s in figure 3.1). Passive stretch during PECO commenced at minute 7 of the protocol.
- 3) CO₂ trial (CO₂), where participants inhaled 5% CO₂ in air prior to commencement of the protocol which was the same as trial 1. This showed responses to passive stretch in hypercapnia.
- 4) CO₂+Exercise (CO₂+EX), where participants inhaled 5% CO₂ in air prior to commencement of the protocol which was the same as trial 2, during which the passive stretch was performed in PECO and with concurrent hypercapnia.

In all 4 trials the cuff was then deflated at 11 minutes into the protocol followed by a 2 minute recovery period. All participants were asked to breathe normally and not to perform any abnormal respiratory manoeuvres throughout the protocol. Participants were also asked to notify the experimenter if at any point they felt pain from the circulatory occlusion and muscle stretch so that the trial would be stopped.

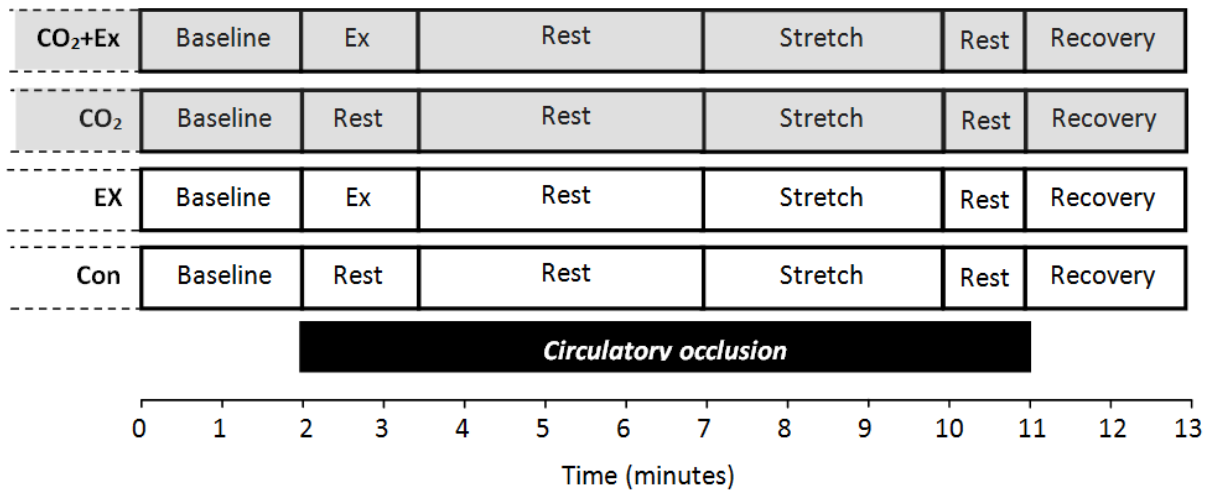


Figure 3.1 Schematic diagram of the protocol. Ex signifies the period of isometric plantaflexion exercise. The period of circulatory occlusion in the 4 trials is shown by the black bar. All participants rested inhaling either air or the 5% CO₂ in air gas mixture in the 5 minutes immediately prior to the start of the above protocol.

Data analysis

The respiratory and cardiovascular variables were recorded throughout the trial as described in chapter 2. Mean averages for minute ventilation (\dot{V}), heart rate (HR), mean arterial pressure (MAP) and $P_{ET}CO_2$ during each period of the trials were calculated. For both cardiovascular and respiratory data, these averages excluded the first 30 seconds after exercise, during which a new post-exercise steady state was established. In addition, for the respiratory data the final recovery value was calculated over the last 30 seconds of the 2 minute recovery period, to allow adequate time for metabolite washout and the associated hyperpnoea to dissipate.

A repeated measures two-way ANOVA, and where appropriate multiple comparison *post hoc* analysis, was used to examine differences in \dot{V} , HR, MAP and $P_{ET}CO_2$ between the four trials and between the six periods within each trial. A repeated measures one-way ANOVA and a students paired *t* test (two-tailed) was used to examine differences between the range of dorsiflexion about the ankle joint during passive stretch and the MVCs achieved between trials respectively. Data are expressed as mean \pm SEM and statistical significance was taken as ($P < 0.05$). Statistical analysis was conducted using a standard statistical package (version 19.0 SPSS, Chicago, IL, USA).

3.3 Results

Contraction and passive stretch torques

The MVCs measured prior to each exercise trial were not significantly different ($P>0.05$) from each other with values of 95.4 ± 8.2 and 99 ± 7.8 Nm being recorded before the CO₂+EX and EX trials respectively. The range of motion about the ankle joint during the passive stretch was not significantly different ($P>0.05$) between trials with values of $32.7 \pm 1.4^\circ$, $33.5^\circ \pm 1.7$, $32.4 \pm 1.4^\circ$ and $32.5 \pm 1.6^\circ$ of dorsiflexion from vertical in the CON, EX, CO₂ and CO₂+EX trials, respectively. There were also no significant differences ($P>0.05$) between the peak torques generated during the passive stretch in each of these trial with values of 38.8 ± 1.4 , 38.1 ± 2.4 , 39.8 ± 2.3 and 39.8 ± 3 Nm respectively, then throughout the stretch period the torque decreased to 64.3%, 65.8%, 67.2% and 67.9% respectively of the peak torque generated in the four trials.

Differences between trials at baseline

The mean \dot{V} , HR and MAP during baseline are presented in Table 3.1. There were no significant differences in any of these variables between the CON and EX trials and also between the CO₂ and CO₂+EX trials. Breathing the normoxic hypercapnic gas mix significantly ($P<0.05$) increased \dot{V} from CON by $+27.67 \pm 1.74$ and $+25.16 \pm 1.89$ l.min⁻¹ in the CO₂ and CO₂+EX trials respectively. MAP was also significantly higher during baseline in the CO₂ trial ($+13 \pm 3.41$ mmHg) and CO₂+EX trial ($+10 \pm 2.66$ mmHg) relative to the CON trial. However HR was not significantly different to the values recorded in the CON trial. The end-tidal partial pressure of CO₂ ($P_{ET}CO_2$) was significantly elevated from control values in the CO₂ trial ($+7 \pm 0.85$ mmHg) and in the CO₂+EX trial ($+8 \pm 0.62$ mmHg; $P<0.05$).

Trial	\dot{V} (l.min ⁻¹)	HR (beats.min ⁻¹)	MAP (mmHg)	$P_{ET}CO_2$ (mmHg)
CON	9.78 ± 0.86	62 ± 2.3	88 ± 2.6	40 ± 0.6
EX	8.97 ± 0.64	63 ± 1.8	89 ± 3.2	41 ± 0.5
CO ₂	$37.44 \pm 1.71^*$	68 ± 2	$101 \pm 3.3^*$	$47 \pm 1^*$
CO ₂ +EX	$34.94 \pm 1.78^*$	69 ± 2.7	$98 \pm 3.3^*$	$48 \pm 0.6^*$

Table 3.1 Mean \dot{V} , HR, MAP and $P_{ET}CO_2$ values recorded during the two minute baseline period in the CON, EX, CO₂, CO₂+EX trials. * Significant difference from CON trial

Respiratory responses

Figure 3.2.1 and 3.2.2 shows changes in mean \dot{V} relative to baseline during each of the four trials. Figure 3.2.1 shows that exercise significantly increased \dot{V} by $+5.74 \pm 0.82$ l.min⁻¹ ($P<0.05$) in room air (EX trial) and it returned to baseline values during circulatory occlusion after exercise. When breathing the hypercapnic gas mixture (CO₂+EX trial) exercise increased \dot{V} by $+6.45 \pm 0.75$ l.min⁻¹ (not different from EX trial, $P<0.05$) but in this case during PECO it remained elevated close to the exercise level ($+7.12 \pm 1.13$, $P<0.05$).

In the CO₂+EX trial the further small increase in \dot{V} during stretch did not reach statistical significant ($P=0.245$). In contrast, figure 3.2.2 shows that without prior exercise (CO₂ trial) stretch caused a significant increase in \dot{V} relative to PECO values ($+2.5 \pm 0.62$ l.min⁻¹; $P<0.05$). \dot{V} was maintained at stretch levels during occlusion phase after stretch in the CO₂ and CO₂+EX trials remaining significantly elevated above baseline ($+3.72 \pm 1.36$ and $+7.65 \pm 1.53$ l.min⁻¹ versus baseline respectively; $P<0.05$). \dot{V} then declined in both of these trials during recovery to levels similar to baseline ($P>0.05$). During the CON trial, \dot{V} was not significantly altered from baseline during any of the experimental periods.

Figure 3.3 shows the mean $P_{ET}CO_2$ values during the different periods of the 4 trial conditions. During the exercise and circulatory occlusion periods prior to stretch the $P_{ET}CO_2$ was not significantly changed from baseline. However, in the CO₂+EX trial there were significant decreases in $P_{ET}CO_2$ from baseline during the stretch and subsequent circulatory occlusion periods ($P<0.05$) with a return to baseline levels during the recovery period ($P<0.05$). In the CO₂ trial there was a significant fall in $P_{ET}CO_2$ during the circulatory occlusion period after stretch. There were no significant changes in $P_{ET}CO_2$ from baseline in any of the periods of the 2 normocapnic trials.

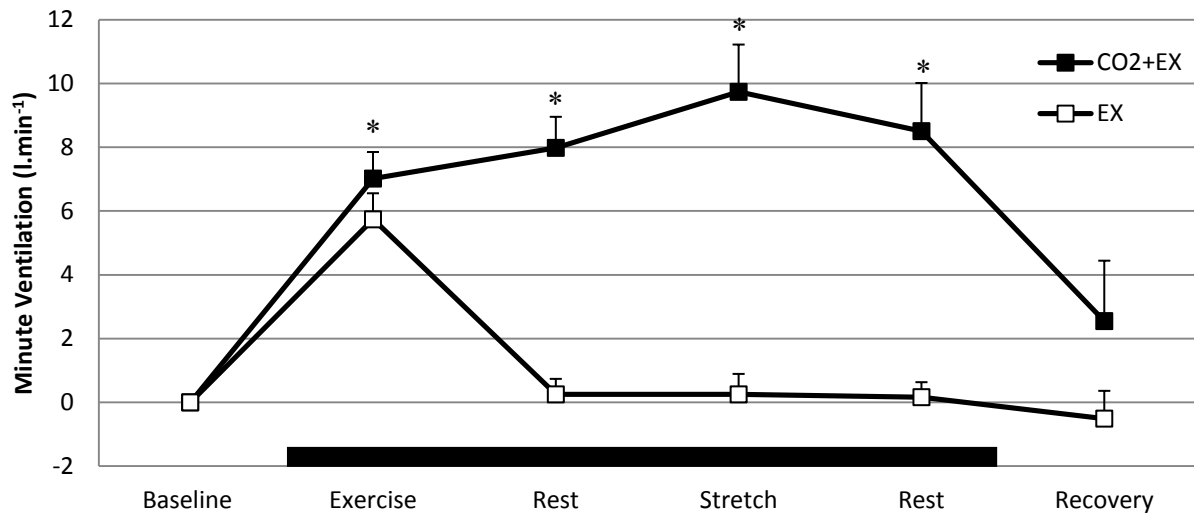


Figure 3.2.1 Change in \dot{V} from baseline during each phase of the CO₂+EX, and EX trials. The black bar shows the periods performed under circulatory occlusion. * Significant difference from Baseline value ($P<0.05$).

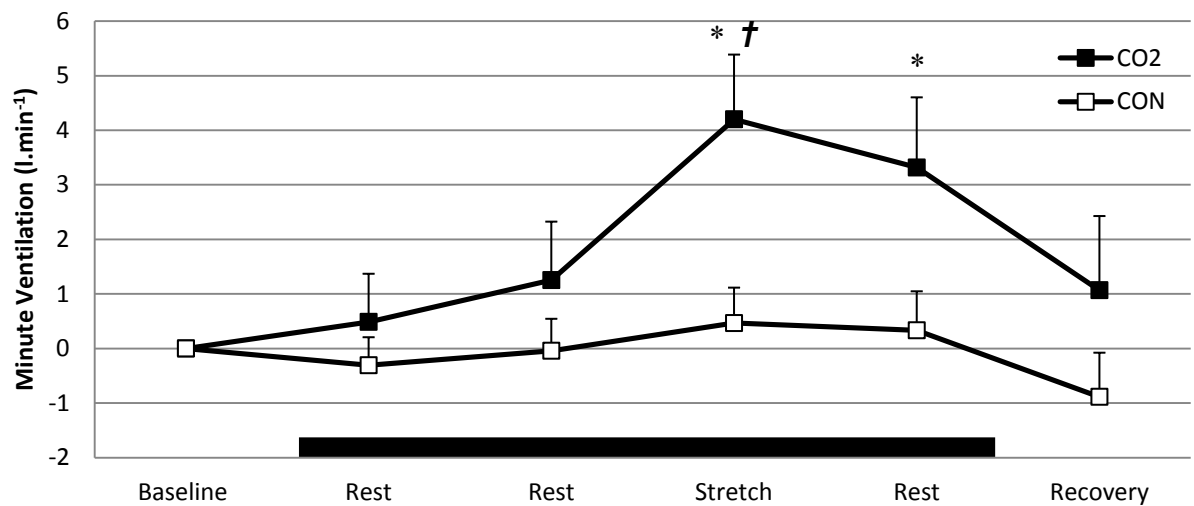


Figure 3.2.2 Change in \dot{V} from baseline during each phase of the CO₂, and CON trials. The black bar shows the periods performed under circulatory occlusion. * Significant difference from Baseline value ($P<0.05$). † Significant difference between the Stretch period and the previous Rest period ($P<0.05$).

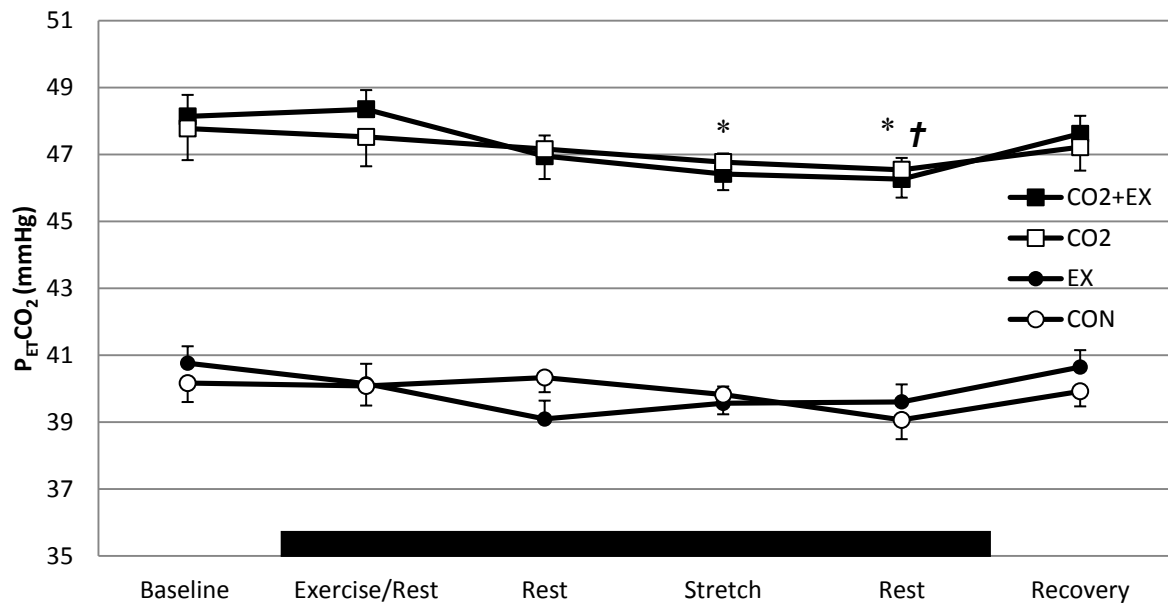


Figure 3.3 $P_{ET}CO_2$ during each phase of the CO_2+EX , CO_2 , EX and CON trials. The black bar shows the periods performed under circulatory occlusion. * Significant difference from Baseline value in $CO_2 + EX$ ($P < 0.05$). † Significant difference from Baseline value in $CO_2 + EX$ and in CO_2 trials ($P < 0.05$).

Cardiovascular responses

Changes in mean HR and MAP, relative to baseline, for each period of the 4 trials are shown in figures 3.4 and 3.6 respectively. Both MAP and HR increased during exercise in both the CO₂+EX trial (12 ± 1.5 mmHg and 14 ± 2.61 beats.min⁻¹; $P < 0.05$ versus baseline) and the EX trial (10 ± 1.1 mmHg and 14 ± 2.6 ; $P < 0.05$ versus baseline). As expected on cessation of exercise MAP fell from exercise levels but still remained significantly elevated above baseline during the subsequent circulatory occlusion period ($P < 0.05$) with no significant difference between trials.

Stretch caused a further significant increase ($P < 0.05$) in MAP from the levels recorded immediately preceding it during circulatory occlusion in the CO₂+EX trial (4 ± 0.85 mmHg) the CO₂ trial (5 ± 1.05 mmHg) and CON trial (4 ± 0.8 mmHg) but not in the EX trial (4 ± 1.2 mmHg; $P = 0.057$). Stretch did not cause a significant overall increase in mean heart rate from preceding circulatory occlusion levels in any trial. However, stretch did cause a significant increase in HR in the first 15 seconds of the stretch period in all 4 trials, with increases ranging 5-6 beats.min⁻¹ (Figure. 3.5; $P < 0.05$). After the initial 15 seconds HR decreased to levels similar to the preceding circulatory occlusion period.

During the recovery periods of each trial MAP and HR values were not significantly different from baseline ($P > 0.05$).

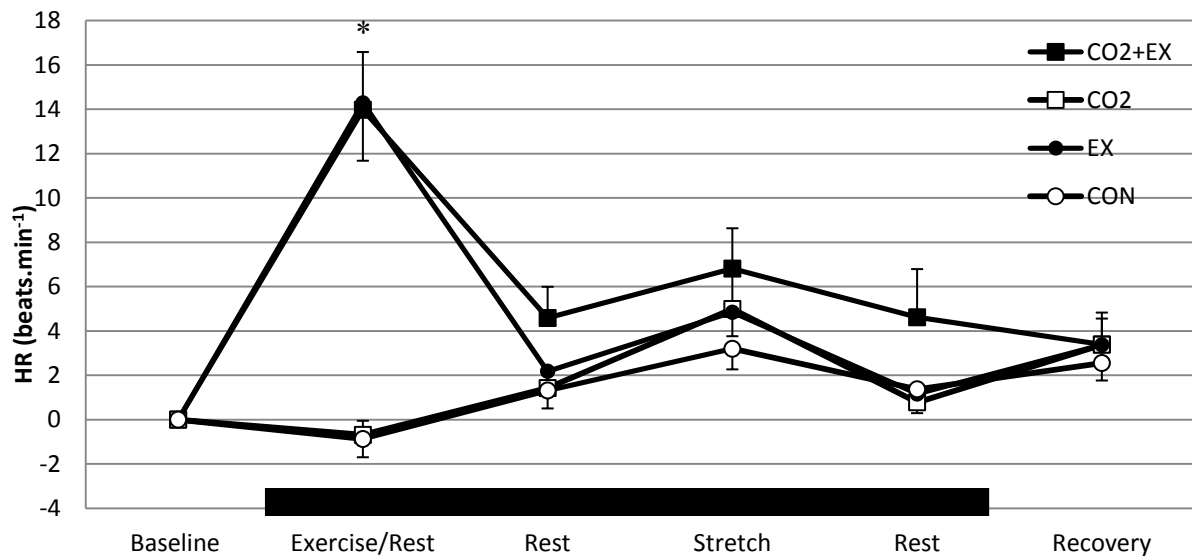


Figure 3.4 Change in HR from baseline during each phase of the CO₂+EX, CO₂, EX and CON trials. The black bar shows the periods performed under circulatory occlusion. * Significant difference from Baseline value ($P < 0.05$).

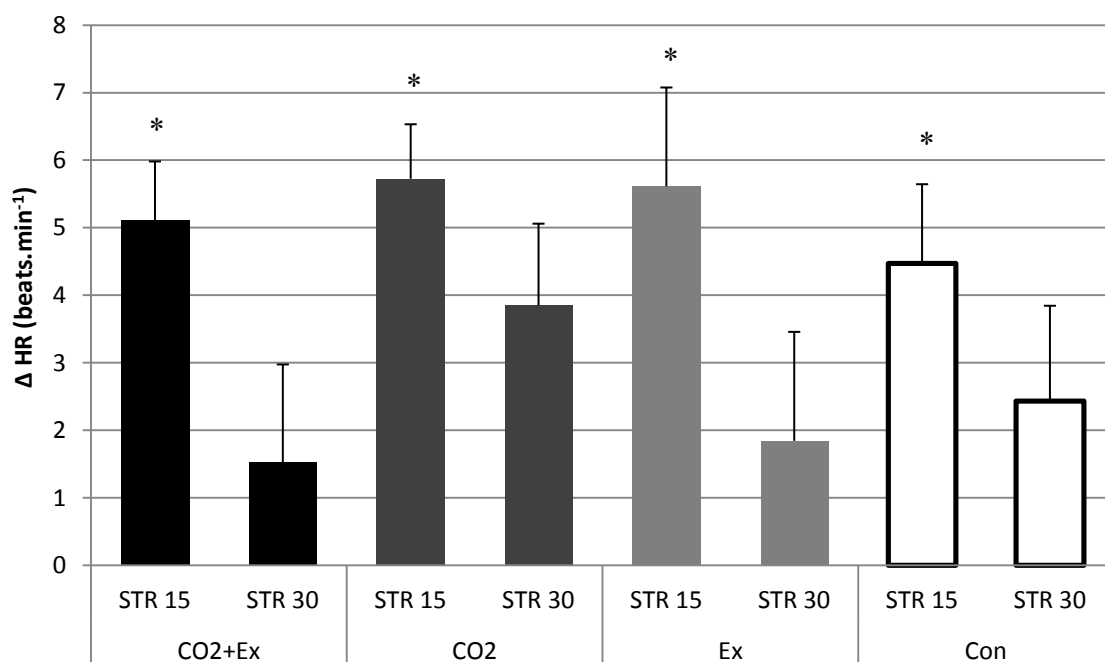


Figure 3.5 Change in HR from levels in the previous period during the first 15 seconds of Stretch (STR 15) and the second 15 seconds of Stretch (STR 30) in the CO₂+EX, CO₂, EX and CON trials. * Significant difference from HR levels in the previous Rest period ($P < 0.05$).

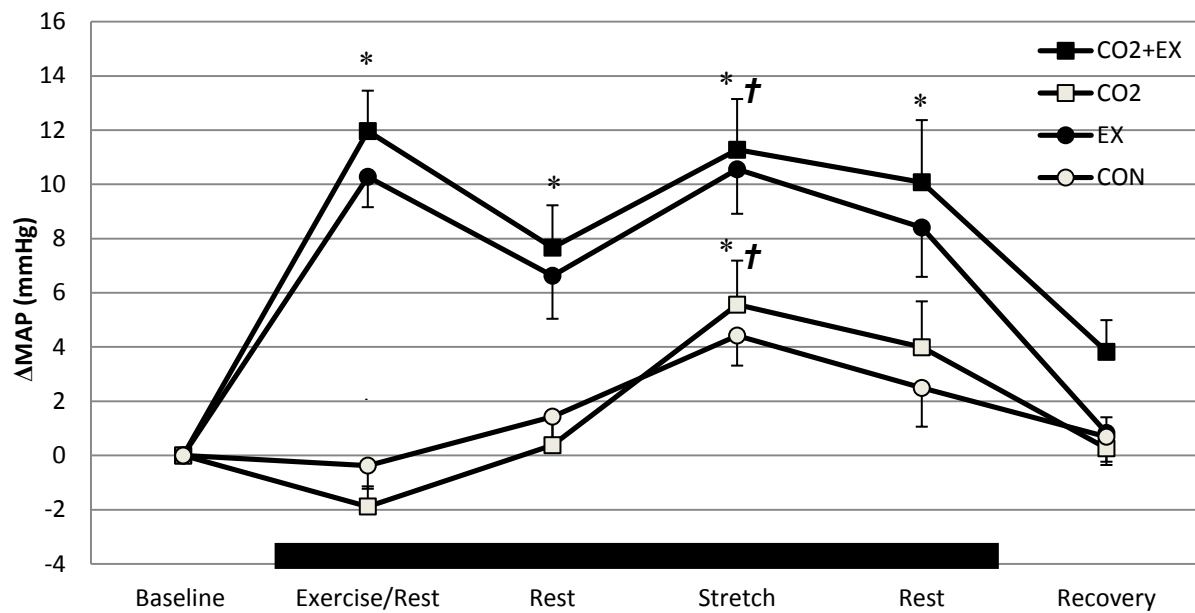


Figure 3.6 Change in MAP from baseline during each phase of the CO₂+EX, CO₂, EX and CON trials. The black bar shows the periods performed under circulatory occlusion. * Significant difference from Baseline value ($P<0.05$). † Significant difference between the Stretch period and the previous Rest period ($P<0.05$).

3.4 Discussion

This investigation examined the respiratory and cardiovascular responses of humans to activation of muscle afferents during concurrent inhalation of a normoxic hypercapnic gas mixture. The main findings are that during conditions of hypercapnia, activation of the muscle metaboreflex using PECO, maintained ventilation at levels recorded during exercise. Furthermore, stimulation of muscle mechanoreceptors by passive muscle stretch during hypercapnia resulted in further increases in ventilation.

Cardiovascular responses to PECO and stretch are reliable and sensitive indicators of muscle afferent activation (Coote *et al.* 1971; McCloskey & Mitchell 1972; Kaufman *et al.* 1983; Fisher *et al.* 2005; Drew *et al.* 2008). Therefore the similar heart rate and blood pressure increases observed during these periods of the normocapnic and hypercapnic trials (Figures 3.4 – 3.6) suggests that muscle afferent activation was well controlled and matched between trials. In addition, there were no significant differences in the exercise intensities performed by the participants and similar stretch torques were applied to the calf muscles in each trial. Therefore, the ventilatory responses observed during the muscle metaboreflex activation (PECO) and mechanoreflex activation (stretch) periods of the hypercapnic trials (Fig 3.2.1 and 3.2.2) is unlikely to be due to increased muscle afferent activation (i.e. from enhanced muscle acidosis). This suggests that a central synergistic interaction between muscle afferent feedback and the hypercapnia induced chemoreflex is more likely. However whether a possible interaction in the peripheral exercised muscle contributes to the observed ventilatory responses requires more direct examination (chapter 4).

It is clear that inhalation of the normoxic hypercapnic gas mixture activated the ventilatory chemoreflex because baseline minute ventilation increased almost 4 fold compared to

control levels. It is also clear that the two experimental procedures, of PECO and passive muscle stretch did indeed stimulate metaboreceptive and mechanoreceptive afferents within the skeletal muscle. Though this study did not directly measure the activity of group III and IV muscle afferents the cardiovascular responses that were observed are well established indicators of their activation. Blood pressure was maintained above baseline (~ 7 mmHg) during PECO whilst HR returned to baseline values in both of the trials involving exercise. These findings are consistent with muscle metaboreflex activation and sustained sympathoexcitation during PECO (Alam & Smirk, 1937; Seals *et al.* 1988; Bull *et al.* 1989; Bell & White, 2005; Fisher *et al.* 2005). That passive stretch produced significant increases in HR in all four trials, is again consistent with previous reports in humans showing that it does stimulate muscle mechanoreceptors, and initiate tachycardia (Gladwell & Coote 2002; Gladwell *et al.* 2005; Drew *et al.* 2008). The initial increase in HR was shown to decline after 15 seconds towards pre-stretch levels. This is likely due to two factors; 1) tendon creep, where there is a progressive decline in tension within the muscle during the stretch and thus a lessening of the stimulus to the mechanoreceptors (in this study stretch torques declined by $\sim 35\%$); and 2) adaptation of mechanoreceptive afferents to the applied stimulus (Kaufman *et al.* 1983; Kaufman & Rybicki, 1987).

The stretch also produced 4-5 mmHg increases in BP from the pre-stretch levels in all four trials. Unlike the HR response to stretch, the BP response was sustained, implying that the stretch stimulated a population of mechanoreceptive afferents which can increase sympathetic vasomotor activity. Again this finding is consistent with other reports in the literature in human (Fisher *et al.* 2005; Drew *et al.* 2008) and in animal studies (Stebbins *et al.* 1988, Potts & Mitchell 1998) which demonstrated a progressive increase in BP during passive muscle stretch.

As expected from many previous studies (Rowell *et al.* 1976, Innes *et al.* 1989, Scott *et al.* 2000, Haouzi *et al.* 2001, Fukuba *et al.* 2007) this study found that when breathing air, ventilation was not maintained at the exercising levels during PECO, rapidly returning to baseline levels when exercise ceased. This suggests that when activated alone, the muscle metaboreflex appears unable to evoke a ventilatory response, in healthy individuals. However, the current study also showed that during PECO with concurrent hypercapnia, ventilation was significantly maintained above baseline, only returning to resting levels upon restoration of circulation to the exercised muscle. This consolidates earlier work from our laboratory showing that in the presence of a sensitising level of CO₂, muscle metaboreflex activation is able to stimulate breathing (Lykidis *et al.* 2010). In this earlier study minute ventilation was maintained at the exercising level throughout 2 minutes of post handgrip exercise circulatory occlusion with concurrent hypercapnia. The present study extends these findings by demonstrating that ventilation is maintained at exercising levels during a much longer period of PECO and following exercise of a larger muscle mass in the lower limb. This provides strong evidence that ventilation can be driven by metaboreflex activation in at least two human muscle groups.

It was also demonstrated that passive stretch of the calf muscle did not produce any change in ventilation while participants were breathing air. In contrast, during hypercapnia the current study has shown for the first time in man, that stretch alone produces a further increase in ventilation. This effect was not dependent on co-activation of the muscle metaboreflex because in the CO₂ trial, no prior exercise was performed to activate it, yet ventilation increased with stretch. Furthermore this observation suggests that a sensitising level of exercise generated metabolites is not necessary for the mechanoreflex to become an effective driver of ventilation. However, combined muscle mechanoreflex activation with a sensitising level of inhaled CO₂ is required to elicit a ventilatory response. One

possible reason for the significant ventilatory response in the CO₂ trial compared to the smaller non-significant increase in the CO₂+EX trial, is that when the stretch was applied in the CO₂+EX trial a small but significant reduction in $P_{ET}CO_2$ had occurred, probably reducing the central stimulus (see limitations).

It is possible that the ventilatory baroreflex had an effect on the respiratory responses observed during the trials with hypercapnia. Stewart *et al.* (2011) have shown that when blood pressure is changed acutely, using the modified Oxford technique, ventilation changes in an inverse relationship with blood pressure. Of particular relevance to the current study is their observation that under hypercapnic conditions this reflex was reset to higher blood pressures but importantly the sensitivity of the reflex remained unchanged. The implications of these new findings for my study are complex but in sum reveal that the effects of muscle afferent activation on ventilation may have been underestimated.

In the hypercapnia trials of the current study (with its associated baseline hypertension) the resetting of the ventilatory baroreflex with maintained sensitivity means that it should still be capable of suppressing a ventilatory response to an acute blood pressure increase in PECO. Given that the current study found equal increases in blood pressure from the respective baseline levels, during PECO and PECO plus stretch both in the air breathing and hypercapnic trials, but only observed a ventilatory response to afferent activation during hypercapnia, it suggests that the afferent input was more effective at elevating ventilation, against the opposing activity of the ventilatory baroreflex in the hypercapnic conditions. This is taken as further indirect evidence for the suggestion that inhalation of the hypercapnic gas mixture has a central synergistic effect with afferent feedback.

There is growing support for the idea that muscle afferent feedback can play a role in exercise hyperpnea. The impressive recent work by Amman *et al.* (2010) where partial blocking of group III and IV afferent input into the spinal cord significantly attenuated the

ventilatory response to dynamic exercise has been particularly influential. Reductions in ventilation were observed even during mild exercise, where there would likely be little muscle metaboreflex activation but some muscle mechanoreflex activation in addition to central command. Likewise the current investigation supports the idea that the stimulation of both muscle mechano and metabosensitive afferents can generate a hyperpnea.

Limitations

Unlike in the trials by conducted by Lykidis *et al.* where $P_{ET}CO_2$ was clamped at 47 mmHg, one limitation with the methodology employed in the current study is that $P_{ET}CO_2$ was not maintained at a constant level throughout the CO_2 and CO_2+Ex trials. Indeed it fell significantly during the periods of muscle stretch due to the resultant hyperventilation. Therefore ventilation would be suppressed during these periods due to reduced afferent feedback from the central/peripheral chemoreceptors. As such the effects of the passive stretch may have an even greater effect on ventilation than those recorded here.

A further issue within the CO_2 and CO_2+EX trials is the slight drifting increase in ventilation that occurred throughout the protocol. It is well known that after the initial increase in ventilation up to a new baseline upon exposure to hypercapnia, ventilation gradually drifts upwards and only reaches a true steady state after 2-3 hours of sustained exposure (Reynolds *et al.* 1972; Tansley *et al.* 1998). In addition it is conceivable that ventilation may have also drifted upwards due to the stimulation of metaboreceptive muscle afferents from any slight and gradual metabolite accumulation in the resting ischemic leg. Therefore ventilation may have slightly drifted upwards throughout both trials involving hypercapnia in the current study. Indeed in the CO_2 trial ventilation had progressively increased to $\sim 1 \text{ l.min}^{-1}$ above baseline in the period immediately prior to

stretching, even though no exercise was performed. As muscle stretching resulted in small increases in ventilation (compared with PECO) any drift makes it more difficult to determine the true contribution of the muscle mechanoreflex alone to the increase in ventilation. However because there was a significant increase in ventilation from this new level (or new baseline) immediately prior to the stretch, and there was also a decrease in ventilation post-stretch, this does imply that activation of the muscle mechanoreflex did enhance ventilation in the CO₂ trial.

Conclusion

In summary the current study has shown that under hypercapnic conditions, stimulation of muscle metaboreceptive and mechanoreceptive afferents stimulates ventilation. Although indirect evidence was produced which suggested that this interaction likely takes place through a central mechanism, further work is required to determine the nature of this interaction.

**CHAPTER 4: MUSCLE
METABOREFLEX CAUSES A
VENTILATORY RESPONSE
DURING CONCURRENT
HYPERCAPNIA IN HUMANS: A
CENTRAL INTERACTION?**

4.1 Introduction

The role of muscle afferent feedback in ventilatory control is controversial as evidenced by classical finding that ventilation returns to resting level during the stimulation of the muscle metaboreceptive afferents via PECO (e.g. Rowell *et al.* 1976). Recently however Lykidis *et al.* (2010) and the findings made in chapter 3 (Bruce & White 2012) demonstrated ventilatory responses to the activation of muscle metaboreflex (PECO) and mechanoreflex (passive muscle stretch) but only during concurrent hyperpnea. It was thought that the contribution of the muscle metabo and mechanoreflex in driving ventilation may have been unmasked by the sensitising effects of the concurrent hypercapnia.

However, the nature of this interaction between muscle afferent feedback and the hypercapnia is still yet to be fully elucidated. It may be that afferent feedback can only be effective in driving ventilation in the presence of a potentially interactive/synergistic input to the central respiratory neuronal pool such as the hypercapnia-induced ventilatory chemoreflex. Alternatively, it is plausible that the acute hypercapnia may exacerbate the exercise-induced muscle acidosis and therefore augment the afferent feedback arising from the muscle. Indeed muscle acidosis does appears to be a factor in stimulating the exercise pressor reflex (Rotto & Kaufman, 1988; Rotto *et al.* 1989; Sinoway *et al.* 1992, 1993) and ventilatory response (Rotto *et al.* 1989) and so could explain the ventilatory responses observed in the studies conducted by Lykidis *et al.* and in chapter 3 (Bruce & White 2012).

Therefore the first aim of this study is to examine whether the site of interaction between muscle afferent feedback and hypercapnia occurs centrally or at the level of the peripheral muscle. This will be achieved by measuring the ventilatory responses to PECO during either systemic hypercapnia or during a local hypercapnia (where hypercapnic blood is

trapped in the exercised muscle only). It is hypothesised that only during systemic hypercapnia activation of the muscle metaboreflex would maintain ventilation at exercise levels, (suggesting a central interaction between muscle afferent feedback and afferent feedback from central and/or peripheral chemoreceptors).

Should this hypothesis be accepted, this study also aims to determine if the interaction occurs through hypercapnia-induced stimulation of peripheral and/or central chemoreceptors. This will be achieved by measuring the ventilatory responses to PECO during hyperoxic hypercapnia, which is considered to be an almost pure central chemoreceptor stimulus in humans (Miller *et al.* 1974, Cunningham *et al.* 1986). It is hypothesised that the ventilatory response to PECO would be unaffected by the hyperoxic hypercapnia suggesting that the central chemoreceptors are the primary driver of this interaction.

4.2 Methods

Participants

10 healthy male participants (21.1 ± 1.4 years old, 78 ± 5.7 kg, 179 ± 4 cm; mean \pm SD) from the University of Birmingham student population volunteered to take part in this study. All participants received written and verbal information regarding the experimental procedures prior to giving informed written consent. All subjects were habituated to all the experimental procedures, which conform to the Declaration of Helsinki and approved by the ethics committee at the University of Birmingham.

Following habituation to the procedures, participants visited the laboratory on 2 occasions for the 2 parts of the study (study 1 and 2). Before each day participants refrained from consuming food and caffeine in the 4 hours before the trial and from performing strenuous physical activity or consuming alcohol in the 12 hours before the trial. This study followed a within-subject design with all subjects participating in all trials. The order of each trial in the study 1 was randomised with a 30 minutes rest period in between.

Study 1: Experimental procedures

Subjects were seated in the isokinetic dynamometer (figure 2.1) and were prepared for experimentation as described in chapter 2. The protocol of the trials are shown in figure 4.1. Both Trials began with a 2-minute rest period. Prior to the start of the experimental portion of the trials (12-22 minutes) the following manoeuvres were performed in order to establish either a local hypercapnia in the right leg or a systemic hypercapnia in the body excluding the right leg (Local trial and Systemic trial respectively).

In the Systemic trial participants rested for a further 5 minutes while inhaling air prior to the inflation of a cuff around the participant's right thigh to 200mmHg by a rapid cuff inflator. Following the circulatory occlusion participants then inhaled a normoxic hypercapnic gas mixture (5% CO₂ in air; BOC gases, Guilford, UK) for 5 minutes and for the rest of the trial. This achieved a systemic hypercapnia (excluding the right leg). Participants inhaled the 5% CO₂ gas mixture for this 5 minutes prior to the start of the baseline recording period in order to establish a steady state in the ventilatory response to the hypercapnic gas mix. Pilot testing demonstrated that 5 minutes was a long enough period to achieve this. In the Local trial, the same procedure was followed but participants first inhaled the hypercapnic gas mix first for 5 minutes and then air (following thigh cuff inflation) for 5 minutes and for the rest of the trial. This achieved a local hypercapnia of the right leg.

Participants inhaled the 5% CO₂ gas mix from a Douglas bag connected to the inspiratory side of a breathing valve and mouthpiece via a 2 metre length of corrugated tubing. Participants inhaled room air through the corrugated tubing which was attached to a filled Douglas bag, but with a tap turned to room air.

Following these 12-minute manoeuvres achieving a systemic or local hypercapnia both trials then consisted of a 2-minute baseline recording period before the 1.5 minute isometric plantarflexion exercise with their right calf. Participants then underwent a 3.5 minute period of PECO before the thigh cuff was deflated and 3 minutes of recovery.

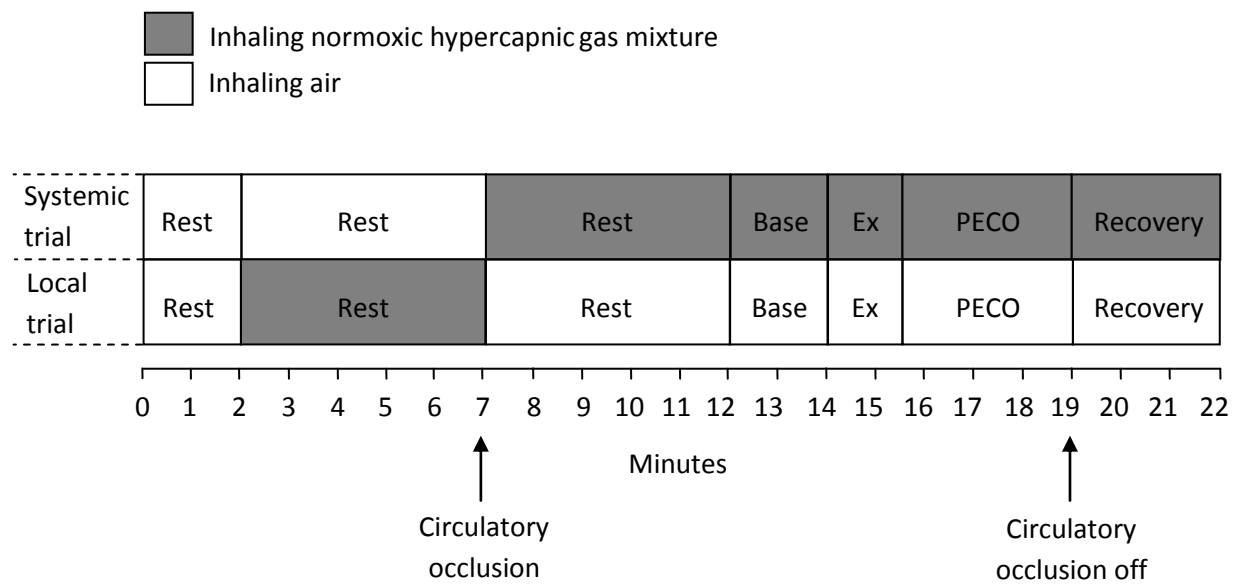


Figure 4.1 Schematic diagram of the protocol for study one. Ex signifies the period of isometric plantarflexion exercise. Base signifies the 2-minute baseline recording period.

Study 2: Experimental procedures

Subjects were seated in the isokinetic dynamometer in the same way as the first experiment. The protocol of this trial (Hyperoxia trial) is shown in figure 4.2. Prior to the start of the 13-minute protocol, participants inhaled a normoxic hypercapnic gas mix (5% CO₂ in air; BOC gases, Guilford, UK) for 5 minutes. Participants inhaled the gas mix from a Douglas bag connected to the inspiratory side of a breathing valve and mouthpiece via a 2 metre length of corrugated tubing. Following this 5-minute preparation period, data gathering commenced (time zero in figure 4.2). Baseline measurements were taken for 2 minutes while the participants continued to rest. At 1min 55seconds of this period a cuff around the participant's right thigh was inflated to 200 mmHg by a rapid cuff inflator to achieve circulatory occlusion. At 2 minutes into the trial participants performed 1.5 minutes of isometric plantarflexion exercise (50% MVC) with their right leg (between minutes 2 - 3.30 in figure 4.2). Participants then rested for 3.5 minutes at which point they were switched to inhale a hyperoxic hypercapnic gas mixture (95% O₂ and 5% CO₂; BOC gases, Guilford, UK) for 1 minute. This 1 minute time period was chosen to avoid the paradoxical phenomenon known as hyperoxic hyperventilation which arises 1-2 minutes after exposure to hyperoxia (see Dean et al. 2004 for a short review). Following this they were switched back to the normoxic hypercapnic gas mix which they inhaled until the end of the trial. At minute 10 of the trial the thigh cuff was deflated and participants recovered for 3 minutes.

All participants were asked to breathe normally and not to perform any abnormal respiratory manoeuvres throughout all trials in study 1 and 2. Participants were also asked to notify the experimenter if at any point they felt pain from the circulatory occlusion so that the trial would be stopped.

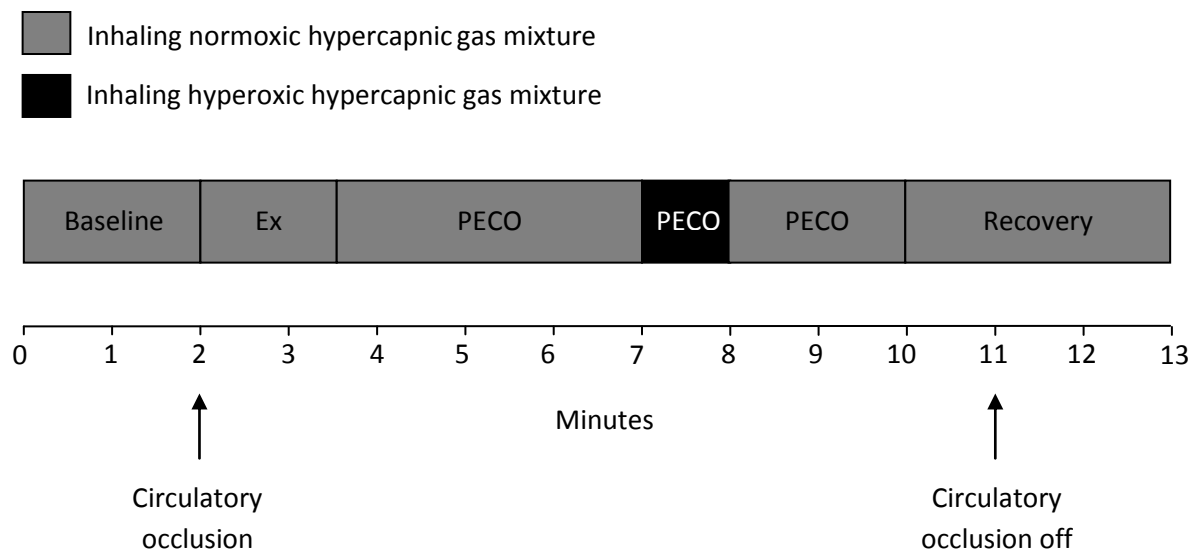


Figure 4.2 Schematic diagram of the protocol for study two (hyperoxia trial). Ex signifies the period of isometric plantarflexion exercise.

Data analysis

The respiratory and cardiovascular variables were recorded throughout the trial as described in chapter 2. Mean averages for minute ventilation (\dot{V}), heart rate (HR), mean arterial pressure (MAP) and $P_{ET}CO_2$ during each period of the trials were calculated. For all measurements, these averages excluded the first 30 of the PECO period so a new steady state post-exercise could be established and the recovery period is a mean of the final 1 minute to allow adequate time for metabolite washout post occlusion. A repeated measures two-way ANOVA, and then when appropriate multiple comparison *post hoc* analysis, was used to examine differences in \dot{V} , HR, MAP and $P_{ET}CO_2$ between the two trials of study 1, and between the periods within the experimental portions of the trials in study 1 and 2. A repeated measures one-way ANOVA was used to examine differences in \dot{V} , HR, MAP and $P_{ET}CO_2$ between the periods of study 2 and to also examine the differences in the MVCs and the baseline \dot{V} , HR, MAP and $P_{ET}CO_2$ between the Central, Local and Hyperoxia trials. Data are expressed as mean \pm SEM and statistical significance was taken as ($P < 0.05$). Statistical analysis was conducted using a standard statistical package (SPSS, version 19, Chicago, IL, USA).

Data are expressed as mean \pm SEM and statistical significance was taken as ($P < 0.05$). Statistical analysis was conducted using a standard statistical package (SPSS, version 19, Chicago, IL, USA).

4.3 Results

Maximal voluntary contraction torques

The MVCs measured prior to each trial of study 1 were not significantly different ($P>0.05$) from each other with mean values of 84.2 ± 4.2 and $86.1 \pm 2.9 \pm \text{Nm}$ recorded before the Systemic and Local trials respectively. Furthermore these MVCs were not significantly different from those recorded prior to the Hyperoxia trial, with a mean value of $82 \pm 4.6 \text{ Nm}$.

Differences between trials at baseline

The mean \dot{V} , HR, MAP and $P_{ET}CO_2$ during baseline are presented in Table 4.1. There were no significant differences in any of these variables between the systemic trial and Hyperoxia trial. Breathing the normoxic hypercapnic gas mixtures in the systemic trial and Hyperoxia trial significantly ($P<0.05$) increased baseline \dot{V} from the levels measured whilst breathing room air (local trial) by $+21.5 \pm 3.5 \text{ l.min}^{-1}$ and $+22.6 \pm 3.1 \text{ l.min}^{-1}$, respectively. Furthermore MAP was also significantly higher during baseline in both the systemic trial ($+10 \pm 4.3 \text{ mmHg}$) and Hyperoxia trial ($+7 \pm 3.6 \text{ mmHg}$) relative to the local trial. However HR was not significantly different to the values recorded in the local trial. The $P_{ET}CO_2$ was significantly elevated in the systemic trial ($+11 \pm 1.3 \text{ mmHg}$) and in the Hyperoxia trial ($+10 \pm 0.9 \text{ mmHg}$; $P<0.05$) compared with the values recorded in the local trial.

Trial	$\dot{V} (\text{l.min}^{-1})$	HR (beats.min ⁻¹)	MAP (mmHg)	$P_{ET}CO_2$ (mmHg)
Local	11.7 ± 1.4	71 ± 2.1	96 ± 3.4	39 ± 1.7
Systemic	$33.3 \pm 3.8^*$	74 ± 3.1	$106 \pm 5.2^*$	$50 \pm 1.2^*$
Hyperoxia	$34.3 \pm 2.7^*$	73 ± 3.7	$103 \pm 4.6^*$	$49 \pm 0.6^*$

Table 4.1 Mean \dot{V} , HR MAP and $P_{ET}CO_2$ values recorded during the 2 minute baseline period in the Local, Systemic and Hyperoxia trials. * Significant difference from Local trial ($P<0.05$).

Respiratory responses

Figure 4.3 shows changes in mean \dot{V} relative to baseline during the 2 trials of the study 1. Exercise significantly increased \dot{V} by $+3.9 \pm 1.1 \text{ l.min}^{-1}$ ($P < 0.05$) in room air (Local trial) and it returned to baseline values during PECO. When breathing the normoxic hypercapnic gas mixture (Systemic trial) exercise increased \dot{V}_I by $+4.5 \pm 0.8 \text{ l.min}^{-1}$ to a similar level as in Local trial but during PECO it remained elevated significantly above baseline ($+4.9 \pm 0.8 \text{ l.min}^{-1}$; $P < 0.05$). \dot{V} then declined in the systemic trial post cuff deflation during the recovery period to levels similar to baseline ($P > 0.05$).

Figure 4.4 shows the mean $P_{\text{ET}}\text{CO}_2$ values during the different periods of the Local and Systemic trials. Throughout the Systemic trial $P_{\text{ET}}\text{CO}_2$ remained significantly elevated above the values recorded in the Local Trial ($P < 0.05$), but within each trial $P_{\text{ET}}\text{CO}_2$ did not significantly alter from baseline levels ($P > 0.05$).

Figure 4.5 shows changes in mean \dot{V} relative to baseline during the Hyperoxia trial. Exercise resulted in a significant increase in \dot{V} from baseline ($+4.7 \pm 1.2 \text{ l.min}^{-1}$; $P < 0.05$) and this was not significantly different from the responses to exercise in either the Systemic or Local trial. \dot{V} remained significantly above baseline during PECO ($+4.3 \pm 1 \text{ l.min}^{-1}$; $P < 0.05$) and did not significantly change from this level whilst participants inhaled the hyperoxic hypercapnic gas mixture (PECO + hyperoxia period). \dot{V} then declined in the Hyperoxia trial post cuff deflation during the recovery period to levels similar to baseline ($P > 0.05$). Figure 4.6 shows the mean $P_{\text{ET}}\text{CO}_2$ values during the different periods of the 95% trial. Throughout the trial $P_{\text{ET}}\text{CO}_2$ did not significantly alter from baseline levels ($P > 0.05$).

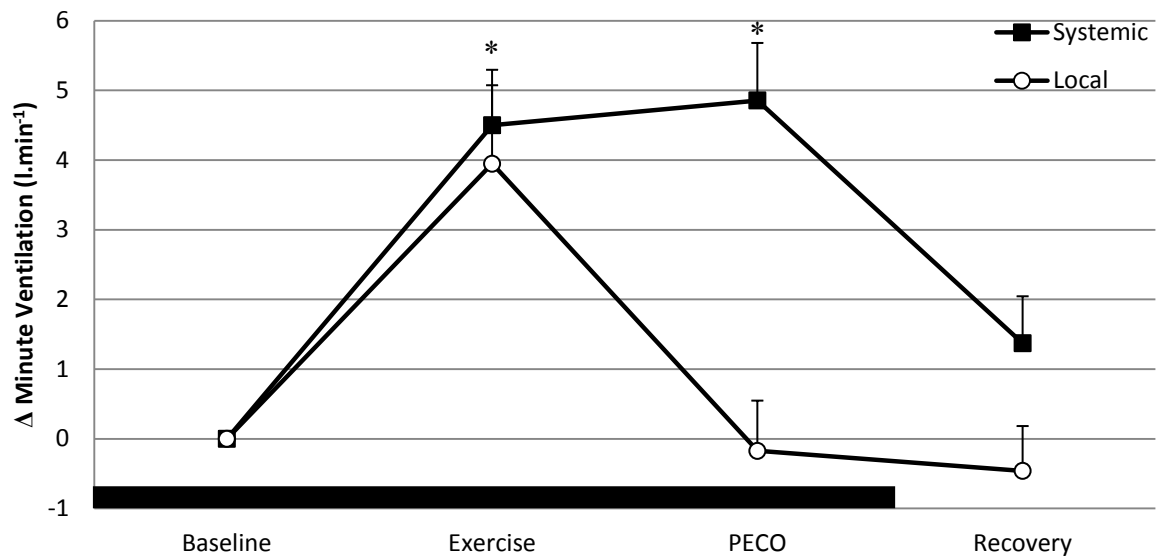


Figure 4.3 Changes in \dot{V} relative to baseline in the Systemic and Local trials. The black bar indicates the phases of the experimental protocol where circulation of the leg was occluded. * significant difference from baseline value ($P < 0.05$).

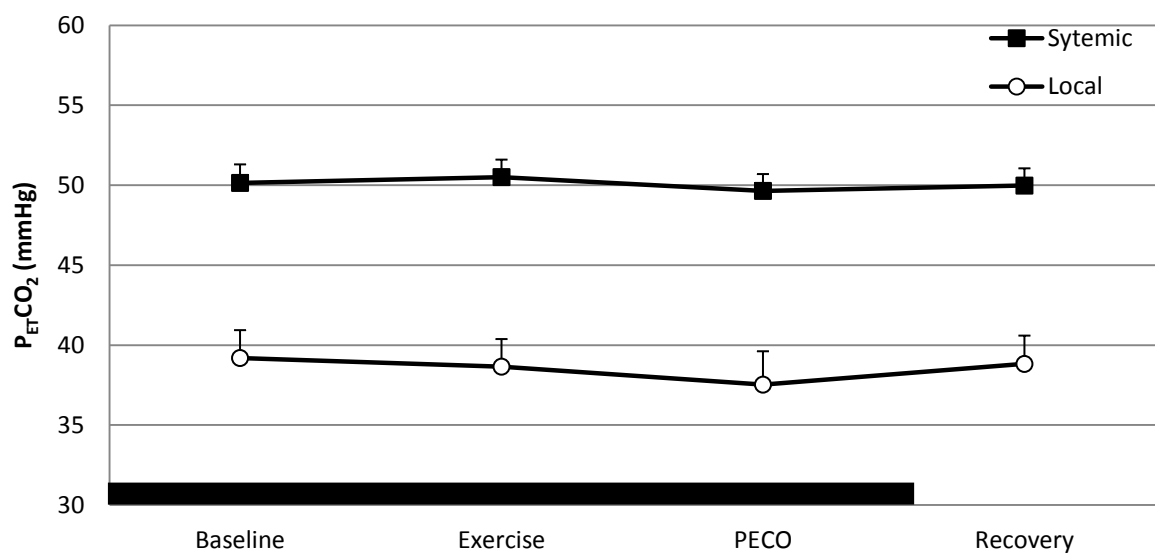


Figure 4.4 P_{ETCO_2} during the Systemic and Local trials. The black bar indicates the phases of the experimental protocol where circulation of the leg was occluded.

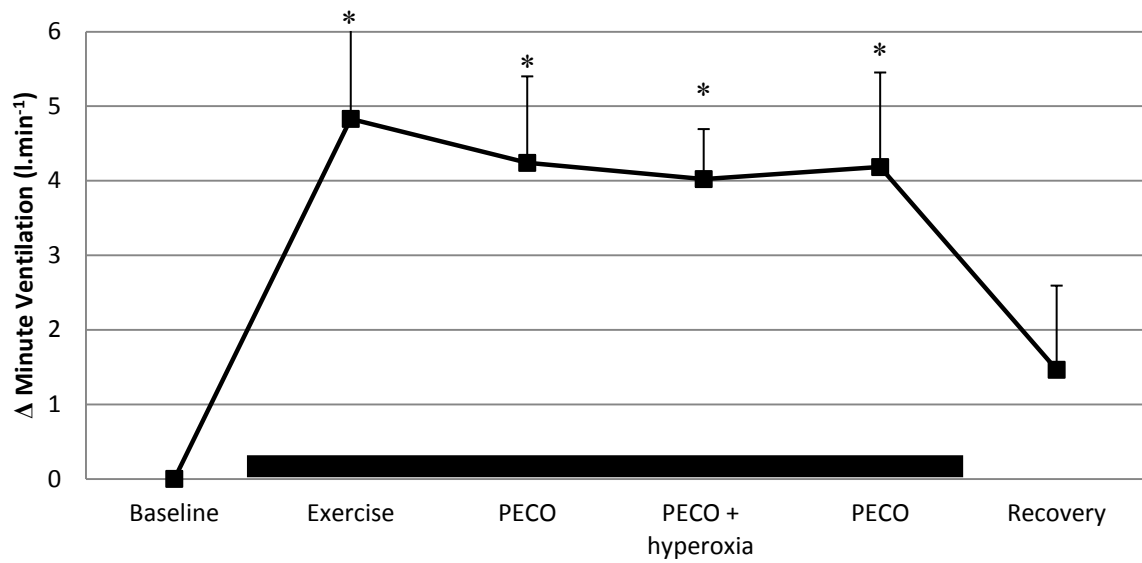


Figure 4.5 Changes in \dot{V} relative to baseline in the Hyperoxia trial. The black bar indicates the phases of the experimental protocol where circulation of the leg was occluded. * significant difference from baseline value ($P < 0.05$).

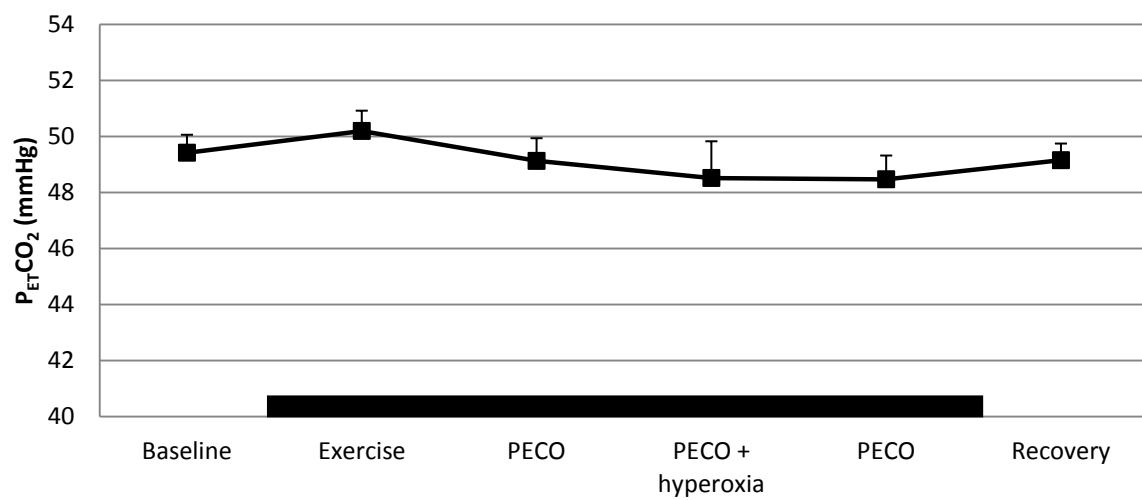


Figure 4.6. $P_{ET}CO_2$ during the Hyperoxia trial. The black bar indicates the phases of the experimental protocol where circulation of the leg was occluded.

Cardiovascular responses

Changes in mean MAP and HR relative to baseline, in the 2 trials of the study 1, are shown in figures 4.7 and 4.8, respectively. Both MAP and HR increased during exercise similarly in both the Local trial (15 ± 2 mmHg and 15 ± 3 beats.min⁻¹; $P < 0.05$ versus baseline) and in the Systemic trial (15 ± 4 mmHg and 16 ± 5 ; $P < 0.05$ versus baseline). As expected on cessation of exercise MAP fell from exercise levels but still remained significantly elevated above baseline during PECO ($P < 0.05$) with no significant difference between trials. During the recovery periods of each trial MAP and HR values were not significantly different from baseline ($P > 0.05$).

Changes in mean MAP and HR relative to baseline, in the Hyperoxia trial of study 2, are shown in figures 4.9 and 4.10 respectively. Both MAP and HR increased during exercise in the Hyperoxia trial (14 ± 3 mmHg and 15 ± 2 beats.min⁻¹; $P < 0.05$ versus baseline) and these values were not significantly different to those recorded in the Local and Systemic trials of study 1. Again on cessation of exercise MAP reduced from exercise levels but remained significantly elevated above baseline during PECO ($P < 0.05$). Furthermore MAP did not significantly alter from this level while participants inhaled the hyperoxic hypercapnic gas mix in PECO. During the recovery periods MAP and HR values were not significantly different from baseline ($P > 0.05$).

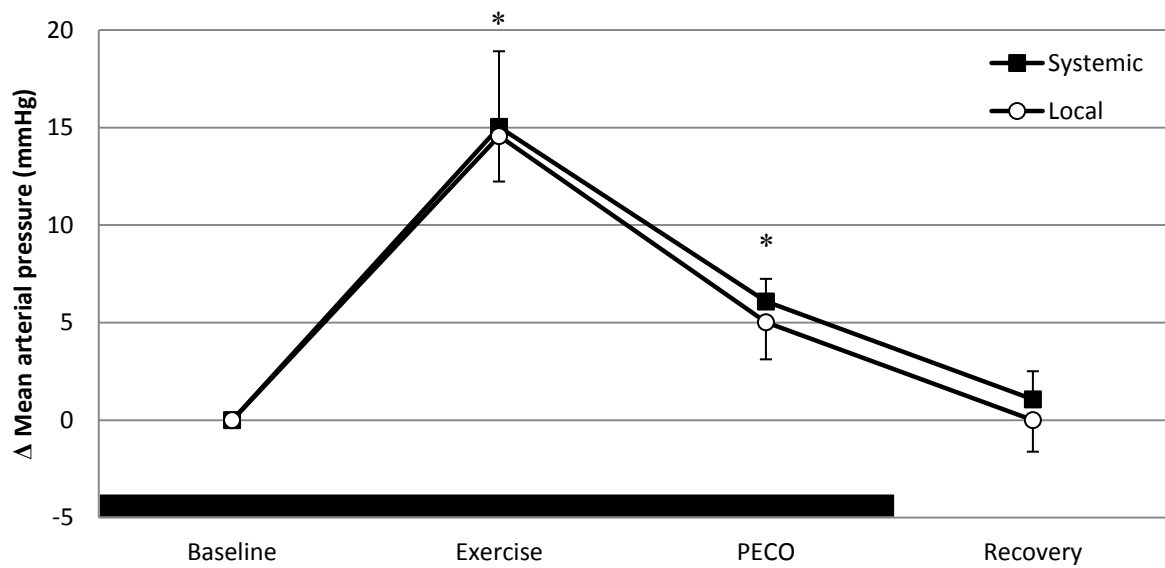


Figure 4.7 Changes in MAP relative to baseline in the Systemic and Local trials. The black bar indicates the phases of the experimental protocol where circulation of the leg was occluded. * significant difference from their respective baseline values ($P<0.05$).

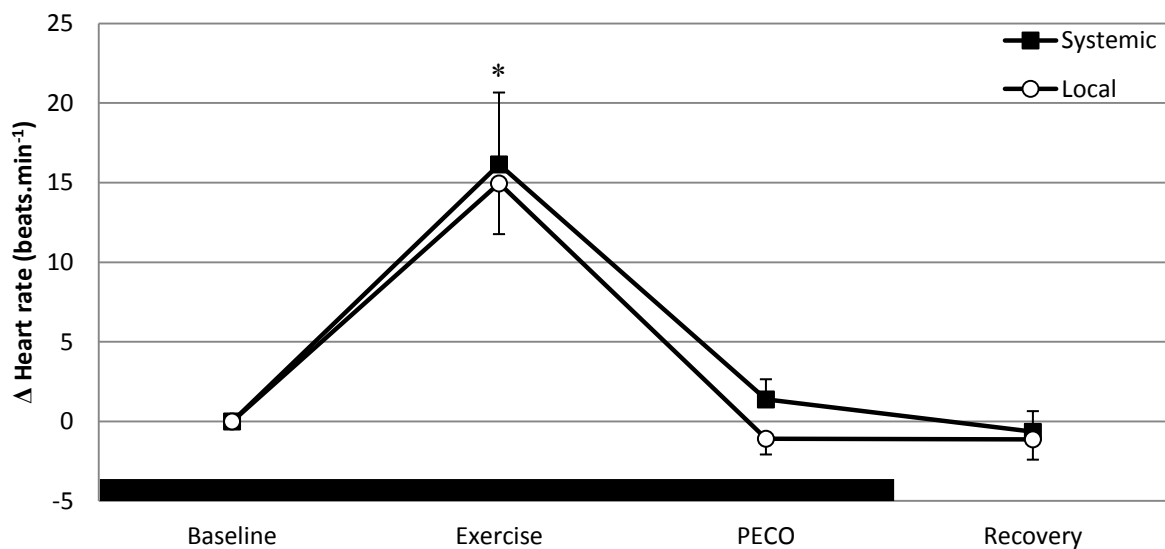


Figure 4.8 Changes in HR relative to baseline in the Systemic and Local trials. The black bar indicates the phases of the experimental protocol where circulation of the leg was occluded. * significant difference from their respective baseline values ($P<0.05$).

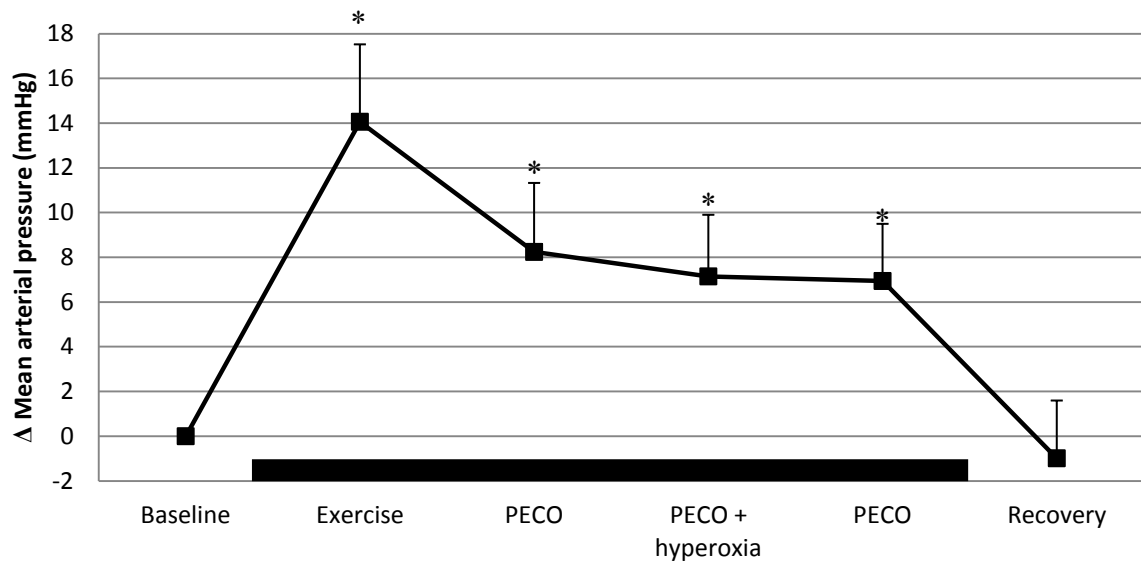


Figure 4.9 Changes in MAP relative to baseline in the Hyperoxia trial. The black bar indicates the phases of the experimental protocol where circulation of the leg was occluded.

* significant difference from baseline value ($P<0.05$).

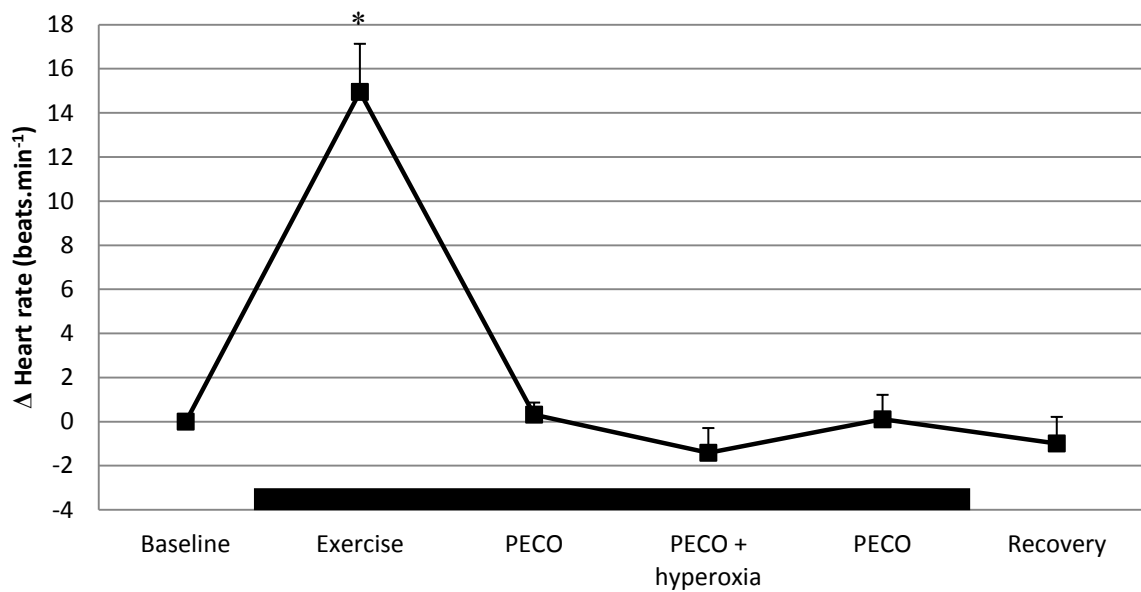


Figure 4.10 Changes in HR relative to baseline in the Hyperoxia trial. The black bar indicates the phases of the experimental protocol where circulation of the leg was occluded.

* significant difference from baseline value ($P<0.05$).

4.4 Discussion

This study investigated the ventilatory and cardiovascular responses of humans to the activation of the muscle metaboreflex during either a systemic or local (restricted to the exercised muscle) normoxic hypercapnia. The main finding from this experiment was that only during systemic hypercapnia did activation of the metaboreflex, using PECO, maintain ventilation at the levels recorded during exercise. This suggests a central interaction between skeletal muscle afferent feedback and the afferent feedback from central and/or peripheral chemoreceptors. Furthermore, this ventilatory response during PECO was not altered by the inhalation of a hyperoxic hypercapnic gas mixture suggesting that the central chemoreceptors are the primary driver of this interaction.

The cardiovascular responses to PECO are known to be a sensitive and reliable indicator of muscle afferent activation (Coote *et al.* 1971; McCloskey & Mitchell, 1972; Kaufman & Rybicki, 1987) and in all three trials blood pressure during PECO was maintained significantly above baseline (~6 mmHg), while heart rate returned to pre-exercise levels. This is consistent with muscle metaboreflex activation and sustained sympathoexcitation during PECO (Alam & Smirk, 1937; Seals *et al.* 1988; Bull *et al.* 1989; Bell & White. 2005; Fisher *et al.* 2005). Furthermore, the similar heart rate and blood pressure responses recorded in all three trials suggest that the level of muscle afferent activation was similar between each trial.

It has been consistently demonstrated that when breathing air ventilation rapidly returns to baseline levels during PECO (Rowell *et al.* 1976, Innes *et al.* 1989, Scott *et al.* 2000, Haouzi *et al.* 2001, Fukuba *et al.* 2007). These results suggest that activation of the muscle metaboreflex alone is unable to stimulate ventilatory responses. However, it has since been shown that metaboreflex activation can produce ventilatory responses during concurrent

hypercapnia (Lykidis *et al.* 2010, chapter 3). The current study has shown that this interaction is not due to the hypercapnia exacerbating the exercise induced muscle acidosis and therefore augmenting the afferent feedback arising from the muscle. This is evidenced by the finding that when the hypercapnic blood was restricted to the exercised muscle no ventilatory responses to PECO were observed.

Muscle metaboreflex activation through PECO was only able to stimulate ventilatory responses during systemic hypercapnia. These results suggest a central synergistic interaction between muscle afferent feedback and the afferent signals from central and/or peripheral chemoreceptors as the ventilatory responses were greater than the sum of the two individual mechanisms. Indeed it is well known that there are several converging signals that can stimulate respiratory neurones within the brainstem (see Daly, 1995 for review). In the present study (and the study in the previous chapter) three of these signals have been manipulated; 1) skeletal muscle afferent feedback through PECO 2) feedback from both central and peripheral chemoreceptors through the inhalation of the hypercapnic gas mix and 3) feedback from the ventilatory baroreflex. The observations made in this investigation could be explained by a synergistic interaction between the enhanced feedback from chemoreceptors during hypercapnia and skeletal muscle afferents. Therefore the breathing responses to muscle afferent stimulation (PECO) can only be observed under the hypercapnic conditions when the combined actions of muscle afferent feedback and chemoreflex overcome the opposing action of the ventilatory baroreflex. So in effect the chemoreflex has perhaps unmasked the involvement of muscle afferents in ventilatory control.

Indeed an interaction could well take place in NTS, a vital integrating site in the dorsal brainstem and key for autonomic regulation including that of respiration (Paton, 1999). Both peripheral chemoreceptor and skeletal muscle afferents are known to project there

(Kalia *et al.* 1981, Donoghue *et al.* 1984, Li *et al.* 1998, Potts *et al.* 1999, Paton *et al.* 2001), and it has further been established that the NTS may be one of several sights for central chemoreception (Nattie & Li 2002, 2009; Feldman 2003). Therefore, the ventilatory responses observed may be the result of the integration between chemoreceptor and muscle afferent feedback through the activation of NTS neurones.

The current study has also established that the central chemoreceptors appear to be the primary driver of this interaction. This is because the inhalation of a hyperoxic, hypercapnic gas mixture (an almost pure central chemoreceptor stimuli, Miller *et al.* 1974; Cunningham *et al.* 1986). had no effect on the ventilatory responses recorded during muscle metaboreflex activation. However, it must be conceded that there may be an element of redundancy and so peripheral chemoreceptors might still play a role in this interaction. However the findings do suggest the central chemoreceptors alone are at least adequate to stimulate the full response independently.

Implications

These results may have possible implications for the control of ventilation in exercise. Of course in normal exercise arterial blood gases are not altered, and in heavy exercise individuals may even become hypocapnic. However because a central interaction was observed in the current study it has become possible to speculate that synergistic interactions of a comparable nature occur with other medullary inputs active in exercise (e.g. central command, which like the chemoreflex can also increase central respiratory drive). This will be examined in chapter 5 where I will investigate whether augmented levels of muscle afferent feedback can increase the ventilatory responses during exercise and/or PECO. If augmented ventilatory responses are only observed in exercise then this

again implies that the stimulation of muscle afferents seem to be only able to generate ventilatory responses in the presence of other possibly interactive/synergistic inputs that are active in exercise. If augmented responses are observed in both exercise and PECO then this would imply that muscle afferent stimulation alone can produce ventilatory responses but it requires a more powerful activation of the muscle metaboreflex via PECO, perhaps to overcome the ventilatory baroreflex.

Conclusion

During systemic normoxic hypercapnia activation of the metaboreflex using PECO maintains ventilation at the levels recorded during exercise suggesting an interaction between skeletal muscle afferent feedback and the afferent feedback from central and/or peripheral chemoreceptors. Furthermore, this ventilatory response during PECO was not altered by the inhalation of a hyperoxic hypercapnic gas mixture suggesting that the central chemoreceptors are the primary driver of this interaction.

CHAPTER 5: MUSCLE

METABOREFLEX ACTIVATION

INCREASES VENTILATION

DURING EXERCISE BUT NOT

DURING PECO

5.1 Introduction

The control of the human exercise hyperpnea is still not fully understood, yet the feed-forward signals from higher brain centres known as central command, which concurrently activates motor and cardiorespiratory control areas of the brain, is widely considered to contribute. The most compelling evidence for this in humans comes from studies that manipulate the level of central command input required to perform a given exercise task and a corresponding alteration in ventilation is observed (Asmussen *et al.* 1965 Goodwin *et al.* 1972, Galbo *et al.* 1987, Innes *et al.* 1992). Furthermore several possible sites of the neurocircuitry involved in central command have been identified (Thornton *et al.* 2001, Green *et al.* 2007).

The role played by group III and IV skeletal muscle afferent fibres in controlling the exercise hyperpnea has also been examined. Recently it has been shown that the blockade of skeletal muscle afferent feedback, through the intrathecal injection of the μ -opiate agonist fentanyl into the L3-L4 vertebral space, significantly reduced the ventilatory and cardiovascular responses of humans to a dynamic cycling exercise. However when muscle afferents have been stimulated in isolation through the technique of PECO ventilation returns to baseline levels immediately upon the cessation of exercise (Rowell 1976, Innes *et al.* 1989, Scott *et al.* 2000, Haouzi *et al.* 2001, Fukuba *et al.* 2007).

It is possible that the effects of muscle afferent activation can only be observed during exercise where the neural feedback from active muscles is working in combination with other potentially synergistic inputs that increase central respiratory drive in exercise, e.g. central command. Indeed interactions between muscle afferent activation and the hypercapnia-induced ventilatory chemoreflex have been observed (Lykidis *et al.*, 2010;

Chapters 3 and 4), so it is possible that there are other neural interactions that occur with muscle afferent feedback in exercise and cause ventilatory responses.

One method that has tested this is the application of circulatory occlusion *during* dynamic exercise. As such the metabolic by-products of exercise are trapped within the exercising muscle, and provide additional stimulus for muscle metaboreceptive afferents, where they would normally be washed away during the relaxation phase of the rhythmic muscle contractions. Asmussen & Nielsen (1964) occluded the circulation to the legs of human subjects with upper thigh cuffs for 3 minutes during a 60 W cycling exercise task. During occlusion ventilation increased from a steady state of approximately 25 l.min⁻¹ to a maximum of 45 l.min⁻¹ at the end of the 3 minute occlusion period. Heart rate (+35 beats.min⁻¹) and blood pressure (+30 mmHg) increases during the occlusion phase were also observed. Similar effects of circulatory occlusion during dynamic cycling exercise have also been shown elsewhere (Sargeant *et al.* 1981; Stanley *et al.* 1985), where the significant increases in ventilation and reductions in $P_{ET}CO_2$ were maintained until cuff deflation.

However as ventilation returns to normal upon the cessation of exercise despite sustained muscle ischemia Asmussen & Nielsen concluded that perhaps the ventilatory responses recorded during occlusion are not due to muscle metaboreflex activation but are a reflection of greater central motor neural drive and central command activation being required to compensate for the reduced capability of the ischemic muscle. Indeed the use of surface EMG suggested that more motor units were active during the occlusion implying that central command input may have been increased (Asmussen & Nielsen, 1964).

Nevertheless, the augmented ventilatory response during exercise could also be explained by an increased activation of the metaboreflex, suggesting that muscle afferent feedback

can stimulate ventilatory responses. As ventilation returns to baseline in PECO this would imply that muscle afferent stimulation can only increase ventilation during exercise, perhaps while other synergistic inputs such as central command are active and elevating central respiratory drive. However, one concern with these studies is that the vast metabolic build up that presumably took place during circulatory occlusion does not happen in normal dynamic exercise. As such these studies do not provide strong evidence that the metaboreflex plays a role in stimulating the normal exercise hyperpnea.

Therefore the protocol of the current study aims to resolve these issues. It involves the dynamic exercise of a single leg with free flowing circulation followed by PECO of that leg immediately upon cessation of the exercise. Once ventilation has returned to baseline then the contralateral leg will perform dynamic exercise. It is hypothesised that the ventilatory response to the exercise of the contralateral leg will be augmented by the metaboreflex activation from PECO of the first leg. This protocol is advantageous not only because the level of metabolite build up may more closely reflect that during normal exercise but also because the level of neural drive (central command) required to activate the second leg will not be affected by the ischemia of the first.

If augmented ventilatory responses to exercise are observed from the enhanced muscle metaboreflex activity a secondary aim is to determine whether there are any differences between the ventilatory responses to PECO of one leg vs. two legs. Indeed ventilatory responses to PECO have been demonstrated but only if the muscle mass and consequential local metabolite accumulation is great enough (Iellemo *et al.* 1999). If augmented ventilatory responses were found in response to greater metaboreflex activation during exercise *and* PECO then this would imply that the cause of the enhanced response was due solely to the greater metaboreceptor stimulation. Conversely, if this response is *only* observed during exercise then this implies that the effects of muscle afferent feedback on

ventilation may have been unmasked by a possible synergistic interaction with another control mechanism of the exercise hyperpnea (e.g. central command).

5.2 Methods

12 healthy male participants (21.7 ± 2.1 years old; 76.2 ± 7.8 kg; 175 ± 9.7 cm; mean \pm SD) from the University of Birmingham student population took part in this study. All participants received written and verbal information regarding the experimental procedures prior to giving informed written consent. All subjects were habituated to all the experimental procedures, which conform to the Declaration of Helsinki and were approved by the local ethics committee. Participants visited the laboratory for one day following habituation to perform the trials. Before the trial day participants refrained from consuming food and caffeine in the 4 hours before the trials and from performing strenuous physical activity or consuming alcohol in the 12 hours before the trial. This study followed a within subject design with all subjects participating in both trials. The order of each trial was randomised with a 30 minutes rest period in between.

Experimental procedures

Participants were seated in the customised electrically braked cycle ergometer (figure 2.3) and were prepared for the experiment as described in chapter 2. The experimental protocol is shown in figure 5.1. Prior to the start of each 17-minute trial, participants sat quietly for 5 minutes so that the cardiorespiratory variables reached a steady state. Following this 5 minute period, data gathering commenced (time zero in figure 5.1). Baseline measurements were taken for 2 minutes while the participants continued to rest. Participants then performed a right-legged cycling exercise task for 2 minutes. The 2 trials which then followed were:

- 1) Control trial (CON), where participants rested for 4 minutes and then performed a 2 minute left-legged cycling exercise task. Upon cessation of exercise a cuff around the

participant’s left upper thigh was inflated to 200 mmHg by a rapid cuff inflator to achieve circulatory occlusion. This period of PECO continued for 4 minutes until the cuff was deflated.

2) PECO trial (PECO), where upon cessation of the right legged exercise a cuff around the participant’s right upper thigh was inflated to 200 mmHg. This period of PECO continued until the 14th minute of the protocol. Then identically with the control trial at 8 minutes participants performed the 2-minute left-legged cycling exercise task and upon cessation of exercise a cuff around the participant’s left upper thigh was inflated to 200 mmHg. At the 14th minute of the trial both thigh cuffs were deflated.

In both trials after the cuff/s was deflated participants rested in a 3 minute recovery period. All participants were asked to breathe normally and not to perform any abnormal respiratory manoeuvres throughout the protocol. Participants were also asked to notify the experimenter if at any point they felt pain from the circulatory occlusion so that the trial would be stopped.

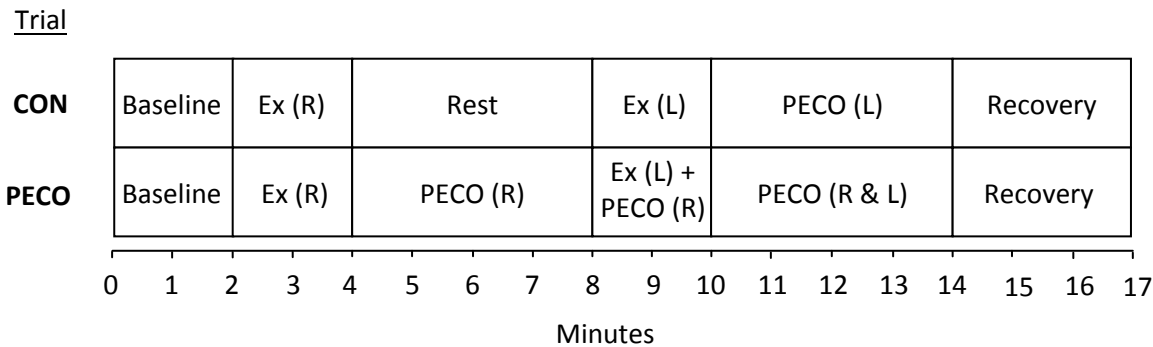


Figure 5.1 Schematic diagram of the protocol. Ex signifies the period of one-legged cycling exercise. PECO signifies the period of post-exercise circulatory occlusion. R and L signifies whether the right or left leg respectively is performing the exercise or undergoing PECO.

Data analysis

The respiratory and cardiovascular variables were recorded as described in chapter 2. Mean averages for each \dot{V} , HR, MAP and $P_{ET}CO_2$ during each minute of the protocols were calculated. A repeated measures two-way ANOVA, and where appropriate multiple comparison *post hoc* analysis, was used to examine differences in \dot{V} , HR, MAP and $P_{ET}CO_2$ within the two trials and between each minute of the two trials. Data are expressed as mean \pm SEM and statistical significance was taken as ($P < 0.05$). Statistical analysis was conducted using a standard statistical package (SPSS, Chicago, IL, USA).

5.3 Results

Differences between trials at baseline

The mean \dot{V} , HR, MAP and $P_{ET}CO_2$ during baseline are presented in Table 5.1. There were no significant differences in any of these variables between the CON and PECO trials.

Trial	\dot{V} (l.min ⁻¹)	HR (beats.min ⁻¹)	MAP (mmHg)	$P_{ET}CO_2$ (mmHg)
CON	6.87 ± 1.1	68 ± 2.1	84 ± 2.7	40 ± 0.4
PECO	6.7 ± 1.2	67 ± 1.6	83 ± 1.7	40 ± 0.3

Table 5.1 Mean \dot{V} , HR MAP and $P_{ET}CO_2$ values recorded during the two-minute baseline period in the CON and PECO trials.

Respiratory responses

Figure 5.2 shows changes in mean \dot{V} relative to baseline during the CON and PECO trials. Right leg exercise significantly increased \dot{V} from baseline in both trials and by the second minute of exercise it increased by $+14.8 \pm 1$ l.min⁻¹ and 14.1 ± 0.7 l.min⁻¹ ($P < 0.05$) in the CON and PECO trials, respectively. \dot{V} returned back to baseline levels following exercise. Left leg exercise then significantly increased \dot{V} in both the CON ($+14.2 \pm 0.8$ l.min⁻¹, $P < 0.05$ versus baseline) and PECO trials ($+16.9 \pm 1.4$ l.min⁻¹, $P < 0.05$ versus baseline). This increase in \dot{V} during exercise was significantly greater during the second minute of the PECO trial compared with the CON trial ($+2.8 \pm 0.7$ l.min⁻¹, $P < 0.05$). \dot{V} then returned back to baseline levels following exercise in both trials. Upon cuff deflation and metabolite washout, \dot{V} increased significantly in the first minute of the recovery period of PECO trial ($P < 0.05$) but not the CON trial.

Table 5.2 shows the mean $P_{ET}CO_2$ values during the different periods of the 2 trial conditions. Throughout both trials, there was no significant change in $P_{ET}CO_2$ from baseline levels and no difference between the 2 trials.

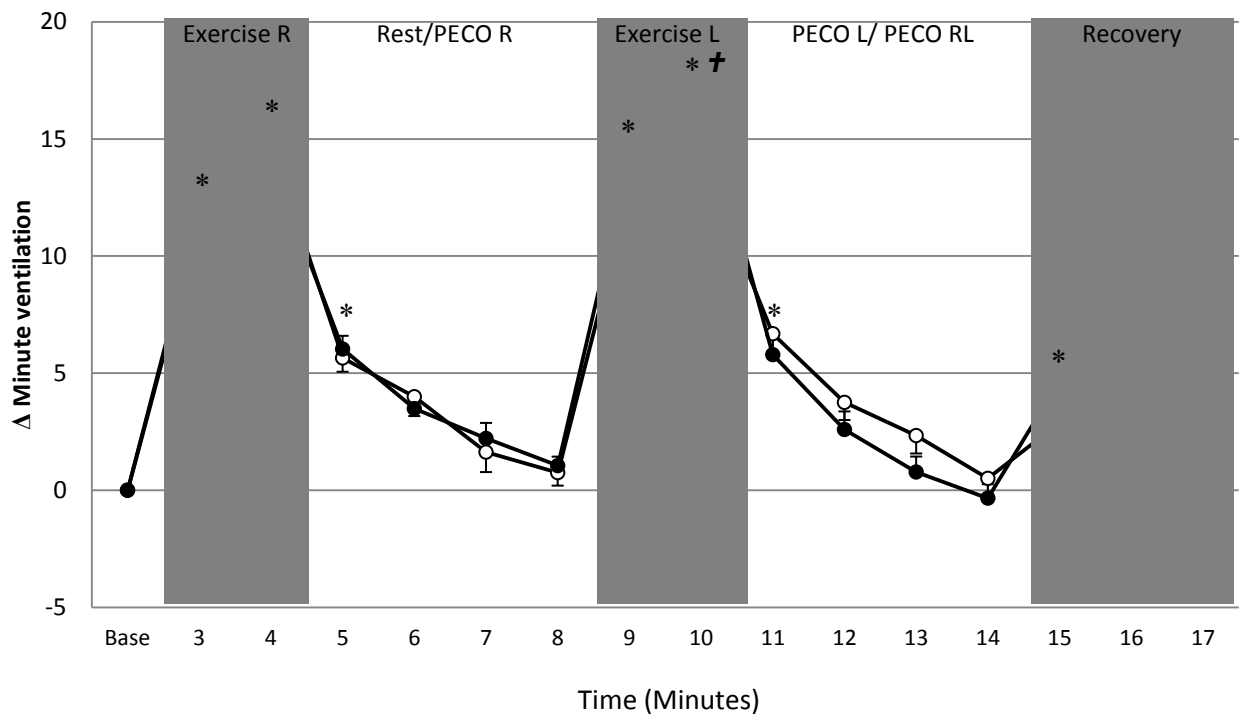


Figure 5.2. Change in \dot{V} from baseline (Base) during each minute of the CON and PECO trials. * Significant difference from Baseline value ($P < 0.05$). † Significant difference between trials.

Trial	Baseline	Exercise R	Rest/ PECO R	Exercise L	PECO L/ PECO LR	Recovery
CON	40 ± 0.4	39 ± 0.6	41 ± 0.4	39 ± 0.4	41 ± 0.6	39 ± 0.6
PECO	40 ± 0.4	39 ± 0.4	41 ± 0.3	37 ± 0.4	40 ± 0.3	40 ± 0.6

Table 5.2 $P_{ET}CO_2$ during each phase of the CON and PECO trials. * Significant difference from Baseline value ($P < 0.05$).

Cardiovascular responses

Changes in mean MAP and HR relative to baseline in the 2 trials are shown in figures 5.3 and 5.4, respectively. Right-legged exercise increased HR and MAP in both trials similarly and by the second minute of exercise they increased by (12 ± 1 mmHg and 32 ± 4 beats.min⁻¹; $P < 0.05$ versus baseline) in the CON trial and (13 ± 2 mmHg and 33 ± 2 ; $P < 0.05$ versus baseline) in the PECO trial. As expected on cessation of exercise HR fell from exercise levels and returned to baseline during the last 3 minutes of the Rest/PECO R period in both trials. MAP also returned to baseline in the CON trial, however in the PECO trial MAP remained significantly elevated above baseline during the PECO R period ($P < 0.05$ versus baseline).

During left-legged exercise HR again increased in both the CON trial (32 ± 1 beats.min⁻¹; $P < 0.05$ versus baseline) and in the PECO trial (38 ± 2 ; $P < 0.05$ versus baseline). This increase in heart rate in response to the exercise was significantly greater during the second minute of the PECO trial compared with the CON trial ($+6 \pm 2$ beats.min⁻¹, $P < 0.05$). MAP also significantly increased in the CON trial during the left legged exercise (13 ± 1 mmHg; $P < 0.05$ versus baseline) and from an elevated level MAP increased in the PECO trial (16 ± 2 mmHg; $P < 0.05$). This exercise produced a similar increase in MAP from the previous period (Rest/PECO R) in both the CON (12 ± 2 mmHg) and PECO trials (10 ± 2 mmHg).

Following the exercise of the left leg HR returned to baseline in the CON and PECO trials. However, MAP remained significantly elevated above baseline during both the trials ($P < 0.05$). Furthermore MAP was significantly higher during the PECO L/PECO RL period in the PECO trial compared with the CON trial ($P < 0.05$). During the recovery period of each trial, MAP and HR values were not significantly different from baseline

($P>0.05$) except during the first minute of the recovery period in the PECO trial where HR was transiently increased.

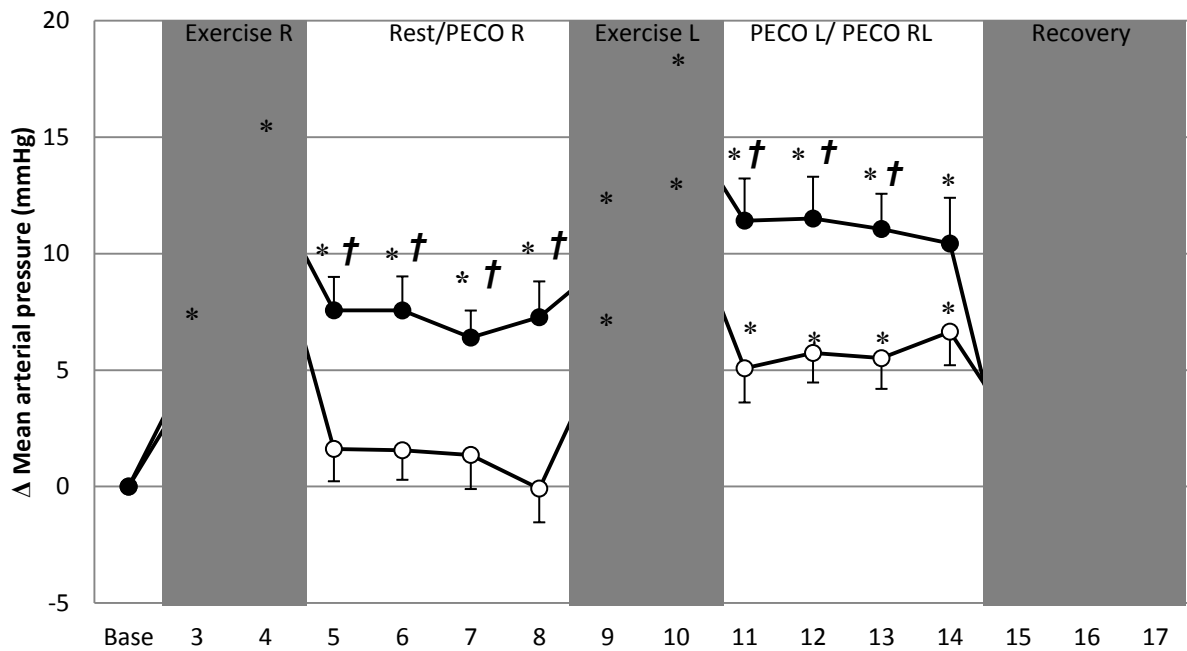


Figure 5.3. Change in MAP from baseline (Base) during each minute of the CON and PECO trials. * Significant difference from Baseline value ($P<0.05$). † Significant difference between trials.

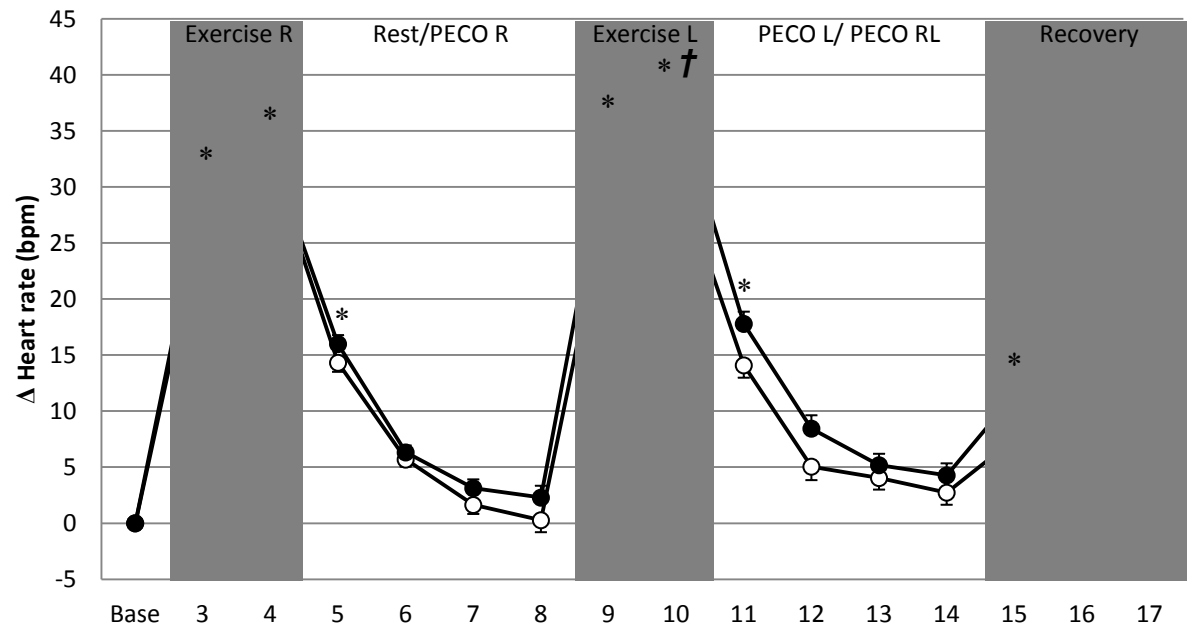


Figure 5.4. Change in HR from baseline (Base) during each minute of the CON and PECO trials. * Significant difference from Baseline value ($P<0.05$). † Significant difference between trials.

5.4 Discussion

This investigation examined the respiratory and cardiovascular responses of humans to exercise during enhanced muscle afferent activation. The main findings are that during conditions of increased muscle metaboreflex activity, through PECO of one leg, the ventilatory and heart rate responses to the exercise of the contralateral leg were augmented. This suggests that during exercise the stimulation of metabolically sensitive afferent receptors in skeletal muscle contribute to the ventilatory and heart rate responses.

Furthermore both ventilation and heart rate returned to baseline levels upon cessation of exercise despite the continued circulatory occlusion which activated the muscle metaboreflex in isolation. Therefore as ventilation and heart rate were only augmented by additional metaboreflex activation during exercise, it suggests a possible synergistic interaction between the neural feedback from the working muscle and other inputs active during exercise that can increase central respiratory drive and heart rate.

Muscle afferent activation

Although the activity of group III and IV muscle afferents were not directly measured, the cardiovascular responses to PECO are reliable and sensitive indicators of muscle afferent activation (Coote *et al.* 1971; McCloskey & Mitchell, 1972; Kaufman *et al.* 1983). Therefore the blood pressure increases and heart rate recovery observed during PECO of a single leg clearly suggests that metabolically sensitive muscle afferent fibres were stimulated during the circulatory occlusion and resulted in a sustained sympathoexcitation. (Alam & Smirk, 1937; Rowell *et al.* 1976, Seals *et al.* 1988; Bull *et al.* 1989; Bell & White, 2005) In addition, the blood pressure response to PECO of both legs was significantly higher still and therefore suggests a greater metaboreflex activation.

Respiratory responses

The results above show that the PECO-induced stimulation of muscle metaboreceptors in one leg potentiates the ventilatory response to exercise of the contralateral leg. This clearly suggests that metabolically sensitive muscle afferents can stimulate increases in ventilation during exercise. These results are supported by studies that applied circulatory occlusion during concurrent dynamic cycling exercise (Asmussen & Nielsen, 1964; Sargeant *et al.* 1981; Stanley *et al.* 1985). They demonstrated significant elevations in ventilation while the circulatory occlusion of the exercising muscles persisted and then returned to steady state after circulation was restored. An alternate method of examining the role of human muscle afferent activation in the control of ventilation is lower body positive pressure (LBPP). This technique reduces muscle blood flow to the lower limbs (Rowell *et al.*, 1991) and slows the washout of exercise induced metabolites. It has been shown that during LBPP, the ventilatory responses to dynamic exercise are increased and can be graded proportionately to the level of pressure (Williamson *et al.*, 1993). This again implies that an augmented metabolic build up stimulates ventilation and supports the concept that muscle afferents can contribute to the exercise ventilatory response.

However the design of the current investigation is advantageous as circulatory occlusion of each leg took place immediately upon cessation of the dynamic cycling exercise and therefore the level of metabolite build up may more closely reflect that during normal exercise. Furthermore, the circulatory occlusion or reduction in blood flow through LBPP of the exercising muscle may result in a greater level of neural drive (i.e. central command) being required to compensate for any reduced functional capability of the ischemic muscle. Therefore a greater level of central command activation may account for the greater ventilatory responses observed by the studies above. However in the current investigation the level of central motor drive required to activate the contralateral leg will

likely not be affected by the post-exercise ischemia of the first. Consequently any change in the ventilation (and heart rate) response to exercise will arguably not be due to any alteration in central command activation.

In the current investigation minute ventilation during exercise of the left leg was ~15% greater during PECO of the right leg. Conversely Asmussen & Nielson (1964) found that minute ventilation almost doubled by the end of their 3 minute circulatory occlusion period. The difference in the augmented responses between studies could be due to several reasons. Firstly, as described above, there may be differences in the levels of central command activation and metabolic build up in the muscle. In addition some group III afferents display polymodal characteristics and as such their responses to mechanical stimuli can be potentiated by metabolic by products of muscle contraction (Hayes *et al.* 2006). Therefore circulatory occlusion during exercise may result in an increased discharge of these afferents for the same mechanical stimulus. In the current investigation this sensitisation is not possible as the circulation of the resting leg was occluded.

However not all studies have observed an increase in minute ventilation to the circulatory occlusion of dynamically exercising muscles. For example Fordyce *et al.* (1982) found no change in the ventilatory response to a rhythmic foot dorsiflexion exercise. In this study, the duration of this “mild” exercise was only 30 seconds and the ventilatory response to it was relatively small compared to the studies of Asmussen & Nielsen, Sargeant *et al.* and Stanley *et al.* Because only a small muscle mass was exercise at a low intensity and for a short duration it is likely that an inadequate metabolic build-up developed in order for a difference in the ventilatory response between trials to be observed.

When developing the current protocol, static calf plantarflexion exercise was used in a pilot study. Interestingly, a small increase in minute ventilation was observed, similar to that seen by Fordyce *et al.* but there was no augmented ventilatory response to single-

legged exercise during PECO of the contralateral leg (for reference this data is shown in appendix, figure 8.2). This trial followed an almost identical protocol to that of the current study except that the exercise was a 2-minute isometric calf plantarflexion at 50% MVC. Because of the exercise intensity/duration this null finding is unlikely due to an inadequate metabolic stimulus. Indeed there was a difference of ~5mmHg in the MAP response to exercise of the left leg between trials. Rather it is probably more likely that ventilatory response to static exercise was not large enough for a small percentage difference to be detected.

In the present study, during exercise the circulatory occlusion of the previously exercised leg caused increased ventilatory responses and yet as expected during PECO alone (one and two legged) ventilation returned to baseline levels. This latter finding is consistent with many previous reports (Rowell 1976, Innes et al. 1989, Scott et al. 2000, Haouzi et al 2001, Fukuba et al. 2007). So why was a ventilatory response seen during exercise but not during PECO?

Metaboreflex activation through PECO seems to give precedence to a blood pressure response, presumably to maintain adequate perfusion to the muscle and facilitate the washout of trapped metabolites. However, the resultant pressor response to PECO, in the absence of central command, may suppress ventilation through the ventilatory baroreflex. So perhaps only during exercise where the muscle metaboreflex is operating in combination with other possibly synergistic inputs to central respiratory neuronal pool (e.g. central command) can the stimulation of metabolically sensitive afferents cause an increase in ventilation against the opposing actions of the ventilatory baroreflex.

In contrast to the findings of the current study, Iellamo et al. (1999) has provided evidence that metaboreflex activation via PECO can induce ventilatory responses. These responses however only manifested when a larger muscle mass (static knee extension vs. static

handgrip) was exercised at a higher intensity (30% vs. 15% MVC for 3 minutes), so that the resultant metabolite accumulation was great enough. As such it seems that PECO can cause ventilatory rate responses if the metaboreflex activation is robust enough. If not then the current study has provided evidence that the muscle metaboreflex can operate in combination with other possibly synergistic inputs active during exercise (e.g. central command) and cause ventilatory responses.

Indeed there is evidence that central interactions between central command and muscle afferent feedback might be possible. Intriguing new evidence in humans has been produced suggesting a potential role of the PAG in the integration of central command and muscle afferent feedback. The PAG is thought to be a component in the neurocircuitry of central command as the stimulation of the dorsal PAG increases blood pressure (Green *et al.* 2005) and the anticipation to exercise and actual exercise causes increases in the neural activity of the PAG (Green *et al.* 2007). Now it has been shown that both blood pressure and the neural activity of the PAG can also increase in response to muscle afferent activation via PECO (Basnayake *et al.* 2011). Therefore the PAG is an area of the brainstem where synergistic interactions like those described above could conceivably occur.

Heart rate responses

Although this study was principally concerned with ventilation, augmented heart rate responses were also produced to the left legged exercise during the PECO of the contralateral leg. This suggests that metabolically sensitive muscle afferents can also stimulate increases in heart rate during exercise. Indeed increased heart rate responses to cycling exercise have also been found by Asmussen & Nielsen (1964) during the

circulatory occlusion of the exercising muscle. These current findings are in contrast to much of the literature which suggests that central command (Krogh & Lindhard 1913; Goodwin *et al.* 1972) and the muscle mechanoreflex (Gladwell & Coote, 2002; Gladwell *et al.* 2005) are the major contributors to the increase in heart rate during exercise, whereas the muscle metaboreflex plays little or no role as classically evidenced by the return of heart rate to resting levels during PECO (Rowell *et al.* 1976).

However the elimination of cardiac parasympathetic tone, with glycopyrrolate, results in the incomplete recovery of heart rate during circulatory occlusion following a moderate intensity static handgrip exercise (25% MVC; Fisher *et al.* 2010). These results suggest that the PECO induced metaboreflex can increase cardiac SNA in humans and elicit heart rate response, but it is usually masked by parasympathetic reactivation post exercise. This reactivation is likely due to the loss of central command and mechanoreflex input and also the influence of the baroreflex on the heart.

The results of the current investigation may support this conclusion as heart rate responses to the additional metaboreflex activation in the PECO trials were only shown *during* the exercise of the contralateral leg. Therefore only during concurrent central command and muscle mechanoreflex activation, where parasympathetic activity will be withdrawn, could the metaboreflex induced increases in cardiac SNA be manifested as a tachycardia. Moreover there is evidence in animals and humans that in addition to a further upward and rightward resetting of the cardiac baroreflex, its sensitivity is decreased in response to augmented metaboreflex activation during dynamic exercise (Sala-Mercado *et al.* 2007, 2010; Hartwich *et al.* 2011). All together these findings provide evidence that metaboreflex activation can drive heart rate during exercise.

In the current investigation no heart rate responses were observed to PECO alone. However, Fisher *et al.* also found that circulatory occlusion following a higher intensity

static handgrip exercise (40% MVC), where there will be a greater degree of local metabolite accumulation, resulted in the incomplete recovery of heart rate. As this was not affected by parasympathetic blockade it provides evidence that a stronger muscle metaboreflex activation is capable of increasing cardiac SNA (and heart rate) and overcome the cardiac parasympathetic reactivation post exercise itself.

Limitations

Ideally greater exercise intensities would also be used to assess the effects of muscle metaboreflex activation at a wider range of minute ventilations and heart rates. However due to the study design we are limited to the current exercise intensity (50 watts for 2 minutes) as a maximum. This is because the greater the intensity the more time required for ventilation and heart rate to return back to baseline levels following the initial right legged exercise. This means that the period of PECO of the right leg would need to be extended beyond 10 minutes and based upon our own observations this is approximately the maximum length of time before the circulatory occlusion following a cycling exercise becomes uncomfortable or even painful for the participant.

One further limitation with the methodology employed is that $P_{ET}CO_2$ was not maintained at a constant level throughout the trials and slightly fell during the left-legged exercise period of the PECO trial, (although this remained a statistically non-significant change). As a result ventilation could potentially be suppressed through reduced feedback from central and/or peripheral chemoreceptors and so the effects of the additional metaboreflex activity during exercise may have an even greater effect on ventilation than that recorded here. Because in normal exercise $PaCO_2$ does not change then any future studies should

perhaps employ a methodology that clamps $P_{ET}CO_2$ at resting levels so a truer contribution of muscle afferent feedback might be observed.

Conclusion

This current investigation examined the ventilatory responses of humans to exercise during enhanced muscle afferent activation. It was shown that during conditions of increased muscle metaboreflex activity, the ventilatory responses to the exercise were augmented. However when the metaboreflex was activated in isolation, via PECO, blood pressure remained elevated but ventilation returned to baseline levels, possibly due to the effects of the ventilatory baroreflex. These findings suggest that only during exercise where the muscle metaboreflex is operating in combination with other possibly synergistic inputs, such as central command, can the stimulation of metabolically sensitive afferents drive increases in ventilation.

**CHAPTER 6: THE VENTILATORY
AND CARDIOVASCULAR
RESPONSES TO MUSCLE
METABOREFLEX ACTIVATION IN
COPD**

6.1. Introduction

COPD is an umbrella term for the occurrence of both chronic bronchitis and emphysema which commonly co-exist and classically cause increases in airflow resistance and lung hyperinflation. Chronic bronchitis is associated with a narrowing of the airways resulting in airflow limitation and the inadequate ventilation of the lungs. The damage to alveolar walls in emphysema results in the enlargement of these air spaces and therefore the surface area available for gas exchange is reduced. Emphysema also reduces the elasticity of the lung which reduces the support for the airways making them likely to collapse and further limit airflow. Airflow limitation and reduced elastic recoil of the lungs slows the rate at which the lungs empty in expiration. Hence during exercise, where respiratory rate is increased, air can remain trapped in the lungs resulting in lung hyperinflation and increases in the mechanical work required to breathe.

One of the most common symptoms of COPD is that of exercise intolerance which is in part caused by the physiological respiratory impairments described above and the consequential psychological sensations of dyspnea (O'Donnell & Webb 2008; O'Donnell *et al.* 2009). Dyspnea (or breathlessness) arises during exercise due to the combined effects of the increased central respiratory drive and the already impaired respiratory function (O'Donnell *et al.* 2009). However, the weak correlation between FEV₁ and exercise capacity suggests that factors other than impaired lung function must also be involved (Killian *et al.* 1992; Gosselink *et al.* 1996). Indeed there is considerable evidence that in addition to respiratory impairments exercise can be limited by skeletal muscle weakness/fatigue, so abnormalities in skeletal muscle function also seems to significantly contribute to the exercise intolerance of COPD patients (Killian *et al.* 1992; Hamilton *et al.* 1996, Gosselink *et al.* 1996, Serres *et al.* 1998 Aliverti & Macklem, 2001; Casaburi *et al.* 2001; Man *et al.* 2003).

Skeletal muscle atrophy is common in COPD and results in the significant reductions in strength associated with the disease (Gosselink *et al.* 1996; Bernard *et al.* 1998; Engelen *et al.* 2000). In terms of the contractile and metabolic characteristics of skeletal muscle, it is well documented that there is a slow to fast transition of muscle fibre types (Whittom *et al.* 1998; Jobin *et al.* 1998; Gosker *et al.* 2002), that glycolytic enzyme activity is increased (Jakobsson *et al.* 1995; Gea *et al.* 2001) and that mitochondrial density and (accordingly) oxidative enzyme activity is reduced (Jakobsson *et al.* 1995; Gosker *et al.* 2002, 2007). These maladaptations may be important determinants in reducing fatigue resistance commonly found in COPD (Serres *et al.* 1998; Coronell *et al.* 2004; Allaire *et al.* 2004; Saey *et al.* 2005). During exercise the increased glycolytic capacity and shift away from oxidative metabolism results in an early onset lactic acidosis and decreased muscle pH. (Casaburi *et al.* 1991; Kutsuzawa *et al.* 1992; Maltais *et al.* 1996). Furthermore this lactic acidosis has been shown to increase the ventilatory response to a given exercise intensity in COPD (Casaburi *et al.* 1991) and so may contribute to the sensations of dyspnea and exercise intolerance.

The factors which underlie this skeletal muscle dysfunction have also been examined and muscular disuse caused by the physical inactivity generally displayed by COPD patients likely contributes (Serres *et al.* 1998). Indeed this concept is part of the dyspnea spiral (figure 6.1) described by Prefaut *et al.* (1995). Patients avoid physical exertion to prevent the unpleasant sensations of dyspnea. This however has the consequence of causing further deconditioning of skeletal muscles along with the associated metabolic changes in muscle and early lactic acidosis in exercise. Hence this provides a stimulus to exaggerate the exercise ventilatory response further and exacerbate the sensations of dyspnea. In this way, dyspnea and exercise tolerance can spiral downwards over time.

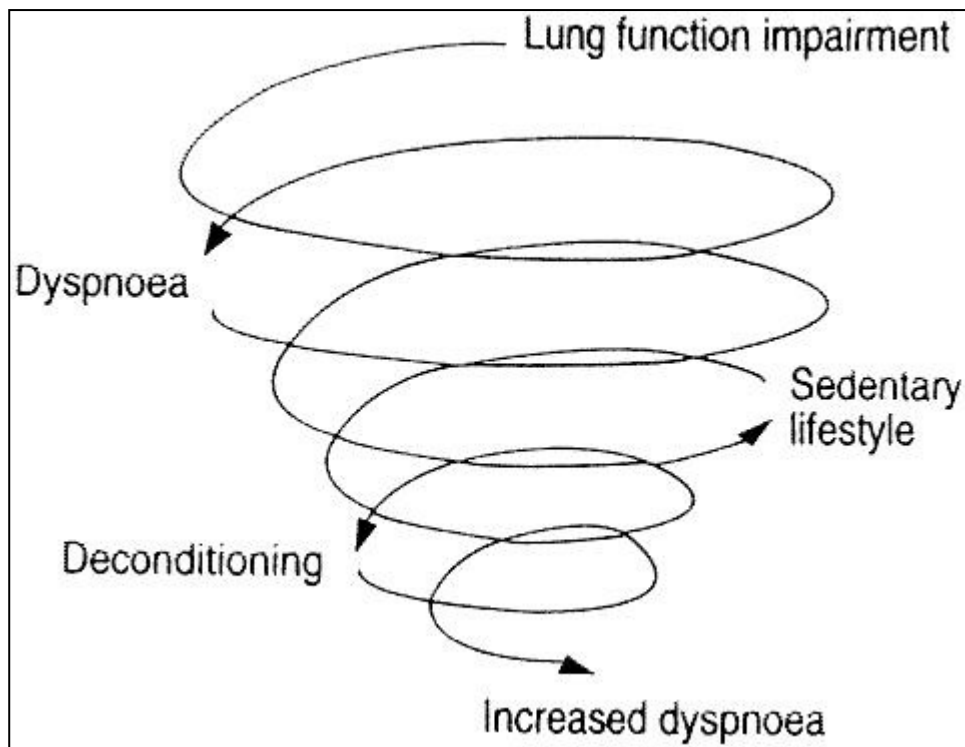


Figure 6.1 Dyspnea spiral from Serres *et al.* (1998a).

Furthermore the chronic hypoxemia, caused by the mismatch between alveolar ventilation and perfusion (Kent *et al.* 2011), and resultant muscle tissue hypoxia is also a possible factor in the derangements of skeletal muscle function and metabolism. It has been demonstrated in humans that chronic exposure to hypoxia results in the increase in glycolytic enzymes and decrease in oxidative enzymes within skeletal muscle (Howald *et al.* 1990, Hoppeler *et al.* 2003) and also causes muscle atrophy (Hoppeler *et al.* 2003). In addition, malnutrition and the use of corticosteroids in the treatment of exacerbations have also been implicated as possible factors in the development of muscle atrophy and consequential weakness. For review see Wüst & Degens, (2007) and Man *et al.* (2009).

The features of the skeletal muscle dysfunction described above appear to be similar to that shown in CHF (Franssen *et al.* 2002) which are likely caused by factors such as

chronic under perfusion of the muscle (due to increased sympathetic vasomotor activity) and the deconditioning associated with inactivity (Piepoli *et al.* 2008; Rehn *et al.* 2012). In addition to the derangements in central haemodynamics, these muscle abnormalities are thought to be a key factor in the exercise intolerance caused by the disease (Rehn *et al.* 2012). During exercise CHF is associated with excessive sympathoexcitation and cardiovascular responses and also with the sensations of dyspnea from excessive ventilatory responses, both of which contribute to exercise limitation (Sinoway & Li, 2005; Piepoli *et al.* 2008). Augmented skeletal muscle afferent feedback is widely considered to be a contributing factor to these abnormal responses to exercise, but it is still hotly debated whether this is due to increased stimulation of muscle metaboreceptors or greater activation/sensitivity of muscle mechanoreceptors (Sterns *et al.* 1991; Piepoli *et al.* 1996; Silber *et al.* 1998, Notarius *et al.* 2001; Middlekauff *et al.* 2004; Middlekauff & Sinoway, 2007; Piepoli & Coats, 2007).

Nevertheless there is considerable reproducible evidence that CHF patients can display exaggerated muscle metaboreflex activity to PECO, after both handgrip exercise (rhythmic and static) and cycling exercise, resulting in augmented ventilatory and cardiovascular responses (Piepoli *et al.* 1996, 1999; Silber *et al.* 1998, Scott *et al.* 2000, 2002; Ponikowski *et al.* 2001; Notarius *et al.* 2001; Crisafulli *et al.* 2007; Olson *et al.* 2010). Indeed an exaggerated metaboreflex may provide a ‘neural link’ between the known skeletal muscle dysfunction in these patients and their exercise intolerance. Thus a ‘muscle-hypothesis’ of exercise intolerance was developed (Piepoli *et al.* 1999). It is proposed that the abnormalities in skeletal muscle (e.g. decreased oxidative capacity, early muscle acidosis, impaired muscle perfusion) may enhance metabolite accumulation in exercise, and as such increase the stimulation of muscle metaboreceptive afferents resulting in exaggerated increases in ventilatory drive and sympathetic vasomotor

activation. This may contribute to the common symptoms of early onset fatigue and dyspnea during physical activity and so exacerbate the skeletal muscle abnormalities further due to the intolerance to exercise resulting in inactivity and deconditioning of the muscle. This process is described as a “vicious cycle” and it of course has obvious parallels to the aforementioned dyspnea spiral in COPD.

It is therefore plausible that an exaggerated metaboreflex response may provide a neural link between skeletal muscle abnormalities and the sensations of dyspnea and exercise intolerance in COPD, and as such may play a role in the “dyspnea spiral”. However, whether these patients have an augmented metaboreflex and/or a metaboreflex induced ventilatory response is still yet to be fully examined. Therefore the first aim of this study is to examine whether patients with moderate to severe COPD have greater ventilatory responses to PECO in comparison to healthy controls

Some patients with COPD retain CO_2 and are chronically hypercapnic, the mechanisms of which have been reviewed elsewhere (Roussos & Koutsoukou, 2003). In brief the airflow resistance and ventilation/perfusion mismatch do not always correlate well with PaCO_2 and other mechanisms have been implicated. These include a desensitisation of central chemoreceptors to CO_2 and impairments in respiratory mechanics. However because many chronically hypercapnic patients can voluntarily ventilate more to become normocapnic (Robin & O'Neill, 1963) this led to the concept of the “wise fighter” where some patients may ‘choose’ to hypoventilate, presumably to prevent respiratory muscle fatigue.

This incidence of a chronically elevated PaCO_2 , which can further increase during exercise (Jones, 1966; Light *et al.* 1988), is another possible factor involved in generating the sensations of dyspnea upon exertion in COPD. It has been shown in patients with a wide ranging severity of COPD and who also retain CO_2 , that the most important stimulus for dyspnea in these patients, during incremental exercise on a cycle ergometer, is an

increased arterial PCO_2 (Cloosterman *et al.* 1998). It has recently been shown in healthy individuals that ventilation can remain elevated during PECO but only under conditions of acute hypercapnia (Lykdis *et al.* 2010; Bruce & White, 2012). Therefore it is possible that the ventilatory response to metaboreflex activation will be augmented further in chronically hypercapnic COPD patients and contribute to sensations of dyspnea. As such the secondary aim is to examine whether there are any differences in these responses to PECO between patients who are hypercapnic ($PaCO_2 > 45$ mmHg) and patients who are normocapnic.

6.2 Methods

18 patients with stable moderate to severe COPD were recruited from the outpatient clinics in the respiratory medicine department at Heartlands Hospital. Patients were not considered stable and excluded from participation if in the previous 6 weeks they had been hospitalised or had exacerbations. Patients with heart failure/disease or an active malignancy revealed by medical history were also excluded. COPD and its severity were defined according to Global Initiative for Obstructive Lung Disease (GOLD) criteria. Hence COPD was defined as FEV₁/FVC ratio <70% after the application of bronchodilator medication. Moderate COPD was defined as a FEV₁% predicted between 50%-79% and severe COPD between 30%-49%. 9 healthy age and gender matched controls also volunteered for this study. Patient pulmonary function data and participant characteristics are shown in table 6.1. All participants received written and verbal information regarding the experimental procedures prior to giving informed written consent. All subjects were habituated to all the experimental procedures, which conform to the Declaration of Helsinki and were approved by an NHS ethical committee.

Participants visited the laboratory for 1 trial day following habituation. On the trial day participants refrained from consuming food and caffeine in the 4 hours before the trial and from performing strenuous physical activity or consuming alcohol in the 12 hours before the trial. This study followed a within subject design with all subjects participating in both trials. The order of each 8-minute trial was randomised with a 30 minutes rest period in between.

Experimental procedures

Participants were seated in an upright position, held a custom-made handgrip dynamometer (figure 2.4) with their right hand and were prepared for the experiments as

described in chapter 2. The experimental protocol is shown in figure 6.2. Prior to the start of each trial participants rested for 5 minutes in order to establish a steady-state in ventilation and cardiovascular variables. Both trials then consisted of a 2-minute baseline recording period before a 2-minute rhythmic handgrip exercise task. Participants then either:

- 1) Rested for a further 4 minutes (**Control trial**) or
- 2) A cuff was rapidly inflated to 200mmHg around the upper right arm by a rapid cuff inflator immediately after exercise to achieve circulatory occlusion and so participants went through a 2-minute period of PECO. Then the cuff was deflated and participants rested for 2 minutes (**PECO trial**)

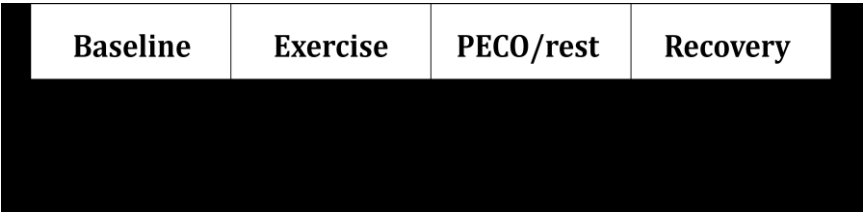


Figure 6.2. Schematic diagram of the 8-minute protocol for the Control and PECO trials.

Data analysis

Respiratory and cardiovascular variables were recorded as described in chapter 2. Mean averages for \dot{V} , HR, MAP and $P_{ET}CO_2$ during each minute of the trials were calculated. A repeated measures two-way ANOVA, and then when appropriate multiple comparison *post hoc* analysis, was used to examine the differences in \dot{V} , HR, MAP and $P_{ET}CO_2$ responses within each of the trials (baseline and minutes 3-8) and also the differences between trials (COPD vs. Healthy trials and also COPD hypercapnic vs. COPD normocapnic trials). A Student's unpaired samples t-test (two-tailed) was used to examine differences between the group means shown in table 6.1 and 6.3. Data are expressed as mean \pm SEM and statistical

significance was taken as ($P<0.05$). Statistical analysis was conducted using a standard statistical package (SPSS, version 19, Chicago, IL, USA).

6.3 Results

Participant characteristics

Participant characteristics are presented in Table 6.1 COPD patients produced significantly lower handgrip forces ($P<0.05$).

	Healthy	COPD
N	9 (5 male)	18 (10 male)
Age	67.7 ± 6.1	65.1 ± 5.1
Height (cm)	167.1 ± 4.9	162.2 ± 3.2
Weight (Kg)	71.8 ± 3.7	75.6 ± 3.1
Handgrip Force (N)	298 ± 45	246 ± 37*
Resting SBP (mmHg)	125 ± 3.2	119 ± 2.4
Resting DBP (mmHg)	78 ± 2.6	75 ± 1.9
Resting MAP (mmHg)	93 ± 3.1	89 ± 1.6
Resting HR (bpm)	71 ± 1.6	68 ± 1.3
FEV ₁ (L)	-	1.06 ± 0.3
FEV ₁ (% predicted)	-	42 ± 5.7
FVC (L)	-	2.35 ± 0.9
FEV ₁ /FVC (%)	-	45 ± 3.4
PaO ₂ (mmHg)	-	67.4 ± 9.2
PaCO ₂ (mmHg)	-	43.4 ± 6.5
pH	-	7.41 ± 0.03
HCO ₃ (mEq.L ⁻¹)	-	27.8 ± 3.1

Table 6.1 Participant characteristics (± SD). * Significant difference from Healthy Control participants ($P<0.05$). FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; HCO₃, arterial bicarbonate content.

Differences between trials at baseline

The mean \dot{V} , HR, MAP and $P_{ET}CO_2$ during baseline are presented in Table 6.2 There were no significant differences in any of these variables between the Control and COPD participants.

Trial	\dot{V} (l.min ⁻¹)	HR (beats.min ⁻¹)	MAP (mmHg)	$P_{ET}CO_2$ (mmHg)
Healthy Control	8.72 ± 0.92	68 ± 1.3	93 ± 3.1	39 ± 1.6
Healthy PECO	9.09 ± 1.02	69 ± 1.4	95 ± 3.8	39 ± 0.9
COPD Control	9.94 ± 0.6	71 ± 1.6	89 ± 1.6	45 ± 2.2
COPD PECO	10.37 ± 0.54	72 ± 1.6	90 ± 1.9	44 ± 2.3

Table 6.2 Mean \dot{V} , HR MAP and $P_{ET}CO_2$ values recorded during the two-minute baseline period in the Healthy Control, Healthy PECO, COPD Control and COPD PECO trials.

Respiratory responses

Changes in mean \dot{V} relative to baseline that were recorded during each of the 4 trials are shown in figure 6.3.1 and 6.3.2. Exercise caused a significant increase in \dot{V} during the first and second minute of exercise in all trials. During the second minute of exercise \dot{V} increased by $6.33 \pm 0.82 \text{ l.min}^{-1}$ and $5.64 \pm 0.68 \text{ l.min}^{-1}$ in the Healthy Control and Healthy PECO trials respectively. In the COPD Control and COPD PECO trials \dot{V} increased by $8.1 \pm 0.64 \text{ l.min}^{-1}$ and $8.38 \pm 0.81 \text{ l.min}^{-1}$ respectively. The differences in the ventilatory response to exercise between the Healthy Control and COPD Control trials and between the PECO and COPD Control trials both reached significance in the second minute of exercise ($P < 0.05$). \dot{V} returned to baseline levels in both the Healthy Control and Healthy PECO trials following exercise. \dot{V} remained significantly above baseline during the first minute after exercise in the COPD Control trial ($2.23 \pm 0.33 \text{ l.min}^{-1}$) but returned to baseline in the second minute. During the COPD PECO trial \dot{V} remained significantly elevated above baseline during both minutes post exercise ($3.82 \pm 0.68 \text{ l.min}^{-1}$ and $2.78 \pm 0.51 \text{ l.min}^{-1}$) and only returned to baseline after cuff deflation. \dot{V} during PECO in the COPD PECO trial was significantly higher than that in the Healthy PECO trial. During the recovery period, \dot{V} was not significantly different from baseline in all 4 trials ($P > 0.05$). Figure 6.4 shows the mean P_{ETCO_2} values during throughout the 4 trials. There was no significant change in P_{ETCO_2} from baseline levels within each trial and no difference between the trials.

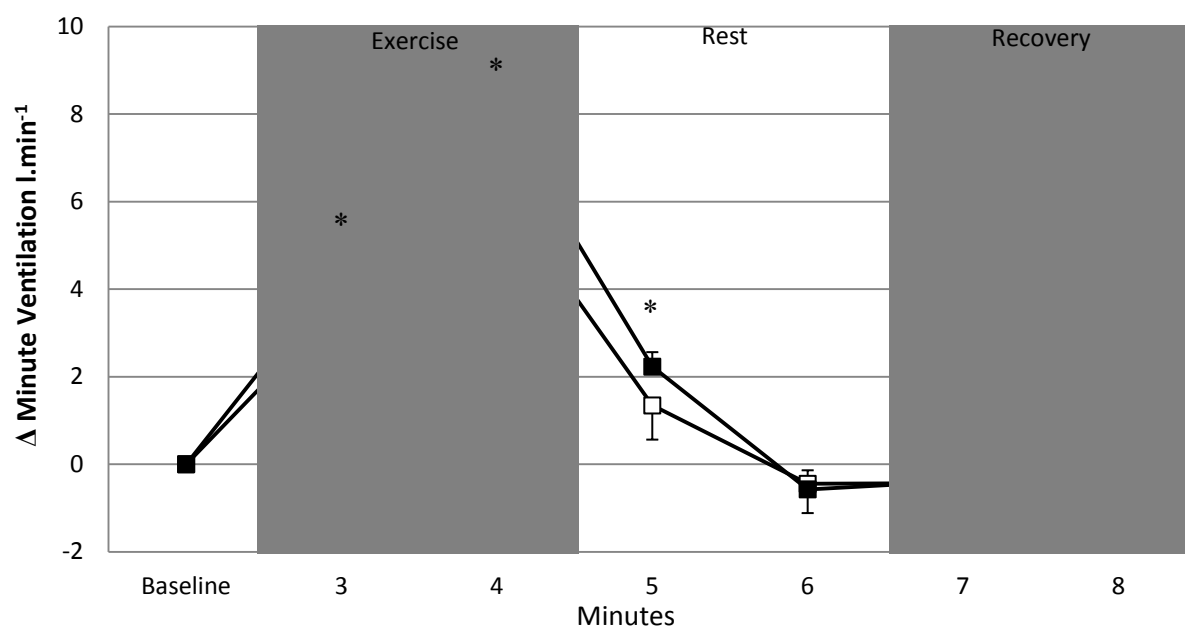


Figure 6.3.1 Change in \dot{V} from baseline during each phase of the Healthy Control and COPD Control trials. * Significant difference from respective baseline values ($P<0.05$).

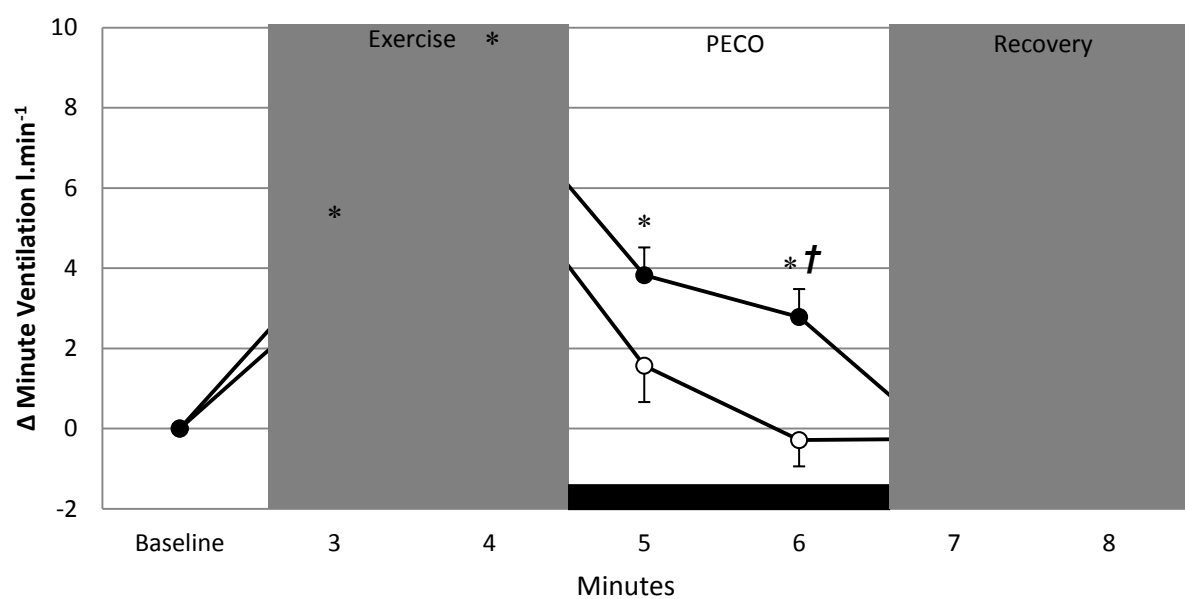


Figure 6.3.2 Change in \dot{V} from baseline during each minute of the Healthy PECO and COPD PECO trials. The black bar shows the periods performed under circulatory occlusion. * Significant difference from respective baseline values ($P<0.05$). † Significant difference between COPD and Healthy trials.

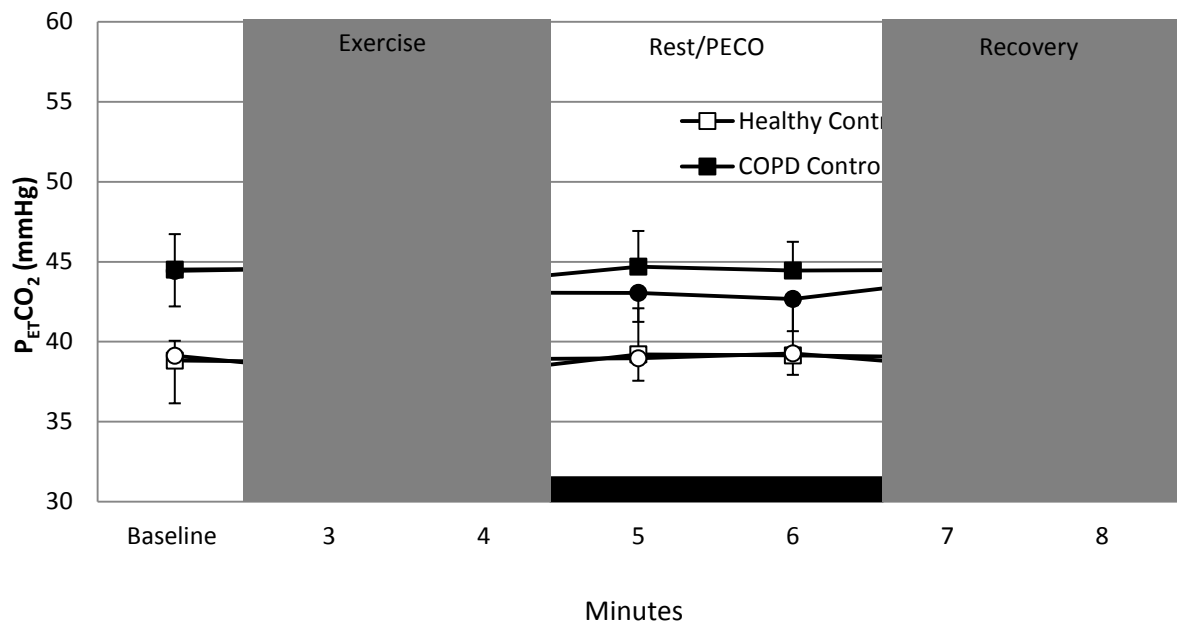


Figure 6.4 $P_{ET}CO_2$ during the Healthy Control, Healthy PECO, COPD Control and COPD PECO trials. The black bar shows the periods performed under circulatory occlusion in the Control PECO and COPD PECO trials.

Cardiovascular responses

Changes in mean HR and MAP relative to baseline during the 4 trials are shown in figures 6.5-6.6. Both MAP and HR significantly increased during exercise in the Healthy Control trial (13 ± 2.3 mmHg and 12 ± 1.5 beats.min⁻¹; $P < 0.05$ versus baseline), in the Healthy PECO trial (13 ± 1.6 mmHg and 14 ± 1 ; $P < 0.05$ versus baseline), in the COPD Control trial (14 ± 1.1 mmHg and 13 ± 1 beats.min⁻¹; $P < 0.05$ versus baseline) and in the COPD PECO trial (13 ± 1 mmHg and 12 ± 1.2 beats.min⁻¹; $P < 0.05$ versus baseline). MAP and HR returned to baseline levels after exercise in the Healthy Control and COPD Control trials. However in both the PECO trials MAP fell from exercise levels but remained significantly elevated above baseline during the 2 minutes post exercise ($P < 0.05$) and only returned to baseline after cuff deflation. There were no significant differences in the MAP responses during PECO in both the Healthy PECO and COPD PECO trials. During the recovery period, both MAP and HR values were not significantly different from baseline in all 4 trials ($P > 0.05$).

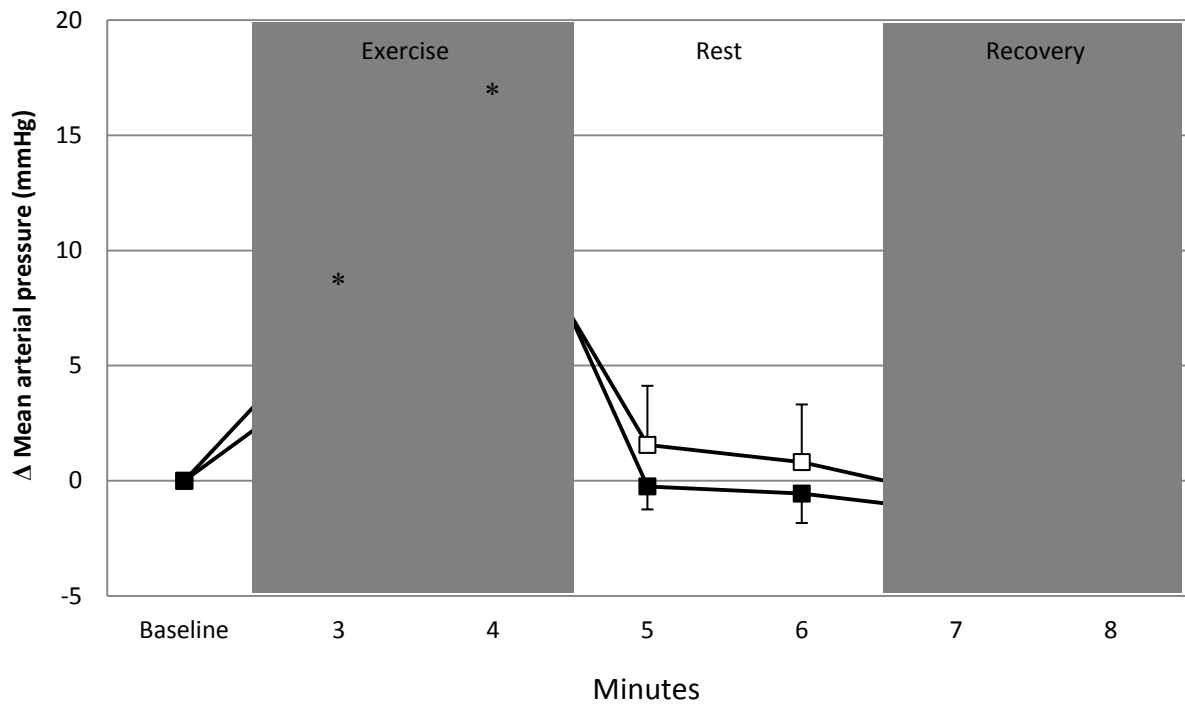


Figure 6.5.1 Change in MAP from baseline during each minute of the Healthy Control and COPD Control trials. * Significant difference from respective baseline values ($P<0.05$).

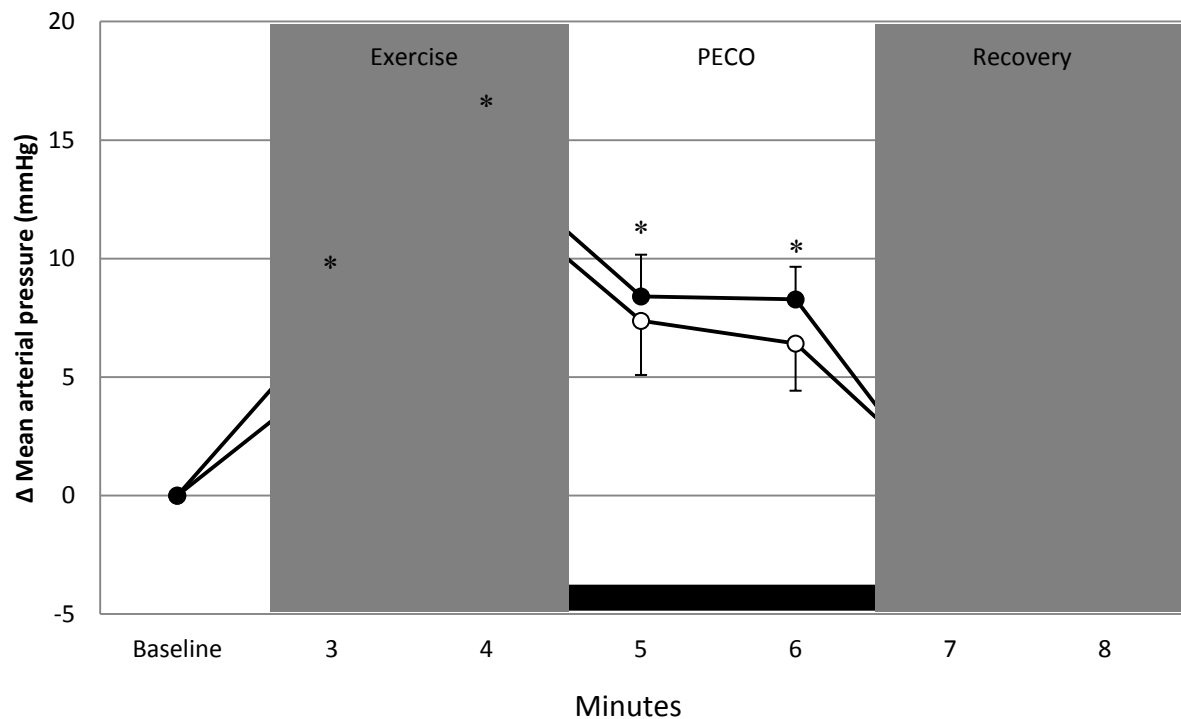


Figure 6.5.2 Change in MAP from baseline during each minute of the Healthy PECO and COPD PECO trials. The black bar shows the periods performed under circulatory occlusion.

* Significant difference from respective baseline values ($P<0.05$).

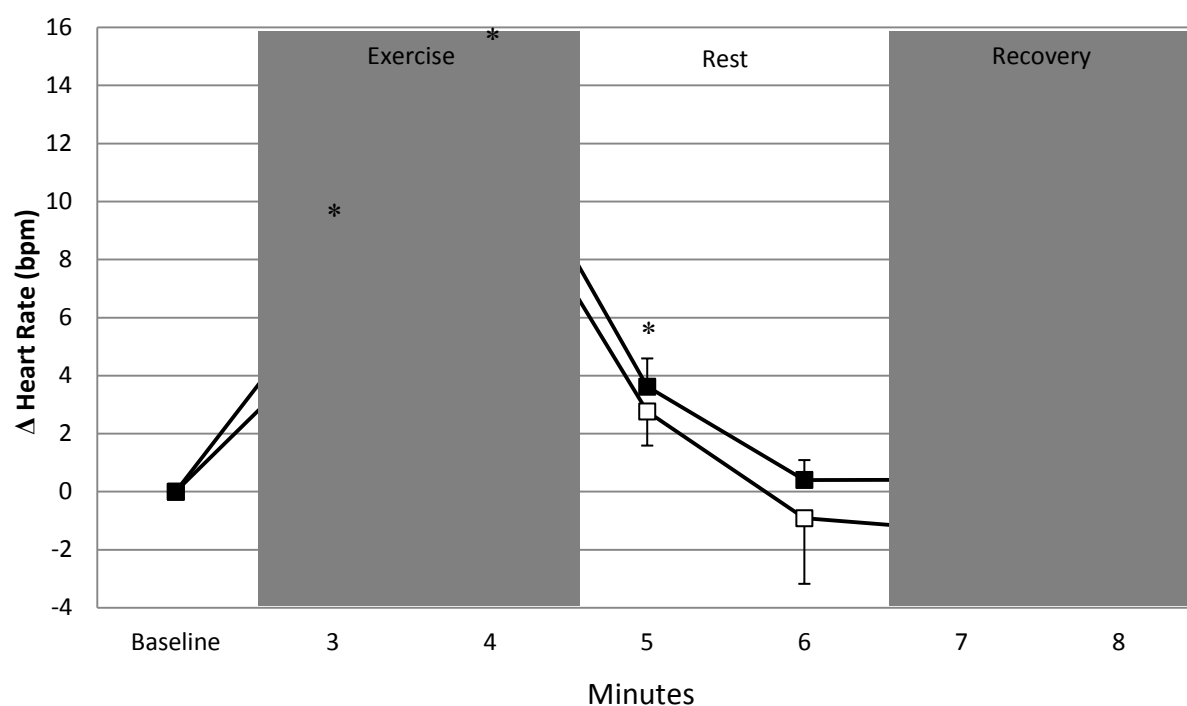


Figure 6.6.1 Change HR from baseline during each minute of the Healthy Control and COPD Control trials. * Significant difference from respective baseline values ($P < 0.05$).

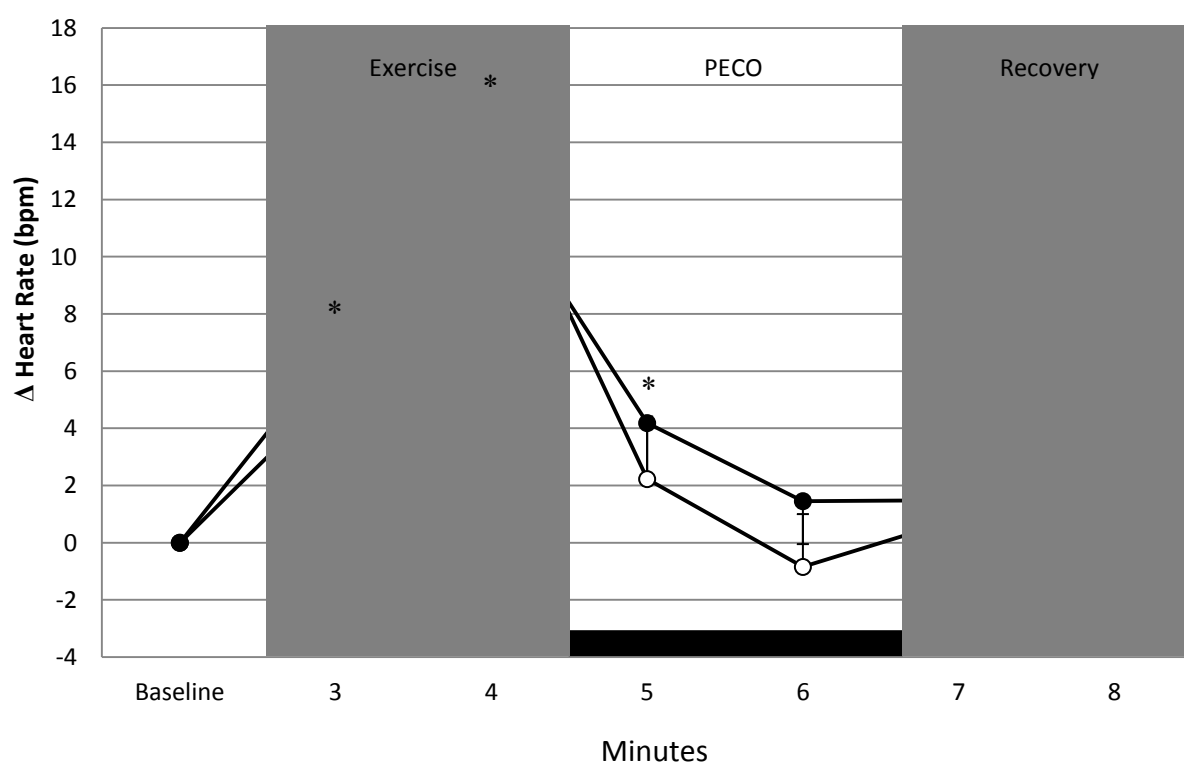


Figure 6.6.2 Change in HR from baseline during each phase of the Healthy Control and COPD PECO trials. The black bar shows the periods performed under circulatory occlusion.

* Significant difference from respective baseline values ($P < 0.05$).

Hypercapnic vs. Normocapnic COPD patients

COPD patients have been separated into two groups for further analysis: Hypercapnic patients ($\text{PaCO}_2 > 45\text{mmHg}$) and normocapnic patients

	COPD normocapnic	COPD hypercapnic
N	9 (5 male)	9 (5 male)
Age	64.3 ± 6.1	67.6 ± 5.1
Height (cm)	160.1 ± 3.8	164.6 ± 3.2
Weight (Kg)	76.8 ± 3.7	74.6 ± 3.1
Handgrip Force (N)	239 ± 56	250 ± 51
Resting SBP (mmHg)	116 ± 4.6	121 ± 3.9
Resting DBP (mmHg)	79 ± 2.6	74 ± 1.9
Resting MAP (mmHg)	91 ± 3.2	89 ± 3.6
Resting HR (bpm)	71 ± 1.6	67 ± 1.3
FEV ₁ (L)	1 ± 0.2	1.13 ± 0.3
FEV ₁ (% predicted)	43.5 ± 5.1	40.5 ± 6.4
FVC (L)	2.24 ± 0.7	2.45 ± 0.8
FEV ₁ /FVC (%)	44 ± 5.1	47 ± 4.3
PaO ₂ (mmHg)	71.6 ± 5.3	$63.3 \pm 6.4^*$
PaCO ₂ (mmHg)	37.7 ± 3.3	$49.2 \pm 2.7^*$
pH	7.41 ± 0.04	7.4 ± 0.03
HCO ₃ (mEq.L ⁻¹)	25.3 ± 1.4	$30.2 \pm 1.6^*$

Table 6.3 Characteristics of patients (\pm SD) separated into hypercapnic and normocapnic groups. * Significant difference from Normocapnic participants ($P < 0.05$). FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; HCO₃, arterial bicarbonate content.

Table 6.3 shows that participants in the hypercapnic group had a significantly higher PaCO_2 , arterial bicarbonate content and lower PaO_2 values than those in the normocapnic group ($P<0.05$). No other significant differences were observed.

Respiratory and cardiovascular responses during the PECO trial

Changes in mean \dot{V} relative to baseline in hypercapnic and normocapnic COPD patients during each minute of the PECO trial are shown in figure 6.7. No significant differences in \dot{V} were observed between the hypercapnic and normocapnic patients at any time point in the trial ($P>0.05$). As expected the P_{ETCO_2} of hypercapnic patients was significantly higher than normocapnic patients during baseline ($+15 \pm 1.3$ mmHg) and throughout the trial ($P<0.05$).

Changes in mean HR and MAP relative to baseline in hypercapnic and normocapnic COPD patients during each minute of the PECO trial are shown in figure 6.8 and 6.9. No significant differences in HR and MAP were observed between the hypercapnic and normocapnic patients at any time point in the trial ($P>0.05$).

The mean \dot{V} , P_{ETCO_2} , MAP and HR recorded during each minute of the Control and PECO trials in hypercapnic and normocapnic COPD patients are shown in the appendix (table 8.2).

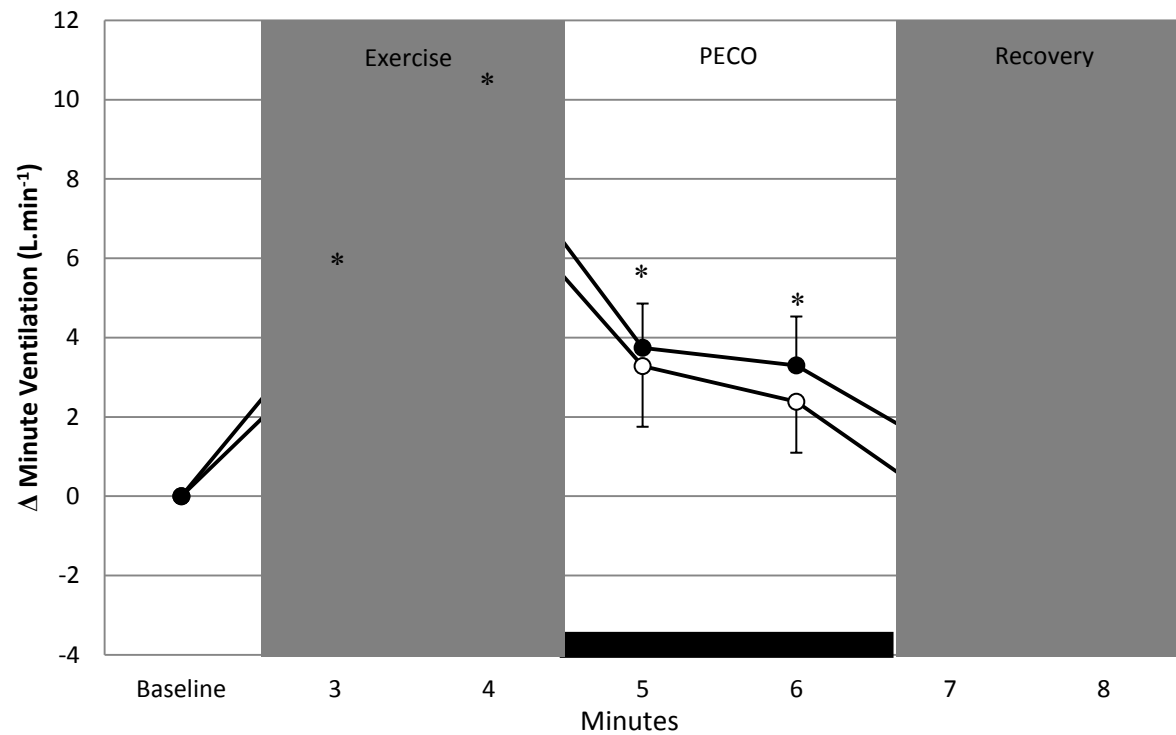


Figure 6.7 Change in \dot{V} from baseline during each minute of the PECO trial in hypercapnic and normocapnic COPD patients. The black bar shows the periods performed under circulatory occlusion. * Significant difference from respective baseline values ($P < 0.05$).

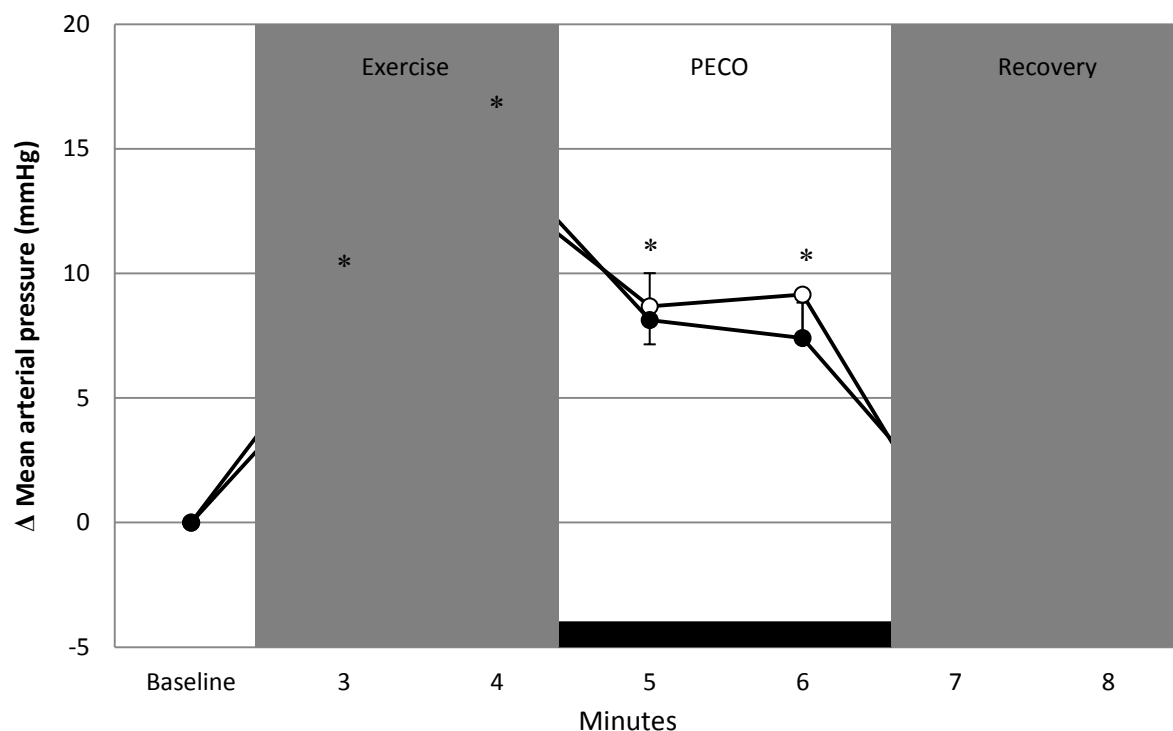


Figure 6.8 Change in mean arterial pressure from baseline during each minute of the PECO trial in hypercapnic and normocapnic COPD patients. The black bar shows the periods performed under circulatory occlusion. * Significant difference from respective baseline values ($P < 0.05$).

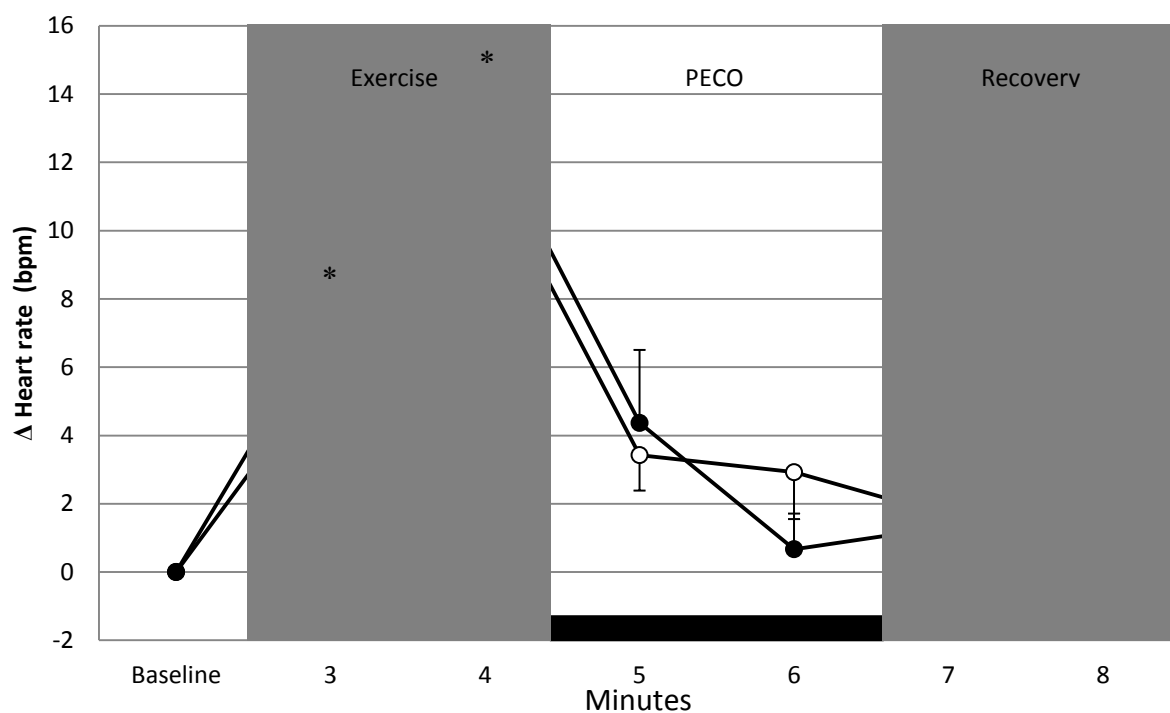


Figure 6.9 Change in heart rate from baseline during each minute of the PECO trial in hypercapnic and normocapnic COPD patients. The black bar shows the periods performed under circulatory occlusion. * Significant difference from respective baseline values ($P < 0.05$).

6.4 Discussion

This study investigated the ventilatory and cardiovascular responses of patients with moderate-severe COPD and healthy aged matched controls to activation of the muscle metaboreflex. The main findings were that activation of the metaboreflex via PECO maintained ventilation significantly above baseline in COPD patients but not in the healthy controls. However, there was no difference in this response between hypercapnic and normocapnic COPD patients.

The cardiovascular responses recorded during PECO are known to be reliable indicators of muscle afferent activation (Coote *et al.* 1971, McCloskey & Mitchell 1972, Kaufman *et al.* 1983). Therefore the blood pressure increases observed during PECO in the current study suggests that the muscle metaboreflex had been activated. Hence the ventilatory responses of COPD patients observed throughout the period of PECO are likely caused by muscle metaboreflex activation. This is supported by the findings that ventilation only returned back to baseline after arm cuff deflation and the consequential reduction in blood pressure and metaboreflex activity. Furthermore ventilation was not maintained above baseline after exercise if circulatory occlusion was not applied. Similar ventilatory responses during circulatory occlusion following a rhythmic handgrip task has been reported by Grieve (2008). However no control subjects were used this study making it impossible to determine if this is a normal response to PECO in their study or if it is an abnormal response related to the disease.

In the current study age-matched control subjects did participate and by contrast they did not display any ventilatory responses to the activation of the muscle metaboreflex during PECO. This finding is consistent with several studies with healthy individuals using circulatory occlusion following handgrip and cycling exercise (Rowell *et al.* 1976, Innes *et*

al. 1989, Scott *et al.* 2000; Haouzi *et al.* 2001, Fukuba *et al.* 2007 and chapters 3, 4 and 5). Therefore it seems likely that the ventilatory response to PECO found in COPD patients is related to the disease.

The current study also demonstrated that the COPD patients tended to produce a greater ventilatory response *during* the rhythmic handgrip exercise task compared with healthy aged matched controls, although this difference failed to reach statistical significance ($P=0.07$ in the Control trial and $P=0.11$ in the PECO trial). Nevertheless the disproportionate exercise ventilatory response in COPD is well documented and is thought to be caused by factors such as the greater mechanical work required to ventilate the lungs, impaired gas exchange and early onset of lactate production (Levison & Cherniack, 1968; O'Donnell, 2001). However because these patients displayed an augmented ventilatory response to PECO compared with healthy controls it is possible that metaboreflex activation contributes to this excessive exercise ventilatory response.

The differences in the ventilatory response to PECO found between COPD patients and healthy individuals in this study could be explained a greater stimulation of muscle metaboreceptive afferents in COPD, possibly because of a greater local metabolite accumulation in the muscle for the same relative workload. This explanation is plausible as COPD is associated with the down regulation of skeletal muscle oxidative enzyme activity, an increased lactic acidosis and a lower muscle pH in exercise (Casaburi *et al.* 1991 Kutsuzawa *et al.* 1992, Gosker *et al.* 2002). Indeed Kutsuzawa *et al.* found an exaggerated decrease in the pH of forearm muscles during a handgrip exercise in patients with COPD compared with healthy controls. In anaesthetised cats it has been shown that the arterial injection of lactic acid/ H^+ causes an increase in the discharge of group III and IV muscle afferent fibres (Rotto & Kaufman 1988) and an increase in ventilation and blood pressure (Rotto *et al.* 1989). This is likely mediated, in part at least, by the stimulation of ASICs

located on the free nerve endings of muscle afferent fibres (Li *et al.* 2004; Hayes *et al.* 2008). Therefore the skeletal muscle dysfunction associated with COPD may result in the increased stimulation of these metabosensitive receptors and an augmented metaboreflex. Thus the abnormal ventilatory response to PECO may be consequently observed, as the enhanced muscle afferent feedback could provide a greater stimulus to respiratory control centres in the brain and increase ventilatory drive.

Furthermore similar augmented ventilatory responses to PECO have been observed in patients with chronic heart failure and are thought to be caused by an enhanced activation of the muscle metaboreflex (Piepoli *et al.* 1996, 1999; Scott *et al.* 2000, 2002; Ponikowski *et al.* 2001; Crisafulli *et al.* 2007; Olson *et al.* 2010). Heart failure is known to result in similar abnormalities in skeletal muscle to that described above in COPD (Franssen *et al.* 2002), so it is possible that this ventilatory response to PECO in heart failure patients, and COPD alike, is a result of an abnormal skeletal muscle milieu.

However in the current study the blood pressure responses of the COPD patients to PECO were not significantly different to that of healthy controls. This indicates that the level of muscle afferent activation may have been similar between these two groups. These findings are consistent with that of Roseguini *et al.* (2008) and Sherman *et al.* (2011) who also found similar blood pressure responses in both COPD patients and controls to circulatory occlusion following an isometric handgrip exercise task. Although no respiratory data was collected in either study these combined findings suggest that the ventilatory responses of COPD patients to PECO in the current investigation were not caused by an augmented muscle metaboreflex. Instead it may imply that the respiratory control centres in the brainstem are by some means more sensitive to muscle afferent feedback and as such a greater ventilatory response is generated for a given metabolic stimulus.

There is evidence however that chronic hypoxemia, which is commonly found in COPD patients, can blunt the vascular responsiveness to a sympathetic stimuli. Heisted *et al.* (1972) observed that forearm vasoconstriction to lower body negative pressure was significantly attenuated in patients who were chronically hypoxemic. Furthermore evidence in animals has shown that chronic hypoxemia can result in the attenuation of the vasoconstriction response to direct sympathetic nerve stimulation (Coney *et al.* 2004). Although the degree of hypoxemia displayed by the patients of the current investigation ($\text{PaO}_2 = 68.9 \text{ mmHg}$) was significantly less severe than those in the studies above (e.g. $\sim 45 \text{ mmHg}$ in Heisted *et al.*), it is possible that hypoxemia attenuated the blood pressure response to metaboreflex activation in these patients. Furthermore, much like with CHF, patients with COPD appear to display a tonic activation of the sympathetic nervous system (Heindl *et al.* 2001) which in turn has been associated with reduced α -adrenergic vasoconstrictor responsiveness (Seals *et al.* 2004). All together these findings suggest that the muscle metaboreflex could be enhanced in COPD but any augmented blood pressure responses to PECO may be cancelled out by a blunted vascular responsiveness to sympathetic stimuli.

An investigation recording MSNA during exercise and PECO would obviously clarify the issue. However, more simply, a further investigation with the leg muscles of COPD patients may also help to elucidate this point. Although it has been demonstrated that the glycolytic enzyme activity of leg (vastus lateralis) and arm (deltoid) muscles are increased in COPD (Jakobsson *et al.* 1995, Gea *et al.* 2001), there is evidence that only leg muscle oxidative enzyme activity appears significantly reduced (Jakobsson *et al.* 1995, Gosker *et al.* 2002) as it seems to be relatively well maintained in arm muscles (Gea *et al.* 2001). As such muscular exercise may generate a greater stimulus (e.g. lactic acid/ H^+) for metabolically sensitive muscle afferents. One obvious reason as to why COPD appears to

cause greater changes in the metabolic characteristics of leg muscles in comparison to upper body muscles is the different levels of disuse that these muscles will likely experience as a consequence of the disease.

Therefore it is possible that differences in the blood pressure response to PECO between COPD and healthy controls may be better observed following a leg exercise (e.g. cycle ergometry, calf plantarflexion, knee extensions), and so a new investigation involving leg exercise may help establish whether the muscle metaboreflex is enhanced by COPD. An added benefit of this new investigation is that it might find even greater and more functionally significant ventilatory responses to muscle metaboreflex activation, due to the differences in the metabolic characteristics and muscle mass of leg compared to arm muscles. Furthermore these findings may provide greater insight into the intolerance of day to day activities, e.g. walking, stair climbing.

Implications

Regardless of the precise mechanism, this study has demonstrated that activation of the muscle metaboreflex via PECO causes ventilatory responses in COPD but not in healthy controls. This may have important implications for these patients as it could contribute to the augmented exercise ventilatory response and sensations of dyspnea that are associated with the disease. Indeed recently Gagnon *et al.* (2012) blocked the neurotransmission of group III and IV muscle afferents from the lower exercising limbs of COPD patients by injecting the μ -opiate agonist fentanyl intrathecally into the L3-L4 vertebral space. During this spinal anaesthesia the exercise ventilatory response was significantly reduced. Furthermore exercise duration at a constant work rate significantly increased from approximately 7 minutes to 10.5 minutes, which was strongly related to the delay in the

ventilatory response and sensations of dyspnea. This study clearly suggests that skeletal muscle afferent feedback may limit exercise capacity in COPD patients and that if these neural signals can be attenuated the exercise ventilatory response and sensations of dyspnea can be reduced and so improve exercise tolerance.

In addition the study by Gagnon *et al.* provides a further possible explanation as to why the exercise training of COPD patients reduces the ventilatory requirements to exercise, along with the sensations of dyspnea, and enhance exercise tolerance (Casaburi *et al.* 1991; Serres *et al.* 1997; Casaburi *et al.* 1997; O'Donnell *et al.* 1998; Gigliotti *et al.* 2003). Endurance training in COPD patients has shown to improve the metabolic efficiency of skeletal muscle by increasing oxidative enzyme activities and reducing lactic acid production and skeletal muscle acidosis in exercise (Casaburi *et al.* 1991; Maltias *et al.* 1996; Whittom *et al.* 1998; Sala *et al.* 1999). An improvement in skeletal muscle oxidative capacity and increased muscle pH may reduce the stimulation of metabolically sensitive receptors in skeletal muscle and thus reduce the central respiratory drive and ventilatory requirements of the exercise. As such the sensations of dyspnea upon exertion may be attenuated and exercise tolerance may increase. Indeed a 6 week training programme of the forearm muscles in CHF has shown to reduce the ventilatory and cardiovascular responses to PECO following rhythmic handgrip exercise (Piepoli *et al.* 1996), and this was thought to be achieved through improvements in skeletal muscle metabolism and the reduced stimulation of the muscle metaboreflex (Piepoli *et al.* 2008). However similar studies are yet to be conducted in COPD patients to determine if training can reduce the ventilatory responses to metaboreflex activation in this patient group.

As an alternative to (or in combination with) exercise training, future work could examine the effects of pharmacological substances that alters the metabolism within skeletal muscle. For example the infusion of Dichloroacetate in healthy humans, which reduces

lactic acid production by stimulating pyruvate dehydrogenase activity, has shown to reduce the sympathetic nerve responses to both static exercise and PECO (Ettinger *et al.* 1991). Because its infusion reduces the discharge of group III afferent fibres to electrically induced static contractions of the hindlimb of cats (Sinoway *et al.* 1993), it was suggested that this reduced sympathetic activity was a result of a blunted lactic acidosis and a consequential reduction in muscle afferent activation. It is therefore possible that the application of Dichloroacetate in COPD patients may reduce the stimulation of metabolically sensitive afferents and result in a reduced ventilatory response to exercise and PECO.

Hypercapnic vs. normocapnic COPD patients

The current study also examined whether there would be differences in the ventilatory responses to muscle metaboreflex activation via PECO between chronically hypercapnic and normocapnic patients with COPD. It has been previously demonstrated that the ventilation of healthy individuals can remain elevated above baseline during PECO under conditions of acute hypercapnia but not normocapnia (Lykidis *et al.* 2010, Chapters 3 and 4). In the current investigation the blood pressure responses to PECO were similar in both the hypercapnic and normocapnic COPD patients suggesting that the level of metaboreflex activation was matched between groups. However we found no differences in the ventilatory responses to PECO between hypercapnic and normocapnic COPD patients. There are several explanations why these findings with *chronic* hypercapnia seemingly differ from those recorded during *acute* hypercapnia.

Firstly, ventilation was maintained significantly above baseline during PECO in normocapnic COPD patients, which was likely a consequence of the disease as healthy

normocapnic participants display no ventilatory responses to the PECO stimulus. It is therefore possible that hypercapnia will have no additional impact as the entire (or a large proportion of the) ventilatory response to muscle metaboreflex activation is observed in COPD under normocapnic conditions

Second, it was demonstrated in chapter 4 that the ventilatory responses to PECO during acute hypercapnia likely occur via a central interaction between the muscle afferent feedback and the hypercapnia-induced stimulation of the central chemoreceptors. Chronic hypercapnia however is associated with a desensitisation of central chemoreceptors to CO₂ (Richerson & Boron, 2005). It is therefore less likely that a central interaction between the muscle metaboreflex and a hypercapnia-induced chemoreflex occurs in these patients.

Finally, although the effects of chronic hypercapnia on skeletal muscle function and metabolism is poorly understood (Man *et al.* 2009), the effects of acute hypercapnia has been more widely examined. There is evidence that acute hypercapnia results in skeletal muscle weakness in healthy humans (Vianna *et al.* 1990) and derangements in the muscle metabolism in COPD patients with acute hypercapnic respiratory failure (Gertz *et al.* 1977; Fiaccadori *et al.* 1987). The mechanism for these maladaptations was thought to be related to the resultant intracellular acidosis¹. However as chronic hypercapnia in COPD results in the expected slow renal compensation and increases in bicarbonate concentration, pH is usually maintained or only slightly decreased (Bruno & Valenti, 2012). In accordance with this, the hypercapnic patients in the current investigation had significantly higher arterial bicarbonate concentrations with no difference in pH. As such it is unlikely that muscle

¹ It is worth noting that the skeletal muscle dysfunction observed in acute hypercapnia will not alter the interpretation of the findings in chapter 3 and 4 as the ventilatory responses to PECO were observed during systemic hypercapnia and not the local hypercapnia of the exercised leg.

metaboreceptor stimulation could be enhanced by a chronic hypercapnia induced muscle acidosis or any resultant abnormalities in skeletal muscle metabolism. Therefore this may provide an additional reason why the ventilatory responses to PECO appear not to be altered by chronic hypercapnia.

The lack of clinical evidence regarding the contribution of *chronic* hypercapnia on skeletal muscle dysfunction in COPD is largely because it primarily co-exists with confounding variables such as chronic hypoxemia. Indeed the hypercapnic COPD patients in the current study showed a significantly lower PaO_2 , compared with normocapnic patients, despite no difference in FEV₁% predicted (Table 6.3). These observations are consistent with several other investigations (Van Meerhaeghe & Sergysels, 1983; Scano *et al.* 1995; Topeli *et al.* 2001). As such this may confound the findings of the current study as a greater degree of chronic hypoxemia could result in the development of further abnormalities in skeletal muscle function and metabolism (see introduction). Therefore the data must be interpreted with caution as we may not be examining the true effects of chronic hypercapnia, in isolation, on the magnitude of the ventilatory response to PECO. Clearly further work with animals will be required to elucidate this issue where a sustained normoxic hypercapnia can be induced, and so should be able to determine with more confidence whether chronic hypercapnia alone alters the ventilatory responses to muscle afferent stimulation. Nevertheless, the data from the current investigation does at least clearly suggest that ventilatory responses to PECO are generated in both normocapnic and hypercapnic COPD patients alike.

COPD severity

Ideally the patients in the current study would have been stratified into groups depending on the severity of their COPD in order to assess any differences in the ventilatory responses to PECO via post-hoc analysis. However in the current investigation the patients examined only had a small range in disease severity (35-53 FEV₁ % predicted) where 15 of the 18 patients had severe COPD and the remaining 3 were moderate as defined by GOLD criteria. Conversely the studies by Grieve (2008) and Sherman *et al.* (2011) utilised patients with a larger range in FEV₁ % predicted values. However they observed that the magnitude of the blood pressure and ventilatory response to PECO was not related to disease severity. So why has no relationship been observed?

Firstly, the wrong muscles may have been examined in these studies. As described above arm muscles are relatively well preserved in COPD whereas leg muscles appear to exhibit a greater degree of dysfunction which is thought to be related to differing degrees of disuse. Therefore the metaboreflex may be up-regulated more in leg muscles and so the cardiorespiratory responses to circulatory occlusion following a leg exercise may be more related to disease severity. Secondly, the severity of COPD was defined by the patients FEV₁ % predicted. However FEV₁ % predicted seems to correlate poorly with exercise capacity and physical activity (Killian *et al.* 1992, Gosselink *et al.* 1996) whereas dyspnea, using the Medical Research Council (MRC) breathlessness scale, has been shown to relate more strongly (Fletcher *et al.* 1959, Bestall *et al.* 1999). Therefore perhaps future investigations should examine whether the ventilatory and cardiovascular responses to metaboreflex activation are related to the sensations of dyspnea.

Conclusion

In conclusion this study demonstrated that the activation of the metaboreflex via PECO maintains ventilation significantly above baseline in COPD patients but not in the healthy controls. However these ventilatory responses to PECO were not related to PaCO₂. The findings of this study add to the emerging concept that skeletal muscle afferent feedback may contribute to the augmented ventilation and sensations of dyspnea that is associated with COPD during exercise.

CHAPTER 7: GENERAL CONCLUSIONS

The cardiovascular responses to exercise are known to be regulated by both central and peripheral neural mechanisms. This includes the feedforward control mechanism of central command and also the neural feedback from group III and IV skeletal muscle afferent which responds to mechanical and metabolic stimuli in the exercising muscle. In contrast the control mechanisms of the exercise hyperpnea are still not fully understood, yet central command is widely considered to play a role. Recently however evidence has suggested that muscle afferent feedback is also capable of driving ventilatory responses in humans as: the inhibiting its neurotransmission of group III and IV muscle afferents reduces the exercise hyperpnea, and the stimulation of muscle afferents via PECO in combination with the sensitising effects of hypercapnia causes ventilatory responses. However the latter piece of evidence requires further examination which was the aim of the first two chapters of this thesis.

The aim of the first study (chapter 3) was to examine whether the stimulation of muscle metaboreceptive afferents (via PECO) and muscle mechanoreceptive afferents (via passive calf stretching) results in ventilatory responses during concurrent hypercapnia and normocapnia. It was shown that under hypercapnic conditions ventilation was maintained significantly above baseline levels during the stimulation of metabolically sensitive afferents. In addition the stimulation mechanoreceptive afferents generated further increases in ventilation during concurrent hypercapnia. However no ventilatory responses were produced to both PECO and passive calf muscle stretching during normocapnia.

The studies in chapter 4 attempted to determine whether this apparent interaction between muscle afferent feedback and the concurrent hypercapnia occurs centrally (i.e. in the CNS) or peripherally in the exercised muscle. During an isolated local hypercapnia of the peripheral exercised muscle, PECO had no effect on ventilation. However during systemic hypercapnia, PECO maintained ventilation significantly above baseline. This provides

evidence for a central synergistic interaction between muscle afferent feedback and the afferent signals from central and/or peripheral chemoreceptors as the ventilatory responses were greater than the sum of the two individual mechanisms. Furthermore, this ventilatory response during PECO was not altered by the inhalation of a hyperoxic hypercapnic gas mixture suggesting that the central chemoreceptors are the primary driver of this interaction.

During exercise PaCO_2 does not increase, but as the studies of chapter 4 demonstrate that central synergistic interactions with muscle afferent feedback are possible perhaps interactions of a similar nature occur with other inputs active in exercise (e.g. central command, which like the chemoreflex can also increase central respiratory drive). This concept was examined in chapter 5 where a study investigated whether augmented levels of muscle afferent feedback can increase the ventilatory responses during exercise and/or PECO. It was shown that during conditions of increased muscle metaboreflex activity, through PECO of one leg, the ventilatory responses to the exercise of the contralateral leg were augmented. This suggests that during exercise the stimulation of metabolically sensitive afferent receptors in skeletal muscle can contribute to the ventilatory response. However ventilation returned to baseline levels upon cessation of exercise despite the continued circulatory occlusion which activated the muscle metaboreflex in isolation. Therefore as ventilation was only augmented by additional metaboreflex activation during exercise, it suggests a possible synergistic interaction between the neural feedback from the working muscle and other inputs active during exercise that can increase central respiratory drive (central command).

The enhanced ventilatory responses to metaboreflex activation during hypercapnia observed in chapters 3 and 4 could have important implications for patients with COPD. COPD is associated with abnormalities in skeletal muscle metabolism but whether this

leads to exaggerated ventilatory responses to muscle metaboreflex activation was unknown. Furthermore as some patients are chronically hypercapnic this might conceivably also enhance the ventilatory response to muscle metaboreflex activation? The study in chapter 6 demonstrated that PECO-induced metaboreflex activation in COPD patients, but not healthy aged matched controls, resulted in ventilation being significantly maintained above baseline until cuff deflation. However this ventilatory response to PECO was not associated with the disease-related chronic hypercapnia. The exaggerated respiratory response might be related to enhanced muscle afferent stimulation during PECO, but further work is required to elucidate this possibility.

Figure 7.1 illustrates the possible integration of inputs to respiratory control areas of the brainstem which together may explain some of the findings in this thesis. In healthy individuals under normal conditions ventilation consistently returned to baseline levels during the stimulation of metaboreceptive afferents alone through PECO. This might be incorrectly interpreted that activation of the muscle metaboreflex has no effect on ventilation. Metaboreflex activation through PECO seems to give precedence to increasing sympathetic vasomotor activity and blood pressure, presumably to maintain adequate perfusion to the muscle and to wash out trapped metabolites. However this increase in blood pressure will suppress ventilation through the ventilatory baroreflex. Therefore any stimulatory effects that the metaboreflex has on ventilation may be cancelled by inhibitory inputs from the ventilatory baroreflex. Indeed this may also be much the same with the stimulation of mechanoreceptive afferents, as sustained passive calf stretching results in blood pressure increases but no ventilatory response under normal conditions.

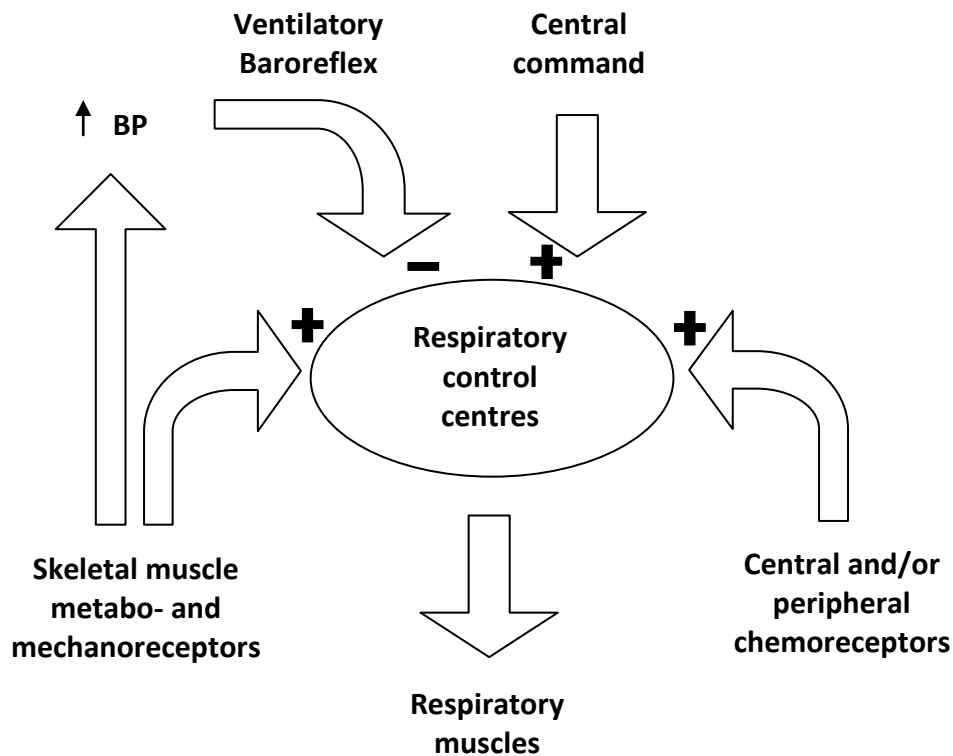


Figure 7.1 A simplified diagram illustrating the possible integration of central command, ventilatory baroreflex inputs, afferent feedback from skeletal muscle metabo/mechanoreceptors and neural feedback from central and/or peripheral chemoreceptors in respiratory control centres in the brainstem. + is a stimulatory input; - is an inhibitory input.

However under conditions of enhanced central respiratory drive, through systemic hypercapnia, ventilation was maintained significantly above baseline levels during PECO and further increased in response to sustained passive muscle stretching. This suggests a synergistic interaction between muscle afferent feedback and the afferent signals from

central and/or peripheral chemoreceptors. It may be that afferent feedback is only effective in driving ventilation in the presence of a synergistic input to the central respiratory neuronal pool. So in effect the chemoreflex may have unmasked the involvement of muscle afferents in ventilatory control.

The precise nature of this interaction is currently unknown. However it was suggested by Coote, (2012) that the hypercapnia-induced chemoreflex may ...

“increase the subliminal fringe of the central neuronal circuits by increasing the excitability of central respiratory neurones.... This enabled the central synaptic effects to summate sufficiently to activate the respiratory effectors. The results convincingly show that facilitation of central neurones by hypercapnia results in a significant increase in ventilation when either muscle mechanoreceptors alone or muscle metaboreceptors alone are activated”.

In other words the observations made in this investigation could be explained by the enhanced feedback from chemoreceptors during hypercapnia increasing the receptivity of respiratory neurones to inputs from muscle afferents. Therefore only under the hypercapnic conditions can the effects of muscle afferent feedback be observed when the combined actions of muscle afferent feedback and chemoreflex overcome the opposing action of the ventilatory baroreflex.

An alternative explanation could be that the feedback from skeletal muscle afferents may modulate the medullary input of the central and/or peripheral chemoreceptors and enhance their sensitivity to CO₂. This would result in a greater level of ventilatory drive for a given level of CO₂ and so would explain the ventilatory responses to PECO during concurrent

hypercapnia. However, although this is possible, it has been shown by several investigators (and in this thesis) that during exercise where muscle afferents are activated, CO₂ inhalation only has additive effects on ventilation, with no observable changes in CO₂ sensitivity reported (Asmussen & Nielson, 1957; Clark *et al.* 1980; Duffin *et al.* 1980; Poon *et al.* 1987).

Therefore a mechanism akin to that described by Coote (2012) is perhaps more likely, where the neural inputs from the chemoreceptors may sufficiently enhance the excitability of central respiratory neurones so that muscle afferent activation alone is able to stimulate ventilation. No enhanced ventilatory responses to exercise are observed during concurrent hypercapnia probably because muscle afferent feedback already appears able to stimulate breathing in exercise (Amann *et al.* 2010). Thus the hypercapnic stimulus may have no additional effect.

Despite the fact that the precise nature of this interaction is speculative, the findings of chapter 3 and 4 clearly suggest that: 1) muscle afferent feedback can stimulate ventilation (under some conditions) and 2) central interactions with muscle afferent feedback are possible. By attempting to apply these findings to more normal physiological conditions we then observed that PECO of one leg can cause increases in ventilation during the exercise of the contralateral leg. This also implies a possibly similar synergistic interaction to that described above between muscle afferent feedback and other inputs to the central neuronal pool active in exercise. These findings clearly suggest that muscle afferents are capable of driving ventilation in exercise. Indeed they seem to compliment the work of Amann *et al.* (2010) as at an equivalent work load for a two-legged cycling exercise the inhibition of muscle afferent neurotransmission attenuated the increase in ventilation, whereas this study has shown that the additional stimulation of metabolically sensitive muscle afferents enhances it. As central command can increase central respiratory drive,

and is active in exercise, this might be an input that plays a comparable role to that of the chemoreflex above.

Interactions between muscle afferent feedback, the chemoreflex and/or central command, shown in figure 7.1, are possible at different sites in the brainstem and two of these are illustrated in figure 7.2. The NTS is a vital integrating site in the dorsal brainstem for autonomic and respiratory regulation (Paton, 1999) and both peripheral chemoreceptor and skeletal muscle afferents are known to project there (Kalia et al. 1981, Donoghue et al. 1984, Li et al. 1998, Potts et al. 1999, Paton et al 2001). Furthermore it has been established that the NTS may be a site for central chemoreception (Nattie & Li 2002, 2009; Feldman 2003). As such the NTS may be a region where an interaction between muscle afferent feedback and neural feedback from chemoreceptors can occur.

However more recent evidence has suggested a role for the PAG as a potentially important site of neural integration in the brainstem for cardiorespiratory control (see Paterson, 2013 for review). Findings in humans made by Green et al (2005, 2007) suggests that the PAG may form part of the neurocircuitry of central command as the stimulation of the dorsal PAG can increase blood pressure and the anticipation to exercise and actual exercise causes increases in the neural activity of the PAG. It has also been established that the neural activity of the PAG can also be increased in response to muscle afferent activation via PECO (Basnayake *et al.* 2011). Therefore the PAG may also be a potential site of integration for central command and muscle afferent feedback in eliciting cardiorespiratory responses. Furthermore it has recently been shown that the PAG may also be a site sensitive to CO_2/H^+ as partial lesions of this area in a rats brainstem results in a significant reduction in the ventilatory response to hypercapnia (Lopes *et al.* 2012). All together these findings suggest that the PAG could conceivably be a site where muscle

afferents, central command and chemoreceptor activity can integrate and generate the respiratory responses observed in the chapters 3-5.

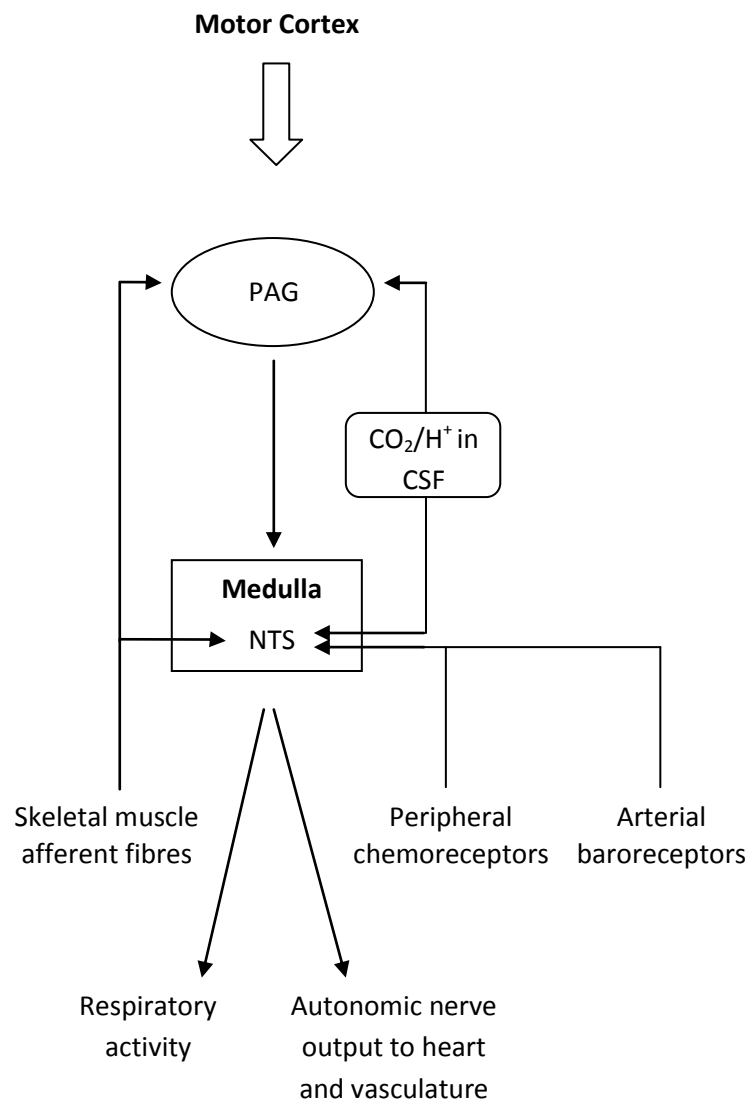


Figure 7.2 Possible sites of integration in the brainstem between central command, muscle afferent feedback and neural feedback from central/peripheral chemoreceptors. PAG, Periaqueductal gray; NTS, nucleus tractus solitarius; CSF, cerebral spinal fluid.

However there is evidence that under some conditions PECO alone can induce ventilatory responses if the metabolite accumulation and metaboreflex activation is great enough. For example in healthy individuals ventilation can remain just above baseline in PECO if the preceding exercise is at high enough intensity and in a large enough muscle mass (Iellamo *et al.* 1999). In addition patient groups, that have been associated with abnormalities in skeletal muscle metabolism, have also shown ventilatory responses to PECO, which includes CHF (e.g. Piepoli *et al.* 1996) and now COPD (chapter 6). These findings are seemingly incompatible with integration of inputs and resultant ventilatory responses described above and illustrated in figures 7.1 and 7.2, as if metaboreflex activity is greater then arguably so will the effects of the ventilatory baroreflex. However the diseased states above tend to display a tonic activation of the sympathetic nervous system which is associated with reduced α -adrenergic vasoconstrictor responsiveness (Seals *et al.* 2004). This could result in an attenuated blood pressure response for a given level of metaboreflex activity. Furthermore in healthy or diseased states, once metaboreflex activity and blood pressure is great enough the ventilatory baroreflex may hit its saturation point where no further increase in blood pressure results in a suppression of ventilation. As such from this point onwards metaboreflex activation may be able to drive increases in ventilation. One way this could be examined experimentally would be to measure the ventilatory responses to PECO while manipulating blood pressure, perhaps via the modified Oxford technique.

Final conclusion

In summary stimulation of skeletal muscle metabo and mechanosensitive afferents with PECO and passive muscle stretching do not consistently stimulate ventilation. Used in isolation these techniques may not be appropriate methods for examining the role of muscle afferents in ventilatory control which may in part be due to the effects of the ventilatory baroreflex. During systemic hypercapnia both PECO and passive muscle stretching stimulates ventilation suggesting a synergistic interaction between muscle afferent feedback and the afferent signals from central and/or peripheral chemoreceptors. It is suggested that feedback from the ventilatory chemoreflex may alter the receptivity/sensitivity of respiratory neurones to inputs from muscle afferents. Therefore only under the hypercapnic conditions can the effects of muscle afferent feedback be observed when the combined actions of muscle afferent feedback and chemoreflex overcome the opposing action of the ventilatory baroreflex. Similar interactions may exist between muscle afferents and inputs active during exercise (e.g. central command) as additional metaboreflex activation can augment the ventilatory response to exercise but not PECO. However the activation of the metaboreflex in isolation via PECO maintains ventilation significantly above baseline in COPD patients. These ventilatory responses to PECO were not related to $PaCO_2$. These findings may add to the emerging concept that skeletal muscle afferent feedback may contribute to the augmented ventilatory responses and sensations of dyspnea associated with COPD during exercise, but further work is required.

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APPENDIX

The ventilatory and cardiovascular responses to augmented muscle metaboreflex activation during a static calf plantarflexion exercise

This Data was taken from 8 participants performing a similar protocol to that in chapter 5 except that a 2 minute static calf plantaflexion exercise was employed (Figure 8.1).

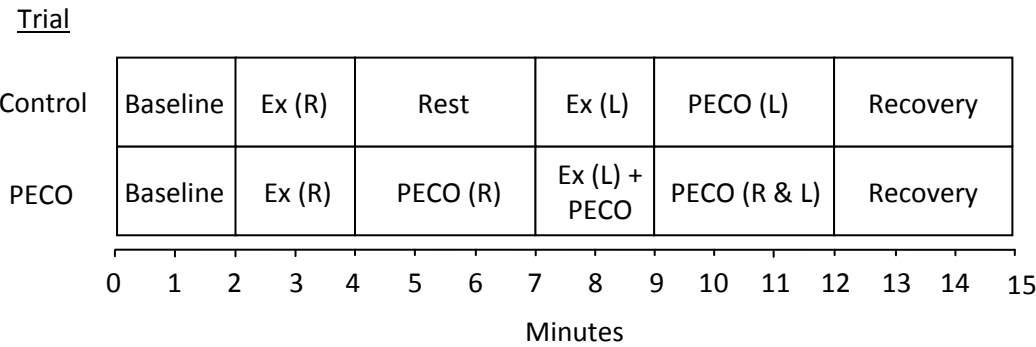


Figure 8.1 Schematic diagram of the protocol. Ex signifies the period of one static calf plantarflexion exercise. PECO signifies the period of post exercise circulatory occlusion. R and L signifies whether the right or left leg respectively is performing the exercise or undergoing PECO.

Differences between trials at baseline

The mean \dot{V} , HR, MAP and $P_{ET}CO_2$ during baseline are presented in Table 1. There were no significant differences in any of these variables between the Control and PECO trials.

Trial	$V_I(l.min^{-1})$	HR (beats.min ⁻¹)	MAP (mmHg)	$P_{ET}CO_2$ (mmHg)
Control	9.77 ± 1.4	72 ± 2.9	91 ± 3.4	38 ± 1
PECO	10.48 ± 0.7	71 ± 2.2	94 ± 3.9	39 ± 1

Table 8.1 Mean values recorded during the two minute baseline period. * Significant difference from Control trial ($P<0.05$).

Respiratory responses

Figure 2 shows changes in mean minute ventilation relative to baseline during the control trial and PECO trial. Left leg exercise significantly increased Minute ventilation from baseline by $+4.2 \pm 1 l.min^{-1}$ and $4.4 \pm 0.8 l.min^{-1}$ ($P<0.05$) in the Control and PECO trials respectively. Minute ventilation returned back to baseline levels following exercise. Right leg exercise then again significantly increased Minute ventilation in both the Control ($+4.3 \pm 0.6 l.min^{-1}$; $P<0.05$ versus baseline) and PECO trials ($4.5 \pm 0.7 l.min^{-1}$; $P<0.05$ versus baseline). Minute ventilation returned back to baseline levels. There was no significant difference in minute ventilation between the trials.

Figure 3 shows the mean $P_{ET}CO_2$ values during the different periods of the 2 trial conditions. Throughout both trials, there was no significant change in $P_{ET}CO_2$ from baseline levels and no difference between the two trials

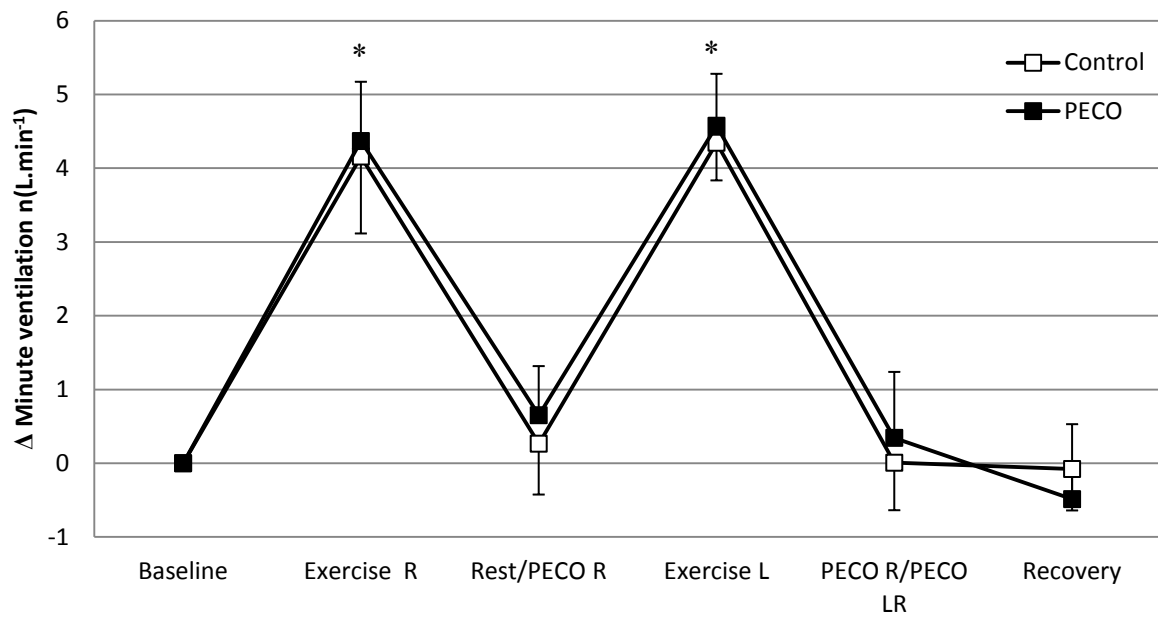


Figure 8.2 Change in Minute ventilation from baseline during each phase of the Control and PECO trials. * Significant difference from Baseline value ($P<0.05$).

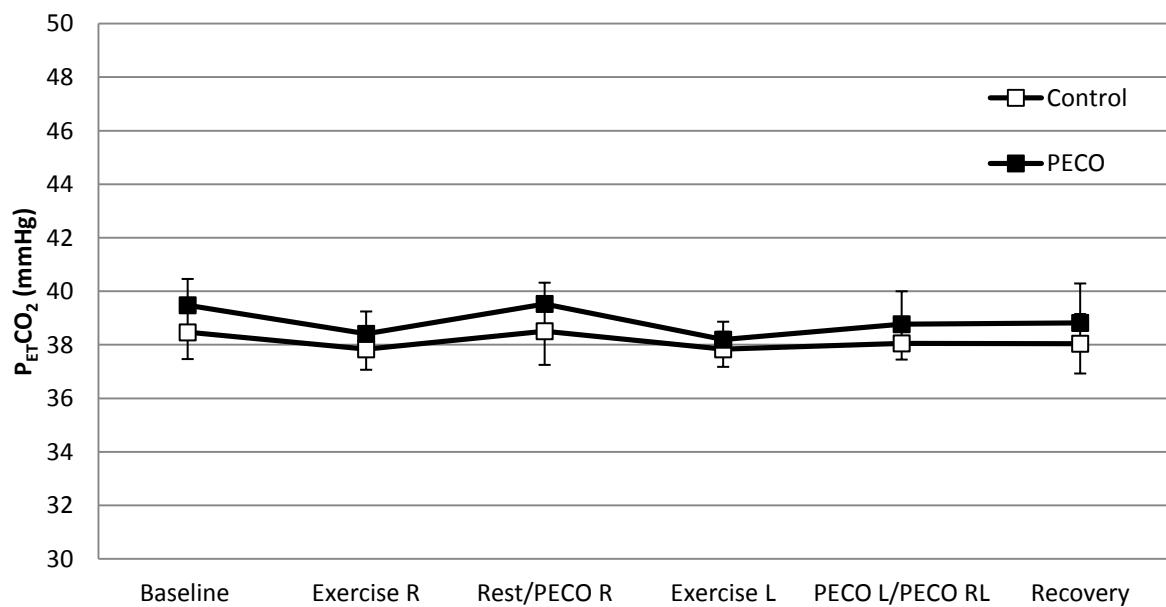


Figure 8.3 P_{ETCO_2} during each phase of the Control and PECO trials. * Significant difference from Baseline value ($P<0.05$).

Cardiovascular responses

Changes in mean MAP and HR relative to baseline in the 2 trials are shown in figures 4 and 5 respectively. Both MAP and HR during Exercise R increased similarly in both the Control trial (15 ± 2 mmHg and 18 ± 3 beats.min⁻¹; $P < 0.05$ versus baseline) and in the PECO trial (15 ± 3 mmHg and 19 ± 2 ; $P < 0.05$ versus baseline). As expected on cessation of exercise HR fell from exercise levels and returned to baseline in the Control trial and PECO trials and MAP also returned to baseline in the Control trial. However in the PECO trial MAP remained significantly elevated above baseline during PECO R ($P < 0.05$).

During Exercise L both HR again increased similarly in both the Control trial (19 ± 2 beats.min⁻¹; $P < 0.05$ versus baseline) and in the PECO trial (21 ± 2 ; $P < 0.05$ versus baseline). MAP also significantly increased in the Control trial (15 ± 3 mmHg; $P < 0.05$ versus baseline) and from an increased level MAP also increased in the PECO trial (19 ± 4 mmHg; $P < 0.05$). The exercise of the left leg produced a similar increase in MAP from the previous period (Rest/PECO R) in both the Control (12 ± 3 mmHg) and PECO trials (11 ± 3 mmHg).

Following the exercise of the left leg HR returned to baseline in the Control trial and PECO trials. However MAP remained significantly elevated above baseline during both the Control and PECO trials ($P < 0.05$). During the recovery periods of each trial MAP and HR values were not significantly different from baseline ($P > 0.05$).

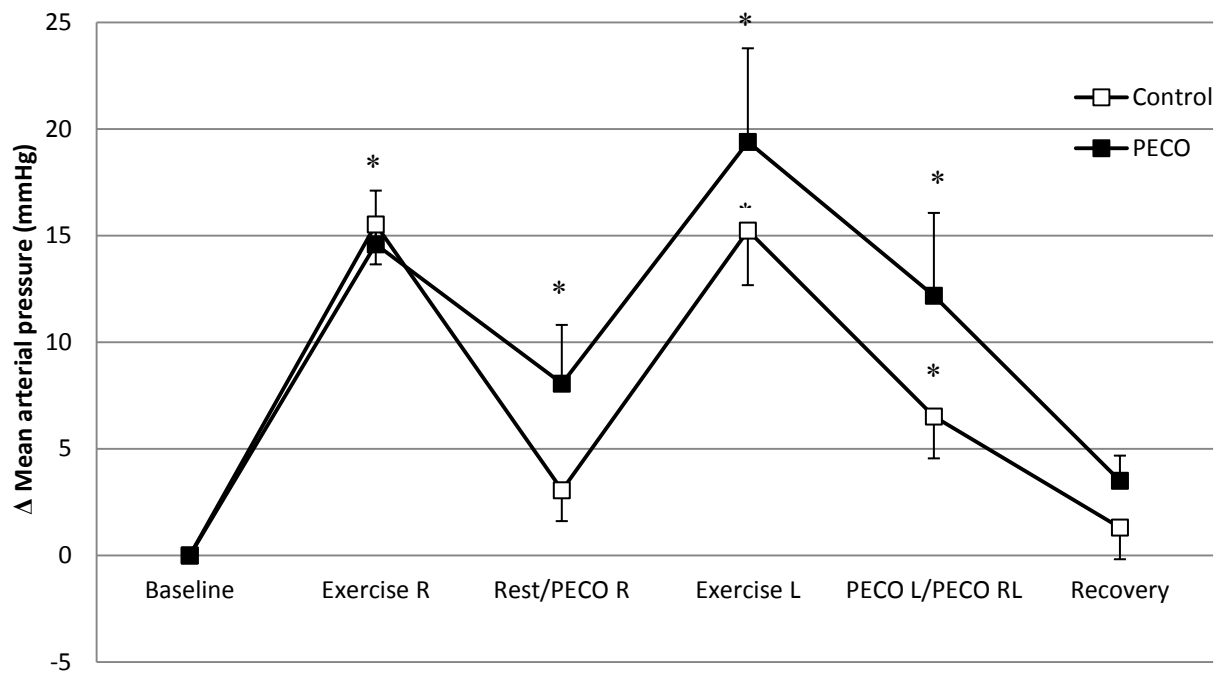


Figure 8.4 Change in Mean arterial pressure from baseline during each phase of the Control and PECO trials. * Significant difference from Baseline value ($P<0.05$).

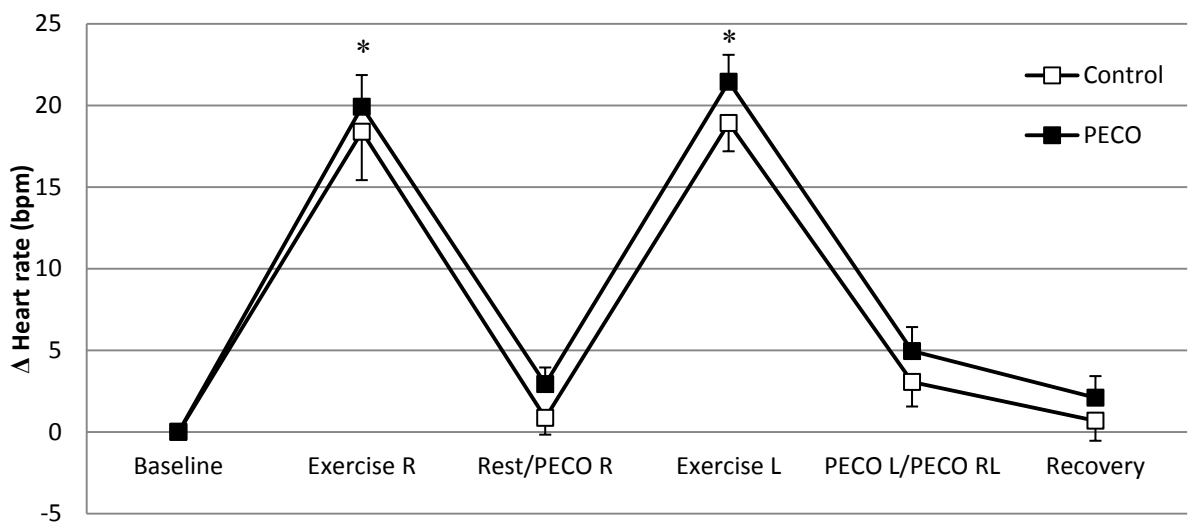


Figure 8.5 Change in Heart rate from baseline during each phase of the Control and PECO trials. * Significant difference from Baseline value ($P<0.05$).

The ventilatory and cardiovascular responses to muscle metaboreflex activation in COPD

Table 8.2 shows additional \dot{V} , HR, MAP and $P_{\text{ET}}\text{CO}_2$ data from the 18 COPD patients when divided in to normocapnic/hypercapnic subgroups.

Trial	\dot{V} (l.min ⁻¹)	HR (beats.min ⁻¹)	MAP (mmHg)	$P_{ET}CO_2$ (mmHg)
COPD Control (normocapnic)				
<i>Baseline</i>	8.86 ± 0.97	69 ± 2.9	91 ± 1.7	37 ± 1.4
<i>Exercise 3</i>	13.37 ± 1.13	77 ± 2.1	99 ± 2.3	38 ± 2
<i>Exercise 4</i>	16.8 ± 1.29	82 ± 1.6	106 ± 1.1	37 ± 2.8
<i>Rest/PECO 5</i>	10.86 ± 0.63	76 ± 1.4	91 ± 1.3	39 ± 2.6
<i>Rest/ PECO 6</i>	7.85 ± 0.45	69 ± 1	90 ± 1.6	37 ± 1.6
<i>Recovery 7</i>	7.82 ± 0.98	69 ± 0.9	90 ± 2.1	37 ± 1.5
<i>Recovery 8</i>	7.97 ± 0.89	69 ± 1.6	89 ± 2.4	38 ± 1.6
COPD PECO (normocapnic)				
<i>Baseline</i>	9.67 ± 0.9	70 ± 2.6	93 ± 2.9	37 ± 1.6
<i>Exercise 3</i>	14.07 ± 0.93	78 ± 1.2	99 ± 1.6	38 ± 2.1
<i>Exercise 4</i>	17.56 ± 1.63	82 ± 2.4	106 ± 1.9	36 ± 2.8
<i>Rest/PECO 5</i>	12.78 ± 1.18	74 ± 2	101 ± 2.6	37 ± 2.2
<i>Rest/ PECO 6</i>	12.6 ± 0.98	72 ± 1.5	101 ± 1.1	36 ± 2.3
<i>Recovery 7</i>	9.36 ± 0.82	72 ± 1.7	91 ± 1.3	37 ± 1.2
<i>Recovery 8</i>	8.42 ± 0.57	71 ± 1.5	91 ± 3	38 ± 3
COPD Control (hypercapnic)				
<i>Baseline</i>	10.62 ± 0.55	73 ± 2.2	87 ± 3.2	52 ± 1.1
<i>Exercise 3</i>	14.73 ± 0.93	79 ± 2.9	93 ± 0.9	52 ± 2.9
<i>Exercise 4</i>	18.38 ± 1.63	87 ± 3.2	101 ± 2.6	51 ± 3.2
<i>Rest/PECO 5</i>	13.08 ± 0.72	77 ± 1.2	87 ± 1.9	51 ± 4.1
<i>Rest/ PECO 6</i>	10.49 ± 1.11	74 ± 1	85 ± 1.4	52 ± 3.6
<i>Recovery 7</i>	10.64 ± 1.25	74 ± 2.1	85 ± 1.6	52 ± 3.8
<i>Recovery 8</i>	10.05 ± 1.31	74 ± 2.4	86 ± 3.1	52 ± 2.2
COPD PECO (hypercapnic)				
<i>Baseline</i>	11.08 ± 0.97	74 ± 1.9	89 ± 3.6	51 ± 1.6
<i>Exercise 3</i>	15.23 ± 0.89	79 ± 1.2	97 ± 1.5	51 ± 2.3
<i>Exercise 4</i>	19.71 ± 1.69	87 ± 2.3	103 ± 1.8	50 ± 3.4
<i>Rest/PECO 5</i>	15.01 ± 1.13	78 ± 3	97 ± 1.3	49 ± 4.1
<i>Rest/ PECO 6</i>	13.8 ± 0.92	75 ± 2.9	96 ± 1.9	49 ± 4.8
<i>Recovery 7</i>	12.14 ± 0.85	75 ± 1.3	89 ± 2.1	50 ± 2.1
<i>Recovery 8</i>	10.37 ± 0.69	73 ± 1.6	89 ± 2.9	51 ± 1.8

Table 8.2. Mean \dot{V} , HR, MAP and $P_{ET}CO_2$ during each minute of the control and PECO trials.

Participants have been separated into hypercapnic and normocapnic groups.