The Role of the Muscle Metaboreflex in Patients with Chronic Disease

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Summary

Exercising muscle needs a constant supply of oxygen for the aerobic metabolism of carbohydrate and fat, and regulation of the blood supply to muscle during exercise is therefore critical. Heart rate, stroke volume and minute ventilation all increase during exercise, and sympathetic vasoconstriction diverts blood to exercising muscle. It is well recognised that receptors in skeletal muscle play a vital role in the regulation of blood flow, including receptors sensitive to products of anaerobic metabolism such as lactate and hydrogen ions: *metaboreceptors*. Activation of the muscle metaboreflex signals the need for an increase in blood flow, and leads to an increase in cardiac output, ventilation and sympathetic vasoconstriction to non-essential organs.

Exercise intolerance is one of the most disabling symptoms in patients with a range of cardiorespiratory diseases. Abnormalities of skeletal muscle favouring anaerobic metabolism have been documented in both chronic heart failure and chronic obstructive pulmonary disease (COPD), and this is thought to be relevant to exercise limitation in these diseases. Studies looking at patients with chronic heart failure have demonstrated an increase in muscle metaboreflex activity. It is thought that abnormal skeletal muscle generates greater quantities of anaerobic metabolites, leading to increased metaboreceptor activation. This in turn causes an increased sympathetic nervous system and ventilatory response to exercise. Patients with COPD have been shown to demonstrate similar skeletal muscle abnormalities, so we hypothesised that we would also find an increase in muscle metaboreflex activity in this group.

It is possible to quantify muscle metaboreflex activity by exercising a small muscle group to fatigue then isolating it from the rest of the circulation with a sphygmomanometer cuff. This traps the metabolic products of exercise in the muscle and leads to prolonged stimulation of metaboreceptors. This can be measured as a sustained increase in blood pressure and ventilation when compared with control recovery without cuff inflation.

The aims of this thesis were as follows: (i) to assess if it is possible to quantify the muscle metaboreflex in a group of patients with COPD and to determine whether muscle metaboreflex activity is increased in patients with more severe disease, (ii) to determine whether supplementation with oral creatine monohydrate alters muscle metaboreflex activity, upper limb strength or endurance and respiratory muscle strength in patients with COPD, (iii) to assess the effects of diabetic autonomic neuropathy on muscle metaboreflex function, and (iv) to evaluate whether pulse transit time is of use in the measurement of muscle metaboreflex activity.

In our first study, we looked at a group of patients with stable COPD and found that rhythmic forearm exercise followed by post-exercise forearm ischaemia led to a sustained increase in blood pressure and minute ventilation when compared with control recovery. These findings are in keeping with previously published observations in normal subjects and in patients with chronic heart failure. We found that there was no difference in muscle metaboreflex activity between the groups of patients with moderate or severe disease.

We then performed a randomised, double-blind, placebo-controlled, crossover trial looking at the effects of loading a group of patients with stable COPD with creatine monohydrate. We demonstrated a small increase in body weight and an increase in peak inspiratory and

expiratory mouth pressures, but there were no effects on muscle metaboreflex activity or forearm muscle strength, endurance or recovery.

A group of patients with type I diabetes mellitus was then used to study the effects of autonomic neuropathy on muscle metaboreflex function. We found that there was no difference in metaboreflex activity between subjects with diabetic autonomic neuropathy and subjects with diabetes but no evidence of autonomic neuropathy, suggesting that the afferent and efferent limbs of the muscle metaboreflex were intact.

Our final study evaluated whether pulse transit time could be used to assess muscle metaboreflex activity. Pulse transit time is defined as the time taken for a pulse wave to travel between two arterial sites, and can be easily and non-invasively measured. It is thought to reflect blood pressure and arterial tone. In a group of healthy subjects, we found that pulse transit time fell with rhythmic handgrip exercise, and post-exercise muscle ischaemia led to a sustained fall in pulse transit time when compared with control recovery. Pulse transit time therefore shows promise in the measurement of muscle metaboreflex activity, but further studies are required. Studies comparing pulse transit time with more invasive measurements such as muscle sympathetic nerve activity would be of particular interest.

List of Contents

Contents

Chapter 1   Introduction and literature review

1.1   Regulation of oxygen delivery to exercising muscle

1.2   A review of the muscle metaboreflex

Chapter 3   The muscle metaboreflex in patients with stable chronic obstructive pulmonary disease

Chapter 6   The effects of creatine supplementation on patients

with stable COPD

Chapter 8   General discussion

List of Tables and Figures

Chapter 4   The assessment of the muscle metaboreflex using pulse transit time

Chapter 5   The muscle metaboreflex in patients with diabetic

autonomic neuropathy

Chapter 6   The effects of creatine supplementation on patients
with stable COPD

Chapter 7   Methodological issues

Publications

Abstracts

Grieve DA, Cotton MM. The ergoreflex in patients with stable chronic obstructive pulmonary disease. Poster discussion session at the meeting of the American Thoracic Society, Orlando, USA, 2004; A903.

Grieve DA, Cotton MM, Wilson LE. Assessment of muscle metaboreflex activity using pulse transit time. Poster presentation at the meeting of the International Union of Physiological Sciences, San Diego, USA, 2005.

Grieve DA, Mukhopadhyay B, Fisher BM, Wilson LE, Cotton MM. The muscle metaboreflex in patients with diabetic autonomic neuropathy. Poster presentation at the meeting of the International Union of Physiological Sciences, San Diego, USA, 2005.

Grieve DAA, Mukhopadhyay B, Cotton MM, Fisher BM. The muscle metaboreflex in patients with diabetic autonomic neuropathy. Poster presentation at the meeting of Diabetes UK, Glasgow, UK, 2005.

Cotton MM, Grieve DAA, Lean MEJ. The effects of short-term creatine loading in stable chronic obstructive pulmonary disease. Poster presentation at the meeting of the American Thoracic Society, San Diego, USA, 2006; A460.

Abbreviations

ADP	adenosine diphosphate	**HbA$_{1C}$**	haemoglobin A$_{1C}$
ANOVA	analysis of variance	**IGF-1**	insulin-like growth factor 1
ATP	adenosine triphosphate	**IL-6**	interleukin 6
BDI	baseline dyspnoea index	**kg**	kilograms
BMI	body mass index	**kg/m^2**	kilograms per metre squared
BP	blood pressure		
bpm	beats per minute	**l**	litres
CHF	chronic heart failure	**LED**	light emitting diode
cm	centimetres	**min**	minutes
cmH$_2$O	centimetres of water	**ml**	millilitres
CO$_2$	carbon dioxide	**mmHg**	millimetres of mercury
COPD	chronic obstructive pulmonary disease	**mmol**	millimoles
		μmol	micromoles
DAN	diabetic autonomic neuropathy	**min**	minutes
		MRC	medical research council
ECG	electrocardiogram	**ms**	milliseconds
EMG	electromyography	**MSNA**	muscle sympathetic nerve activity
FEV$_1$	forced expiratory volume in 1 second	**MVC**	maximal voluntary contraction
FVC	forced vital capacity		
g	grams	**nNOS**	neuronal nitric oxide synthetase
GOLD	global initiative for obstructive lung diseases	**ns**	non-significant

NYHA	New York Heart Association criteria	**RCO**	regional circulatory occlusion
pCO$_2$	partial pressure for carbon dioxide	**RPM**	revolutions per minute
		s	seconds
PCr/P$_i$	ratio of phosphocreatine to inorganic phosphate	**SEM**	standard error of the mean
		TNF-α	tumour necrosis factor alpha
PEP	pre-ejection period		
pg	picograms	**VCO$_2$**	carbon dioxide production per minute
PH-RCO	post-handgrip regional circulatory occlusion		
		VE	minute ventilation
^{31}P NMR	^{31}P nuclear magnetic resonance spectroscopy	**VO$_2$**	oxygen uptake per minute
		VO$_2$ max	uptake of oxygen at maximal exercise
pO$_2$	partial pressure for oxygen		
PTT	pulse transit time		

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Declaration

This study represents original work carried out by the author, and has not been

submitted in any form to any other University.

Douglas Grieve

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Chapter 1

Introduction and Literature Review

1.1 Regulation of oxygen delivery to exercising muscle

1.1.1 General principles

The human body has a remarkable ability to adapt to the demands placed upon it during exercise. Exercising skeletal muscle requires a constant source of energy, and the ability to increase energy supply immediately is essential. The conversion of phosphocreatine to creatine through the creatine kinase reaction provides an immediate source of adenosine triphosphate (ATP), although stores are limited and this is only the major source of energy during the first few seconds of intense exercise . The anaerobic metabolism of muscle glycogen then becomes important, although this is inefficient with the production of only 3 mmol of ATP per mmol of glycogen . Oxidative metabolism of carbohydrate and free fatty acids is a much more efficient form of energy production, with 1mmol of glycogen providing 36mmol of ATP, and 1mmol of lipid (eg palmitate) providing 130 mmol of ATP . As exercise progresses, skeletal muscle therefore relies on the oxidative metabolism of carbohydrates and free fatty acids, and this necessitates a vast increase in oxygen delivery. This is achieved through the following mechanisms:

- Cardiac output increases up to 6-fold through an increase in heart rate and stroke volume, increasing pulmonary blood flow and blood flow to exercising muscle .
- An increase in sympathetic nervous system activity causes vasoconstriction of the vascular bed of non-essential organs (eg intestine) and non-exercising muscle .

- Minute ventilation increases up to 30-fold through an increase in respiratory rate and tidal volume, thus increasing the delivery of oxygen to the pulmonary capillaries .

- Local factors play a role, with the increase in temperature and reduction in pH associated with exercising muscle assisting oxygen unloading from haemoglobin through a rightward shift in the oxygen-haemoglobin dissociation curve .

A feedback mechanism must exist to match oxygen availability to the demand of the exercising muscle. If demand for oxygen exceeds availability, either through a high intensity of exercise or impairment of blood flow or oxygenation (as may happen in certain disease states), anaerobic metabolism of glycogen leads to the production of lactate and the onset of intramuscular acidosis with the creation of an oxygen debt . Accumulation of lactate and acidosis are associated with the onset of muscular fatigue .

1.1.2 Central command

The exact mechanisms behind this feedback mechanism are controversial, and mechanisms driving ventilation and cardiovascular responses during exercise differ. "Central command" is important during the initial stages of exercise in humans . Anticipation of exercise leads to a withdrawal of resting parasympathetic tone and a resultant increase in heart rate and cardiac output . The idea of central command was first suggested by Krogh and Lindhard in 1913 , who talked about the "irradiation" of the command to exercise from the cerebral cortex to the cardiovascular and respiratory

centres. This idea is supported by experiments looking at partial neuromuscular blockade both in man and in animal models, where the increased central command to generate equivalent work causes an increased blood pressure and heart rate response to exercise . Further insight into the importance of central command has been gained from experiments on a series of patients with Brown-Séquard syndrome, where hemisection of the spinal cord leads to sensory loss on one side of the body and paralysis on the other side . Attempts to contract the leg with a motor deficit led to the highest ratings of perceived exertion and the greatest elevations in blood pressure and heart rate, despite generation of the lowest force. Central command does not, however, appear to play an important part in the increase in sympathetic nerve activity with exercise. It has been observed that during forearm exercise, muscle sympathetic nerve activity (MSNA) does not start to increase until about 2 minutes of exercise has been completed, well after the initial heart rate and blood pressure response usually attributed to central command . Studies using partial neuromuscular blockade to increase central command suggest that near maximal levels of central command are necessary to evoke an increase in muscle sympathetic nerve activity . Other factors are therefore involved in the generation of an increase in MSNA with exercise, with central command merely initiating the efferent activity to the heart and blood vessels in parallel with recruitment of motor units to perform work.

1.1.3 Afferents arising from exercising muscle

With the progression of exercise, cardiovascular responses are modulated by a feedback mechanism arising from working muscle. *Mechanoreceptors* probably contribute to circulatory regulation at the start of exercise, and are sensitive to pressure and tension stimuli. Dejours in 1964 postulated that a reflex from moving limbs, probably originating in the muscle spindle, could be responsible for the fast adaptation of the cardiorespiratory system to exercise. Mechanoreflex activation would appear to have rapid effects on the cardiovascular system, with static contraction of triceps surae increasing renal sympathetic nerve activity after a delay of only 1 second . Passive stretch of triceps surae has been shown to lead to rapid increases in heart rate and blood pressure in animals: the immediate onset and rapid recovery of group III afferent nerve activity is consistent with mechanoreceptor activation . It has, however, been shown that muscle spindles and Golgi tendon organs, the afferent fibres of which are large group I and II afferents, do not elicit cardiovascular reflexes , and blockade of group I and II afferents does not affect cardiovascular responses to muscle contraction . Evidence suggests that mechanoreceptors are particularly important during mild handgrip exercise .

Metaboreceptors are the other main form of receptor within skeletal muscle involved in cardiorespiratory responses to exercise. These sense metabolic changes within the working muscle.

This thesis will concentrate on the role of the muscle metaboreflex.

1.2 A review of the muscle metaboreflex

1.2.1 The history of the muscle metaboreflex

The first mention of the possibility of the existence of muscle metaboreceptors was by Zuntz and Geppart in 1886 . They postulated that any mismatch between muscle blood flow and metabolism would change the concentration of metabolites within the muscle, which would be detected by chemosensitive afferent fibres in the muscle. The efferent arm of the reflex, the sympathetic nervous system, would increase blood pressure, increasing blood flow to muscle, which would reduce the concentration of accumulated metabolites. This theory, however, was not tested until 1937, when Alam and Smirk performed their classic experiments looking at the arrest of blood flow following localised exercise of a small group muscles. They inflated a sphygmomanometer cuff round one upper arm and then asked the subject to perform repetitive exercise of the forearm muscles. Blood pressure increased with exercise, and fell rapidly to resting levels on cessation of exercise. If the cuff remained inflated beyond cessation of exercise, they discovered that blood pressure remained at peak exercise levels until the cuff was deflated. This experiment produced similar results when repeated on the lower limb, and when circulatory arrest was achieved by exercising the forearm in a bath of mercury. They also observed that the rise in blood pressure during exercise with circulatory occlusion depended on the total work done, and that the maintained elevation in blood pressure with circulatory occlusion post-exercise occurred in the absence of pain, and was independent of the degree of pain. The conclusion drawn was that the arrest of the circulation on cessation of exercise led to the accumulation of

metabolic products of exercising muscle and the stimulation of afferent nerves, causing a sustained increase in blood pressure.

The physiological responses to the arrest of blood flow following exercise were revisited by Asmussen and Nielsen in 1963 . They asked healthy volunteers to exercise on a cycle ergometer until steady state was reached, then inflated sphygmomanometer cuffs round both thighs to 300mmHg. Subjects were asked to continue exercising for 5 minutes with the cuffs inflated. During this period, p_ACO_2 was kept constant by addition of CO_2 to inspired air. VO_2 fell by 50% due to the isolation of exercising muscle from the circulation: the muscle was therefore reliant on anaerobic metabolism to provide energy. Ventilation steadily increased throughout this period, as did blood pressure and peripheral resistance. The conclusions drawn were that either anaerobic metabolism led to stimulation of intramuscular chemoreceptors, or that a reduction in tension and shortening in each muscle fibre led to an increase in recruitment of muscle fibres. The possibility of muscle chemoreceptor activation stimulating the respiratory control centres was raised. It was noted that pain was not experienced during the experiments, thus discounting the possibility of pain during cuff inflation stimulating ventilation and the pressor response.

1.2.2 Neural mechanisms of the muscle metaboreflex

Much of our insight into the mechanisms of the muscle metaboreflex has been gained from experiments on anaesthetised animals, allowing us to separate responses to exercise from other factors such as voluntary control and responses to pain or discomfort. McCloskey and Mitchell established beyond any doubt the existence of a neural signal arising from exercising muscle in 1972 using anaesthetised cats . Isometric exercise of the hindlimb muscles was elicited by stimulating ventral spinal roots L7-S1, which led to a rise in blood pressure, ventilation and heart rate. Blood pressure and ventilatory responses were abolished by severing the dorsal (sensory) roots, which demonstrated the importance of afferent signals from muscle, but did not confirm the nature of the receptors. The investigators then exercised the triceps surae muscle by ventral root stimulation with the femoral blood vessels occluded during and after exercise. Blood pressure remained elevated after cessation of exercise until the occlusion was removed, though heart rate and ventilation were not affected. This led to the conclusion that a local metabolite of exercise was eliciting these responses. Injection of an irritant in the form of either 5% sodium chloride or isotonic potassium chloride into the muscle led to similar cardiovascular and ventilatory responses as isometric exercise, though this was not seen with isotonic sodium chloride: again these responses were abolished by severing the dorsal roots. The final experiment demonstrated that preferential blockade of unmyelinated and small myelinated nerve fibres abolished cardiovascular and respiratory responses, but blockade of large nerve fibres did not. The conclusion drawn was that the metaboreceptor response to exercise is mediated by group III and IV (small myelinated and unmyelinated) nerve fibres. Experiments by Tibes on anaesthetised dogs support these conclusions, as does work

by Kaufman *et al* , who demonstrated that ischaemic contraction of cat hindlimb muscle leads to an increased discharge of group IV afferents compared with non-ischaemic contraction despite the same amount of work being performed. Ischaemic contraction was presumed to have led to an increased production of metabolites of anaerobic metabolism with a resultant increase in metaboreflex activation. Although it is clear that muscle ischaemia during exercise potentiates the metaboreflex, a work stimulus is required, with muscle ischaemia alone insufficient to generate a pressor response .

Many of the animal experiments looking at the metaboreflex have used electrical stimulation of ventral roots or peripheral nerves. Adreani *et al* noted that this leads to recruitment of α-motoneurones with the fastest conduction velocities first, the opposite of what is seen during dynamic exercise . They addressed this issue by looking at electrical stimulation of the mesencephalic locomotor region in the midbrain to simulate dynamic exercise in cats. It was found that group III afferents discharged in synchrony with muscle contraction, consistent with mechanoreflex activation, unlike group IV afferents, which probably responded to a metabolic stimulus.

1.2.3 The muscle metaboreflex: mechanisms of activation

As the metaboreflex has evolved to sense the need to increase oxygen delivery to skeletal muscle, it follows that it must be a product of muscle metabolism that stimulates the metaboreceptors. What that metabolic stimulus is has been a source of much debate. Rotto & Kaufman attempted to address this question in a cat model through the intra-arterial injection of exogenous substances believed to be the metabolic products of muscular contraction. Substances used were chosen on the basis of studies looking at contraction of skeletal muscle and which metabolites are elevated in the venous outflow of working muscle. Only lactic acid and arachidonic acid had excitatory actions on the discharge of group III and IV afferents innervating the triceps surae muscle. Lithium, sodium lactate and adenosine did not have a substantial effect. Further work by the same group suggested that arachidonic acid potentiates the responses of group III muscle afferents to static contractions, probably through increased sensitivity of the mechanoreflex. Indomethacin and aspirin, both inhibitors of cyclo-oxygenase, appear to attenuate the response of group IV afferents to static contraction , and the prostaglandins PGE_2 and $PGF_{1\alpha}$ in effluent blood from exercised muscle haven been shown to correlate with metaboreflex activity in patients with heart failure . Adenosine has also been implicated in the generation of the metaboreflex in patients with heart failure: caffeine, an adenosine receptor antagonist, attenuates the rise in muscle sympathetic nerve activity (MSNA) with isometric handgrip exercise . The role of interstitial potassium, as suggested by Wildenthal *et al* and Rybicki *et al* has been called into question following experiments on humans documenting the lack of correlation between venous potassium from the exercising forearm and MSNA

response during static and dynamic handgrip exercise . A recent review promoted the idea that ATP could be important in evoking the metaboreflex .

Hydrogen ion and the development of acidosis within skeletal muscle are thought to stimulate the metaboreflex. Hydrochloric acid injected into the arterial supply of triceps surae of the cat increases heart rate, blood pressure and ventilation . Equimolar lactic acid had an even more potent effect on cardiorespiratory reflexes, though injection of sodium lactate at neutral pH did not have any effect. Lactic acid is a metabolite of anaerobic glycolysis, so it would make sense that it has a part to play in the generation of a signal that more oxygen is required. With the advent of ^{31}P Nuclear Magnetic Resonance Spectroscopy (^{31}P NMR), it has become possible to test this hypothesis on humans. Victor *et al* asked 11 healthy human volunteers to perform static and rhythmic handgrip exercise whilst recordings of ^{31}P NMR spectra and MSNA were made. During the first 2 minutes of exercise, ADP increased and PCr/P_i (ratio of phosphocreatine to inorganic phosphate) declined with no change in pH or MSNA. During the 3rd and 4th minutes of exercise, there was little further change in PCr/P_i, but pH decreased. MSNA increased progressively as pH decreased, suggesting that the development of acidosis is a potent stimulant of MSNA. Prolonged bouts of rhythmic handgrip exercise at a low workload, however, can lead to a progressive rise in MSNA without the development of significant muscle acidosis , suggesting that the mechanoreflex is perhaps more important in this setting.

Interesting insight into the role of lactic acid and acidosis has been gained from a group of patients with McArdle's disease, who do not produce lactic acid due to a hereditary deficiency of myophosphorylase. Even this has proven to be controversial. Pryor *et al*

found that there was an abnormal MSNA response to static handgrip exercise in patients with McArdle's disease, and Fadel *et al* produced similar results, whilst demonstrating that the MSNA response to other reflex stimuli (for example the cold pressor test) is intact. These data suggest that the glycogenolytic pathways are necessary for metaboreflex-mediated sympathoexcitation to occur during static exercise in humans. This is contradicted by the work of Vissing *et al* , who found that patients with McArdle's disease had a normal MSNA response to static handgrip exercise to fatigue, despite muscle pH not falling with exercise as happened in the healthy control subjects. They also studied one patient with mitochondrial myopathy, who developed a profound muscle acidosis with exercise, but did not have an enhanced MSNA response to exercise. It could be argued that MSNA in patients with McArdle's disease is stimulated by an increase in central command or mechanoreflex activation, though if this were the case, an immediate increase in MSNA would be expected: the time course of the MSNA response to exercise more closely resembles that of a metaboreflex mediated increase in MSNA. Further work by the same group looking at post-exercise muscle ischaemia, which causes prolonged metaboreceptor activation, found that metaboreflex activity was normal in this group despite a failure to produce lactate . Finally, it was noted in one study on healthy human volunteers that muscle pH continued to fall after cessation of exercise, whereas MSNA, heart rate and blood pressure all rapidly returned to baseline : this was taken as evidence that intramuscular acidosis is not the sole factor driving cardiovascular responses to exercise.

1.2.4 The function of the muscle metaboreflex: whole body versus small muscle group exercise

Although it is clear that combined mechanoreflex and metaboreflex activation during systemic exercise serves to divert blood flow away from non-essential organs such as kidney and gut, it is more controversial whether an increase in MSNA causes vasoconstriction which may actually limit blood flow to exercising skeletal muscle. This probably depends on the type of exercise that is being performed. It is recognised that maximal vasodilatation of the vascular bed of all skeletal muscle can theoretically lead to a muscle blood flow that would exceed maximal cardiac output . Given that blood pressure does not fall with exercise, is can be assumed that muscle blood flow is therefore regulated by tonic vasoconstriction during exercise which is driven by metaboreceptors, as hypothesised by Rowell .

The situation is different in the case of exercise of a small muscle group. Maximal exercise, and therefore maximal blood flow to that muscle group, will obviously not exceed the ability of the heart to increase cardiac output. It could therefore be argued that the metaboreflex limits exercise performance in this setting by limiting muscle blood flow unnecessarily, as it has been documented that exhausting handgrip exercise reduces blood flow to the exercising calf muscle , and that activation of the muscle metaboreflex in the forearm using ischaemic handgrip leads to an increase in vascular resistance in the exercising calf . Kardos *et al* attempted to address this question by looking at a group of patients before and after thoracoscopic sympathetic trunkotomy for idiopathic hyperhidrosis. It was found that there was an increase in forearm exercise capacity, a decrease in muscle acidification and phosphocreatine depletion at

matched workloads and a decreased pressor response to exercise following thoracic sympathetic trunkotomy. This suggests that it is possible to improve exercise performance of a small muscle group by attenuating the sympathetic nervous system response to exercise. It is also possible to augment blood flow to exercising skeletal muscle by blocking sympathetic afferents . Metaboreflex downregulation may play a part in responses to training of a small muscle group. It is known that exercise conditioning increases skeletal muscle vasodilator capacity and reduces sympathetic vasoconstrictor responses . It is not unreasonable to suggest that improved muscle bioenergetics with training leads to a reduction in metaboreflex activity, a reduction in reflex MSNA and an increase in forearm blood flow. This in turn may further improve muscle oxygenation during exercise. Mostoufi-Moab *et al* , however, were unable to show an improvement in deep venous oxygen saturation with exercise training despite an attenuation of the metaboreflex.

1.2.5 Functional sympatholysis

As discussed above, it is possible that vasoconstriction during exercise due to metaboreflex activation and an increase in sympathetic activity compromises blood flow to exercising muscle. It is clear, however, that local vasodilator effects offset the metaboreflex-mediated vasoconstriction to some extent. In the 1960's, Remensnyder *et al* coined the term "functional sympatholysis" to describe the relative insensitivity of the exercising muscle vascular bed to sympathetic activation . They applied negative pressure to the carotid sinus to simulate systemic hypotension, which led to an increase in sympathetic nervous system activity. It was found that despite an increase in

systemic blood pressure, blood flow to the resting limb was reduced due to sympathetically mediated vasoconstriction. Conversely, blood flow to the exercising limb increased in proportion to the increase in systemic blood pressure. This was thought to be due to the local release of substances that impair noradrenaline release or α-receptor responsiveness such as adenosine and nitric oxide when skeletal muscle contracts. In a more recent study by Tschakovsky *et al* , tyramine was injected into the brachial artery to stimulate endogenous release of noradrenaline. It was found that tyramine infusion evoked a vasoconstrictor response that was blunted by forearm exercise in an exercise intensity-dependant manner. Sodium nitroprusside administration further attenuated the vasoconstrictor response to tyramine, suggesting that nitric oxide is an important mediator of functional sympatholysis in humans. Given that type II (fast twitch) skeletal muscle fibres fatigue more rapidly than type I (slow twitch) fibres, it is not a surprise that functional sympatholysis is more important in fast twitch muscle in rats , and that neuronal nitric oxide synthetase (nNOS) is preferentially located in type II fibres .

The concept of functional sympatholysis has been called into question by Shoemaker *et al* , who performed experiments on humans looking at rhythmic handgrip exercise during lower body negative pressure. Lower body negative pressure causes pooling of blood in the lower limbs and a consequent reduction in venous return: this leads to an increase in sympathetic nervous system output to counteract this. It was found that during forearm exercise and recovery following the application of lower body negative pressure, forearm blood flow velocity, venous oxygen saturation and pH were lower and lactate was higher than with control forearm exercise without lower body negative pressure. They drew the conclusion that the vasodilatatory effects of local metabolites

produced during forearm exercise were unable to counteract the sympathetic vasoconstriction that was a result of the lower body negative pressure. The conclusion drawn was that functional sympatholysis during exercise of a small muscle group is not important in humans.

1.2.6 Baroreflex modulation of the muscle metaboreflex

Post-exercise muscle ischaemia, as first studied by Alam and Smirk , can be used to evaluate the contribution of the metaboreflex to the effects of exercise on cardiovascular regulation. By exercising a small muscle group and then isolating it from the rest of the circulation during recovery, metabolites produced during exercise are trapped within the muscle, and cause prolonged metaboreflex activation. As the subject is now at rest, the effects of the mechanoreflex and central command are no longer important, and any differences in heart rate or blood pressure relative to recovery without muscle ischaemia are due to metaboreflex activation. This technique has been used widely to assess metaboreflex activity .
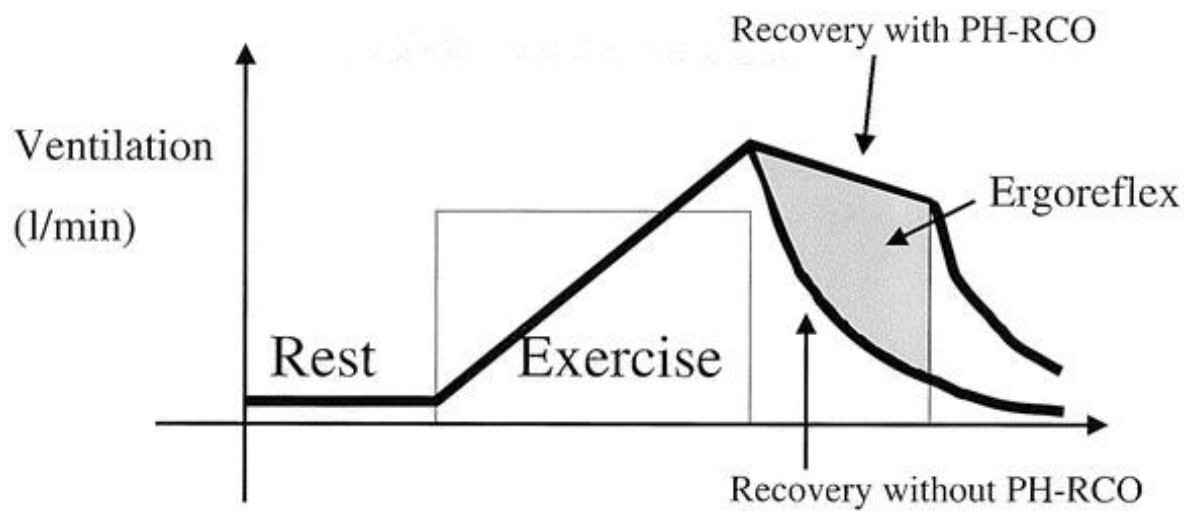
Figure 1.1 Schematic diagram of the measurement of the ergoreflex (muscle metaboreflex) in humans: this could also apply to blood pressure, heart rate and muscle sympathetic nerve activity (MSNA). PH-RCO= Post-Handgrip Regional Circulatory Occlusion. Diagram reproduced from Scott *et al* .

A striking finding of most studies looking at post-exercise muscle ischaemia is that although blood pressure remains elevated as a result of continued metaboreflex activation, heart rate very rapidly recovers to resting levels . This could be considered surprising, given that metaboreflex activation causes an increase in sympathetic activity, and this in turn should cause a sustained increase in heart rate. This observation can be explained by the effect that the metaboreflex-mediated rise in blood pressure has on the arterial baroreflex.

Arterial baroreceptors are located in the carotid sinus and aortic arch, and they alter both cardiac function and vasomotion in response to acute changes in arterial blood pressure . Arterial baroreflex unloading causes a reflex tachycardia and peripheral vasoconstriction to restore blood pressure to the "set point": loading of the baroreflex has the opposite effects. During exercise, the baroreflex is probably reset to a new set point to allow a rise in blood pressure . Dogs lacking an arterial baroreflex (following sinoaortic denervation) do not exhibit an immediate rise in blood pressure at the onset of exercise . As it is central command that initiates the initial rise in blood pressure, this suggests that resetting of the baroreflex at the start of exercise is also modulated by central command. The baroreceptors then perceive resting blood pressure to be "hypotensive" relative to its new operating point with heart rate and systemic vascular resistance increasing as a consequence .

The immediate increase in heart rate that is seen at the onset of exercise is not thought to be metaboreflex mediated . As discussed before, central command causes the initial rise in heart rate through withdrawal of resting parasympathetic tone, with metaboreflex activation and an increase in sympathetic nerve activity only becoming important as exercise progresses . Work looking at sustained passive stretch of triceps surae in humans suggests that small fibre muscle mechanoreceptors also inhibit cardiac vagal activity and therefore increase heart rate .

During post-exercise muscle ischaemia, central command has been withdrawn, and parasympathetic tone therefore returns to resting levels. This has an inhibitory effect on heart rate and therefore partially offsets the positively chronotropic effects of metaboreflex activation on heart rate. The withdrawal of central command probably

also means that the baroreflex "set point" returns to resting levels, and the metaboreflex mediated elevation in blood pressure is perceived as abnormal, leading to a further increase in parasympathetic activity and a negatively chronotropic effect . It is the combination of these two mechanisms that leads to the rapid fall in heart rate during post-exercise muscle ischaemia. The sustained elevation in blood pressure is maintained by increased sympathetic nervous system activity from continued metaboreflex activation. In support of this idea, it has been observed that administration of atropine during metaboreflex activation in dogs causes a reflex tachycardia, with the inhibition of the return of resting parasympathetic tone leading to unopposed sympathetic activity . It has also been observed that there is an increase in heart rate variability in humans during post-exercise muscle ischaemia, reflecting an increase in parasympathetic nervous system activity during activation of the muscle metaboreflex .

Baroreceptor activation also appears to alter the metaboreflex-driven changes in blood pressure and MSNA that are seen during exercise. Work by Cui *et al* suggests that during metaboreceptor activation, the sensitivity of baroreflex control of MSNA in humans is elevated and the baroreflex curve is reset, and drew the conclusion that this allows "finer tuning" of blood pressure control during exercise . Attempting to alter pharmacologically the rise in blood pressure seen with handgrip exercise also appears to have an effect on the baroreflex, with suppression of the blood pressure response with nitroprusside augmenting the rise in heart rate and MSNA by 300% and administration of phenylephrine to accentuate the increase in blood pressure having the opposite effect . The inhibitory effects of baroreceptor activation on MSNA are underlined by the observation that baroreceptor denervation in dogs causes a greater

pressor response to exercise following graded reductions in hindlimb perfusion. The mechanism of the blood pressure increase with metaboreceptor activation also appears to change with baroreflex denervation, with the increase in blood pressure mediated through peripheral vasoconstriction rather than increased cardiac output .

1.2.7 Metaboreflex control of ventilation: a source of controversy.

Although there is clear evidence that the metaboreflex is intimately involved with the regulation of blood supply to muscle during exercise in humans, the mechanism of the control of ventilation is less clear.

In healthy human subjects at sea level, ventilation at rest is controlled very tightly by p_aCO_2. A small rise in p_aCO_2 leads to diffusion of carbon dioxide across the blood brain barrier into the cerebrospinal fluid, creating an acidosis, which is a potent stimulus of central chemoreceptors near the ventral surface of the medulla . Even during exercise, when VCO_2 may increase 15-fold, there is an almost perfect straight-line relationship between ventilation and VCO_2 . It is therefore frequently assumed that a rise VCO_2 is the main stimulus to increase ventilation at the start of exercise. This would, however, require a rise in p_aCO_2 to initiate the feedback loop, which does not happen : in healthy subjects, p_aCO_2 remains steady during exercise and decreases when exercising beyond the lactate threshold to correct the resultant metabolic acidosis . It has also been stated that the control of ventilation during heavy exercise cannot be explained fully by lactic acid stimulation of arterial chemoreceptors . Central command and mechanoreceptors may play a role in the sudden increase in ventilation at the start of exercise, and it has

been suggested that the increase in pulmonary blood flow caused by an increase in cardiac output at the start of exercise is sensed by receptors in the lungs , though a study by Grucza *et al* calls this idea into question, by showing no relationship between cardiac output and ventilation in the early phase of dynamic exercise and rhythmic-static exercise .

Metaboreflex control of ventilation has been studied extensively in animal models, and the general consensus is that the metaboreceptor activation does cause a reflex increase in ventilation . It is not possible to conduct such detailed studies on humans, and research has therefore focussed on the effects of post-exercise muscle ischaemia on ventilation. Clark *et al* asked healthy volunteers to run on a treadmill with sphygmomanometer cuffs inflated round the thighs to arrest blood flow . It was observed that the slope relating ventilation and VCO_2 (the ventilatory equivalent) was steeper than during control exercise (without thigh cuffs), and this result was taken as evidence of a metabolic stimulus arising from working skeletal muscle. The same group also demonstrated that post-exercise muscle ischaemia in healthy subjects leads to an increase in ventilation compared with control recovery , and it has been shown that restriction of blood flow to exercising muscle using lower body positive pressure causes an increased ventilatory response to exercise, even before the production of substantial quantities of lactate . Oelberg *et al* again addressed this issue using lower body negative pressure during leg exercise , and found that this increased ventilatory response to exercise correlated strongly with quadriceps pH, with the conclusion that metaboreflex-induced hyperventilation is stimulated by muscle acidosis. It is also interesting to note that the small increase in systemic arterial lactate seen with this form of exercise was not nearly enough to account for the large increase in ventilation when

compared with previously published data. Electromyographic (EMG) studies in humans have shown that the increase in ventilation seen with ischaemic forearm exercise is concomitant with the EMG signs of neuromuscular fatigue in the absence of the release of metabolites into the systemic circulation, suggesting that neural pathways arising from the fatigued muscle cause the ventilatory response .

Many of the studies that cast doubt on the role that the muscle metaboreflex plays in the control of ventilation during exercise involve either differential anaesthetic blockade or patients with a sensory neuropathy . With anaesthetic blockade, it is difficult to effect a complete sensory loss whilst leaving all motor fibres intact, and this raises the question of whether subjects exhibit increased levels of central command to compensate, leading to an increased ventilatory response. Strange *et al* exercised a group of patients following epidural anaesthesia using electrical stimulation of leg muscle . The ventilatory response to electrical stimulation remained intact even though blood pressure responses were abolished. This could be construed as evidence against a ventilatory metaboreflex, although is raises the possibility of the ventilatory metaboreflex being a redundant control mechanism that is only important in humans under certain circumstances . It should also be noted that electrical stimulation of skeletal muscle leads to a different recruitment order of nerve fibres to voluntary exercise, calling into question the validity of experiments using electrical nerve stimulation .

1.2.8 Post-exercise muscle ischaemia: potential confounding factors

It is important to deal with the subject of muscle discomfort during post-exercise muscle ischaemia, as one of the criticisms of the metaboreflex theory of ventilatory control during exercise is that occluding the blood supply to a working muscle can be uncomfortable and may therefore cause a reflex increase in MSNA and ventilation . The numerous experiments on anaesthetised animals go some way to addressing this issue, but these data can not necessarily be directly applied to human subjects. This question was dealt with as early as 1938 by Alam and Smirk , who noted that discomfort was not necessary to elicit a pressor response to post-exercise muscle ischaemia. Experiments looking at graded muscle ischaemia in humans also demonstrated a pressor response to post-exercise muscle ischaemia in the absence of pain . The effects of pain on MSNA have not been extensively studied , although it has been documented that painful electrical skin stimulation does not have an effect on MSNA . Studies on patients with McArdle's syndrome also address this question with MSNA falling rapidly to resting levels despite the sensation of intense muscle pain continuing post-exercise . It is still possible that muscle discomfort could have an effect on ventilation during post-exercise muscle ischaemia, as ventilation is under voluntary control to a greater extent than either MSNA or blood pressure.

1.3 The muscle metaboreflex in the context of chronic disease

1.3.1 Introduction

The possibility that abnormal metaboreflex function could contribute to exercise limitation in chronic disease has been attracting attention. The first study looking at the metaboreflex in disease was probably conducted by Lorentsen in 1972 . Patients with unilateral intermittent claudication were asked to perform leg exercise, and it was found that the blood pressure response to exercise was greatly enhanced in the diseased limb compared with the normal side. In contrast, the exercise-induced increases in heart rate were similar between the two legs. This is consistent with flow limitation during exercise leading to anaerobic metabolism of glycogen, the development of a muscular acidosis and metaboreflex overactivation. All of the recent studies looking at the metaboreflex in disease have, however, concentrated on patients with chronic heart failure.

1.3.2 Exercise limitation in heart failure: peripheral factors

Chronic heart failure is a syndrome in which cardiac output is unable to match the metabolic demands of the body due to pump failure. Exercise limitation is a hallmark of chronic heart failure, and the cause of exercise limitation would appear straightforward with a reduction in cardiac output causing inadequate blood flow and oxygen delivery to exercising muscle. There is, however, a lack of correlation between

exercise capacity and traditional indices of cardiac function such as cardiac output ,
suggesting that this is not the whole story. In addition, pharmacological interventions
aimed at acutely improving cardiac output and femoral artery blood flow do not
immediately improve leg exercise capacity , and cardiac transplant does not improve
exercise tolerance immediately . This raises the possibility that it is not cardiac output
that limits exercise tolerance in heart failure, but it is a failure of skeletal muscle to take
up oxygen .

Numerous studies have documented skeletal muscle abnormalities in patients with
chronic heart failure. There is a reduction in muscle bulk and strength and a shift in
fibre type towards type 2 (fast twitch glycolytic) . There are also alterations in muscle
biochemistry with a reduction in enzymes required for oxidative metabolism of
glycogen . This leads to increased reliance on anaerobic metabolism, with earlier
production of lactate and premature muscle fatigue . In addition, interventions aimed at
conditioning the skeletal muscle such as cardiac rehabilitation programmes have been
shown to improve quality of life and exercise capacity by improving peripheral muscle
function rather than indices of cardiac function .

1.3.3 The "muscle hypothesis" in chronic heart failure

It has been observed that patients with chronic heart failure demonstrate an exaggerated sympathetic nervous system and ventilatory response to exercise . This has led some authors to speculate that anaerobic metabolism in skeletal muscle during exercise causes early build-up of metabolites such as lactate and hydrogen ion, which stimulates muscle metaboreceptors and leads to an increase in MSNA and ventilation . The increase in sympathetic nervous system activity increases afterload on the already failing heart, which has further deleterious effects. This vicious cycle has been termed the "muscle hypothesis" of exercise limitation in heart failure (see fig. 1.2) .
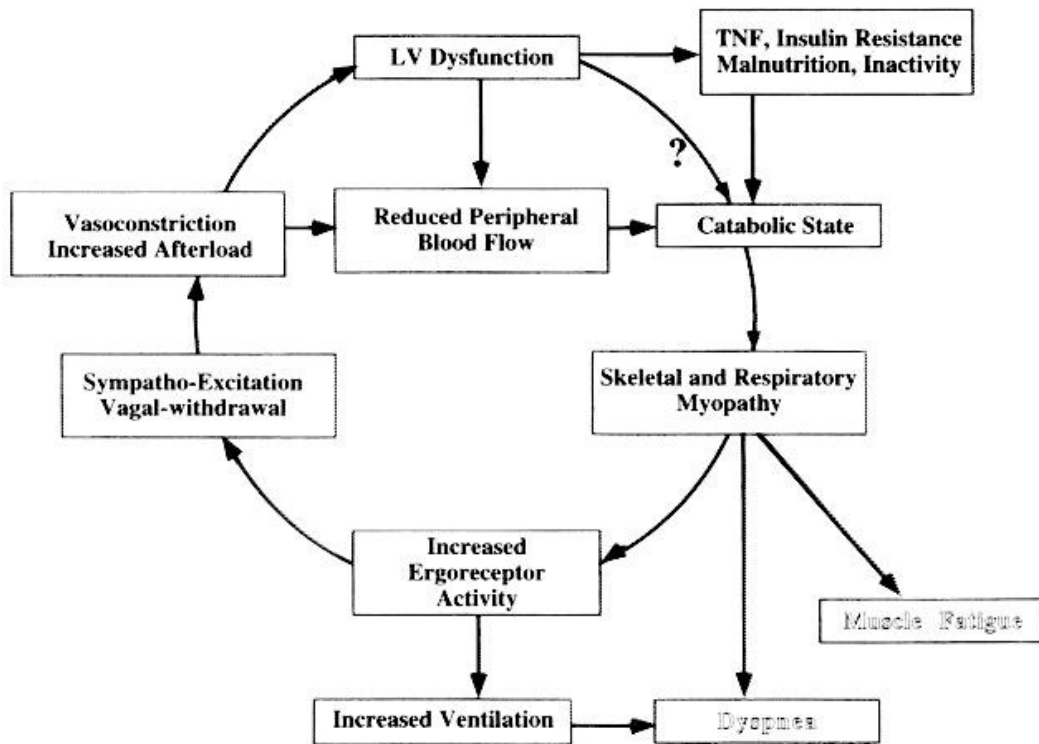
Figure 1.2 The "muscle hypothesis" of chronic heart failure. Reproduced from Piepoli *et al* . This is an attempt to link the muscle metaboreflex to the pathophysiology of the development of skeletal muscle dysfunction and exercise intolerance in chronic heart failure. LV = Left Ventricular, TNF = Tumour Necrosis Factor.

1.3.4 The muscle metaboreflex in chronic heart failure

Piepoli *et al* studied the metaboreflex in patients with chronic heart failure using rhythmic handgrip exercise to fatigue followed by post-exercise circulatory occlusion with a sphygmomanometer cuff . It was found that the subjects with chronic heart failure demonstrated an increased metaboreflex contribution to ventilation and blood pressure during post-exercise muscle ischaemia compared with healthy control subjects. Leg vascular resistance was assessed using strain-gauge plethysmography, and this was also found to be increased during forearm post-exercise muscle ischaemia, suggesting an increase in sympathetic nervous system activity. All subjects were then asked to train the forearm muscles for 6 weeks using a "gripper" device, and it was found that training led to a reduction in muscle metaboreflex activity in both groups, and this was more marked in the group with chronic heart failure. The conclusion drawn was that increased anaerobic metabolism led to an increase in metaboreceptor stimulation, and that this could be partially reversed by training the muscle. More recent studies have demonstrated an increase in metaboreflex activity in the lower limb muscles of patients with chronic heart failure , that there is a correlation between upper and lower limb metaboreceptor activity in heart failure , and that the technique of post-exercise muscle ischaemia to assess the metaboreflex in heart failure is reproducible on a different day . Other work by the same group looks at metaboreflex activity in relation to symptoms as defined by New York Heart Association (NYHA) class of heart failure . It was found that metaboreflex activity correlated with severity of symptoms and VO_2 max: there was only a very weak correlation between left ventricular ejection fraction and VO_2 max. The observation that increased metaboreceptor sensitivity is a strong predictor of baroreflex impairment and sympathetic nervous system activation would appear to

suggest that a blunted baroreceptor response in chronic heart failure may result in a reduction of baroreceptor-mediated inhibition of the metaboreflex, leading to further increases in sympathetic activity. The authors suggest that metaboreflex overactivation may initially be a beneficial compensatory mechanism that assists in the physiological response to exercise, but in the chronic disease state, excessive stimulation of the metaboreflex leads to sympathetic activation and blunted baroreflex control, which may be deleterious in the long term with increased cardiac afterload and fluid retention.

Other centres have come to contrasting conclusions regarding the muscle metaboreflex in chronic heart failure. Sterns *et al* studied MNSA responses to static handgrip exercise and post-handgrip circulatory arrest in subjects with heart failure and healthy controls. They found a marked attenuation in MSNA response to post-exercise muscle ischaemia in subjects with heart failure despite ^{31}P NMR confirming a similar degree of muscle acidosis. It was concluded that heart failure leads to the attenuation of the muscle metaboreflex, perhaps causing a less efficient use of cardiac output during exercise. Shoemaker *et al* assessed the metaboreflex by asking patients to perform rhythmic handgrip exercise whilst the whole exercising forearm was subjected to 50mmHg of positive pressure . It was found that metaboreflex contribution to the rise in blood pressure was normal in heart failure, despite increased production of lactate and hydrogen ions, and the authors speculated that chronic metaboreflex activation leads to desensitisation of the nerve fibres, which is offset by the increased production of lactate. Interestingly, it was also found that the augmented blood pressure brought about by activation of the metaboreflex did not lead to an improvement in venous oxygen saturation or venous metabolite concentrations, again suggesting that this might be a redundant control mechanism.

Silber *et al* re-visited the observation that the MSNA rise during non-fatiguing rhythmic handgrip exercise is not metaboreflex mediated . They studied a group of patients with heart failure and asked them to perform rhythmic handgrip exercise at 25% of maximum voluntary contraction (MVC) . It was found that this exercise protocol did lead to the development of a muscular acidosis in the heart failure patients, but not in the control subjects, and it was therefore not surprising that post-handgrip muscle ischaemia led to an increased MSNA in this group. Notarius *et al* also came to the conclusion that metaboreflex activity is increased in heart failure and that this may further impair exercise performance and accelerate disease progression .

In summary, muscle metaboreflex activity is increased in patients with chronic heart failure and may contribute to the pathophysiology of exercise limitation and disease progression. Whether chronic over-stimulation of the metaboreflex by the metabolites of anaerobic exercise leads to desensitisation of the metaboreflex is a matter that has been disputed. Sinoway and Li, in a recent review article, summarise the issues nicely by stating that "whether the muscle metaboreflex is attenuated or accentuated may depend on the relative degree of muscle metabolic abnormalities, the degree of metaboreceptor desensitisation, and the mode of exercise being performed (rhythmic vs. static)" . The last point is important given the important physiological differences between rhythmic and static exercise, and between exercise of a small muscle group and whole body exercise. Experiments on dogs using graded reduction of terminal aortic blood flow to stimulate the metaboreflex reveal that heart failure causes a change in the mechanism of metaboreflex-mediated increases in blood pressure . Healthy dogs increased blood pressure through an increase in cardiac output, whereas dogs with heart

failure, unable to increase cardiac output, responded through an increase in peripheral vasoconstriction, presumably through stimulation of the arterial baroreflex. It can, however, be assumed that metaboreflex mechanisms will be different in the context of small muscle group exercise in heart failure, when cardiac output will not limit the ability to maintain blood pressure.

1.4 Exercise limitation in chronic obstructive pulmonary disease

1.4.1 Pathophysiology of chronic obstructive pulmonary disease (COPD)

Chronic Obstructive Pulmonary Disease (COPD) is one of the major causes of death and disability in Scotland, and it is predicted to become the 3rd most important cause of death globally by 2020 . It is caused by an inflammatory response to inhaled irritants, particularly cigarette smoke. Neutrophilic infiltration and cytokine production lead to the development of airway inflammation, and inhaled free radicals cause a disturbance of the protease/ anti-protease balance and the development of emphysema . The physiological consequences are progressive airflow limitation and lung hyperinflation, the hallmarks of the disease. It is disappointing that there are no pharmacological interventions that influence disease progression. Inhaled bronchodilators only provide symptomatic relief , although there is some debate over whether inhaled corticosteroid may slow the decline in FEV_1 in severe disease .

1.4.2 Exercise limitation in COPD: the role of peripheral skeletal muscle

Exercise limitation is probably the most distressing symptom of COPD, and as lung damage is often severe by the time dyspnoea is an important feature, current treatments could be considered palliative. It is often assumed that exercise limitation is caused by dyspnoea, with airflow limitation and air trapping causing increased work of breathing and dynamic hyperinflation , but it is noteworthy that a significant proportion of patients with COPD are limited by peripheral factors such as muscle fatigue . This raises the question of whether exercise is limited by abnormalities in the peripheral skeletal muscle . Similar to the story in chronic heart failure, where exercise capacity correlates poorly with indices of haemodynamic function, exercise tolerance in COPD is poorly correlated with FEV_1. A much stronger correlation exists between exercise capacity and leg muscle mass , and muscle strength is also a good predictor of exercise tolerance . A recent study by Saey *et al* found that half of their group of patients with COPD developed contractile fatigue following a constant work-rate cycle ergometer test to exhaustion, and those who developed contractile fatigue did not improve their exercise performance following bronchodilator administration despite a 15% improvement in FEV_1 . The conclusion drawn was that a significant number of patients with COPD are limited by peripheral factors and not ventilatory constraints. Lactate production by exercising muscle is also of importance. Patients with COPD demonstrate a reduced anaerobic threshold, beyond which lactate enters the systemic circulation . The resultant acidosis is a potent stimulus to ventilation, further increasing the load on the respiratory system. Casaburi *et al* demonstrated that an exercise training

programme leads to a lower ventilatory requirement at a given level of work, and this is in proportion to the reduction in lactic acidosis .

Although there is no doubt as to the existence of skeletal muscle abnormalities in COPD, it should be noted that there is considerable debate as to whether this is simply due to deconditioning and whether skeletal muscle dysfunction truly limits exercise tolerance .

1.4.3 Skeletal muscle abnormalities in COPD

Functional, histological and metabolic abnormalities of skeletal muscle have all been described in patients with COPD. Fat free mass may be reduced with preservation of body weight , and a reduction in quadriceps muscle cross sectional area has been reported . Numerous studies document a reduction in skeletal muscle strength , endurance and fatigability compared with control subjects. Upper limb muscles appear to be better preserved than lower limb muscles , possibly due to a greater reduction in activity of the lower limbs. Reduction in strength appears to be proportional to the degree of muscle atrophy . Structural abnormalities include a reduction in the proportion of type I (oxidative) fibres, an increase in the proportion of type II (glycolytic) fibres and a reduction in muscle capillarity compared with control subjects . Changes in muscle metabolism all favour anaerobic glycolysis: quadriceps muscle biopsies have demonstrated reductions in oxidative enzyme capacity in COPD patients , and ^{31}P NMR studies suggest early depletion and prolonged recovery of phosphocreatine in the quadriceps muscle following exercise. Oxygen kinetics are slow

in COPD , and lactic acidosis is seen earlier in exercise in patients with COPD , placing a further load on the respiratory system .

The various causes of skeletal muscle dysfunction in COPD are a source of much discussion. The abnormalities of muscle function and metabolism are similar to those seen in healthy subjects following acute deconditioning . This has led some observers to speculate that skeletal muscle dysfunction in COPD is simply a "side effect" of a reduction in activity forced upon patients by ventilatory constraints. Acute exacerbations of COPD are known to cause a reduction in skeletal muscle strength , and corticosteroid therapy may cause a myopathy . Other possible contributors include the effects of hypoxia and hypercapnia, malnutrition and oxidative stress. Couillard *et al* demonstrated that exhaustive quadriceps exercise induces local oxidative stress in patients with COPD by obtaining muscle biopsies before and 48 hours after quadriceps exercise. They also found a reduction in post-exercise antioxidant activity (as measured by glutathione peroxidase activity) in patients with COPD compared with healthy control subjects . Muscle oxidative stress in the patients with COPD in this study was associated with reduced quadriceps endurance.

1.4.4 Exercise training in COPD

The benefits of exercise training in COPD further underline the importance of peripheral skeletal muscle in the development of exercise intolerance. In the past, it has been believed that the benefits of exercise training in COPD are simply psychological, as ventilatory constraints would surely prevent the attainment of the critical training intensity required to train skeletal muscle . It is now clear that exercise-based pulmonary rehabilitation programmes lead to an improvement in exercise capacity and quality of life and prescription of such programmes is now part of routine care in patients with COPD . Exercise training programmes reduce lactate production at a given workload and may therefore also reduce the "burden" on the ventilatory system. Benefits have been seen with both strength and endurance training , and high intensity endurance training has been shown to provide greater benefit than low intensity training . Troosters *et al* studied a method of distinguishing patients who will respond to exercise training from those who will not . It was found that ventilatory reserve and peripheral muscle strength are predictors of the training response, perhaps suggesting that patients with no ventilatory reserve do not achieve a muscle training effect and may therefore not benefit from pulmonary rehabilitation. Studies using inspiratory and bilevel pressure support suggest that such interventions may "offload" the respiratory muscles to allow an increase in training intensity, with patients subsequently deriving a greater training benefit, but further studies are required. Heliox, a mixture of helium and oxygen which is lower density than room air, does not appear to enable patients to increase training intensity .

1.4.5 Cachexia in COPD

The development of cachexia in COPD is an important and ominous sign. Schols *et al* looked retrospectively at body mass index (BMI) and survival in COPD and found that low BMI is an independent predictor of mortality . Body composition also appears to correlate with exercise performance, with fat free mass more important than total body weight . BMI may in fact be a misleading measurement, as it fails to distinguish between body fat and lean mass. Subjects with a normal BMI may have a significantly reduced fat free mass , and muscle mass appears to be a better predictor of outcome in COPD than body weight . The potential causes of weight loss in COPD are somewhat diverse, and include reduced calorie intake, increased energy expenditure due to increased oxygen cost of breathing and mechanical inefficiency of skeletal muscle , acute exacerbations of COPD and systemic inflammation. Reduced skeletal muscle mass as determined by creatine-height index has been associated with increased circulating levels of IL-6 and TNF-α and increased levels of acute phase proteins have been found in the serum of patients with COPD and a raised resting energy expenditure with reduced fat free mass . Systemic inflammation may also contribute to the increased cardiovascular mortality seen in patients with COPD .

If cachexia and muscle dysfunction in COPD are such strong predictors of mortality, do interventions aimed at increasing body mass index lead to an improvement in outcome? Studies have looked at both dietary and pharmacological intervention. The effects of dietary intervention have been disappointing, with a number of studies reporting negative results. A meta-analysis by Ferreira *et al* concluded that nutritional supplementation is probably not of benefit in patients with COPD in terms of

anthropometric measures or functional exercise capacity . A more recent study by Steiner *et al* looked specifically at nutritional supplementation during an exercise-based pulmonary rehabilitation programme, when a negative calorie balance is likely. It was found that the supplemented group gained weight, but only the well nourished patients (body mass index > 19kg/m^2) demonstrated a benefit in terms of exercise performance. It is possible that the malnourished patients did not respond because of the systemic inflammatory response: Creutzberg *et al*, in a study looking at nutritional supplementation in 24 patients with COPD and fat free mass depletion, discovered that an elevated systemic inflammatory response was associated with failure to gain weight . Post-hoc analysis of an earlier study by Schols *et al* looking at the effects of nutritional supplementation on patients with COPD suggested that those who did manage to put on weight over the course of the study had a reduced mortality .

Attempts at pharmacological intervention have also been disappointing. Burdet *et al* studied the effects of growth hormone in a placebo-controlled study, and found that IGF-1 levels increased, but there were no benefits in terms of functional parameters, even though lean body mass increased to a greater extent in the treated group compared with the placebo group . Schols *et al* investigated the effects of the anabolic steroid nandrolone in combination with nutritional supplementation in a placebo-controlled trial on 217 patients with COPD: there was a greater increase in fat-free mass in the nandrolone-treated group, but no effects on functional status . Other studies looking at stanozolol and oxandrolone have demonstrated similar results, with increases in lean body mass with no improvement in functional status.

A recent placebo controlled trial in our department looked at the effects of creatine monohydrate supplementation, administered in conjunction with an exercise based pulmonary rehabilitation programme. It was found that creatine supplementation led to significant improvements in fat free mass and peripheral skeletal muscle strength and endurance compared with placebo . More importantly, a clinically significant improvement in quality of life was seen, as assessed by the St George's Respiratory Questionnaire.

1.5 Creatine supplementation: a therapeutic intervention?

1.5.1 Creatine metabolism

Creatine is a naturally occurring amino acid found in abundance in skeletal muscle, mainly in the form of phosphocreatine . In healthy adults, the liver and kidneys synthesise 1-2 grams per day, supplemented by 1-2 grams daily as part of the normal diet : meat and fish have a particularly high content . The body excretes about 1-2 grams per day in the form of creatinine, which is cleared by the kidneys. There is evidence that creatine supplementation suppresses endogenous creatine production , and excess ingested creatine is simply excreted. Creatine plays an integral role in skeletal muscle energy metabolism, with phosphocreatine acting as a high energy buffer: the creatine kinase reaction converts phosphocreatine to creatine, donating a phosphate molecule to ADP to create ATP . It is therefore of particular importance during high intensity exercise.

1.5.2 Creatine supplementation in healthy subjects

Creatine has become one of the most widely used nutritional supplements amongst athletes, based on the results of numerous trials in the 1990's suggesting benefit. It was initially shown in 1993 that short-term creatine supplementation increases the muscle creatine pool and improves ability to perform rapid intermittent muscle contractions . Numerous trials since then have suggested improvements in athletic performance in

sprint events , and it is therefore commonly used as an ergogenic aid in many sports. It is not currently on the International Olympic Committee (IOC) list of banned substances. There are, however, many negative trials which counter those suggesting benefit. In particular, creatine supplementation does not appear to have an effect on endurance exercise. Ingestion of 20g of creatine daily for 5 days has been shown to increase muscle creatine concentration by 20% in healthy subjects, although there is significant inter-subject variability in muscle uptake. Addition of carbohydrate solution to creatine supplements increases uptake by skeletal muscle through the effects of increased insulin secretion on creatine transport .

1.5.3 Creatine supplementation in disease

It has also been demonstrated that creatine supplementation may benefit patients with chronic diseases affecting the skeletal muscles.   Several months of creatine supplementation attenuated loss of dominant hand muscle strength and increased fat free mass in patients with Duchenne muscular dystrophy , though this was not seen in a group of patients with type I myotonic dystrophy or Huntingdon's disease . The specific effects of creatine supplementation on respiratory muscles were evaluated in patients with amyotrophic lateral sclerosis. There were no differences in functional score, forced vital capacity or maximum voluntary ventilation between creatine and placebo . Short term creatine supplementation in a group of 81 patients with a variety of neuromuscular diseases led to an increase in body weight and handgrip strength, though this study was not placebo- controlled for the first part, and only single-blinded for the second part . Creatine supplementation has been evaluated in patients with

congestive cardiac failure, with significant increases in skeletal muscle total creatine and lower limb muscle function following 10 days of supplementation in the absence of an exercise training programme . Creatine has also been shown to attenuate the abnormal skeletal muscle response to exercise seen in chronic heart failure, with a reduction in lactate at a given work rate .

1.6 Could the muscle metaboreflex be relevant in COPD?

1.6.1 Chronic heart failure and COPD: a similar story

The above literature review highlights some of the striking similarities in the pathogenesis of exercise intolerance between chronic heart failure and COPD . Both diseases are now recognised to cause more than simple single-organ failure leading to exercise dysfunction, with traditional markers of disease severity (ejection fraction and FEV_1) shown to be poor predictors of exercise capacity. Similar skeletal muscle functional and metabolic abnormalities have been described with similar improvements in quality of life and exercise performance with exercise-based rehabilitation programmes. The development of cachexia conferring a poor prognosis and the role of systemic inflammation has been recognised in both diseases. Even the "dyspnoea spiral" proposed by Préfaut *et al* , reproduced in figure 1.3, bears similarities to the "muscle hypothesis" proposed in cardiac failure by Piepoli *et al* , with a cyclical model of exercise intolerance due to dyspnoea and early muscle fatigue with subsequent avoidance of physical activity and deconditioning leading to further exercise intolerance.
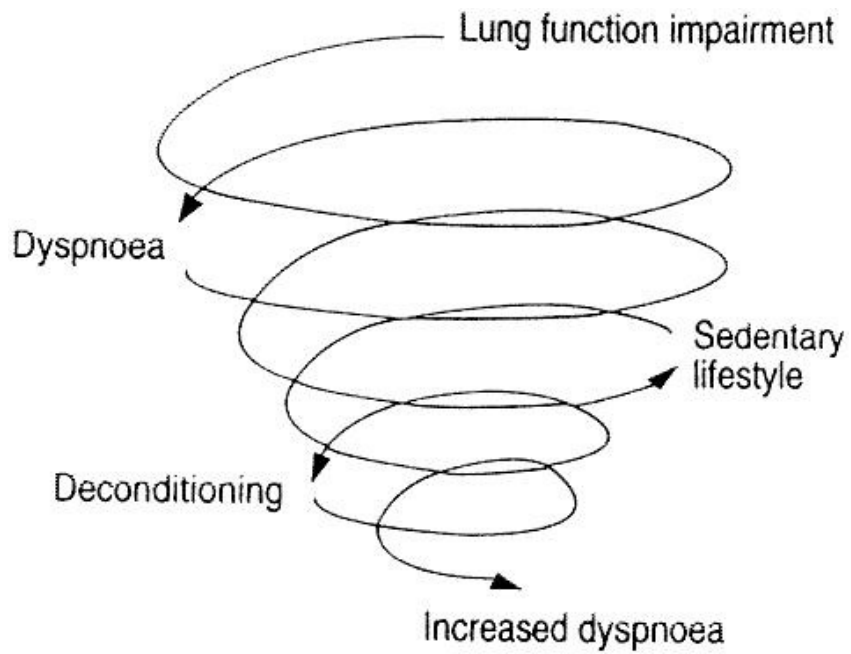
Figure 1.3 The dyspnoea spiral of COPD. Reproduced from Serres *et al* .

1.6.2 Autonomic nervous system function in COPD

Another important area in which there are similarities between chronic heart failure and COPD concerns the development of abnormalities of the autonomic nervous system. Several studies have documented reduced heart rate variability in patients with COPD, suggesting a loss of resting parasympathetic tone . Heindl *et al* studied 11 patients with chronic respiratory failure and noted an increase in heart rate and MSNA at rest compared to control subjects . It was found that oxygen inhalation decreased MSNA, which is consistent with the observation that acute hypoxaemia is known to increase muscle sympathetic nerve activity (MSNA) in healthy humans through stimulation of arterial chemoreceptors . This would appear to suggest that increased sympathetic nervous system activity is due to chronic hypoxaemia, but it has also been speculated that increased work of breathing in COPD leads to stimulation of the muscle metaboreflex. A study by St Croix *et al* looked at the effects of fatiguing inspiratory muscle work in healthy humans demonstrated an increase in MSNA in the leg as fatigue approached . The same group then demonstrated that similar exercise causes an increase in leg vascular resistance . It was noted in the discussion that the diaphragm is richly innervated by group III and IV afferent nerve fibres, which led to the conclusion that this increase in MSNA was due to activation of the metaboreflex in the diaphragm muscle. It is interesting to note that metaboreflex activation may also have effects on the respiratory system, with one study showing that metaboreflex activation leads to a reduction in airway resistance following methacholine-induced bronchospasm in a group of asthmatic patients, presumably also mediated by the sympathetic nervous system .

Excessive sympathetic nervous system activation in COPD, together with the observation that there are similar skeletal muscle abnormalities as are seen in chronic heart failure, leads to the intriguing possibility that there is up-regulation of muscle metaboreflex activity in COPD. Creatine supplementation improves skeletal muscle function in COPD, which raises the question of whether it also leads to a reduction in metaboreflex activity.

1.7 The Aims of this thesis.

The muscle metaboreflex has been shown to be of importance in the regulation of oxygen delivery to exercising skeletal muscle through its effects on ventilation, blood pressure and systemic vascular resistance. Studies on metaboreflex activity in chronic heart failure, a disease characterised by alterations in muscle structure and metabolism, have suggested that metaboreflex over-activity may contribute to the pathophysiology of exercise limitation. As patients with chronic obstructive pulmonary disease (COPD) suffer from similar abnormalities of skeletal muscle, this thesis aims to evaluate further the metaboreflex in COPD and to determine whether it is possible to alter metaboreflex activity in COPD by supplementation with creatine monohydrate.

A review of the literature has identified the following research questions:

1. Is it possible to measure the muscle metaboreflex in patients with COPD using post-exercise forearm muscle ischaemia, and is the muscle metaboreflex up-regulated in patients with more severe COPD?

2. Can pulse transit time be used to measure muscle metaboreflex activity during post-exercise muscle ischaemia?

3. Is the muscle metaboreflex attenuated in patients with autonomic nervous system dysfunction secondary to diabetes mellitus?

4. Does creatine loading have any effects on forearm strength or endurance, respiratory muscle strength or body weight in patients with COPD?

5. Does creatine loading have any effects on forearm muscle metaboreflex activity in patients with COPD?

Chapter 2

Materials and Methods

Materials and Methods

2.1 The muscle metaboreflex in patients with stable COPD

The aim of the initial study was to measure the muscle metaboreflex in a group of subjects with COPD and to determine whether markers of disease severity correlate with ergoreflex activity. The protocol described is adapted from that of Piepoli *et al* .

2.1.1 Ethical considerations

Ethical approval for this study was obtained from the Glasgow Royal Infirmary Local Research Ethics Committee before this study commenced. All patients provided written informed consent prior to participation in the study.

2.1.2 Patient recruitment

Sixteen subjects were recruited from the outpatient clinics of the Department of Respiratory Medicine at Glasgow Royal Infirmary. Adverts were placed in the clinics to alert medical staff of the study, and permission was sought from patients to contact them by telephone at a later date. Patients with stable Chronic Obstructive Pulmonary Disease (COPD) were invited to participate in the study, which involved two visits to the Pulmonary Function Testing laboratory- one for a standard set of pulmonary

function tests (as part of routine care) and one for muscle metaboreflex measurement. We defined "stable" COPD as the absence of hospitalisation, oral corticosteroid or antibiotic use or exacerbation in the preceding 6 weeks. COPD was defined according to the criteria of the Global Initiative for Obstructive Lung Disease (GOLD): FEV_1/FVC ratio <70% with less than 15% bronchodilator reversibility . All patients had a smoking history of at least 20 pack years. A clinical history was taken and hospital casenotes were reviewed to exclude patients with unstable cardiac disease or cardiac failure, significant neurological or locomotor disease, active malignancy or advanced renal or hepatic disease.

2.1.3 Pulmonary function testing

Pulmonary Function Testing was performed as part of routine care, and organised from the outpatient clinic. All equipment was calibrated in accordance with the manufacturers' instructions on the morning of the visit. Patients were asked to refrain from taking inhaled or nebulised therapy on the morning of the visit, and spirometry was performed according to standard procedures (V6200 Autobox, SensorMedics Corp, Anaheim, USA). The best of three efforts was taken for FEV_1 and FVC, ensuring that there was less than 10% variability between the three tests. Reversibility testing was then performed using 5mg of nebulised salbutamol. Spirometry was repeated after 15 minutes. Standard protocols were used to measure lung volumes by body box plethysmography (V6200 Autobox, SensorMedics Corp, Anaheim, USA) and single breath transfer factor for carbon monoxide (Transflow system, Model 540, Morgan Medical, Kent, UK).

2.1.4 Patient information

A full medical history was taken from all patients, including past medical history, recent use of corticosteroids, smoking history, medication history and Baseline Dyspnoea Index . A brief physical examination was also conducted, and height and weight were measured with shoes removed.

2.1.5. Muscle metaboreflex assessment

The activity of the muscle metaboreflex was assessed using the method of forearm exercise followed by post-exercise muscle ischaemia. Forearm exercise to fatigue leads to the production of metabolites such as lactate, hydrogen ion, prostaglandins and adenosine, which have been shown to stimulate the muscle metaboreflex. Occlusion of the circulation to and from the exercised muscle on cessation of exercise traps such metabolites in the muscle, leading to sustained metaboreflex activation for as long as the occlusion is maintained. This manifests itself as a sustained rise in blood pressure and ventilation, which can be measured non-invasively. A direct comparison can then be made with "control" recovery (exercise followed by recovery without circulatory occlusion), to allow estimation of metaboreflex activity. We also assessed the effects of circulatory occlusion at rest to exclude the possibility that this was having an effect on measured variables.

Subject preparation

Subjects were asked to refrain from drinking caffeine or smoking in the 4 hours preceding the visit. Where possible, subjects were also asked to refrain from taking inhaled beta agonists during this period. The protocol was explained in detail to the patient, following which there was an opportunity for familiarisation with the exercise and monitoring equipment.

Exercise

The forearm muscles of the dominant arm were exercised using a hand strengthener device designed to train the muscles of rock climbers (Gripmaster Hand Strengthener, DMM Products, Pawcatuck, CT, USA). Subjects were asked to squeeze the device to the beat of a metronome set at 40 beats per minute. Encouragement was given to squeeze the device as hard as possible, and the forearm was supported on the arm of a chair to avoid the use of other muscles. Subjects were asked to exercise to fatigue, and verbal encouragement was given to this effect throughout the exercise run. On cessation of exercise, instructions were given to relax completely, and the handgrip device was taken away. The time exercised was noted, and subjects were asked to exercise for the same time during the second exercise run.

Measurements made

Throughout all parts of the protocol, blood pressure and heart rate were measured every 30 seconds using an automated sphygmomanometer with the cuff on the non-dominant arm.   Results were documented by hand.   A metabolic cart (Medical Graphics Corporation, St Paul, Minnesota) was used to measure minute ventilation, respiratory rate, VCO_2, and VO_2 continuously.   The nose was sealed with a clip, and an analyser sampled expiratory gas continuously from the mouthpiece.   Results were displayed online on a computer screen, and downloaded to an Excel spreadsheet at the end of the test.   Familiarisation with the rubber mouthpiece took place before the study commenced, and it was ensured that all variables were stable for at least 2 minutes before the start of exercise.

Protocol

There were 3 parts to the protocol:

1. Cuff inflation at rest.

   This always took place at the start of the study to allow familiarisation with the equipment. Once all variables to be measured had been stable for 2 minutes, the sphygmomanometer cuff was inflated to 200mmHg (or 20mmHg above systolic blood pressure, whichever was greater) for 3 minutes, using a hand pump. The mercury column of a manual sphygmomanometer was connected to the cuff with oxygen tubing, and this was used to ensure that the pressure was maintained. The pressure was topped up if necessary using the hand

pump. The cuff was then deflated, and measurements continued for a further 3 minutes. The subject was encouraged to relax at all times, and it was recorded at the end whether there had been any pain or discomfort.

2. Control exercise.

Once all measured variables had been stable for 2 minutes, the subject was given the handgrip device and asked to start exercising as described above. Encouragement was given to exercise for as long as possible and not to move any part of the body except the exercising muscle. When the patient gave a pre-arranged hand signal suggesting an inability to continue, the handgrip device was taken away and measurements continued for a further 6 minutes.

3. Exercise followed by cuff occlusion.

Once all measured variables had been stable for 2 minutes, the same exercise protocol as described above was followed. At peak exercise, the sphygmomanometer cuff was inflated round the upper part of the exercising arm using a hand pump, and the subject was asked to relax completely. The pressure in the sphygmomanometer cuff was monitored using a mercury column, and care was taken to maintain the pressure at 200mmHg or 20mmHg above systolic blood pressure, whichever was higher. The hand pump was used to top up the pressure in the sphygmomanometer cuff as necessary. Cuff occlusion was maintained for 3 minutes, at which point the cuff was deflated and measurements continued for a further 3 minutes.

Cuff inflation at rest took place at the start of the study, and the other 2 parts of the protocol followed in random order. We allowed 30 minutes of rest between the two parts of the protocol involving forearm exercise.

2.1.6. Data analysis

Data were tested for normality using a Shapiro-Wilk test. Results are all given as mean ± standard error of the mean (SEM). Resting values were taken as the mean of the 60 seconds before the start of exercise. Peak exercise values are the mean of the last 30 seconds of exercise. To evaluate the effects of regional circulatory occlusion (RCO) (and therefore metaboreflex contribution to measured variables), the area under the curve was calculated for the 3 minutes of post-exercise RCO and the equivalent 3 minute period of control recovery. Resting values were subtracted, and this figure was expressed as a percentage of the difference between resting and peak exercise measurements. The following formula was therefore used:

% Metaboreflex contribution = (Peak exercise value – Resting value) divided by (Mean of period of RCO – Resting value) multiplied by 100

To compare the effects of cuff inflation with control recovery, either a paired Student's *t*-test or a Wilcoxon signed-rank test was used after testing for normality with a Shapiro-Wilk test (GB-Stat version 8, Dynamic Microsystems Inc, Silver Spring, MD,

USA). To compare the effects of post-exercise RCO between patient groups, either an unpaired Student's t-test or a Mann-Whitney U test was used, depending on normality. Changes in each variable across time were compared using two-way ANOVA followed by Newman-Keuls *post hoc* analysis when significant F-values were obtained. A p value of <0.05 was considered statistically significant.

2.2. The Assessment of the muscle metaboreflex using pulse transit time

The aim of this study was to determine whether Pulse Transit Time (PTT) can be used to measure muscle metaboreflex activity.

2.2.1 Pulse transit time measurement

Pulse Transit Time is defined as the time taken for a pulse wave to travel between two arterial sites. The physiological basis of pulse transit time is discussed in more detail in chapter 4. It is difficult to measure a pulse wave at two sites, so the "R" wave of the ECG tracing can be used as a surrogate for one site, with a pulse oximetry probe on a finger or toe detecting the pulse wave at a peripheral site. Commercial software is available to measure the time difference between such signals. One such device is the RM 60, manufactured by DeVilbiss, and this is designed for use in the field of sleep medicine (RM60; DeVilBiss, France). Electrocardiogram leads are attached to stickers on both shoulders and the area of the chest overlying the apex beat of the heart, and a pulse oximetry probe is placed on a finger or toe. Both devices are then plugged into a battery-operated box and measurements of heart rate, oxygen saturations and PTT are made continuously. The data is then downloaded onto a computer and it is possible to access the raw data for further analysis (figure 2.1).

Some improvements were made to the equipment used for this experiment. To achieve more rapid sphygmomanometer cuff inflation following exercise, a foot pump was connected to the circuit using oxygen tubing. This enabled inflation of the cuff from 0mmHg to 200mmHg in about 0.5 seconds, preventing some escape of metabolites from the forearm circulation.

We also used a different method of forearm exercise. The Medical Physics department at the Southern General Hospital, Glasgow made us a handgrip device to our own specifications (figure 2.2).

A commercially available spring loaded handgrip device (Power Hand Grip, Rehabmart, Winterville, GA, USA) was fitted with a sensor which measured the distance between the two arms of the device. This was connected to an electronic box which converted this signal to an LED display of 10 lights, allowing the operator to visualise the force generated by squeezing the handgrip device. To calibrate the handgrip device, it was clamped horizontally to a table and weights were suspended from the upper arm. A dial allowed us to calibrate the LED display according to which weights were used. A careful record was kept of how the device was calibrated before each patient visit to ensure reproducibility.

The handgrip device was calibrated at the start of each patient visit as follows. The subject was familiarised with the equipment and encouraged to try gripping the device. The subject was then asked to grip the device as hard as possible with the dominant hand, and at maximum force, the dial on the side of the box was turned until all of the LED lights lit up. On relaxation, all of the LED lights went out. Subsequent

contractions then lit up a number of the lights according to the force generated- it was therefore possible to ensure that each contraction represented a fixed percentage of maximum voluntary contraction.

Figure 2.1 Recording Pulse Transit Time

RM60 Data Recorder

Pulse Oximetry Probe

ECG Pads
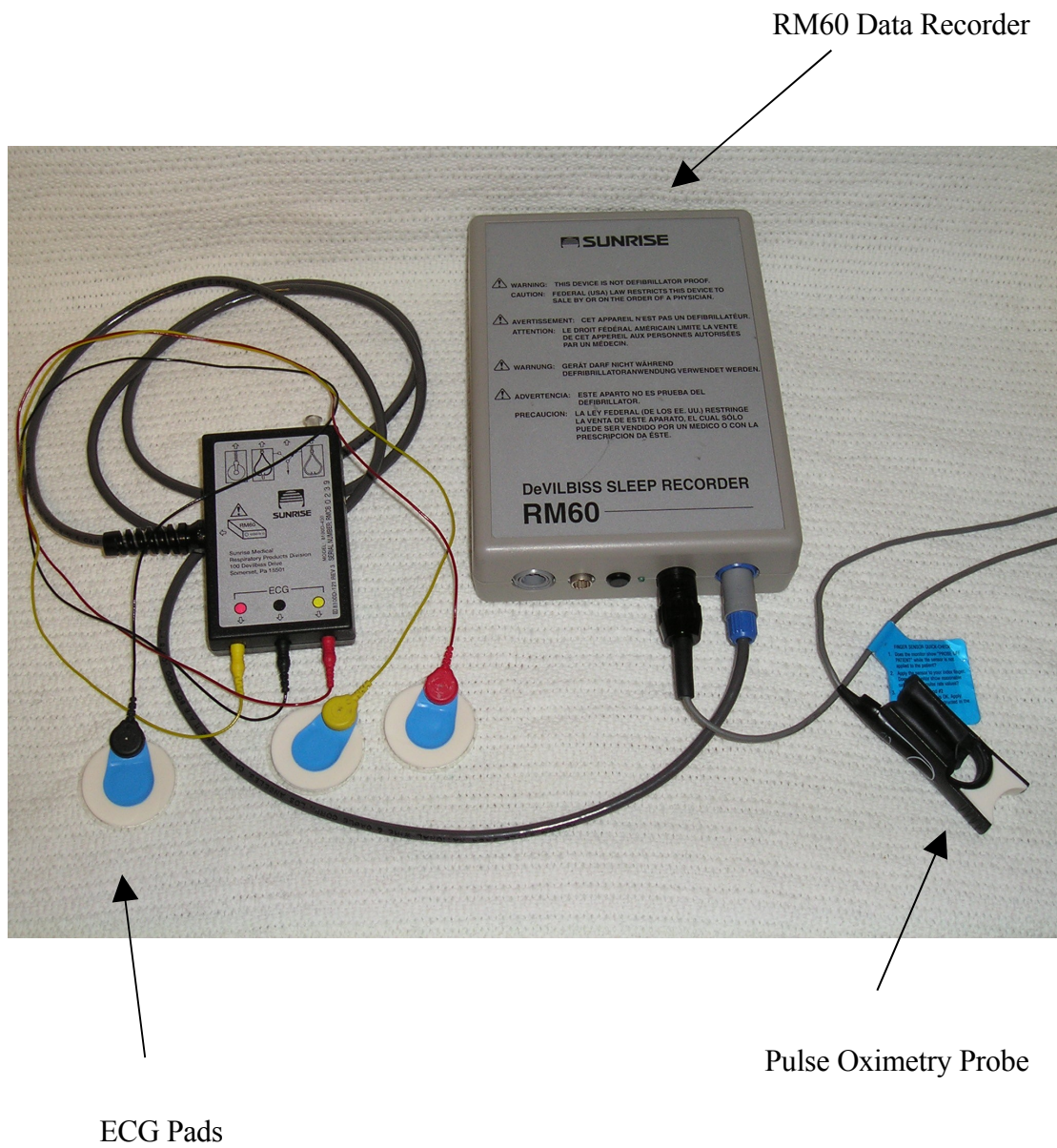
Photograph of the DevilBiss RM60 equipment used to measure pulse transit time. The pulse oximetry probe and ECG electrodes both plug in to the data box. Data can be viewed "online" on a laptop computer.

Figure 2.2 Handgrip exercise device

LED Display (1-10)   Sensor

Arms of handgrip device
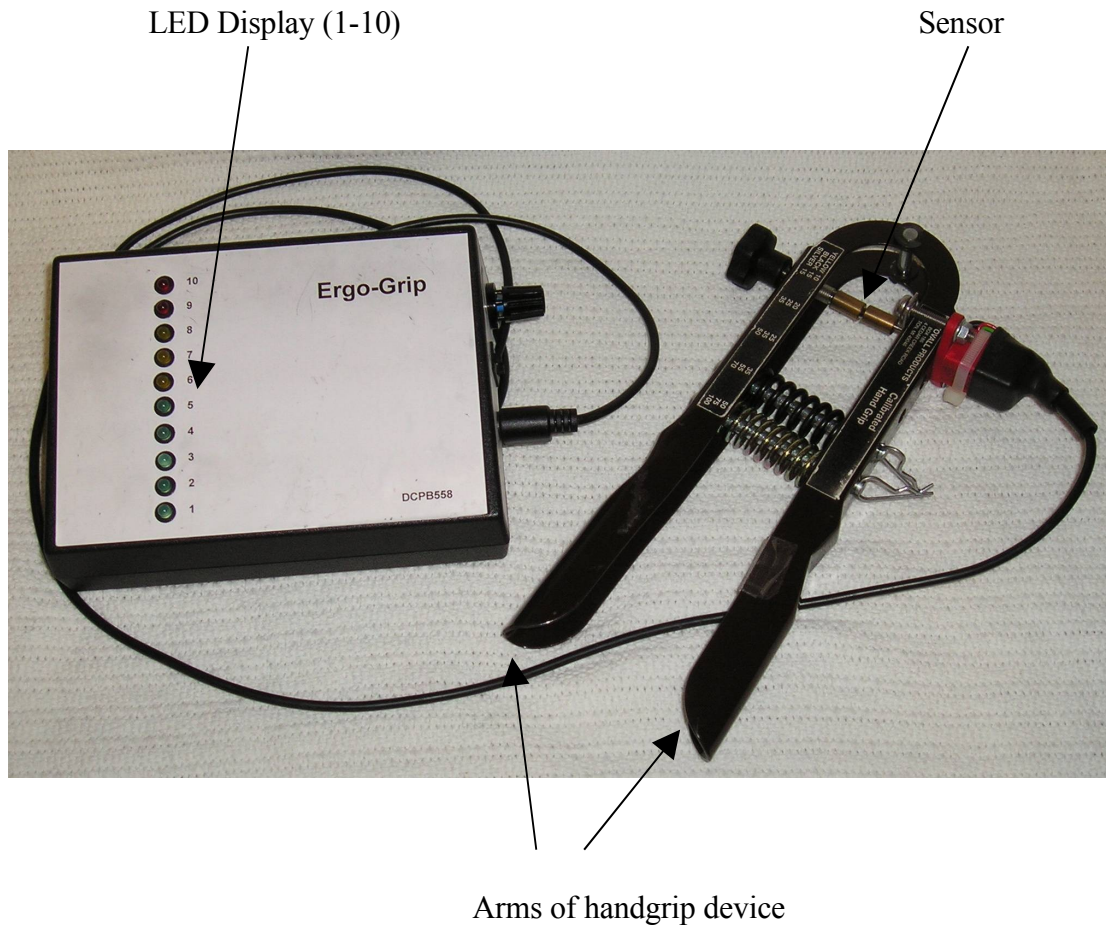
Photograph of the forearm exercise equipment, custom-built by the Department of Medical Physics at the Southern General Hospital, Glasgow.

2.2.2 Ethical considerations

Ethical approval for this study was obtained from the Glasgow Royal Infirmary Local Research Ethics Committee before this study commenced. All patients provided written informed consent prior to participation in the study.

2.2.3 Subject recruitment

We recruited eight healthy volunteers for this study, which involved one 2-hour visit to our laboratory. All subjects were members of staff at Glasgow Royal Infirmary. A medical history was taken to exclude subjects with significant cardiac, respiratory, neurological, locomotor or neoplastic disease. Subjects were all non-smokers.

2.2.4 Exercise protocol

Subject preparation

The protocol was thoroughly explained to the subject, and there was an opportunity for familiarisation with the exercise equipment. The handgrip device was then calibrated as described above, with the subject performing a maximal voluntary contraction with the dominant arm whilst seated: the forearm was supported on the arm of the chair. The RM60 was then set up using a laptop computer: the time on the computer was synchronised with a wristwatch, and data recording commenced. With the subject seated upright on a chair, the pulse oximetry probe was placed on the 2nd toe of the right

foot. A blanket was wrapped round the foot to secure the probe and ensure adequate circulation, and it was plugged into the data recorder. Three ECG electrodes were fixed to the chest wall: on the anterior of the right and left shoulders over a bony prominence, and over the apex of the heart at the 5th intercostal space in the mid-clavicular line. A wrist sensor was then placed over the radial artery of the non-dominant arm to measure blood pressure. Blood pressure was measured every 10-15 seconds using the principle of arterial tonometry. The wrist device intermittently applies gentle pressure to the radial artery to create a waveform, from which blood pressure is calculated (Vasotrac 205A; Medwave, St Paul, Minnesota). This machine has been validated against invasive blood pressure monitoring, with excellent correlation . The machine was calibrated each time it was used as per the instructions of the manufacturer. The subject was asked not to move this arm for the entire duration of the experiment.

Exercise

The subject was seated comfortably with the dominant forearm rested horizontally on a table. At the commencement of exercise, the metronome was started at a rate of 45 beats per minute and the handgrip device was placed in the subject's dominant hand. The subject was asked to squeeze the device intermittently to the beat of the metronome, and encouragement was given to achieve "7" on the LED display with each contraction. Exercise was terminated when a pre-arranged signal was given to suggest fatigue, or when "7" was not reached on the display on 3 consecutive contractions despite verbal encouragement.

Protocol

There were 3 parts to the protocol: cuff inflation at rest, control exercise, and exercise followed by cuff occlusion. The protocol was identical to that followed in section 1, where it is described in detail. Cuff inflation at rest took place at the start of the study, and the other 2 parts of the protocol followed in random order to allow for any potential effects of low frequency muscle fatigue on the second exercise run. Thirty minutes of rest was allowed between the two parts of the protocol involving forearm exercise.

Measurements made

Blood pressure and heart rate were measured every 10-15 seconds with the "Vasotrac", and readings were stored in the memory for later analysis. Pulse transit time was monitored continuously with the RM60. At the end of each visit, the RM60 was connected to a laptop, and the results were downloaded using dedicated software. Blood pressure and heart rate readings were entered into an excel spreadsheet direct from the "Vasotrac".

2.2.5   Data analysis

Data from the RM60 was displayed in a dedicated programme as a graph, with time on the "x" axis and pulse transit time, heart rate and oxygen saturation on the "y" axis. A readout of the exact pulse transit time at any given time was displayed to the left hand side of the graph: moving the cursor along the "x" axis generated a PTT reading every second. It was not possible to download the raw data directly onto a spreadsheet, so the data was entered manually.

To obtain resting values, readings were averaged over the 60 seconds before the commencement of exercise. Peak exercise values were obtained by averaging readings over the last 30 seconds of exercise. To quantify the effects of regional circulatory occlusion (RCO), the area under the curve was taken for each variable for the 3 minutes following peak exercise during cuff inflation. This was compared with the corresponding 3 minute period following peak exercise during the protocol without RCO. For the control cuff inflation, resting values were taken as described above and compared with the area under the curve for the duration of cuff inflation.

Data were checked for normality using a Shapiro-Wilk test. Statistical analysis was then carried out using a paired Student's t-test or repeated-measures analysis of variance where appropriate (GB-Stat version 8, Dynamic Microsystems Inc, Silver Spring, MD, USA).

Figure 2.3 Sample of Pulse Transit Time Tracing
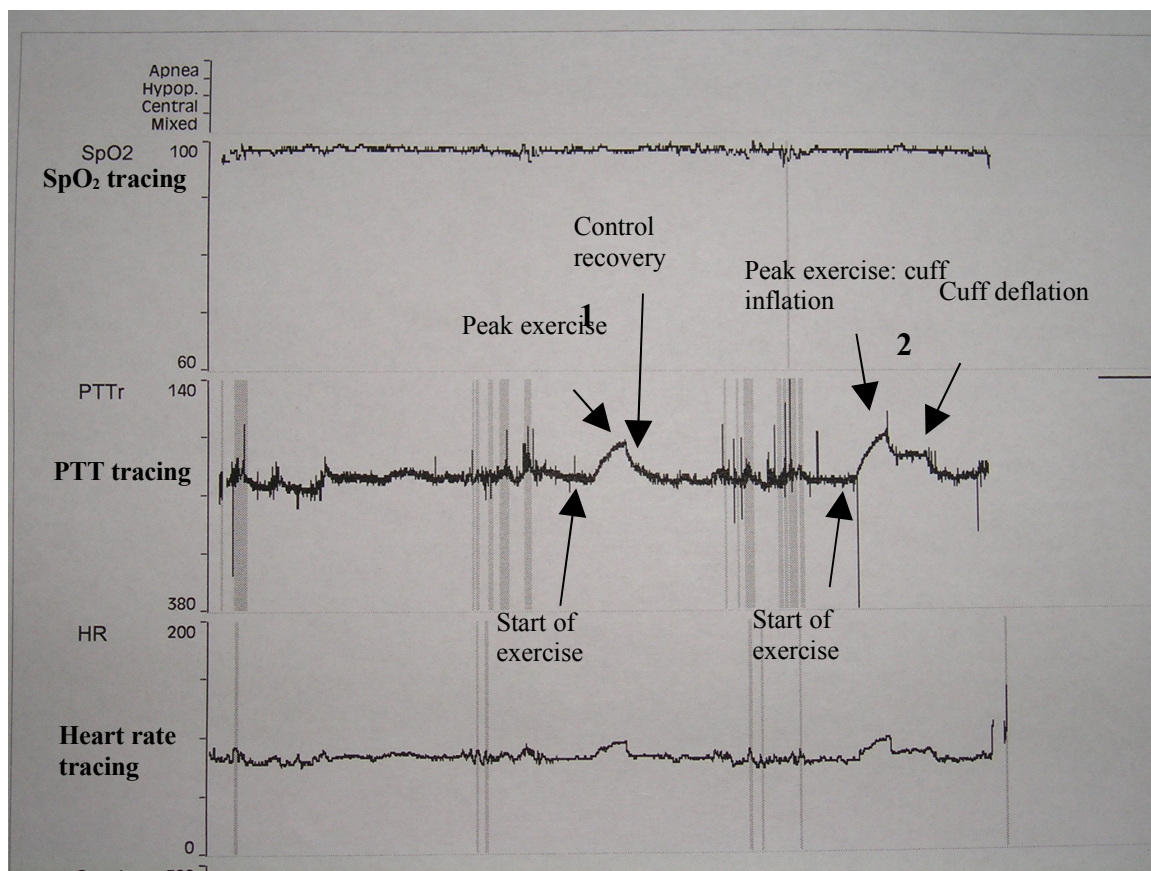
Sample tracing printed out from the computer screen. Oxygen saturations, pulse transit time and heart rate are displayed along the "y" axis, and time is displayed along the "x" axis. This chart displays PTT so that a fall in PTT leads to movement up the "y" axis.

1. The effects of handgrip exercise followed by control recovery: there is a gradual fall in PTT throughout the duration of exercise, which then returns to baseline during recovery.

2. The effects of post-exercise cuff inflation: PTT again falls with handgrip exercise, and remains below baseline values for the duration of sphygmomanometer cuff inflation. PTT then returns to baseline with cuff deflation.

2.3 The Muscle Metaboreflex in Patients with Diabetic Autonomic Neuropathy

The aim of this study was to determine whether the muscle metaboreflex is attenuated in patients with diabetic autonomic neuropathy.

2.3.1 Ethical considerations

This study was approved by the Glasgow Royal Infirmary Local Research Ethics Committee, and all subjects provided written informed consent prior to participation in the study.

2.3.2 Subject recruitment

We recruited patients from the outpatient department of the Department of Diabetes and Endocrinology at Glasgow Royal Infirmary. Patients with autonomic neuropathy were selected from a database and invited to participate in the study. A group of patients with diabetes and no symptoms of autonomic neuropathy was recruited from the clinic. As far as possible the two groups were matched for age and duration of diabetes mellitus.

Subjects with a definite diagnosis of Type I diabetes mellitus were included in the study. The nature of this group of patients meant that a significant number had other complications of diabetes such as hypertension, diabetic retinopathy and ischaemic

heart disease. We excluded subjects with unstable ischaemic heart disease, atrial fibrillation, cardiac failure, respiratory disease, neoplastic disease or locomotor disease which precluded exercise testing. All patients on beta blockers or other rate limiting cardiac drugs were excluded.

2.3.3 Baseline data collection

Baseline blood tests were taken as part of routine clinical care. Casenotes were reviewed for recent urea and electrolytes and haemoglobin A_{1C}. A detailed history was taken of duration of diabetes, smoking habits, past medical history and current medications.

2.3.4 Protocol

The study consisted of 2 visits. The first visit lasted an hour and included consent, medical history and autonomic function testing. Assessment of the muscle metaboreflex took place during the second visit, which lasted about 90 minutes.

Visit 1. Autonomic function testing.

The protocol that we used was that of Ewing and Clarke . This is a series of 5 tests designed to assess the sympathetic and parasympathetic components of the autonomic nervous system. Testing took place in a quiet room, and the subject was asked to lie on

the couch and relax for 10 minutes before testing. There were 5 components to the autonomic testing:

- Heart rate variation during deep breathing. This depends on an intact parasympathetic nervous system. With the patient lying supine, 12-lead ECG electrodes were fitted in the standard manner. The ECG recording commenced, and the subject was asked to breathe deeply at 6 breaths per minute (5 seconds in, 5 seconds out). Recording continued for 1 minute. The maximum and minimum R-R intervals during each breathing cycle were then measured with a ruler and converted to beats per minute: the result was expressed as the mean of the difference between maximum and minimum heart rates for the six measured cycles. "Normal" was defined as >14 beats per minute (bpm), "borderline" as 11-14 bpm and "abnormal" as <11 bpm.

- Heart rate response to Valsalva manoeuvre. This is again a test of parasympathetic function. During the strain period of the Valsalva manoeuvre, the blood pressure drops and the heart rate rises: after release, the blood pressure rises and "overshoots" leading to a fall in heart rate mediated by the vagus nerve. The subject was asked to sit on the couch at 45 degrees and ECG recording commenced. The subject was then given a mouthpiece attached to a sphygmomanometer and asked to blow into the mouthpiece, maintaining the pressure at 40mmHg for 15 seconds. This test was performed 3 times with one minute of rest in between. The result was expressed as the Valsalva ratio, the ratio of the longest R-R interval after the manoeuvre to the shortest R-R interval

during the manoeuvre. "Normal" was defined as a ratio of >1.20, "borderline" as 1.10-1.20 and "abnormal" as <1.10.

- Heart rate response to standing. On standing, there is an immediate reflex tachycardia, which should be followed by a relative overshoot bradycardia mediated by the vagus nerve. The subject was asked to lie supine on the couch during continuous ECG monitoring, then stand up unaided: this point was marked on the ECG. The shortest R-R interval at or around the 15th beat and the longest R-R interval at or around the 30th beat after standing were measured with a ruler. This was expressed as the 30:15 ratio: "normal" was defined as >1.04, "borderline" as 1.00-1.04 and "abnormal" as <1.00.

- Blood pressure response to standing. Pooling of blood in the legs on standing causes a drop in blood pressure, which should be rapidly offset by peripheral vasoconstriction mediated by the sympathetic nervous system. Before the heart rate response to standing test described above, blood pressure was measured using an automated sphygmomanometer. Blood pressure was then repeated after the subject stood up. A normal result was defined as a fall in systolic blood pressure of less than 10mmHg, "borderline" was 10-29mmHg and "abnormal" was >29mmHg.

- Blood pressure response to sustained handgrip. During sustained handgrip there should be a rise in blood pressure due to a sympathetically-mediated heart rate-dependent increase in cardiac output. Blood pressure was measured 3 times at rest. A handgrip dynamometer was then used to determine maximal voluntary

contraction (MVC) of the dominant arm whilst seated. The subject was then asked to maintain handgrip at 30% of MVC for a total of 5 minutes, and blood pressure was recorded from the non-dominant arm at 1 minute intervals. The result was expressed as the difference between the highest diastolic blood pressure during handgrip exercise and the mean of the three resting readings. A normal result was defined as a rise in diastolic blood pressure of more than 15mmHg, "borderline" was 11-15mmHg and "abnormal" was less than 11mmHg.

There were two doctors present during autonomic function testing: one to operate the ECG machine and mark the tracings, and one to instruct the patient. All tracings were analysed at the end of the study so that the doctor performing visit 2 was blinded from the results of the autonomic testing. All ECG tracings were analysed by Dr Babulyeb Mukhopadhyay, a Specialist Registrar in Diabetes and Endocrinology at Glasgow Royal Infirmary. Autonomic neuropathy was defined as definite abnormalities in 2 or more of the above tests.

Visit 2. Metaboreflex assessment.

Assessment of the metaboreflex followed a similar protocol to that described above. Subjects were asked to refrain from smoking or caffeine during the 4 hours prior to the visit. Repetitive forearm exercise to fatigue was carried out at 70% of maximum voluntary contraction at a rate of 45 contractions per minute. Blood pressure and heart rate were measured every 10-15 seconds by arterial tonometry (Vasotrac 205A; Medwave, St Paul, Minnesota). Minute ventilation, VO_2, VCO_2 and respiratory rate

were measured continuously from exhaled gas sampled from a rubber mouthpiece by a metabolic cart (Medical Graphics Corporation, St Paul, Minnesota). There were again 3 parts to the protocol, the order of which was varied randomly:

- Cuff inflation at rest: 3 minutes of resting measurements, following which a sphygmomanometer cuff was rapidly inflated, using a foot pump, to 200mmHg (or 20mmHg above systolic blood pressure) for 3 minutes. Three minutes of recovery.

- Forearm exercise followed by control recovery: 3 minutes of rest followed by repetitive forearm exercise to fatigue. This was followed by 6 minutes of normal recovery.

- Forearm exercise followed by cuff occlusion: 3 minutes of resting measurements followed by repetitive forearm exercise to fatigue. At peak exercise, a sphygmomanometer cuff was inflated round the upper part of the exercising arm to 200mmHg (or 20mmHg above systolic blood pressure) for 3 minutes and the subject was asked to relax. Following this, there was a further 3 minutes of recovery.

The control cuff inflation always took place first, followed by the two parts involving exercise in random order.

2.3.5 Data analysis

Results are all given as mean ± standard error of the mean. Resting values were taken as the mean of the 60 seconds before the start of exercise. Peak exercise values are the mean of the last 30 seconds of exercise. To evaluate the effects of regional circulatory occlusion (RCO), a mean was taken of the 3 minutes of post-exercise RCO and compared with the equivalent period of control recovery. As the two exercise runs were performed on the same group of patients, a paired Student's t-test was used after testing for normality using a Shapiro-Wilk test. To compare the effects of post-exercise RCO between groups, an unpaired Student's t-test was used after checking for normality. Changes in each variable across time were compared using two-way ANOVA followed by Newman-Keuls *post hoc* analysis when significant F-values were obtained (GB-Stat version 8, Dynamic Microsystems Inc, Silver Spring, MD, USA). A p value of <0.05 was considered statistically significant.

2.4 The effects of creatine supplementation on patients with stable COPD

The purpose of this study was to determine the effects of creatine supplementation on a group of patients with stable COPD. Primary outcome measure was muscle metaboreflex activity, and other variables measured included body weight, forearm muscle strength, forearm muscle endurance and respiratory muscle strength.

2.4.1 Ethical considerations

This study was approved by the Glasgow Royal Infirmary Local Research Ethics Committee, and all subjects provided written informed consent prior to participation in the study.

2.4.2 Subject recruitment

Fifteen patients were recruited from the outpatient department of the Department of Respiratory Medicine at Glasgow Royal Infirmary. All patients had a diagnosis of COPD as per the guidelines of the Global Initiative for Obstructive Lung Diseases (GOLD) . Patients who had suffered an exacerbation during the previous 6 weeks were excluded: we defined an exacerbation as a deterioration in symptoms requiring hospitalisation, a visit to primary care or a course of antibiotics or oral corticosteroids. Other exclusion criteria were unstable cardiac disease or cardiac failure, significant neurological or locomotor disease, active malignancy or advanced renal or hepatic disease as determined by hospital casenote review, patient history and clinical

examination. We also excluded patients with diabetes mellitus because of the glucose load in the creatine supplements.

2.4.3 Patient screening

Suitable patients were identified at outpatient clinics and followed up through telephone contact. A series of questions was asked to confirm suitability, and pulmonary function tests were conducted as part of routine clinical care to confirm the diagnosis of COPD, as described above. At the first visit, a full medical history was taken, including smoking history, current and previous medications, exacerbation history and corticosteroid use.

2.4.4 Study design

This was designed as a randomised, double-blind, placebo-controlled crossover study looking at the effects of loading with creatine monohydrate for 10 days compared with placebo. There was at least a 2 week washout period between the 2 arms of the study (figure 2.4).

2.4.5 Creatine supplementation

A dose of creatine monohydrate was chosen based on previous studies demonstrating benefit . We chose to use 5 grams of creatine monohydrate three times daily for 10 days. The addition of glucose to creatine during a period of loading has been shown to augment creatine retention by skeletal muscle through the actions of insulin . We therefore elected to mix 30 grams of glucose monohydrate with each dose. This also allowed a placebo to be developed consisting of 30 grams of glucose monohydrate alone.

The creatine monohydrate used in this study was obtained from Flamma pharmaceuticals (Flamma, Bergamo, Italy), and a certificate provided by the company confirmed its purity. The creatine was delivered by Arena Pharmaceuticals (Arena Pharmaceuticals Ltd, Buckingham, UK) and given to our hospital pharmacy for storage. The pharmacy department at the Western Infirmary, Glasgow prepared the creatine supplements and the placebo, which were stored until use by the pharmacy at Glasgow Royal Infirmary. Each individual supplement was dispensed in a brown medicine bottle, and the bottles holding the creatine supplement and the placebo were identical in appearance. Each patient "pack" therefore consisted of 30 identical bottles, enough for 10 days of supplementation. Randomisation was carried out by the pharmacist at the Western Infirmary, and the "break codes" were held in a sealed envelope until completion of the study.

Figure 2.4 Design of the creatine study

| Placebo arm | → | Washout | → | Creatine arm |
| (10 days) | | (2 weeks) | | (10 days) |

↗

Randomisation

↘

| Creatine arm | → | Washout | → | Placebo arm |
| (10 days) | | (2 weeks) | | (10 days) |

↑   ↑   ↑   ↑

VISIT 1   **VISIT 2**   **VISIT 3**   **VISIT 4**
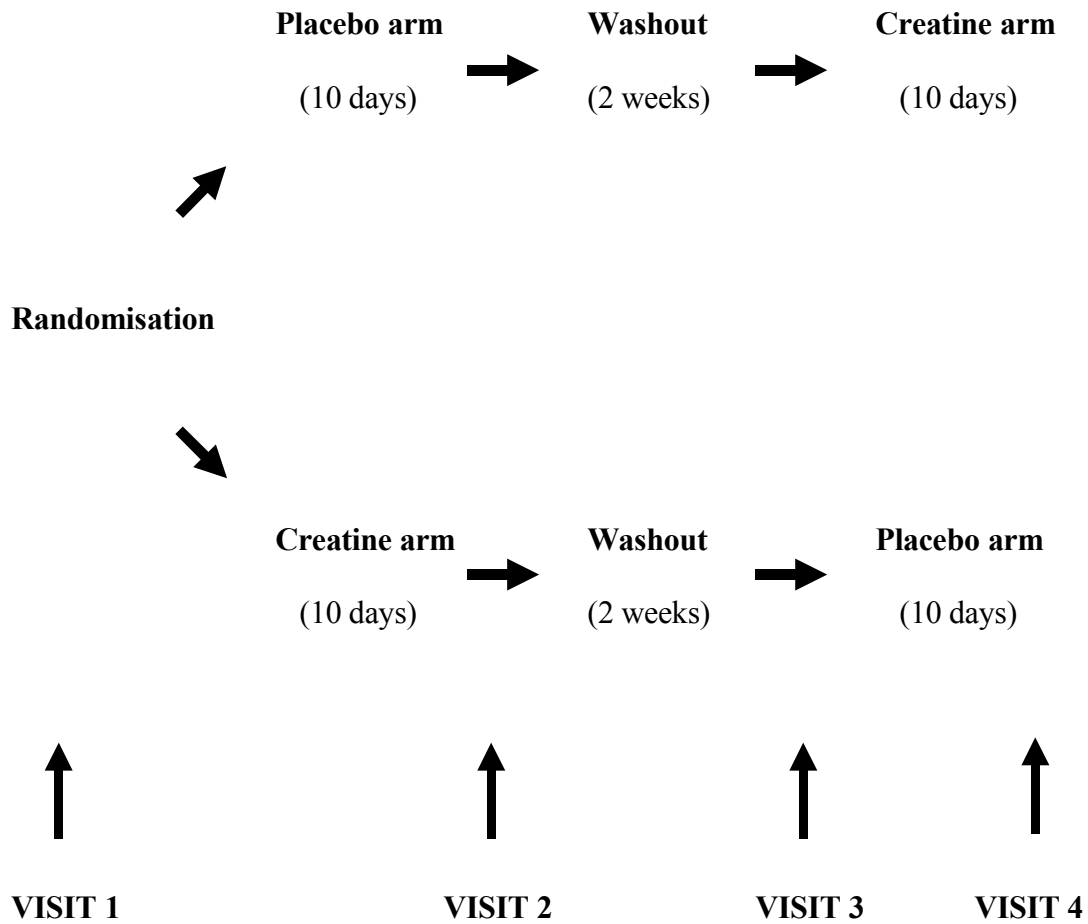
This was a randomised, double-blind, Placebo-controlled crossover study with a 2 week washout period between the two arms.

2.4.6 Study protocol

Each patient visit was identical, and tests were always carried out in the same order. As far as was possible, all patient visits took place at the same time of the day and in the same laboratory. The visit was divided up as follows:

At the start of the visit, questions were asked about recent symptoms, medication use and any exacerbations. At visits 2 and 4, specific questions were asked about side effects and compliance. All bottles were returned and counted to confirm compliance. Height and weight were then taken with jacket and shoes off using the equipment in the pulmonary function lab, calibrated regularly according to the instructions of the manufacturer.

Venesection

The non-dominant arm was used for venesection. 20ml of blood was withdrawn from the antecubital fossa using a vacutainer system with a tube containing clot activator. The blood was left to stand for 30 minutes, then centrifuged at 3500rpm for 15 minutes. The plasma was immediately separated using a pipette and divided into 4 aliquots. The samples were then stored in a -70ºC freezer for future analysis.

Forearm muscle function

This part of the protocol was aimed at testing strength, endurance and recovery of the muscles of the forearm. Tests were always performed in the same order.

i) Strength:

The subject was seated comfortably on a chair and asked to relax. A handgrip dynamometer (Grip-A, Takei Scientific Instruments Co, Tokyo, Japan) was placed in the dominant hand and the subject was asked to pull the lever as hard as possible with the arm extended towards the floor. After a short rest, this was repeated with the non-dominant hand and the readings were recorded. This test was repeated 5 times with each hand, with time for recovery in between each test. The highest recording of the 5 readings was taken as maximum handgrip strength.

ii) Endurance:

The subject was asked to sit with the dominant forearm resting on a table with the palm facing the ceiling. Detailed instructions were given before the test commenced and there was the opportunity to have a "trial run". A metronome was set to a beat of 40 per minute and instructions were given to pull and release the lever of the dynamometer to each beat of the metronome. Before the start of the test, the subject was told what the target was for each pull of the handgrip, corresponding to 70% of the maximum handgrip. Verbal encouragement was given during the test to continue for as long as possible and to achieve the target strength with each pull of the lever. The test was discontinued when the subject failed to achieve the target with three consecutive pulls of the lever. The number

of repetitions managed was recorded and the test was repeated with the non-dominant forearm after an adequate period of rest.

iii) <u>Muscle Recovery:</u>

This test immediately followed the endurance testing. The subject had been informed of the protocol before commencement of the endurance test. At the end of the endurance test, a stopwatch was reset and the clock was started. The subject was asked to perform maximum handgrips at set points over the next 5 minutes to document muscle recovery. Time points chosen were as follows (reference point being the end of the endurance test): 10 seconds, 20s, 40s, 80s, 160s, 300s. This part of the protocol was performed with both the dominant and the non-dominant forearms and readings were recorded for later analysis.

Respiratory muscle strength

This was tested using a hand-held mouth pressure meter (Pearson Medical, North Yorkshire, UK). The subject was asked to stand up and the protocol was explained. A disposable mouthpiece was attached to the meter and it was zeroed. The lips were placed round the mouthpiece in maximal inspiration and the subject was asked to blow as hard as possible. This was repeated, with adequate rest in between tests, until there were 3 readings with less than 10% variability. Inspiratory muscle strength was then tested by asking the subject to suck through the mouthpiece as hard as possible from maximal expiration. This was also repeated until there were 3 readings with less than

10% variability. The highest readings were taken as peak inspiratory and peak expiratory pressure.

Muscle Metaboreflex Measurement

Assessment of the metaboreflex followed a similar protocol to that described earlier in the chapter. An identical exercise protocol was used, with repetitive forearm exercise to fatigue at 70% of maximum voluntary contraction at a rate of 45 contractions per minute. Blood pressure and heart rate were measured every 10-15 seconds by arterial tonometry (Vasotrac 205A; Medwave, St Paul, Minnesota). Minute ventilation, VO_2, VCO_2 and respiratory rate were measured continuously from exhaled gas sampled from a rubber mouthpiece by a metabolic cart (Medical Graphics Corporation, St Paul, Minnesota). Pulse Transit Time was measured using the RM60 (DevilBiss, France) as described in chapter 2.2.1. There were again 3 parts to the protocol:

- Cuff inflation at rest:

  3 minutes of resting measurements, following which a sphygmomanometer cuff was rapidly inflated (<1 second) to 200mmHg (or 20mmHg above systolic blood pressure) using a foot pump for 3 minutes. Three minutes of recovery.

- Forearm exercise with control recovery:

  3 minutes of resting measurements followed by repetitive forearm exercise to fatigue. This was followed by 6 minutes of normal recovery.

- <u>Forearm exercise followed by cuff occlusion:</u>

    3 minutes of resting measurements followed by repetitive forearm exercise to fatigue. At peak exercise, a sphygmomanometer cuff was inflated round the upper part of the exercising arm to 200mmHg (or 20mmHg above systolic blood pressure) for 3 minutes and the subject was asked to relax. Following this, there was a further 3 minutes of recovery.

The control cuff inflation at rest always took place first, followed by the two parts involving exercise in random order. During the first visit, it was noted which order the tests were performed in, and this order was repeated at subsequent visits.

It was decided that it was important to ensure that the same total amount of work was performed during metaboreflex assessment at each visit. Time to fatigue was therefore recorded and the subject was asked to exercise for the same time at subsequent visits. Following the first visit, the settings of the handgrip device used for exercise were recorded. At the start of subsequent visits, the device was calibrated with weights to ensure identical settings at each visit.

Creatine Administration

At the end of each visit, a package containing the creatine supplements or placebo was collected from pharmacy and given to the patient. A prescription was filled out for each course, and pharmacy issued a package according to patient number and patient visit. Instructions were given to take one dose first thing in the morning, one dose at

lunch time and one dose just before bed. Patients were asked to add the whole pot of powder to a cup of lukewarm water, stir well and consume the drink as quickly as possible. If there was still residue left in the cup, it was to be rinsed with a small quantity of warm water and the contents consumed. Visits were planned so that visit 2 or 4 took place on the day of the final dose of creatine or placebo.

2.4.7   Plasma analysis

Stored plasma was analysed at the end of the study for C-reactive protein (using a highly sensitive assay), interleukin-6 and homocysteine.

2.4.8   Data analysis

Normality of distribution of data was assessed using a Shapiro-Wilk test (GB-Stat version 8, Dynamic Microsystems Inc, Silver Spring, MD, USA). All data are presented as mean ± standard error of the mean. Pre- and post-loading data were compared using a paired Student's *t*-test. The effects of creatine supplementation were compared to the effects of placebo using a paired Student's *t*-test.

For assessment of the muscle metaboreflex, resting values are the mean of the 60 seconds before the start of exercise. Peak exercise values are the mean of the last 30 seconds of exercise. To evaluate the effects of regional circulatory occlusion (RCO), the area under the curve was calculated for the 3 minutes of post-exercise RCO and

compared to the equivalent period of control recovery using a paired Student's *t*-test. Changes in any variable across time were assessed using two-way analysis of variance (ANOVA) followed by Newman-Keuls *post-hoc* analysis when significant *F*-values were obtained. A *p* value of <0.05 was considered statistically significant.

Chapter 3

The Muscle Metaboreflex in Patients with Stable Chronic Obstructive Pulmonary Disease

3.1 Chapter introduction

Chronic Obstructive Pulmonary Disease (COPD) is one of the most common causes of death and disability in the Western World . Inhaled irritants such as cigarette smoke lead to chronic inflammation of the airways and the development of progressive airflow limitation. Disturbance of the protease/ anti-protease equilibrium causes destruction of the alveolar membrane with a loss of elastic tissue . The physiological consequences of fixed airflow obstruction, increased lung compliance and reduced alveolar membrane surface area include flow limitation, impairment of gas exchange and dynamic hyperinflation on exercise . These are all thought to contribute to increased work of breathing and exercise intolerance, the most distressing symptom of COPD. It has, however, been noted that a significant proportion of patients with COPD cite muscle fatigue as the reason for terminating an incremental exercise test , and there has therefore been substantial interest in the role of peripheral skeletal muscle dysfunction in exercise limitation, with atrophy, a change in fibre type and altered metabolism described .

It has been speculated that exercise limitation in COPD and chronic heart failure may have similar contributing causes, and there are striking similarities in the changes seen in skeletal muscle . Traditional indices of disease severity (FEV_1 and ejection fraction) correlate poorly with exercise capacity and exercise-based rehabilitation classes partially reverse muscle abnormalities whilst improving exercise performance and quality of life . Excessive ventilation for a given workload and an increased ventilatory equivalent (VE/VCO_2) have been noted in both COPD and chronic heart failure, and these abnormalities can be partially reversed with exercise training .

The muscle metaboreflex has been of particular interest in patients with chronic heart failure. Studies have looked at assessing the muscle metaboreflex using forearm exercise followed by post-exercise circulatory occlusion, as previously described in chapter 1 of this thesis. It has been shown that there is an increase in muscle metaboreflex activity in patients with chronic heart failure , with activity correlating with NYHA symptom classification . This observation has been attributed to abnormalities in muscle metabolism, with an increase in lactate production and intramuscular acidosis causing increased metaboreceptor stimulation . It has been speculated that this contributes to the excessive sympathetic nervous system and ventilatory response to exercise in chronic heart failure . It has also proven possible to reduce metaboreflex activity in patients with chronic heart failure through training of skeletal muscle . As patients with COPD suffer from similar skeletal muscle abnormalities , this chapter set out to investigate whether it is possible to measure the muscle metaboreflex in patients with stable COPD, and whether patients with more severe disease demonstrate an increase in metaboreflex activation.

3.2 Research questions

The aims of this study were to answer the following research questions:

i)   Is it possible to measure muscle metaboreflex activity in patients with stable COPD?

ii)   Is there a difference in muscle metaboreflex activity between patients with moderate and severe COPD?

3.3 Patient characteristics and pulmonary function testing

Sixteen patients with stable Chronic Obstructive Pulmonary Disease (COPD) were recruited into this study: all met the criteria described in chapter 2. Metaboreflex assessment followed the protocol described in chapter 2. Two patients were unable to complete the protocol: one subject could not tolerate the rubber mouthpiece of the metabolic cart, and one subject found the sphygmomanometer cuff too uncomfortable to tolerate. Subject characteristics are described in table 3.1. This was a group of patients with significant airflow limitation, as seen by the mean FEV_1 of $46.4 \pm 3.7\%$ of predicted and the mean $FEV_1/$ FVC ratio of $37.9 \pm 3.0\%$. There was also evidence of hyperinflation at rest and impaired diffusion capacity. Table 3.2 outlines what medications the patients were taking.

3.4   The effects of exercise and post-exercise regional circulatory occlusion: whole group

3.4.1   The effects of rhythmic handgrip exercise

Rhythmic handgrip exercise led to a significant increase in blood pressure, heart rate, ventilation, VCO_2 and VO_2. There was no significant difference between resting or peak values between the control exercise run and the exercise followed by RCO (table 3.3, figures 3.1-3.6).

3.4.2   The effects of post-exercise regional circulatory occlusion

Regional circulatory occlusion (RCO) at the end of rhythmic handgrip exercise led to a sustained increase in systolic blood pressure ($p<0.005$), diastolic blood pressure ($p<0.0001$), ventilation ($p<0.005$), VO_2 ($p<0.005$) and VCO_2 ($p<0.001$) when compared with control recovery (table 3.4). Post-exercise RCO had no effect on heart rate.

3.4.3   The effects of control regional circulatory occlusion at rest

Control regional circulatory occlusion at rest did not affect blood pressure, heart rate or ventilation (table 3.5, figures 3.1-3.6). There was a significant fall in VO_2 and VCO_2 during RCO, confirming "isolation" of the forearm muscle from the rest of the circulation.

Table 3.1 Subject characteristics

Male/ Female (n)	8/ 6 (14)
Age (years)	63.7±3.1
Height (cm)	164 ± 2.6
Weight (kg)	73.3±4.8
Body mass index (kg/m^2)	27.5±1.7
Smoking history (pack years)	57.3±9.1
MRC dyspnoea score	2.9±0.2
FEV$_1$ (litres)	1.18±0.12
FEV$_1$ (% predicted)	46.4±3.7
FEV$_1$/ FVC	37.9±3
Bronchodilator reversibility (%)	7.1±2.7
Bronchodilator reversibility (ml)	67.1±27.2
Corrected TLCO (% predicted)	50.6±5.3
Residual volume (% predicted)	174.7±15.5
Total lung capacity (% predicted)	122.7±6.1
Maximum handgrip (kg)	30.8±2.7
Maximum handgrip (% predicted)	93.4±4.2
Peak inspiratory pressure (cmH$_2$O)	74.9±7.1
Peak inspiratory pressure (% predicted)	107.8±11.6
Peak expiratory pressure (cmH$_2$O)	71.6±4.2
Peak expiratory pressure (% predicted)	69+5.1
Exacerbations in previous year	3.5±1.0

Age, height, weight, sex and lung function of this subject group. Data are presented as mean ± standard error of the mean. Standard formulae and tables were used to calculate predicted mouth pressures and handgrip strength .

Table 3.2 Medication history

Medication	Total	Moderate group	Severe group
N	14	7	7
Seretide	8	5	3
Symbicort	1	0	1
Nebulised pulmicort	1	1	0
Salbutamol inhaler	10	4	6
Serevent	4	2	2
Tiotropium	7	4	3
Theophylline	4	2	2
Oral Beta-2 agonist	1	1	0
Carbocysteine	1	0	1
Nebulised salbutamol	8	4	4
Nebulised atrovent	5	2	3
Combivent inhaler	2	2	0
Aspirin	3	1	2
Calcium channel blocker (non-rate limiting)	2	1	1
Statin	1	0	1
ACE inhibitor	3	2	1

This table details the inhaled and oral COPD medications that were taken by patients.

Cardiovascular medications are also listed.

Table 3.3 The effects of rhythmic handgrip exercise to fatigue on this group

	Control exercise run		Exercise followed by RCO	
	Rest	**Peak exercise**	**Rest**	**Peak exercise**
Systolic BP (mmHg)	128.0±5.0	156.1±5.5*	129.3±4.5	160.5±5.2*
Diastolic BP (mmHg)	77.5±3.7	102.1±5.3*	77.8±2.9	95.7±4.4*
Heart rate (bpm)	80.6±3.5	91.2±3.5*	79.2±3.0	89.2±3.0*
Ventilation (l/min)	10.7±0.4	18.1±0.7*	10.8±0.4	17.6±0.8*
VO_2 (ml/min)	187±10	301±18*	188±11	300±19*
VCO_2 (ml/min)	183±10	344±22*	182±10	323±22*

All data are presented as mean ± standard error of the mean. To compare resting and peak exercise values, statistical significance was tested using a paired Student's *t*-test after checking for normality. *$p < 0.0001$ compared with resting values.

Table 3.4 The effects of post-exercise regional circulatory occlusion

	Post-exercise recovery	Post-exercise RCO
Systolic BP (mmHg)	132.5±4.0	153.5±5.7*
Diastolic BP (mmHg)	79.1±3.3	94.6±3.6††
Heart rate (bpm)	80.9±3.6	81.5±3.5
Ventilation (l/min)	12.4±0.5	14.7±0.8*
VO$_2$ (ml/min)	205±13	231±15**
VCO$_2$ (ml/min)	220±15	261±19†

Values given represent the "area under the curve" of the 3 minutes following peak exercise, either during recovery from exercise without regional circulatory occlusion (RCO) or during post-exercise RCO. Statistical significance was assessed using a paired Student's *t*-test after checking for normality using a Shapiro-Wilk test. Comparison between control recovery and RCO: *p<0.005, **p<0.0005, †p<0.001, ††p<0.0001.

Table 3.5 The effects of regional circulatory occlusion at rest

	Control rest period	RCO at rest
Systolic BP (mmHg)	130.6±4.2	131.9±4.5
Diastolic BP (mmHg)	78.1±3.2	79.0±3.0
Heart rate (bpm)	79.4±3.3	79.3±3.3
Ventilation (l/min)	11.1±0.5	11.1±0.5
VO_2 (ml/min)	193±10	184±10**
VCO_2 (ml/min)	193±11	183±11*

Values given represent the "area under the curve" of the 3 minutes of either a control period of rest or regional circulatory occlusion (RCO) at rest. Statistical significance was assessed using a paired Student's t-test after checking for normality using a Shapiro-Wilk test. Comparison between control rest and RCO at rest: *$p<0.05$, **$p<0.005$.

Figure 3.1 The effects of exercise followed by RCO on systolic blood pressure
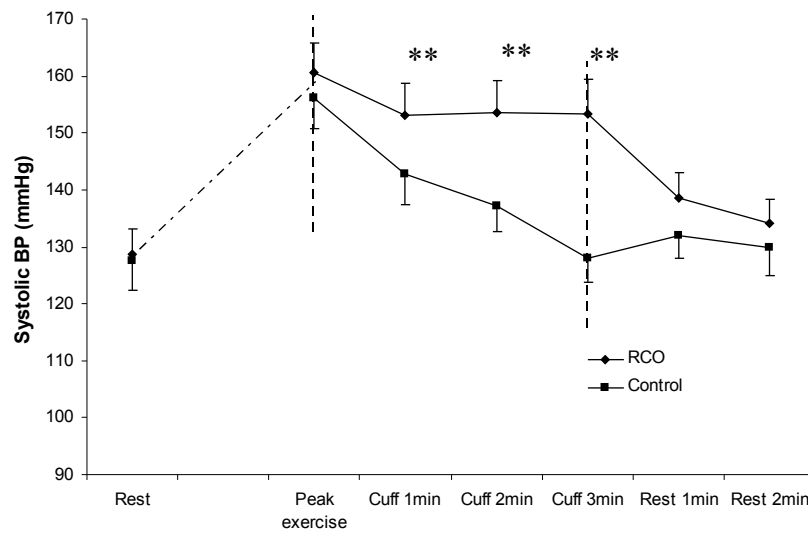
Figure 3.2 The effects of exercise followed by RCO on diastolic blood pressure
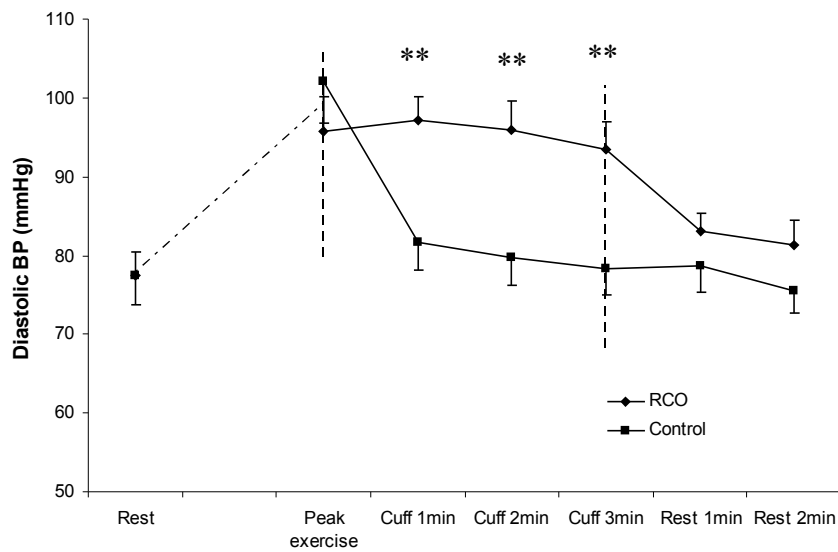
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: $*p<0.05$, $**p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 3.3 The effects of exercise followed by RCO on heart rate
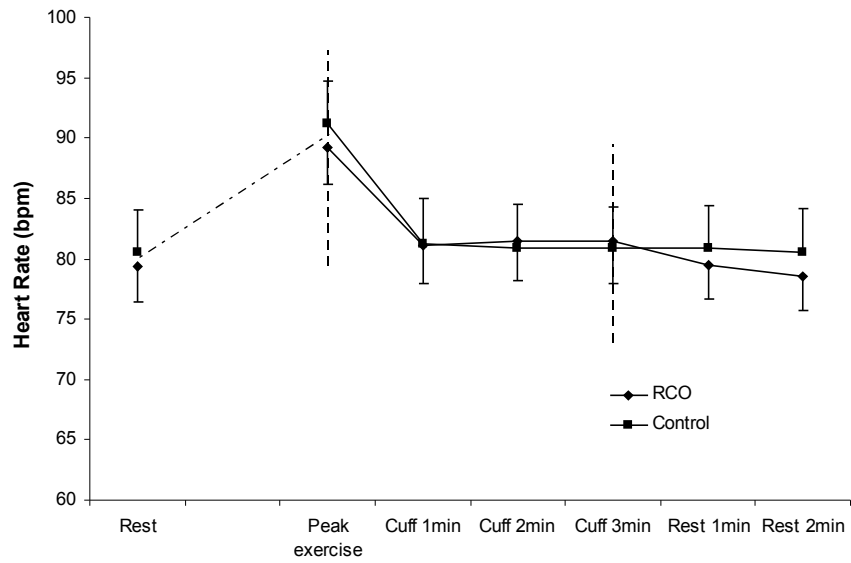
Figure 3.4 The effects of exercise followed by RCO on ventilation
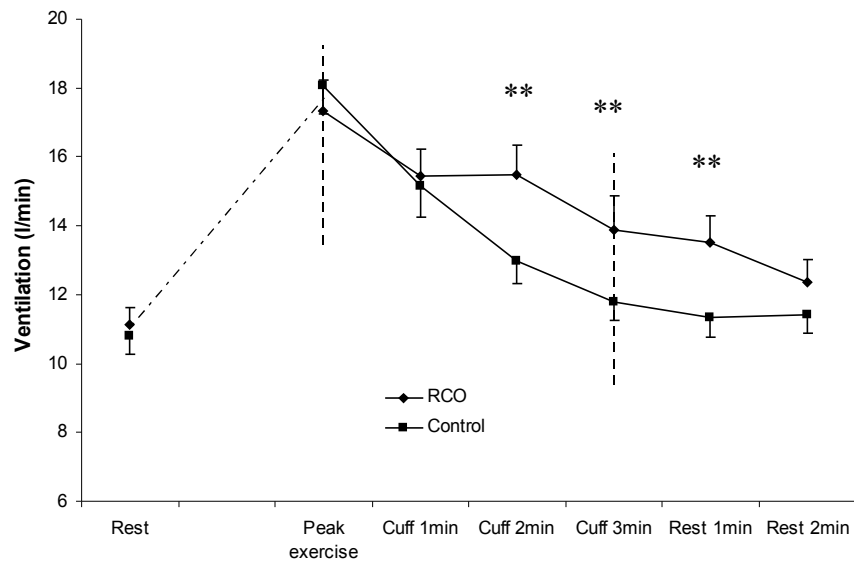
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: $*p<0.05$, $**p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 3.5 The effects of exercise followed by RCO on oxygen consumption (VO$_2$)
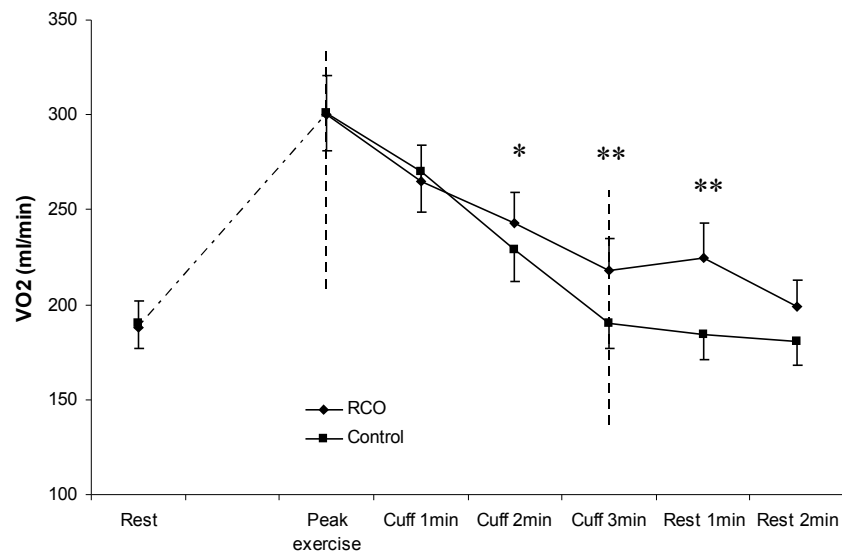
Figure 3.6 The effects of exercise followed by RCO on carbon dioxide production (VCO$_2$)
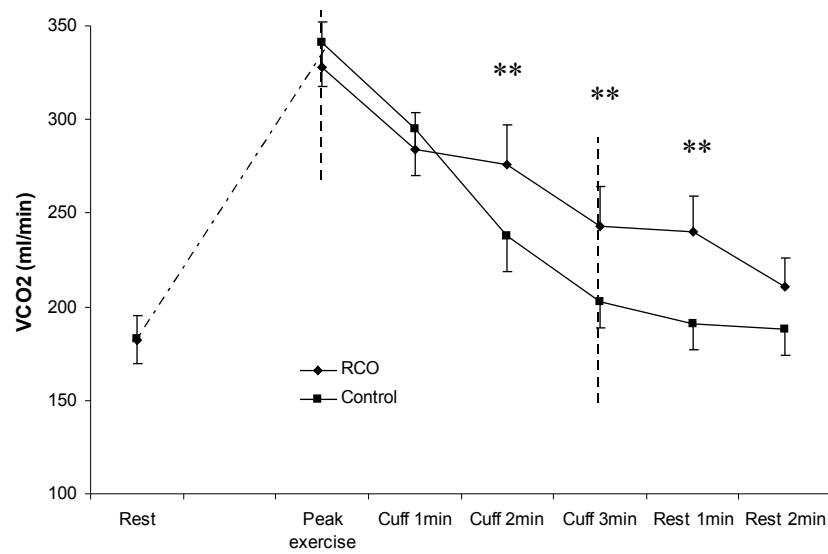
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *$p < 0.05$, **$p < 0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

3.5 Stratification into moderate and severe COPD

Analysis of results was performed following stratification of patients into 2 groups. It should be pointed out that stratification occurred after completion of the study: this is therefore a *post-hoc* analysis. FEV_1 was used to determine severity of COPD. The seven patients with the lowest FEV_1 were put in the "severe" group, and the remaining patients were put in the "moderate" group. Characteristics of the 2 groups are shown in table 3.6, and a medication list is given in table 3.2.

3.5.1   Anthropometry

There was no significant difference in age or height between the two groups. The "severe" group was significantly lighter than the "moderate" group (62.2 ± 4.1kg vs. 84.4 ± 6.3kg, p<0.05), with a lower body mass index (22.4 ± 1.4kg/m^2 vs. 32.7 ± 1.4kg/m^2). Patients in the "severe" group had a trend towards a heavier smoking history, a greater number of exacerbations in the previous year and greater functional impairment as measured by the baseline dyspnoea index, although these small differences were not statistically significant.

3.5.2   Pulmonary function testing

The "severe" group of patients had a significantly greater degree of airflow limitation than the "moderate" group, as seen by the lower FEV_1 and FEV_1/FVC ratio (p<0.05). Diffusion capacity for carbon monoxide was impaired in both groups, more so in the "severe" group ($60.2\pm6.2\%$ predicted vs. $41\pm5.1\%$ predicted, p=0.06). The "severe"

group had a greater degree of air trapping, with a residual volume of 204.1% of predicted compared with 140.3% of predicted in the "moderate" group (p<0.05). There was a trend towards greater impairment of respiratory muscle strength in the "severe" group, although this did not reach statistical significance. This cannot be explained by a difference in height, as the "severe" group was slightly taller. There was no difference in handgrip strength between the groups.

3.6   The effects of exercise and post-exercise regional circulatory occlusion: stratified groups

3.6.1   The effects of rhythmic handgrip exercise

There were no significant differences in resting blood pressure, heart rate, ventilation, oxygen consumption or carbon dioxide production between the "moderate" and "severe" groups (Table 3.7). Rhythmic handgrip exercise to fatigue led to a significant increase in blood pressure, heart rate, ventilation, oxygen consumption and carbon dioxide production in both groups (all $p<0.005$). Both groups achieved similar peak exercise blood pressure and heart rate. The exercise-induced increase in ventilation, oxygen consumption and carbon dioxide production was smaller in the "severe" group, although this did not achieve statistical significance. Resting ventilatory equivalent (ventilation (VE) divided by carbon dioxide production (VCO_2)) was significantly greater in the "severe" group (62.8 ± 3.4 vs. 53.6 ± 3.4, $p<0.05$), presumably reflecting a combination of increased dead space ventilation and reduced muscle bulk.

3.6.2   The effects of post-exercise regional circulatory occlusion

Systolic blood pressure, diastolic blood pressure, ventilation, oxygen consumption and carbon dioxide production were all significantly greater during post-exercise RCO compared with control recovery in both the "moderate" and "severe" groups (tables 3.8 and 3.9, figures 3.7-3.18). There was no difference in the magnitude of the effects of post-exercise RCO between the groups. Post-exercise RCO did not significantly affect heart rate in either group.

Stratification of the patients with COPD by handgrip strength, MRC dyspnoea score and body mass index was carried out. There was no correlation between muscle metaboreflex activity and any of these variables (data not shown).

Table 3.6 Subject characteristics

	Moderate COPD	Severe COPD
N	7	7
Age	66.3±3.4	61.1±5.4
FEV$_1$ (litres)	1.3±0.2	1.0±0.1
FEV$_1$ (% predicted)	56.1±4.9	36.6±1.6*
FEV$_1$/FVC	46.1±2.9	29.7±2.8*
TLCO (corrected, % predicted)	60.2±6.2	41±5.1
Residual volume (% predicted)	140.3±18.9	204.1±11.8*
Total lung capacity (% predicted)	108±5.8	135.3±5.7
Smoking (pack years)	50.8±10.0	62.9±15.4
Baseline dyspnoea index (BDI)	3.0±0.4	3.7±0.8
Exacerbations in previous year	3.4±1.6	3.6±1.5
Height (cm)	160.1±4.2	166.9±2.7
Weight (kg)	84.4±6.3	62.2±4.1*
Body mass index (kg/m^2)	32.7±1.4	22.4±1.4**
Max handgrip (kg)	29.6±4.9	32.1±2.4
Max handgrip (% predicted)	98.8±6.0	87.1±5.5
Peak inspiratory pressure (cmH$_2$O)	85.1±10.6	64.6±8.4
Peak inspiratory pressure (% predicted)	125.4±15.6	90.3±15.3
Peak expiratory pressure (cmH$_2$O)	76.4±4.7	66.7±6.8
Peak expiratory pressure (% predicted)	76.8±4.0	61.2±8.6

Comparison of age, height, weight and lung function between the "moderate" and "severe" groups. Data are presented as mean ± standard error of the mean. *$p<0.05$, **$p<0.005$ for between-group comparisons. Statistical significance was assessed using an unpaired Student's *t*-test after checking for normality. Predicted values for mouth pressures and handgrip strength were taken from standard formulae and tables .

Table 3.7 Comparison of the effects of rhythmic handgrip exercise

	Moderate COPD		Severe COPD	
	Rest	**Peak exercise**	**Rest**	**Peak exercise**
Heart rate (bpm)	81.3±5.3	91.3±4.9**	79.9±5.1	91.1±5.4**
Systolic BP (mmHg)	135.3±5.9	158.3±4.2**	120.7±7.5	154±10.5**
Diastolic BP (mmHg)	78±3.3	100.7±5.7**	76.9±7	103.6±9.5**
Ventilation (l/min)	10.2±0.4	18.9±1.1**	11.4±0.6	17.3±0.8**
VO_2 (ml/min)	198.1±17.3	331.6±30**	181.7±13	279.6±16**
VCO_2 (ml/min)	190.3±16.1	378.4±35.7**	181.4±14	309.3±20.1**
VE/VCO_2	53.6±3.4	49.9±4	62.8±3.4†	55.9±1.4

All data are presented as mean ± standard error of the mean. To compare resting and peak exercise values, a paired Student's *t*-test was used after checking for normality using a Shapiro-Wilk test. To compare values between groups, an unpaired Student's *t*-test was used after checking for normality. *p<0.05, **p<0.005, rest vs peak exercise; †p<0.05, resting values between groups.

Table 3.8 Comparison of the effects of post-exercise RCO between groups: absolute values

	Moderate COPD		Severe COPD	
	Control	RCO	Control	RCO
Systolic BP (mmHg)	142.9±4.6	156±2.8*	129±6.5	150.8±11.2
Diastolic BP (mmHg)	80.9±3.2	98.6±2.6**	79±6.1	92.4±6.0
Heart rate (bpm)	81.4±5.6	82.3±4.8	80.55.0	80.4±5.0
Ventilation (l/min)	13.1±0.9	15.5±1.2	13.5±0.7	14.3±0.9
VO₂ (ml/min)	244.4±23.8	257.1±26.0	208.6±14.2	226.7±14.3
VCO₂ (ml/min)	263.7±28.1	291.7±29.9	226.4±16.2	243.4±20.9

Values represent the "area under the curve" of the three minutes of post-exercise RCO compared with the equivalent period of control recovery. All data are presented as mean ± standard error of the mean. To compare control and RCO values, a paired Student's t-test was used after checking for normality using a Shapiro-Wilk test. To compare values between groups, an unpaired Student's t-test was used after checking for normality. *$p<0.05$, **$p<0.005$, rest vs peak exercise.

Table 3.9 Comparison of the effects of post-exercise RCO between groups: percentage values

	Moderate COPD		Severe COPD	
	Control	RCO	Control	RCO
Systolic BP (%)	37.2±16.5	73.8±9.2	27.4±8.5	70.7±13.8*
Diastolic BP (%)	-10.4±19.4	122.3±19.2**	-6.3±17.6	114.4±23.0**
Heart rate (%)	-23.9±21.6	20.6±17.1	11.0±10.9	11.8±30.7
Ventilation (%)	34.7±8.9	69.1±30.8*	34.4±4.2	56.6±7.3*
VO$_2$ (%)	35.6±5.7	52.3±8.8	23.9±6.6	42.5±14.9
VCO$_2$ (%)	42.2±7.4	64.6±10.7	33±5.0	59.3±10.3

This time, values following either three minutes of post-exercise RCO or the equivalent period of control recovery are expressed as a percentage of the effects of exercise (see text in chapter 2 for further information). All data are presented as mean ± standard error of the mean. To compare control and RCO values, a paired Student's *t*-test was used after checking for normality using a Shapiro-Wilk test. To compare values between groups, an unpaired Student's *t*-test was used after checking for normality. *p<0.05, **p<0.005, rest vs peak exercise.

Figure 3.7 The effects of exercise followed by RCO on systolic blood pressure: moderate COPD
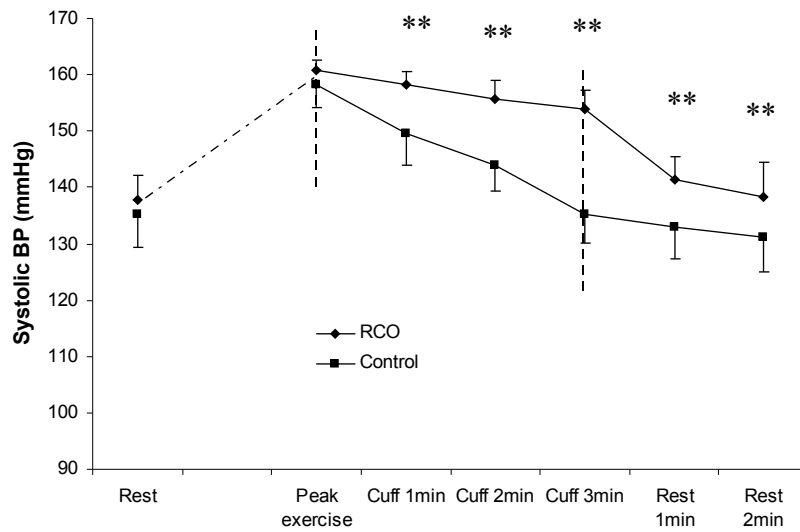
Figure 3.8 The effects of exercise followed by RCO on systolic blood pressure: severe COPD
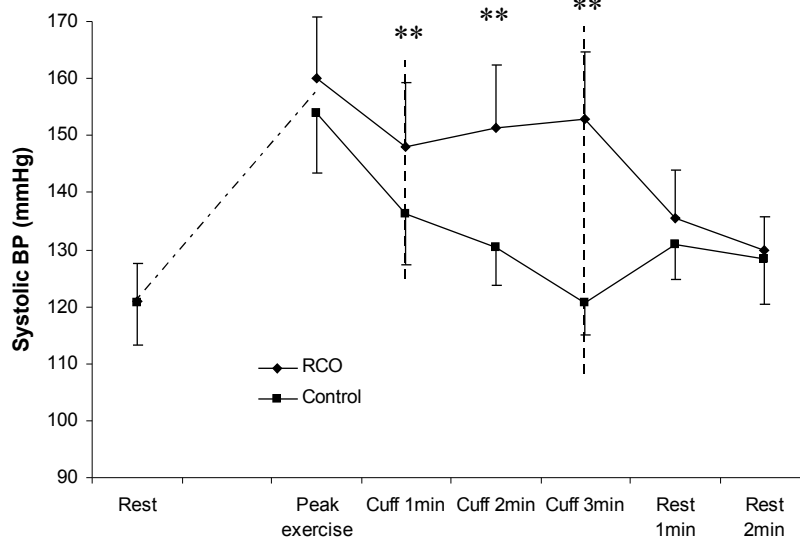
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: $*p<0.05$, $**p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 3.9 The effects of exercise followed by RCO on diastolic blood pressure: moderate COPD
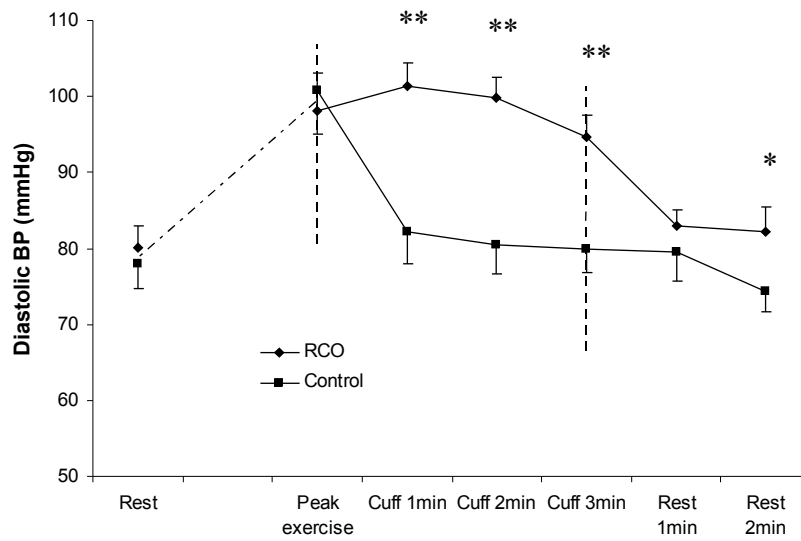
Figure 3.10 The effects of exercise followed by RCO on diastolic blood pressure: severe COPD
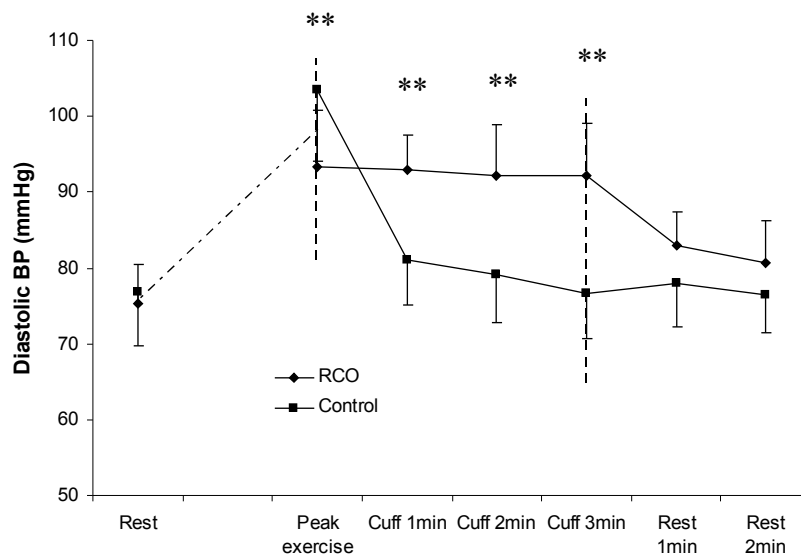
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 3.11 The effects of exercise followed by RCO on heart rate: moderate COPD
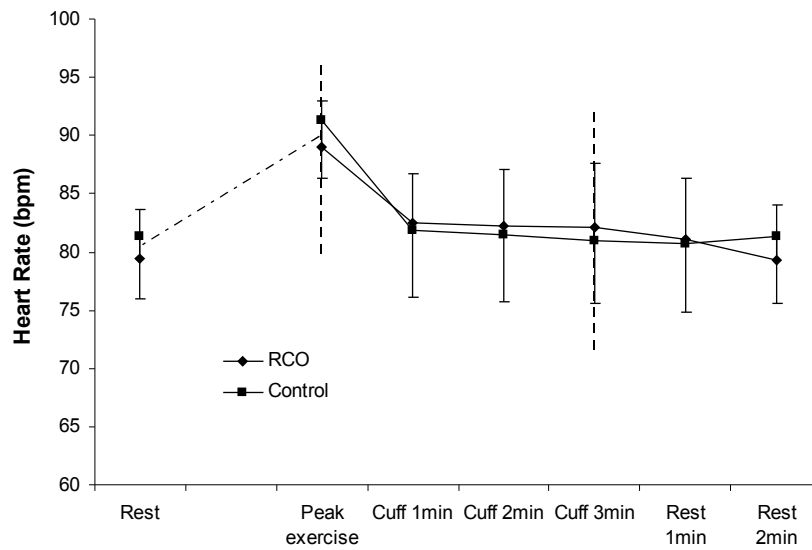
Figure 3.12 The effects of exercise followed by RCO on heart rate: severe COPD
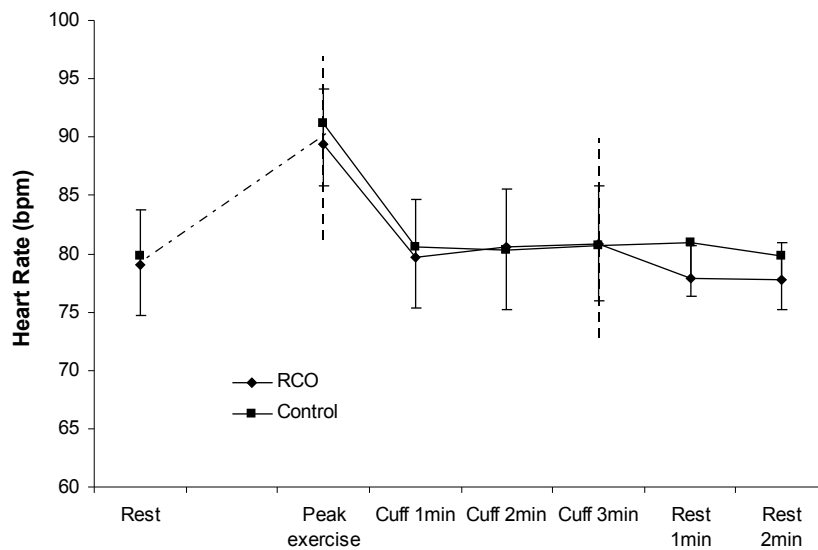
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *$p<0.05$, **$p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 3.13 The effects of exercise followed by RCO on ventilation: moderate COPD
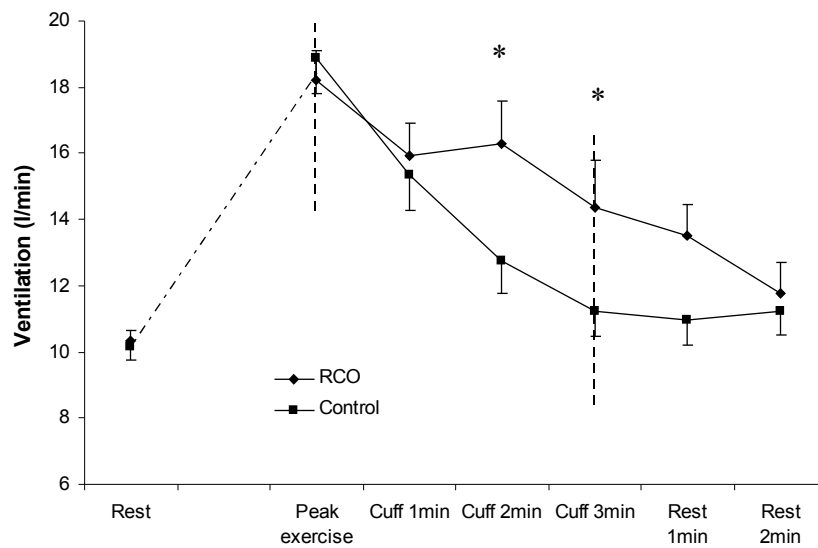
Figure 3.14 The effects of exercise followed by RCO on ventilation: severe COPD
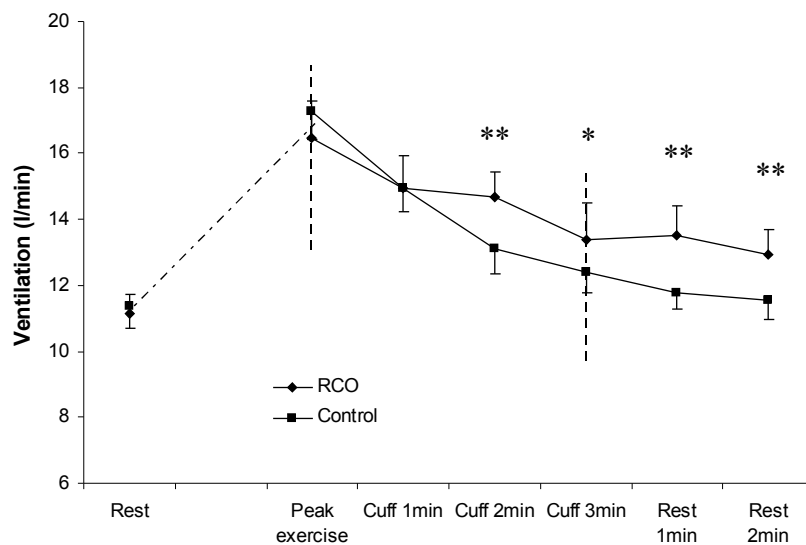
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 3.15 The effects of exercise followed by RCO on oxygen consumption (VO$_2$): moderate COPD
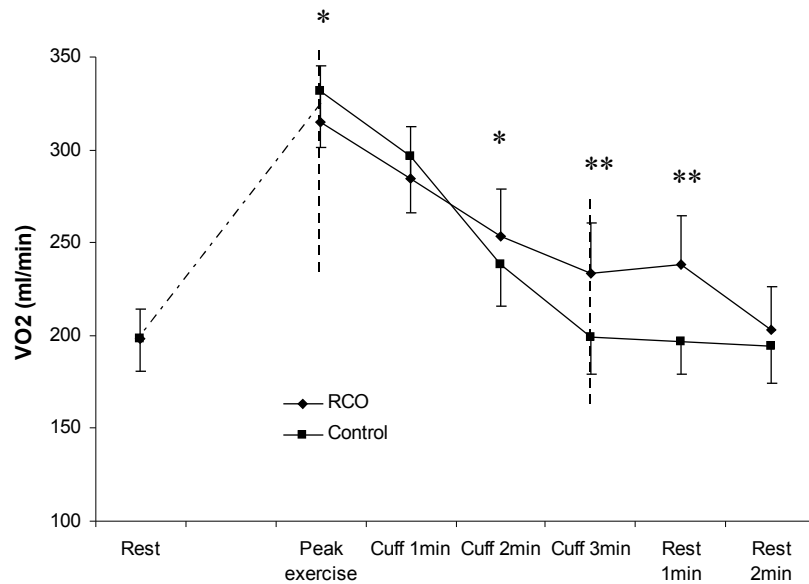
Figure 3.16 The effects of exercise followed by RCO on oxygen consumption (VO$_2$): severe COPD
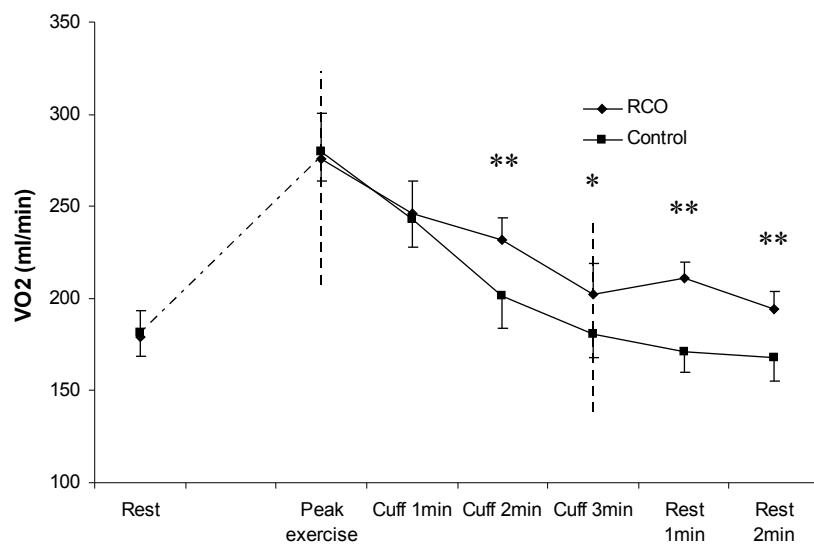
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *$p < 0.05$, **$p < 0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 3.17 The effects of exercise followed by RCO on carbon dioxide production (VCO₂): moderate COPD
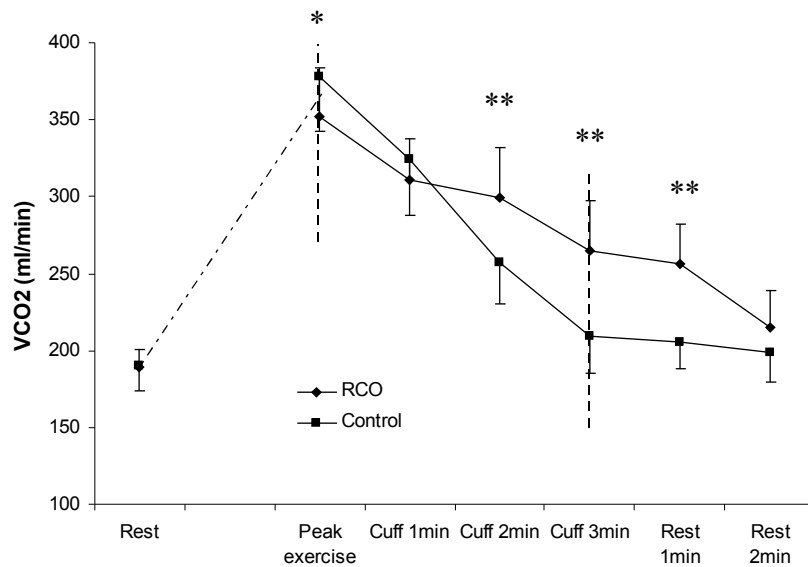
Figure 3.18 The effects of exercise followed by RCO on carbon dioxide production (VCO₂): severe COPD
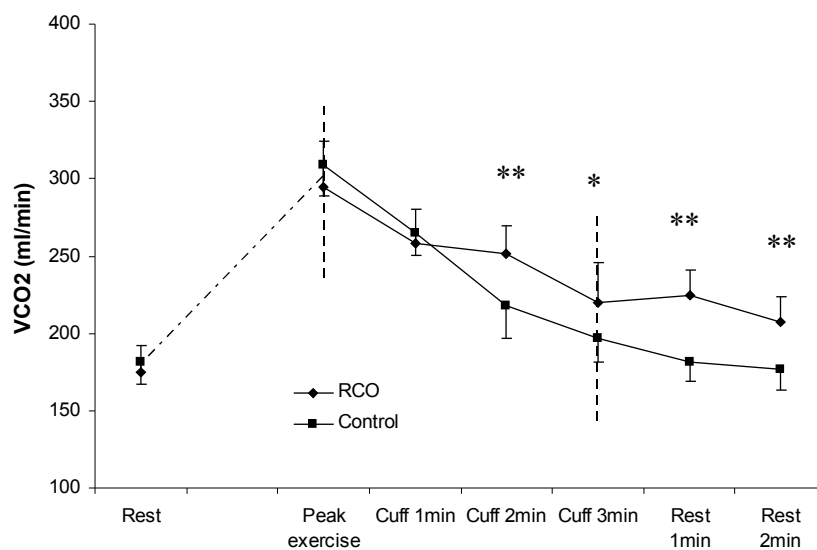
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: $*p<0.05$, $**p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

3.7 Chapter discussion

The aim of this study was to evaluate the contribution of the muscle metaboreflex to ventilatory and pressor responses to forearm exercise in patients with moderate and severe COPD. We have shown that post-exercise regional circulatory occlusion causes a sustained increase in ventilation and blood pressure, but not heart rate, when compared with control recovery without circulatory occlusion. This supports our hypothesis that the skeletal muscle plays an important role in the control of ventilatory and pressor responses to exercise. When comparisons were made between patients with moderate and severe COPD, there was no difference in exercise responses or duration of exercise, although patients with severe disease had a non-significantly lower peak VO_2, presumably reflecting decreased skeletal muscle bulk and impaired aerobic capacity. There was no difference in metaboreflex activity between the 2 groups. The efficacy of regional circulatory occlusion is supported by the fall in resting VO_2 seen with cuff inflation at rest, reflecting "isolation" of that muscle group from the rest of the circulation.

Further stratification of patient groups into categories of body mass index, MRC dyspnoea score and handgrip strength did not reveal any relationships between these variables and muscle metaboreflex activity.

The presence of a pressor reflex arising from exercising skeletal muscle is not a new idea, having first been demonstrated by Alam & Smirk in 1937 . It was thought that metabolites produced by exercising muscle were trapped by circulatory arrest, stimulating receptors in the muscle, with the sympathetic nervous system forming the

efferent arm of the reflex loop. The metaboreflex is abolished in anaesthetised animals by severing the dorsal nerve root , and it has since been shown that small myelinated and unmyelinated group III and IV afferent nerves mediate the reflex . Metaboreflex activity is attenuated in patients with Brown-Séquard syndrome , confirming the importance of the sensory nervous system. Hydrogen ion, lactate, potassium and prostaglandins, produced by exercising muscle, have all been implicated in metaboreflex activation , and intramuscular acidosis (measured using ^{31}P NMR) appears to correlate with metaboreflex activity in normal subjects .

The role of the muscle metaboreflex in the control of ventilation during exercise has been more controversial, although animal studies suggest that it is of importance . Human studies have also shown that the ventilatory response to dynamic handgrip exercise persists during post-exercise regional circulatory occlusion . The contribution of muscle fatigue (as determined by EMG) to metaboreflex activation and consequent hyperventilation has been documented in normal humans, with measurement of venous lactate and potassium during circulatory occlusion demonstrating the efficacy of isolating the exercised muscle from the rest of the circulation .

Our observation that post-exercise circulatory occlusion has no effect on heart rate has previously been noted in normal subjects. The increase in heart rate at the start of exercise is predominantly due to central command (or "anticipation" of exercise), mediated through withdrawal of resting vagal tone . It is the increase in sympathetic activation that is thought to be metaboreflex driven. With post-exercise circulatory occlusion, metaboreceptor stimulation by trapped metabolites causes a sustained increase in sympathetic activity, and consequently an increase in peripheral vascular

resistance and therefore blood pressure. However, the chronotropic effects of the sympathetic nervous system are offset by the activation of the baroreflex and the withdrawal of central command, leading to an increase in vagal tone. This hypothesis is supported by the observations that R-R variation increases during post-exercise RCO and administration of atropine during post-exercise RCO causes a persistent tachycardia .

All previous work looking at the potential clinical importance of the metaboreflex has been carried out in patients with chronic heart failure, who are known to demonstrate an exaggerated ventilatory and sympathetic nervous system response to exercise. Metaboreflex overactivity in chronic heart failure appears to correlate with disease severity when assessed symptomatically with the New York Heart Association criteria . A six week forearm exercise training programme attenuates the abnormal metaboreflex response in heart failure, confirming that this is a reversible phenomenon . In view of the similarities in muscle metabolic abnormalities between advanced COPD and heart failure, we hypothesised that we would also see an increase in metaboreflex activity in patients with severe COPD.

Our failure to detect a difference in muscle metaboreflex activity between patients with moderate and severe COPD could be for several reasons. We chose FEV_1 to define patients with severe disease, and it is known that FEV_1 correlates poorly with exercise capacity . This parallels the picture seen in cardiac failure with left ventricular ejection fraction showing a poor correlation with exercise capacity . Severity of symptoms appears to be more important, with NYHA class correlating with metaboreflex activity , so further work should perhaps look at MRC dyspnoea score as a marker of symptom

severity. This study was not large enough to allow us to stratify according to MRC dyspnoea score. Another possibility is that we studied the wrong muscle group. It is well documented that the upper limb muscles in patients with COPD are relatively preserved compared to the leg muscles , presumably reflecting relatively greater disuse of the locomotor muscles of the lower limbs. It is therefore possible that the metaboreflex would be more active in the lower limbs: the metaboreflex has already been shown to be upregulated in the legs of patients with chronic heart failure . It is also possible that the difference in nutrition between the groups is important, with the muscle metaboreflex having been shown to be attenuated in obese subjects . One final confounding factor could be the presence of abnormalities of the autonomic nervous system. Patients with COPD are known to develop an autonomic neuropathy , perhaps contributing to the increase in resting heart rate and muscle sympathetic nervous activity seen at rest. Presence of a neuropathy could potentially attenuate an augmented metaboreflex, negating any difference between moderate and severe groups.

One potential source of criticism of this chapter is the lack of a "normal" control group for comparison. We had initial difficulty recruiting activity-matched control subjects, so decided instead to compare patients with moderate and severe COPD. It could be argued that many of the changes seen in skeletal muscle with advanced COPD are due to "detraining", with a lack of physical activity leading to muscle deconditioning. It has been shown that training a muscle group attenuates the metaboreflex response, so it could be argued that it is not valid to include a group of control subjects unless they are age and activity matched.

3.8 Chapter conclusions

In conclusion, the muscle metaboreflex appears to contribute to blood pressure and ventilatory responses to rhythmic handgrip exercise in COPD, although we have not been able to show a difference in metaboreflex activity between moderate and severe disease when stratified by FEV_1. Larger studies are needed to determine whether the metaboreflex is as relevant to symptom generation and exercise limitation in COPD as it appears to be in chronic heart failure. The following additional research questions are raised:

1. Do indices of functional disability such as MRC dyspnoea score correlate with muscle metaboreflex activity (as is the case with chronic heart failure and NYHA class)?

2. Is autonomic nervous system dysfunction important in the measurement of the muscle metaboreflex in patients with COPD?

3. Do patients who are not "ventilatory limited" on maximal exercise testing exhibit exaggerated muscle metaboreflex activity?

4. Does muscle fibre type and oxidative capacity determine muscle metaboreflex activity?

Chapter 4

The Assessment of the Muscle Metaboreflex using

Pulse Transit Time

4.1 Chapter introduction

Measurement of blood pressure during exercise can be achieved through use of a traditional mercury sphygmomanometer, although this method is cumbersome and unable to provide continuous readings. Other methods, such as arterial tonometry (used in this study) and finger blood-volume clamping (eg the Finapres) can provide more continuous readings, although the equipment is expensive and susceptible to movement artefact. The "gold standard" for continuous blood pressure monitoring involves arterial cannulation, which is uncomfortable and potentially risky. An alternative is measurement of the transit time of the pulse pressure wave as it moves through the arterial tree, termed the "pulse transit time". Pulse transit time (PTT) has been shown to correlate inversely with blood pressure . It can be measured by timing the pressure wave at two different sites (e.g. the finger and the toe) using infrared photoplethysmography. Alternatively, the electrocardiograph "R" wave, which correlates with ventricular depolarisation, can be used as a proximal starting point: PTT measured using this technique is known as the rPTT . It should be noted, however, that the rPTT also includes the pre-ejection period (the PEP), the time between ventricular depolarisation and the onset of ventricular contraction , thus introducing another potential source of error.

The ability of pulse transit time to detect sudden changes in blood pressure has led to its use in the field of sleep medicine. Patients with obstructive sleep apnoea suffer from "microarousals" following apnoeic episodes, which lead to a burst of sympathetic nervous system activity and a rise in blood pressure. The ability of pulse transit time to detect such microarousals has led to the development of commercially available

portable equipment designed to be worn overnight by a patient. A pulse oximetry probe is worn on the finger and three ECG electrodes are attached to the chest. These are fed into a battery operated unit which records PTT, heart rate and oxygen saturations. The data can then be downloaded onto a laptop computer as either a continuous tracing or raw data. Pulse transit time is dependent on vascular tone, so an increase in sympathetic output causes arterial wall stiffening and a decrease in pulse transit time . Other areas of research in PTT have included its use as a measure of sympathetic nervous system activity in panic disorder . Sympathetic blockade with epidural bupivocaine has been shown to increase pulse transit time through a decrease in arterial wall tone, with the change in pulse transit time detecting sympathetic blockade with a higher reliability than the routine methods of skin temperature and arterial blood pressure .

The effects of exercise on pulse transit time have not previously been documented, neither have the effects of muscle metaboreflex activation. We hypothesised that pulse transit time would fall with rhythmic handgrip exercise, and that post-exercise circulatory occlusion, in causing sustained activation of the muscle metaboreflex, would also affect PTT. The potential value of this finding would be that PTT could be used as a method of evaluating the muscle metaboreflex continuously and non-invasively without the need for expensive and cumbersome blood pressure monitoring equipment. We also set out to investigate the relationship between rPTT and blood pressure during rhythmic handgrip exercise and post-exercise RCO.

4.2 Research questions

The aims of this study were to answer the following research questions:

i)   What are the effects of rhythmic handgrip exercise and muscle metaboreflex activation on pulse transit time?

ii)   Is there a relationship between pulse transit time and blood pressure during rhythmic handgrip exercise and muscle metaboreflex activation?

4.3 Subject characteristics

We recruited 7 healthy male volunteers from the staff of Glasgow Royal Infirmary (height 179.6 ± 2.7cm, weight 82.6 ± 2.8kg, age 32.3 ± 2.2 years, all values mean ± standard error of the mean (SEM). None had significant cardiovascular, neurological, respiratory or metabolic diseases, and none were taking any medication.

4.4 The effects of exercise and post-exercise regional circulatory occlusion

All subjects completed the three parts of the test. There was no difference in exercise duration between the 2 bouts of handgrip exercise: 132.4 ± 13.8 seconds during the control run and 132.7 ± 13.7 seconds during the run followed by post-exercise regional circulatory occlusion (RCO).   Four subjects described post-exercise RCO as uncomfortable, and one subject described light-headedness after the cuff was released.

4.4.1   The effects of post-exercise regional circulatory occlusion at rest

There were no significant changes from resting levels in blood pressure, heart rate or pulse transit time when the cuff was inflated round the upper arm for 3 minutes at rest (see table 4.2, figures 4.1-4.4).

4.4.2   The effects of rhythmic handgrip exercise

Handgrip exercise caused an increase in systolic blood pressure, diastolic blood pressure and heart rate. Pulse transit time fell from 312.2 ± 17.8 milliseconds (ms) to 287.2 ± 17.4 ms with handgrip exercise. There was no significant difference in any measured variables between peak exercise during the control run and peak exercise during the run followed by RCO (table 4.3, figures 4.1-4.4).

4.4.3   The effects of post-exercise regional circulatory occlusion

Following control exercise, blood pressure, heart rate and pulse transit time all fell rapidly towards baseline. We calculated the area under the curve for all variables for the 3 minutes of post-exercise RCO and compared it with the area under the curve for the corresponding 3 minutes during the control exercise run.   RCO caused a significantly increased systolic and diastolic blood pressure compared with normal recovery, as previously reported, but did not affect recovery of heart rate towards resting levels.   Pulse transit time remained near peak exercise levels until the circulatory occlusion was released, and this was statistically significant compared with control recovery from exercise (table 4.4, figures 4.1-4.4).

Table 4.1 Subject characteristics

Male/ Female (*n*)	7/0
Age (years)	32.3±2.2
Height (cm)	179.6±2.7
Weight (kg)	82.6±2.8

Age, sex, height and weight for the subject group. Data presented as mean ± standard error of the mean.

Table 4.2 The effects of post-exercise regional circulatory occlusion at rest

	Rest	RCO	Rest
Systolic BP (mmHg)	124.9 ± 7.3	126.8 ± 7.3	125.2 ± 7.9
Diastolic BP (mmHg)	67.4 ± 2.2	69.3 ± 2.4	67.3 ± 3.0
Heart Rate (mmHg)	70.2 ± 4.6	69.4 ± 4.1	70.4 ± 3.6
Pulse Transit Time (ms)	310.1 ± 18.3	310.1 ± 16.9	310.9 ± 16.7

Data presented as mean ± standard error of the mean. For comparison of rest and control regional circulatory occlusion (RCO), a paired Student's *t*-test was used after checking for normality using a Shapiro-Wilk test.

Table 4.3 The effects of handgrip exercise to fatigue on this group

	Rest	Peak exercise	3 min normal recovery
Systolic BP (mmHg)	127.1 ± 6.1	158.9 ± 5.5*	130.2 ± 7.2
Diastolic BP (mmHg)	67.9 ± 4.4	92.3 ± 4.7*	72.0 ± 5.0
Heart Rate (bpm)	70.0 ± 4.3	87.6 ± 6.1*	69.9 ± 4.5
Pulse Transit Time (ms)	312.2 ± 17.8	287.2 ± 17.4*	303.1 ± 17.5

Comparison between resting and peak exercise values: *$p<0.0001$. Statistical significance was assessed using a paired Student's *t*-test after checking for normality using a Shapiro-Wilk test. All values are mean ± standard error of the mean.

Table 4.4 The effects of post-exercise regional circulatory occlusion

	Rest	Peak exercise	3 min RCO
Systolic BP (mmHg)	126.0 ± 6.1	160.2 ± 6.3*	157.6 ± 8.3††
Diastolic BP (mmHg)	68.1 ± 4.2	94.7 ± 4.9*	89.0 ± 5.6†
Heart Rate (bpm)	68.9 ± 3.6	90.1 ± 5.0*	74.6 ± 4.8
Pulse Transit Time (ms)	314.8 ± 16.1	285.6 ± 16.9*	289.9 ± 17.9†

Values for RCO are taken as the "area under the curve" for the three minutes of post-exercise RCO. Comparison of rest and peak exercise *$p<0.0001$. Comparison of RCO and equivalent period of control recovery (from table 4.3) †$p<0.005$, ††$p<0.001$. Statistical significance was assessed using a paired Student's *t*-test after checking for normality using a Shapiro-Wilk test.

Figure 4.1 The effects of post-exercise RCO on systolic blood pressure
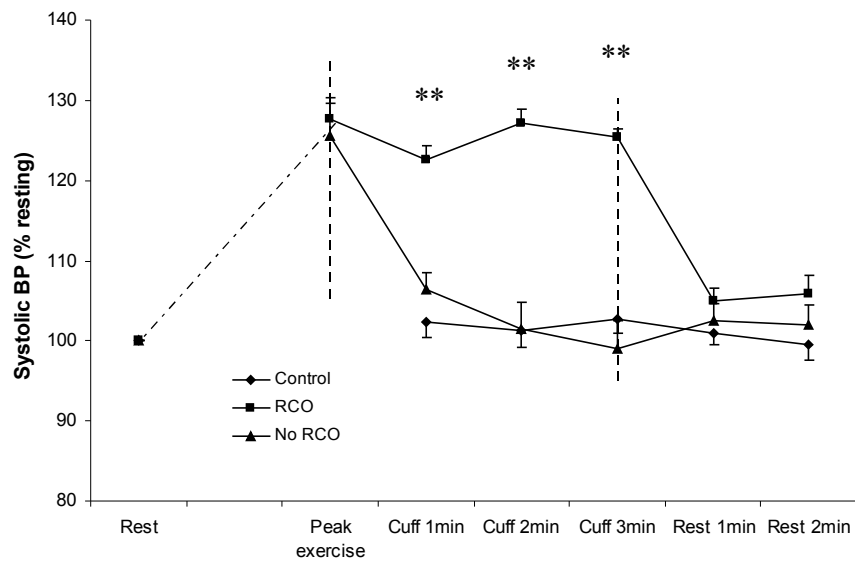
Figure 4.2 The effects of post-exercise RCO on diastolic blood pressure
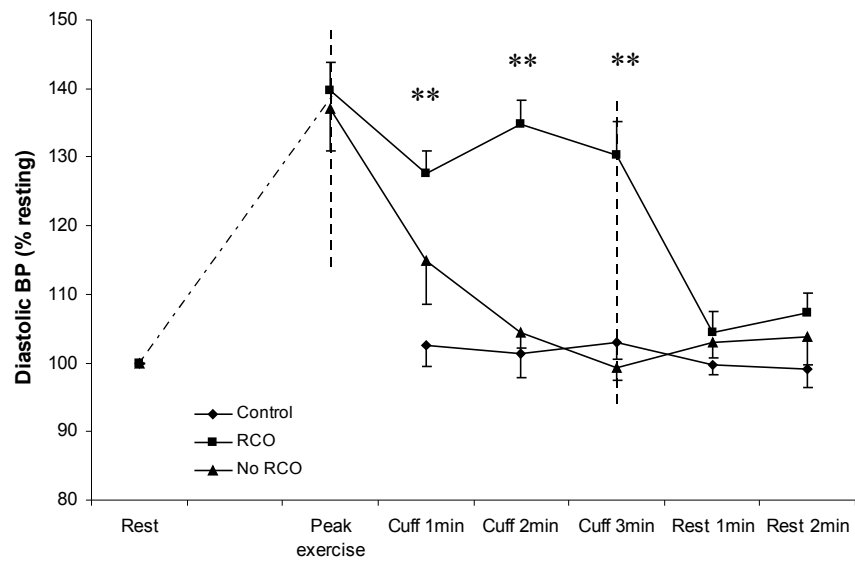
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: $*p<0.05$, $**p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures. The diamonds signify the non-exercise run with control cuff inflation.

Figure 4.3 The effects of post-exercise RCO on heart rate
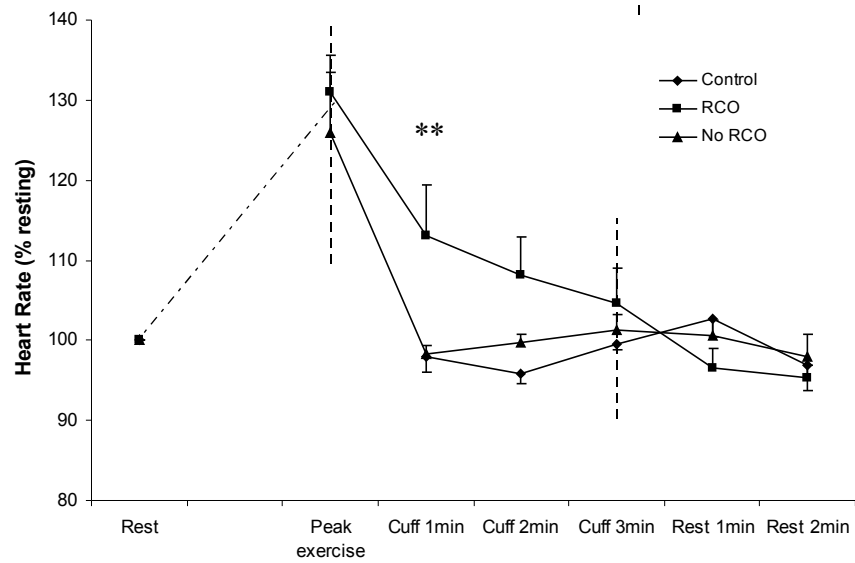
Figure 4.4 The effects of post-exercise RCO on pulse transit time
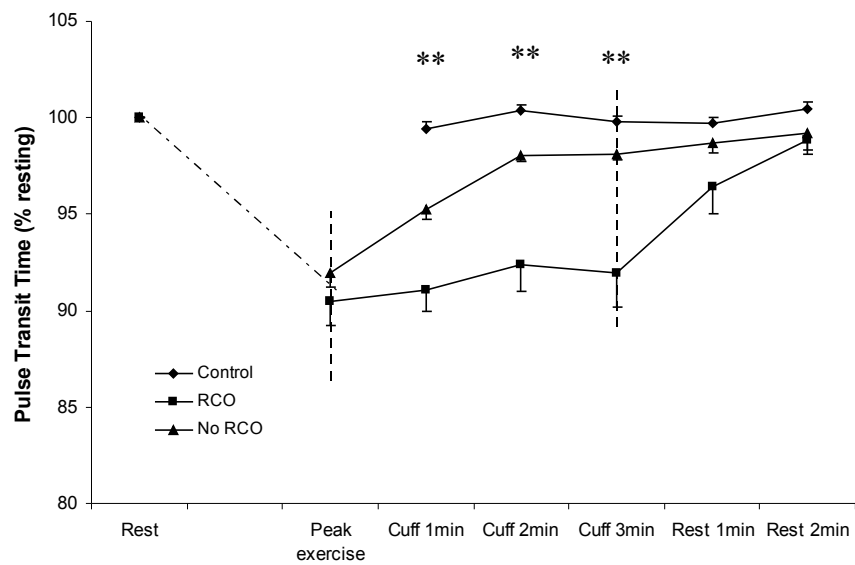
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures. The diamonds signify the non-exercise run with control cuff inflation.

4.5 The relationship between blood pressure and pulse transit time

The relationships between pulse transit time and systolic, diastolic and mean arterial blood pressure are shown in table 4.5 and figures 4.5- 4.8. In all subjects, there was a significant correlation between pulse transit time and blood pressure. Individual "r" values for each subject are shown in table 4.5, and figures 4.5- 4.8 illustrate the relationship graphically in two typical subjects (numbers 1 and 7).

Table 4.5 The relationship between pulse transit time and blood pressure

Subject no.	No. of measurements	Systolic BP	Diastolic BP	Mean arterial BP
1	99	-0.66	-0.76	-0.79
2	126	-0.77	-0.74	-0.78
3	63	-0.86	-0.82	-0.84
4	64	-0.74	-0.73	-0.74
5	74	-0.77	-0.73	-0.75
6	132	-0.68	-0.63	-0.66
7	109	-0.69	-0.68	-0.69

This table looks at the relationship between pulse transit time and blood pressure for each individual subject. Pearson's regression analysis was used to calculate the correlation coefficient for each subject.

Figure 4.5 The relationship between PTT and systolic blood pressure (subject 1)
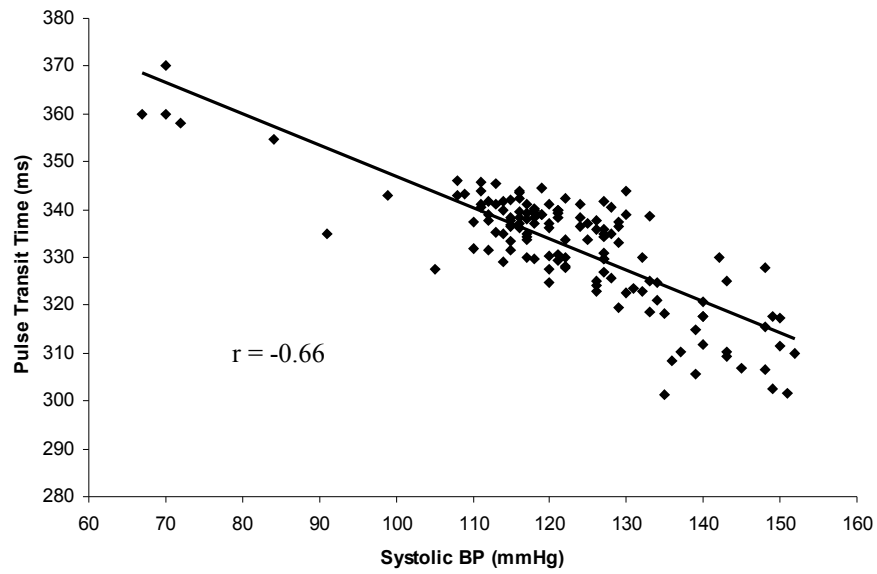
Figure 4.6 The relationship between PTT and diastolic blood pressure (subject 1)
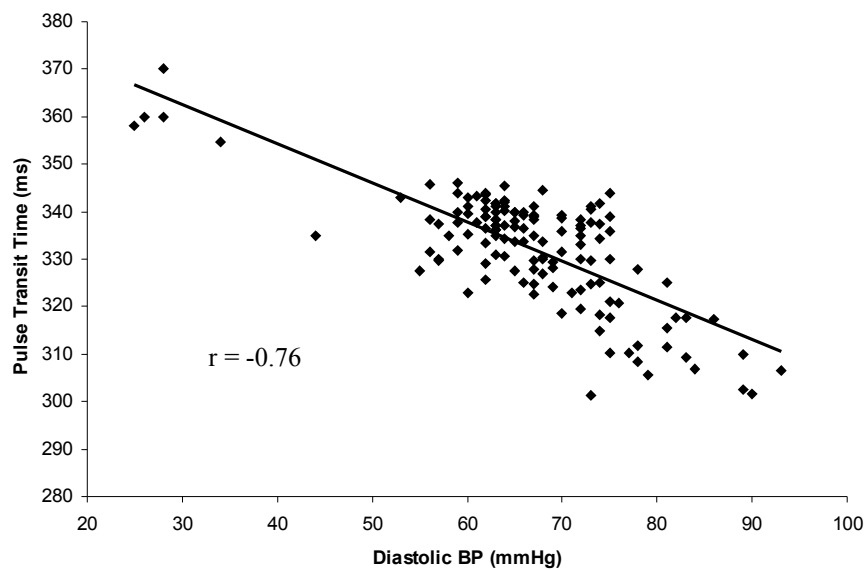
These graphs show the relationship between pulse transit time and systolic (top) and

diastolic blood pressure for subject 1 during testing for the muscle metaboreflex.

Figure 4.7 The relationship between PTT and systolic blood pressure (subject 7)
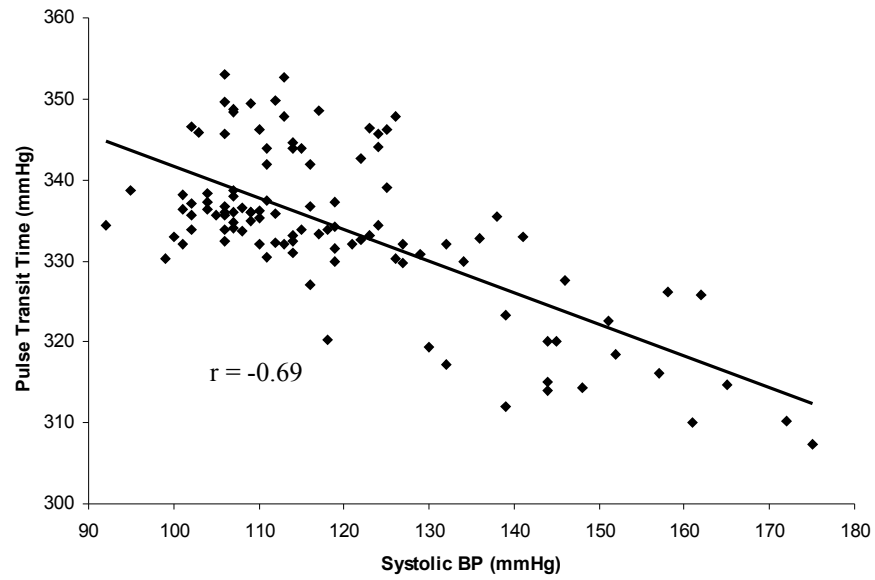
Figure 4.8 The relationship between PTT and diastolic blood pressure (subject 7)
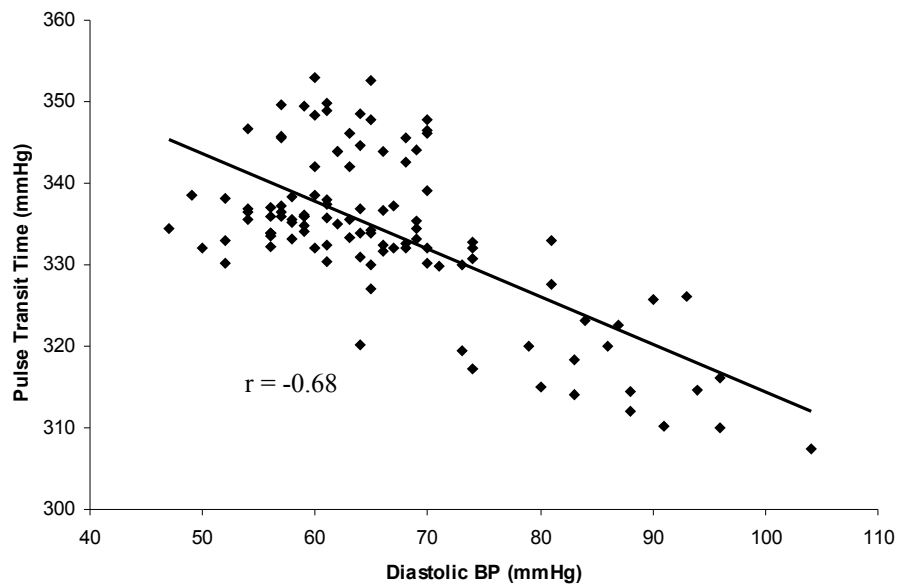
These graphs show the relationship between pulse transit time and systolic (top) and

diastolic blood pressure for subject 7 during testing for the muscle metaboreflex.

4.6 Chapter discussion

This study set out to investigate the use of pulse transit time in the measurement of the muscle metaboreflex in a group of normal subjects.

Our finding that post-exercise muscle ischaemia led to a sustained increase in systolic and diastolic blood pressure compared with control recovery is in keeping with previous studies (see chapter 1 for a detailed discussion). We also found that post-exercise muscle ischaemia did not significantly affect heart rate recovery. This is again in keeping with previous findings, with the sympathetic activation mediated by muscle metaboreflex activation being offset by the negatively chronotropic effects of the baroreceptor-mediated increase in vagal tone (see chapter 1).

The main findings of our study are the effects that handgrip exercise and post-exercise RCO have on pulse transit time. There was a clear fall in rPTT with rhythmic handgrip exercise. This could be explained by the rise in blood pressure leading to an increase in vascular tone. It should, however, be remembered that rPTT also depends on the pre-ejection period, which will also fall with exercise due to the increase in heart rate. Pulse transit time also appears to behave in much the same way as blood pressure during post-exercise RCO (figures 4.1, 4.2, 4.4), with a sustained fall in PTT for the duration of circulatory occlusion. It could be argued that the pre-ejection period during post-exercise RCO should be similar to the pre-ejection period during control recovery, as heart rate recovers in much the same way. This would suggest that the effects of

post-exercise RCO on PTT are mediated through changes in vascular tone rather than pre-ejection period.

There has been interest in muscle sympathetic nerve activity (MSNA) in the evaluation of the metaboreflex. MSNA can be measured by inserting a tungsten microelectrode into the peroneal nerve in the popliteal fossa . It has consistently been shown to remain elevated in the non-exercising limb during post-exercise RCO . This causes vasoconstriction of blood vessels to non-essential organs, diverting blood flow to the exercising muscle, where vasoconstriction is probably offset by local mediators . It is also interesting to note that during fatiguing inspiratory muscle work, resting limb MSNA increases . Increased neural activity is seen in group IV afferents from the fatiguing diaphragm of the anaesthetised rat , so it is hypothesised that metaboreceptor stimulation in the diaphragm mediates this rise in MSNA. The measurement of MSNA, however, is a technique requiring specialist training and equipment, so PTT is perhaps another method of assessing trends in sympathetic activity. Further work is needed in this field, and it would be interesting to compare trends in MSNA with trends in PTT during exercise followed by post-exercise RCO. Studies looking at pPTT (which is rPTT following correction for pre-ejection period) in addition to rPTT during metaboreflex activation would also be useful.

A detailed study looking at the effects of various vasoactive drugs on both rPTT and pPTT was carried out by Payne *et al* . It was found that there was a good correlation between rPTT and systolic blood pressure, but not diastolic blood pressure. Diastolic and mean arterial blood pressure correlated well with pPTT, and this was thought to be consistent with the fact that arterial stiffness, and therefore vascular PTT (i.e. pPTT), is

dependent on mean arterial pressure rather than systolic blood pressure. It was also noted that PEP contributed significantly to rPTT (12% to 35%), which would make rPTT inappropriate for use purely for the measurement of arterial tone. Perhaps future studies comparing MSNA with PTT in assessment of the muscle metaboreflex should either correct rPTT for PEP to obtain the pPTT, or directly measure PTT using two arterial sites. It is also possible to use the phonocardiogram to time cardiac ejection for analysis along with a peripheral pulse, which would avoid the need for correction for PEP.

Our findings that there was a reasonable correlation between rPTT and systolic blood pressure during exercise are therefore not surprising, although we demonstrated a closer correlation between rPTT and diastolic blood pressure than Payne *et al*. It should be emphasised that our correlation between blood pressure and PTT is by no means perfect, and highlights the fact that factors other than blood pressure have an effect on PTT.

4.7 Chapter conclusions

In conclusion, muscle metaboreflex activation using the method of rhythmic forearm exercise followed by post-exercise muscle ischaemia has a clear effect on pulse transit time, likely to be due to a combination of effects on cardiac output, pre-ejection period and vascular tone. Pulse transit time shows some promise in the assessment of muscle metaboreflex activity as it is easy to measure, non-invasive and provides a continuous reading. Further work needs to be done looking at whether PTT shows any meaningful correlation with MSNA in this setting, and it is possible that pPTT is a more appropriate parameter to measure.

Chapter 5

The Muscle Metaboreflex in Patients with

Diabetic Autonomic Neuropathy

5.1 Chapter introduction

Muscle metaboreflex control of ventilation and blood pressure during exercise is complex, and depends on intact sensory and autonomic nervous systems. As discussed in chapter 1, the sensory arm of the reflex loop is mediated by group III and group IV afferent nerves, and the efferent arm predominantly relies on the sympathetic nervous system. Sympathetic nervous system activation leads to an increase in cardiac output (through an increase in heart rate and stroke volume) and vasoconstriction of vessels supplying non-exercising muscle and non-essential organs, leading to an increase in blood pressure.

This leads to the interesting question of what happens to the muscle metaboreflex when either the sensory or autonomic nervous systems are damaged through injury or disease. This was touched on in chapter 1, where it was noted that severing of the dorsal spinal roots in anaesthetised animals abolishes the metaboreflex . It has also been shown that a Brown-Séquard spinal lesion leads to attenuation of the metaboreflex on the side affected by sensory loss . To our knowledge, there have been no studies to date assessing the muscle metaboreflex in patients with autonomic nervous system dysfunction.

Autonomic neuropathy is an important complication of diabetes mellitus, and can often be detected prior to the development of symptoms . It is also of prognostic significance: impaired heart rate recovery following exercise is an independent predictor of mortality , and autonomic neuropathy is associated with an increased risk of sudden cardiac death .

Subjects with diabetic autonomic neuropathy are recognised to demonstrate impaired cardiovascular responses to exercise. Reduced VO_2 max and attenuated heart rate and blood pressure responses to exercise have both been documented, even after the exclusion of subjects with occult ischaemic heart disease . A reduction in myocardial contractility and failure of peripheral vasoconstriction during exercise are probably both important. It has been noted that hepato-splanchnic vascular resistance does not increase on exercise to the same degree in patients with diabetic autonomic neuropathy as in control subjects, presumably a manifestation of impaired sympathetic nervous system function . The possibility that an abnormal metaboreflex contributes to abnormal cardiovascular exercise responses in diabetic autonomic neuropathy has not been studied. This chapter therefore set out to investigate whether patients with diabetic autonomic nervous system dysfunction had altered muscle metaboreflex function in comparison to a group of diabetic subjects without autonomic dysfunction.

5.2 Research questions

The aims of this study were to answer the following research questions;

    i)   Is it possible to detect muscle metaboreflex activity in patients with impairment of the autonomic nervous system secondary to type I diabetes mellitus?

    ii)   Is there a difference in muscle metaboreflex activity between patients with diabetic autonomic neuropathy and patients with type I diabetes mellitus who do not have diabetic autonomic neuropathy?

5.3 Patient characteristics

Fifteen patients with a history of type I diabetes mellitus were entered into the study. They were stratified into 2 groups: those with autonomic dysfunction and those without autonomic dysfunction (see chapter 2 for criteria). All subjects completed all parts of the protocol. Equipment failure during an important part of the protocol meant that we were unable to include any meaningful results for one of the patients with autonomic dysfunction. This patient was unwilling to repeat the protocol and is therefore excluded from further analysis. Subject characteristics are described in table 5.1.

It can be seen from table 5.1 that there were no significant differences in age, weight or body mass index between the patient groups. There was a trend towards a greater duration of diabetes and a higher serum creatinine in the group with autonomic

neuropathy, although this was not statistically significant. Mean HbA$_{1C}$ was 8.6 in both groups, suggesting sub-optimal diabetic control.

5.4 Characteristics of autonomic dysfunction

Autonomic dysfunction in patients with diabetes mellitus is a heterogeneous mix of abnormalities of sympathetic and parasympathetic nervous system dysfunction. It is, however, necessary to define a "cut-off" to allow stratification into 2 groups. As discussed in chapter 2, we used the criteria of Ewing and Clarke to define autonomic dysfunction, namely an abnormality of 2 or more of the five tests carried out. We have reported in detail the results of the autonomic tests for each patient in table 5.2.

It can be seen from table 5.2 that seven of the subjects had clear abnormalities in two or more of the autonomic tests carried out. As mentioned earlier, subject 3 was excluded from further analysis due to equipment failure during testing. It is noteworthy that only one subject (subject 2) had a completely normal set of tests. Eleven out of the fourteen subjects had some evidence of parasympathetic nervous system dysfunction.

Table 5.1 Subject characteristics

	Control	DAN	*p* value
n	8	6	
Age	46.3 ± 4.5	53 ± 2.7	NS
Sex (M/F)	4 / 4	3 / 3	
Weight (kg)	70.8 ± 4.8	67.8 ± 2.1	NS
Body mass index (kg/m^2)	25.1 ± 1.4	26 ± 1	NS
Diabetes duration (years)	23.5 ± 2	27 ± 2.7	NS
Ischaemic heart disease (*n*)	1	1	
Peripheral vascular disease (*n*)	1	2	
Sensory Neuropathy (*n*)	3	5	
Retinopathy (*n*)	5	4	
Serum creatinine (μmol/l)	94 ± 4	101 ± 6	NS
HbA$_{1C}$ (%)	8.6 ± 0.5	8.6 ± 0.3	NS

Comparison of age, height, weight and characteristics of diabetes between the groups with and without diabetic autonomic neuropathy (DAN). Data are presented as mean ± standard error of the mean. Statistical significance was assessed using an unpaired Student's *t*-test after checking for normality using a Shapiro-Wilk test. NS = not statistically significant.

Table 5.2. Autonomic function testing.

Patient	Test 1	Test 2	Test 3	Test 4	Test 5	Classification
1	A	B	N	N	N	Normal
2	N	N	N	N	N	Normal
3	A	A	B	B	A	DAN
4	N	N	N	N	B	Normal
5	A	B	A	B	N	DAN
6	A	N	N	N	N	Normal
7	A	A	A	B	B	DAN
8	A	A	A	A	A	DAN
9	A	B	A	A	A	DAN
10	A	B	N	B	A	DAN
11	A	N	N	B	N	Normal
12	N	N	N	B	N	Normal
13	A	N	N	B	N	Normal
14	N	N	N	N	N	Normal
15	A	A	A	N	N	DAN

Detailed results of autonomic function testing for each individual subject. Tests 1-5 are as follows: 1. Heart rate response to deep breathing, 2. Heart rate response to the Valsalva manoeuvre, 3. Heart rate response to standing, 4. Diastolic blood pressure response to standing, 5. Diastolic blood pressure response to sustained handgrip. N = normal, A = Abnormal, B = borderline.

5.5 Resting and exercise measurements

Subjects with DAN had a lower resting systolic and diastolic blood pressure and a higher resting heart rate than patients without DAN at rest, although this did not reach statistical significance (see table 5.3). This was also true of the peak exercise values, with only diastolic blood pressure reaching statistical significance ($p < 0.05$). Rhythmic handgrip exercise led to a significant rise in blood pressure, heart rate, minute ventilation and VO_2 in both groups. There was no difference in the magnitude of the blood pressure response to exercise between the groups, but the DAN group did have a significantly smaller heart rate response to exercise when expressed in absolute values (9.7 ± 1.3 beats per minute (bpm) vs. 15.7 ± 2 bpm, $p < 0.05$).

5.6 The effects of regional circulatory occlusion at rest

Regional circulatory occlusion following 20 minutes of rest did not have any effect on any of the variables measured (see figures 5.1-5.12, raw data not reported).

5.7 The effects of post-exercise regional circulatory occlusion

Post-exercise regional circulatory occlusion led to a sustained increase in systolic and diastolic blood pressure until the sphygmomanometer cuff was deflated (see table 5.4 and figures 5.1-5.12). This achieved statistical significance in both groups of patients. There was no difference in the magnitude of the response between the patient groups (see table 5.5). Post-exercise RCO did not affect heart rate recovery in either group.

It was found that measurement of ventilation was susceptible to the effects of hyperventilation after exercise whether or not the sphygmomanometer cuff was inflated. During post-exercise RCO, there was a trend towards a higher VO_2 compared with control recovery in the DAN group, but not in the control group (table 5.4 and 5.5, figures 5.9 and 5.10).

5.8 Pulse transit time

Due to the nature of the recording equipment, it was not possible to tell if there were problems with the pulse transit time tracing obtained until it had been downloaded to computer at the end of the session. Vascular insufficiency may make it difficult to obtain a good pulse oximetry signal at the toe. Despite this, we managed to obtain very good tracings from all patients with the exception of subject 3 (already excluded) and subject 6. Figures 5.13 and 5.14 show two sample tracings, with the control exercise run superimposed upon the exercise run with post-exercise RCO. There is a clear muscle metaboreflex effect on pulse transit time in subject 10 (figure 5.14), and this is less obvious, though still present, in subject 1 (figure 5.13). Signal averaging of the 9

seconds around each point has been performed to allow clearer graphical illustration (figures 5.13 and 5.14): each point on the graph represents the mean of that point and the 4 points either side of it. All tracings used for analysis were of similar quality to these graphs. It is reassuring that pulse transit time tracings are almost identical for the two exercise runs in each patient with identical peak exercise values, suggesting reproducibility of the method of exercise and the method of PTT measurement.

Pulse transit time fell during rhythmic handgrip exercise in both groups ($p < 0.05$). Pulse transit time was lower during post-exercise RCO than during the equivalent recovery period following control exercise in both groups, although this was only significant during the last 2 minutes of post-exercise RCO (table 5.4, figures 5.11 and 5.12).

5.9 Comparison with normal subjects

The results of muscle metaboreflex testing of the normal control group used in chapter 4 are given in table 5.6. There was no difference in the muscle metaboreflex effect on blood pressure, heart rate or pulse transit time between any of the three groups.

Table 5.3 Comparison of the effects of rhythmic handgrip exercise

	Control		Autonomic dysfunction	
	Rest	Peak exercise	Rest	Peak exercise
Systolic BP (mmHg)	138.9±7.5	178.8±7.8**	123.8±6.5	156.9±5.8**
Diastolic BP (mmHg)	71.8±3.5	98.5±5.1**	63.1±3.3	84.9±2.3**†
Heart Rate (bpm)	80.1±1.4	96.0±1.6*	88.1±5.4	97.8±4.7**
Ventilation (l/min)	8.4±0.7	13.6±0.9**	8.3±1.1	12.1±1*
VO₂ (ml/min)	143.7±15.7	219.5±23.5**	117±14.7	193.8±16.3**
PTT (ms)	278.2±12.7	240.1±12.8**	292.5±8.3	264.0±7.3**

All data are presented as mean ± standard error of the mean. To compare resting and peak exercise values, a paired Student's t-test was used after checking for normality using a Shapiro-Wilk test. To compare values between groups, an unpaired Student's t-test was used after checking for normality. $*p<0.05$, $**p<0.005$, rest vs peak exercise; $†p<0.05$, values between groups.

Table 5.4 The effects of post-exercise regional circulatory occlusion: absolute values

	Control		Autonomic dysfunction	
	Control	**RCO**	**Control**	**RCO**
Systolic BP (mmHg)	142.8±9	166.9±5.9**	129.8±6.7	153.6±6.8*
Diastolic BP (mmHg)	73.3±4.9	87.3±4.0**	65.8±3.4	80.3±2.9*
Heart Rate (bpm)	77.4±3.0	79.5±3.2	88.0±6.1	89.7±4.8
Ventilation (l/min)	10.7±0.7	11.3±1.0	10.8±1.2	13.4±1.7
VO$_2$ (ml/min)	172.9±13.7	165.8±17.8	140.7±14.4	147.7±16.5
PTT (ms)	261.8±12.3	255.2±12.3	281.7±8.0	269.3±6.5*

Values given represent the "area under the curve" of the 3 minutes following peak exercise, either during control recovery or during post-exercise regional circulatory occlusion (RCO). Statistical significance was assessed using a paired Student's t-test after checking for normality using a Shapiro-Wilk test. Comparison between control recovery and RCO: *p<0.05, **p<0.005.

Table 5.5 The effects of post-exercise regional circulatory occlusion: percentage values

	Control		Autonomic dysfunction	
	Control	RCO	Control	RCO
Systolic BP	12.8±8.7	71.1±6.4**	14.7±7.7	77.5±10.0**
Diastolic BP	3.5±7.0	59.7±5.0**	14.0±8.1	74.1±11.4*
Heart Rate	3.7±5.8	18.3±14.2	4.3±12.1	11.0±13.6
Ventilation	47.0±10.5	50.7±9.5	70.9±42.0	287.1±118.0
VO$_2$	42.3±8.0	26.2±10.4	31.8±4.6	40.3±9.2
PTT	43.8±7.2	59.9±12.6	33.2±4.8	78.0±12.9*

This time, values following either three minutes of post-exercise RCO or the equivalent period of control recovery are expressed as a percentage of the effects of exercise (see text in chapter 2 for further information). All data are presented as mean ± standard error of the mean. To compare control and RCO values, a paired Student's *t*-test was used after checking for normality using a Shapiro-Wilk test. To compare values between groups, an unpaired Student's *t*-test was used after checking for normality. *p<0.05, **p<0.005, rest vs peak exercise.

Table 5.6 The effects of post-exercise regional circulatory occlusion on the normal control group used in chapter 4 (percentage values)

	Normal control subjects	
	Control	RCO
Systolic BP	11.7±6.3	95.2±11.0**
Diastolic BP	16.0±6.2	82.2±9.0**
Heart Rate	1.1±5.8	20.3±12.8
PTT	38.7±4.7	92.5±14.1*

Values following either three minutes of post-exercise regional circulatory occlusion (RCO) or the equivalent period of control recovery are expressed as a percentage of the effects of exercise (see text in chapter 2 for further information). All data are presented as mean ± standard error of the mean. To compare control and RCO values, a paired Student's *t*-test was used after checking for normality using a Shapiro-Wilk test. To compare values between groups, an unpaired Student's *t*-test was used after checking for normality. *p<0.05, **p<0.005, rest vs peak exercise.

Figure 5.1 The effects of exercise followed by RCO on systolic blood pressure: control group
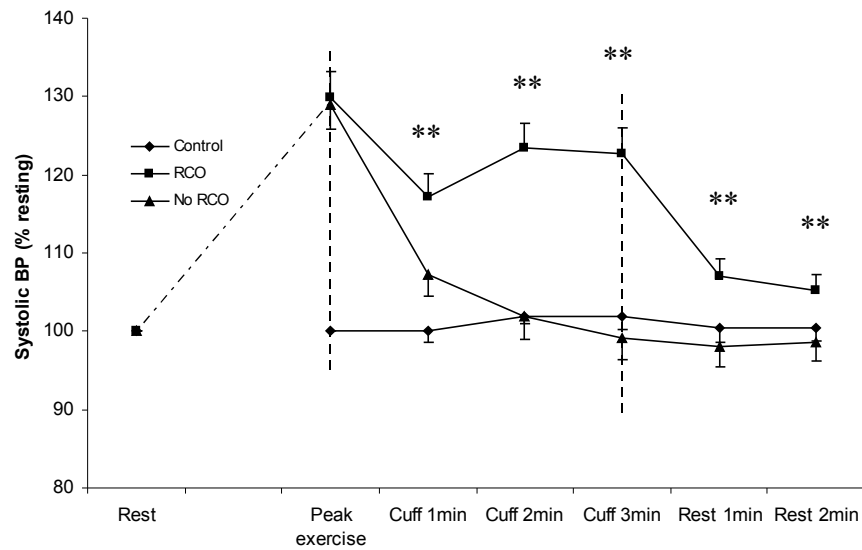
Figure 5.2 The effects of exercise followed by RCO on systolic blood pressure: diabetic autonomic neuropathy group
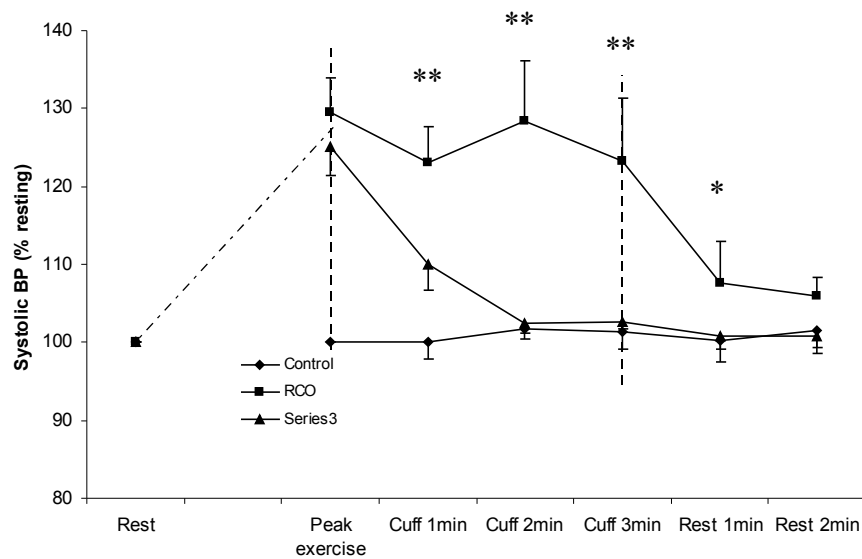
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 5.3 The effects of exercise followed by RCO on diastolic blood pressure: control group
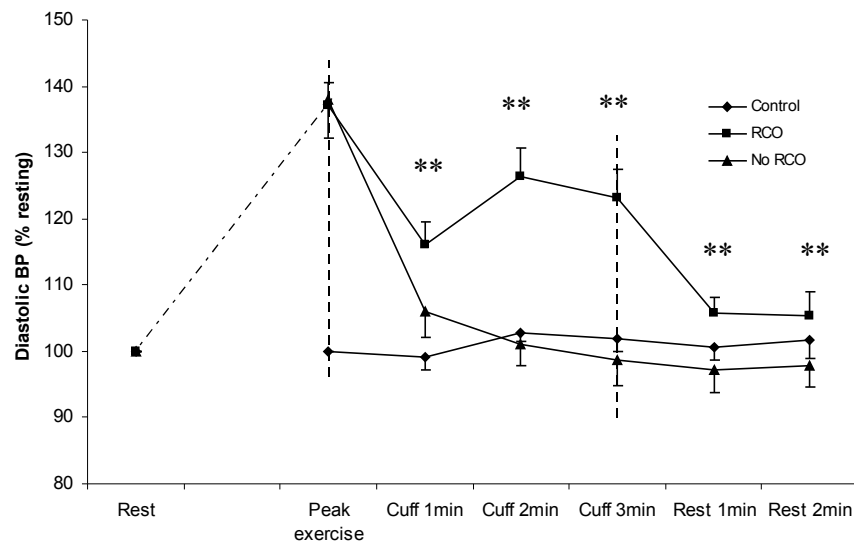
Figure 5.4 The effects of exercise followed by RCO on diastolic blood pressure: diabetic autonomic neuropathy group
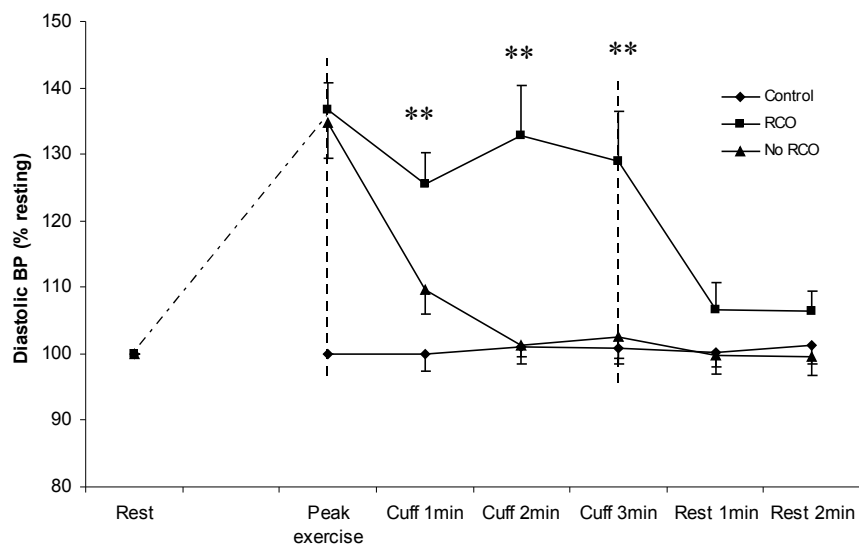
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 5.5 The effects of exercise followed by RCO on heart rate: control group
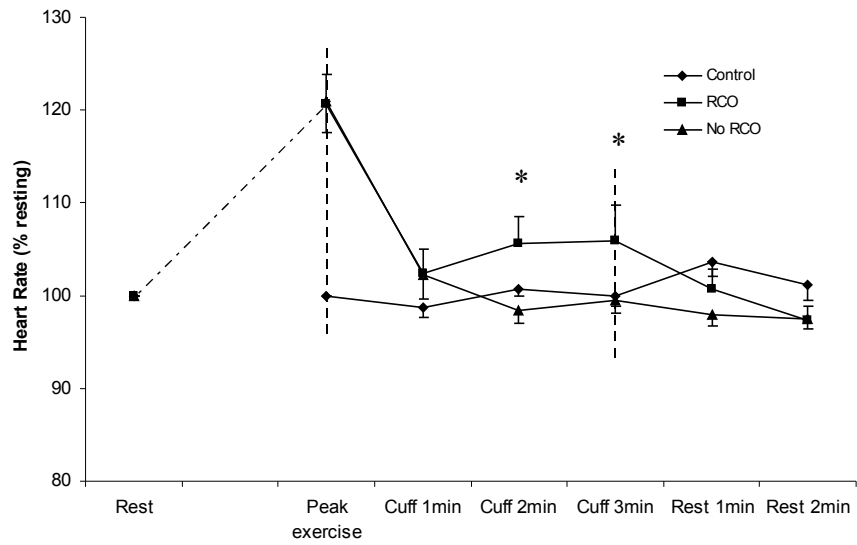
Figure 5.6 The effects of exercise followed by RCO on heart rate: diabetic autonomic neuropathy group
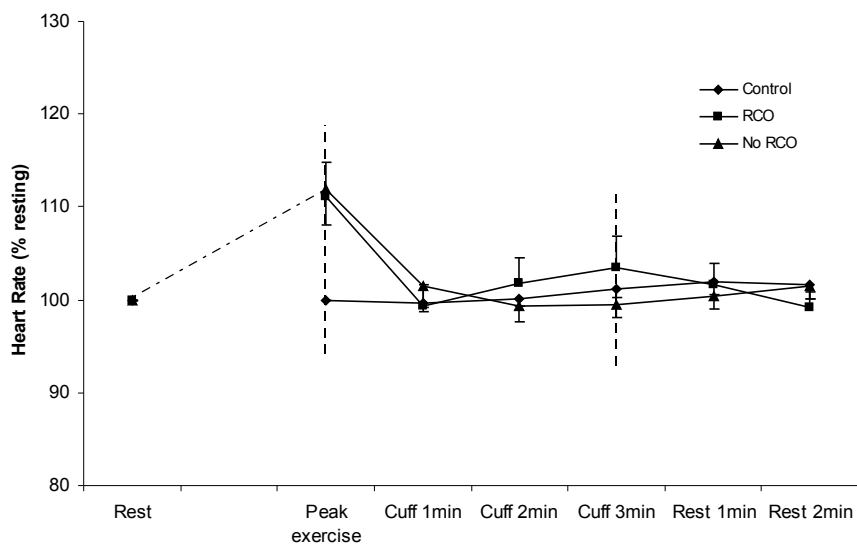
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 5.7 The effects of exercise followed by RCO on ventilation: control group
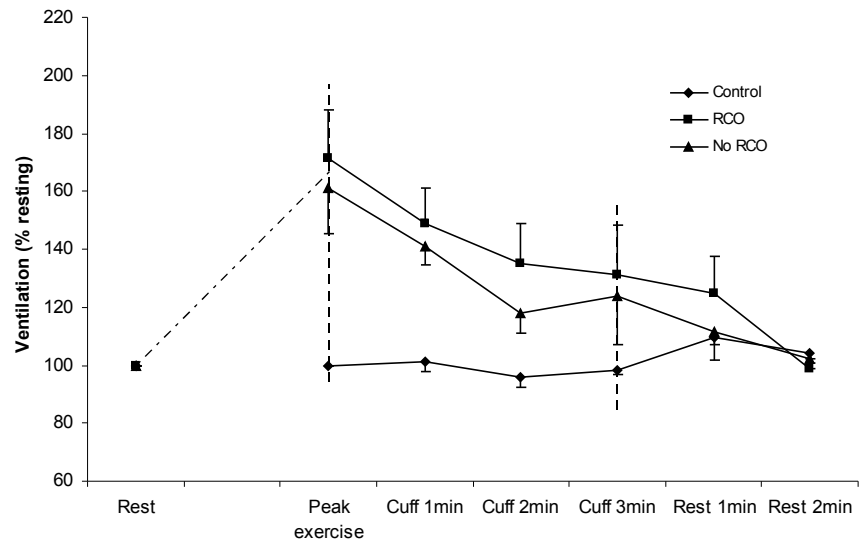
Figure 5.8 The effects of exercise followed by RCO on ventilation: diabetic autonomic neuropathy group
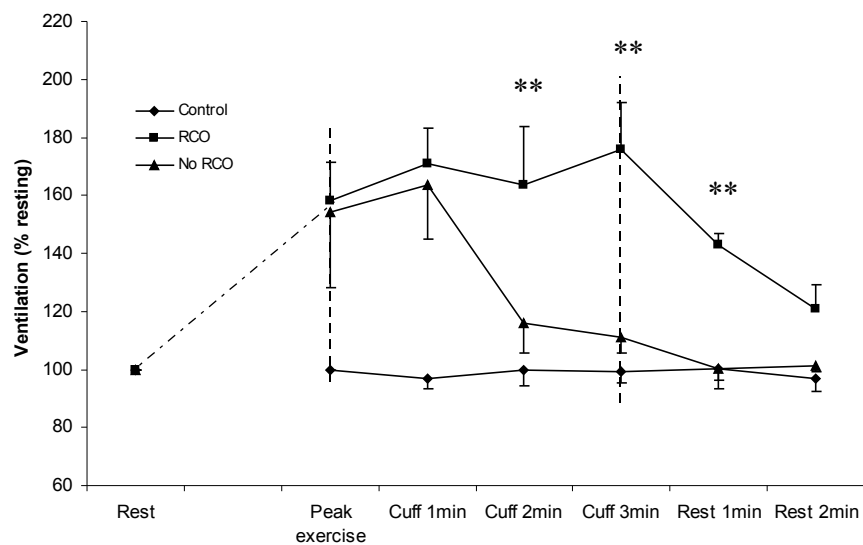
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *$p<0.05$, **$p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 5.9 The effects of exercise followed by RCO on oxygen consumption (VO₂): control group
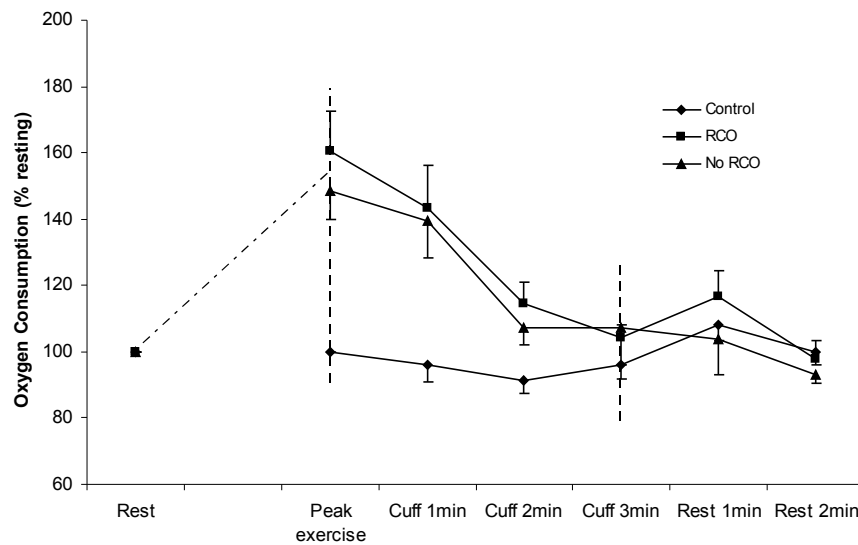
Figure 5.10 The effects of exercise followed by RCO on oxygen consumption (VO₂): diabetic autonomic neuropathy group
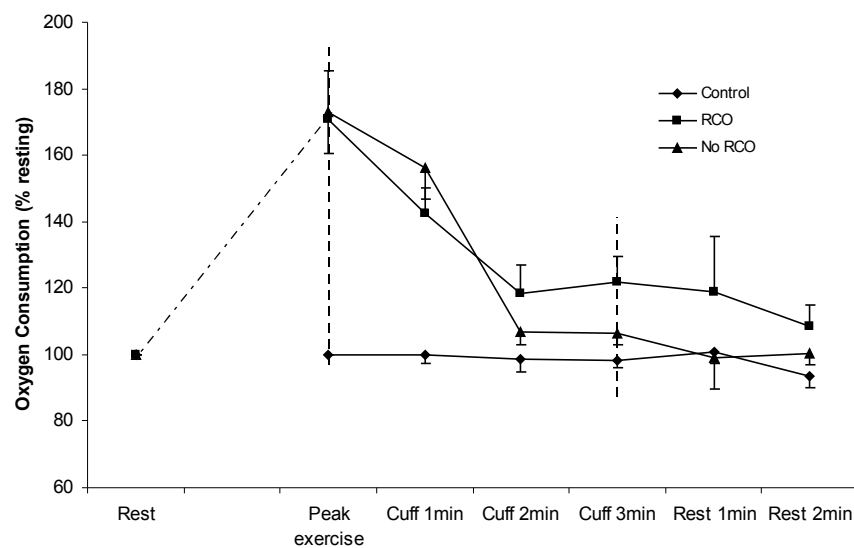
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 5.11 The effects of exercise followed by RCO on pulse transit time: control group
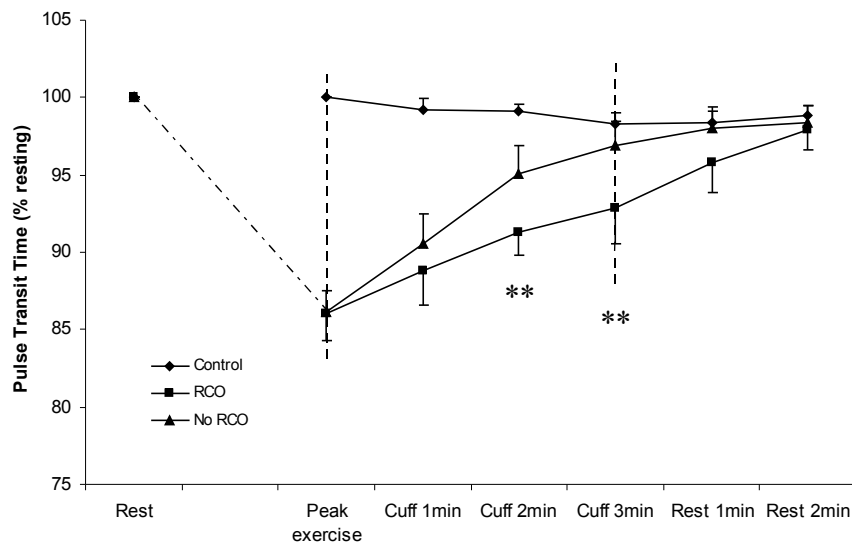
Figure 5.12 The effects of exercise followed by RCO on pulse transit time: diabetic autonomic neuropathy group
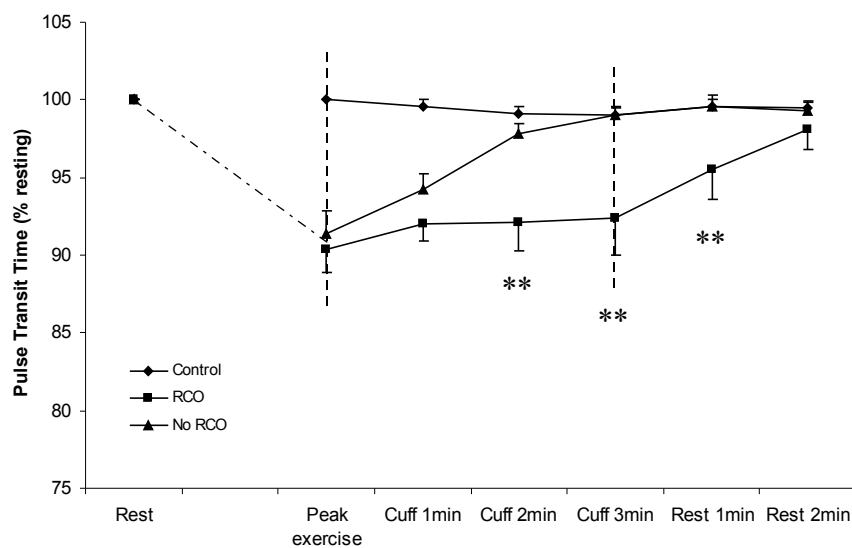
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 5.13 Pulse transit time tracing for subject 1
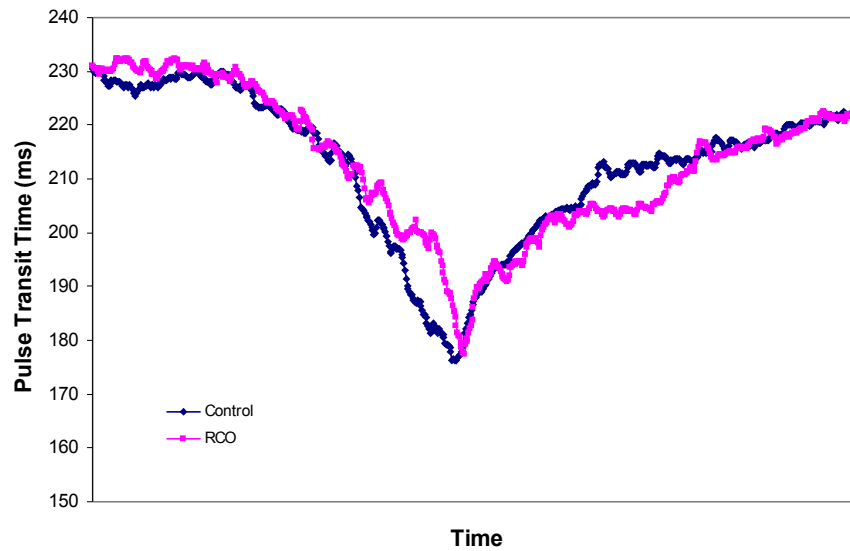
Figure 5.14. Pulse transit time tracing for subject 10
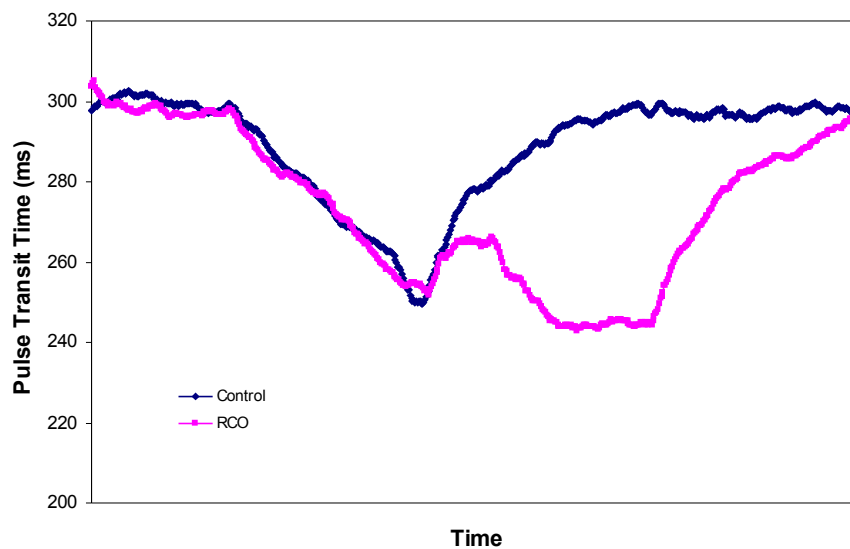
Graphs looking at changes in pulse transit time during rhythmic handgrip exercise followed by regional circulatory occlusion (pink lines) and control recovery (blue lines) for subjects 1 and 10. To allow greater clarity, each point on the graph represents the mean of that point plus the 4 points to either side to give a moving average.

5.10Chapter discussion

To our knowledge, this is the first attempt to assess muscle metaboreflex function in a group of patients with diabetic autonomic neuropathy. Previous studies looking at the muscle metaboreflex in patients with neurological dysfunction have always centred on the sensory nervous system, as documented earlier in the chapter.

The principal finding of this study is that there appears to be no difference in metaboreflex function between patients with diabetic autonomic neuropathy (DAN) and a group of control subjects with diabetes who do not have DAN as per the criteria of Ewing and Clarke .

Diabetic neuropathy would be expected to cause damage to both the afferent and efferent limbs of this reflex. It is therefore surprising that we were so convincingly able to demonstrate normal metaboreflex function in our patient groups. Our patients with DAN had a normal blood pressure response to rhythmic handgrip exercise, and post-exercise regional circulatory occlusion led to a similar sustained increase in blood pressure as in the control diabetic group and the group of non-diabetic subjects used in chapter 4. This raises the possibility that patients with diabetic neuropathy suffer from selective damage to some sensory and autonomic pathways but not others. It is also possible that the diagnosis of autonomic neuropathy by the criteria of Ewing and Clarke has selected a group of patients with predominantly parasympathetic dysfunction, so it is therefore perhaps not a surprise that the metaboreflex, which is sympathetically mediated, is intact in our group. Our finding of intact metaboreflex function is in keeping with the observation that phase I oxygen kinetics are similar in patients with

autonomic neuropathy to those with normal autonomic function and control subjects ,
suggesting adequate delivery of oxygen to skeletal muscle and adequate regulation of
oxygen supply.

It is noteworthy that post-exercise circulatory occlusion, which caused a sustained
increase in blood pressure, did not affect heart rate recovery in either group, with heart
rate falling rapidly to resting levels. Resting heart rate was slightly higher in the DAN
group, presumably due to the loss of normal resting parasympathetic tone. This would
explain the smaller heart rate response to exercise, with both groups achieving the same
peak heart rate.   These findings are in keeping with previous work looking at
metaboreflex control of heart rate. As discussed in chapter 1, the initial heart rate
response to exercise is mediated through central command and a withdrawal of
parasympathetic tone.   During post-exercise muscle ischaemia, the withdrawal of
central command, along with the effects of relative hypertension on the baroreflex, lead
to a rapid fall in heart rate to resting levels .   We had hypothesised that the
parasympathetic dysfunction in our DAN group would lead to an unopposed
metaboreflex-mediated increase in heart rate during post-exercise RCO.   This was
clearly not the case, with heart rate rapidly falling to resting levels. The implication is
that baroreflex control of heart rate in this group of subjects was intact, and raises the
possibility that the baroreflex is "reset" to a higher resting heart rate.

There are many potential confounding factors when performing a study on subjects
with diabetic autonomic neuropathy. Patients with neuropathy due to diabetes mellitus
are a heterogeneous group who appear to suffer from varying degrees of both sensory

neuropathy and parasympathetic and sympathetic dysfunction. All of these abnormalities could potentially affect metaboreflex function. As 5 of the 6 patients with autonomic neuropathy also had sensory neuropathy, we could conclude that the presence of a sensory neuropathy does not affect the afferent (sensory) limb of the metaboreflex. It is also clear that many patients with long-standing diabetes mellitus suffer from subclinical autonomic dysfunction that is only evident on detailed testing . It is therefore very difficult to recruit a group of subjects matched for age and duration of diabetes that have got no evidence of autonomic dysfunction. It is also true that peripheral neuropathy tends to affect the longer nerves in the legs before the shorter nerves in the arms are involved. Any abnormalities in metaboreflex function would therefore presumably affect the leg muscles before affecting the forearm muscles that we tested.

5.11 Chapter conclusions

In conclusion, subjects with diabetic autonomic neuropathy appear to have normal metaboreflex function in the forearm, with a normal heart rate recovery during post-exercise RCO suggesting normal baroreflex buffering of the metaboreflex.

Chapter 6

The Effects of Creatine Supplementation on Patients with

Stable Chronic Obstructive Pulmonary Disease

6.1 Chapter introduction

Creatine is a naturally occurring compound found in abundance in skeletal muscle, mainly in the form of phosphocreatine. As discussed in chapter 1, it plays an integral role in skeletal muscle energy metabolism, with phosphocreatine acting as a crucial energy buffer to maintain supplies of adenosine tri-phosphate (ATP) . It is of particular importance during high intensity, short-term exercise. Creatine supplementation in healthy subjects is known to improve athletic performance in sprint events and increase fat free mass , and it is therefore legally used as an ergogenic aid in "power" events in athletics. It has also been demonstrated that creatine supplementation may benefit patients with neuromuscular diseases and congestive cardiac failure . Other studies have failed to demonstrate benefit in patients with neuromuscular diseases .

The multiple causes of exercise limitation in chronic obstructive pulmonary disease (COPD) were dealt with in chapter 1, and it is clear that abnormalities in skeletal muscle play an important role. With the additional importance attached to nutrition and the development of cachexia in COPD, one can see why there has been some interest in creatine supplementation. It was shown by Fuld *et al* that creatine supplementation in patients with stable COPD during an exercise-based pulmonary rehabilitation programme led to an improvement in skeletal muscle strength and endurance, health-related quality of life and fat free mass. In this study, there was also an increase in fat free mass and peripheral muscle strength following creatine loading without exercise training.

It has been shown in the past that it is possible to alter muscle metaboreflex activity in patients with chronic heart failure. Piepoli *et al* studied the effects of physical training and found that a 6 week programme of handgrip endurance training significantly reduced muscle metaboreflex activity. The same group showed that it is possible to attenuate the muscle metaboreflex acutely through infusion of sodium bicarbonate, lending weight to the argument that intramuscular acidosis leads to metaboreceptor activation . It is reasonable to hypothesise that creatine supplementation may also lead to attenuation of the metaboreflex. An increase in the intramuscular stores of phosphocreatine during exercise should improve the rate of ATP regeneration and availability of energy during intensive exercise .

It has been suggested that creatine may have some beneficial effects on inflammatory pathways. *In vitro* studies looking at the effects of creatine on endothelial cells suggest that creatine supplementation inhibits endothelial permeability, neutrophil adhesion and adhesion molecule expression on cultured endothelial cells . Creatine may also have direct antioxidant effects, with a study by Lawler *et al* suggesting that creatine displays a significant ability to act as an antioxidant scavenger against radical ions. Santos *et al* studied the effects of creatine supplementation *in vivo* in a group of amateur athletes participating in a 30 kilometre race. It was found that creatine supplementation led to significant attenuation of the post-race increase in markers of muscle damage and inflammation such as creatine kinase, prostaglandin E_2 and TNF-α. All of these findings are of potential relevance in patients with COPD and skeletal muscle dysfunction. Couillard *et al* found that local quadriceps exercise performed to exhaustion led to evidence of markers of oxidative stress in the plasma of a group of patients with COPD, but not in healthy control subjects. The same group followed this

research up by demonstrating that exercise caused oxidative stress in the quadriceps muscle of patients with COPD, and this was associated with reduced quadriceps endurance . If creatine does have anti-inflammatory and anti-oxidant properties, this may partially explain the benefits seen in patients with chronic disease.

Creatine supplementation may also affect the metabolism of homocysteine. Creatine is synthesised in the human body by two metabolic steps. Guanidinoacetate is synthesised from glycine and arginine in the kidney, and this reaction is catalysed by the enzyme arginine: glycine amidinotransferase. Guanidinoacetate is then transported to the liver where it is methylated to creatine by guanidinoacetate-methyltransferase . There is a daily loss of 2% of total body creatine in the urine, which is compensated for by dietary supplementation and endogenous creatine synthesis. Methylation of guanidinoacetate to form creatine has been estimated to account for up to 70% of the transmethylation reactions in the body, and homocysteine is a byproduct of this process (see figure 6.1) . It is known that creatine supplementation suppresses arginine: glycine amidinotransferase biosynthesis and endogenous production of creatine, so it could be hypothesised that creatine supplementation should reduce the production of homocysteine. Studies looking at haemodialysis patients and normal young females have failed to prove this hypothesis, and it is clear that further larger studies are required. It is known that elevated plasma homocysteine concentrations are a risk factor for the development of atherosclerotic disease . Patients with COPD are known to suffer an excess of deaths from vascular disease (even after correction for other factors such as cigarette smoking) , so it is possible that this is a group of patients that would benefit from a reduction in plasma homocysteine levels.

Figure 6.1 The interaction between homocysteine and creatine metabolism.
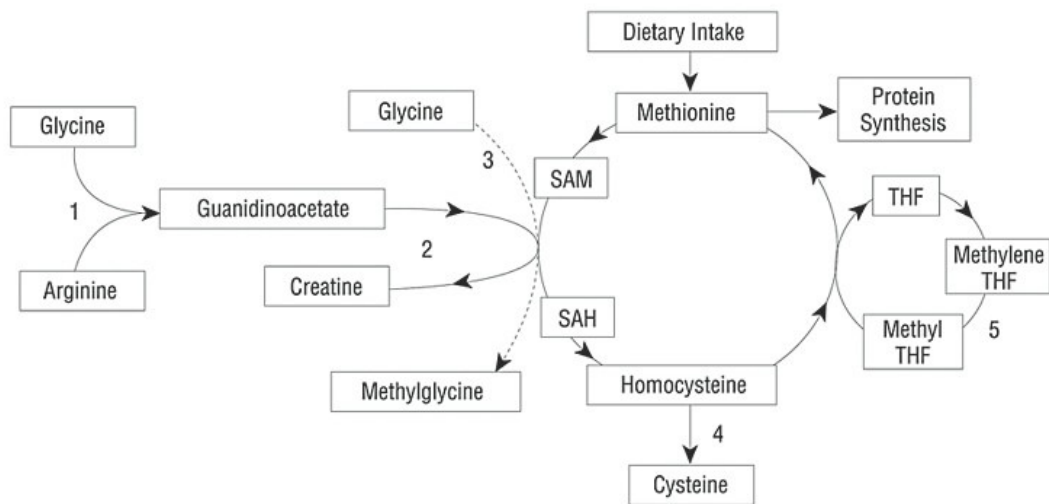
SAM = S-adenosylmethionine
SAH = S-adenosylhomocysteine
THF = Tetrahydrofolate

Guanidinoacetate is synthesised from glycine and arginine using the enzyme arginine: glycine amidinotransferase (1). This is methylated in the liver to form creatine (2). Homocysteine is also formed from methionine as a byproduct of the methylation process. Exogenous creatine supplementation suppresses synthesis of arginine: glycine amidinotransferase and the endogenous production of creatine. (Diagram taken from Steenge *et al*).

6.2 Research questions

The aims of this study were to answer the following primary research questions:

i)   Does loading with creatine attenuate muscle metaboreflex activity in patients with stable COPD?

ii)   Does loading with creatine lead to any improvements in forearm muscle strength, endurance or recovery in patients with COPD?

iii)   Does loading with creatine improve inspiratory or expiratory mouth pressures in patients with stable COPD?

iv)   Does loading with creatine lead to a reduction in levels of plasma homocysteine?

v)   Does loading with creatine lead to a reduction in markers of systemic inflammation (C-reactive protein or interleukin 6)?

We also had the secondary research question:

i)   Can we apply our findings relating to the use of pulse transit time to a study looking at the effects of an intervention on the muscle metaboreflex?

6.3 Patient characteristics

Patient characteristics are given in table 6.1. It can be seen that this is a group of patients with moderate to severe airflow obstruction, impaired diffusion capacity and significant air trapping at rest. This is an overweight group of patients, as can be seen by the mean body mass index of $28.4\pm1.8kg/m^2$. Five of the patients in the creatine study had previously participated in the study looking at the muscle metaboreflex in COPD (chapter 3).

Four subjects out of 15 withdrew from the study prior to completion. Subject 7 suffered from an exacerbation of COPD following visit 3. She therefore completed the creatine arm but not the placebo arm. We were unable to bring her back for the rest of the study due to expiry of the supplements. Subject 8 withdrew half way through the first arm due to vomiting (this turned out to be the "creatine" arm). He blamed the sweet taste of the supplements and did not wish to take part any further. Subject 11 developed mild diarrhoea during the first part of the study, but completed the course of supplements. This turned out to be the placebo arm, but she withdrew consent as she did not wish to go through further exercise testing. Subject 15 developed a mild exacerbation shortly after visit 2 and did not take any supplements. He was therefore withdrawn from the study.

Eleven subjects therefore completed all parts of the study. In addition, one subject completed the placebo arm only and one subject completed the creatine arm only. Compliance was excellent, and the correct number of empty bottles was returned on every occasion. There were no side effects reported other than those described above.

Table 6.1 Patient characteristics.

	Total	Subjects who completed study
Male/ Female (_n_)	7/ 8	5/6
Age (years)	64.9±2.2	65.2±2.7
Height (cm)	161.7±2.6	163.0±3.4
Weight (kg)	73.9±4.6	74.3±6.0
Smoking history (pack years)	53.7±6.3	50.9±7.7
Body mass index (kg/m^2)	28.4±1.8	28.2±2.4
FEV$_1$ (litres)	1.26±0.16	1.35±0.21
FEV$_1$ (% predicted)	53.3±5.2	56.7±6.9
FEV$_1$/ FVC	42.3±2.7	43.4±3.5
Corrected TLCO (% predicted)	52.4±6.5	54.1±8.0
Residual volume (% predicted)	165.8±11.9	165.9±15.2
Total lung capacity (% predicted)	122.3±6.2	124.7±7.8
Maximum handgrip right hand (kg)	27.9±2.8	28.3±3.7
Maximum handgrip (% predicted)	89.2±4.4	91.3±4.9
Peak inspiratory pressure (cmH$_2$O)	67.8±8.0	65.1±9.5
Peak inspiratory pressure (% predicted)	98.4±11.1	95.1±14.2
Peak expiratory pressure (cmH$_2$O)	60.8±4.2	60.8±5.3
Peak expiratory pressure (% predicted)	61.3±3.6	60.8±4.3

Baseline characteristics of this patient group. Data are presented as mean ± standard error of the mean. The right hand column excludes patients who did not complete the study. Predicted values for handgrip strength and peak mouth pressures were obtained from standard formulae and tables .

6.4 Weight

Weight increased by 0.6±0.3kg in the creatine group (p<0.05), and by 0.3±0.2kg in the placebo group (p=ns). These data are given in table 6.2.

6.5 Handgrip strength, endurance and recovery

Creatine supplementation did not affect handgrip strength or endurance in this group of patients (see table 6.2). There was also no improvement in muscle recovery following endurance exercise (see tables 6.3 and 6.4, figures 6.2 and 6.3). Placebo did not have any effects on these measurements.

6.6 Respiratory muscle strength

Creatine supplementation led to a statistically significant increase in peak inspiratory pressure (from 68.0±8.8cmH$_2$O to 73.8±8.9cmH$_2$O, p<0.05) and peak expiratory pressure (from 62.0±3.6cmH$_2$O to 70.3±4.0cmH$_2$O, p<0.01). Supplementation with placebo did not affect respiratory muscle strength (see table 6.2, figures 6.4-6.7).

Table 6.2 The effects of creatine loading and placebo.

	Placebo arm		Creatine arm	
	Pre-loading	**Post-loading**	**Pre-loading**	**Post-loading**
Weight (kg)	74.4±6.0	74.7±6.1	74.1±5.9	74.7±6.0 *
Handgrip strength Right (kg)	27.9±3.9	28.1±3.8	29.2±4.5	29.0±4.2
Handgrip strength Left (kg)	26.2±3.9	26.3±3.9	27.1±4.2	27.4±4.3
Handgrip endurance Right (repetitions)	30.5±3.4	34.5±3.8	29.4±4.9	32.6±5.4
Handgrip endurance Left (repetitions)	27.1±1.7	30.5±3.1	33.5±5.3	35.0±4.7
Peak inspiratory pressure (cmH$_2$O)	68.8±8.9	67.8±7.7	68.0±8.8	73.8±8.9 *
Peak expiratory pressure (cmH$_2$O)	61.4±4.4	60.6±3.7	62.0±3.6	70.3±4.0 **

This table shows the effects of creatine loading and placebo on weight, mouth pressures and forearm muscle strength and endurance. Data are presented as mean ± standard error of the mean. Statistical significance was tested using a paired Student's *t*-test after checking for normality using a Shapiro-Wilk test. For comparison of pre-loading with post-loading: *p<0.05 **p<0.01.

Table 6.3 The effects of creatine and placebo on right forearm muscle recovery

	Placebo arm		Creatine arm	
	Pre-loading	Post-loading	Pre-loading	Post-loading
10s recovery	22.7±3.2	22.4±2.6	23.6±3.3	23.7±3.0
20s recovery	23.6±3.0	23.1±2.6	24.1±3.4	23.9±3.0
40s recovery	24.9±3.1	24.2±3.1	24.8±3.6	24.6±3.1
80s recovery	26.2±3.0	25.6±3.1	26.0±3.9	26.2±2.9
160s recovery	26.9±3.4	25.6±3.3	28.0±4.0	27.3±3.2
300s recovery	27.4±3.7	27.0±3.3	28.6±4.5	27.3±3.5

Table 6.4 The effects of creatine and placebo on left forearm muscle recovery

	Placebo arm		Creatine arm	
	Pre-loading	Post-loading	Pre-loading	Post-loading
10s recovery	20.7±3.1	21.5±2.8	22.1±3.7	21.0±3.0
20s recovery	21.1±3.0	21.9±2.8	22.5±3.4	21.3±3.2
40s recovery	21.5±3.1	22.4±2.8	23.1±3.5	22.1±2.9
80s recovery	22.5±3.2	23.4±2.9	24.1±3.6	23.8±3.3
160s recovery	22.3±3.4	24.8±3.1	25.3±3.8	25.1±3.2
300s recovery	24.0±3.4	25.6±3.4	26.5±3.9	25.5±3.5

These tables show the effects of creatine and placebo on forearm muscle recovery following endurance testing for the right (top) and left (bottom) arms. Data are presented as mean ± standard error of the mean.

Figure 6.2 The effects of placebo on forearm muscle recovery
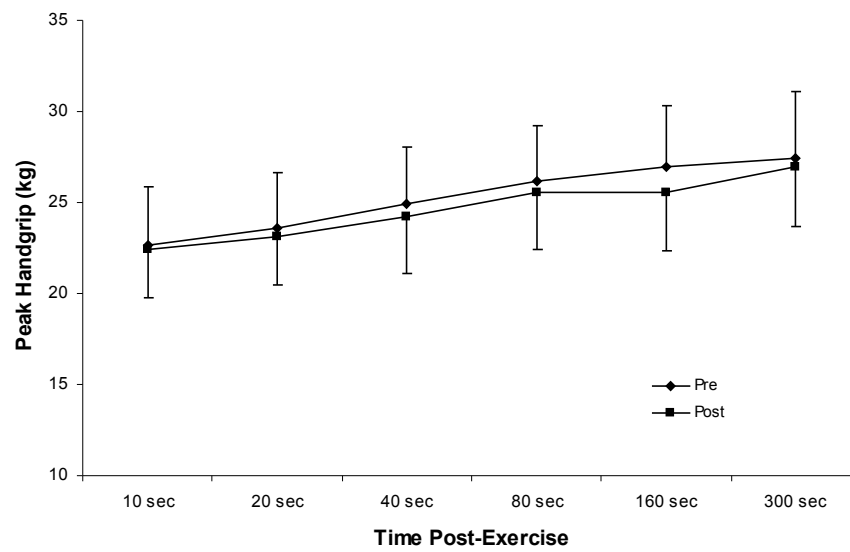
Figure 6.3 The effects of creatine on forearm muscle recovery
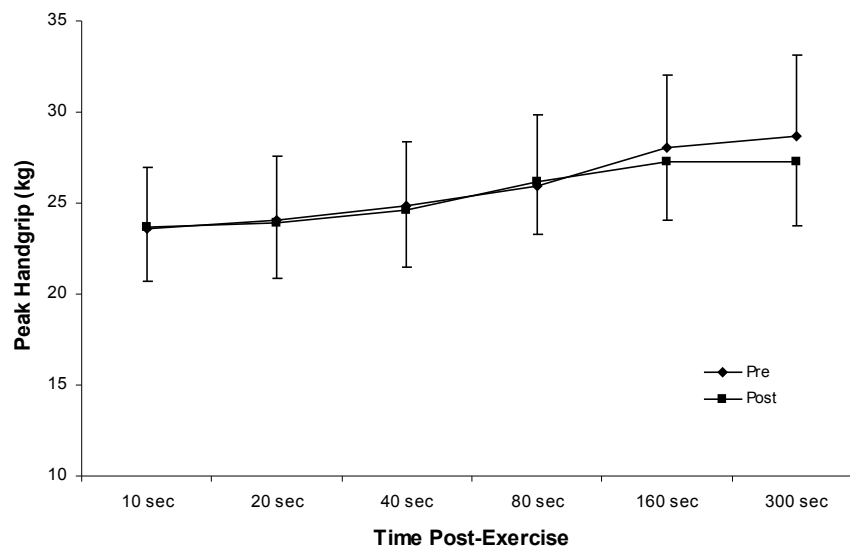
These graphs show the effects of supplementation with placebo (top) and creatine (bottom) on right arm forearm muscle recovery after endurance testing. Error bars represent the standard error of the mean.

Figure 6.4 The effects of placebo on peak inspiratory mouth pressure
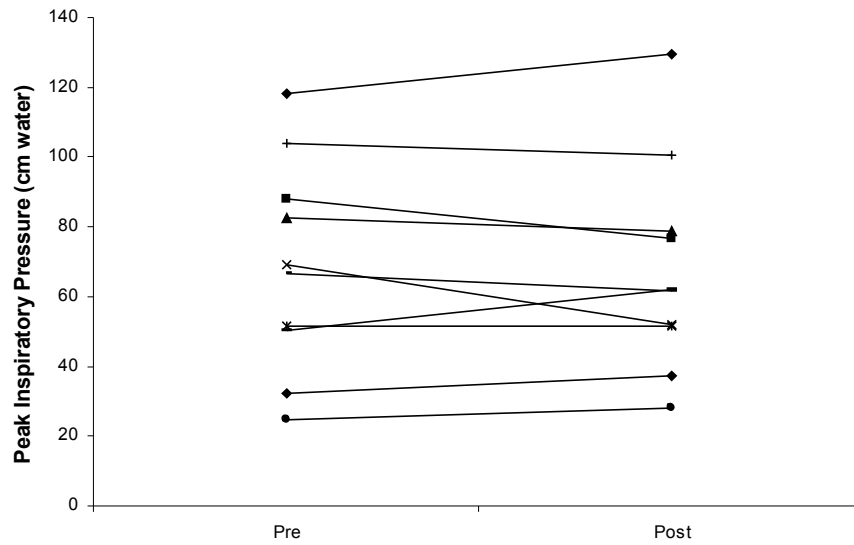
Figure 6.5 The effects of creatine on peak inspiratory mouth pressure
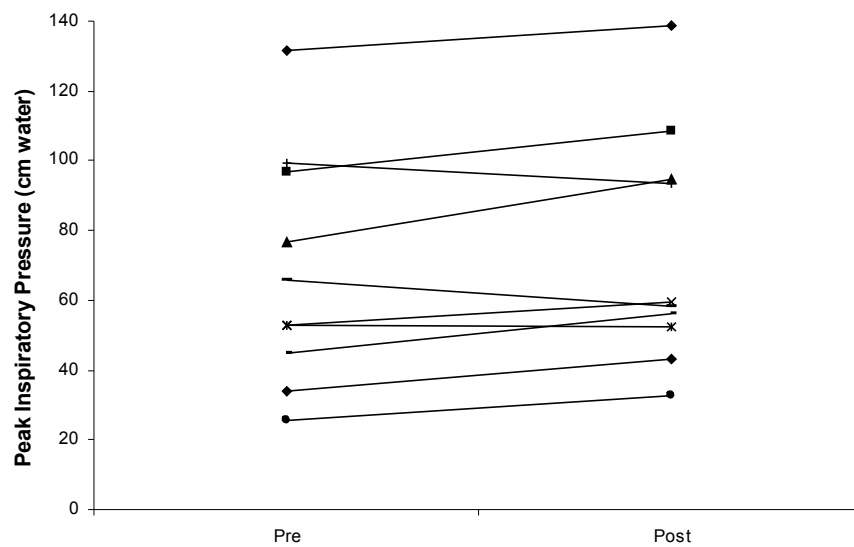
These graphs illustrate the effects of supplementation with placebo (top) and creatine (bottom) on peak inspiratory mouth pressure.

Figure 6.6 The effects of placebo on peak expiratory mouth pressure
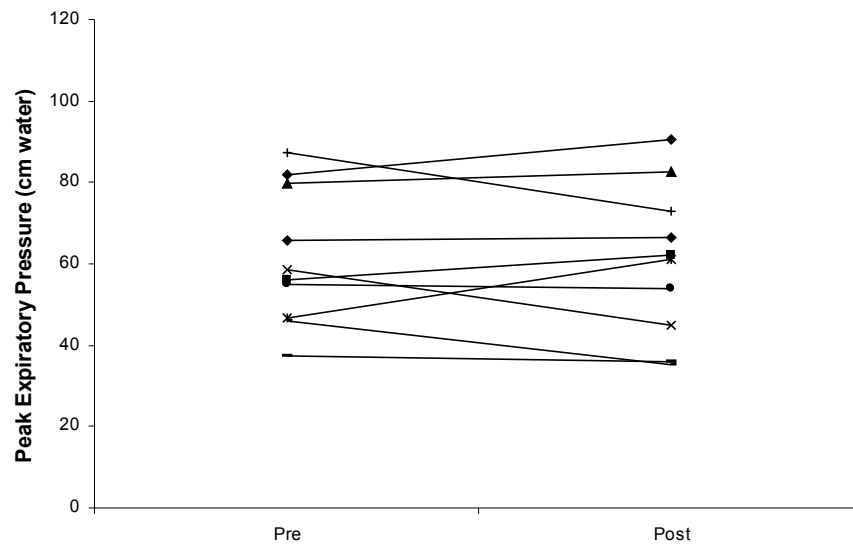
Figure 6.7 The effects of creatine on peak expiratory mouth pressure
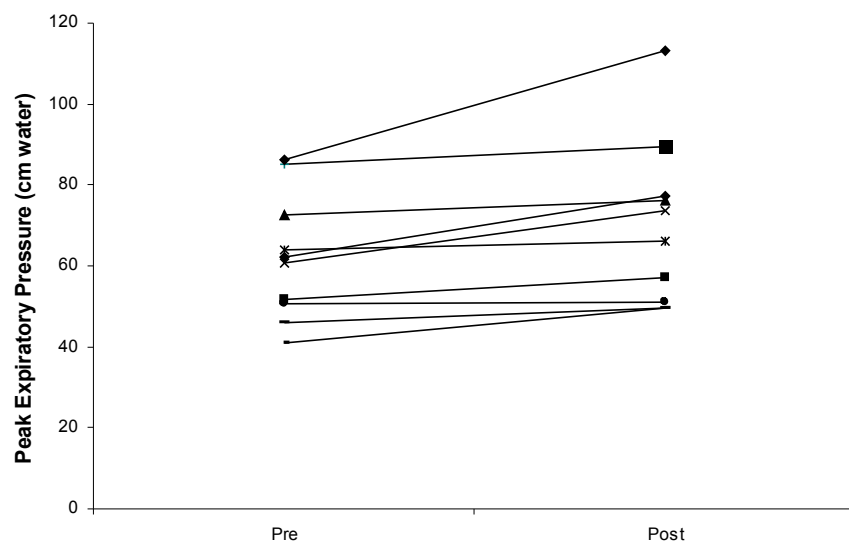
These graphs illustrate the effects of supplementation with placebo (top) and creatine (bottom) on peak expiratory mouth pressure.

6.7 The muscle metaboreflex

Rhythmic handgrip exercise led to significant increases in heart rate, ventilation, blood pressure and oxygen consumption (data not presented). These changes were of similar magnitude to those seen in chapter 3. There was also a significant reduction in pulse transit time, as seen with control subjects in chapter 4. There was no difference in resting or peak exercise measurements between any of the four exercise runs in each patient (data not shown). Control regional circulatory occlusion (RCO) following a period of rest did not affect any of the variables measured, as demonstrated previously in chapters 3-5.

Post-exercise RCO led to a sustained increase in systolic blood pressure ($p<0.0005$) and diastolic blood pressure ($p<0.0001$) compared with control recovery without RCO. These differences were of a similar magnitude to those seen in the patients in chapter 3 (table 6.5, figures 6.8 and 6.9). Post-exercise RCO did not affect heart rate recovery, ventilation or oxygen consumption (table 6.5, figures 6.10 and 6.12). This is at odds with the data in chapter 3, where a significant effect on ventilation was seen. The fall in pulse transit time seen with exercise was sustained during post-exercise RCO compared with control recovery ($p<0.0001$, see table 6.5 and figure 6.11). It is of note that good pulse transit time tracings (suitable for analysis) were obtained in all subjects except subject 3. All of the PTT tracings from subject 3 were of poor quality: possible reasons include tremor obscuring the "R" wave of the ECG, poor peripheral circulation and low oxygen saturations.

Table 6.5 Baseline measurement of the muscle metaboreflex in all patients.

	Rest	Peak exercise	3min recovery	3min RCO
Heart rate	77.2±1.7	87.4±2.0*	78.3±2.1	78.0±2.1
Systolic BP (mmHg)	127.4±3.3	164.1±5.4*	135.3±4.3	161.2±5.7†
Diastolic BP (mmHg)	64.1±1.7	86.8±3.2*	67.5±2.4	82.4±3.0††
Ventilation (l/min)	9.9±0.7	13.2±0.9*	12.8±1.1	12.4±0.8
VO_2 (ml/min)	143.8±9.8	204.0±15.8*	180.5±12.8	169.6±11.8
Pulse transit time (ms)	271.9±6.0	242.2±6.5*	260.0±5.9	247.7±6.1††

All data are presented as mean ± standard error of the mean. To compare resting and peak exercise values, statistical significance was tested using a paired Student's *t*-test or after checking for normality using a Shapiro-Wilk test. *p<0.0005 compared with resting values. Values given for post-exercise recovery and regional circulatory occlusion (RCO) represent the "area under the curve" for the 3 minute time period. To compare these values, statistical significance was assessed using a paired Student's *t*-test after checking for normality: †p<0.0005 ††p<0.0001.

Figure 6.8 The effects of exercise followed by RCO on Systolic blood pressure
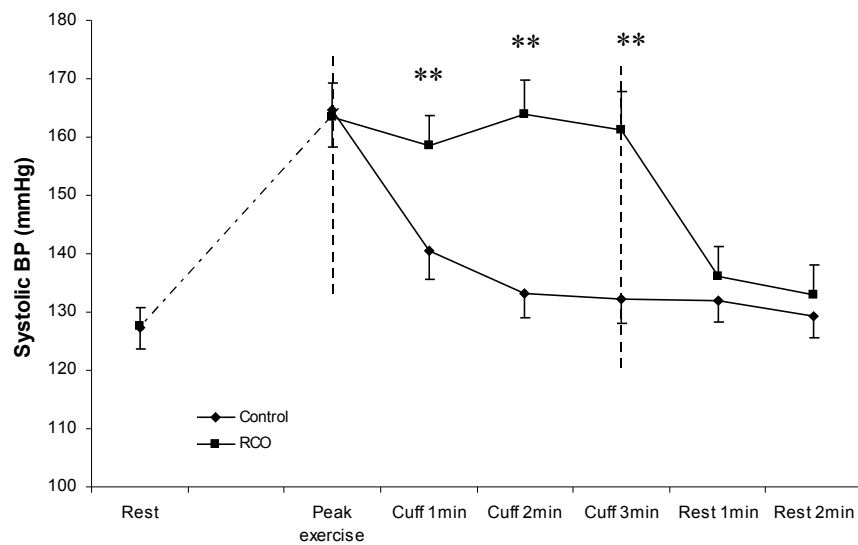
Figure 6.9 The effects of exercise followed by RCO on diastolic blood pressure
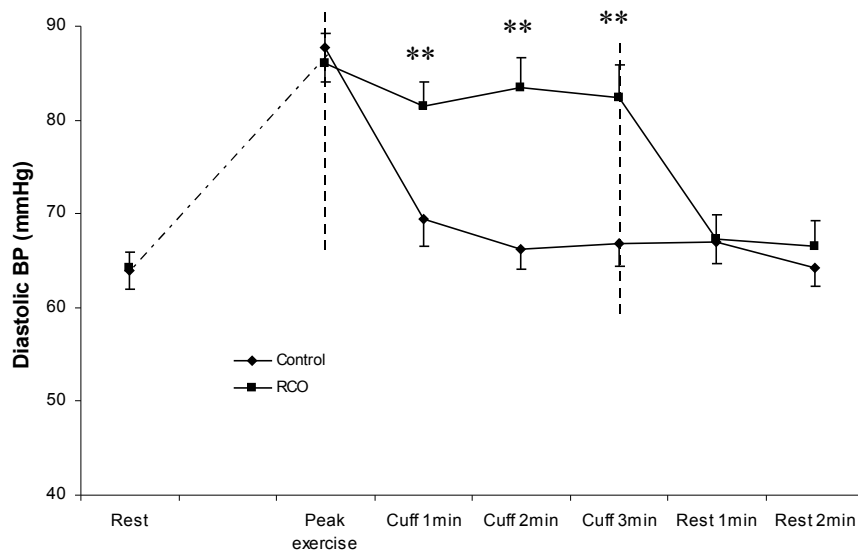
These are the results of baseline testing. Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 6.10 The effects of exercise followed by RCO on heart rate
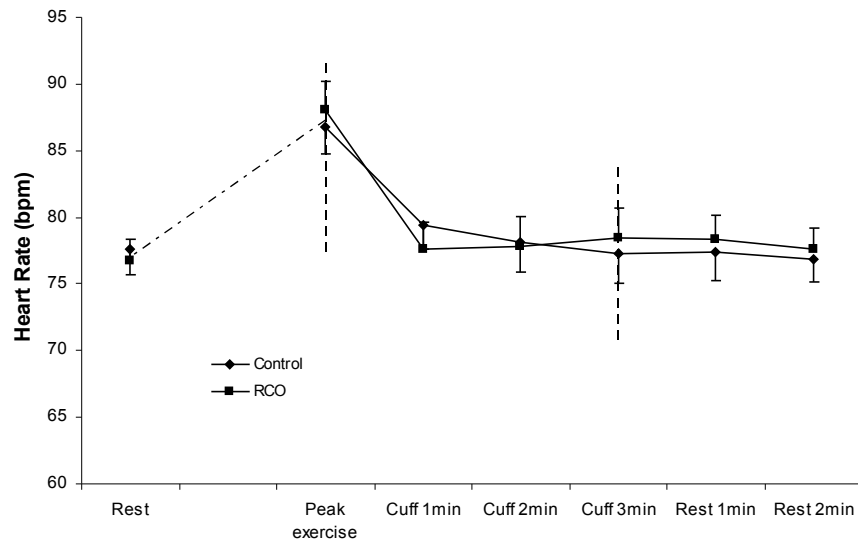
Figure 6.11 The effects of exercise followed by RCO on pulse transit time
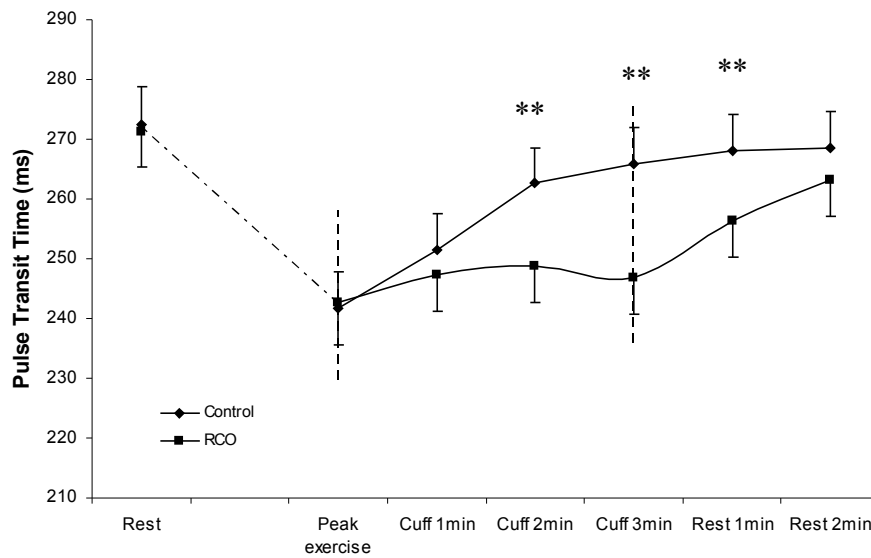
These are the results of baseline testing. Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 6.12 The effects of exercise followed by RCO on ventilation
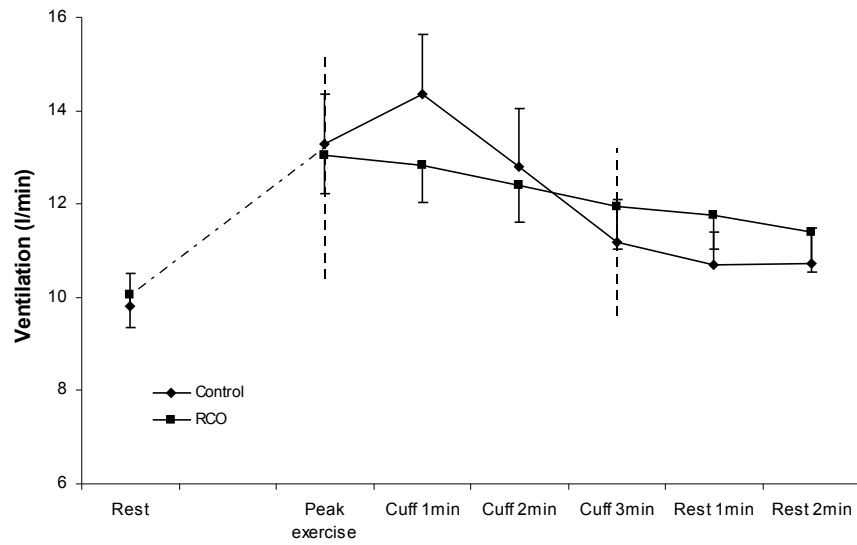
These are the results of baseline testing. Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

6.8 The effects of creatine supplementation on the muscle metaboreflex

Resting and peak exercise blood pressure, heart rate, ventilation, pulse transit time and oxygen consumption were not affected by either creatine supplementation or placebo (data not shown). Duration and intensity of exercise was deliberately kept the same for all exercise runs to allow comparison of the muscle metaboreflex at a similar level of work.

Tables 6.6-6.9 show the effects of creatine and placebo supplementation on muscle metaboreflex activity. Figures 6.13-6.18 graph exercise responses and the muscle metaboreflex pre- and post-creatine loading. Post-exercise RCO had significant effects on systolic and diastolic blood pressure compared with control recovery during all exercise runs. Post-exercise RCO had a significant effect on PTT during all except the post-placebo exercise runs (this did not quite achieve statistical significance). Again, ventilation and oxygen consumption were not significantly affected by post-exercise RCO in this group. Creatine supplementation had no effect on the muscle metaboreflex contribution to any of the measured variables. This also applied to supplementation with placebo.

6.9 Pulse transit time measurement

It should be noted that there were some difficulties with a few of the pulse transit time tracings. Thirty nine of the 49 tracings taken were of sufficiently good quality to analyse. There were two problems in particular:

1. Loss of the oximetry signal due to poor circulation or cold feet occasionally led to a gap in the tracing. It was not possible to view the data online, so problems could not be rectified as they arose. One significant gap in the tracing would prevent analysis of any of the data from that visit. This problem has been resolved by obtaining the software for the RM60 and using a laptop to view the data during testing.

2. Variable PTT tracing. Patients with COPD often have tremor secondary to inhaled β-agonist or theophylline use. The hyperinflated chest can also contribute to a poor ECG signal. As PTT depends on identification of the "R" wave of the ECG, this can lead to analysis problems and a variable PTT tracing.

Complete data were available for ten of the patients before and after the creatine arm of the study. There was data missing from five of the patients for the placebo arm, and these subjects are therefore excluded from analysis. This explains why the decrease in PTT with post-exercise RCO did not achieve statistical significance following placebo supplementation.

Table 6.6 The effects of placebo on the muscle metaboreflex: pre-loading

	3min recovery	3min RCO	Absolute difference
Heart rate (beats/min)	78.0±2.8	76.4±4.4	-1.6±0.9
Systolic BP (mmHg)	137.8±6.0	161.6±5.2**	23.8±5.8**
Diastolic BP (mmHg)	68.7±3.8	82.4±3.2**	13.7±3.8**
Ventilation (l/min)	12.3±1.2	12.3±1.2	0.0±0.7
VO$_2$ (ml/min)	180.5±15.1	169.6±13.9	-10.9±7.3
Pulse transit time (ms)	272.6±12.8	260.0±13.1**	-12.6±2.6**

Table 6.7 The effects of placebo on the muscle metaboreflex: post-loading

	3min recovery	3min RCO	Absolute difference
Heart rate (beats/min)	76.6±1.5	76.5±1.7	-0.1±1.9
Systolic BP (mmHg)	130.2±5.2	154.8±6.5**	24.6±4.1**
Diastolic BP (mmHg)	65.1±3.1	79.5±4.0**	14.4±2.7**
Ventilation (l/min)	10.9±0.7	11.8±1.1	1.0±5.5
VO$_2$ (ml/min)	163.2±12.3	174.3±14.5*	11.1±5.5*
Pulse transit time (ms)	273.5±10.7	265.1±13.7	-8.4±4.9

These tables look at the muscle metaboreflex before (top) and after (bottom) placebo. The figures given represent the "area under the curve" for the three minutes of post-exercise regional circulatory occlusion (RCO) and the equivalent period of control recovery. Statistical analysis was carried out using a paired Student's *t*-test to compare RCO with control recovery and to compare pre- and post- loading values. Normality was tested using a Shapiro-Wilk test. *p<0.05, **p<0.005.

Table 6.8 The effects of creatine on the muscle metaboreflex: pre-loading

	3min recovery	3min RCO	Absolute difference
Heart rate (beats/min)	80.5±2.5	79.6±2.4	-0.9±1.2
Systolic BP (mmHg)	133.6±3.5	163.9±7.3**	30.3±5.4
Diastolic BP (mmHg)	64.2±1.4	82.5±3.4**	18.2±3.0
Ventilation (l/min)	11.5±1.1	11.8±1.0	0.3±0.9
VO$_2$ (ml/min)	189.5±12.9	188.4±14.8	-1.2±5.5
Pulse transit time (ms)	262.7±6.9	252.4±7.5*	-10.3±3.8

Table 6.9 The effects of creatine on the muscle metaboreflex: post-loading

	3min recovery	3min RCO	Absolute difference
Heart rate (beats/min)	74.1±1.4	74.5±1.9	0.4±1.1
Systolic BP (mmHg)	131.0±3.6	151.6±3.3**	20.6±3.3
Diastolic BP (mmHg)	62.3±1.4	74.4±2.2**	12.0±2.3
Ventilation (l/min)	11.1±0.8	11.7±1.2	0.6±0.8
VO$_2$ (ml/min)	177.0±17.1	176.5±14.9	0.5±4.0
Pulse transit time (ms)	271.3±8.6	258.2±9.1**	-13.1±1.9

These tables look at the muscle metaboreflex before (top) and after (bottom) creatine. The figures given represent the "area under the curve" for the three minutes of post-exercise regional circulatory occlusion (RCO) and the equivalent period of control recovery. Statistical analysis was carried out using a paired Student's *t*-test to compare RCO with control recovery and to compare pre- and post- loading values. Normality was tested using a Shapiro-Wilk test. *p<0.05, **p<0.005.

Figure 6.13a The effects of exercise followed by RCO on systolic blood pressure: before creatine loading
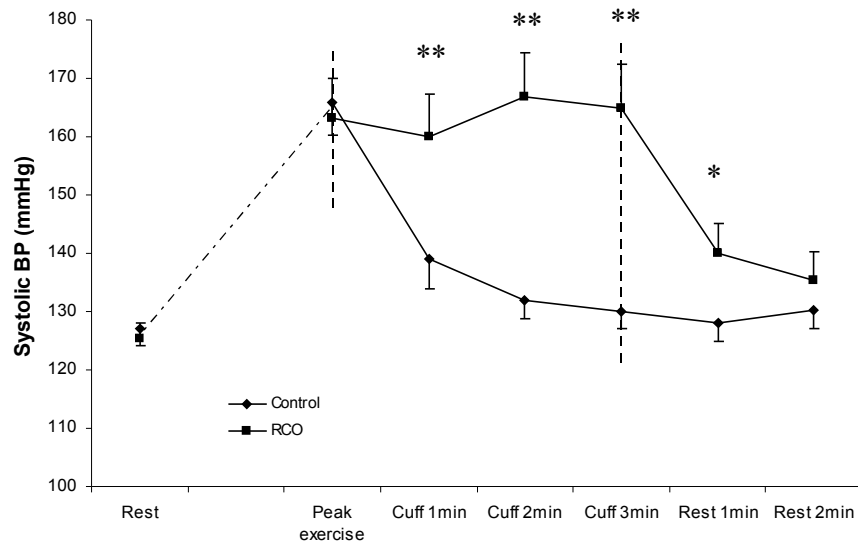
Figure 6.13b The effects of exercise followed by RCO on systolic blood pressure: after creatine loading
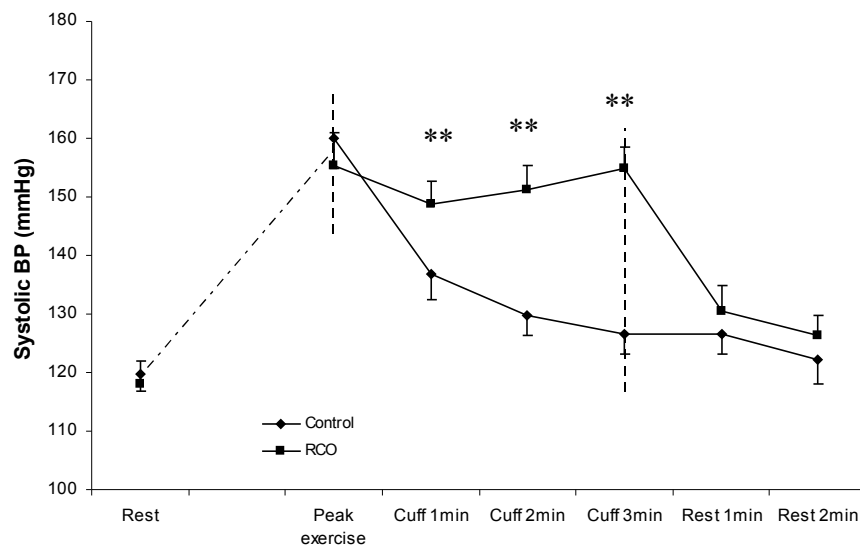
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 6.14a The effects of exercise followed by RCO on diastolic blood pressure: before creatine loading
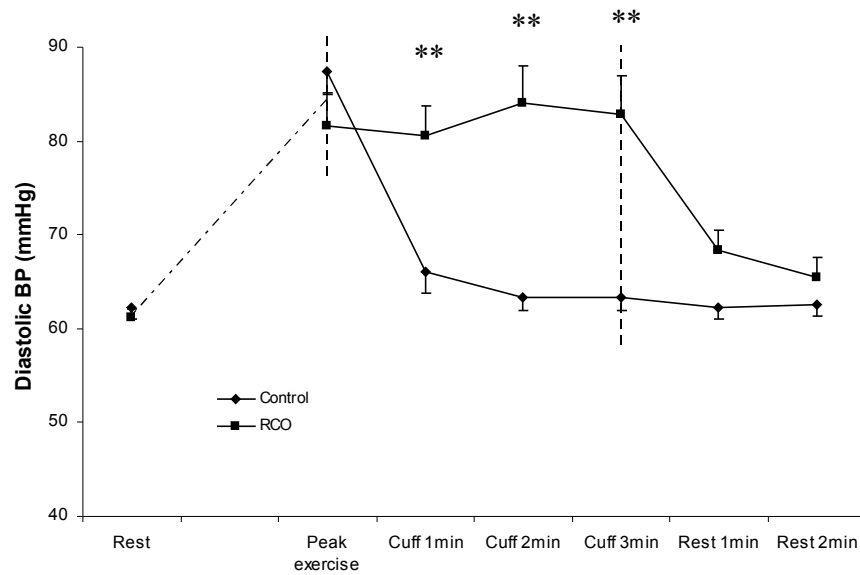
Figure 6.14b The effects of exercise followed by RCO on diastolic blood pressure: after creatine loading
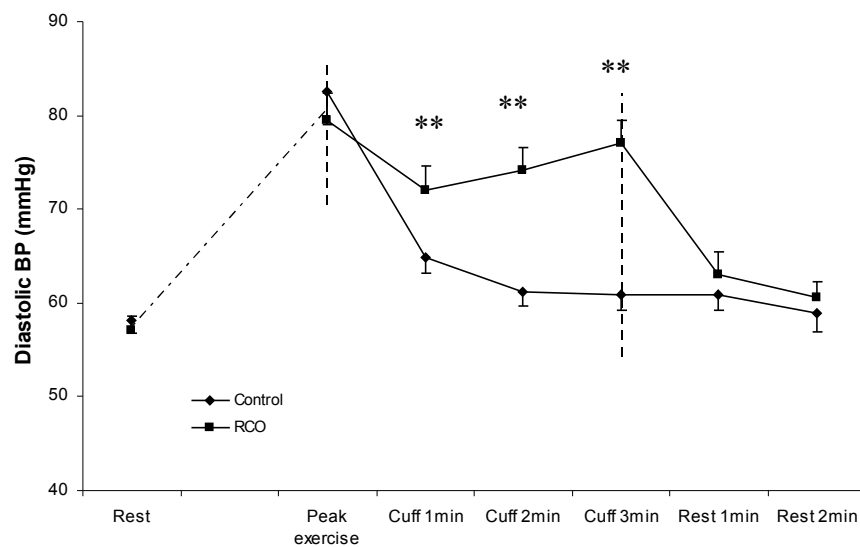
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: $*p<0.05$, $**p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 6.15a The effects of exercise followed by RCO on heart rate: before creatine loading
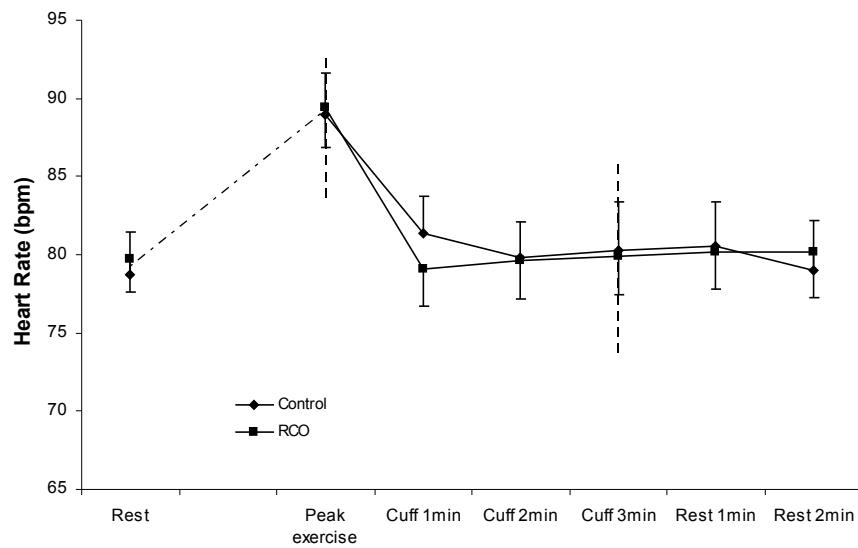
Figure 6.15b The effects of exercise followed by RCO on heart rate: after creatine loading
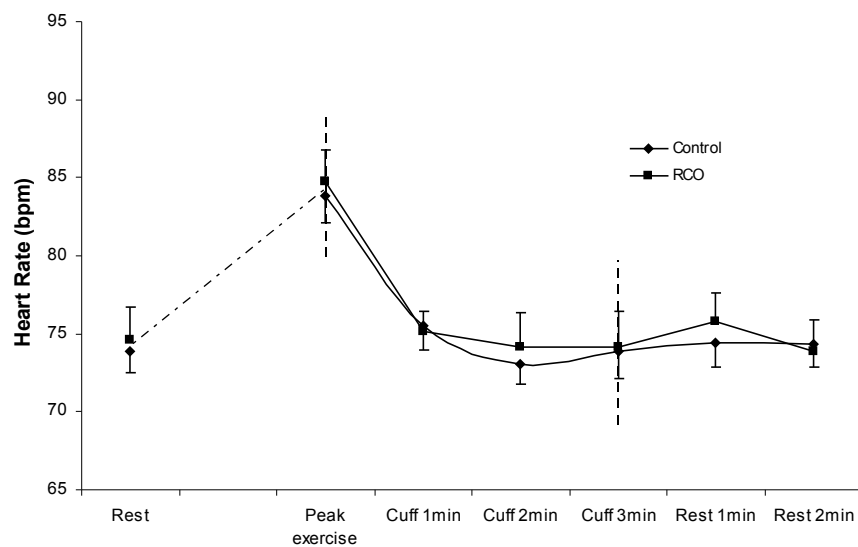
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: $*p<0.05$, $**p<0.01$. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 6.16a The effects of exercise followed by RCO on pulse transit time: before creatine loading
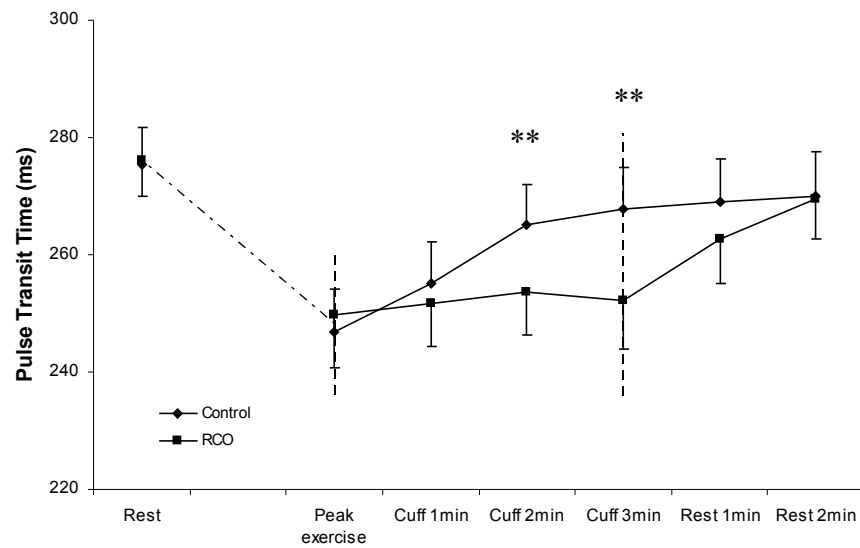
Figure 6.16b The effects of exercise followed by RCO on pulse transit time: after creatine loading
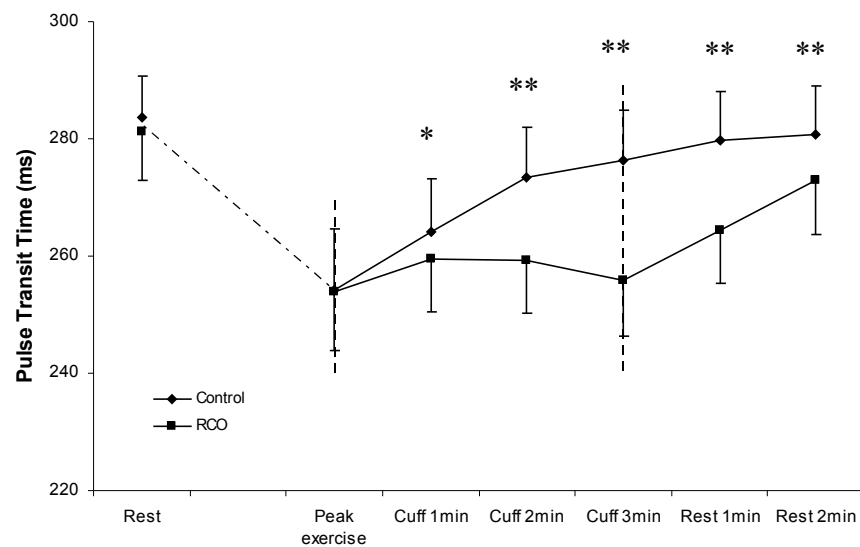
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 6.17a The effects of exercise followed by RCO on ventilation: before creatine loading
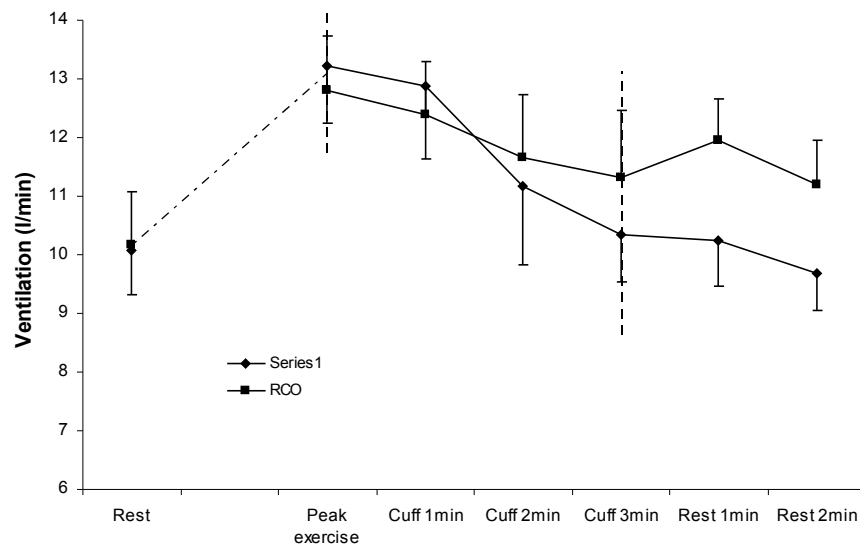
Figure 6.17b The effects of exercise followed by RCO on ventilation: after creatine loading
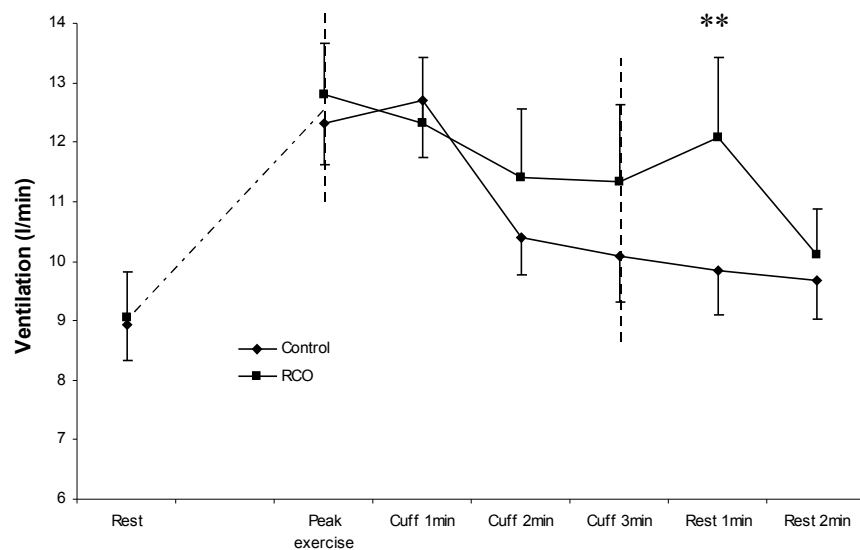
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

Figure 6.18a The effects of exercise followed by RCO on oxygen consumption: before creatine loading
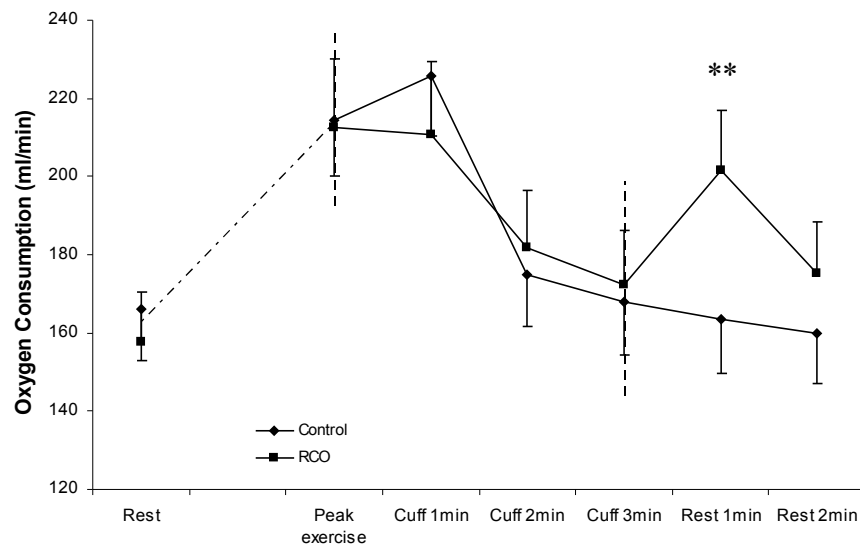
Figure 6.18b The effects of exercise followed by RCO on oxygen consumption: after creatine loading
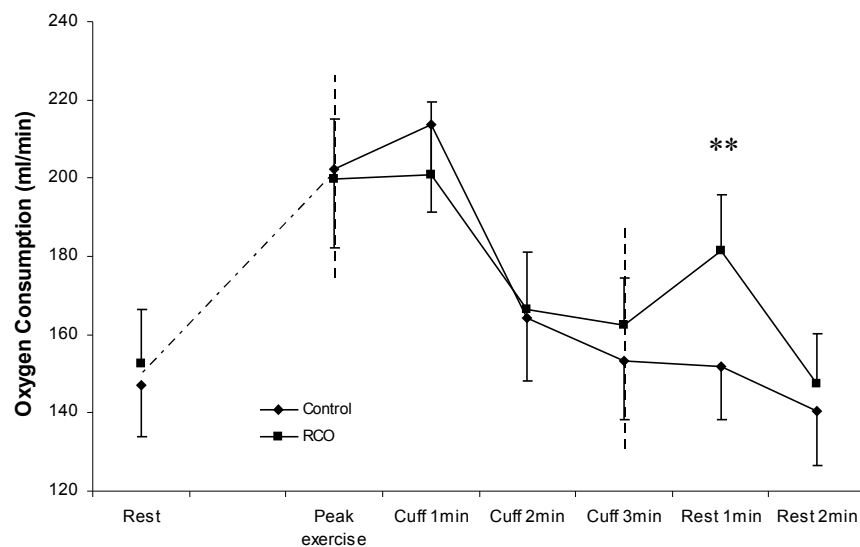
Error bars represent standard error of the mean. Comparison between "control" exercise run and "RCO" exercise run: *p<0.05, **p<0.01. Statistical significance was assessed using analysis of variance for repeated measures.

6.10 Analysis of serum

Baseline measurements of interleukin 6, C-reactive protein and homocysteine are given in table 6.10 for all patients except patient 12 (see below).

It was clear looking at the raw data that patient 12 had very abnormal results during visit 2. C-reactive protein was 112.31mg/l at visit 2 and less than 10mg/l at subsequent visits. Interleukin 6 was also much higher than expected. It was not clear whether this was due to an error of analysis or a recent infective or inflammatory episode. We therefore withdrew this patient's results from analysis and the graphic representation.

Creatine loading did not have any significant effects on C-reactive protein, interleukin 6 or homocysteine. These results are given in table 6.11 and figures 6.19-6.21. As expected, placebo supplementation did not affect any of these variables.

Table 6.10 Baseline CRP, IL-6 and homocysteine

	Baseline
C-reactive protein (mg/l)	5.9±1.1
Interleukin 6 (pg/ml)	4.5±0.30
Homocysteine (µmol/l)	19.5±3.0

Baseline measurements of C-reactive protein, interleukin 6 and homocysteine. All data are given as mean ± standard error of the mean. Patient 12 was excluded from the analysis for reasons that have been explained in the text.

Table 6.11 The effects of creatine loading on CRP, IL-6 and homocysteine

	Placebo arm		Creatine arm	
	Pre-loading	Post-loading	Pre-loading	Post-loading
C-reactive protein (mg/l)	5.7±1.5	6.4±1.7	4.6±1.0	4.1±1.0
Interleukin 6 (pg/ml)	4.24±0.43	4.92±0.81	4.48±0.53	4.70±0.49
Homocysteine (µmol/l)	18.0±1.5	19.1±4.1	21.8±5.1	19.9±7.2

Data are given as mean ± standard error of the mean. Comparison of pre- and post-loading values was carried out using a paired Student's *t*-test after checking for normality using a Shapiro-Wilk test.

Figure 6.19a The effects of placebo on C-reactive protein
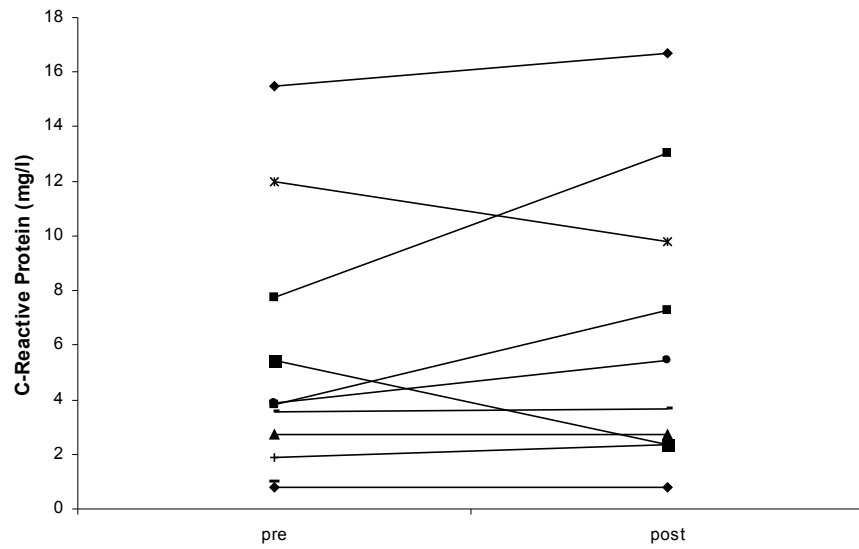
Figure 6.19b The effects of creatine on C-reactive protein
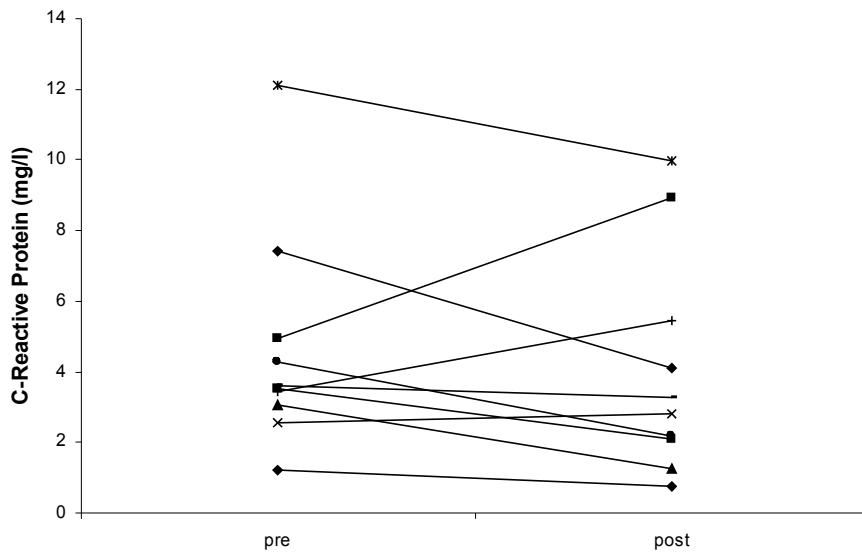
These graphs illustrate the effects of supplementation with placebo (top) and creatine (bottom) on C-reactive protein.

Figure 6.20a The effects of placebo on interleukin 6
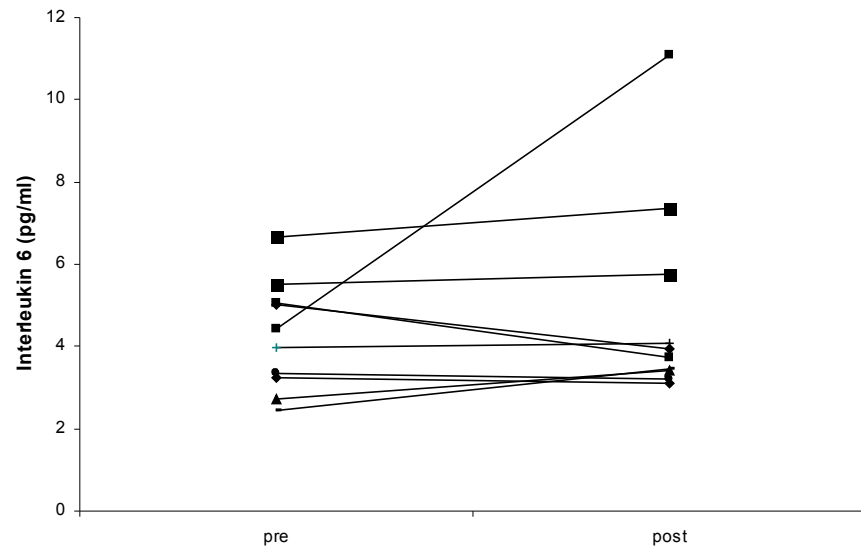
Figure 6.20b The effects of creatine on interleukin 6
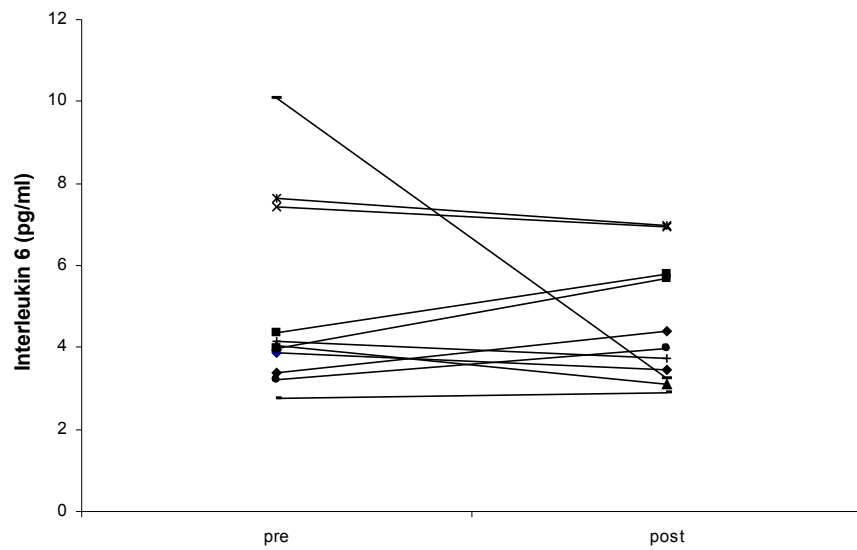
These graphs illustrate the effects of supplementation with placebo (top) and creatine (bottom) on interleukin 6 (IL-6).

Figure 6.21a The effects of placebo on homocysteine
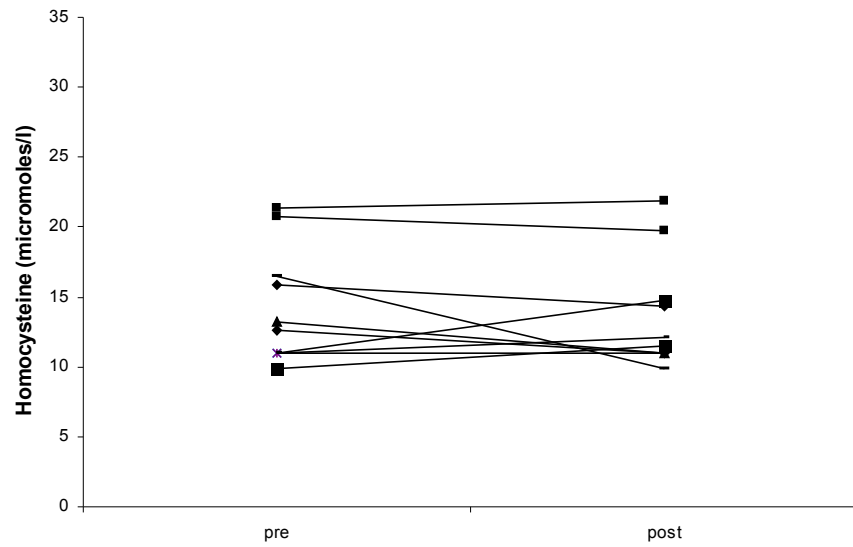
Figure 6.21b The effects of creatine on homocysteine
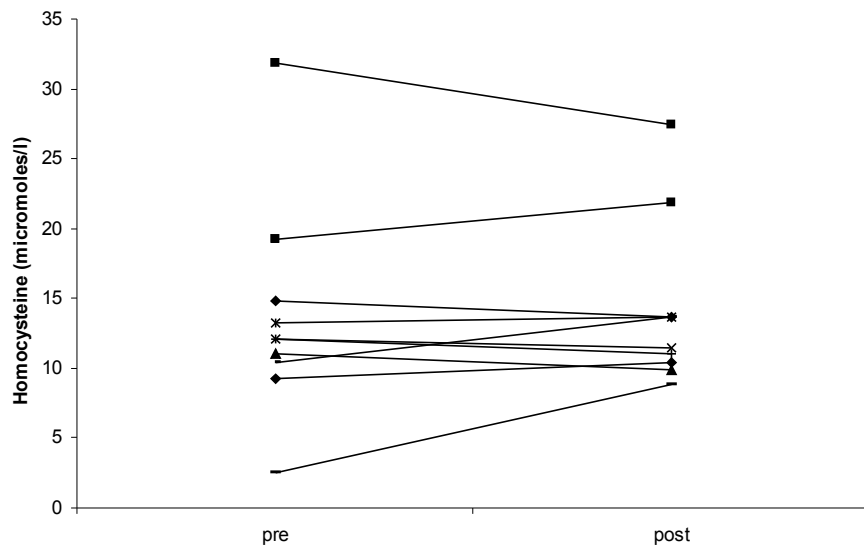
These graphs illustrate the effects of supplementation with placebo (top) and creatine (bottom) on homocysteine.

6.11 Chapter Discussion

6.11.1 The muscle metaboreflex

The primary research question of this study was whether creatine supplementation had any effect on muscle metaboreflex activity in a group of patients with COPD. As previously discussed, COPD is a disease characterised by histological, metabolic and functional abnormalities of skeletal muscle function very similar to those in patients with chronic heart failure. We wished to test the hypothesis that creatine loading, which has already been shown to improve skeletal muscle function in patients with stable COPD, attenuates muscle metaboreflex activity.

It can be seen from the data presented that our exercise protocol was able to detect muscle metaboreflex activity in this patient group, with post-exercise regional circulatory occlusion leading to a sustained increase in systolic and diastolic blood pressure, but not heart rate. The results obtained for these variables are very similar to those presented in chapter 3 using a different group of patients with COPD. Our data on the use of pulse transit time are also encouraging, with very good tracings obtained in most subjects. Once we figured out how to view the pulse transit time data during exercise testing, it was possible to see when there was a problem with the tracing which could then be rectified. As a result, pulse transit time recording was much more reliable in the later stages of the study.

It is worth noting that we did not see a sustained increase in ventilation during post-exercise RCO in this study. This is at odds with the data in chapter 3 of this thesis, and

the previously published data in chronic heart failure. As discussed in chapter 1, the contribution of the muscle metaboreflex towards ventilatory control is still somewhat controversial. We found that measuring minute ventilation "online" with a metabolic cart led to variability in results which made them hard to interpret. Breathing pattern often changes when breathing through a mouthpiece, even when allowing familiarisation time with the equipment. When exercise commenced, it was often found that minute ventilation fell as the subject started to concentrate on the handgrip display. As handgrip exercise did not have a great effect on minute ventilation (compare the effects of total body exercise, where minute ventilation may increase 30-40 fold), the effects of such voluntary control of ventilation are magnified. This makes it difficult to pick out the effects of handgrip exercise and post-exercise RCO over the "noise" of other factors which affect ventilation. Blood pressure and pulse transit time therefore appear to be more robust variables by which to measure muscle metaboreflex activity.

6.11.2 The effects of creatine supplementation on peripheral skeletal muscle

It is clear from our data that creatine supplementation did not have an effect on forearm skeletal muscle function or muscle metaboreflex activity in this group of patients. There were no improvements in maximum handgrip strength, forearm muscle endurance or forearm muscle recovery following 10 days of creatine supplementation. These results are disappointing, given the previously documented improvements in skeletal muscle strength seen in patients with chronic heart failure and COPD following supplementation with creatine. Gordon *et al* demonstrated that 10 days of creatine

loading in a group of patients with chronic heart failure led to an improvement in lower limb skeletal muscle performance . A study looking at 5 days of creatine loading in a similar group of patients by Andrews *et al* demonstrated improvements in ability to perform repetitive forearm muscle contractions at 75% of maximum voluntary contraction . A reduction in ammonia and lactate production per muscle contraction was also seen. This finding is potentially relevant to our study given that lactate has been implicated as an activator of the metaboreflex. The effects of creatine loading in patients with COPD in the study by Fuld *et al* were described in the introduction . In contrast to this, Gosselink *et al* found that creatine loading followed by maintenance dosing in patients with COPD did not improve the training effect seen with an exercise-based pulmonary rehabilitation programme. It should be noted that creatine was administered without carbohydrate in this study: carbohydrate administration has been shown to augment the uptake of creatine by skeletal muscle .

The quantity of creatine used in our study would appear adequate, as ingestion of 20g of creatine daily for 5 days by healthy volunteers has been shown to lead to an increase in muscle creatine concentration of 20%, the majority of tissue creatine uptake occurring during the initial few days of supplementation . Compliance appeared to be excellent, though it is possible that not all supplements were taken. Creatine supplementation did lead to a significant increase in body weight not seen in the placebo arm, in keeping with previous studies.

It is possible that our study was looking at the wrong muscle group. The forearm muscles of patients with COPD have been shown to be relatively well preserved in comparison with the lower limb muscle groups . This is thought to relate to greater disuse of the lower limb muscles. It is feasible that creatine supplementation has a

greater effect on the larger muscles of the legs, and we should perhaps have looked at muscle metaboreflex activity in the legs.

It is well documented that there is significant inter-subject variability in response to creatine supplementation. Possible factors include nutritional status, creatine uptake by skeletal muscle and creatine excretion. It has been reported that 20-30% of healthy subjects do not respond to creatine supplementation, in that no increase in muscle total creatine is seen . In the study looking at creatine supplementation in chronic heart failure by Gordon *et al*, only the subjects with a low muscle total creatine demonstrated a significant response to supplementation . Improvement in muscle function appeared to occur primarily in this group of patients. The multi-system disease that is COPD encompasses a heterogeneous group of patients with varying degrees of airflow limitation, gas exchange abnormalities, skeletal muscle dysfunction and systemic inflammation. It is therefore possible that creatine supplementation benefits a "sub-group" of patients with COPD that a small study such as this will not identify. It should also be noted that our study looked at a group of patients who were overweight (mean body mass index of 28.4±1.8), perhaps the group least likely to benefit from creatine supplementation. Further studies should perhaps look at muscle biopsies and analysis of urinary excretion of creatine, and select a group of undernourished patients with COPD limited by "peripheral" rather than "ventilatory" factors.

The failure of creatine supplementation to attenuate muscle metaboreflex activity in this group of patients is therefore not surprising. Creatine supplementation did not appear to alter any objective measurement of peripheral skeletal muscle function. Attenuation in muscle metaboreflex activity would imply a change in muscle bioenergetics, which should also manifest as an improvement in muscle function.

It is possible that the reason that creatine supplementation does not have an effect on the muscle metaboreflex is that it targets the wrong metabolic process. The measurement of the muscle metaboreflex uses post-exercise regional circulatory occlusion to "fix" the metabolic state of skeletal muscle at the point of fatigue following endurance exercise. Creatine supplementation is known to improve muscle performance in "strength" and "sprint" events rather than "endurance" events. It is inevitable that metabolic products of anaerobic metabolism will have accumulated at the point of fatigue, and it is difficult to see what impact creatine supplementation would have on the "metabolic environment" surrounding the metaboreceptors at this point. In the context of a study looking at creatine supplementation, it would perhaps be more appropriate to measure the muscle metaboreflex following a very short period of intense exercise in a large muscle group rather than a longer period of forearm endurance exercise. The importance of this concept is highlighted by looking at the only two studies demonstrating that an intervention can have an effect on the muscle metaboreflex. Bicarbonate infusion, as studied by Scott *et al*, has an immediate effect on intramuscular acidosis, one of the main drivers of the muscle metaboreflex . Forearm muscle training, as studied by Piepoli *et al*, has an effect on endurance exercise and the ability of skeletal muscle to avoid anaerobic metabolism . The main effects of creatine supplementation, however, are on the performance of skeletal muscle during "short burst" exercise: cardiorespiratory control and regulation of muscle blood flow are not mediated by the muscle metaboreflex in such circumstances.

6.11.3 The effects of creatine supplementation on respiratory muscle function

The novel finding of this study was that there was a statistically significant improvement in peak inspiratory and peak expiratory mouth pressures following creatine supplementation. This improvement was not seen in the placebo arm. This is an interesting and potentially important finding. Respiratory failure is a serious consequence of an acute exacerbation of COPD, and ventilatory support in the form of invasive ventilation is often deemed inappropriate. It can also be difficult to wean patients from a ventilator following a pronged period of invasive ventilation. Such patients often have a negative protein balance, with loss of muscle mass due to disuse, muscle paralysis, poor nutrition and the acute inflammatory response. It would be interesting to study creatine supplementation in this setting to see if it has any effect on muscle recovery. It must be remembered, however, that the documented effects of creatine supplementation relate to "short burst" exercise rather than endurance: the muscles of respiration could be considered to be the ultimate muscles of endurance.

6.11.4 The effects of creatine supplementation on markers of systemic inflammation

Creatine supplementation did not affect C-reactive protein or interleukin 6 levels in this patient group. This presumably reflects the small numbers studied, and a larger study is needed to look at the effects of creatine supplementation on the systemic inflammatory response. The baseline variability of such markers makes a longitudinal study difficult in such a small group. A longer period of supplementation is perhaps also needed.

The potential effects of creatine supplementation on plasma homocysteine levels were discussed in the chapter introduction. It is difficult to say what should be regarded as a "normal" homocysteine level, but it has been suggested that the normal range is between 5 and 16μmol/l . The same paper suggested that 10μmol/l should be considered an achievable target, given the risk of atherosclerotic disease in patients with hyperhomocysteinaemia . Only 2 of our 15 subjects had a plasma homocysteine level <10μmol/l. Four subjects could be considered to have "moderate" (16-30μmol/l) elevations in plasma homocysteine, and a further 2 subjects had "medium" elevations (30-100μmol/l) . It is possible that dietary factors are important given that intake of folate and vitamins B_6 and B_{12} appear to affect levels of homocysteine: the West of Scotland has a poor dietary record with very low consumption of fresh fruit and vegetables. It was disappointing that creatine supplementation in this study did not have an effect on the elevated plasma homocysteine seen in our patient group. It is possible that studies looking at longer term supplementation in a larger group of patients are needed.

6.12 Chapter conclusions

In conclusion, the effects of creatine supplementation in this group of patients were disappointing, particularly in light of the encouraging results of the study by Fuld *et al* in COPD and the studies looking at creatine supplementation in chronic heart failure . It is possible that creatine affects skeletal muscle in a way that will not be picked up by measurement of the muscle metaboreflex using this protocol. The effects of creatine on respiratory muscle dysfunction are of particular interest, and perhaps merit further detailed study.

Chapter 7

Methodological issues

The research undertaken for this thesis has raised a number of important issues regarding the methodology of these, and any future studies. This chapter aims to address some of these issues.

7.1 Control subjects

When undertaking physiological research into a group of patients with a certain medical condition, it is important to know how a population of normal healthy subjects behaves: a control group. Our failure to include a group of control subjects in chapter 3 of this thesis could be criticised, although we had significant difficulties in obtaining a group of age-matched control subjects locally. This explains our decision to split the COPD patients into moderate and severe groups to see if there were any differences, although this was done *post-hoc*. A substantial body of literature does, however, exist studying the muscle metaboreflex in a normal population.

One of the difficulties with selecting a group of normal control subjects to compare with a diseased population is that there are often many differences between the groups that may or may not be related to disease. It is well known that patients with COPD perform less physical activity than an age-matched healthy population, and other confounding factors may include cigarette smoking, the effects of certain medications and genetic susceptibility. There is often fierce debate as to whether the changes that are seen in skeletal muscle in patients with COPD are part of the disease process or are simply down to disuse and deconditioning. Skeletal muscle abnormalities in COPD resemble those seen in an unfit population , albeit the changes are more pronounced.

We did study a group of normal healthy subjects in our study looking at pulse transit time (chapter 4), and it is therefore possible to undertake a limited comparison between this group and our patients with COPD.

Table 7.1 Subject characteristics

	Control group	COPD group
Male/ Female (n)	7/0	8/6
Age (years)	32.3±2.2	63.7±3.1**
Height (cm)	179.6±2.7	164±2.6
Weight (kg)	82.6±2.8	73.3±4.8
Body mass index (kg/m²)	25.6±1.2	27.4±1.7
FEV_1 (litres)	-	1.18±0.12
FEV_1 (% predicted)	-	46.4±3.7
FEV_1/ FVC	-	37.9±3

Values given are mean ± standard error of the mean. Comparison was made between groups using unpaired Student's t-test after checking for normality using a Shapiro-Wilk test. We did not have pulmonary function data on the control group. *p<0.001.

Table 7.2 Resting and peak exercise measurements

	Control group		COPD group	
	Rest	**Peak exercise**	**Rest**	**Peak exercise**
Systolic BP (mmHg)	127.1 ± 6.1	158.9 ± 5.5*	128.0±5.0	156.1±5.5*
Diastolic BP (mmHg)	67.9 ± 4.4	92.3 ± 4.7*	77.5±3.7	102.1±5.3*
Heart rate (bpm)	70.0 ± 4.3	87.6 ± 6.1*	80.6±3.5	91.2±3.5*

All values are mean ± standard error of the mean. Statistical significance within each group was assessed using a paired Student's t-test after checking for normality using a Shapiro-Wilk test. *$p<0.0001$ compared with resting values.

Table 7.3 The effects of post-exercise regional circulatory occlusion

	Control group		COPD group	
	3 min recovery	**Post-exercise RCO**	**3 min recovery**	**Post-exercise RCO**
Systolic BP (mmHg)	130.2 ± 7.2	157.6 ± 8.3**	132.5±4.0	153.5±5.7*
Diastolic BP (mmHg)	72.0 ± 5.0	89.0 ± 5.6*	79.1±3.3	94.6±3.6**
Heart rate (bpm)	69.9 ± 4.5	74.6 ± 4.8	80.9±3.6	81.5±3.5

Values given represent the "area under the curve" of the 3 minutes following peak exercise, either during recovery from exercise without regional circulatory occlusion (RCO) or during post-exercise RCO. Statistical significance was assessed using a paired (within groups) or unpaired (group comparison) Student's t-test after checking for normality using a Shapiro-Wilk test. *p<0.005, **p<0.001 (control recovery versus RCO). No statistically significant difference between groups.

It can be seen from table 7.1 that there was a significant difference in age between the control group and the group of patients with COPD. Table 7.2 details the blood pressure and heart rate responses to rhythmic handgrip exercise to fatigue. We were not able to obtain data on ventilation or gas exchange on the control group. It can be seen that absolute blood pressure responses to exercise were almost identical between the groups. There was a trend towards a higher resting heart rate in the COPD group (p=0.08). This could reflect the age of the two groups, the younger control group having a higher resting parasympathetic tone. It is also recognized that patients with COPD have an elevated resting heart rate, with possible explanations including hypoxaemic stimulation of peripheral chemoreceptors, activation of the respiratory metaboreflex or beta adrenergic medications .

The effects of muscle metaboreflex activation on blood pressure and heart rate are documented in table 7.3. Rhythmic handgrip exercise followed by post-exercise regional circulatory occlusion led to a sustained elevation in systolic and diastolic blood pressure in both groups, suggesting muscle metaboreflex activation. There was no effect on heart rate, reflecting a normal baroreflex-mediated return of parasympathetic tone and withdrawal of central command (discussed in chapters 1 and 3). Comparison of the muscle metaboreflex effect on blood pressure and heart rate between controls and subjects with COPD did not reveal any significant differences.

7.2 Historical control data

There are some limitations to the above comparisons between our healthy control group and our group of patients with COPD. We did not obtain data on ventilation and gas exchange on our control group, as they had been selected to participate in a different study, during which we did not have access to a metabolic cart. The control group was also significantly younger, although there has been one study suggesting that there is no difference in muscle metaboreflex activity between young subjects and a healthy older group . Studies looking at the muscle metaboreflex in patients with chronic heart failure have, however, used age-matched control groups, and this allows some further comparisons to be made.

When selecting a study with which to compare our data, it is important to choose a study using a similar exercise protocol. On review of the literature studying the muscle metaboreflex in patients with chronic heart failure, there are important differences in how the muscle metaboreflex is measured. Some studies use static rather than dynamic handgrip exercise , some look at lower rather than upper limb , and some use handgrip exercise to a pre-determined point in time rather than exercise to fatigue . The potential importance of these issues is discussed later in this chapter.

Piepoli *et al* studied the effects of a forearm exercise training programme on muscle metaboreflex activity in healthy control subjects and in patients with chronic heart failure . The protocol used for metaboreflex assessment in this study was similar to our protocol: rhythmic handgrip exercise to fatigue using a handgrip dymamometer, with 40 contractions per minute at 50% of the pre-determined maximal voluntary

contraction. Sphygmomanometer cuff inflation took place just before the end of exercise at 30mmHg above systolic blood pressure for 3 minutes. Tables 7.4 and 7.5 therefore compare our COPD population with the control and chronic heart failure subjects in this study.

It is obviously impossible to perform any statistical analysis on these groups due to the lack of access to the raw data from the study by Piepoli *et al*. Resting parameters are similar between the chronic heart failure and COPD groups, with the exception of minute ventilation, which could be explained by increased dead space ventilation and V/Q mismatch in the COPD group. Our group of patients with COPD appeared to have a reduced heart rate and systolic blood pressure response to exercise, which may reflect slight variation in the exercise protocol and work undertaken rather than any true difference between groups.

Table 7.4 Comparison of the characteristics of control and chronic heart failure groups with our COPD group

	Control group	CHF group	COPD group
Number	10	12	14
Age (years)	59.2±3.5	59.6±1.7	63.7±3.1
Weight (kg)	75.9±3.3	78.3±3.6	73.3±4.8
Body mass index (kg/m²)	Unclear	Unclear	27.4±1.7
Resting VE (l/min)	7.6±0.1	7.5±0.1	10.7±0.4
Peak exercise VE (l/min)	14.3±1.0	20.2±1.9	18.1±0.7
Resting SBP (mmHg)	132.3±1.6	128.1±1.4	128.0±5.0
Peak exercise SBP (l/min)	189.6±6.6	176.0±7.3	156.1±5.5
Resting DBP (l/min)	73.8±0.6	77.4±0.7	77.5±3.7
Peak exercise DBP (l/min)	99.4±4.1	101.8±2.7	102.1±5.3
Resting HR (bpm)	67.1±0.7	77.9±0.5	80.6±3.5
Peak exercise HR (bpm)	92.2±3.7	96.2±2.7	91.2±3.5
Resting VO$_2$ (ml/min)	235.5±5.0	215.0±4.1	187.0±10.0
Peak exercise VO$_2$ (ml/min)	446.6±20.0	375.5±13.7	301.0±18.0

CHF = chronic heart failure, COPD = chronic obstructive pulmonary disease. All data are given as mean ± standard error of the mean. CHF data and control data are taken from Piepoli *et al* . Further statistical analysis was not performed due to lack of access to the raw data.

Table 7.5 Comparison of the effects of post-exercise regional circulatory occlusion (RCO) on control, CHF and COPD subjects

	Control group	CHF group	COPD group
Systolic BP (mmHg)			
• **Control recovery**	135.4±9.2	129.0±6.2	132.5±4.0
• **RCO**	166.2±8.1*	171.5±5.5*	153.5±5.7*
• **Percentage**	59.2	90.6	90.7
Diastolic BP (mmHg)			
• **Control recovery**	75.1±2.7	77.7±2.7	79.1±3.3
• **RCO**	91.0±4.1*	101.5±2.4*	94.6±3.6*
• **Percentage**	67.2	98.8	69.5
Heart rate (bpm)			
• **Control recovery**	70.6±3.1	80.3±2.6	80.9±3.6
• **RCO**	70.8±5.4	82.0±3.1	81.5±3.5
• **Percentage**	14.7	22.4	8.5
Ventilation (l/min)			
• **Control recovery**	8.3±0.5	8.6±0.8	12.4±0.5
• **RCO**	11.6±0.9*	20.2±2.3*	14.7±0.8*
• **Percentage**	59.7	100	54.1
VO$_2$ (ml/min)			
• **Control recovery**	251.2±10.4	219.4±11.6	205.0±13.0
• **RCO**	240.0±16.0	219.7±16.1	231.0±15.0*
• **Percentage**	2.1	2.9	38.6

CHF = chronic heart failure, COPD = chronic obstructive pulmonary disease. All data are given as mean ± standard error of the mean. Control and CHF data are taken from Piepoli *et al* . Statistical analysis between groups was performed using paired Student's *t*-test after checking for normality using a Shapiro-Wilk test. *p<0.005.

Table 7.5 looks at muscle metaboreflex activity in a healthy control group, a group of patients with stable chronic heart failure and our group of patients with COPD. It is difficult to compare the effects of post-exercise regional circulatory occlusion (RCO) on absolute values due to the differences in baseline and peak exercise variables between the groups. That explains why percentage values are reported in table 7.5, which reflect the effect of post-exercise RCO on the change in a variable from rest to peak exercise, and these values can be directly compared across different groups.

It can be seen that there does not appear to be a difference in metaboreflex effect on ventilation between the healthy control group and our COPD group. This is in keeping with our finding in chapter 3 of no differences between the group of patients with moderate COPD and the group with more severe airflow obstruction. The data on blood pressure do, however, raise the possibility that there is a heightened muscle metaboreflex contribution to exercise blood pressure responses in patients with COPD. Further statistical analysis is not possible, so we cannot draw any further conclusions from this.

7.3. Method of exercise

It is difficult to compare studies on the muscle metaboreflex in humans due to the wide range of exercise protocols that can be employed. There is considerable debate as to the contribution of the muscle metaboreflex to ventilation in humans, and different groups argue that the metaboreflex is more active or less active in patients with cardiac

failure. Some of this debate will no doubt reflect the variation in exercise protocols, as highlighted by the following discussion.

7.3.1 Static vs rhythmic forearm exercise

Many research groups appear to favour static rather than dynamic handgrip exercise when studying the muscle metaboreflex. Sinoway's group, who propose that the muscle metaboreflex is attenuated in heart failure, used static handgrip exercise at 30% of maximal voluntary contraction prior to cuff inflation . Piepoli's group, who propose that the muscle metaboreflex is upregulated in heart failure, use rhythmic handgrip exercise to fatigue . There are important physiological differences between such exercise protocols that make comparison between such experiments very difficult.

During static exercise, an increase in intramuscular pressure will be transferred to intramuscular blood vessels, preventing blood flow into and within the muscle . It can therefore be assumed that there is relatively little oxidative metabolism of muscle glycogen during such exercise. During rhythmic handgrip exercise, blood flow is possible during the relaxation phase, thus allowing oxygen delivery to exercising skeletal muscle. Total work performed will be greater with dynamic exercise, leading to some aerobic and anaerobic metabolism of muscle glycogen. It could be argued that rhythmic exercise allows more meaningful study of the oxidative capabilities of skeletal muscle. Our hypothesis does, after all, relate to impaired oxidative metabolism during exercise leading to metaboreflex upregulation. There is surprisingly little literature

studying the effects of static and dynamic exercise on measurement of the muscle metaboreflex, and this certainly merits further research.

7.3.2   *Timing of cuff inflation*

Many of the studies on the muscle metaboreflex use forearm exercise to fatigue followed by cuff inflation. Some studies appear to use an arbitrary cut-off point such as "5 minutes after commencement of exercise" . There are clear difficulties with both approaches. The use of an arbitrary point in time risks some subjects not managing to reach this point. On the other hand, skeletal muscle is bound to demonstrate an acidosis at the point of fatigue, and this will clearly lead to muscle metaboreflex activation. It could therefore be argued that fatigue is not the correct point at which to measure metaboreflex activity if we are to demonstrate differences between subject groups. In the creatine study (chapter 6), we were careful to ensure that subjects exercised at the same work rate for the same time during each leg of the study to avoid this confounding problem.

It is clear that the method of exercise to fatigue followed by cuff inflation will lead to supra-maximal stimulation of the muscle metaboreflex. This may not be representative of the way in which the reflex behaves during normal day to day activity, and it is therefore possible that lower intensity exercise should be employed when studying the metaboreflex.

Skeletal muscle fatigue may also explain why we could not detect differences in metaboreflex activity between our diabetic autonomic neuropathy group and diabetic control group (chapter 5): perhaps muscle fatigue generates a supra-physiological signal which overcomes any subtle abnormalities of the sensory or autonomic nervous systems.

7.3.3 Exercise intensity

Choice of exercise intensity is also of importance. A heavy exercise load will cause rapid muscle fatigue and development of an intramuscular acidosis. A very light exercise load will cause recruitment of a different muscle fibre type , and this may lead to barely perceptible increases in measured variables, with the effects of exercise lost in "noise". It is also known that there are differences in reflex physiological responses to different degrees of forearm exercise intensity. Batman *et al* showed that a prolonged bout of low intensity rhythmic handgrip exercise led to a gradual increase in muscle sympathetic nerve activity in the absence of an intramuscular acidosis, suggesting that mechanoreceptor activation is of greater importance in this context.

7.3.4   Arm exercise vs leg exercise

Most muscle metaboreflex research in humans has concentrated on forearm muscle exercise. The main reason for this is that it is far easier to exercise an isolated group of arm muscles without using "accessory" muscles that will confound the results. It is also much simpler to isolate exercised forearm muscle from the rest of the body with a sphygmomanometer cuff than it is to isolate leg muscle: the bulkier quadriceps muscles make it difficult to occlude the vasculature reliably.

The question of whether to study arm muscle or leg muscle is important when looking at the muscle metaboreflex in disease. It is known that the arm muscles are relatively preserved in patients with COPD when compared with the leg muscles . This presumably reflects greater disuse and deconditioning of the leg muscles. It would therefore be of interest to study the muscle metaboreflex in the leg in patients with COPD. Although it is more difficult to study the lower limb, it has been done in patients with heart failure , and Scott *et al* found a correlation between muscle metaboreflex activity in the upper and lower limbs of patients with heart failure.

7.4. Other factors

There are some other potential confounding factors that may affect studies looking at the muscle metaboreflex in humans.

7.4.1   Ventilatory data

We found that there was a metaboreceptor contribution to ventilation in our first study looking at patients with COPD (chapter 3), but failed to find this in the group of subjects with COPD in chapter 6. As already discussed, there is considerable debate as to whether the muscle metaboreflex is relevant to the control of ventilation during exercise . It is also possible that the metaboreflex is one of a number of mechanisms which control ventilation, some of which are redundant in some subjects, and that this may explain inter-subject variability.

We had some difficulty in the measurement of ventilation, which may explain some of our results. When a mouthpiece is inserted, breathing pattern often changes, although we did allow time for breathing pattern to normalise. It is also true that the relatively small increase in ventilation that we were trying to detect may be lost in the "noise" of variations in breathing pattern. The contribution of voluntary control to ventilation is significant when compared with other measured variables such as heart rate and blood pressure.

7.4.2   *Reproducibility of technique*

The potential contribution of voluntary control to ventilation calls into question the reproducibility of the technique of forearm exercise followed by post-exercise circulatory occlusion. This has been studied by Scott *et al* in a group of subjects with chronic heart failure . It was found that there was satisfactory reproducibility when the technique was performed 5 days apart.

7.4.3   *Age*

The effects of age on the muscle metaboreflex were studied by Roseguini *et al* in a group of healthy subjects . Three minutes of static handgrip exercise at 30% of maximum voluntary contraction followed by cuff inflation was used to evaluate the metaboreflex in a group of young (mean age 23±3) and a group of older (mean age 62±7 years) subjects. It was found that there was no difference in metaboreflex activity, suggesting that age alone does not lead to attenuation of this reflex.

7.4.4   *Weight*

The effects of obesity on the muscle metaboreflex have been studied , with a suggestion that metaboreflex control of muscle sympathetic nerve activity is impaired in obese women. The authors hypothesized that insulin resistance led to a reduction in glycolysis in skeletal muscle, thus attenuating exercise-induced acidosis.

Quantification of skeletal muscle acidosis was not undertaken, and it is equally possible that difficulty in adequately occluding blood flow to and from the obese arm led to escape of metabolites and attenuation of the reflex.

The effects of muscle bulk may also be important, although this has not been specifically studied. One could speculate that an increase in muscle bulk would simply lead to an increase in ventilatory response to exercise, with no effect on the muscle metaboreflex. Any change in muscle fibre type could conceivably affect the metaboreflex, with a shift towards type 2 (glycolytic) fibres leading to increased production of lactate and increased metaboreflex activation. This merits further study.

7.4.5   Prescribed medications

A final potential confounding factor is the use of prescribed medications. Patients with chronic medical conditions are frequently prescribed a combination of tablets which affect electrolyte balance, the renin-angiotensin system, the glucocorticoid axis and adrenergic or muscarinic receptors. In reality, it is difficult to exclude such subjects, and careful note should be taken of the medication list. One study has suggested that beta blockade in patients with chronic heart failure reduces the ventilatory response to exercise, in keeping with the hypothesis that excessive exercise ventilation in heart failure is mediated through a metaboreflex driven increase in sympathetic nervous system activity . The individual effects of different classes of drugs on the muscle metaboreflex merits further research. We were careful to exclude patients on beta blockers or other rate-limiting cardiac medications from all of our studies.

7.5. Statistical issues

The failure to include a control group in chapter 3 led to a decision to test the hypothesis that muscle metaboreflex activity would be affected by disease severity in a group of patients with COPD. This decision was taken after completion of the study, and the data analysis was therefore performed *post-hoc*. In future, it would be both desirable and more statistically valid to select patients according to disease severity at the point of recruitment. How disease severity is determined should also be given consideration for future studies. Although FEV_1 is the traditional marker of disease severity, it is a relatively poor predictor of exercise performance. Perhaps the BODE index , which also considers nutritional status, dyspnoea and exercise tolerance, would be a more valid parameter to use. New York Heart Association symptom class has been shown to correlate with muscle metaboreflex activity in heart failure , so Medical Research Council dyspnoea score may be worth studying, although a much larger subject group would be needed.

Chapter 6 looked at the effects of creatine supplementation on a number of variables. The effects of creatine supplementation were disappointing, although there appeared to be statistically significant effects on inspiratory and expiratory mouth pressures. In retrospect, we should have brought this group of patients back for one final "washout" visit to see if mouth pressures returned to baseline. Time constraints prevented us from measuring lung volumes at each visit, which would have allowed us to determine whether the change in mouth pressures was due to a change in lung volumes rather than

muscle strength. It is also possible that the chance element of performing multiple Student's *t*-tests could explain our results: use of analysis of covariance would be more statistically robust in this situation, removing the element of chance.

7.6. How big should future studies be?

Although our creatine study (chapter 6) was largely negative, our data does allow us to perform a more detailed power calculation to guide planning for future studies. The numbers recruited for this study were based on previous studies looking at the effect of forearm muscle training on the muscle metaboreflex, and we had similarly hoped to detect a 25% change in muscle metaboreflex activity . We did find that there was more variation in metaboreflex activity between subjects, which would have affected power calculations. This probably reflects greater patient heterogeneity in our COPD group.

Using the data from the study by Piepoli *et al* along with the standard deviations from our COPD population, it can be estimated how many subjects would be needed to detect a similar difference between our COPD group and a control group. To have a 90% chance of detecting a similar difference at a 95% significance level, we would need between 18 and 27 subjects in each group, depending on the variable used. This does suggest that our study was underpowered due to the greater variation in muscle metaboreflex activity seen in our COPD group.

It is difficult to predict what magnitude of a difference in muscle metaboreflex we should expect between two groups of diabetic patients (with and without autonomic

neuropathy). Looking at the standard deviations from our data, power calculations suggest that we would need 9 subjects in each group to have a 90% chance of detecting a 30% difference in muscle metaboreflex activity at a 95% significance level.

7.7 Chapter conclusion

In conclusion, our research has highlighted a number of methodological issues that should be considered before designing any further research projects looking at the muscle metaboreflex in patients with COPD.

Chapter 8

General Discussion

8.1 General points

The question of how the human body matches the varying demands of exercising skeletal muscle with a constant supply of oxygen has long been debated by physiologists. Although central (voluntary) command and mechanoreceptors sensitive to movement and stretch are of clear importance, it is now established beyond doubt that the muscle metaboreflex plays a vital part in this feedback loop . The individual contribution of central command, the mechanoreflex and the metaboreflex to ventilation, blood pressure, sympathetic nervous system activity and heart rate would appear to differ, and this remains a source of some controversy. As an example, it is well established that the immediate increase in heart rate at the start of exercise is due to a centrally mediated withdrawal of resting vagal tone , but it is less clear whether ventilation is controlled by the metaboreceptor or mechanoreceptor activation, venous distension or higher cortical centres .

There is also debate surrounding the question of the importance of such reflexes in human disease. This was highlighted in a recent Point: Counterpoint article in the Journal of Applied Physiology, with one group arguing that the exaggerated exercise pressor response in heart failure is due to an increase in mechanoreceptor activity, and the other group countering that it is due to an increase in metaboreflex activity . Both groups were able to support a substantial body of evidence supporting their respective causes. As highlighted in chapter 1, the question of whether the metaboreflex is attenuated in patients with McArdle's disease is also controversial .

8.2 The muscle metaboreflex in patients with stable COPD

The first study in this thesis looked at whether we could measure muscle metaboreflex activity in patients with chronic obstructive pulmonary disease (COPD). As already discussed, there are many similarities between the syndromes of chronic heart failure and COPD including exercise intolerance, skeletal muscle dysfunction and cachexia . Our data would suggest that there is a metaboreflex contribution to the blood pressure response to exercise in patients with COPD. We also found that muscle metaboreflex activation in this group led to a significant increase in ventilation, in keeping with the work of Piepoli *et al* in patients with heart failure and in normal healthy volunteers . Our lack of a healthy control group makes it difficult to tell whether muscle metaboreflex activity was increased in this population.

We then looked at whether there was any difference in muscle metaboreflex activity between patients with moderate and severe COPD as determined by FEV_1, although this was a *post-hoc* analysis of data and results should therefore be interpreted with caution. We found no difference in metaboreflex contribution to blood pressure, heart rate or ventilation between the two groups. There are several possible reasons for this.

Although FEV_1 is used as an indicator of disease severity in COPD in most international guidelines, it is known to be poorly predictive of exercise tolerance in much the same way as ejection fraction in patients with chronic heart failure . Both measurements only reflect the function of one organ and ignore the other variables that contribute to the body's ability to exercise. It is therefore possible that use of a measurement that reflects functional disability, such as Medical Research Council

dyspnoea score, would be more appropriate. Most of the evidence for increased metaboreflex activity in patients with chronic heart failure centres on NYHA class, which reflects functional status . Our study was too small to stratify patients into four different groups, so perhaps a larger study should look in detail at whether the metaboreflex is more active in patients who are more breathless. We did not find any correlation between muscle metaboreflex activity and any other variable, including body mass index, handgrip strength or transfer factor.

It is also possible that differences in the pathophysiology of exercise limitation between patients with CHF and COPD are important. It has consistently been shown in patients with CHF that acute improvements in cardiac output do not lead to an improvement in exercise tolerance . This was taken as evidence that peripheral factors such as the muscle metaboreflex were of vital importance in causing abnormal exercise responses . Patients with COPD, however, are often limited by ventilatory constraints: about two thirds of patients will reach their predicted maximum minute ventilation during an incremental exercise test. It is therefore possible that the muscle metaboreflex is of secondary importance to ventilatory limitation. It is noteworthy that offloading the respiratory muscles with non-invasive ventilation can lead to an improvement in exercise tolerance , suggesting that central factors are more important. The good relationship between inspiratory capacity, which in turn predicts dynamic hyperinflation, and exercise capacity again supports the argument that central limitation is more important .

This argument does not take account of the significant number of patients with COPD who terminate an exercise test before reaching their predicted maximum ventilation. A

study by Troosters *et al* suggested that patients in this subset are more likely to benefit from an exercise-based pulmonary rehabilitation programme . It is therefore possible that it is this group who are more likely to have abnormal skeletal muscle responses to exercise and an abnormal muscle metaboreflex: any further work should perhaps address this issue.

One more factor which makes study of this population of patients difficult is the heterogeneity of the study population. Two patients with COPD may have similar disease severity when stratified according to FEV_1, but vast differences in other aspects of their disease. Body mass index, muscle dysfunction, systemic inflammatory response, concomitant disease, degree of gas exchange abnormality, smoking status and corticosteroid use could all conceivably affect measurement of the muscle metaboreflex. It is likely that study of a very large group of patients would be necessary to evaluate the metaboreflex properly in COPD. Even selection of a control group is difficult given the considerable debate over whether skeletal muscle abnormalities are simply due to deconditioning- it would be impossible to find a truly activity-matched group of healthy control subjects.

The muscle metaboreflex relies on an intact sensory and autonomic nervous system to function effectively. It is well recognised that patients with COPD develop subtle abnormalities of the autonomic nervous system , and it is therefore possible that this has an effect on the muscle metaboreflex. It should be noted, however, that the similar abnormalities of the autonomic nervous system that exist in patients with cardiac failure do not seem to affect the metaboreflex.

8.3 Creatine supplementation in COPD

Our study looking at the effects of short-term creatine supplementation in patients with COPD followed on from the work of Fuld *et al* , who found that both short term and long term creatine supplementation led to an increase in fat free mass and skeletal muscle strength and endurance. We hypothesised that creatine supplementation would also lead to attenuation of the muscle metaboreflex through an improvement in muscle bioenergetics, and we hoped to find similar improvements in skeletal muscle strength and endurance.

Our main finding was that there was no change in upper limb muscle metaboreflex activity following 10-14 days of creatine supplementation in our patient group. This is perhaps not a surprise, given that we did not see an improvement in forearm muscle strength or endurance with creatine loading. This finding contrasts with the work of Fuld *et al*, who found that quadriceps strength and endurance improved significantly following creatine loading . It is, however, recognised that upper limb skeletal muscle function in COPD is well preserved ; perhaps study of the muscle metaboreflex in the lower limbs would be more appropriate, although regional circulatory occlusion of the leg is technically difficult . It is also possible that a subset of patients with COPD who are "peripherally limited", as discussed above, merits further study. The increase in weight that was seen in this study following creatine supplementation was also less than that seen in previous studies .

Our other finding was of a significant increase in peak inspiratory and expiratory mouth pressures following creatine loading, although as we do not have full lung function data for each visit, we cannot exclude the possibility that this was due to changes in lung volumes. The study by Fuld *et al* found a trend towards an improvement in mouth pressures following creatine loading , but this was not quite statistically significant. This finding is of potential importance, as respiratory failure is often a consequence of an exacerbation of COPD. It would perhaps be interesting to look at creatine supplementation in patients recovering from an exacerbation of COPD or weaning from a ventilator in the intensive care unit, or whether creatine improves respiratory muscle endurance in patients with COPD.

Creatine supplementation did not seem to affect markers of systemic inflammation in this group, but the size of our study is too small to draw any conclusions. The effects of creatine supplementation on homocysteine merit further attention in a larger study.

8.4 Pulse transit time

Pulse transit time would appear to show promise in the measurement of the muscle metaboreflex. Our findings of a reasonable correlation with blood pressure are not new, although it is still a matter of debate as to what pulse transit time is actually measuring. It would be interesting to do more detailed studies comparing pulse transit time with more invasive measurements such as muscle sympathetic nerve activity during exercise. The fact that PTT is able to detect the muscle metaboreflex reliably means that it is potentially of use in other studies. We found measuring blood pressure with arterial

tonometry difficult, and it only gave us a reading every 10-15 seconds. There were occasional difficulties with measurement of blood pressure during exercise, and the wrist probe sometimes slipped out of place necessitating a repeat of the exercise run. Invasive arterial blood pressure monitoring is clearly not appropriate in this setting, and alternatives such as the "Finapres" are very expensive. Pulse transit time has the advantage of ease of use and continuous measurement.

8.5 The autonomic nervous system

The final study sought to investigate what happens to the muscle metaboreflex in a group of patients with autonomic nervous system dysfunction. We studied a group of patients with type I diabetes mellitus, in whom autonomic neuropathy is relatively common. Our finding that muscle metaboreflex activity is unaffected by autonomic neuropathy was a surprise, given the involvement of the sensory and autonomic nervous systems in the metaboreflex loop. This either suggests that the pathways involved in the metaboreflex are not damaged in autonomic neuropathy, or that activation of the muscle metaboreflex in this manner leads to a supra-physiological response which overrides autonomic and sensory nervous system abnormalities.

Although we made an effort to recruit two distinct groups of patients, it is possible that the control group had more subtle peripheral nervous system abnormalities. It should be noted that detailed autonomic function testing revealed this group to have varying degrees of sympathetic and parasympathetic dysfunction, which may make detection of an abnormal muscle metaboreflex in one group difficult. Our group was too small to allow sub-stratification into groups with specific problems such as an abnormal blood

pressure response on standing. It is worth pointing out that the results obtained from both of our patient groups are very similar to those seen using a similar exercise protocol in normal subjects.

One confounding factor is our use of the arm muscles to measure metaboreflex activity. It is recognised that the longer nerves supplying the lower limbs are affected earlier in autonomic neuropathy, so this perhaps needs further work. It is also impossible to select a group of patients without concomitant disease. Although we tried to select patients without overt cardiac disease, it is likely that some had silent cardiac or peripheral vascular disease.

8.6 Pathophysiological relevance of the muscle metaboreflex

The evidence in support of the existence of the muscle metaboreflex is overwhelming, and it is clear that it is one of a number of mechanisms by which the human body regulates blood flow to exercising skeletal muscle. There is also increasing evidence that there are differences in circulatory and ventilatory responses to exercise between patients with chronic disease and healthy subjects. Although the current weight of evidence appears to favour upregulation of the metaboreflex in patients with heart failure, this remains controversial. As previously discussed, there have been few studies looking at the metaboreflex in other diseases. One recent study suggested that calf vasoconstrictor responses to metaboreflex activation are actually blunted in patients with moderate to severe COPD , although the reasons for this are not clear and further

work is needed including the measurement of muscle sympathetic nerve activity during such a protocol. This study also used static rather than dynamic handgrip exercise.

It is important at this stage to consider why alterations in muscle metaboreflex activity may be of clinical or pathological relevance rather than a mere physiological curiosity. Like any other physiological system, the human body seems to support a number of mechanisms and reflexes by which oxygen supply to exercising skeletal muscle is matched with demand. Perhaps the human body would be able to cope without such a reflex, and it is genetic diversity that governs whether the metaboreflex is active or redundant?

To attempt to answer the question of the potential clinical relevance of the metaboreflex, it is worth taking a closer look at the syndrome of chronic heart failure. By conventional wisdom, a failure to increase cardiac output with exercise leads to inadequate oxygen delivery to skeletal muscle . This, in combination with deconditioning, will lead to changes in skeletal muscle favouring anaerobic metabolism, which can be detected as a reduced lactate threshold on exercise testing. The muscle metaboreflex, designed to divert blood flow to exercising muscle, will be active in all exercising muscle groups, leading to an increase in sympathetic nervous system activity, a rise in blood pressure and systemic vasoconstriction. This may initially be a beneficial compensatory mechanism, assisting with the physiological response to exercise . In a chronic disease state, such excessive stimulation of these reflexes may cause persistent sympathetic overactivity, with an increase in cardiac afterload putting extra stress on the failing heart and vasoconstriction leading to a reduction in peripheral blood flow: thus a protective mechanism aimed at preserving

oxygen supply to muscle becomes a deleterious one. This "muscle hypothesis" was illustrated by Piepoli *et al* (figure 1.2) . Muscle training appears to attenuate the metaboreflex in heart failure, perhaps interrupting the cycle of decline: this possibly goes some way to explaining the benefits of exercise rehabilitation programmes.

It would be physiologically interesting to study the metaboreflex in other diseases characterised by skeletal muscle dysfunction, but is it of relevance? One could argue that measured metaboreflex activity is simply a reflection of skeletal muscle fitness, and that it is bound to be altered in such conditions. It is telling that the forearm metaboreflex is attenuated in the very well trained muscle of rock climbers .

It is worth mentioning the muscle metaboreflex in COPD in the context of some more recent research. It is thought that metaboreceptors exist in diaphragmatic muscle, and that diaphragm fatigue causes muscle metaboreflex activation . Reductions in calf blood flow can be seen with fatiguing inspiratory muscle work, reflecting metaboreflex mediated vasoconstriction to non-exercising muscle . It has since been shown that expiratory muscle fatigue causes a reduction in leg exercise capacity, presumably through metaboreflex mediated vasoconstriction to exercising muscle , and that inspiratory muscle training attenuates the human respiratory metaboreflex . When patients with COPD exercise, work of breathing is significantly increased for a number of reasons, and respiratory muscles account for an increased fraction of oxygen consumption. It is possible that increased activation of the respiratory muscle metaboreflex causes a reduction in blood flow to exercising limbs, limiting oxygen delivery. This may partially explain why a plateau in limb blood flow may be seen during incremental exercise , and the observation that unloading the respiratory muscles

with Heliox or non-invasive ventilation improves exercise performance. Here may lie the "missing link" between exercise dyspnoea and limb fatigue in patients with chronic disease.

8.7 Further work

Further work should be done on the muscle metaboreflex in patients with COPD, with attention to the methodological issues described in chapter 7. A large scale study using a similar protocol to the one used here would help answer the question of whether the metaboreflex is of as much importance in COPD as it appears to be in chronic heart failure. Stratification should be done according to MRC dyspnoea score rather than FEV_1, and it would be possible to select out a subset of patients who are not "ventilatory limited" to study further. It would also be interesting to see if an exercise-based pulmonary rehabilitation programme has any effect on metaboreflex activity.

Study of the muscle metaboreflex could also be applied to other chronic diseases. One would suspect that the main problem with studying the metaboreflex in patients with COPD is that most patients seem to be limited by pulmonary mechanics. It would be of interest to study the muscle metaboreflex in patients with idiopathic pulmonary hypertension. It is well recognised that minute ventilation is greater for a given workload in patients with idiopathic pulmonary hypertension than in healthy control subjects, and that there is also an increase in the VE/VCO_2 ratio during exercise . It is not possible to explain this through ventilation/ perfusion mismatch alone, so it is possible that a change in muscle fibre type, with a shift towards anaerobic metabolism and a consequent increase in muscle metaboreflex activity plays an important role.

Patients with type II diabetes mellitus have also been shown to demonstrate a shift in muscle fibre type from type I (oxidative) to type II (glycolytic), favouring anaerobic metabolism . Sympathetic overactivity in the metabolic syndrome and type II diabetes mellitus is well recognized and the possibility that the muscle metaboreflex contributes to this phenomenon may also merit study.

Another group of patients that would be worth studying is those with chronic renal failure who are on haemodialysis. Patients undergoing haemodialysis have rapid shifts in acid-base balance during dialysis. Given that an intramuscular acidosis is thought to be important in activation of the metaboreflex , it would be interesting to measure muscle metaboreflex activity before and after haemodialysis.

Work is ongoing in our department looking at the effects of acetazolamide supplementation on the metaboreflex. Acetazolamide is known to stimulate breathing by creating a mild metabolic acidosis , and has been shown to improve resting p_aO_2 and p_aCO_2 in patients with COPD . Acetazolamide also reduces the ability of skeletal muscle to exercise in a hypoxic environment , and this study is aimed at assessing whether this is due to an increase in metaboreflex activity.

The benefits of creatine supplementation in COPD are unclear. The study of Fuld *et al* suggested that creatine loading and supplementation during a pulmonary rehabilitation are of benefit. Work by Gosselink *et al* contradicts these findings. Perhaps further work should target patients who demonstrate impairment of peripheral muscle dysfunction. It would also be interesting to study whether creatine supplementation is

of benefit following an exacerbation of COPD, during non-invasive ventilation or during weaning from invasive ventilation in an intensive care unit.

The potential of pulse transit time in assessment of the muscle metaboreflex also merits further study. Although it is clear that metaboreflex activation has an effect on pulse transit time, it is less clear exactly what pulse transit time represents. Work could perhaps be carried out looking at the relationship between pulse transit time and muscle sympathetic nerve activity.

8.8   Concluding remarks

In conclusion, it is possible to measure muscle metaboreflex activity in patients with chronic obstructive pulmonary disease, but we could not find any difference in activity between patients with moderate and severe disease. Creatine loading did not alter forearm muscle strength, endurance or muscle metaboreflex activity in this small group of patients, although there was a modest increase in peak inspiratory and expiratory mouth pressures.

Muscle metaboreflex activity appears to be normal in patients with diabetic autonomic neuropathy, suggesting preservation of the afferent and efferent pathways of this reflex in this patient group. In addition, pulse transit time shows promise in the evaluation of the muscle metaboreflex, and this merits further study.

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