

Exploration and Modification of the  
Skeletal Muscle Metabolic Response to  
Exercise in Chronic Obstructive  
Pulmonary Disease

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by  
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## Declaration

The work in this thesis has been done mainly during the period of registration. No part of this work has been submitted in previous application for a higher degree. I have performed all the work towards this thesis except where assistance has been acknowledged. Assistance during exercise tests has been provided by Kamal Flora, Linzy Houchen and Carolyn Sandland and during the muscle biopsy procedures by Rachael Evans and Sarah Deacon.

I give consent for this thesis to be made available for consultation, photocopying and use through other libraries either directly or via the British Lending Library.

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## Chapter One: General Introduction

### 1.1.1 Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) is a common chronic respiratory condition, affecting over 900,000 people in the United Kingdom alone. It is characterised by slowly progressive airflow limitation that does not change markedly over several months, leading to symptoms of breathlessness, wheeze, cough and sputum production [Celli BR *et al*, 2004b]. The diagnosis requires spirometric evidence of airflow obstruction, defined as  $FEV_1 < 80\%$  predicted and  $FEV_1/FVC$  ratio  $< 70\%$ , that does not fully reverse with treatment [British Thoracic Society, 1997; National Collaborating Centre for Chronic Conditions, 2004]. Tobacco smoke is a causal factor in over 90% of cases, and sufferers commonly present in middle age (50 to 80 years).

Originally, COPD was described as two separate disease pathologies [CIBA Guest Symposium, 1959]. Chronic Bronchitis is characterised by small airways disease (obstructive bronchitis) and defined by yearly recurrent periods of sputum expectoration. Emphysema describes a pathological condition consisting of air space destruction, loss of elastic recoil of the lung parenchyma and progressive hyperinflation of the lungs. Nowadays it is clear that patients lie on a spectrum between these two entities, the relative contributions varying between individuals. In addition to airway obstruction, structural changes to the lung parenchyma result in reduced diffusion capacity and impaired gas exchange. It is now apparent that an abnormal inflammatory response within the lung also plays a role in the pathogenesis.

Severity of COPD has traditionally been determined by the degree of lung function impairment and this forms the basis of current Gold criteria [Global Initiative for Chronic

Obstructive Lung Disease, 2007], which classifies COPD as stage 0 (suggestive symptoms but normal FEV<sub>1</sub>), stage I (FEV<sub>1</sub>> 80% predicted), stage II (FEV<sub>1</sub> 50-79%), stage III (FEV<sub>1</sub> 30-49%), and stage IV (FEV<sub>1</sub>< 30%). However, there is increasing evidence that more advanced disease is characterised by significant systemic manifestations such as weight loss, skeletal muscle dysfunction, anaemia and osteoporosis, which have an important impact on morbidity and mortality independent of lung function [Agusti AG, 2005]. It is now recognised that these factors need to be considered in addition to lung impairment when assessing the impact of COPD on sufferers, and new indices that take these factors into account such as the BODE index [Celli BR *et al*, 2004a] are increasingly being used in clinical practice.

### **1.1.2 The burden of COPD**

As the early stages of disease often go unnoticed, many patients with COPD present late when they have already developed severe lung function impairment [Calverley PMA *et al*, 2000]. Consequently, COPD has become a frequent cause of significant disability. The WHO estimates that the burden of COPD will continue to rise in line with worldwide trends in tobacco consumption to become the third leading cause of disability by 2020 [Murray CJ *et al*, 1997]. In addition, many people die prematurely from this disease, which currently accounts for 27,000 deaths each year.

In the United Kingdom, COPD is already an important cause of GP consultation and emergency hospital admission [Hubbard R, 2006]. An ageing population will only lead to an increase in its prevalence with a consequent increase in medical costs. The health consequences and socio-economic impact of COPD are therefore huge and it is easy to understand why COPD represents such a major and growing problem for health care services.

### **1.1.3 Exercise intolerance in COPD**

Exercise intolerance is a frequent complaint for patients suffering with COPD and they struggle to carry out many activities of daily living [American Thoracic Society, 1999]. This may lead to social isolation and depression as patients become increasingly disabled by their impaired functional performance.

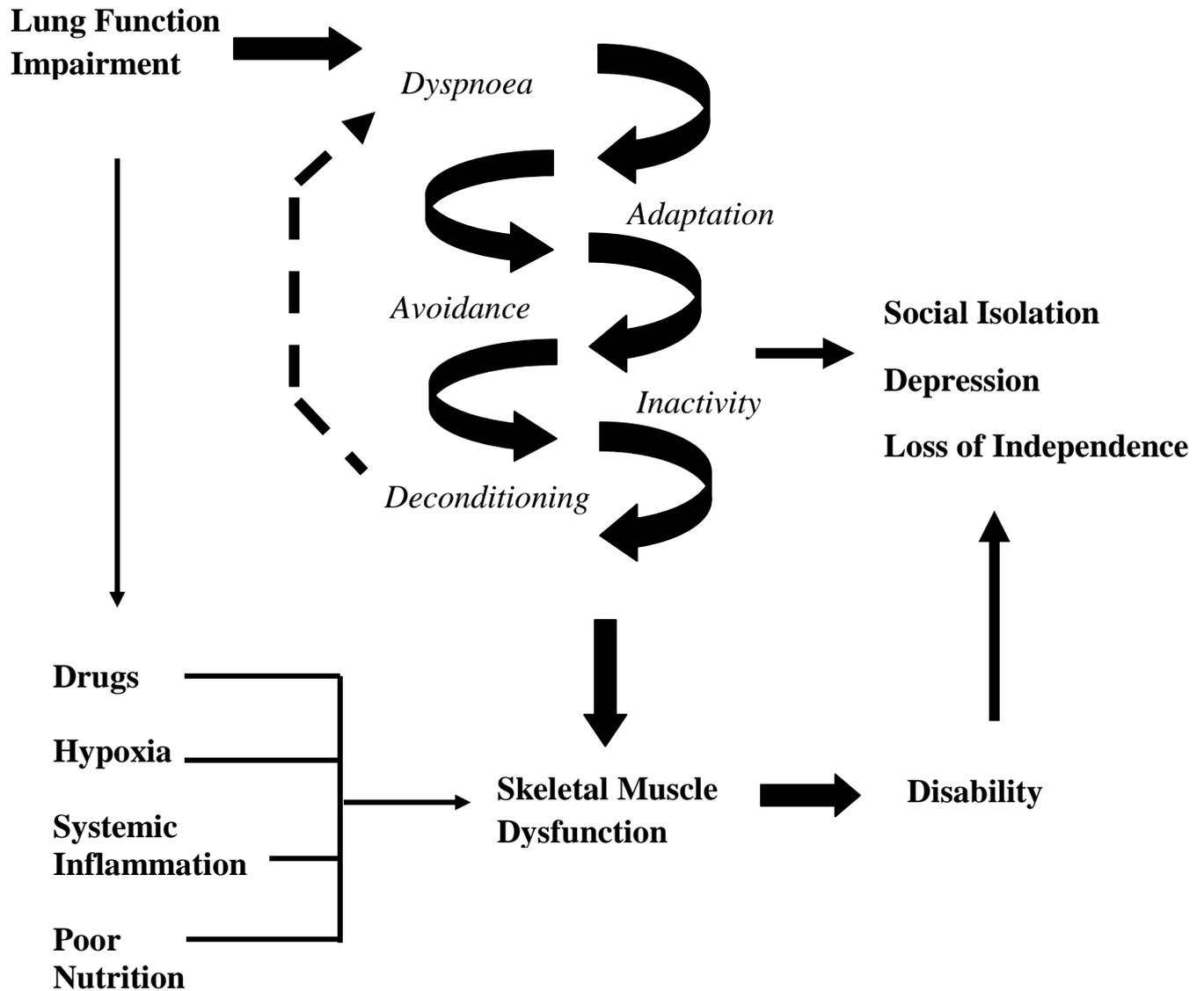
It is evident that limitations in pulmonary ventilation are not sufficient to explain the degree of exercise intolerance for many patients with COPD. The relationship between lung function decline and the development of exercise limitation is not straightforward, and severity of lung impairment is a poor predictor of exercise capacity and disability [Killian KJ *et al*, 1992; Levy RD *et al*, 1993; Low DE *et al*, 1992; Morgan AD *et al*, 1983]. This implies that other factors must contribute to exercise limitation, which appears to be the result of a complex interaction of behavioural and lifestyle factors [**Figure 1.1**].

The reduced functional status and low level of physical activity resulting from exercise intolerance predict poor quality of life and mortality [Garcia-Aymerich J *et al*, 2006]. Improvement of exercise performance is therefore an important therapeutic goal in COPD.

### **1.1.4 Skeletal muscle function in COPD**

There is growing realisation that, in a considerable proportion of patients with COPD, skeletal muscle function is impaired compared to healthy subjects of a similar age [American Thoracic Society, 1999]. The significance of muscle dysfunction as an independent factor limiting exercise performance and contributing to poor quality of life

Figure 1.1: Schema for the long term development of disability in lung disease.



Whilst the initiating event is lung function impairment, long term disability is the result of a more complex chain of events that may involve extrapulmonary disease manifestations, complications of treatment and long term changes to physical activity. This has been termed the “spiral of disability” in chronic lung disease.

and reduced survival is now well established [American Thoracic Society, 1999].

However, there is likely to be a spectrum of disease that overlaps with the frail deconditioned elderly, and detection of pathological state within the muscles can be difficult.

There is compelling evidence that the load imposed on the respiratory system by the skeletal muscles is abnormally increased in COPD patients [Debigare R *et al*, 2008]. This load is represented by the metabolic demands of exercising muscles, which are high due to metabolic inefficiency. This inefficiency also results in failure of energy delivery to meet demand in exercising muscle and may be important in exercise limitation. A comprehensive understanding of the mechanisms behind altered muscle energy delivery in COPD is therefore important if the decline in functional status is to be prevented.

Although reduced capacity of the respiratory system is clearly important, this is difficult to treat and largely irreversible. However, targeting skeletal muscle impairment and reducing the respiratory load during exercise by improving metabolic efficiency can offer valuable therapeutic prospects in this population. This is well illustrated by pulmonary rehabilitation programmes, which achieve clear benefits in increasing exercise capacity and reducing disability despite having no effect on lung function [Casaburi R *et al*, 1997; Vogiatzis I *et al*, 1999].

Measurement of muscle energy metabolism during exercise might be useful to evaluate interventions that specifically target the muscles and identify those patients likely to benefit from such interventions. However, measuring muscle metabolic events during exercise and training is difficult. Therefore the development of more easily measurable outcomes of energy delivery is of research interest and could be clinically relevant.

## **1.2 Thesis aims**

The overall aim of this Thesis was to develop further understanding of skeletal muscle energy delivery during exercise in patients with COPD. The work in this Thesis involved physiological studies, and three specific aims were addressed:

1. To validate plasma ammonia as a measure of energy delivery during exercise in COPD.
2. To explore energy metabolism during cycling and walking exercise by characterising the plasma ammonia response.
3. To determine the effect of physical and pharmacological interventions targeting skeletal muscle on energy delivery during exercise.

The hypothesis tested in this Thesis is that plasma ammonia changes will reflect adenine nucleotide metabolism during exercise in COPD and be sensitive to interventions targeting the skeletal muscles.

Chapters 2 and 3 provide a detailed review of the existing literature. In **Chapter 2**, the normal skeletal muscle structure and metabolic response to exercise is discussed. **Chapter 3** provides an overview of what is currently known about impaired skeletal muscle function and the abnormal metabolic response to exercise in patients with COPD. In **Chapter 4** the methods used in the Thesis studies and the ammonia analysis techniques are described in detail.

A number of techniques have been developed in recent years, which increase our understanding of peripheral muscle function in COPD. However, techniques currently available which measure metabolic capacity in skeletal muscles during exercise are not suitable for use in clinical practice in patients with COPD. New markers of the metabolic

response are therefore needed. Energy is provided by adenine nucleotide metabolism in skeletal muscle. Plasma ammonia accumulates when adenine nucleotide re-synthesis fails to meet the energy demands of exercising muscle. Therefore, plasma ammonia may be a useful measure of energy delivery and metabolic efficiency of the muscles. Blood lactate accumulation is a measure of muscle anaerobic energy production and measured alongside ammonia could provide further insight into the mechanisms of energy delivery during exercise in COPD. In this Thesis, therefore, plasma ammonia response in relation to lactate response to exercise has been considered. In **Chapter 5**, the first study of this Thesis explores the plasma ammonia response to cycle exercise in COPD patients and healthy subjects. In this study, plasma ammonia has been assessed as a marker of adenine nucleotide metabolism within the muscles and compared to muscle biopsy adenine nucleotide changes. The ammonia response to exercise in COPD is explored further in **Chapter 6**, where cycling and walking are compared.

The second part of this Thesis investigates adaptation of the metabolic response to exercise following interventions specifically targeting the skeletal muscles in COPD subjects. In these studies, plasma ammonia response is measured as a marker of skeletal muscle energy metabolism in order to gain insight into metabolic changes within the muscle in relation to improvements gained in exercise performance.

The study in **Chapter 7** uses a pharmacological agent specifically targeting skeletal muscle energy metabolism. This explores a novel concept of using pharmacological therapy to improve metabolic efficiency of the muscles in order to improve exercise performance. Pulmonary rehabilitation is currently used extensively in clinical practice to improve muscle function in patients with COPD. The final study in this Thesis described in **Chapter 8** examines adaptation of the plasma ammonia response to exercise training

through a pulmonary rehabilitation programme. **Chapter 9** comprises a general discussion and provides suggestions for future studies.

Study design and protocols have all been developed as part of this Thesis. I have performed all clinical assessments unless otherwise stated. I performed the laboratory work described in the main text, which includes the ammonia and lactate analysis. Analysis of muscle biopsy samples was performed by another centre. This has been acknowledged in the text and the procedure is described in Appendix 2. Publications arising from the work in this Thesis are described in Appendix 4.

## Chapter Two: Normal skeletal muscle morphology and metabolic function

### **2.1 Introduction**

The primary aim of this Thesis is to explore the plasma ammonia response to exercise and through this gain further insight into energy metabolism within the skeletal muscles of COPD subjects. In order to understand how such changes might lead to impaired exercise capacity and to put this into a clinical context, knowledge of normal skeletal muscle structure and metabolic function is essential. This chapter describes muscle function in healthy subjects. It includes a detailed literature review of the different pathways that contribute to re-synthesis of adenosine 5-triphosphate (ATP) during exercise, which is relevant to some of the later work performed as part of this Thesis. The plasma ammonia response during exercise and its relationship with muscle adenine nucleotide changes in healthy subjects has been well described and is also reviewed in detail in this chapter. The concept of ‘metabolic stress’, a fall in the energy potential of muscle due to adenine nucleotide depletion, during exercise is also discussed. Measurement of blood lactate in exercise is commonly used by athletes to prescribe training, and by clinicians in the management of various medical conditions. The lactate response to exercise can provide information on the aerobic capacity of skeletal muscle and is described for healthy subjects in this chapter. In the subsequent chapter, changes found in the skeletal muscles of patients with COPD are discussed.

## **2.2 Normal muscle morphology**

*Detailed review from Maughan [Maughan R et al, 1997] and Gorselink [Gorselink M et al, 2003].*

### **2.2.1 Muscle Ultra-structure**

The skeletal muscles link parts of the skeleton to which they are attached by tendons. They maintain posture and allow movement to take place, and are under direct conscious control.

The muscles consist of individual long multi-nucleated fibres located in bundles. One nerve innervates a number of fibres, called a motor unit, which contract simultaneously. A network of small blood vessels runs between the fibres to supply nutrients and oxygen and drain end-products of metabolism. Each fibre consists of several hundred to thousands of myofibrils, which together form the contractile machinery. The two contractile protein filaments myosin (which constitutes the thick filament) and actin (which constitutes the thin filament) are arranged in a regular array. Cytoskeletal proteins anchor the thick filament (at the z-line) and keep the contractile machinery in proper spatial arrangement to allow the thin filaments to slide between the thick filaments [Huxley A.F., 1957], leading to muscle contraction. The sliding movement between actin and myosin is achieved through cross-bridge cycling. When an action potential is generated, calcium released from the sarcoplasmic reticulum into the sarcoplasm binds to myosin and initiates cross-bridge activation. These cross-bridges then generate the tension associated with contraction, which is driven by adenosine 5'-triphosphate (ATP) hydrolysis to adenosine 5'-diphosphate (ADP) and inorganic phosphate ( $P_i$ ) [Lynn RW et al, 1971].

In order to cope with a broad spectrum of functions, muscles consist of a mixture of different types of fibres, which can be grouped according to their physiological and

metabolic characteristics [Table 2.1]. Fibre type profile within a muscle is important in determining metabolic capacity. Type I fibres are characterised by slow contraction velocity and high oxidative capacity, making them relatively resistant to fatigue. Type IIb fibres are characterised by fast contraction velocity and low oxidative capacity making them more prone to fatigue. Type IIa fibres are intermediate. Muscle fibre composition varies widely between different muscle groups and different individuals. This is genetically determined but can be altered by exercise training, ageing or disease.

### **2.2.2 Muscle energy metabolism**

Energy for muscle contraction comes from ATP hydrolysis in a reaction catalysed by adenylate kinase:



This reaction is regulated by both ATP concentration and phosphorylation status, determined by the ATP:ADP ratio, within the muscle cell [Atkinson DE, 1968]. A rise in ADP and adenosine 5'-monosphate (AMP) concentrations and a fall in the ATP:ADP ratio activates ATP re-synthesis.

As energy stores within muscle cells are small, ATP must be continually replenished to allow contraction to continue past the first few seconds. Three pathways for ATP re-synthesis exist and become activated at the onset of exercise [Figure 2.1]. One involves oxygen (known as mitochondrial, oxidative or aerobic energy metabolism) and two do not require oxygen (non-oxidative or anaerobic energy metabolism).

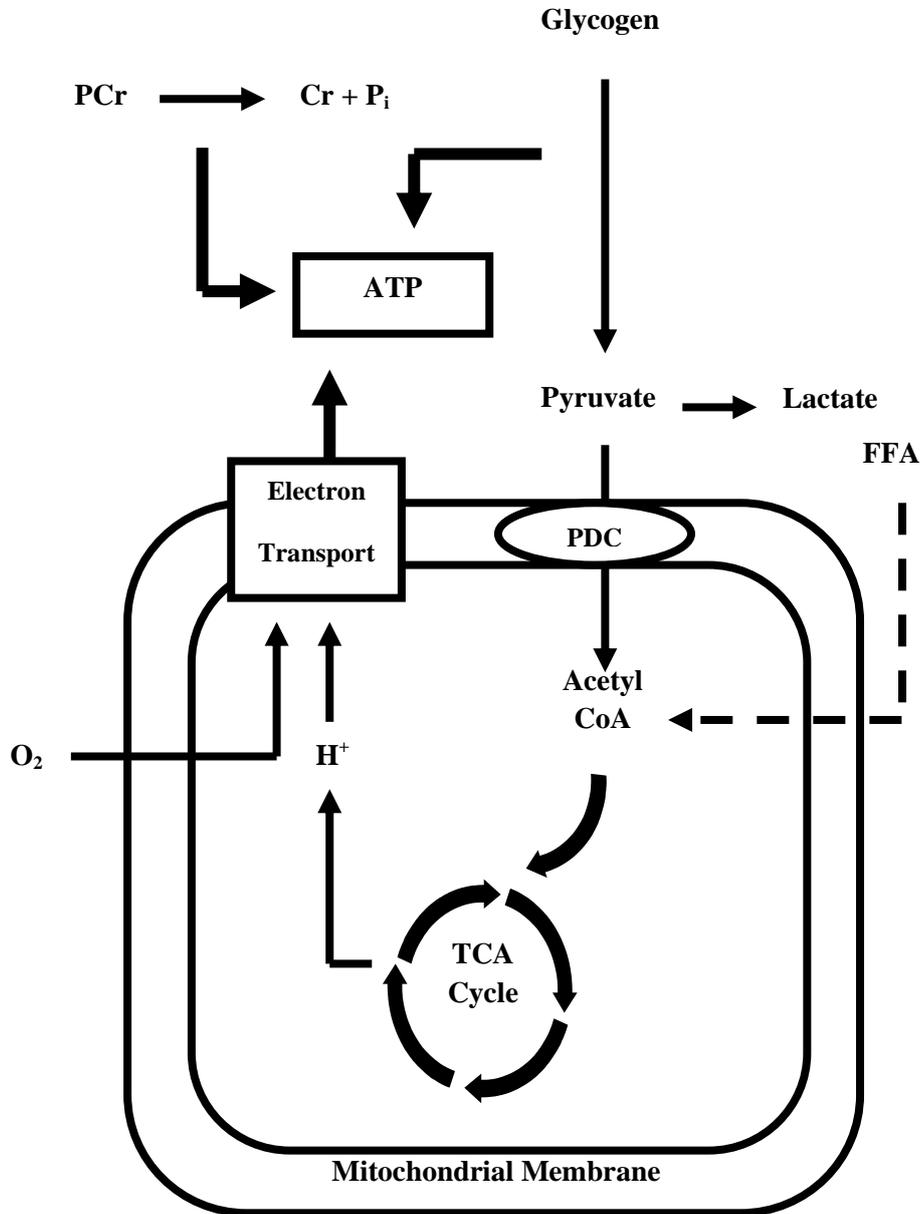
A high-energy phosphate pool in the form of phosphocreatine (PCr) exists in the sarcoplasm, which can rapidly supply ATP anaerobically at a high rate for rapid forceful actions, although the limited stores are quickly depleted.

Table 2.1: Characteristics of human fibre types

	<b>Type I</b>	<b>Type IIa</b>	<b>Type IIb</b>
<b>Structural</b>			
Fibre diameter	Small	Moderate	Large
Capillary density	High	Medium	Low
<b>Biochemical</b>			
Metabolism	Oxidative	Oxidative, glycolytic	Glycolytic
Mitochondrial Density	High	Medium	Low
Fatigability	Low	Low	High
<b>Functional</b>			
Speed	Slow	Fast	Fast
Power	Low	High	High
Endurance	High	Medium	Low

Adapted from Maughan R., Gleeson M. and Greenhaff P.L. *The Biochemistry of Exercise and Training*, Oxford University Press, 1997.

Figure 2.1: Cellular energy metabolism



This is a schematic diagram showing the pathways providing energy for ATP resynthesis. See text for description.

Abbreviations: PCr = Phosphocreatine; Cr = Creatine; P<sub>i</sub> = Inorganic Phosphate; ATP = Adenosine Triphosphate; PDC = Pyruvate Dehydrogenase Complex; FFA = Free Fatty Acids; TCA = Tricarboxylic Acid.

PCr is broken down to creatine (Cr) and inorganic phosphate (Pi) and the phosphate transferred to ADP to form ATP. PCr stores must later be replenished which requires energy.

The conversion of blood glucose or muscle glycogen (the intracellular glucose store) into pyruvate within the sarcoplasm is common for two pathways of ATP re-synthesis and does not require oxygen. This reaction generates ATP but requires removal of pyruvate to proceed. Pyruvate can either enter the mitochondrion to undergo oxidative conversion, or be converted to lactate anaerobically within the cytoplasm. Lactate is then released into the bloodstream. This latter process is called the glycolytic process and produces relatively small amounts of ATP by substrate-level phosphorylation (direct transfer of a phosphate group to ADP from a reactive intermediate). It becomes active instantaneously at the onset of exercise and rate of lactate formation is primarily dependant on relative exercise intensity. This process has a larger capacity for ATP re-synthesis than PCr but occurs at a slower rate, therefore producing less power.

Alternatively, pyruvate can enter the tricarboxylic acid (TCA) in the mitochondria and undergo oxidative phosphorylation. The first step of this process is transport of pyruvate across the mitochondrial membrane and conversion of pyruvate to acetyl CoA, which is the major substrate for the TCA cycle, by a mitochondrial enzyme called pyruvate dehydrogenase complex (PDC). Acetyl CoA is converted through the TCA cycle to oxygen and hydrogen ions with the generation of ATP. The hydrogen ions then enter the electron transport chain, which requires oxygen, allowing oxidative phosphorylation with re-synthesis of ATP. Aerobic metabolism is slower to produce ATP after the onset of exercise than anaerobic mechanisms and ATP production increases exponentially over the first 30-60 seconds of exercise. However, aerobic processes are capable of producing

substantial amounts of ATP (1 molecule of glucose can generate up to 36 molecules of ATP). Blood borne fatty acids are also important substrates for oxidative conversion by  $\beta$ -oxidation to acetyl CoA and generate more ATP per molecule than glucose. Protein (as amino acids) is another substrate for the TCA cycle.

### **2.3 Energy supply during exercise**

*For detailed review see [Maughan R, 1997] and [Gorselink M, 2003].*

The relative contributions that different mechanisms of ATP re-synthesis make to energy production during exercise depend upon exercise intensity and duration, physical conditioning, diet and health [Gorselink M, 2003]. For example, energy for sprint exercise is almost totally provided anaerobically, whilst in marathon runners 99% of muscle energy requirements are provided aerobically. These two scenarios, high intensity exercise (e.g. sprint) and submaximal endurance exercise (e.g. marathon) are considered below:

*High intensity exercise:* In short-burst high intensity exercise PCr degradation can supply energy for around 6secs. The glycolytic anaerobic pathway reaches its maximum rate of ATP re-synthesis at around this time and can maintain maximal intensity exercise for a further 20-30 seconds. However, lactate accumulation and resulting acidosis can lead to reduced muscle cell performance. Therefore as high intensity exercise continues beyond this, aerobic ATP metabolism, which is activated more slowly, becomes more important and makes an increasing contribution to energy production allowing exercise to continue. Without this progressive increase in aerobic metabolism, and thereby reduction of anaerobic energy delivery, onset of muscle fatigue would be markedly accelerated.

*Submaximal endurance exercise:* In low to moderate intensity activity a metabolic steady-state is reached where ATP demand is met by supply and exercise can be sustained

for long periods. Aerobic mechanisms predominate, although in the first 1-2minutes of exercise anaerobic mechanisms will provide ATP requirements whilst the aerobic system is activated. At low intensities glucose and fatty acid are used equally as substrate, as intensity increases carbohydrates become more important.

## **2.4 Blood lactate accumulation during exercise**

### **2.4.1 Pattern of lactate accumulation during exercise**

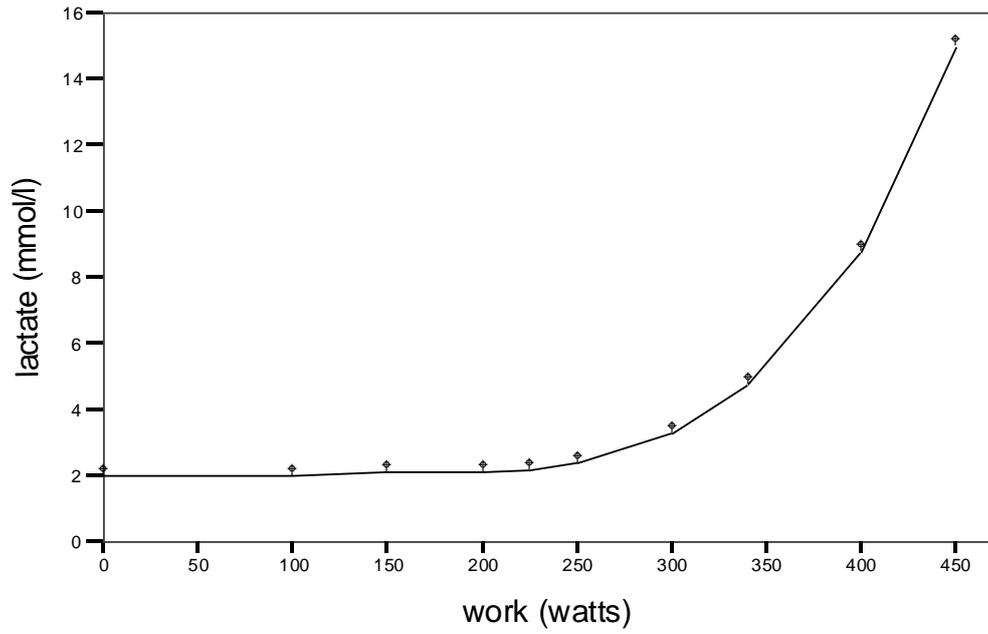
Ryffel first reported that blood lactate increased after exercise in humans a century ago, and subsequently Hill related this lactate rise to activity of an anaerobic component in the body's metabolic processes [Hill AV *et al*, 1924; Loat CE *et al*, 1993]. Since then the lactate response to exercise has been well characterised by numerous authors [Loat CE, 1993]. Although lactate produced by muscle during exercise was traditionally thought to be due to a shortfall in oxygen supply, this concept has been superseded and the current consensus is that lactate accumulation reflects the imbalance between ATP demand and capacity for oxidative ATP replenishment. The activity of PDC controls the incorporation of pyruvate into the TCA cycle (oxidative ATP metabolism) and hence also determines lactate accumulation. If the rate of pyruvate production exceeds the rate of PDC-mediated uptake into the mitochondria, pyruvate enters the glycolytic pathway (anaerobic ATP re-synthesis) and lactate accumulation will occur. Therefore at lower workrates, metabolism is predominantly aerobic and can continue for a prolonged time in physiological steady state. Above a certain workrate aerobic metabolism is increasingly supplemented by anaerobic ATP regeneration with accumulation of lactate in contracting muscles and circulating blood.

Resting blood lactate lies around 0.5mmol/l [Cooper CB *et al*, 2001b; Graham TE, 1984]. During incremental exercise, at low work intensities lactate concentration is steady

or increases slightly as lactate production and disposal remain balanced. As workrate increases, there is a threshold workrate above which lactate accumulates significantly in muscle and blood. The threshold is related to percentage of predicted maximum oxygen consumption ( $\text{VO}_2\text{max}$ ) and typically occurs when around 55-70%  $\text{VO}_2\text{max}$  is achieved in healthy subjects, but is recognised to vary between individuals [Cooper CB, 2001b]. For example, in normal sedentary subjects this threshold will be around 50% predicted  $\text{VO}_2\text{max}$ , but will be higher in aerobically fit individuals and with physical training can reach over 80% predicted  $\text{VO}_2\text{max}$ . The  $\text{VO}_2$  at which significant increase in lactate accumulation is seen has been termed the 'lactate threshold' and is preferably identified as the 'break-point' on a blood lactate accumulation curve [Figure 2.2]. Beyond this threshold, lactate concentration in blood and skeletal muscle continues to rise at a progressively steeper rate in an exponential relationship with  $\text{VO}_2$ . Lactate concentration in blood continues to rise for approximately 2 minutes after the termination of exercise [Babij P *et al*, 1983; Buono MJ *et al*, 1984] and this has been related to the delay in efflux across muscle membranes. The magnitude of lactate accumulation with exercise is governed by several factors including motivation and training. Failure of a lactate response to exercise may be attributed to lack of motivation, non-metabolic cause of exercise limitation, or low tolerance of muscle fatigue.

Blood lactate concentration primarily reflects the balance between rate of production, efflux from muscle to blood and rate of clearance from blood by inactive muscle and other, chiefly visceral, organs (liver, heart, brain) [Cooper CB, 2001b].

Figure 2.2: Blood lactate accumulation during incremental cycling exercise in healthy subjects



Lactate breakpoint with incremental cycle exercise (illustration only)

Although blood lactate does not have a 1:1 relationship with muscle lactate concentration, lactate concentration in blood (arterial, venous or capillary) does give an indirect and reproducible indication of aerobic capacity of exercising muscles and can be used to assess changes in aerobic fitness and as a guide to training intensity in endurance and sprint athletes.

The term ‘anaerobic threshold’ is sometimes used to describe the workrate at which transition between aerobic and anaerobic metabolism occurs [Wasserman K *et al*, 1973]. However, as discussed previously, anaerobic and aerobic metabolism contribute to ATP production in varying degrees throughout exercise without a sudden switch between the two mechanisms, making this term inaccurate [Cooper CB, 2001b]. When the threshold is determined by serial lactate measurements it should be termed ‘lactate threshold’. It can also be determined by gas exchange measurements (commonly by examining the plot of  $VCO_2$  and  $VO_2$ ), in which case it is termed ‘gas exchange threshold’. Lactate buffering with bicarbonate generates carbon dioxide ( $CO_2$ ) such that  $VCO_2$  increases as lactate increases out of proportion to aerobic  $VCO_2$  production and this can be detected on the plot as a breakpoint [Wasserman K *et al*, 1986]. Studies that have examined both methods of threshold determination confirm the relationship between the two measurements [Neary PJ *et al*, 1985].

#### **2.4.2 Energy production at the onset of exercise**

As mentioned previously, lactate accumulation is likely to be determined by the balance of pyruvate production and pyruvate oxidation in the mitochondria. Pyruvate dehydrogenase complex (PDC) is a multi-enzyme complex situated on the mitochondrial inner membrane which regulates the entry of pyruvate into the mitochondrion and TCA cycle by catalysing the irreversible conversion of pyruvate to acetyl CoA [Greenhaff PL *et*

*al*, 1998]. PDC exists in an active and inactive form. Recent evidence suggests that the degree of activation of PDC is a critical predictor of the rate of oxidative metabolism during exercise and, in turn, the degree of non-oxidative metabolism and lactate accumulation [Greenhaff PL *et al*, 2004; Heigenhauser GJ *et al*, 1999]. Exercise itself results in maximal activation of PDC after a few minutes of exercise in healthy subjects.

At the onset of skeletal muscle contraction there is a marked increase in energy demand that must be matched by a rapid increase in ATP re-synthesis. It is widely accepted that in healthy subjects, oxidative ATP production does not increase instantaneously at the onset of exercise but follows an exponential time course. During this period the shortfall in ATP is met by anaerobic processes. Bangsbo showed during high intensity exercise that non-oxidative processes contribute 80% of ATP delivery in the first 30 seconds, 45% by 60-90 seconds and only 30% at 2 minutes [Bangsbo J *et al*, 1990]. These changes were paralleled by an increase in aerobic ATP re-synthesis. Once aerobic ATP re-synthesis is established a metabolic steady-state is attained (i.e. ATP supply and demand is matched).

This lag in aerobic metabolism was initially attributed to a finite rate of increase in oxygen delivery to the skeletal muscles in humans. However, a growing body of evidence indicates that this is not the case, and muscle blood flow and capillary diffusion do not limit oxygen utilisation at the onset of exercise [Grassi B *et al*, 1998a; Grassi B *et al*, 1998b; Greenhaff PL, 2003]. Rather, the inertia in aerobic ATP production appears to reside at the level of the PDC, and exercise-induced PDC activation during the minutes over the transition from rest to steady-state exercise results in an increase in the supply of mitochondrial acetyl CoA paralleled by an exponential rise in aerobic ATP generation [Timmons JA *et al*, 1997; Timmons JA *et al*, 1998a].

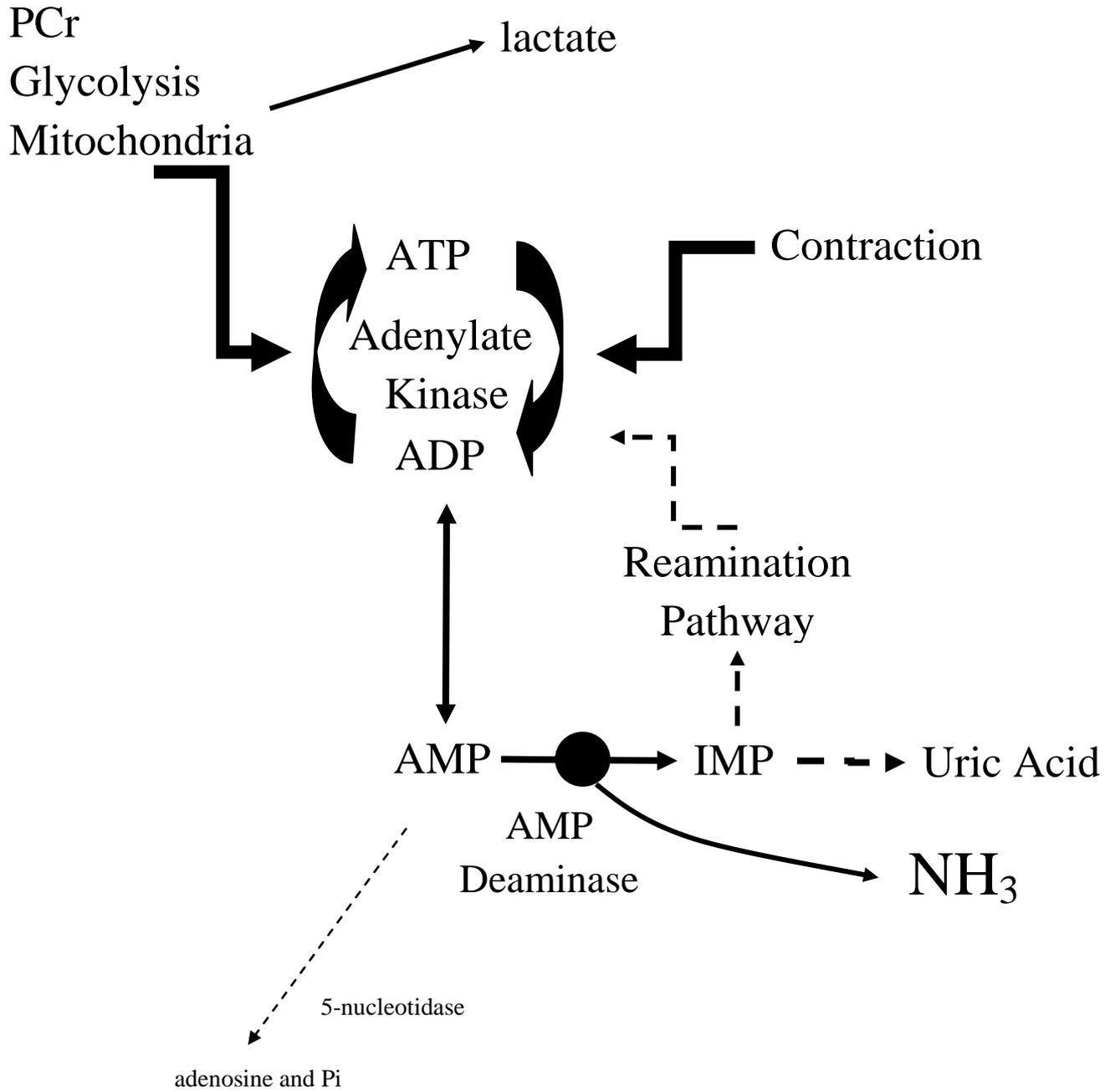
Further evidence comes from in vitro work on canine muscle and human muscle under partly ischemic conditions showing that pharmacological activation of PDC, using the PDC kinase inhibitor Dichloroacetate (DCA), and priming with acetyl CoA prior to exercise markedly reduces lactate accumulation and PCr degradation (markers of anaerobic metabolism) during subsequent intense exercise, particularly in the first 30-120 seconds [Grassi B *et al*, 2002; Greenhaff PL, 1998; Timmons JA *et al*, 1996a; Timmons JA *et al*, 1996b; Timmons JA *et al*, 1998b]. DCA reduced anaerobic ATP re-synthesis by 80% by 1 minute of contraction and 50% by 6 minutes and this was associated with reduced fatigue [Howlett RA *et al*, 1999]. This was directly linked to PDC activation and inherent lag in aerobic metabolism at onset of muscle contraction independent of oxygen delivery [Timmons JA, 1997]. Subsequent studies demonstrated that a lag in acetyl CoA provision occurs which mirrors the lag in PDC activation (called the ‘acetyl group deficit’) but that increased flux of acetyl CoA independent of PDC activation is not responsible for the increased oxidative phosphorylation [Constantin-Teodosiu D *et al*, 1999].

## **2.5 Blood ammonia accumulation during exercise**

### **2.5.1 Adenine nucleotide loss during exercise**

When oxidative and non-oxidative mechanisms of ATP re-synthesis are unable to meet the energy demands of exercising muscle the concentration of ATP will fall. This results in an increase in ADP and AMP (by ADP deamination). AMP can then be metabolised in two different pathways and the relative importance of these differs between fibre types [Maughan R, 1997]. AMP can either be deaminated by AMP deaminase to inosine 5-monophosphate (IMP) and ammonia (this constitutes the first step of the purine nucleotide cycle) or dephosphorylated by 5-nucleotidase to adenosine and Pi [Figure 2.3].

Figure 2.3: Adenine nucleotide metabolism during exercise in skeletal muscle



It is clear from the literature that in humans deamination predominates in type II fibres (which have fewer mitochondria and low oxidative capacity), whereas type I fibres (which are abundant in mitochondria and have high oxidative capacity) have a fourfold lower activation of deamination. AMP deaminase is inactive in resting muscle but becomes activated during high intensity exercise by a fall in energy charge [a measure of the muscles ability to do work and calculated as ratio of  $(ATP + 0.5ADP)/(ATP+ADP+AMP)$ ] or intracellular pH. A rise in AMP and ADP concentration also regulate activation.

The irreversible deamination of AMP leads to accumulation of IMP in exercising muscles and release of ammonia into the bloodstream. IMP is subsequently degraded to inosine, xanthine and uric acid [Sahlin K *et al*, 1989a; Sahlin K *et al*, 1989b] and reamination to adenine nucleotides only occurs over hours or days. By preventing excessive accumulation of ADP and AMP, this process increases the phosphorylation potential of the adenine nucleotide pool and maintains activation of adenylate kinase, thereby enabling contraction to continue in the short term. However, such a situation is not sustainable because of the resulting decline in ATP concentration and availability of adenine nucleotides for phosphorylation. Substantial metabolic stress is said to have occurred under conditions of muscle ATP depletion and IMP accumulation, and blood ammonia increase [Dudley GA *et al*, 1983; Dudley GA *et al*, 1985], and is seen only when high exercise intensities are reached in young healthy adults [Buono MJ, 1984; Graham TE *et al*, 1990].

### **2.5.2 Pattern of ammonia accumulation with exercise**

Resting plasma ammonia concentration in young healthy adults is 30-70 $\mu$ mol/l [Buono MJ, 1984; Graham TE *et al*, 1992; Mutch BJ *et al*, 1983] and will be influenced by factors such as diet (especially protein). Normal values for ammonia at maximum exercise

have not been well established. As with lactate, subject motivation and fitness are important, but a healthy subject typically achieves a maximum ammonia concentration of 70-120 $\mu$ mol/l [Graham TE, 1992]. Similarly, ammonia release during exercise is likely to be influenced by interacting factors although these are not yet fully elicited. Failure to elicit an ammonia rise could be due to lack of effort or non-metabolic cause of exercise limitation.

A number of studies have investigated blood ammonia response to exercise in young healthy subjects, and its relationship to muscle ammonia and IMP accumulation. An increase in blood ammonia has been reported after physical activity in humans using various exercise platforms such as arm work, leg extension, cycling, and treadmill walking [Babij P, 1983; Graham TE, 1990; Harris RT *et al*, 1989; MacLean DA *et al*, 1991]. However, the pattern of response varies with intensity [Babij P, 1983] and duration [Broberg S *et al*, 1989]. Broberg and Sahlin (1989) demonstrated that plasma accumulation was due to increased muscle efflux, and correlated with muscle ammonia accumulation [Broberg S, 1989]. Many other studies have also linked blood ammonia response with muscle IMP accumulation and AMP deaminase activity in exercising vastus lateralis [Dudley GA, 1985; Flanagan WF *et al*, 1986; Goodman MN *et al*, 1977; Graham TE *et al*, 1997; Katz A *et al*, 1986; Meyer RA *et al*, 1980]. Studies in patients with AMP deaminase deficiency demonstrate that these patients do not get an ammonia rise with exercise [Mutch BJ, 1983], supporting the contention that ammonia produced during exercise comes from muscle adenine nucleotide metabolism.

In incremental exercise to exhaustion, there is no change in plasma ammonia until work intensity is above 50%  $VO_{2max}$  [Graham TE, 1992]. Above this load threshold, plasma ammonia then rises with increasing exercise intensity in a curvilinear fashion to

reach a level 3 or 4 times baseline concentration by peak exercise, and continues to rise until 3-5 minutes into the recovery period [Buono MJ, 1984; Graham TE, 1990]. This is similar to the pattern of lactate response to incremental exercise, and a causal relationship appears to exist between lactate and ammonia response [Babij P, 1983; Broberg S, 1989]. Furthermore, the threshold exercise intensity for lactate and ammonia rise is strongly correlated [Buono MJ, 1984; Yuan Y *et al*, 2002].

### **2.5.3 Relationship between blood ammonia accumulation and adenine nucleotide loss**

Venous plasma ammonia has often been used as a non-invasive indication of muscle adenine nucleotide loss during exercise in young healthy subjects [Graham TE *et al*, 1987; Graham TE, 1990; Graham TE, 1992; MacLean DA, 1991]. Plasma ammonia concentration during exercise has consistently been shown to reflect changes in vastus lateralis (thigh) muscle ammonia concentration [Graham TE, 1987; Graham TE, 1990; Graham TE, 1997; Katz A, 1986] although plasma ammonia clearance in recovery is significantly increased. It is postulated that this is due to rapid lung clearance [Graham TE, 1990], uptake from inactive muscle [Bangsbo J *et al*, 1996] or possibly sweat, since clearance through the liver and kidney is much slower. Within the vastus lateralis muscle, the loss of adenine nucleotides is matched by the rise in IMP and ammonia concentrations. Arterial blood levels are also matched in a 1:1 stoichiometric relationship to muscle IMP levels and fall in ATP concentration following exhaustive leg-extension exercise [Graham TE, 1990]. Therefore, in healthy subjects plasma ammonia accumulation closely reflects energy delivery within the muscles, and specifically a mismatch between ATP supply and demand [Graham TE, 1997].

In prolonged steady-state exercise ammonia levels rise continuously and rate of rise is dependent on exercise intensity [Broberg S, 1989; Graham TE, 1984; Graham TE, 1987; Graham TE, 1990; Yuan Y, 2002]. No relationship is seen between lactate and ammonia response to exercise and catabolism of branched-chain amino acids may be an additional source of ammonia production under these conditions [Rennie MJ *et al*, 1981].

#### **2.5.4 Ammonia production and muscle fibre type**

The immediate source of blood ammonia during exercise appears to be the result of AMP deamination. Therefore, fundamental differences that exist in adenylate metabolism between type I and type II fibres, due to different levels of AMP deaminase activity, may explain some of the variability in blood ammonia response to exercise. AMP deaminase activity is more apparent in type II (fast twitch) fibres compared with type I (slow twitch) fibres in young healthy subjects [Dudley GA, 1983; Katz A, 1986]. Slow twitch fibres are recruited at low workloads and fast twitch fibres are recruited as exercise intensity increases. Therefore the pattern of plasma ammonia accumulation with low ammonia production at low workloads and exponential ammonia increase at higher workloads might reflect muscle fibre composition in healthy subjects. This concept is supported by work from Dudley and co-workers using incremental cycle exercise, which demonstrated an inverse relationship between ammonia rise and the proportion of Type I fibres of the vastus lateralis muscle [Dudley GA, 1983]. They concluded that the proportion of slow twitch fibres in exercising skeletal muscle is important in determining the magnitude of ammonia response.

### **2.5.5 Plasma ammonia response to training**

Trained subjects have lower plasma rise with both incremental and endurance exercise and this is a reflection of reduced ammonia production and release from active muscle [Graham TE, 1997]. This appears to be associated with delayed onset of fatigue and increased exercise capacity [Mutch BJ, 1983]. Following 8 weeks cycle endurance training the work intensity threshold for ammonia rise increases [Yuan Y, 2002], although this was not seen after a 1-year low-intensity training programme [Yuan Y *et al*, 2004]. Although the mechanisms of metabolic adaptation to training have not clearly been elicited, it is postulated that this reflects a reduction in AMP deaminase activity following training.

### **2.6 Exercise cessation and muscle fatigue**

There are many reasons why people terminate exercise. In healthy subjects, cessation due to ventilation limits or perception of symptoms such as breathlessness and fatigue often occur before contraction failure.

The development of contractile fatigue in exercising skeletal muscle is multifactorial and leads to a failure of muscle contraction. There is a clear association between adenine nucleotide loss and development of fatigue during both high intensity (maximal) exercise and prolonged submaximal exercise reflecting failure of energy delivery [Sahlin K *et al*, 1998] and depletion of substrate for ATP resynthesis. In such situations the muscles are under 'metabolic stress' and this may contribute to exercise limitation. Muscle lactate accumulation and intracellular acidosis is also important in the development of fatigue [Hultman E *et al*, 1991; Sahlin K, 1992]. Other contributing factors include muscle PCr degradation, and structural and metabolic disorganisation of contractile proteins.

The effect of blood lactate accumulation on the ventilatory response to exercise is twofold. Firstly lactate is buffered in the blood which requires an increase in  $V\dot{C}O_2$ . This puts additional stress on the ventilatory system and is accompanied by changes in ventilation. Eventually there is a metabolic acidosis causing direct stimulation of ventilation through central chemoreceptors.

Plasma ammonia accumulation has been implicated in development of fatigue through stimulation of ventilation and central nervous system [Banister EW *et al*, 1990; Mutch BJ, 1983]. However, this is unlikely to play a significant role under most circumstances since higher plasma ammonia concentrations than usually reached during exercise are required.

## **2.7 Effect of aging on muscle function**

Aging has significant effects on muscle size and function (sarcopenia) [Grimby G *et al*, 1983]. Strength (which is maximal at around 20-30 years) becomes 30% weaker by the 7<sup>th</sup> decade at up to 50% weaker by the ninth decade and this has been associated with disability and mortality [Doherty TJ, 2003; Dutta C *et al*, 1997]. Loss of strength is primarily due to reduced muscle mass associated with atrophy of type II fibres, whilst type I fibres remain relatively preserved [Rogers MA *et al*, 1993]. Oxidative capacity is also lower compared with young subjects [Conley KE *et al*, 2000]. However, muscles maintain the capacity to adapt in response to training [Fiatarone MA *et al*, 1990; Frontera WR *et al*, 1988; Jubrias SA *et al*, 2001; Meredith CN *et al*, 1989]. This suggests physical inactivity may play a role in the decline in muscle function seen with increasing age.

## **2.8 Summary**

In this chapter, normal muscle structure and metabolic function has been explained in order to put into context changes found in skeletal muscles of patients with COPD, which is described in the subsequent chapter. Both the lactate and ammonia responses to exercise have been extensively described in healthy subjects and together help provide an insight into skeletal muscle metabolic response to exercise. Specifically the relationship between plasma ammonia and adenine nucleotide depletion has been explained. Although lactate response to exercise in subjects with COPD is well documented, plasma ammonia response has not been described. However, evidence from studies in healthy subjects suggests that plasma ammonia may be a useful tool in increasing our understanding of the metabolic response in COPD patients.

## Chapter Three: Exercise limitation and impaired skeletal muscle function in COPD

### **3.1 Introduction**

Exercise intolerance is the inability to complete a required physical task adequately, and in the clinical context this means a task that normal subjects would find tolerable. Disability represents the loss of function resulting from this impairment. Exercise intolerance is the main factor limiting participation in activities of daily living in patients with COPD and is closely linked to disability. The symptoms that limit exercise in the majority of patients are dyspnoea and/or fatigue [Killian KJ, 1992].

It was initially thought that patients were limited solely by their lung impairment resulting in inability to increase ventilation adequately to meet the increased metabolic demands of exercise. Pulmonary limitations do exist and include mechanical disadvantage, inadequate gas exchange, expiratory flow limitation, reduced peak oxygen consumption and dynamic hyperinflation [O'Donnell D, 1994]. In these patients maximum voluntary ventilation is often reached or exceeded during exercise and either breathing discomfort or limits of the pulmonary system terminate exercise.

Reduction in the capacity of the respiratory system is clearly important in the development of dyspnoea during exercise. However, reduced lung function fails to account for the degree of exercise limitation in many patients with COPD, which implies that other contributing factors exist. These factors are highly inter-dependent [Antonucci R *et al*, 2003; Cooper CB, 2001a], occur in varying combinations in different patients and importantly cannot be predicted in individual patients from physiological variables, such as FEV<sub>1</sub>, gas transfer factor, cardiac ejection fraction and body mass index, at rest. There is

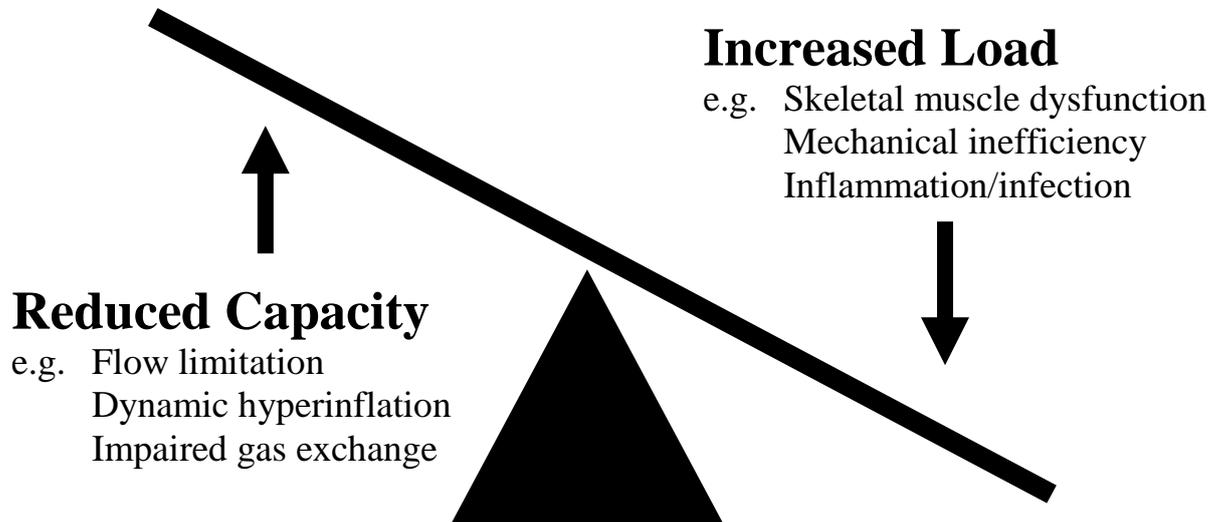
also growing evidence that the mechanisms of exercise intolerance depend on exercise performed. Leg fatigue appears to be particularly important in cycling exercise [Man WD *et al*, 2003].

Breathlessness during exertion can be considered as an imbalance between the capacity of the respiratory system and the load applied by the exercising muscles [**Figure 3.1**]. Traditional assessments of the impact of lung diseases have concentrated on the reduction in lung capacity but there is now compelling evidence that the load imposed on the respiratory system by the skeletal muscles is abnormally increased. This load is represented by the metabolic demands of exercising muscles, which are high due to impaired muscle metabolic efficiency in many patients with COPD.

Over recent years there has been increasing recognition that changes in skeletal muscle structure and function exist in patients with COPD and a number of studies have begun to investigate this both at rest and during exercise. Whilst exercise performance poorly correlates with measures of lung function, significant correlation is seen with leg muscle mass and cross-sectional area [Baarends EM *et al*, 1997]. Impaired skeletal muscle functional capacity is now recognised as an independent limiting factor to exercise performance in COPD patients [American Thoracic Society, 1999] and is likely to occur to some degree in a considerable proportion of the COPD population. However, its direct role in exercise limitation is less clear especially in day to day functional exercise.

The main focus of this chapter examines the evidence for impaired skeletal muscle function and, in particular, an abnormal metabolic response to exercise in patients with COPD. The known effects of training on exercise performance, and muscle performance are also described. The first section in this chapter briefly explains the pulmonary limitations to exercise in COPD.

Figure 3.1: Breathlessness during exercise in COPD



The activation of the respiratory system and the development of breathlessness will depend on the balance between the capacity of the respiratory system and the metabolic demand (load) of the exercising muscles. In COPD both sides of this balance may be affected by the disease process and its longer term consequences.

### **3.2 Physiological limitations to exercise**

Increased ventilatory demand due to exercise can be hard for patients with COPD to tolerate. They often require a higher level of ventilation for a given level of work, on top of increased work of breathing for a given level of ventilation. In most patients many factors can contribute either directly or indirectly and identifying one variable is often impossible. Impaired right ventricular function secondary to lung pathology in COPD can lead to reduced cardiovascular function, which may further limit exercise capacity. These limits to ventilation are particularly important as the ventilatory system can struggle to cope with the additional abnormally high metabolic demands of exercise caused by metabolic inefficiency of the skeletal muscles in this population.

#### **3.2.1 Ventilatory limitation**

Ventilatory limitation occurs when subjects approach ventilatory capacity and is arbitrarily set at 90% maximal voluntary ventilation (MVV) [Cooper CB, 2001b]. MVV can be measured directly or estimated from FEV<sub>1</sub> [Cooper CB, 2001b]. Limitation is not normally expected with exercise (normal individuals use 50-75% of their ventilatory capacity at maximal exercise), but can be identified in patients with COPD when ventilatory capacity is reduced. During exercise, increased ventilatory requirements (mainly secondary to increased ventilation-perfusion mismatch) and abnormal dynamic ventilatory mechanics stresses the already diminished cardiorespiratory reserves. Limitation can also be precipitated by other factors that increase ventilatory requirements for exercise such as inefficient breathing and anxiety.

### **3.2.2 Abnormal ventilatory pattern**

As a consequence of reduced expiratory airflow in COPD, the lungs fail to empty completely on expiration leading to an increase in end-expiratory lung volume. This causes progressive dynamic hyperinflation (a shift towards higher lung volumes and reduced inspiratory capacity) during exercise, which is a potent cause of breathlessness because it puts the respiratory muscles at a mechanical disadvantage.

### **3.2.3 Impaired gas exchange**

Hypoxia and exercise desaturation can be a feature of COPD, particularly emphysema where there is destruction of lung parenchyma with high ventilation-perfusion mismatch. Hypoxia can limit exercise capacity directly by stimulation of chemoreceptors to increase ventilation, or indirectly by stimulation of lactate production.

### **3.2.4 Reduced respiratory muscle function**

Inspiratory muscle weakness and impaired endurance are present in COPD subjects and contribute to hypercapnia, dyspnoea, oxygen desaturation and reduced exercise performance. Maximum inspiratory pressures increase and are related to dynamic hyperinflation during exercise. The diaphragm undergoes adaptations to increase fatigue resistance secondary to chronic overload.

### **3.2.5 Abnormal symptom perception**

Psychometric scales are used to compare symptom response to exercise with accompanying physiological responses [Borg GA, 1982]. In this regard perceived exertion correlates with variables of cardiovascular response and Borg breathlessness score loosely correlates with the proportion of ventilatory capacity used. COPD patients may have symptom increase with exercise out of proportion to underlying physiological changes and

this can become the sole limiting factor during maximal exercise. Anxiety, fear and poor motivation also impact on symptom perception in these patients.

### **3.3 Impaired skeletal muscle function in COPD**

The structural and functional changes in skeletal muscle, especially the vastus lateralis muscle, have been consistently described in COPD [Casaburi R, 2000; Mador MJ *et al*, 2001a; Maltais F *et al*, 2000a] and are summarised in **Table 3.1**. Skeletal muscle impairment is characterised by both a loss of muscle mass and strength and a loss of oxidative capacity, and has been associated with exercise intolerance, poor quality of life, increased utilisation of healthcare resources and reduced survival [Decramer M *et al*, 1997]. These influences are generally independent of impairment in lung function.

#### **3.3.1 Muscle wasting and performance**

A number of studies have demonstrated that when compared with similar-aged healthy subjects, skeletal muscle mass and strength is reduced in COPD [Bernard S *et al*, 1998; Clark CJ *et al*, 2000; Gosselink R *et al*, 1996; Schols AM *et al*, 1991b]. Thigh cross-sectional area on CT is reduced by 30% in moderate to severe disease [Bernard S, 1998] compared with age-matched subjects. Importantly, muscle wasting is an independent prognostic indicator of exercise capacity and mortality in this patient population [Marquis K *et al*, 2002; Schols AM *et al*, 1998; Schols AM *et al*, 2005]. The prevalence of muscle wasting in COPD is around 30%, and these patients are more disabled and have worse health status [Gosselink R *et al*, 1998; Mostert R *et al*, 2000; Schols AM *et al*, 1991c]. Not surprisingly, healthcare utilisation is greater in patients with muscle wasting [Decramer M, 1997].

Table 3.1: Skeletal muscle abnormalities in resting muscle of COPD subjects

Muscle atrophy	
Muscle weakness	
Morphological changes	<p>↓ proportion type I fibres</p> <p>↑ proportion type IIb fibres</p> <p>Atrophy type I and IIa fibres</p> <p>↓ capillarisation</p>
Altered metabolic capacity	<p>↓ mitochondrial enzyme activities</p> <p>↓ ATP concentration</p> <p>↑ IMP concentration</p> <p>↑ muscle lactate</p> <p>↓ intramuscular pH</p>

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Some patients show selective muscle atrophy with relative preservation of the fat mass compartment. Alternatively, muscle wasting may occur as part of a cachexia-like syndrome in COPD in which both muscle and fat mass are depleted [Schols AM *et al*, 1993]. The ‘cachexia syndrome’ has been associated with a systemic inflammatory response in a number of studies [Eid AA *et al*, 2001; Gan WQ *et al*, 2004]. These patients show increases in a number of systemic inflammatory cytokines, particularly TNF $\alpha$  and IL-6 although the source of this inflammatory response remains unclear. Our understanding of the molecular mechanisms underlying muscle wasting in other cachectic syndromes is increasing and has indicated that muscle protein breakdown is centrally involved [Glass DJ, 2003].

Muscle performance is determined by strength and endurance. Strength indicates the capacity to develop maximal force, and endurance the capacity to maintain force over time and resist fatigue. Most studies looking at muscle strength have concentrated on the lower limb musculature. Quadriceps strength is decreased on average by 20-30% in moderate to severe COPD [Decramer M, 1997; Gosselink R, 1996], although there is considerable variability among patients ranging from normal values to reductions of over 50%. Lower limb muscle strength is an important predictor of shuttle walk performance [Steiner MC *et al*, 2005b] and has recently been shown also to be a predictor of mortality independent of severity of lung disease [Swallow EB *et al*, 2007].

A consistent finding is that muscle strength and mass are closely correlated. The contribution of muscle strength to whole body performance is less clear. A number of studies have identified a correlation between isometric muscle strength and laboratory cycle performance [Gosselink R, 1996; Hamilton AL *et al*, 1995]. Strength has been shown to correlate with performance in a self-paced (six-minute) walk test [Gosselink R, 1996]

but not the endurance shuttle walk test [Steiner MC, 2005b]. Measurements of muscle mass have also been related to laboratory measures of whole body performance and self-paced walk tests but appear not to correlate with endurance or incremental shuttle performance [Schols AM, 1991b; Steiner MC, 2005b; Wilson DO *et al*, 1989]. These differences are likely to reside in differences in the patient populations studied but also emphasize subtle differences in the characteristics of these performance tests.

### **3.3.2 Muscle morphology**

Although generalised atrophy of muscle fibres occurs in COPD, type I fibres appear to be preferentially affected. Muscle biopsy samples taken from vastus lateralis muscles of COPD patients at rest have shown atrophy of type I fibres compared with healthy controls, with reductions in the proportion of type I fibres (17-29% compared with 45-50% in sedentary elderly subjects and 60-65% in active elderly subjects), and a reciprocal increase in the proportion of type II fibres, in particular type IIb [Jobin J *et al*, 1998; Whittom F *et al*, 1998]. This is in contrast to changes seen with sarcopenia (aging) where type II fibre atrophy occurs. However, in mild COPD the proportion of type I fibres is usually well preserved. In addition to fibre atrophy, the number of capillaries in relation to type I fibres is reduced in COPD compared with controls [Jobin J, 1998]. Muscle capillarity is an important component of oxidative metabolism.

The shift in fibre type has functional consequences due to different contractile properties. The increased proportion of type IIb fibres helps to preserve strength, although this is at the cost of reduced endurance and increased susceptibility to fatigue. However, the relative proportion of fibre types does not appear to have an independent effect on exercise capacity [Maltais F *et al*, 1999].

### **3.3.3 Metabolic Function**

Many patients with COPD struggle to sustain muscular contraction and terminate exercise because of muscle fatigue. Most studies of exercise responses in COPD have suggested a greater rise in blood lactate at a given exercise intensity compared with age-matched healthy subjects [Casaburi R *et al*, 1991; Maltais F *et al*, 1996b]. These findings imply that skeletal muscle energy metabolism is altered in COPD. Support for this concept comes from studies of muscle samples taken at rest, which have indicated reduced mitochondrial oxidative enzyme concentrations in patients with COPD when compared to similar-aged healthy individuals [Mador MJ, 2001a]. Citrate synthase enzyme, involved in the TCA cycle, and 3-hydroxyacyl CoA dehydrogenase, involved in  $\beta$ -oxidation of fatty acids, are good markers of oxidative capacity and both are significantly reduced in the vastus lateralis muscle [Jakobsson P *et al*, 1995b; Maltais F *et al*, 2000b]. The reduction in oxidative enzyme concentrations appears to be selective. Glycolytic enzyme concentrations are normal and cytochrome oxidase concentration (a key enzyme in the electron transport chain) is increased [Sauleda J *et al*, 1998]. Reductions in oxidative enzyme concentrations correlate with both maximal exercise capacity and the magnitude of the lactate response during exercise [Maltais F, 1996b; Maltais F, 2000b]. This indicates that the observed changes in skeletal muscle are functionally relevant.

Changes in resting energy metabolites observed in the vastus lateralis muscle of patients with COPD are predictive of relatively poor aerobic function. A reduction in resting ATP concentration has been described in the quadriceps muscle of chronically hypoxic patients [Jakobsson P *et al*, 1990; Jakobsson P *et al*, 1995a] and more recently in non-hypoxic patients [Steiner MC *et al*, 2005a]. This may reflect reduced efficiency of ATP re-synthesis due to increased reliance on anaerobic mechanisms in the face of

impaired oxidative capacity. Consistent with this, in the vastus lateralis muscle, PCr concentrations, ATP/ADP ratio and PCR/Cr ratio are lower, and lactate concentrations higher than in healthy, age-matched controls [Fiaccadori E *et al*, 1987; Jakobsson P, 1995a; Moller P *et al*, 1982]. Pouw and colleagues also observed in the anterior tibialis muscle increased IMP concentrations associated with reduced ATP/ADP and PCR/Cr ratios in a subgroup of COPD patients [Pouw EM *et al*, 1998b].

### **3.3.4 Metabolic response to exercise**

Overall the observed muscle changes imply that oxidative metabolism is impaired in subjects with COPD at rest and during exercise, with a resultant increase in non-oxidative glycolytic activity. However, direct information about skeletal muscle bioenergetics during whole body exercise is scanty. Magnetic resonance (<sup>31</sup>P-MRS) is used as an indirect measure of oxidative phosphorylation [Mahler M, 1985] and studies can provide limited data on metabolic events during exercise. Findings have indicated greater PCr breakdown during exercise and slower PCr re-synthesis during recovery [Kutsuzawa T *et al*, 1995; Sala E *et al*, 1999]. This supports the notion of impaired oxidative metabolism and ATP re-synthesis and high reliance on anaerobic processes in COPD. However, <sup>31</sup>P-MRS data makes assumptions about intracellular pH buffering capacity that may not be true for COPD patients and is limited in the mode of exercise that can be studied. In particular whole body exercise is difficult to study within the confines of the equipment.

More recently studies have suggested that energy availability in the form of ATP is compromised at low absolute exercise intensities in COPD patients that may be relevant to their activities of daily living. ATP degradation and inosine 5-monophosphate (IMP) accumulation in skeletal muscle during exercise, suggestive of an imbalance between ATP utilisation and supply or metabolic stress, occurred in subjects with COPD despite

significantly lower absolute workloads than aged-matched controls [Steiner MC, 2005a]. IMP is a degradation product of ADP and its accumulation may suggest impairment of ATP re-synthesis processes during muscular contraction.

### **3.3.5 Exercise-induced lactate accumulation**

When ATP stores cannot be replenished by aerobic metabolism, anaerobic glycolysis may be accelerated in an attempt to provide energy to working muscles. In these circumstances, lactate accumulates as pyruvate produced from glycolysis cannot be completely oxidised in the mitochondria by oxidative phosphorylation [Maughan R, 1997].

Lactate starts to rise at very low workloads during incremental exercise in subjects with COPD compared with healthy subjects [Casaburi R, 1991; Maltais F, 1996b]. Since lactate accumulation is an important cause of premature muscle fatigue this may be a contributing factor to exercise limitation in this population. Maltais and co-workers [Maltais F *et al*, 1998] showed that this premature rise in blood lactate was not related to oxygen delivery or blood flow, which was not lower than in healthy controls, but was likely to be due to increased lactate production. Subsequent studies linked the accelerated lactate rise with reduced oxidative enzyme concentrations and suggest it is likely to be due to reduced oxidative capacity within the muscle resulting in early activation of non-oxidative ATP metabolism [Maltais F, 1996b; Maltais F, 2000b; Saey D *et al*, 2005].

### **3.3.6 Early fatigue in COPD**

Development of contractile fatigue in exercising skeletal muscle is likely to contribute to exercise limitation in COPD [Hamilton AL, 1995]. Recently the direct impact of leg fatigue on exercise response and acute bronchodilation in COPD subjects was evaluated. Saey and co-workers found over half of patients with severe COPD met pre-

determined criteria for quadriceps contractile fatigue to magnetic twitch after sub-maximal cycle exercise to exhaustion [Saey D *et al*, 2003]. In this group, when ventilatory limitation was relieved by bronchodilation, exercise endurance failed to improve. This indicates that muscle fatigue is limiting exercise performance in these patients and provides indirect evidence for the role of peripheral muscle dysfunction in exercise limitation.

In patients with COPD, skeletal muscle changes that could be associated with premature contractile fatigue include reductions in the proportion of type I fibres [Bernard S, 1998; Gosker HR *et al*, 2002a; Whittom F, 1998] and oxidative enzyme concentrations [Kutsuzawa T, 1995; Maltais F, 2000b; Wuyam B *et al*, 1992] leading to reduced oxidative capacity. Increased anaerobic ATP re-synthesis will result in lactate accumulation and premature muscle acidosis, which impairs cell function [Allaire J *et al*, 2004; Maltais F, 1996b]. In patients who are unable to exercise to a sufficient intensity to reach a blood lactate threshold, muscle lactate accumulation may still contribute to intracellular acidosis and fatigue if concentrations are high relative to muscle mass.

Patients with quadriceps fatigue (fall in magnetic twitch force) following exercise have increased muscle LDH activity and reduced muscle capillarisation [Saey D, 2005] compared with non-fatiguers. Correlation between fall in twitch force and LDH activity, blood lactate and capillary/fibre ratio suggests increased reliance on anaerobic metabolism during exercise is associated with contractile fatigue.

In COPD the accelerated lactate response is particularly detrimental to a system working with a reduced ventilatory capacity and becomes an additional determinant in exercise limitation because the lactate-induced acidosis is buffered by the excretion of CO<sub>2</sub> by the lungs. This requires an increase in ventilation by a system already compromised by the presence of airflow obstruction.

### **3.4 Causes of skeletal muscle impairment**

The causes of impaired skeletal muscle function are likely to be multifactorial. We are now beginning to understand some of the mechanisms behind these muscle abnormalities, and deconditioning through long-term reduction in physical activity is probably of central importance in most patients. Whether the changes seen in COPD represent a more severe form of changes seen with healthy aging (accelerated sarcopenia) has also been strongly debated in the literature. Some insight into mechanisms responsible has been gained by considering changes in muscle groups other than those of ambulation. Muscles of respiration do not appear to be affected and in contrast to changes in the quadriceps muscle, there is a fibre shift from type IIb to type I in the diaphragm [Levine S *et al*, 1997]. Relative preservation of upper muscle strength and oxidative enzyme concentrations has also been demonstrated [Bernard S, 1998; Gosselink R, 1996]. These findings provide a strong argument for deconditioning over systemic influences. However, evidence is accumulating in the literature that this cannot provide the whole explanation and a number of other disease factors have been implicated, although these have mostly been explored in cross-sectional studies and their relative contributions remain uncertain. The main factors implicated are poor nutrition, acidosis, hypoxaemia, hypercapnia, chronic systemic inflammation, oxidative stress, medications especially corticosteroids, coexisting heart disease and electrolyte disturbance [Couillard A *et al*, 2005; Mador MJ, 2001a; Maltais F, 2000a]. Some of these are considered in more detail below. The degree to which each of these factors influences impacts on the individual is difficult to quantify and likely to vary widely. Although our knowledge of skeletal muscle impairment is increasing, the molecular mechanisms by which these events lead to changes in muscle structure and function in COPD are largely unknown.

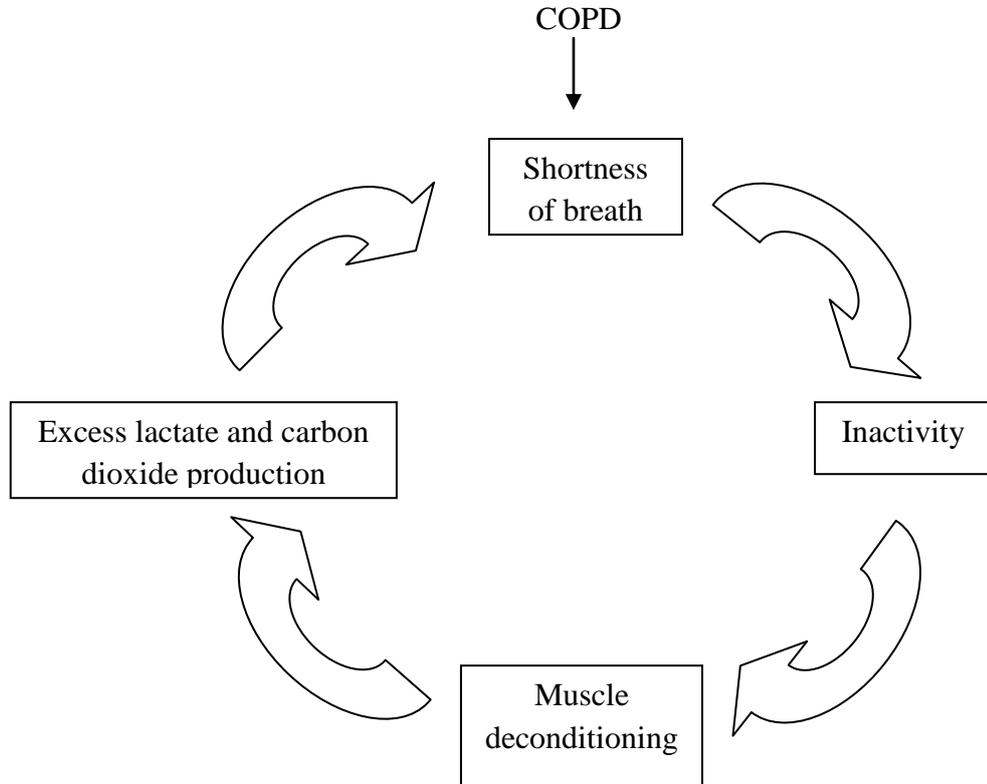
### **3.4.1 Disuse and Deconditioning**

Skeletal muscle is a very plastic tissue with considerable capacity to adapt in response to different patterns of activity and disuse. Due to their disease, patients with COPD have significant and progressive loss of physical activity leading to disuse, which contributes to a number of adaptations within the skeletal muscle from deconditioning. This can lead to a downward spiral of disease with progressively worsening muscle function [Figure 3.2].

Disuse causes muscle atrophy with a relative reduction in the percentage of type I fibres, and a fall in the concentration of enzymes involved in oxidative ATP metabolism [Franssen FM *et al*, 2002]. Functionally this can result in reduced strength and endurance capacity. Therefore, physical inactivity is likely to contribute to the observed loss of muscle mass, fibre shift and reduced oxidative capacity in COPD. These changes are similar to those seen in detrained healthy subjects [Appell HJ, 1990] and subjects with exercise intolerance due to heart failure [Gosker HR *et al*, 2000].

However, physical inactivity and deconditioning can only partly explain the abnormalities observed in skeletal muscle and the early exercise lactate rise in COPD [Engelen MP *et al*, 2000]. There is only weak correlation between physical activity and quadriceps endurance [Serres I *et al*, 1998] or between exercise performance in healthy subjects and COPD subjects with similar activity [Couillard A, 2005].

Figure 3.2: Spiral of disease leading to worsening skeletal muscle function



Furthermore, exercise training to improve physical performance does not fully reverse the metabolic changes [Couillard A, 2005]. It is therefore unlikely that inactivity is the only factor involved in skeletal muscle impairment.

### **3.4.2 Hypoxia**

In subjects with COPD oxygen delivery to peripheral muscles may be insufficient. Tissue hypoxia can lead to adaptive changes although the mechanisms remain uncertain. In addition, hypoxia probably induces oxidative stress through reduced antioxidant status, which in turn may contribute to muscle damage.

Studies of muscle biopsies taken at rest suggest that intramuscular high-energy phosphates and glycogen are lower in hypoxic COPD patients than normoxic controls [Jakobsson P, 1990] and type 1 fibre proportion is smaller [Gosker HR *et al*, 2002b]. Oxygen therapy can significantly improve exercise performance along with aerobic metabolism in appropriate patients [Jakobsson P, 1995a; Leach RM *et al*, 1992; Mannix ET *et al*, 1995]. Further information on muscle adaptive effects from hypoxia comes from studies in healthy subjects living for a few months in conditions where oxygen levels are reduced [Caquelard F *et al*, 2000; Hoppeler H *et al*, 1990]. Cross-sectional area is reduced in the quadriceps muscle due to muscle atrophy, and oxidative activity is reduced due to reduced TCA activity. Hypoxia can also reduce the proportion of type I fibres and this is correlated with arterial oxygen pressure ( $P_{O_2}$ ). Oxygen supplementation improves aerobic capacity but not the fibre shift. The hypoxia-induced changes are associated with reduced muscle strength and endurance.

### **3.4.3 Chronic inflammation**

Some patients with COPD show evidence of an exaggerated systemic inflammatory response and this may cause protein catabolism and contribute to muscle mass depletion [Eid AA, 2001; Pouw EM *et al*, 1998a; Schols AM *et al*, 1996]. Studies have consistently found elevated circulating levels of inflammatory cytokines and enhanced activation of circulating inflammatory cells (neutrophils and lymphocytes) [Gan WQ, 2004]. This has been shown to trigger catabolic/ anabolic imbalance leading to wasting and weakness in this population [Debigare R *et al*, 2001; Debigare R *et al*, 2003]. High levels of TNF $\alpha$  in particular are associated with severe lean muscle wasting leading to cachexia and this may be a distinct form of muscle disease [Di Francia M *et al*, 1994; Schols AM, 1996].

Although chronic inflammation is likely to be implicated in skeletal muscle impairment in COPD and a causal relationship is supported in the literature for CRP, IL8 and TNF $\alpha$  [Couillard A, 2005], considerable uncertainty remains to the prevalence and causes of inflammation and mechanisms by which this leads to loss of muscle mass in this population.

### **3.4.4 Oxidative stress**

Oxidative stress occurs when there is an imbalance between formation of and protection against reactive oxygen or nitrogen species (RONS). There is evidence that oxidative stress occurs in the lung and blood of COPD subjects and contributes to progression of disease [Couillard A *et al*, 2002].

Oxidative stress to muscle can increase significantly during exercise. The main source of free radicals in this situation is oxidative phosphorylation but they are also generated from AMP degradation and inflammation such as TNF $\alpha$ . In health, exercise

triggers protective antioxidant mechanisms and regular exercise increases antioxidant status of muscle. Conversely, disuse has a negative effect on antioxidant status as the usual trigger for antioxidant mechanisms is reduced. This may therefore promote oxidative damage to muscle during occasional bouts of exercise due to enhanced oxygen supply to exercising muscles causing bursts of free radicals that exceed capacity of defence.

Recent data implies that oxidative stress contributes to muscle structural and functional damage through reactive oxygen species [Couillard A, 2002; Droge W, 2002]. Damage by free radicals has been linked to uncoupling of oxidative phosphorylation and impaired oxidative capacity by a number of mechanisms and can result in muscle atrophy. Oxidative stress has been implicated in the aetiology of skeletal muscle dysfunction in COPD [Agusti AG, 2005; Berton E *et al*, 2001]. The purine nucleotide degradation pathway is a potential source of exercise-induced oxidative stress through the activity of xanthine oxidase [Hellsten Y *et al*, 1997b; Hellsten Y *et al*, 1997a]. Recent studies in COPD have shown exercise induces oxidative stress at low intensities that patients are likely to be exposed to on a regular basis [Heunks LM *et al*, 1999]. Further studies are needed to elicit molecular mechanisms behind effects of oxidative stress on muscle performance and its relationship to inflammation and hypoxia.

#### **3.4.5 Nutritional depletion**

Poor nutritional status is common in COPD and an important determinant of exercise capacity and mortality [Engelen MP *et al*, 1994; Gosker HR, 2000; Gray-Donald K *et al*, 1989; Mostert R, 2000]. Dietary intake may be reduced by dyspnoea, fatigue, early satiety and systemic inflammation and is associated with predominant loss of fat mass. Patients can also lose weight due to elevated energy expenditure particularly from increased cost of breathing and this is associated with loss of both fat and lean mass

[Schols AM *et al*, 1991a]. However, loss of muscle mass may be secondary to decline in muscle function due to other factors and it remains unclear to what degree specific nutritional deficiencies contribute to impaired skeletal muscle function.

### **3.4.6 Drugs**

Patients with COPD are frequently treated with drug therapies (systemic and inhaled) with the potential to adversely affect peripheral muscle function. Systemic corticosteroids are known to cause a myopathy and this may be present in some patients [Decramer M *et al*, 1994]. Detrimental effects on skeletal muscle structure and function leading to muscle atrophy and reduced strength has been shown with both high dose and long-term low dose steroids. These effects are not seen with short burst courses unrelated to exacerbations. Beta-agonists are an anabolic stimulus to skeletal muscle [Martineau L *et al*, 1992; Revill SM *et al*, 1998] Although evidence from studies in asthmatics show that inhaled beta-agonists do not have significant effects on performance [Meeuwisse WH *et al*, 1992; Revill SM, 1998] it is difficult to ascertain impact of larger doses used by some COPD subjects on muscle function.

### **3.5 Adaptations to training**

It is well established that exercise capacity can be effectively improved with training in COPD subjects. Depending on the training programme, improvements in both endurance and strength muscle performance can be achieved. Furthermore, when trained at sufficiently high intensities, metabolic and physiological adaptations within the peripheral muscles can be induced. However, understanding of the mechanisms by which exercise training improves performance in COPD is not complete.

### **3.5.1 Pulmonary rehabilitation programmes**

Pulmonary rehabilitation (PR) is a multidisciplinary approach to training that aims to reduce disability in patients with lung disease [British Thoracic Society Standards of Care Subcommittee on Pulmonary Rehabilitation, 2001]. Although exercise is an essential component, most programmes also provide education packages and other services such as occupational therapy, dietician advice and psychological support [Nici L *et al*, 2006]. The benefits of pulmonary rehabilitation are confirmed in large randomised controlled studies [Goldstein RS *et al*, 1994; Griffiths TL *et al*, 2000; Lacasse Y *et al*, 2002] and include improvements in health-related quality of life alongside increased exercise performance, despite no effect of lung pathophysiology. Other aspects such as increased confidence and reduced fear of physical activity are also observed.

The best mode of training has not yet been established and may vary between patients. The focus is often on lower limb endurance since improvements in walking is frequently identified by patients as their most important functional goal. Walking speed closely correlates with oxygen consumption ( $VO_2$ ) and can be used to prescribe exercise [Singh SJ *et al*, 1994]. However, cycling, lower limb strength training, and upper limb strength and endurance training is often included. Training can be performed successfully in the outpatient [Griffiths TL, 2000; Singh SJ *et al*, 1998], inpatient [Goldstein RS, 1994] and home setting [Strijbos JH *et al*, 1996] but supervised exercise is superior to unsupervised exercise [Puente-Maestu L *et al*, 2000]. Training load can be progressed through increased exercise duration [Singh SJ, 1998] or intensity [Griffiths TL, 2000], although direct comparison between these two approaches has not been made. At least two weeks are required for physiological adaptation and most training programmes run for at

least 4 weeks, although it is clear that further improvements can be seen after 7 weeks [Green RH *et al*, 2001; Sewell L *et al*, 2006] and probably beyond [Singh SJ *et al*, 2007].

### **3.5.2 Physiological training response**

Clear physiological training benefits in ventilatory response to exercise have been shown with PR [Casaburi R, 1997]. The greater the intensity of training, the greater the benefits on exercise performance, and patients with COPD are able to train at higher relative intensity (85% peak  $\text{VO}_2$ ) than healthy subjects. Improvements in performance generally occur in the mode of training employed, for example endurance training will lead to increases in endurance.

A variety of performance measures have been used to assess the outcome of training. Peak work in laboratory tests frequently increases and the ventilatory requirements for a given workload decrease. Data on the effect of training on laboratory measurements of peak  $\text{VO}_2$  is conflicting with some studies showing an increase [Ries AL *et al*, 1995] whereas others did not [Lake FR *et al*, 1990; McGavin CR *et al*, 1977]. Sub-maximal endurance performance is generally more sensitive to training (although less reproducible), perhaps unsurprisingly as endurance training is most frequently used in exercise training programmes [Revill SM *et al*, 1999; Weiner P *et al*, 1992]. Timed walk test [Goldstein RS, 1994; Lake FR, 1990] and incremental and endurance shuttle performance [Griffiths TL, 2000; Singh SJ, 1998] is also sensitive to this type of training intervention.

Although reductions in peripheral muscle strength are well documented, strength training is less frequently included in rehabilitation programmes and fewer data are available concerning its effectiveness, although evidence is increasing [O'Shea SD *et al*, 2004; Spruit MA *et al*, 2002]. Studies have shown that progressive weight training can

increase peripheral muscle strength between 16-40% [Bernard S *et al*, 1999; Clark CJ, 2000; Simpson K *et al*, 1992]. However, the translation of these increases to changes in whole body performance and health status is less consistent.

### **3.5.3 Metabolic training response**

Several investigators have shown significant reductions in blood lactate accumulation for a given level of exercise following 12 weeks endurance training [Casaburi R, 1991; Maltais F *et al*, 1997] and these findings suggest benefits to muscle function are likely to contribute to the reduction in ventilatory requirements and dyspnoea seen [Casaburi R, 1997]. There was an associated increase oxidative enzyme concentrations (notably citrate synthase and 3-hydroxyacyl CoA dehydrogenase), type I fibre size and capillarity suggesting improved muscle oxidative capacity [Maltais F *et al*, 1996a]. Glycolytic enzymes were unaffected by PR and changes in oxidative enzyme concentrations correlated inversely with exercise-induced changes in blood lactate concentration. Fatigue resistance and quadriceps endurance increased [Maltais F, 1996a]. These findings suggest greater contribution of oxidative metabolism to energy provision after training. Similar training effects on oxidative capacity are seen in healthy adults [Wibom R *et al*, 1992].

Further evidence supporting these findings comes from studies using <sup>31</sup>P-MRS, which have provided direct information about improved peripheral muscle bioenergetics following training [Sala E, 1999]. The exercise-induced rise in Pi:PCr ratio decreased with training indicating less PCr degradation. PCr re-synthesis following submaximal exercise was faster after training consistent with improved oxidative capacity. However, training fails to completely reverse the metabolic changes seen in COPD. For example, in the studies reviewed, no change in the proportion of type I fibres was seen [Whittom F, 1998]

and the reduction in oxidative enzyme activity (about 38% for citrate synthase) was only increased by around 16% with endurance training [Couillard A, 2005].

Training may have additional benefits through increased antioxidant status and subsequent reduced risk of exercise-induced oxidative stress in COPD subjects, although this remains to be confirmed.

### **3.6 Measuring exercise capacity in COPD**

#### **3.6.1 Physiological variables**

As mentioned at the start of this chapter, exercise intolerance is multi-factorial in patients with COPD and cannot be confidently predicted by cardiac and pulmonary indices at rest. Cardiopulmonary exercise testing (CPET) remains the gold-standard for assessing exercise performance, although the specific test will vary depending on what is being studied. For example, incremental maximal tests are used to objectively measure exercise capacity and quantify physiological limitations, whereas submaximal constant workload tests are often more sensitive in detecting change or response to intervention. Peak oxygen consumption,  $VO_{2peak}$ , is the best available index of aerobic capacity (providing subjects have attained their limit) and is reproducible [Palange P *et al*, 2007]. The ‘anaerobic threshold’ can be determined on an incremental test from either the ‘gas exchange threshold’ or ‘lactate threshold’.

#### **3.6.2 Metabolic variables**

Techniques available for measurement of metabolic capacity in skeletal muscles during exercise in patients with COPD are currently inadequate. Non-invasive measurement using MRS studies provide useful information on muscle biogenetics before and after exercise but are not a direct measure of the muscle response [Constantin-

Teodosiu D *et al*, 1997]. Although muscle biopsies are the gold standard and provide important information on metabolic changes and oxidative capacity, they are invasive and technically difficult to undertake during exercise particularly in this population of patients who commonly are frail and suffering from a number of co-morbidities.

Lactate accumulation can be measured in the muscle or in the blood. Serial blood lactate concentrations can provide indirect but reproducible measurement of muscle aerobic capacity. However, lactate does not reflect adenine nucleotide metabolism directly and therefore cannot provide a complete picture of adenine nucleotide turnover and energy delivery during exercise.

More recently non-volitional techniques for measuring muscle function have been developed using magnetic stimulation of motor nerves [Polkey MI *et al*, 1996]. The drop in twitch force after exercise has also been used as a measure of muscle fatigue [Saey D, 2003]. Reductions in fatigability have been demonstrated after pulmonary rehabilitation using this technique [Mador MJ *et al*, 2001b].

### **3.7 Summary**

Peripheral muscle impairment is an integral component of exercise intolerance in COPD and if managed effectively has the potential to reduce disability in the context of a disease that is essentially irreversible. It is apparent that skeletal muscle impairment in COPD is characterised by altered muscle energy metabolism and is likely to be of importance to a varying degree in exercise limitation in many patients with COPD. Current data indicates a shift in the capacity to re-synthesise ATP within the skeletal muscles from aerobic (oxidative) to anaerobic (non-oxidative) mechanisms. Accelerated lactate production increases ventilatory demands on a system that already has limited capacity, and contributes to muscle contractile failure and early termination of exercise. Biopsy

studies in COPD subjects indicate ATP depletion may occur at low absolute workrates relevant to daily activities. This suggests that failure of energy delivery may be functionally relevant and is worthy of further investigation.

Leg fatigue can relate to impaired peripheral muscle function and contribute to exercise intolerance and symptom limitation. Deconditioning is likely to be the most important mechanism leading to impaired muscle function, but other factors will have varying influences. In support of this notion, exercise training can improve the metabolic adaptations to some degree.

Managing skeletal muscle impairment in COPD patients has become an important clinical goal and tools to evaluate muscle function are required if treatment strategies are to be optimised. However, this is difficult in routine clinical practice because, in contrast to measurements of individual muscle force generation, the muscles are operating as part of an integrated system that is activated when whole body exercise is performed. This system includes the lungs and cardiovascular system, which are also affected by the disease. Muscle biopsies, magnetic resonance spectroscopy and magnetic twitch have provided important data in research studies but are not practical for routine use. The measurement of lactate production during exercise may give an indication of the oxidative potential of the muscles but the magnitude of the lactate response is also influenced by other factors such as the mode of exercise (cycling is generally greater than walking) and the degree of ventilatory limitation.

Targeting the skeletal muscle also offers novel perspectives for performance-enhancing therapies. However, a greater understanding of the pathophysiology of skeletal muscle energy metabolism and its relationship to exercise and muscle performance is currently needed. This might allow us, for example, to predict who will benefit from

exercise training or other performance enhancing therapy and determine what mode of enhancement is most useful for individual patients. Further investigation requires the development of new tools to evaluate muscle events and energy delivery during exercise. One possibility is the measurement of plasma ammonia concentration. From studies in healthy subjects, it is apparent that the ammonia response to exercise can accurately reflect muscle energy metabolic events and be sensitive to metabolic adaptations following therapeutic intervention.

Therefore, plasma ammonia response to exercise in subjects with COPD may give further insight into the abnormal metabolic response and be a useful measure of energy metabolism in this population.

## Chapter Four: Methodology and Validation of Ammonia Analysis

In this chapter the clinical measurements and laboratory procedures are described in detail.

### 4.1 Clinical measurements

#### 4.1.1 Baseline measurements

##### *Lung function*

Spirometry was performed to BTS standards [British Thoracic Society, 1994] on three occasions in the seated position (Vitalograph Model R, Buckingham, UK). FEV<sub>1</sub> and FVC are expressed as %predicted using ERS regression equations [Quanjer PH *et al*, 1993]. Blood gas analyses were also performed in COPD subjects. Arterialised blood samples for PaO<sub>2</sub>, PaCO<sub>2</sub> and PH were obtained at rest, on room air, using the earlobe micromethod (Bayer RapidLab 348, USA). Full lung function studies were performed on COPD subjects in the respiratory physiology department by trained technicians using helium dilution technique (Spiro Air, Medisoft, Belgium).

##### *Body composition*

Body mass index (BMI) was calculated from height (measured by wall mounted stadiometer to the nearest 0.1cm) and weight (measured in light clothing by digital scales to the nearest 0.1 kg (SECA, UK)).

FFM (Kg) was estimated with subjects semi-supine using whole-body single frequency bioelectrical impedance (Bodystat 1500; Bodystat Ltd, Douglas, UK) as described by Lukaski [Lukaski HC *et al*, 1985]. Self-adhesive electrodes were applied over the dorsal surfaces of the right hand and foot. On the foot the electrodes were sited over the second metatarsal and midway between the medial and lateral malleoli. On the hand the

electrodes were placed over the second metacarpal and medial to the distal process of the ulna. A current of 500microamps at 50Hz was passed between these electrodes and the impedance recorded. Measurements were taken after voiding the bladder.

FFM is calculated from impedance measurement using regression equations derived for a number of different populations including patients with COPD and healthy subjects using a variety of reference methods. In this Thesis a disease-specific and gender-specific regression equation derived from a population of COPD patients using deuterium-dilution as a validation method [Schols AMW, University of Maastricht, Maastricht, The Netherlands] was used [Steiner MC *et al*, 2002]. Fat free mass index (FFMI, Kg/m<sup>2</sup>) is calculated as FFM divided by height<sup>2</sup>.

### ***Peripheral muscle strength***

Muscle strength is defined as the maximal force generated by a specific group of muscles. Changes in strength may be important for physical functioning in COPD patients and isometric (static) force is commonly used as an outcome measure to demonstrate change, for example following pulmonary rehabilitation.

Maximum isometric quadriceps force of the dominant leg was evaluated by measuring maximum voluntary contraction (MVC) using a Cybex II Norm dynamometer (CYBEX NORM™ Testing and Rehabilitation System, CYBEX International, New York). The dynamometer was calibrated for each subject and data was calculated with computer software (system version 2.0). Subjects were seated with the backrest at 85<sup>0</sup>, arms crossed over the chest to minimise upper torso movement and stabilizing straps applied across the chest and mid-thigh of the leg being tested. An adjustable lever arm was attached to the leg proximal to the lateral malleolus and its axis of rotation aligned visually to the lateral femoral epicondyle. Gravity correction to torque at 45<sup>0</sup> (leg straight = 0<sup>0</sup>) was calculated

by the dynamometer. Subjects' positioning on the cybex was recorded so that future measurements were taken under identical conditions. MVC was measured with the subject seated at 90° hip flexion and 70° knee flexion. Previous data from our department indicates that isometric measurements performed under these conditions show high reliability and no familiarisation is needed. Subjects were instructed to extend their knee as hard as they could, in a controlled manner, for four seconds or until effort declined. Two sets of three MVCs were performed, with thirty seconds rest between contractions and two minutes rest between sets. Best reproducible result (<5% variability between 2 readings) was recorded.

### ***Physical activity questionnaire***

Physical activity has been shown to have an important impact on physical and mental health [Voorrips LE *et al*, 1991]. Many methods of assessing physical activity are described in literature, but as yet no gold standard exists. In epidemiological and physiological research an activity questionnaire is a practical and widely used approach to assessing physical activity and one has been used in the first study of this Thesis.

Physical activity was assessed using a physical activity questionnaire adapted and validated for the elderly [Voorrips LE, 1991] and used in COPD subjects [Engelen MP, 2000; Serres I, 1998]. The questionnaire is interview-led and references a 1-year period prior to the interview. It is based on the activity questionnaire described by Baeke *et al* [Baecke JA *et al*, 1982] but has been developed for use in the elderly [Voorrips LE, 1991]. Test-retest reliability, and validity compared to 2 independent methods of assessing physical activity (pedometer and 24hour activity recall) in the elderly population has been established [Voorrips LE, 1991].

The questionnaire consists of scores for household activities, sport activities and leisure activities, resulting in an overall activity score (see Appendix 3).

### ***The Self-Reported Chronic Respiratory Questionnaire***

Health status was assessed using the chronic respiratory disease (CRQ) questionnaire [Guyatt GH *et al*, 1987] which was self-reported [Williams JE *et al*, 2001]. This questionnaire is a disease-specific measure of health related quality of life (or health status), which is used as an outcome measure in pulmonary rehabilitation. The questionnaire measures four domains- dyspnoea, fatigue, emotion and mastery (see Appendix 3). Results are presented as mean scores for each domain. The threshold for a clinically significant change in each domain is 0.5 [Juniper EF *et al*, 1994].

#### **4.1.2 Exercise challenge**

All exercise tests on the bike and treadmill were performed with one of the subject's hand placed in a handwarmer on a trolley to the side of the exercise platform [Figure 4.1].

#### ***Incremental exercise test***

The maximal incremental exercise test determines peak exercise work capacity and is performed to symptom-limitation. Subjects exercised on an electrically braked cycle ergometer with electrocardiogram, blood pressure and oxygen saturation monitoring [Ergometric Er900; Ergoline GmbH, Bitz, Germany]. Following 1-2 minutes unloaded cycling (warm-up period), workload increased using a ramp protocol by 10 watts every 1 minute in COPD subjects or 20 watts every 1 minute in healthy subjects. Participants cycled at a constant rate of 40-45rpm and were encouraged to continue cycling at the required rate for as long as possible (exercise-limit was reached if subjects were unable to continue pedalling at this rate). The peak workload achieved was recorded.

**Figure 4.1: Pictures to show patients during exercise test**

Patient performing cycle exercise whilst blood tests and muscle biopsy performed

Ventilation and gas exchange measurements were made throughout the test using a breath-by-breath computerised system (Zan-680 ErgoTest, Zan Messgeraete GmgH, Germany). Maximum voluntary ventilation (MVV) for each subject was calculated as forced expiratory volume in 1 second (FEV<sub>1</sub>) x 35. Peak ventilation was expressed as a percentage of predicted MVV and patients with COPD were deemed ventilatory limited if peak ventilation exceeded 90% MVV [Cooper CB, 2001b]. Breathlessness and perceived exertion were assessed on the modified Borg scale at the end of the test [Borg GA, 1982].

### ***Constant workrate exercise test***

The constant workrate (WR) challenge is a sub-maximal test performed until symptom limitation. The workload for this test was set for individual subjects at 80% of the peak work achieved during the incremental test. In the constant WR test, work increased from rest to target load over 1 minute and subjects continued at this workrate until exhaustion. The work intensity was chosen for several reasons. In particular, this is the intensity chosen for previous work in the department examining skeletal muscle dysfunction during exercise in COPD [Steiner MC, 2005a]. However, it also reflects the intensity at which we ask patients to perform endurance training in rehabilitation and therefore has clinical relevance. Similar work intensities have been demonstrated to stimulate an ammonia response to sub-maximal exercise in healthy subjects whereas lower work intensities do not [Graham TE, 1992]. Participants cycled at a constant rate of 40-45rpm and were encouraged to continue cycling at the required rate for as long as possible (exercise limit was reached if subjects were unable to continue pedalling at this rate). Electrocardiogram, blood pressure and oxygen saturation were monitored throughout the test. Breath-by-breath measurements of ventilation and gas exchange were made throughout exercise and recovery periods (Zan-680 ErgoTest, Zan Messgeraete GmgH,

Germany). Breathlessness and perceived exertion were assessed on the modified Borg scale at the end of the test.

### ***Treadmill test***

The treadmill test, performed to symptom limitation, was undertaken before and after attendance at the rehabilitation programme [RAM 770CE Treadmill; RAM Medical and Industrial Instruments & Supplies, Padova, Italy]. The treadmill protocol was based on the Incremental Shuttle Walk Test (ISWT), a maximal symptom-limited field exercise test used to measure maximum exercise capacity. The ISWT protocol has been extensively used as an outcome measure in pulmonary rehabilitation and is sensitive to this intervention [Singh SJ *et al*, 1992; Singh SJ, 1998]. The walking speed was increased incrementally with subjects walking at each speed for one minute [Table 4.1], until unable to continue or maintain the required speed. Total distance walked and speed that subject reached were recorded. Breath-by-breath measurements of ventilation and gas exchange were made throughout exercise and recovery periods (Zan-680 ErgoTest, Zan Messgeraete GmgH, Germany). Oxygen saturation and pulse rate were also monitored throughout the test. Breathlessness and perceived exertion were assessed on the modified Borg scale at the end of the test.

### ***ISWT and ESWT***

The shuttle tests are externally paced field walking tests, which assess a subject's functional capacity [Singh SJ, 1992]. They are extensively used as outcome assessments in pulmonary rehabilitation and are sensitive to this intervention [Griffiths TL, 2000; Singh SJ, 1998]. For the purpose of this Thesis trained pulmonary rehabilitation practitioners performed these tests. The Incremental Shuttle Walk Test (ISWT) is a symptom-limited maximal test.

Table 4.1: Walking speed, distance and predicted  $VO_{2max}$  for each stage of the 10m ISWT (Singh et al 1992)

Stage	Speed (m/min)	Distance per stage (m)	Total distance (m)	Predicted $VO_{2max}$ (ml/kg/min)
1	30	30	30	4.94
2	40	40	70	5.94
3	50	50	120	7.19
4	60	60	180	8.69
5	70	70	250	10.44
6	80	80	330	12.44
7	90	90	420	14.69
8	100	100	520	17.19
9	110	110	630	19.94
10	120	120	750	22.94
11	130	130	880	26.19
12	140	140	1020	29.69

Performance is predictive of  $VO_{2peak}$  [Singh SJ, 1994] and is reproducible after a single practice test. Patients walk around cones set 10-metre apart (one shuttle) at a speed indicated by a series of beeps from a tape recording, with patients required to walk the distance between the two cones before the next beep [Singh SJ, 1992]. Initial walking speed is 30m/min and increases every minute by 10m/min until patients are unable to continue or maintain the required speed. Total distance of the completed shuttles walked is recorded.

The Endurance Shuttle Walk Test (ESWT) is a constant WR submaximal test of endurance [Revill SM, 1999]. After a 2-minute warm up, patients walk at a speed set around the 10-metre course. Again their speed is guided by beeps similar to the ISWT. The speed is set at the equivalent of 85% of the predicted  $VO_{2peak}$  achieved during the ISWT. Total time walked (excluding warm-up time) is recorded.

#### **4.1.3 Blood sampling**

Half an hour prior to the exercise test a 12g retrograde cannula was inserted into a superficial lower forearm vein and placed inside a hand-warmer, warmed to 50-55°C. Retrograde indicates that the cannula is inserted in the opposite direction from usual, so it is pointing towards the hand rather than away from the hand. The hand-warmer and retrograde cannula enables arterialised-venous blood to be collected, which is representative of arterial blood and is therefore not contaminated by ammonia generated by the hand and forearm muscles. This method has been previously validated and used for plasma ammonia measurements in healthy subjects [Greenhaff PL *et al*, 1991; Greenhaff PL *et al*, 1993; Lambert CP *et al*, 1993]. Arterialised-venous blood samples were taken for ammonia and lactate concentration via the cannula at rest (after subjects had rested on a couch for at least 30 minutes) and throughout exercise. A total of 2mls was taken with a

fresh syringe each time after cannula dead space had been discarded. The cannula was flushed with normal saline following each sampling. Samples were placed immediately on ice (1ml stored in EDTA for ammonia analysis and 1ml in fluoride for lactate analysis).

#### **4.1.4 Muscle biopsy sampling**

Muscle biopsy samples were obtained from the non-dominant Vastus Lateralis muscle at rest and immediately following constant WR exercise using the Bergstrom technique [Bergstrom J, 1975]. This meant that muscle samples were taken following an exercise challenge of the same relative intensity for all subjects. Resting samples were taken after subjects rested on a couch for at least 30 minutes. For peak exercise samples, rapid sampling of the muscle within a few seconds of the completion of exercise was required. Therefore, post-exercise samples were obtained with the subject seated on the bike, no longer than 10 seconds after the end of the exercise test [Bergstrom J, 1975].

Muscle biopsy was performed under aseptic conditions. With subjects lying on the couch, local anaesthesia (1% Lignocaine) was infiltrated to the skin, subcutaneous tissues and muscle fascial sheath. Three 0.5cm incisions were made at least 1cm apart with a scalpel to incise just through the fascial sheath. Two resting muscle biopsies (to ensure adequate sample size for analysis) were taken through two of the incisions using two Bergstrom needles. The open needle was introduced into the muscle, which was simultaneously compressed externally by the hand so that muscle tissue entered the needle. The inner trochar of the needle was used to cut this tissue and take the biopsy whilst suction was applied through the needle by a second person to increase yield. Each needle took a single biopsy and was placed immediately into liquid nitrogen with biopsy tissue still within the needle trochar. The tissue was later removed without thawing. Pressure was then applied to the biopsy sites for at least five minutes. Steristrips and gauze dressing

were then applied prior to the exercise challenge. The third biopsy through the third incision was taken using an identical procedure immediately following completion of exercise with the subject still seated on the bike. A research fellow not involved in the study but trained in the procedure performed the peak-exercise biopsy to enable blood samples for ammonia and lactate analysis to be taken by me at the same time. Steristrips were applied, covered with tegaderm and a compression bandage applied for up to 8 hours. Subjects received written instructions about post-muscle biopsy care with emergency contact details. Muscle samples were immediately frozen and stored at  $-196^{\circ}\text{C}$  in liquid nitrogen.

## **4.2 Laboratory procedures**

### **4.2.1 Ammonia analysis**

Blood for ammonia analysis was centrifuged using a bench-top mini-centrifuge (Spectrafuge, Sigma-Aldrich Company Ltd, UK) at 6000rpm for 2 minutes at room temperature immediately following the exercise test, and plasma stored at  $-196^{\circ}\text{C}$  in liquid nitrogen. Samples were analysed in duplicate usually immediately, and always within 24 hours using an enzyme assay technique (Sigma-Aldrich Co. Ltd, UK). Ammonia measurements have been shown to be accurate if analysed up to 24 hours after storage in liquid nitrogen [Howanitz JH *et al*, 1984; Linder A *et al*, 1993]. The assay technique was developed for the purpose of this Thesis and validated for repeatability and reliability of measurements (see section 4.2.2). The coefficient of variation for ammonia determined from standards was 5%.

The ammonia analysis technique was developed under the supervision of John Fox, Tim Constantinou and Prof. Paul Greenhaff, (Department of Biomedical Sciences, Nottingham University), who have considerable experience in plasma ammonia analysis.

The laboratory techniques required were learnt in Nottingham and a protocol for ammonia analysis using the Sigma assay (see below) established. The protocol was based on a validated technique that has been used in publications on healthy subjects [Greenhaff PL, 1991; Lambert CP, 1993]. The technique can be used to determine ammonia concentrations in the range of 2-880 $\mu$ mol/l.

The ammonia analysis kit (Sigma-Aldrich Company Ltd, UK) used in this Thesis is produced for quantitative enzymatic determination of ammonia concentration in biological samples. Ammonia ( $\text{NH}_4^+$ ) reacts with  $\alpha$ -ketoglutaric acid (KGA) and reduced nicotinamide adenine dinucleotide phosphate (NAPDH) in the presence of L-glutamate dehydrogenase (GDH) to form L-glutamate and oxidised nicotinamide adenine dinucleotide phosphate ( $\text{NADP}^+$ ) as follows



L-glutamate dehydrogenase reacts specifically with ammonia. Therefore, the decrease in absorbance at 340nm, due to oxidisation of NAPDH, is proportional to the ammonia concentration.

All work conditions were kept free from ammonia sources to prevent contamination. This included ammonia present in water absorbed from the atmosphere (double distilled fresh water used). Each vial of ammonia assay reagent (dry reagent contains  $\alpha$ -ketoglutaric acid, NAPDH, buffers and stabilisers) was reconstituted with 10ml double distilled water and mixed by gentle inversion. Other reagents were used as provided in the kit.

A standard curve consisting of high and low ammonia concentration (each in duplicate) was performed with every set of sample assays analysed to ensure reliability of results. Ammonia standard solution was provided at concentration 10µg/ml (588µM ammonium sulphate) and diluted with double distilled water to provide required ammonia concentrations each time a standard curve was assayed. If values obtained with the ammonia standard solution were within 5% of the stated concentration, the test performance was considered acceptable.

Plasma samples were defrosted in a waterbath at 25<sup>0</sup>C and mixed on a vortex immediately before use. Each assay consisted of a blank (fresh double distilled water), a high standard and a low standard, and the plasma samples from between 1 to 3 subjects' exercise tests. All samples/ blanks/ standards were assayed in duplicate. Using a 100µl pipette 50µl of sample/ blank/ standard was added into a 1cm acrylic cuvette. 1000µl reconstituted reagent was added to each cuvette and mixed gently to prevent any droplet loss on a vortex. The cuvettes were then covered with foil and incubated in the dark at 18-35<sup>0</sup>C for 45minutes. Initial absorbance (A1) of each cuvette was measured using a spectrophotometer (Ultrospec III, Pharmacia LKB Biochrom Ltd, Cambridge, UK) at 340nm having first blanked the spectrophotometer using 1ml double distilled water in an acrylic cuvette.

Using a positive displacement pipette 10µl L-glutamate dehydrogenase was then added to each cuvette and mixed well with a plastic stirring rod (the glycerol in L-glutamate dehydrogenase will sink to the bottom of the cuvette). The cuvettes were incubated for up to 30minutes at 18-35<sup>0</sup>C until the reaction was complete (reaction kinetics were monitored using the highest standard concentration). Final absorbance (A2) of each cuvette was measured at 340nm after re-blanking the spectrophotometer.

**Calculation of ammonia concentration**

$$\text{ammonia concentration } (\mu\text{mol/l}) = A1 - A2 - \Delta B1 \times k \times 58.8$$

$$\text{where } k = \frac{\text{total volume in cuvette} \times \text{weight of 1 mol of NH}_3}{\text{mM absorptivity of NADPH at 340nm} \times \text{sample volume}}$$

$$\text{i.e. } k = \frac{1060 \times 17}{6.22 \times 50}$$

therefore

$$\text{ammonia concentration } (\mu\text{mol/l}) = A1 - A2 - \Delta B1 \times 3407$$

A1= first absorbance reading of sample

A2= final absorbance reading of sample

$\Delta B1$  = first absorbance reading of blank - final absorbance reading of blank

Considering the resolution of the assay, stating ammonia concentrations to one decimal point is not appropriate and values in the following sections should be viewed accordingly.

**4.2.2 Repeatability and validation of ammonia assay**

The assay was validated for test-retest reliability and reproducibility in the laboratories at the Institute for Lung Health, Glenfield Hospital.

In seven separate experiments, standard curves were produced in duplicate by dilution of a known ammonia concentration. The data are given in Table A1.1, Appendix 1. The coefficient of variation across these standard curves was 1.5% for both high and low standard concentrations.

Blood samples were then taken from young healthy volunteers. Each sample was analysed on 2 separate occasions on the same day. This data is given in **Table 4.2**.

Table 4.2: Blood samples from healthy subjects (n=10)

Each sample has been analysed on 2 separate occasions (analysis 1 and analysis 2) to establish reproducibility of ammonia analysis technique.

Subject	Ammonia concentration in analysis 1 ( $\mu\text{mol/l}$ )	Ammonia concentration in analysis 2 ( $\mu\text{mol/l}$ )	Difference between 2 measurements ( $\mu\text{mol/l}$ )
2	34.1	34.1	0.0
3	44.3	44.3	0.0
4	13.6	20.4	6.8
5	23.8	20.4	-3.4
6	44.3	40.9	-3.4
7	23.8	20.4	-3.4
8	64.7	68.1	3.4
9	88.6	88.6	0.0
10	47.7	44.3	-3.4

Difference was calculated by subtracting the first measurement from the second. Data has been used to calculate paired t-test and intra-class correlation (ICC). ICC gives an expression of how within patient variability compares with between patient variability (Altman, 1991). A value for ICC of 0.7 or greater indicates acceptable reproducibility.

Ammonia concentration for each sample was not significantly different between the 2 separate analyses [mean (SD) difference 0.3(3.4) $\mu\text{mol/l}$ , paired t-test  $p=0.758$ ] and demonstrated excellent level of agreement [intra-class correlation, ICC=0.99 (95%CI 0.96,1.00)]. This indicates that the ammonia analysis technique used has good repeatability and test-retest reliability.

#### **4.2.3 Analysis of biological variability in ammonia measurement**

Normal variability for resting ammonia was determined in 11 sedentary healthy subjects (mean (SD) age 41(8)years, 6 male). Plasma ammonia was measured on two occasions 20 minutes apart, and analysed in duplicate to determine biological variability. Variability over 20 minutes was thought to be relevant because during each study in this Thesis, measurements for ammonia concentration throughout an exercise challenge were taken over a similar time-span. The plasma ammonia concentrations obtained are given in **Table 4.3**. Mean (SD) difference between repeat measurements for ammonia concentration in resting sedentary healthy volunteers over 20 minutes was 1.2 (7.8) $\mu\text{mol/l}$ . Using a paired t-test there was no significant difference between the 2 readings ( $p=0.615$ ). The ICC between the measurements from the normal volunteers was 0.91 (95%CI 0.7, 0.97) demonstrating good agreement. Limits of agreement (mean  $\pm$  2SD of difference) were analysed to determine repeatability error of the test in order to assess the variability in resting ammonia concentration and were +14.4 and  $-16.8\mu\text{mol/l}$ .

#### **4.2.4 Determination of ammonia response to exercise**

In my first study, I observed *post hoc* two separate ammonia responses to exercise, namely a rise above or below 15 $\mu\text{mol/l}$ . I defined this as ammonia response and non-response respectively and have subsequently investigated this prospectively. The ammonia

concentration cut off at 15 $\mu$ mol/l clinically distinguishing the groups is a similar concentration to that I observed statistically in analysis of biological variability. This supports the biological plausibility of my clinical findings, which approximate to the reproducibility of the test.

#### **4.2.5 Lactate analysis**

Whole blood lactate concentrations were analysed in duplicate immediately following exercise using a bench-top analyser (YSI 1500 sport l-lactate analyser, YSI Inc, USA). The coefficient of variation for lactate determined from standards was 2%. The lactate machine was calibrated daily and calibration repeated every 20 samples analysed using a standard of known concentration (5mmol/L). Standard curves to ensure linearity using a high and low standard of known concentration (5mmol/l and 15mmol/l) were performed each time the machine was used.

#### **4.2.6 Muscle biopsy analysis**

Whilst the analysis and interpretation of muscle biopsy data was performed myself, I did not perform the laboratory work. Samples were stored and transferred to University of Nottingham on completion of the study for analysis. Methodology is described in Appendix 2.

### **4.3 Statistical analysis**

Statistical advice has been sought from the Trent Institute [Altman DG, 1980; Altman DG, 1991; Bland JM *et al*, 1986; Chinn S, 1991; Matthews JN *et al*, 1990; Stratford PW, 2004]. Statistical analysis has been performed using SPSS version 14/15 (SPSS Inc Chicago, USA).

Table 4.3: Plasma ammonia concentration ( $\mu\text{mol/l}$ ) at rest, taken at time 0 and 20 minutes later in 11 sedentary healthy volunteers [mean (SD) age 41(8)years]

Subject	Ammonia concentration at time 0mins ( $\mu\text{mol/l}$ )	Ammonia concentration at time 20mins ( $\mu\text{mol/l}$ )	Average ammonia concentration ( $\mu\text{mol/l}$ )	Ammonia change ( $\mu\text{mol/l}$ )
1	44.3	34.1	39.2	-10.2
2	32.4	39.2	35.8	6.8
3	81.8	86.9	84.4	5.1
4	44.3	35.8	40.1	-8.5
5	64.7	64.7	64.7	0.0
6	39.2	35.8	37.5	-3.4
7	59.6	47.7	53.7	-11.9
8	63.0	57.9	60.5	-5.1
9	59.6	54.5	57.1	-5.1
10	39.2	47.7	43.5	8.5
11	76.6	86.9	81.8	10.3

Ammonia change is calculated by subtracting the first measurement from the second.

## Chapter Five: The plasma ammonia response to cycle exercise in COPD

In the first study of this Thesis, I have used the ammonia analysis technique that I have developed (see section 4.2) to characterise the pattern of plasma ammonia response to cycle exercise in COPD patients. The purpose of this approach was to develop plasma ammonia as tool that could be used as a marker of energy metabolism during exercise in COPD, and the plasma ammonia changes with exercise have therefore been compared to muscle adenine nucleotide changes using muscle biopsies.

### **5.1 Introduction**

Abnormal peripheral muscle function has been identified as an important contributor to exercise intolerance in patients with COPD. This is independent of disease severity and linked to disability, poor quality of life and survival [American Thoracic Society, 1999]. Quadriceps muscle samples, taken from COPD patients at rest, show reductions in oxidative enzyme activity and the proportion of type I fibres compared with age-matched healthy controls [Gosker HR, 2002a; Maltais F, 2000b; Whittom F, 1998]. An accelerated rise in blood lactate during exercise in COPD has been reported compared to control subjects, and is associated with a reduction in quadriceps muscle mitochondrial enzyme activity [Maltais F, 1996b]. This implies that resynthesis of adenosine 5-triphosphate (ATP) during muscle contraction from oxidative sources is impaired in COPD with a consequent increase in non-oxidative metabolism and presumably fatigue during exercise. Importantly, this impairment in skeletal muscle energy metabolism may be a remediable feature of an otherwise largely irreversible pulmonary disease.

It has previously been shown in COPD that ATP degradation and inosine 5-monophosphate (IMP) accumulation in skeletal muscle during exercise occurs despite the significantly lower absolute workrates that these individuals can achieve [Steiner MC, 2005a]. This suggests that metabolic stress occurs in patients with COPD at these low absolute workrates that may be relevant to their activities of daily living. However, there was significant inter-individual variability in the magnitude of the metabolic response, and further understanding of the characteristics and mechanisms underlying the skeletal muscle metabolic response to exercise is currently required. Measurement of metabolic events during exercise may be an important investigational tool, but obtaining muscle biopsies during exercise is technically difficult in this frail elderly population and therefore not practical for larger clinical trials. Alternative methods for studying the metabolic response are therefore needed.

During intense exercise, ATP degradation occurs when oxidative and non-oxidative ATP re-synthesis fail to meet ATP demand. This is associated with accumulation of IMP as a result of irreversible deamination of adenosine 5-monophosphate (AMP) in exercising skeletal muscle. This process has been described in the literature as metabolic stress [Dudley GA, 1985; Pouw EM, 1998b]. During this reaction, ammonia is produced in stoichiometry with IMP and released into the bloodstream. In young healthy adults blood ammonia concentration has been shown to increase during incremental exercise only when high intensities are reached [Buono MJ, 1984; Graham TE, 1990] and this has been implicated in development of fatigue and physical exhaustion [Mutch BJ, 1983]. Although plasma ammonia has been shown to closely reflect muscle adenine nucleotide metabolism in healthy subjects [Mutch BJ, 1983], the ammonia response to exercise in subjects with COPD has not been reported.

I hypothesised that, in COPD subjects, changes in ammonia concentration during exercise would reflect adenine nucleotide metabolism within skeletal muscle, and provide a useful marker of skeletal muscle energy metabolism that is less invasive than obtaining a muscle biopsy. In this study I have examined the plasma ammonia response to both incremental and constant workrate (WR) cycle exercise in COPD, and explored the relationship between plasma ammonia concentration and skeletal muscle adenine nucleotide changes.

## **5.2 Methods**

Stable patients with COPD (aged 50-85 years, n=25) who met GOLD criteria [Rabe KF *et al*, 2007] were recruited from outpatient clinics. Patients were excluded if taking maintenance oral corticosteroids, were unable to perform exercise tests, demonstrated exercise desaturation ( $\text{SaO}_2 < 85\%$ ), had significant cardiac dysfunction, an exacerbation of COPD within the previous 6 weeks or pulmonary rehabilitation within the last year. Similar-aged healthy controls (n=13) were recruited by local advertisement and screened for abnormal lung function and significant cardiac or respiratory disease. Full approval was obtained from the Leicestershire Research Ethics Committee and all participants provided informed written consent.

*Study design:* Participants attended an initial visit to collect baseline data and familiarise with the exercise test. Spirometry, BMI, FFM, isometric quadriceps force and physical activity score were measured as described in Chapter 4. On a subsequent visit at least 72 hours later, subjects performed a maximal (symptom-limited) incremental exercise test on an electrically braked cycle ergometer. A week later subjects performed a constant WR exercise challenge. The details of the exercise tests are provided in Chapter 4. Arterialised-venous blood samples were taken at rest (subjects rested on couch for 30

minutes), every minute during exercise to peak exercise, and at two and five minutes after exercise. Blood was analysed for ammonia and lactate as described in Chapter 4.

Muscle biopsies of the vastus lateralis (Bergstrom technique [Bergstrom J, 1975]) were taken at rest and immediately post-exercise following the constant WR challenge. This meant that muscle samples were taken following an exercise challenge of the same relative intensity for all subjects. Muscle samples were stored and analysed as described in Chapter 4.

*Statistical analysis:* Between-group comparisons were made using the Student's unpaired t-test or Mann Whitney-U test when not-normally distributed. Within-group comparisons are made using paired t-tests. Correlations between parameters were calculated with Pearson's or Spearman's correlation tests. Data were analysed using SPSS package version 14.0 (SPSS Inc Chicago, USA). Significance was assumed at  $p < 0.05$ .

### **5.3 Results**

#### ***Patient characteristics***

A total of 25 patients with COPD and 13 similar-aged controls were included. One COPD patient dropped out after familiarisation and was not included in analyses. Missing data in the incremental test were due to equipment failure (2 controls, 2 COPD) and insufficient blood for accurate analysis (1 control). Missing data in the constant WR test were due to equipment failure (1 control, 3 COPD) intolerance of procedure (2 COPD), and insufficient biopsy material (3 control, 7 COPD). Baseline characteristics for COPD and control subjects are shown in **Table 5.1A** and are presented as mean when normally distributed and median when not. Demographically the groups were well matched apart from FEV<sub>1</sub> and physical activity score, which were expected.

Table 5.1: Baseline characteristics (A) and exercise data from incremental cycle test (B) for similar-aged control subjects and COPD subjects

A	Controls n=13	COPD n=25
Age	68 (7)	69 (7)
Gender	10 m	20 m
FEV <sub>1</sub> (% pred)	101 (16)	47 (12)*
FEV <sub>1</sub>	2.88 (0.71)	1.21 (0.29)*
Isometric quadriceps strength (Nm)	155 (52)	130 (47)
FFMI (Kg/m <sup>2</sup> )	19 (3)	18 (2)
BMI (Kg/m <sup>2</sup> )	26 (4)	27 (4)
Physical activity score <sup>‡</sup>	15.7 (10.3)	5.7 (5.8)*

B

Heart rate (% pred)	93 (7)	73 (10)*
Peak WR (watts)	156 (46)	67 (20)*
VO <sub>2 peak</sub> (ml/kg/min)	28.50 (8.25)	17.21 (3.41)*
Peak V <sub>E</sub> (L/min)	74 (21)	37 (10)*
Peak V <sub>E</sub> (% MVV)	75 (18)	91 (23) <sup>†</sup>
Peak RQ	1.14 (0.15)	0.96 (0.07)
Peak PE <sup>‡</sup>	16 (6)	16 (9)

Expressed as mean (SD) unless stated: <sup>‡</sup> Median (IQR)

<sup>†</sup> p<0.05, \* p<0.001 compared with controls

BMI= body mass index; FFMI= fat free mass index; VO<sub>2 peak</sub>= peak oxygen uptake; Peak V<sub>E</sub> = peak ventilation; Peak RQ= peak respiratory quotient; Peak PE= perceived exertion at peak exercise, MVV= maximum voluntary ventilation (calculated as FEV<sub>1</sub> x 35), WR = workrate.

### ***Incremental exercise***

Data from the incremental exercise test is shown in **Table 5.1B**. Peak WR [mean (SD)] was significantly lower in subjects with COPD [67.2(20.5)Watts] than similar-aged controls [156.2(45.7)Watts],  $p<0.001$ . Peak ventilation was significantly increased in subjects with COPD compared with similar-aged controls, and 10 COPD subjects were ventilatory limited.

**Tables 5.2A** and **5.2B** show mean (SD) plasma ammonia and blood lactate responses to incremental cycle exercise. Resting plasma ammonia concentrations were similar for COPD and similar-aged subjects and within published ranges [Babij P, 1983; Buono MJ, 1984; Dudley GA, 1985]. Plasma ammonia concentration increased during exercise in subjects with COPD ( $p<0.001$ ) and similar-aged subjects ( $p<0.001$ ) and continued to increase at 2 minutes after exercise before declining towards baseline at 5 minutes after exercise.

In COPD subjects two distinct patterns of response appeared when the plasma ammonia increase with incremental exercise was plotted against peak oxygen uptake [**Figure 5.1A**]. In one group of COPD subjects plasma ammonia increased significantly with exercise, and change in ammonia concentration correlated with peak oxygen uptake [Pearson correlation  $r=0.56$ ,  $p=0.03$ ]. The ammonia increase in control subjects also correlated with oxygen consumption [ $r= 0.56$ ,  $p=0.07$ ]. In a second group of subjects with COPD, ammonia did not rise with exercise despite subjects achieving similar peak oxygen uptake [mean(SD) 17.1(4.2)ml/kg/min versus 17.3(3.0)ml/kg/min ( $p=0.88$ ) in group with ammonia rise] and peak WR [63(19)Watts versus 69(21)Watts ( $p=0.45$ ) in group with ammonia rise].

Table 5.2: Plasma ammonia and blood lactate concentrations at rest and in response to incremental exercise in all COPD subjects (n=24) and age-matched controls (n=12) [A] and in COPD subjects with [group 1:n=15, peak work 69(21) Watts] and without [group 2:n=9, peak work 63(19) Watts] an ammonia increase with exercise [B].

A		Rest	Peak exercise	2min recovery	5min recovery	Peak change <sup>++</sup>
<b>Ammonia</b> <b>Mean (SD)</b> <b>µmol/l</b>	Control	63.7 (16.5)	106.2 (30.9)*	109.3(26.7)*	95.8(26.6) *	55.5 (7.0)
	All COPD	56.5 (13.4)	80.4 (21.3)*	81.9(21.8)*	70.0(18.1) *	28.7(4.3) <sup>+</sup>
<b>Lactate</b> <b>Mean (SD)</b> <b>mmol/l</b>	Control	0.64 (0.16)	2.96 (0.73)*	3.67(0.83)*	3.44(1.2) *	3.17 (0.27)
	All COPD	0.72 (0.25)	1.94(0.83)*	2.28(0.91)*	2.05(0.84) *	1.64(0.82) <sup>+</sup>
<b>B</b>						
<b>Ammonia</b> <b>Mean (SD)</b> <b>µmol/l</b>	COPD group 1	55.9 (12.5)	91.3 (18.4)*	93.0 (19.2)*	77.1(18.7) *	42.8 (3.3)
	COPD group 2	60.8 (14.4)	62.1 (10.5)	64.5 (12.2)	58.2(8.7)	5.1 (1.1) <sup>++</sup>
<b>Lactate</b> <b>Mean (SD)</b> <b>mmol/l</b>	COPD group 1	0.70 (0.23)	2.00 (0.89)*	2.36 (0.97)*	2.18(0.91) *	1.78 (0.21) <sup>+</sup>
	COPD group 2	0.77 (0.30)	1.84 (0.73)**	2.16 (0.88)*	1.81(0.68) *	1.39 (0.30) <sup>+</sup>

Expressed as mean (SD). Group 1= COPD subjects with ammonia response during exercise, Group 2= COPD subjects without ammonia response during exercise

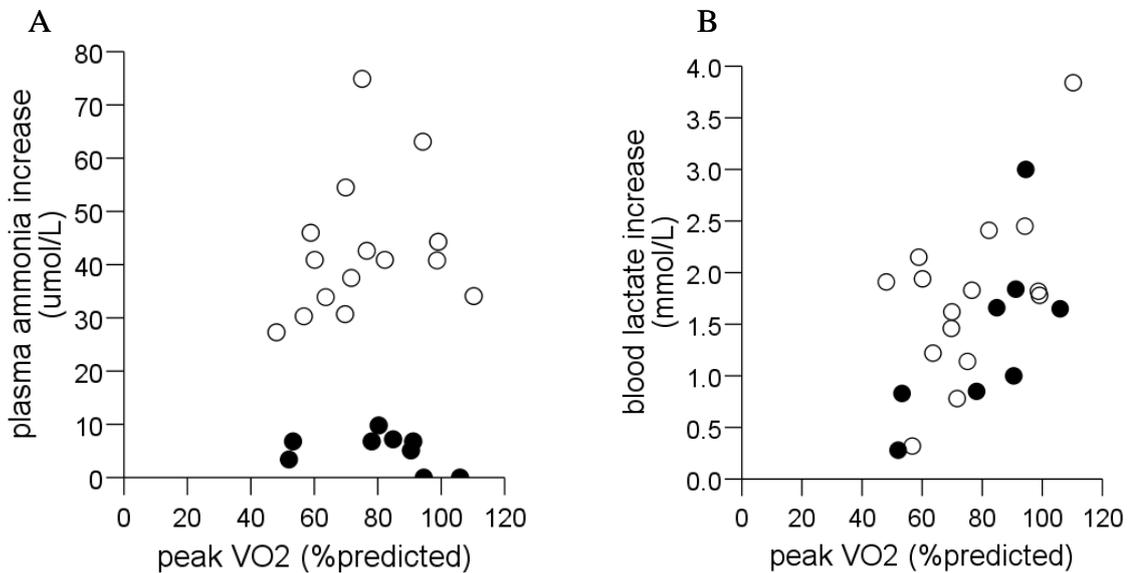
\*p<0.001 \*\*p<0.01 Within group, compared with resting

<sup>+</sup>p<0.001 Between COPD and control group analysis of peak change

<sup>†</sup>p<0.001 Between COPD group1 and group2 analysis of peak change

<sup>++</sup>Calculated as peak concentration minus resting concentration

Figure 5.1: Plot of peak oxygen uptake ( $\text{VO}_2$  in ml/kg/min expressed as % predicted) and maximum plasma ammonia change ( $\mu\text{mol/l}$ ) [A] or maximum blood lactate change (mmol/l) [B] following maximal incremental cycle exercise in COPD subjects ( $n=24$ ). Subjects with an ammonia response are indicated by open circles ( $n=15$ ); subjects without an ammonia response are indicated by closed circles ( $n=9$ )

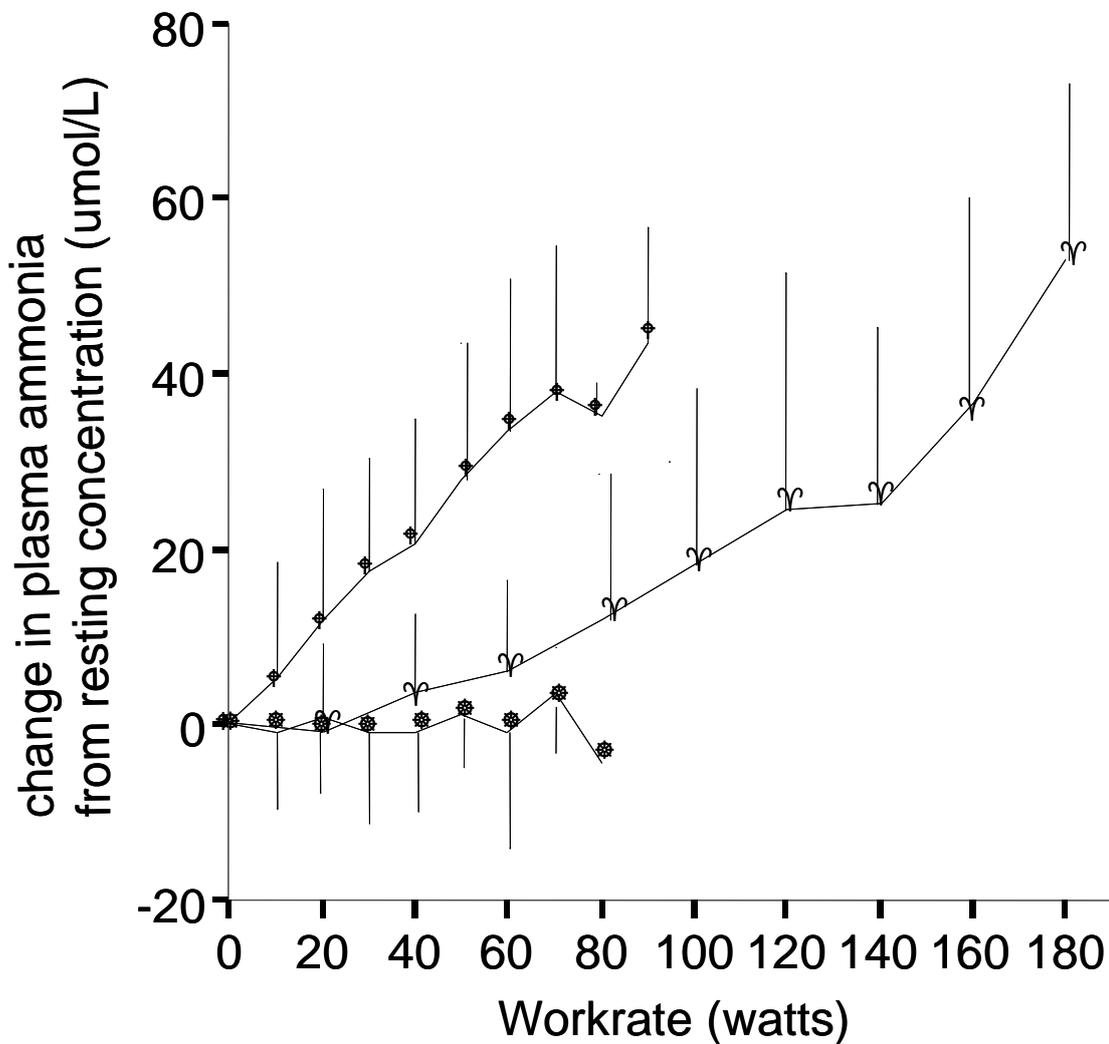


However, no such differential response was seen with blood lactate [**Figure 5.1B**], and the change in blood lactate during exercise was not significantly different between the two groups of COPD subjects ( $p=0.30$ ) [**Table 5.2B**]. The magnitude of blood lactate accumulation was correlated with peak oxygen uptake as expected [ $r= 0.55$ ,  $p=0.007$ ]. The differential ammonia response in COPD subjects could not be predicted from demographic variables, medication or exercise parameters, including limitations to exercise and ventilatory limitation, which were not significantly different between the two groups.

**Figure 5.2** shows the pattern of change in plasma ammonia from resting concentration to peak WR during incremental exercise for all control subjects, COPD subjects with an ammonia rise and COPD subjects with no ammonia rise. In age-matched controls, plasma ammonia concentration remained near resting concentration at low WRs. At higher intensity exercise, ammonia concentration increased with increasing WR. In COPD subjects with an ammonia response, plasma ammonia concentration increased from the onset of exercise and continued to rise with increasing WR.

The increase in plasma ammonia concentration during exercise [mean (SE)  $42.8(3.3)\mu\text{mol/l}$ ] in COPD subjects with a response ( $n=15$ ) was not significantly different to similar-aged controls [mean (SE)  $55.5(7.0)\mu\text{mol/l}$ ] ( $p =0.12$ ), despite the significantly lower absolute peak WR achieved [**Tables 5.2A and 5.2B**]. However, the increase in blood lactate in these COPD subjects was significantly lower than control subjects ( $p<0.001$ ). In the COPD group with no measurable ammonia increase ( $n=9$ ), the change in plasma ammonia concentration was within repeatability of the measurement (see section 4.2.3).

Figure 5.2: Mean (SD) change in plasma ammonia concentration ( $\mu\text{mol/l}$ ) from resting concentration during incremental exercise in COPD subjects with ammonia increase (closed circle,  $n=15$ ), COPD subjects without ammonia increase (closed square,  $n=9$ ) and similar-aged controls (closed diamond,  $n=12$ )



Unlike my findings in similar-aged subjects, where there was a linear relationship between peak ammonia and lactate concentrations [ $r = 0.61$ ,  $p = 0.046$ ], there was no relationship between plasma ammonia and blood lactate concentration in all subjects with COPD [ $r = 0.02$ ,  $p = 0.938$ ].

### ***Constant WR exercise***

Subjects with or without a plasma ammonia increase in the incremental exercise challenge had a consistent response in the constant WR exercise challenge. Change in plasma ammonia concentration during incremental exercise strongly correlated with plasma ammonia change during constant WR exercise in all subjects with COPD [**Figure 5.3**:  $r = 0.88$ ,  $p < 0.001$ ].

*Biopsy data:* ATP degradation and IMP accumulation occurred in skeletal muscle during constant WR exercise in all COPD subjects [ $n = 14$  mean (SD) change  $-3.11(1.41)$  mmol/kg dry weight,  $p = 0.046$  and  $0.58(0.23)$  mmol/kg dry weight,  $p = 0.029$ , respectively] and similar-aged controls [ $n = 9$ , mean(SD) change  $-4.44(1.42)$  mmol/kg dry weight,  $p = 0.019$ , and  $2.86(0.81)$  mmol/kg dry weight,  $p = 0.01$ , respectively]. The absolute WRs were significantly different between COPD subjects and similar-aged controls [mean (SD)  $52(17)$  Watts and  $128(38)$  Watts respectively,  $p < 0.001$ ]. PCr and PCR/Cr ratio fell significantly and to a similar extent in COPD subjects and controls. PCr concentrations pre and post exercise were  $72.1(12.8)$  mmol/kg and  $53.4(18.3)$  mmol/kg dry weight respectively in COPD subjects and  $70.9(7.4)$  mmol/kg and  $40.0(10.3)$  mmol/kg dry weight respectively in controls. PCr/Cr ratios pre and post exercise were  $1.36(0.33)$  and  $0.80(0.36)$  respectively in COPD subjects and  $1.43(0.23)$  and  $0.53(0.21)$  respectively in controls. Insufficient tissue was available for analysis of other purine nucleotide derivatives.

Figure 5.3: Scatter to show correlation between maximum plasma ammonia change ( $\mu\text{mol/l}$ ) during maximal incremental and sub-maximal constant workrate cycle exercise in COPD subjects ( $n=21$ );  $r=0.875$ ,  $p<0.001$ . Open circles indicate COPD subjects with ammonia response ( $n=12$ ), closed circles indicate COPD subjects without ammonia response ( $n=9$ )

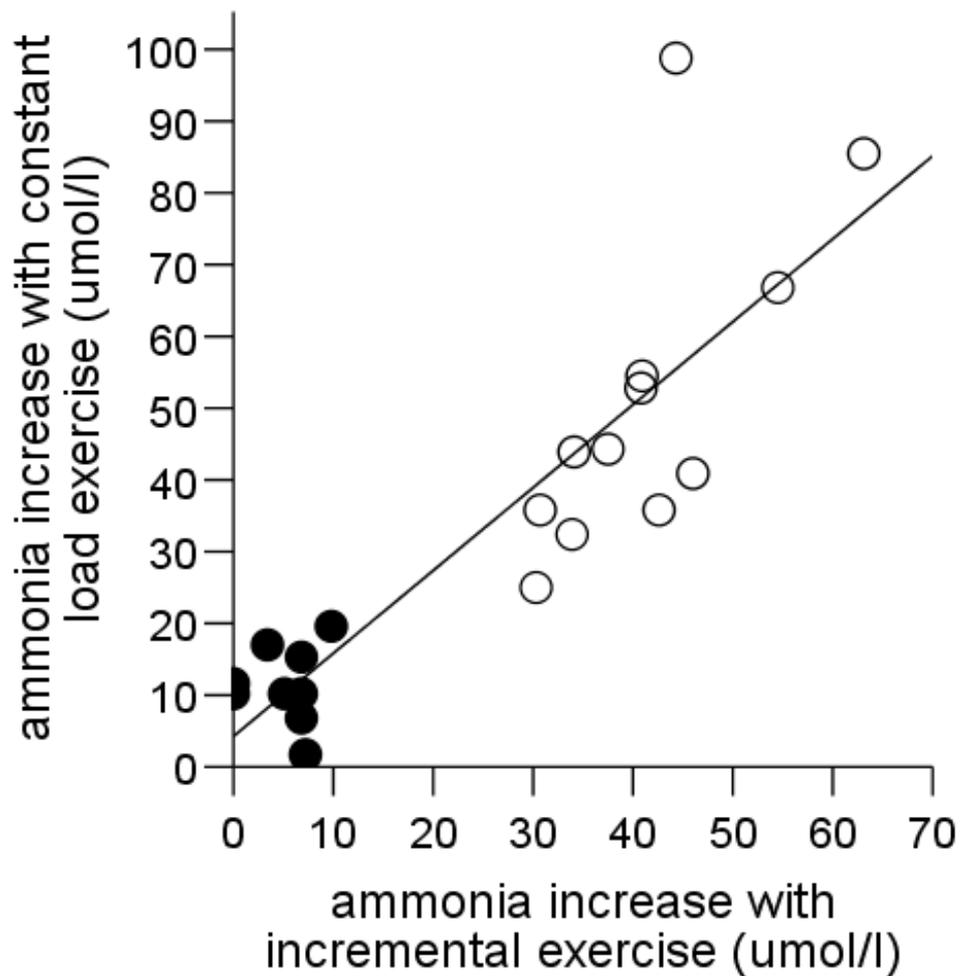
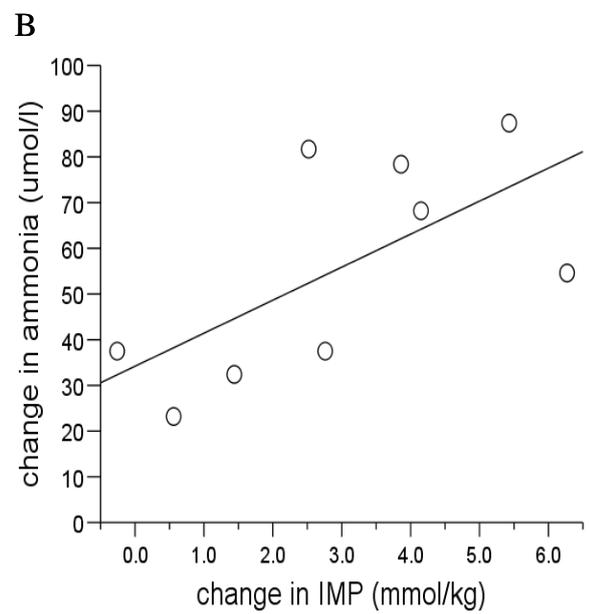
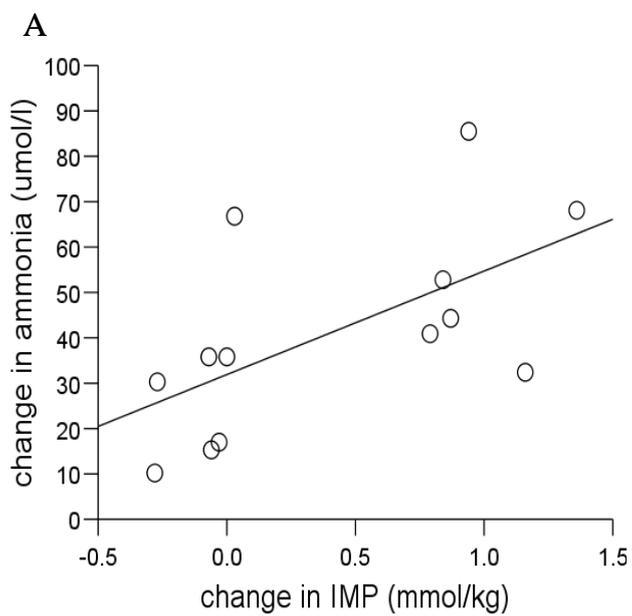


Figure 5.4: Correlation between change in plasma ammonia concentration and quadriceps muscle IMP accumulation in response to sub-maximal constant workrate cycle exercise

A: COPD subjects (n=13),  $r = 0.61$ ,  $p = 0.029$

B: Similar-aged control subjects (n=9),  $r = 0.66$ ,  $p = 0.055$



No statistically significant differences were seen in the exercise-induced change in muscle metabolites between the two COPD subgroups (with and without an ammonia response), although because of missing biopsy data, numbers were small.

Correlation existed between muscle IMP accumulation and plasma ammonia increase in subjects with COPD [ $r = 0.61$ ,  $p = 0.029$ ] and similar-aged controls [ $r = 0.66$ ,  $p = 0.055$ ]. **Figures 5.4A and 5.4B** demonstrate these correlations graphically. No correlation was found between plasma ammonia increase and muscle ATP degradation or between muscle IMP accumulation and ATP degradation in either COPD or control subjects.

#### **5.4 Discussion**

This study is the first to describe the plasma ammonia response to cycle exercise in COPD. Overall, I found a significant exercise-induced increase in plasma ammonia concentration, which began early in exercise and peaked 2 minutes after exercise. Similar-aged controls displayed a curvilinear ammonia response to incremental exercise, which was similar to findings documented in the literature for young healthy subjects [Buono MJ, 1984; Graham TE, 1990]. However, there was a differential ammonia response to exercise in the COPD cohort. In one subgroup, the increase in plasma ammonia from rest to end-exercise was similar to controls despite significantly lower peak WRs. The other subgroup of COPD subjects did not demonstrate an increase in plasma ammonia concentration despite having a rise in blood lactate concentration.

Failure of energy delivery by oxidative and anaerobic ATP re-synthesis to meet the demands of muscle force generation results in an increase in ADP and AMP, and activation of AMP deaminase. The irreversible deamination of AMP leads to accumulation of IMP in exercising muscles and release of ammonia into the bloodstream. In the short

term, by preventing excessive accumulation of ADP and AMP, this increases the phosphorylation potential of the adenine nucleotide pool allowing the adenylate kinase reaction and contraction to continue. Such a situation is not sustainable because of the resulting accumulation of ADP and decline in ATP availability. Substantial metabolic stress is said to have occurred under these conditions [Dudley GA, 1985; Steiner MC, 2005a] and is associated with fatigue in healthy subjects [Broberg S, 1989; Sahlin K, 1989a]. It has recently been shown that ATP loss and IMP accumulation in muscle occurs at significantly lower absolute WRs in subjects with COPD than healthy subjects [Steiner MC, 2005a]. Data from the present study supports these findings and demonstrates a rise in ammonia with exercise at lower absolute WRs compared with similar-aged control subjects. This data supports previous observations that skeletal muscles in subjects with COPD are working under conditions of metabolic stress at low absolute work intensities similar to those required for activities of daily living [Steiner MC, 2005a]. The increase in plasma ammonia correlated with muscle IMP accumulation in constant WR exercise supporting my hypothesis that plasma ammonia may be a useful marker of the nucleotide metabolic response within skeletal muscle.

The main source of plasma ammonia produced in skeletal muscles during intense exercise is from deamination of AMP, which constitutes part of the purine nucleotide cycle (PNC). It has been demonstrated in humans that the activity of the PNC and blood ammonia production is predominantly a reflection of fast twitch (type II) fibre activity during short-term intense exercise [Graham TE, 1992]. Dudley and co-workers reported an inverse relationship between the proportion of slow twitch (type I) fibres of the vastus lateralis muscle and ammonia increase during intense exercise in healthy subjects [Dudley GA, 1983]. My findings of an early increase in plasma ammonia in incremental exercise in

subjects with COPD suggest that fast twitch fibre recruitment is occurring at low WRs in this population. Atrophy of type I fibres and an increased proportion of type II fibres in skeletal muscle samples taken at rest in patients with COPD is well recognised [Gosker HR, 2002a; Whittom F, 1998].

A reduction in oxidative enzyme concentrations in muscles of subjects with COPD has been demonstrated at rest [Maltais F, 1996b; Maltais F, 2000b]. This implies either reduced oxidative ATP metabolism and/or increased reliance on anaerobic ATP resynthesis during exercise, or a preferential atrophy of oxidative muscle fibres. Increased reliance on glycolytic metabolism has recently been associated with contractile fatigue following cycle exercise [Saey D, 2005]. Further support for this is provided by Maltais and colleagues, who showed an early and accelerated blood lactate accumulation during incremental exercise in severe COPD [Maltais F, 1996b]. Although blood lactate concentration can be used as a marker of metabolic response to exercise, plasma ammonia may more closely reflect changes in adenine nucleotide metabolism occurring under conditions of metabolic stress.

It was unclear from my data why some subjects with COPD failed to display an ammonia response during exercise. I was unable to identify differences in demographics, disease severity or the pattern of exercise response between these patients and those who did show a rise in ammonia. Missing biopsy samples rendered interpretation of exercise-induced metabolite changes difficult between the two COPD subgroups because of the small sample size. However, an increase in blood lactate does not necessarily have to be matched by an increase in plasma ammonia. One possibility is that in patients without a rise in ammonia, the ATP demands of contraction were being met, and fatigue was attributable to another factor not associated with the failure of energy delivery. An

alternative explanation is that these patients may have differed in muscle fibre composition, such that considerably less ammonia was generated. In this respect, human slow twitch muscle fibres are known to have considerably less deamination of AMP to IMP, and therefore less ammonia generation. Previous literature in COPD has suggested a shift in fibre composition towards a greater proportion of type II fibres [Gosker HR, 2002a; Whittom F, 1998] but this phenomenon may vary considerably across the COPD population and it is possible that patients not showing a rise in ammonia were those with better preservation of type I (slow twitch) fibres. This was a post-hoc analysis and as such does need to be confirmed in future studies together with measurements of muscle fibre composition, blood flow and oxidative enzyme concentrations to explain these observations.

A number of limitations to the current study are acknowledged, particularly in interpreting the muscle biopsy data. As found in previous studies on COPD subjects, tissue from biopsies taken immediately post-exercise was small and in some cases inadequate for complete analysis. This highlights the technical difficulties and limitations in using muscle biopsies to investigate the metabolic response to exercise in COPD. Biopsies were taken following constant WR exercise, which has been shown in previous work [Steiner MC, 2005a] to induce skeletal muscle metabolic stress. Because the WR for the constant WR test was determined by performance during the incremental test, the metabolic response to exercise measured in the muscles will have been influenced by the limit to maximal performance. This highlights some of the problems with standardising sub-maximal exercise tests for studies. However, an intensity of 80% work achieved in the maximal incremental test was felt to be appropriate for several reasons. It reflects the intensity at which patients perform endurance training in pulmonary rehabilitation

programmes and therefore has some practical relevance. In addition, similar work intensities have been demonstrated to stimulate an ammonia response to sub-maximal exercise in healthy subjects [Graham TE, 1992]. Although I did not measure muscle ammonia directly in this study, a strong relationship has been shown to exist between muscle adenine nucleotide loss and plasma ammonia accumulation [Harris RC *et al*, 1991]. Measurements from forearm arterialised blood may be less sensitive than those taken from femoral venous blood as this directly drains the exercising muscles, but the aim of this study was to evaluate less invasive measurements of ammonia that may be practical as an investigational tool in clinical studies. For this reason I did not undertake femoral venous cannulation. Finally, whilst my control group were not physically well trained and were representative of the healthy elderly population, they were significantly more active than the COPD group. Thus I was unable to distinguish the effects of loss of fitness due to inactivity from other aspects of the disease on the aetiology of my observations.

COPD is a leading cause of disability worldwide and places an increasing burden on healthcare resources [Rabe KF, 2007]. Peripheral muscle dysfunction and in particular impaired energy metabolism may prove an important remediable source of exercise intolerance in this population despite largely irreversible lung impairment. In healthy subjects training increases ammonia workload threshold [Yuan Y, 2002] and a reduction in blood ammonia concentration appears to delay onset of fatigue and increase duration of intense exercise [Mutch BJ, 1983; Yuan Y, 2002]. It is feasible that similar results with training could be achieved in COPD patients who have an observed ammonia increase with exercise. Plasma ammonia may be a marker of metabolic stress in the skeletal muscles and therefore could be used as an outcome when assessing the impact of interventions targeting skeletal muscle energy metabolism such as pulmonary rehabilitation.

In conclusion, I have shown that plasma ammonia concentration increases during incremental and constant WR cycle exercise in subjects with COPD. Compared with similar-aged controls, similar peak exercise ammonia concentrations are reached despite significantly lower peak WRs. Plasma ammonia accumulation reflects muscle adenine nucleotide changes, suggesting it can be used as a surrogate to examine muscle energy metabolism. The observed differential ammonia response to cycle exercise appears to be distinct from the lactate response and may provide a useful clinical marker for investigating differences in skeletal muscle energy metabolism during exercise in COPD patients.

## Chapter Six: Plasma Ammonia Response to Cycling and Walking in COPD

In the second study of this Thesis, I have compared the ammonia response to cycling and walking, two exercise modalities that produce different metabolic loads. A secondary aim of this study was to confirm *ad hoc* the subgroup response identified in the previous study.

### **6.1 Introduction**

Exercise intolerance is a major factor limiting participation in activities of daily living in patients with COPD. It is now appreciated that lower limb skeletal muscle function has a significant impact on exercise capacity in COPD [American Thoracic Society, 1999]. Resting muscle biopsy and magnetic resonance studies have suggested that quadriceps muscle fibre type composition and mitochondrial function are altered in COPD implying that skeletal muscle energy delivery during exercise may be compromised [Gosker HR, 2002b; Gosker HR *et al*, 2007; Heunks LM, 1999; Maltais F, 2000b; Sala E, 1999]. In keeping with this, significant skeletal muscle adenine nucleotide loss and accumulation of purine nucleotide derivatives occurs during submaximal cycle exercise in COPD patients [Steiner MC, 2005a]. Intramuscular adenine nucleotide depletion occurs if adenosine 5-triphosphate (ATP) utilisation is not matched by its resynthesis. In healthy adults this occurs only during high intensity exercise and has been associated with contractile fatigue due to the failure of energy delivery. These circumstances have been referred to as ‘metabolic stress’ because the situation of imbalance between ATP utilisation and resynthesis is unsustainable [Dudley GA, 1985; Mutch BJ, 1983]. Importantly, the magnitude of adenine nucleotide loss observed in COPD patients was comparable to

similar aged healthy subjects, suggesting that the failure of energy delivery may contribute to fatigue in COPD despite the low absolute exercise workrates these individuals achieved.

A by-product of adenine nucleotide loss is the accumulation of blood ammonia resulting from the irreversible deamination of AMP to form IMP. Thus the accumulation of ammonia in the blood is an indicator of the energy status of the muscle [Babij P, 1983; Broberg S, 1989; Graham TE, 1992]. I have shown in the last chapter that significant blood ammonia accumulation occurs during incremental and constant load cycle exercise in COPD patients and that the magnitude of this rise was similar to healthy subjects, despite the substantially lower absolute workrates at which patients with COPD were exercising.

It is therefore conceivable that normal activities of daily living and the regular high intensity exercise entailed in pulmonary rehabilitation represent repeated bouts of skeletal muscle metabolic stress for some COPD patients.

An important factor in understanding the clinical and physiological implications of these observations is the exercise platform chosen to study these responses. Previous studies have indicated that the physiological and contractile responses to walking and cycling differ significantly [Cockcroft A *et al*, 1985; Man WD, 2003; Mathur RS *et al*, 1995; Palange P *et al*, 2000]. Cycling may be an unfamiliar mode of exercise for many patients in the UK whereas walking is not only more relevant to daily function but also the most frequent mode of exercise employed in outpatient pulmonary rehabilitation. However, the degree to which walking exercise imposes skeletal muscle metabolic stress in COPD has not been established. I therefore aimed to compare the skeletal muscle energy response to walking and cycling in a cohort of COPD patients with significant exercise limitation. To avoid the need for muscle biopsy immediately post-exercise (a technically challenging and invasive procedure) I used blood ammonia accumulation as a marker of

skeletal muscle adenine nucleotide depletion. In my previous study of the ammonia response I observed in a post-hoc analysis a subgroup of patients who did not demonstrate a significant rise in blood ammonia during exercise despite showing a rise in blood lactate. A second aim of this study was to determine whether this observation was reproducible and transferable between exercise platforms.

## **6.2 Methods**

*Study population:* Stable patients who met GOLD criteria for COPD [Rabe KF, 2007] were recruited from the pulmonary rehabilitation waiting list at Glenfield hospital. Patients were excluded if taking maintenance oral corticosteroids, had significant cardiac dysfunction, or had undergone pulmonary rehabilitation within the last two years. Full approval was obtained from the Leicestershire Research Ethics Committee and all participants provided informed written consent.

*Study design:* Subjects attended an initial visit for baseline measurements (spirometry and BMI) and familiarisation tests on the cycle and treadmill were performed. On a second visit at least 72 hours later, subjects performed a maximal symptom-limited incremental exercise test on an electrically-braked cycle ergometer. Subjects re-attended after a further 72 hours or more to perform a maximal symptom-limited treadmill exercise test. Blood was sampled for ammonia and lactate concentration during cycle and treadmill exercise tests at rest, at 1 minute and 2 minutes of exercise, at peak exercise, and at two minutes after exercise cessation. Timing for blood tests was determined from my previous study. Exercise tests and techniques for blood analysis are described in Chapter 4.

*Statistical analysis:* Normality of data was confirmed with Kolmogorov-Smirnov test. Significance was assumed at  $p < 0.05$ . Paired t-tests were used to evaluate differences

between cycle and treadmill. Correlations between parameters were calculated with Pearson's correlation tests (SPSS package version 15.0, SPSS Inc, Chicago, USA). Subgroups were defined *a priori* by exercise-induced ammonia rise with cycling above (group 1) or below (group 2) 15 $\mu$ mol/l. This was based on the 95% limit of agreement for resting variability of repeat measures of plasma ammonia (see Chapter 4). Between-group comparisons were made using independent sample t-tests.

### **6.3 Results**

#### ***Subject characteristics and exercise parameters***

Twenty-nine subjects (21 male) with COPD completed the study. Mean (SD) age was 68(7)years, FEV<sub>1</sub> 50(19)% predicted and 1.27(0.50)L, and BMI 28(7) kg/m<sup>2</sup>. Twenty subjects were ex-smokers and nine were current smokers. All patients reported breathlessness on exertion (MRC breathlessness score 5 [n=4] 4 [n=7], 3 [n=10] or 2 [n=8]).

Exercise data is shown in **Table 6.1**. In the cycle exercise test, 11 subjects were deemed ventilatory limited (defined as >90% maximum voluntary ventilation). The main cause of symptom limitation during cycling was either dyspnoea (n=11) or leg fatigue (n=12). In the walking exercise test, 12 subjects were deemed ventilatory limited and main cause of symptom limitation was dyspnoea (n=12) with fewer subjects complaining of leg fatigue (n=6).

#### ***Ammonia and lactate response to exercise***

**Table 6.2** shows plasma ammonia and blood lactate responses to cycle and treadmill walking exercise. In the COPD group as a whole, there was a significant (p<0.001) plasma ammonia and blood lactate rise with both cycling and treadmill walking.

Table 6.1: Exercise data for cycle and treadmill walking in COPD subjects (n=29)

	Bike	Treadmill
Peak workload (watts)	57 (20)	-
Distance (metres)	-	284 (175)
Exercise time (secs)	353 (141)	310 (124)
VO <sub>2</sub> max (ml/min/kg)	15.52 (4.56)	16.83 (4.16)*
V <sub>E</sub> max (L/min)	35.2 (11.0)	36.0 (9.8)
V <sub>E</sub> max (% MVV)	84 (23)	86 (20)
Heart rate (% predicted)	73 (11)	72 (9)
Peak oxygen saturation (%)	93 (4)	91 (5)**
Peak RER	0.96 (0.05)	0.89 (0.06)**
Peak BS ‡	4 (3-5)	4(4-7)
Peak PE ‡	15(14-17)	15(13-17)

Expressed as mean (SD) unless stated: ‡ Median (IQR)

\*p=0.019; \*\*p<0.001

VO<sub>2</sub>max= maximum oxygen uptake; V<sub>E</sub>max = Maximum ventilation; MVV=maximum voluntary ventilation (calculated as FEV<sub>1</sub> x 35); Peak RER= peak respiratory quotient; Peak BS=breathlessness measured by Borg score at peak exercise; Peak PE=perceived exertion at peak exercise

Table 6.2: Plasma ammonia and blood lactate concentrations at rest and in response to exercise in all COPD subjects (n=29)

		Rest	1minute exercise	2minute exercise	Peak exercise
<b>Ammonia concentration</b> <b>μmol/l</b>	Cycle	52.6 (14.6)	62.3 (17.9)*	68.7 (18.6)*	84.0 (23.5)*
	Treadmill	49.1 (16.3)	58.4 (21.5)*	62.4 (21.3)*	71.5 (25.1)* <sup>††</sup>
<b>Lactate concentration</b> <b>mmol/l</b>	Cycle	0.73 (0.15)	0.90 (0.21)*	1.01 (0.26)*	1.91 (0.67)*
	Treadmill	0.71 (0.19)	0.81 (0.26)*	0.92 (0.32)*	1.39 (0.68)* <sup>†</sup>

		2minute of recovery	Change over rest concentration <sup>+</sup>
<b>Ammonia concentration</b> <b>μmol/l</b>	Cycle	79.6 (23.3)*	35.2 (4.27)*
	Treadmill	63.7 (24.7)* <sup>††</sup>	24.7 (3.83)* <sup>††</sup>
<b>Lactate concentration</b> <b>mmol/l</b>	Cycle	2.07 (0.76)*	1.43 (0.12)*
	Treadmill	1.54 (0.81)* <sup>†</sup>	0.88 (0.14)* <sup>†</sup>

Mean (SD) values unless stated. <sup>+</sup> Mean (SEM)

\*p<0.001 Within group, compared with resting value

<sup>†</sup> p<0.001, <sup>††</sup>p<0.01 Between group cycle vs treadmill analysis

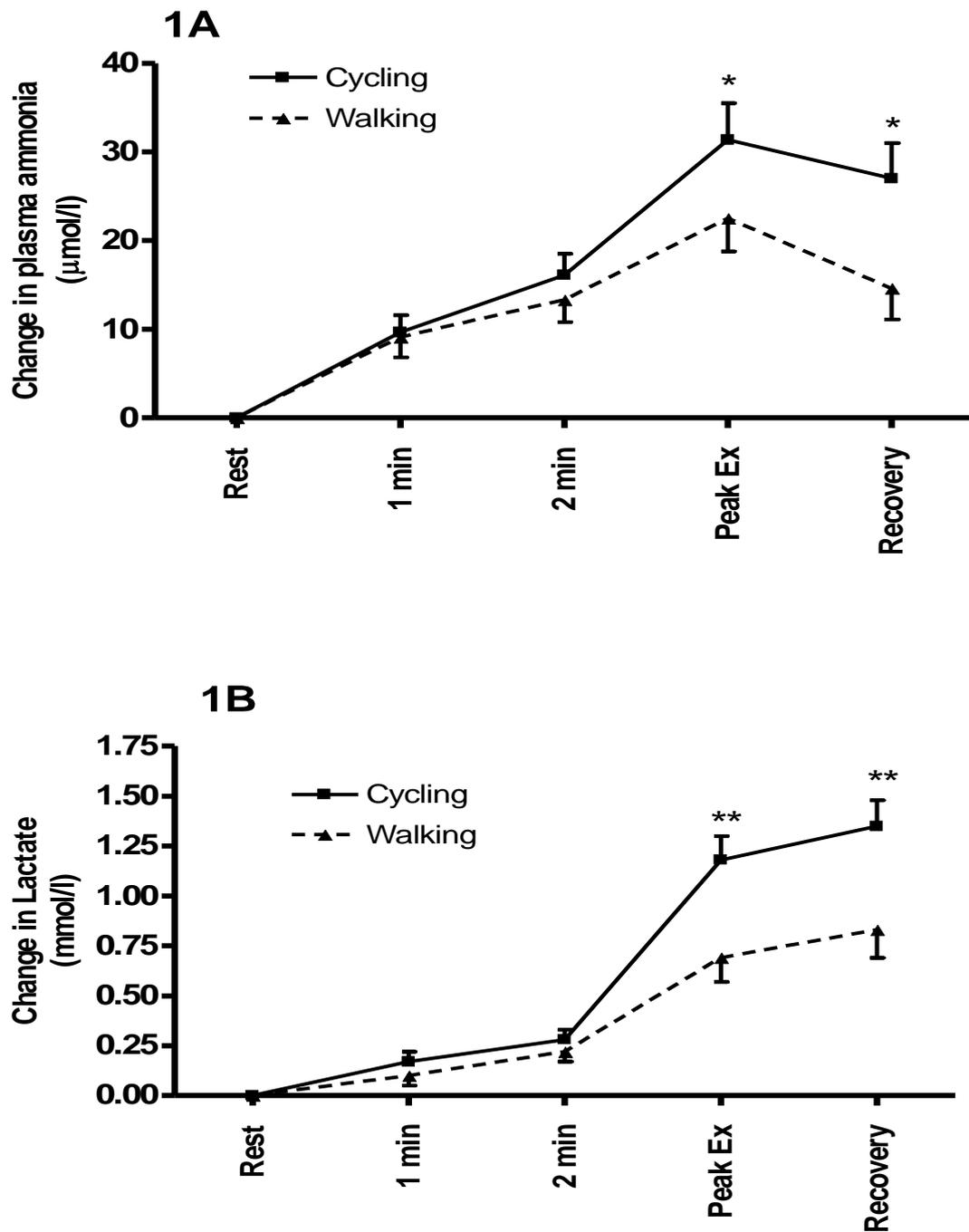
The pattern of plasma ammonia and blood lactate rise was similar for both exercise modalities, although peak exercise concentrations were statistically lower in treadmill walking (**Figure 6.1**). The rise in ammonia concentration correlated with peak work on cycle ( $r=0.66$ ,  $p<0.001$ ) and distance on treadmill ( $r=0.43$ ,  $p=0.02$ ). There was correlation between cycle and treadmill tests in ammonia rise ( $r=0.61$ ,  $p<0.001$ ) and lactate rise ( $r=0.74$ ,  $p<0.001$ ). There were no differences between patients who did or did not reach ventilation limitation in peak exercise indices, ammonia and lactate responses for both cycling and walking.

### ***Subgroup analysis***

Subjects who displayed an ammonia response to cycle exercise (group 1,  $n=22$ ) had a consistent response in treadmill walking except in three cases where exercise-induced ammonia change was below  $15\mu\text{mol/l}$  with walking. All subjects without an ammonia rise with cycling (group 2,  $n=7$ ) similarly did not have a response with walking (**Figure 6.2**). Peak work was significantly lower in group 2 in cycling but not in walking. There were no other significant differences in any measured demographic variable, medication, exercise parameter, ventilatory limitation or exercise-induced lactate rise between the 2 subgroups (**Table 6.3**).

Subgroup comparison of the ammonia and lactate response to exercise is shown in **Figure 6.3**. The mean(SEM) ammonia rise from resting values in group 1 was  $45.3(3.4)\mu\text{mol/l}$  (cycle) and  $31.9(3.9)\mu\text{mol/l}$  (treadmill). In group 2 ammonia change with exercise was  $3.5(1.3)\mu\text{mol/l}$  (bike) and  $2.3(2.3)\mu\text{mol/l}$  (treadmill). Blood lactate accumulation with exercise was not significantly different between group 1 and group 2 [mean(SEM)  $1.51(0.15)\text{mmol/l}$  and  $1.17(0.22)\text{mmol/l}$  respectively with cycling, ( $p=0.24$ ), and  $0.94(0.18)\text{mmol/l}$  and  $0.70(0.12)\text{mmol/l}$  respectively, ( $p=0.47$ ), with walking].

Figure 6.1: Mean (SE) change from resting concentration for plasma ammonia ( $\mu\text{mol/l}$ ) [1A] and blood lactate ( $\text{mmol/l}$ ) [1B] during incremental exercise in COPD subjects ( $n=29$ ) with cycling (closed square) and treadmill walking (closed triangle).



\* $p < 0.01$ , \*\* $p < 0.001$ , between cycle and treadmill analysis

Figure 6.2: Scatter to show exercise-induced change in ammonia concentration for individual subjects (n=29) achieved in cycling and treadmill exercise. The dotted line represents the threshold for definition of a significant ammonia response (see methods for more detail).

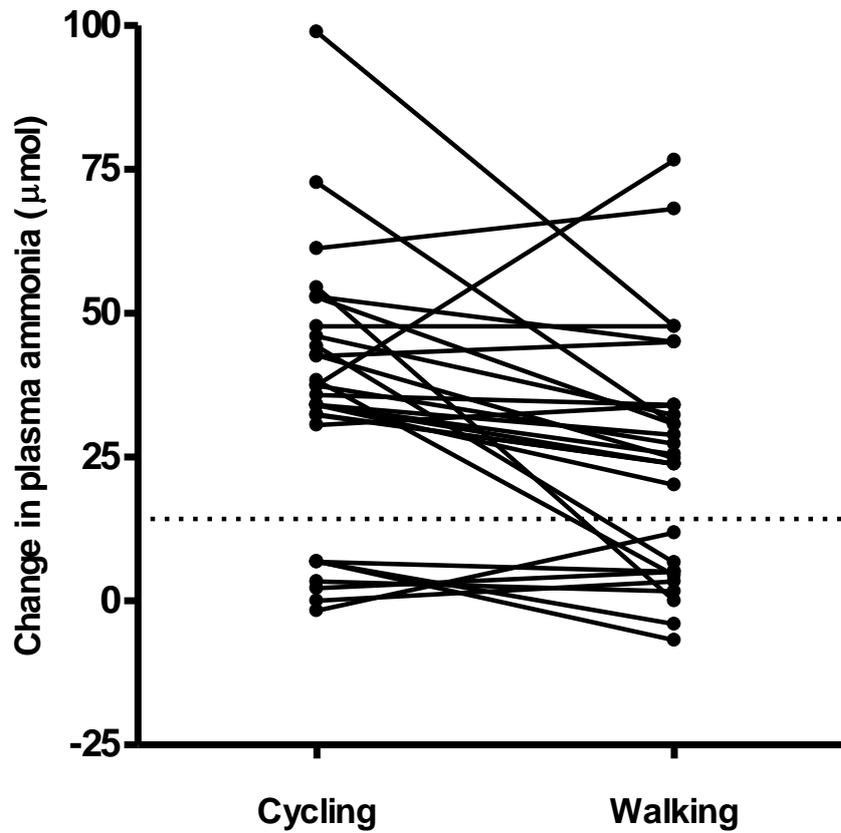


Table 6.3: Baseline characteristics and exercise parameters for COPD subjects with an ammonia response to cycle exercise (group 1, n=22) and without an ammonia response to exercise (group 2, n=7)

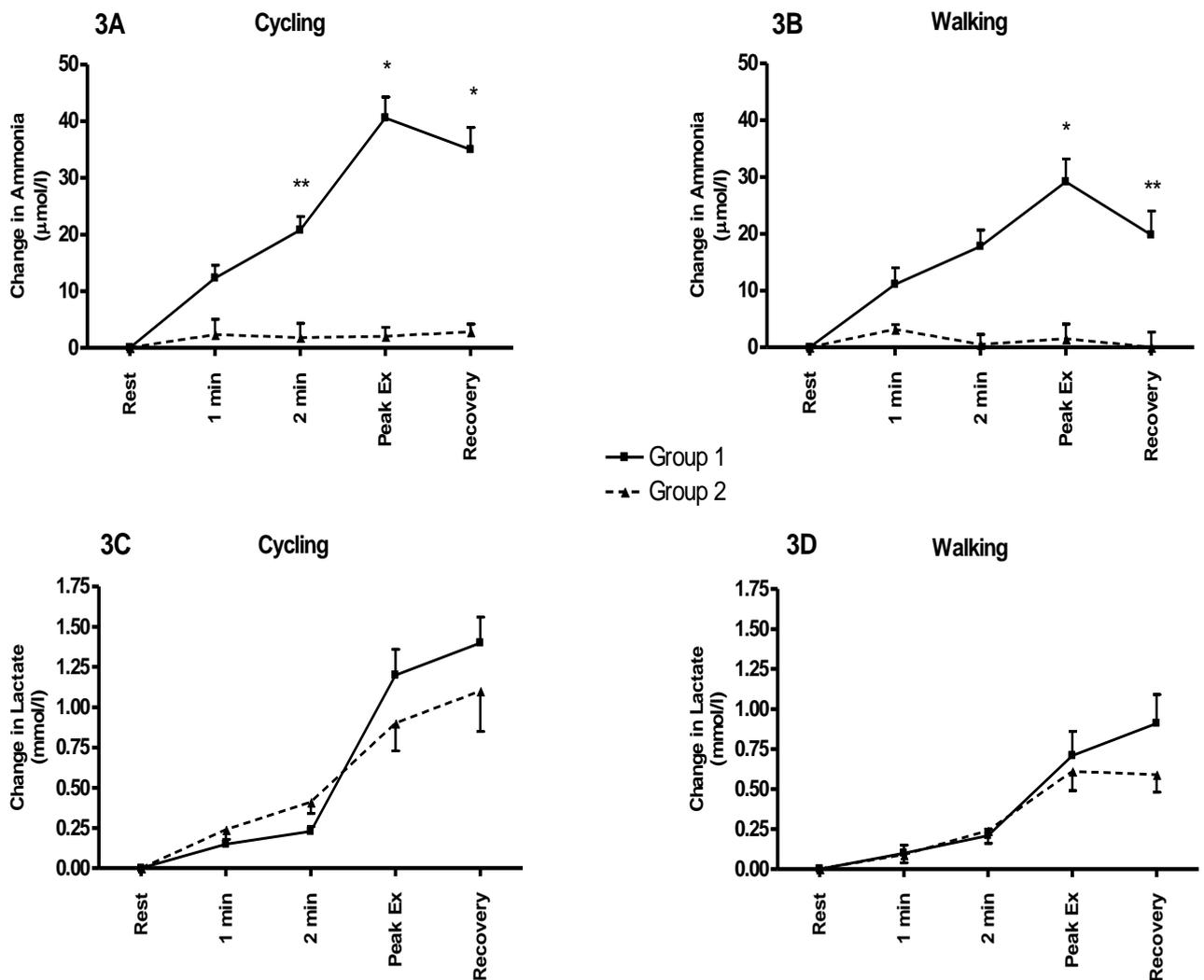
	Group 1 (n=22)	Group 2 (n=7)
Age (years)	68 (6)	69 (10)
FEV <sub>1</sub> (% predicted)	50 (19)	52 (19)
FFMI (kg/m <sup>2</sup> )	18 (2)	19 (3)
Oxygen saturation at rest (%)	96 (2)	96 (1)
<i>Cycle</i>		
VO <sub>2max</sub> (ml/min/kg)	16.1 (4.8)	13.6 (3.3)
Peak workload (watts)	61 (20)	43(14)*
Peak V <sub>Emax</sub> (% MVV)	86 (22)	78 (28)
Heart rate (% predicted)	73 (11)	76 (10)
Lactate rise (mmol/l)	1.51 (0.15)	1.17 (0.22)
<i>Treadmill</i>		
VO <sub>2max</sub> (ml/min/kg)	17.0 (4.4)	16.4 (3.5)
Distance(metres)	309(184)	205(123)
Time (seconds)	329(124)	248(109)
V <sub>Emax</sub> (% MVV)	85 (21)	89 (21)
Heart rate (% predicted)	72 (10)	74 (6)
Lactate rise (mmol/l)	0.94(0.18)	0.70(0.12)

Expressed as mean (SD)

\* p=0.043

FEV<sub>1</sub>= forced expiratory volume in 1 second; FFMI= fat free mass index; VO<sub>2peak</sub>=peak oxygen uptake; V<sub>Emax</sub> = maximum ventilation; MVV=maximum voluntary ventilation (calculated as FEV<sub>1</sub> x 35)

Figure 6.3: Mean (SE) change from resting concentration for plasma ammonia ( $\mu\text{mol/l}$ ) [3A&3B] and blood lactate ( $\text{mmol/l}$ ) [3C&3D] during incremental cycling and treadmill walking exercise in COPD subjects with an ammonia response (group 1, closed square,  $n=22$ ), and COPD subjects without an ammonia response (group 2, closed triangle,  $n=7$ ).



\* $p < 0.001$ , \*\* $p = 0.055$ , between group 1 and group 2 analysis

## **6.4 Discussion**

In this study I have compared the skeletal muscle energy response to incremental cycle and walking exercise in a cohort of patients with COPD. I observed a rise in plasma ammonia with both cycle and walking exercise suggesting that muscle adenine nucleotide depletion occurs with both exercise platforms. My results suggest that failure of skeletal muscle energy delivery may contribute to exercise limitation in walking as well as cycling in this population. The lower rise in plasma ammonia with walking was consistent with previous studies indicating that the two exercise platforms are not interchangeable.

The energy for muscular contraction is provided by the dephosphorylation of ATP to form ADP in the adenylate kinase reaction. The accumulation of dephosphorylated adenine nucleotides stimulates glycolytic and oxidative metabolic pathways providing the energy for ATP resynthesis and allowing contraction to continue [Atkinson DE, 1968]. At higher exercise intensities, the utilisation of ATP is not matched by its resynthesis resulting in the accumulation of dephosphorylated adenine nucleotides which are inhibitory to the adenylate kinase reaction. Under these conditions significant adenine nucleotide loss occurs through the deamination of ADP to form IMP and ammonia [Broberg S, 1989; Sahlin K, 1989a]. This temporarily allows contraction to continue through an increase in the phosphorylation status of the adenine nucleotide pool but is detrimental to prolonged contraction because of a reduction in overall adenine nucleotide availability. Thus the release of ammonia and its accumulation in the blood is an indicator of muscle adenine loss during exercise. The condition of adenine nucleotide loss has been termed 'metabolic stress' because of its detrimental impact on sustained muscle contraction [Dudley GA, 1985; Mutch BJ, 1983].

In COPD patients, although there is evidence of altered skeletal muscle mitochondrial capacity and fibre composition, it has been unclear whether sufficient exercise workrates can be achieved to cause significant skeletal muscle metabolic stress particularly in patients with evidence of a ventilatory limit to exercise. However, it has recently been confirmed that adenine nucleotide loss does occur during cycle exercise in COPD patients and that its magnitude was comparable to that observed in similar aged healthy subjects despite substantially lower absolute work rates being achieved [Steiner MC, 2005a]. I subsequently demonstrated that significant blood ammonia accumulation occurs during constant load cycle exercise in COPD and confirmed that this was related to the accumulation of muscle IMP. In keeping with previous observations, the magnitude of ammonia rise was similar to that seen in similar aged healthy subjects. The current study extends these observations by comparing the pattern of ammonia accumulation during walking and cycling in another cohort of COPD patients. This is important because walking may be more functionally relevant for activities of daily living and is frequently chosen as the exercise platform for outpatient pulmonary rehabilitation. The results show that adenine nucleotide loss as indicated by a rise in blood ammonia does occur during walking exercise but at a lower magnitude to that seen during cycling. These findings suggest that the failure of skeletal muscle energy delivery and the consequent development of metabolic stress may contribute to exercise limitation in COPD patients. There was little difference in ammonia and lactate responses between patients defined as ventilatory limited and non-ventilatory limited, suggesting that metabolic stress develops in the ambulatory muscles even in patients with an apparent ventilatory limit to exercise.

My observations are consistent with previous reports in demonstrating a higher maximum  $\text{VO}_2$  and ventilation but lower lactate accumulation during walking. Palange and

co-workers reported higher ventilatory demand with walking, explained in part by a larger degree of lung gas exchange inefficiency [Palange P, 2000]. Metabolic differences did not explain these findings and blood lactate levels were significantly lower with walking compared with cycling. A similar pattern of ventilatory and metabolic differences has been noted by others [Christensen CC *et al*, 2004; Cockcroft A, 1985; Mathur RS, 1995]. More recently, Man and colleagues found no quadriceps fatigue to magnetic twitch following incremental walking, although twitch tensions fell significantly following cycling [Man WD, 2003]. They suggest patients terminate walking due to excess ventilatory demand before quadriceps fatigue develops.

Whilst I observed a similar overall pattern of lactate and ammonia accumulation during exercise, I reproduced my previous observation of dissociation of the ammonia and lactate response in some patients. In other words, some subjects did not demonstrate a blood ammonia rise during exercise despite showing a rise in blood lactate. This was broadly consistent between the two exercise platforms although there were three subjects in whom ammonia rose significantly during cycling but did not during walking. The reasons for these differences cannot be determined from my data. One possibility is that patients without an ammonia response were unable to exercise to sufficient intensity to cause adenine nucleotide loss. In support of this hypothesis, exercise capacity was lower in this group and the magnitude of lactate accumulation also lower although this was not statistically significant. These individuals may have terminated exercise for other reasons, for example ventilatory limitation, although I did not observe significantly higher peak ventilation rates in this group and the proportion of patients with ventilation limitation were similar between the two groups. Alternatively, this group may have had better preserved mitochondrial function or type I fibre proportions than those who did

demonstrate a rise in ammonia. This would result in more efficient adenine nucleotide rephosphorylation and therefore less AMP deamination. In support of this explanation, previous studies in healthy subjects have demonstrated that Type II fibres are more prone to adenine nucleotide loss [Dudley GA, 1985]. Previous literature in COPD has suggested a shift in fibre composition towards a greater proportion of type II fibres [Gosker HR, 2002b; Whittom F, 1998]. The preponderance of type II fibres may explain the rise in ammonia at low absolute workrates in the overall COPD cohort whereas conversely, the absence of an ammonia rise may identify patients in whom type I fibres are better preserved.

I acknowledge limitations to the interpretation of my data. I did not obtain muscle biopsies to directly measure adenine nucleotide loss during the exercise challenge, relying on the less invasive measurement of blood ammonia. However numerous previous investigations in healthy subjects have confirmed the relationship between AMP deamination and blood ammonia accumulation [Graham TE, 1990; MacLean DA, 1991; Mutch BJ, 1983; Sahlin K, 1989a] and I have also demonstrated this in COPD patients in Chapter 5. My observations are limited to maximal incremental exercise performance and may not be transferable to submaximal exercise. Precise standardisation of submaximal exercise on different exercise platforms is difficult to achieve and I believe that my choice of exercise challenge was appropriate to the question I wished to address in this study.

I can draw only limited clinical implications from my data but this and previous work have consistently indicated that skeletal muscle metabolic stress may occur during low absolute exercise workrates in patients with COPD. Thus the condition of metabolic stress may be a more frequent occurrence during daily activities in these individuals compared with healthy subjects. My data suggest these circumstances may arise during

walking exercise, an activity that is of functional importance to most individuals with COPD. It may also be relevant that the purine nucleotide degradation pathway is a potential source of exercise-induced oxidative stress through the activity of xanthine oxidase [Hellsten Y, 1997a]. It is possible therefore that regular metabolic stress is a trigger for free radical production, which may have an impact on wider cellular function. Previous studies have indicated that exercise in COPD increases oxidant stress and that this can be ameliorated by the xanthine oxidase inhibitor allopurinol [Heunks LM, 1999]. Further research is required to confirm these relationships and explore their clinical relevance.

In summary, I have demonstrated that plasma ammonia concentration increases significantly during incremental treadmill walking as well as cycling in subjects with COPD. Although the pattern of response is similar, the magnitude of rise was lower with walking indicating that the two exercise platforms cannot be used interchangeably to assess cellular metabolic stress in the skeletal muscles in response to exercise in COPD. My data suggest that limitations to skeletal muscle energy delivery may be a factor limiting both cycling and walking exercise.

## Chapter Seven: Dichloroacetate enhances performance and reduces blood lactate during maximal cycle exercise in COPD

The second half of this Thesis investigates adaptation of the ammonia response to exercise following interventions specifically targeting skeletal muscle. In this study I examined the adaptation of the metabolic response to cycle exercise following a pharmacological intervention known to act on skeletal muscle enzymes involved in energy metabolism.

### **7.1 Introduction**

Improving physical performance is an important therapeutic goal in COPD. Recent evidence has demonstrated that muscle mitochondrial (oxidative) capacity is reduced in COPD and that this contributes to exercise intolerance in this population [American Thoracic Society, 1999]. Importantly, impairment in skeletal muscle function may be a remediable feature of an otherwise largely irreversible disease. Currently there are no pharmacological therapies that specifically target skeletal muscle oxidative energy metabolism, but such interventions have therapeutic potential for improving disability in COPD and other chronic diseases where exercise limitation due to skeletal muscle dysfunction is a key feature.

Several studies indicate that mitochondrial oxidative energy production is reduced in the skeletal muscles of COPD patients [Gosker HR, 2002b; Maltais F, 1996b; Maltais F, 2000b; Whittom F, 1998]. Recently significant muscle adenine nucleotide loss during exercise was demonstrated in subjects with COPD. This suggests that ATP resynthesis is unable to meet the energy demands of exercise even at the low absolute exercise intensities patients with COPD can achieve [Steiner MC, 2005a].

Mitochondrial ATP production does not increase instantaneously at the onset of exercise, resulting in the reliance on non-oxidative sources of energy production to meet this shortfall in ATP supply in the early stages of exercise. Recent evidence suggests that this inertia in mitochondrial ATP production resides at the level of the pyruvate dehydrogenase complex (PDC), a mitochondrial multi-enzyme complex which catalyses the conversion of pyruvate to mitochondrial acetyl CoA [Greenhaff PL, 2004]. This period of metabolic inertia may be particularly relevant in conditions such as COPD where the capacity for mitochondrial energy delivery is already reduced.

Dichloroacetate (DCA) activates PDC by inhibiting the kinase responsible for inactivating the enzyme complex [Stacpoole PW *et al*, 2003]. DCA has been shown in animal studies [Roberts PA *et al*, 2002; Timmons JA, 1997] and studies involving healthy humans [Howlett RA, 1999; Timmons JA, 1998a] to reduce reliance on non-oxidative production of ATP in muscle, particularly in the first 30-120 seconds of exercise, thereby reducing muscle lactic acidosis. DCA has also been shown to reduce exercise-induced muscle lactate accumulation under ischaemic conditions in healthy volunteers [Timmons JA, 1998b] and in contracting heart muscle [Bersin RM *et al*, 1994].

The aim of this study was to determine the effects of an infusion of DCA at rest on maximal exercise performance and on blood lactate and ammonia accumulation during exercise in patients with COPD. I hypothesised that DCA, by activating PDC, would reduce the inertia in mitochondrial ATP production at the onset of exercise. The net effect of this would be better matching of the ATP demands of contraction by mitochondrial ATP generation, thereby reducing reliance on non-oxidative sources of ATP and consequently blood lactate accumulation. I also hypothesised that this increase in mitochondrial ATP production would result in the reduction of muscle adenine nucleotide loss resulting in

reduced accumulation of blood ammonia (a product of AMP deamination). Finally, I hypothesised that overcoming metabolic inertia by activation of PDC would result in increased maximal exercise performance.

## **7.2 Methods**

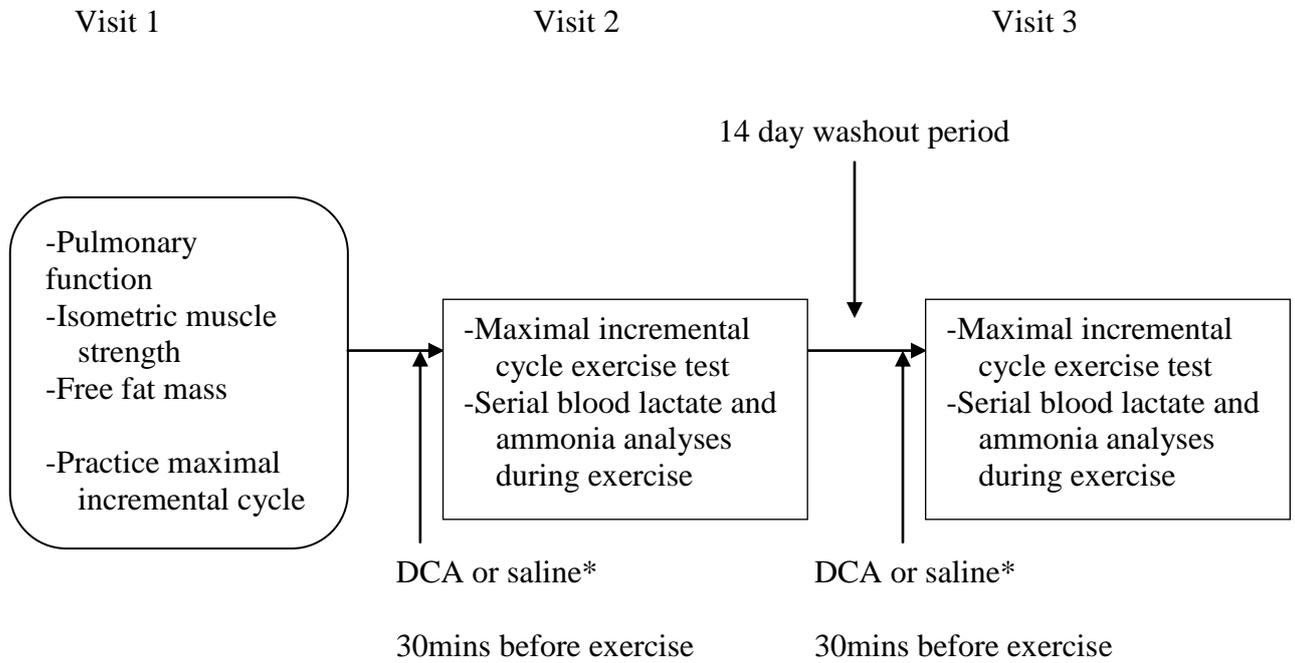
Eighteen stable patients (aged 50-85 years) who met clinical and spirometric GOLD criteria for COPD [Quanjer PH, 1993; Rabe KF, 2007] were recruited from outpatient clinics at Glenfield Hospital. Full approval was obtained from the Leicestershire Research Ethics Committee and all participants provided informed written consent. Patients were excluded if taking maintenance oral corticosteroids, were unable to perform the exercise tests, demonstrated significant exercise desaturation ( $\text{SaO}_2 < 80\%$ ), had significant cardiac dysfunction, an exacerbation of COPD within the previous 6 weeks, or pulmonary rehabilitation within the last year.

*Study design:* This was a double-blind cross-over study with a two week wash-out period, comparing Dichloroacetate (DCA) infusion with control (normal saline), given immediately before a standardised maximal exercise challenge (**Figure 7.1**).

*Outcome measures:* The primary outcome for this study was peak exercise blood lactate concentration. Secondary outcomes were peak exercise workload and oxygen consumption, and exercise-induced changes in blood lactate and ammonia concentrations.

Participants attended an initial visit to collect baseline data and familiarise with the exercise test. Spirometry, BMI, FFM and isometric quadriceps force were measured as described in Chapter 4. On a subsequent visit at least 1 week later, subjects performed a maximal (symptom-limited) incremental exercise test on an electrically braked cycle ergometer.

Figure 7.1: Flow diagram of study protocol



\*Administered in random order

Arterialised-venous blood samples were collected at rest, 1 minute and 2 minutes following the onset of exercise, peak exercise, and 2 minutes following cessation of exercise and analysed for ammonia and lactate concentrations (see Chapter 4). After a two-week washout period, subjects repeated the exercise challenge. Prior to exercise participants received either 50mg/kg body mass of Dichloroacetate (DCA; 25mg/ml, sodium salt) or an equivalent volume of normal saline as an intravenous infusion into a forearm vein over 45minutes. Following the infusion, subjects rested for 30 minutes before undergoing exercise to ensure PDC activation was achieved. The order subjects received DCA and placebo was randomised and all investigators and research participants were blinded. Unblinding of the study did not occur until the last patient had completed their final assessment.

*Preparation of DCA/placebo:* DCA was purchased from Fluorochem Ltd (UK) and prepared for sterile infusion (50mg/kg body mass; 25mg/ml, sodium salt) by the pharmacy production and quality control departments at University Hospitals of Nottingham, UK. This dose of DCA has previously been demonstrated to result in a three-fold increase in muscle PDC activation and acetylation of the muscle carnitine pool (demonstrating increased PDC flux) *in vivo* in humans at rest [Timmons JA, 1998a]. Allocation of randomisation after baseline measurements and dispensing of DCA/placebo was performed independently by staff from the Pharmacy Department at University Hospitals of Leicester, who were not involved with the conduct of the study.

*Statistical analysis:* The study was powered at 80% to detect a 0.8mmol reduction in peak exercise blood lactate at 5% level of significance, giving a sample size of 18 (STPLAN, Double Precision Study Planning Calculations software package, Department of Biostatistics & Applied Mathematics, University of Texas, USA). A standard deviation

of 0.82mmol was taken from my previous studies on COPD subjects. Data were originally analysed with SPSS version 14 (SPSS Inc Chicago, USA) using paired t-tests to look for any differences between drug and placebo conditions (significance assumed at p-value <0.05). Statistical advice for subsequent analysis was obtained from J Bankart (RDSU). Shapiro-Wilk tests were carried out on each difference score to assess the normality of the drug minus placebo differences, and in each case the test failed to reject normality (p>0.05). Data were subsequently analysed using SAS Proc Mixed (version 9.1) and for each dependent variable the treatment effect was adjusted for a period effect and also for a treatment by period interaction.

### **7.3 Results**

#### ***Patient characteristics***

Baseline characteristics are shown in **Table 7.1**. Subjects were either ex-smokers (n=11) or current smokers (n=7). All patients reported breathlessness on exertion (MRC breathlessness score 4 [n=10], 3 [n=5] or 2 [n=3]). No adverse events occurred during DCA infusions and no side effects were reported. For all data, the treatment effect was significant and there was no evidence of either a significant period effect or a significant period by treatment interaction.

#### ***Blood lactate and ammonia (Table 7.2)***

DCA infusion resulted in a small but statistically significant reduction in blood lactate concentration at rest compared with control [mean(SE) difference 0.18(0.04)mmol/l, p<0.001]. Plasma ammonia concentration at rest was not affected [mean(SE) difference 2.1(2.4) $\mu$ mol/l, p=0.4].

Table 7.1: Baseline characteristics for COPD subjects (n=18)

Age (years)	68 (7)
Gender	16 male
FEV <sub>1</sub> (% predicted)	45 (15)
FEV <sub>1</sub> (L)	1.16 (0.47)
BMI (kg/m <sup>2</sup> )	29 (6)
FFMI (kg/m <sup>2</sup> )	19 (3)
Isometric leg strength (Nm)	138 (44)
Oxygen saturation at rest (%)	95 (2)

Expressed as mean (SD)

FEV<sub>1</sub>= forced expiratory volume in 1second; BMI= body mass index:

FFMI= fat free mass index

Table 7.2: Blood lactate and plasma ammonia concentrations at rest and response to incremental exercise in all COPD subjects (n=18) after DCA and Saline infusion

	Lactate Mean (SD) mmol/l		Ammonia Mean (SD) $\mu$ mol/l	
	Saline	DCA	Saline	DCA
<b>Rest</b>	0.69 (0.19)	0.51 (0.15)*	56.0(11.7)	53.9 (11.5)
<b>1 minute exercise</b>	0.90(0.25)	0.51(0.14)*	66.9(11.5)	55.5 (13.5) <sup>++</sup>
<b>2 minute exercise</b>	1.04(0.35)	0.58(0.16)*	72.7(11.8)	58.0 (14.4) <sup>+</sup>
<b>Peak exercise</b>	1.85 (0.70)	1.50 (0.53) <sup>+</sup>	85.1(17.4)	71.1(12.8)*
<b>2 minutes post exercise</b>	2.09(0.76)	1.64(0.63) *	79.0(13.4)	65.7(12.9)*
<b>Exercise-induced change</b>	1.46(0.71)	1.16(0.57) <sup>**</sup>	31.4(14.5)	19.3(10.4)*

Expressed as mean (SD). \*p<0.001, \*\*p=0.02, <sup>+</sup>p=0.003, <sup>++</sup>p=0.004

At all time points during exercise absolute blood lactate concentration was significantly reduced by DCA infusion compared with control [**Figure 7.2A**]. Peak blood lactate concentration was reduced by 20% [mean(SE) difference 0.48(0.11)mmol/l,  $p<0.001$ ]. The exercise-induced rise in blood lactate above the resting concentration was also significantly reduced by DCA [mean(SE) difference 0.30(0.12)mmol/l,  $p=0.02$ ]. The largest reduction in blood lactate concentration with DCA was seen during the first two minutes of exercise (39% at 1minute and 41% at 2 minutes). DCA infusion also significantly reduced exercise-induced plasma ammonia accumulation compared with control [**Figure 7.2B**], and this effect was greatest at peak exercise [15% reduction, mean (SE) difference 14.2(2.9) $\mu$ mol/l,  $p<0.001$ ].

### ***Exercise parameters***

Data from the incremental exercise test performed after DCA and normal saline infusion is shown in **Table 7.3**. DCA improved indices of exercise performance, but peak heart rate and perceived breathlessness and exertion were similar. Peak workload increased significantly from mean (SD) 67(26) watts under control conditions to 75(27) watts after DCA, (mean (SE) difference 8 (1) watts, 12.5%,  $p<0.001$ ). Both peak oxygen consumption and peak ventilation increased significantly after DCA infusion compared with control [mean (SE) difference 1.2(0.5) ml/kg/min, 8%,  $p=0.03$  and 3.7(1.4) L/min, 11%,  $p=0.018$  respectively]. **Figure 7.3** shows the changes in the mean oxygen consumption (7.3A) and ventilation (7.3B) during exercise after DCA and control. Isotime values (measurements recorded at the equivalent time that exercise ceased in the control test) for the DCA group are shown to allow comparison with peak exercise values for the control group. Fifteen subjects showed an increase in exercise performance following DCA compared with placebo, two showed a slight decrease and one showed no change.

Table 7.3: Exercise data from maximal cycle test in COPD subjects (n=18) after DCA and saline infusion

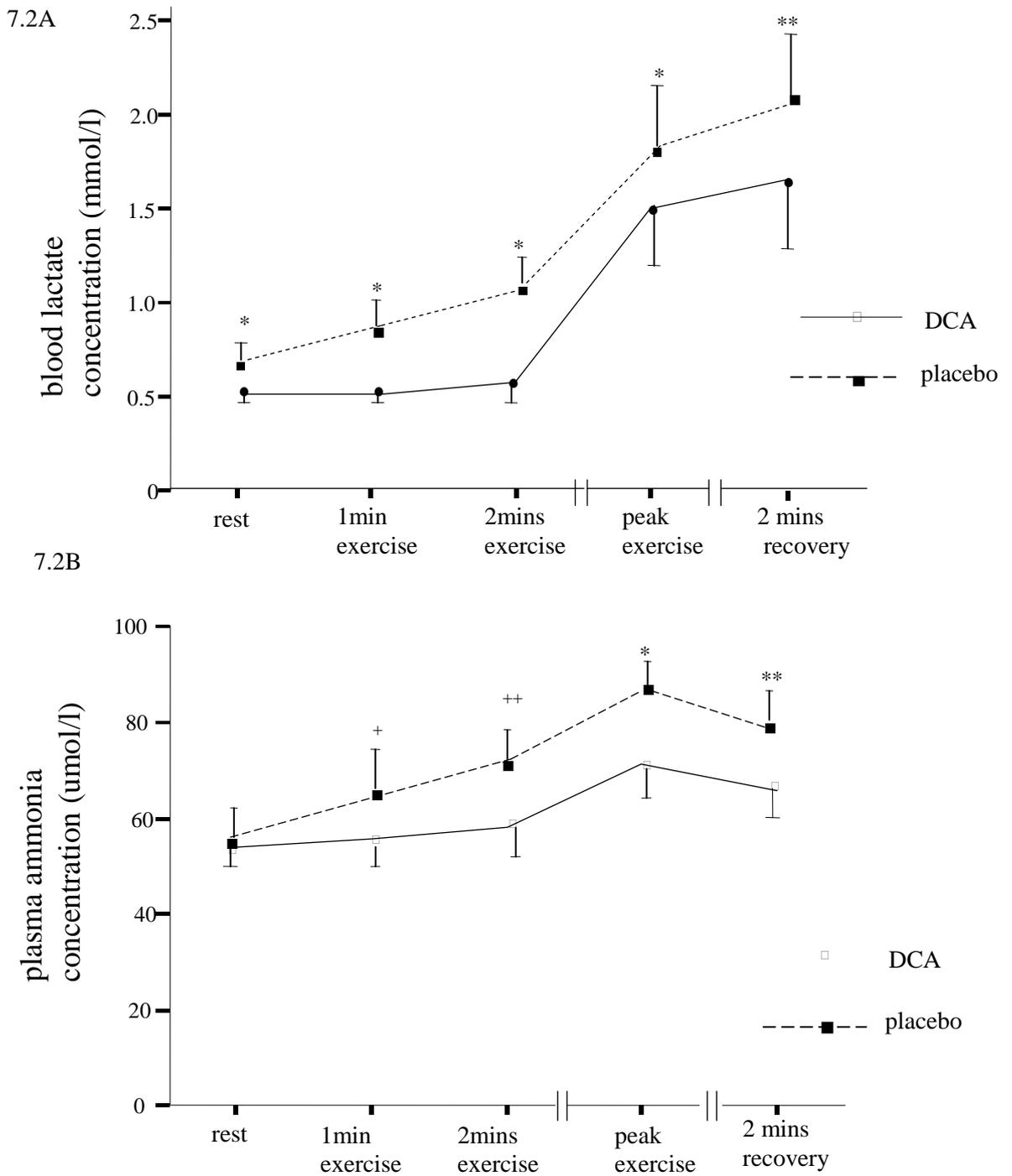
	Saline	DCA
Exercise duration (secs)	410 (155)	467 (181)**
Peak workload (watts)	67 (26)	75 (27)*
VO <sub>2 peak</sub> (ml/kg/min)	15.9 (4.2)	17.0 (4.2)**
Peak V <sub>E</sub> (L/min)	36 (13)	39 (14) <sup>+</sup>
Peak V <sub>E</sub> (% MVV)	91 (27)	98 (28) <sup>+</sup>
Peak RER	0.92 (0.06)	0.96 (0.06)*
Peak PE <sup>‡</sup>	17(16-17)	17 (16-17)
Peak heart rate (% pred)	74 (9)	73 (9)

Expressed as mean (SD) unless stated: <sup>‡</sup> Median (IQR)

\*p<0.001, \*\*p<0.01, <sup>+</sup>p=0.018, <sup>++</sup>p=0.03

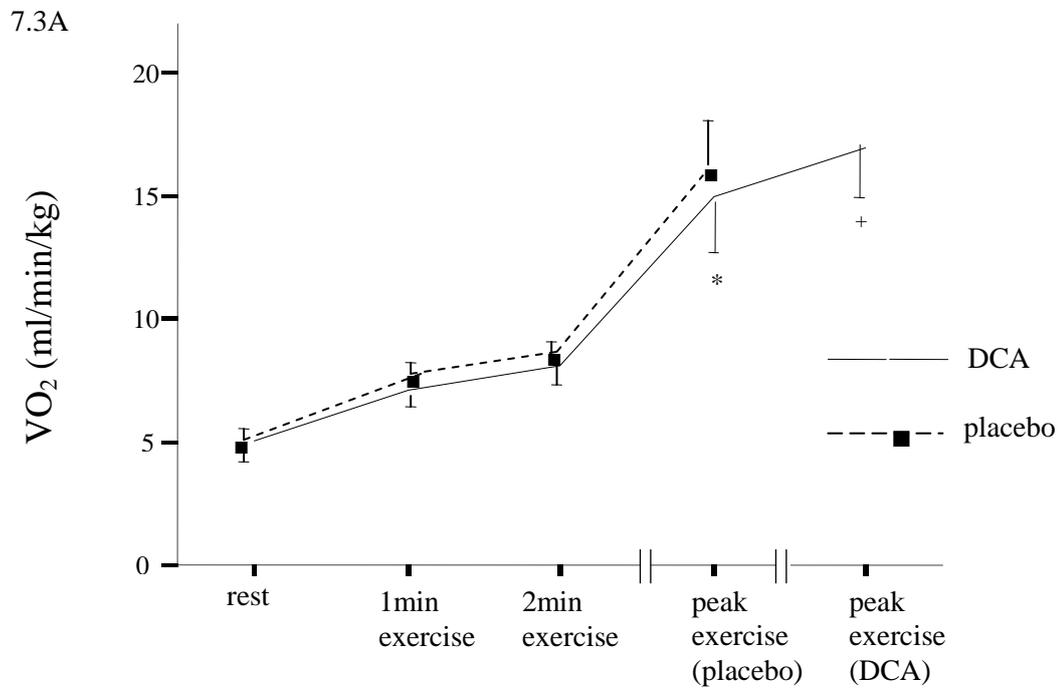
VO<sub>2 peak</sub>=peak oxygen uptake; Peak V<sub>E</sub>=peak ventilation; Peak RER= peak respiratory exchange ratio; Peak PE=perceived exertion at peak exercise, MVV=maximum voluntary ventilation (calculated as FEV<sub>1</sub> x 35). Predicted peak HR calculated as 220-age.

Figure 7.2: Graph to show mean (SD) lactate accumulation (A) and mean (SD) ammonia accumulation (B) with exercise following DCA and saline infusion in COPD patients

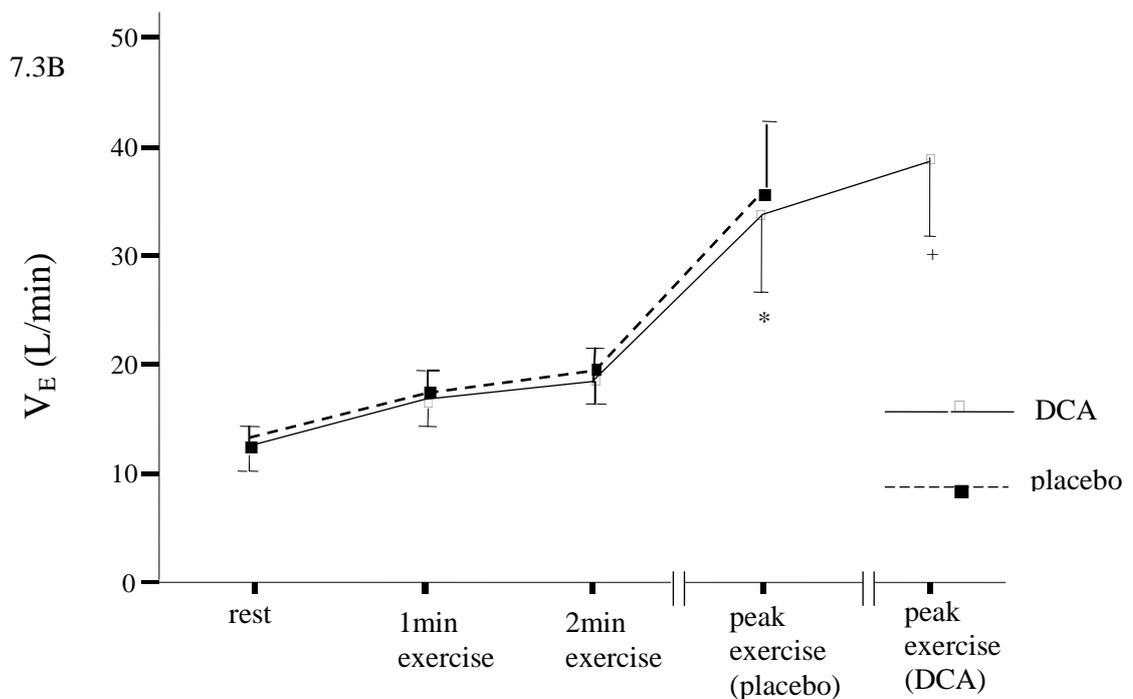


Expressed as mean (SD). \*p<0.001, \*\*p=0.001, +p=0.003, ++p=0.004

Figure 7.3: Graph to show mean (SD) oxygen consumption,  $VO_2$ , (A) and mean (SD) ventilation,  $V_E$ , (B) at rest and during exercise following DCA and saline in COPD patients.



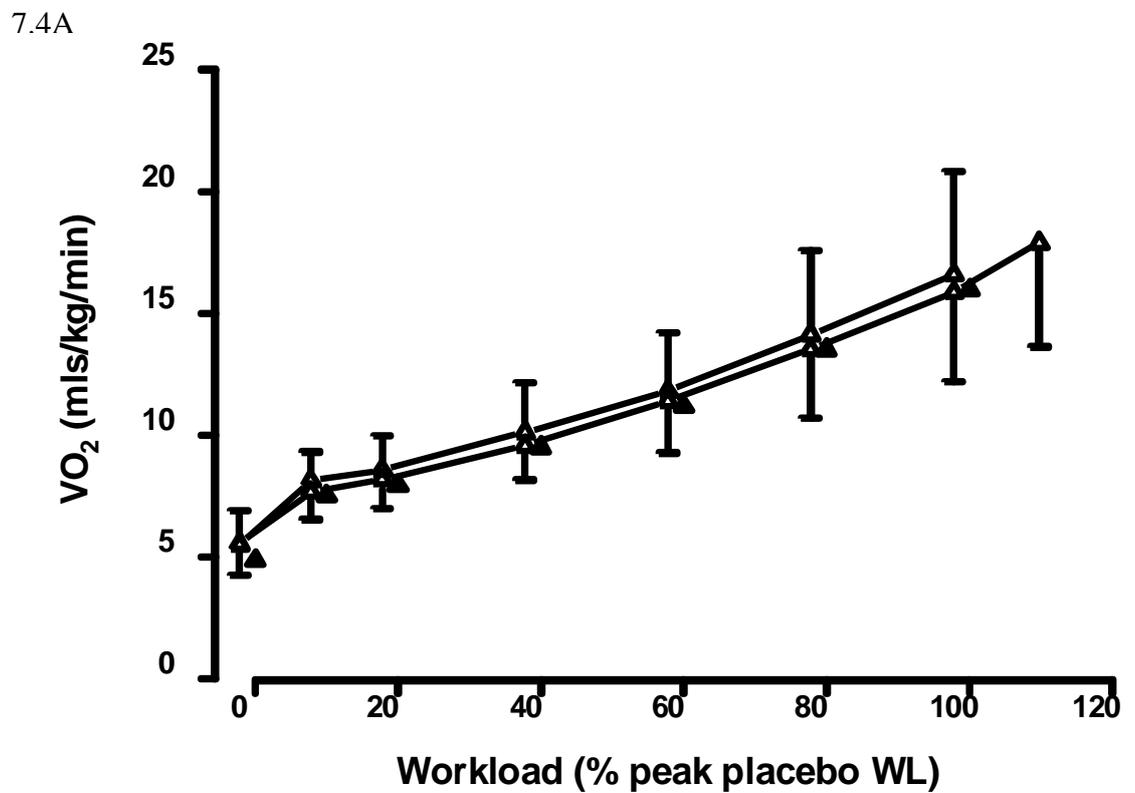
Expressed as mean (SD). Between group \* $p=0.059$  Within group  $^+p=0.03$



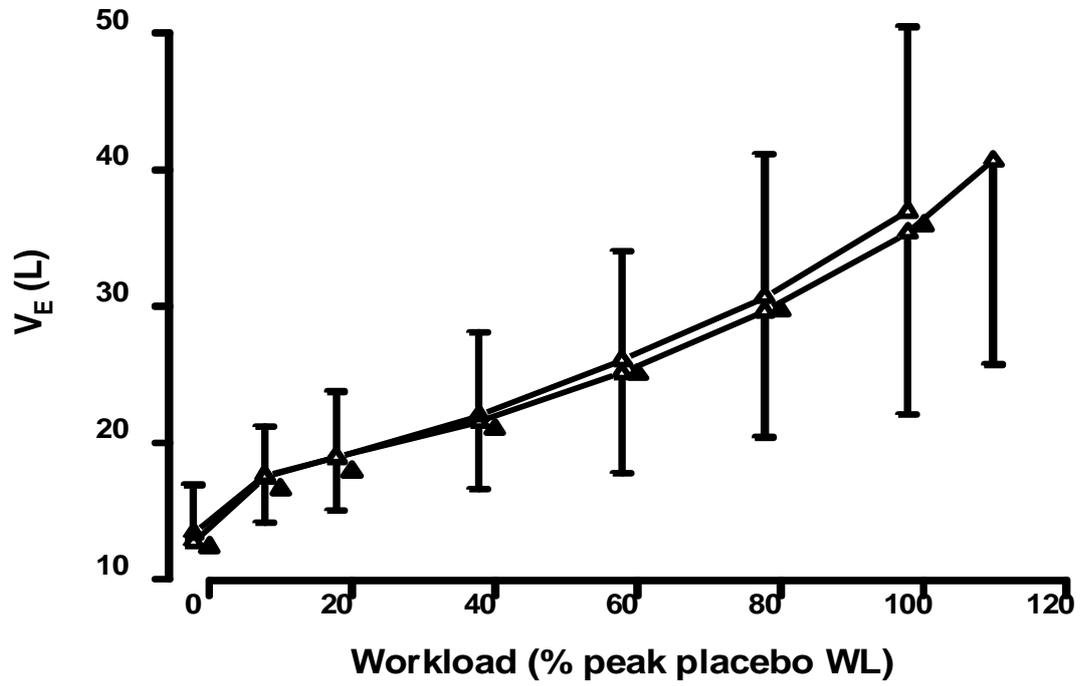
Expressed as mean (SD). Between group \* $p=0.044$  Within group  $^+p=0.018$

For the individual who showed no change, isotime and peak values are identical. For the individuals showing a decrease in performance, peak values are included but isotime values are missing. For both  $\text{VO}_2$  and  $V_E$  there was a small reduction in isotime data with DCA [mean (SE) difference for oxygen consumption 1.0(0.5)ml/kg/min,  $p=0.059$  and for ventilation 1.8(0.8)L/min,  $p=0.044$ ]. The trajectory of change of  $\text{VO}_2$ ,  $V_E$  and  $\text{VCO}_2$  during exercise was not different between the DCA and placebo tests. Plots of changes in these variables standardised for peak placebo performance are shown in **Figures 7.4 A to C**. There was no statistically significant difference between absolute values in the placebo and DCA groups at rest or during exercise for these variables, although peak values are higher in the DCA group (**Table 7.3**). RER at peak exercise was significantly greater following DCA (**Table 7.3**). Isotime values for RER were not significantly different between placebo and DCA exercise tests.

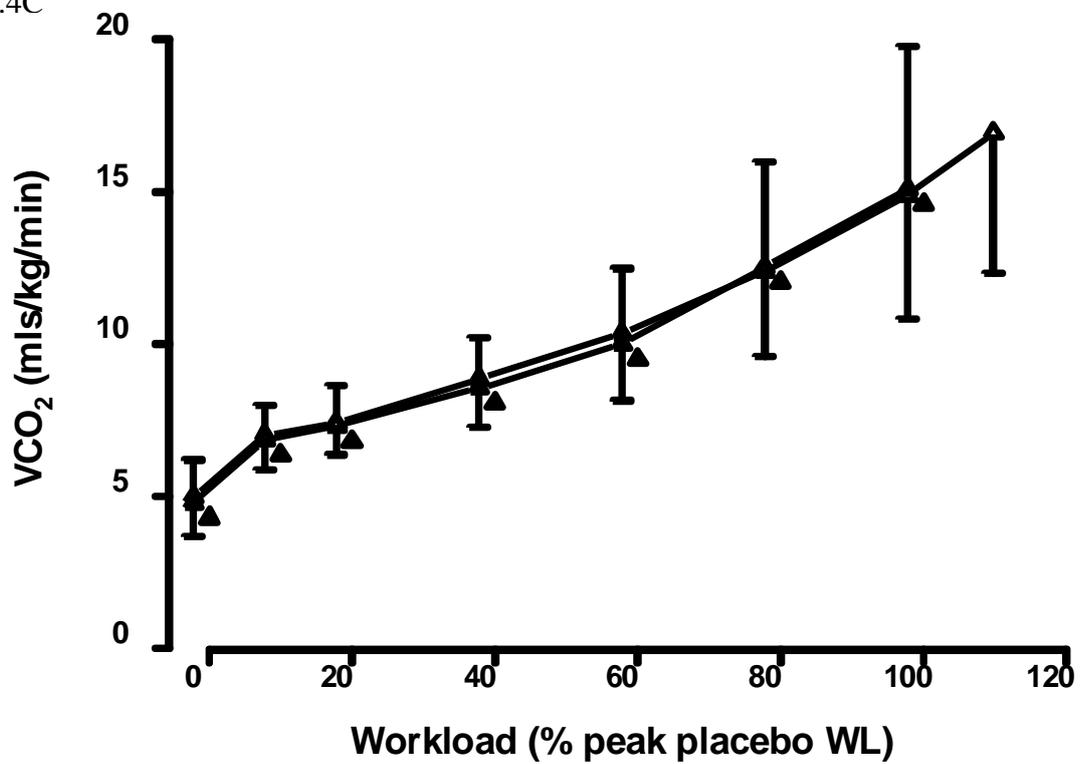
Figures 7.4A, B and C: Mean (SD) changes in  $VO_2$  (A),  $V_E$  (B) and  $VCO_2$  (C) measured during incremental exercise following DCA (open symbols) and Saline (closed symbols) infusions. Workload is standardised and expressed as a percentage of peak work achieved during incremental exercise following saline. No statistical difference between the two groups was found.



7.4B



7.4C



## **7.4 Discussion**

In this study I have demonstrated for the first time that a pharmacological intervention known to reduce the inertia in mitochondrial (oxidative or aerobic) ATP generation and the reliance on anaerobic (non-oxidative) ATP resynthesis at the onset of exercise can improve maximal exercise performance in patients with COPD. Following DCA infusion, blood lactate and ammonia accumulation decreased at all stages of exercise, and maximal cycling workload and maximum oxygen consumption increased. The results of this study indicate that PDC could be a novel therapeutic target for improving exercise capacity and hence disability in this population.

The likely explanation for my observations is that DCA-mediated activation of PDC overcame the inertia in mitochondrial ATP production at the onset of exercise through an increase in the provision of acetyl CoA for mitochondrial utilisation. This results in increased mitochondrial ATP resynthesis rates and reduced muscle ADP and AMP accumulation. Consequently, there is reduced activation of anaerobic glycolysis and thus lower blood lactate accumulation [Bangsbo J, 1990; Roberts PA, 2002; Timmons JA, 1997]. This is supported by my observation of reduced blood ammonia accumulation following DCA. Ammonia is a product of adenine nucleotide deamination, which occurs during intense exercise when muscle ATP resynthesis rates cannot meet demand. Blood ammonia accumulation has been shown to occur during intense exercise in healthy humans [Babij P, 1983; Graham TE, 1990] and I have confirmed that this also occurs during maximal incremental exercise in patients with COPD.

My results indicate that in COPD, overcoming metabolic inertia at the onset of exercise can increase maximal exercise performance. Mitochondrial ATP production does not increase instantaneously at the onset of exercise and recent evidence suggests that this

inertia resides at the level of PDC. The activation of PDC during the transition from rest to exercise results in an increase in the supply of mitochondrial acetyl CoA paralleled by an exponential rise in mitochondrial ATP production [Roberts PA, 2002; Timmons JA, 1997; Tschakovsky ME *et al*, 1999]. This initial period of metabolic inertia may be particularly relevant in conditions such as COPD where the capacity for mitochondrial energy delivery is already reduced and where the short duration and low intensity of exercise that patients can customarily achieve may not achieve full activation of PDC. As a result of a single DCA infusion I observed an increase in peak workload and peak oxygen consumption of 8 watts and 1.2ml/kg/min respectively compared to placebo. Whilst these differences were modest in absolute terms, they represented a 12.5% and 8% improvement respectively relative to control exercise performance. Large improvements in maximal exercise performance are difficult to achieve in COPD patients. Whilst the wider and longer-term clinical benefits of activating PDC are unknown, I believe my data justify further investigations in this area.

The majority of patients in the current study demonstrated ventilatory limitation during maximal exercise. I had expected improvements in exercise performance following DCA infusion to be associated with a reduction in ventilation at a given workload because of reduced blood lactate levels. However, whilst I observed a small reduction in isowork ventilation following DCA infusion compared to placebo, I also found that the increase in peak workload was associated with an increase in peak ventilation in the DCA group. In other words, following DCA infusion patients were able to exercise further into their ventilatory reserve despite most patients almost reaching predicted maximal voluntary ventilation. This may have been due to a reduction in lower limb muscle fatigue due to an increase in oxidative ATP delivery and a concomitant reduction in phosphocreatine

hydrolysis, inorganic phosphate (Pi) accumulation, anaerobic glycolysis and lactate accumulation. However, I cannot rule out other mechanisms such as improvements in mitochondrial ATP delivery in the respiratory muscles that would allow greater maximum ventilation. I also found that RER increased at peak exercise following DCA. Whilst a reduction in peak lactate might be expected to reduce RER, this observation may be explained by the fact that the DCA mediated activation of PDC will result in the preferential utilisation of carbohydrate during exercise [Stacpoole PW, 1989; Timmons JA, 1997]. This increase in carbohydrate oxidation will reduce fat oxidation and hence produce a decline in the oxygen cost of substrate oxidation and the observed increase in the respiratory exchange ratio.

This study is the first to report the effects of DCA on muscle energy metabolism in patients with exercise limitation due to COPD. Several studies indicate that the capacity of mitochondrial oxidative energy production is reduced in the skeletal muscles of COPD patients, particularly in the ambulatory muscles [Gosker HR, 2002b; Maltais F, 1996b; Maltais F, 2000b; Steiner MC, 2005a; Whittom F, 1998]. The action of DCA in activating PDC and thereby reducing the lag in mitochondrial ATP production at the onset of exercise means that in healthy subjects its effects are generally restricted to the first few minutes of exercise [Howlett RA, 1999; Roberts PA, 2002; Timmons JA, 1998a; Timmons JA, 1998b]. In contrast to this, I observed a reduction in blood lactate and ammonia not only after 1 and 2 minutes of exercise but also at peak workload. I speculate that in COPD, where mitochondrial oxidative capacity is reduced and maximum exercise intensity and duration is low (the mean duration of exercise following control was 403s), exercise fails to completely activate PDC thus providing scope for DCA to exert its effects even at peak exercise.

I acknowledge some limitations to the current study. I did not directly measure the activity of PDC in the current study but numerous previous reports in humans have confirmed the efficacy of DCA in this respect [Greenhaff PL, 2004; Heigenhauser GJ, 1999; Timmons JA, 1998a]. I was unable to correlate improvements in exercise performance with changes in blood lactate or ammonia and therefore cannot make definitive conclusions about the physiological mechanisms underpinning my observations. The effects of DCA may be of greatest magnitude during high intensity exercise where reliance on anaerobic sources of energy is important. This may not necessarily translate into clinically significant benefits during other exercise modalities such as constant workrate exercise. Further investigation will be needed to explore these issues and provide greater understanding of the underlying mechanisms.

There is increasing recognition of the importance of the systemic consequences of COPD and in particular the role of skeletal muscle dysfunction in exercise limitation and disability [American Thoracic Society, 1999]. For many patients with COPD currently available therapies aimed at improving lung function do not adequately relieve their symptoms. Because lung function impairment is largely irreversible in COPD, alternative approaches are needed and targeting the systemic features of the disease such as impaired skeletal muscle function may be of therapeutic benefit. The established benefits of pulmonary rehabilitation in improving skeletal muscle function and exercise performance in this population confirm that this approach can be successful. My findings suggest that inertia in mitochondrial ATP production during exercise is of functional significance in COPD and that interventions to reduce this phenomenon, such as DCA, may have therapeutic value. DCA is safe when given as a single intravenous dose and although it has been used in oral form to treat congenital lactic acidosis [Stacpoole PW *et al*, 2006]

concerns remain over its longer-term safety as chronic dosing has been associated with the development of a peripheral neuropathy in some patients [Kaufmann P *et al*, 2006]. However, my results indicate that clinical trials of DCA or other agents that activate PDC are warranted to evaluate the efficacy and safety of such an intervention. The integration of such interventions with established therapies such as exercise training also requires investigation. Importantly, any therapeutic advances are likely to have a wide application given the similarities seen in skeletal muscle abnormalities between COPD and other chronic diseases such as heart failure and peripheral vascular disease where exercise limitation is a key feature.

In summary, I have demonstrated that DCA infusion given before a maximal exercise challenge reduces exercise-induced blood lactate and ammonia accumulation and increases whole body exercise performance in COPD patients. Pharmacological modulation of the skeletal muscle metabolic response to exercise may be of therapeutic benefit in the treatment of reduced functional capacity and disability in COPD.

## Chapter Eight: Skeletal muscle energy delivery improves following pulmonary rehabilitation in COPD

Pulmonary rehabilitation (PR) is an important clinical intervention in COPD management and is known to have beneficial effects on exercise performance. In the final study of this Thesis, the effect of pulmonary rehabilitation in modifying the ammonia response to both cycle and treadmill exercise is examined.

### **8.1 Introduction**

Peripheral skeletal muscle metabolic inefficiency and impaired oxidative capacity has been identified as an important contributor to exercise intolerance [Maltais F, 1996b; Maltais F, 2000b; Whittom F, 1998] independent of disease severity in patients with COPD [American Thoracic Society, 1999].

Adenine nucleotide depletion occurs in exercising muscle when ATP re-synthesis fails to meet demand, and results in accumulation of AMP, which is subsequently deaminated to IMP and ammonia [Dudley GA, 1985; Pouw EM, 1998b]. Plasma ammonia therefore accumulates during exercise when there is inadequate muscle ATP re-synthesis in relation to utilisation [Graham TE, 1990]. In healthy adults blood ammonia accumulation during exercise has been implicated in the development of fatigue [Mutch BJ, 1983]. Skeletal muscle ATP depletion and plasma ammonia accumulation have previously been shown to occur during exercise in COPD subjects despite the low absolute exercise intensities these individuals achieve [Steiner MC, 2005a]. Plasma ammonia, as a measure of the efficacy of ATP supply during exercise in COPD patients, therefore has potential as a tool for investigating the impact of interventions such as PR on skeletal muscle energy delivery. I have already demonstrated the utility of plasma ammonia

measurement in evaluating pharmacological intervention targeting muscle energy metabolism.

Exercise training in the context of pulmonary rehabilitation (PR) is an integral part of clinical management of COPD [Celli BR, 2004b; Nici L, 2006]. The clinical efficacy of PR in improving exercise performance in patients with COPD is well established but the degree to which improvement in energy delivery underpins these improvements is less well understood [Griffiths TL, 2000; Lacasse Y, 2002]. Measurement of the adaptation in energy delivery following exercise training could provide further insight into these mechanisms and allow refinement and targeting of performance enhancing therapy in order to achieve maximal functional benefits.

The aim of this study was to determine the effects of exercise training as part of a pragmatic outpatient PR programme on exercise-induced plasma ammonia accumulation in patients with COPD. I hypothesised that PR would attenuate the exercise-induced rise in plasma ammonia due to positive effects on skeletal muscle oxidative ATP production and reduction of exercise-induced adenine nucleotide loss. In previous studies I observed a subgroup of COPD patients that did not appear to have an ammonia rise with exercise. An additional aim of this study therefore was to determine whether these subjects responded differently to PR.

## **8.2 Methods**

Patients referred to the PR programme at Glenfield Hospital who met GOLD clinical and spirometric criteria for COPD [Rabe KF, 2007] consecutively assessed for inclusion in the study. Medical therapy was optimised in outpatient clinic prior to PR. Patients were excluded from the programme if considered unsuitable for the exercise component due to musculoskeletal impairment, significant medical conditions such as unstable coronary

artery disease or when compliance with PR was not possible. Additional study exclusion criteria were maintenance oral corticosteroids, cardiac failure, PR within the last two years or exacerbation requiring hospitalisation within the last 6 weeks. Approval was obtained from the Leicestershire Research Ethics Committee and all participants provided informed written consent.

*Study design:* All subjects participated in the standard 7 week outpatient PR programme at University Hospitals of Leicester NHS Trust [Deacon SJ *et al*, 2008; Sewell L, 2006]. Study assessments were made prior to commencing, and within 2 weeks of completing PR. Disease specific health status was measured using the Self-Reported Chronic Respiratory Diseases Questionnaire (CRQ-SR) [Williams JE, 2001]. Maximal and sub-maximal field exercise capacity was assessed by trained PR practitioners, before and after PR using the incremental (ISWT) [Singh SJ, 1992] and endurance (ESWT) shuttle walk tests respectively [Revill SM, 1999].

On an initial visit baseline measurements were collected and familiarisation tests on cycle and treadmill performed. On two subsequent visits at least 72 hours apart, subjects performed a maximal symptom-limited incremental exercise test on an electrically-braked cycle ergometer and a maximal symptom-limited treadmill exercise test respectively. At least 72 hours following completion of PR, subjects attended a further 2 visits to repeat the cycle and treadmill tests. Blood was sampled for ammonia and lactate concentration during exercise tests at rest, at 1 minute and 2 minutes of exercise, at peak exercise, and at two minutes following exercise. During the post-PR assessments an extra blood sample was taken at the isotime of peak exercise achieved in the pre-PR tests. Timing of blood sampling was based on my previous studies. See Chapter 4 for more details.

*Pulmonary rehabilitation programme:* Patients attended PR at Glenfield Hospital, University Hospitals of Leicester NHS Trust, twice weekly for a total of 14 sessions over at least 7 weeks. The exercise component consisted of endurance (walking) and strength training with 1 hour supervised exercise in each session. For endurance exercise, patients walked at speed equivalent to 85% peak VO<sub>2</sub> predicted from baseline ISWT performance. Patients were also given a daily home walking programme. Walking times were increased progressively during the course of the programme, and new targets set at each session. Patients also underwent 5 minutes cycle exercise at each session. Cycle resistance was increased as Borg breathlessness score reduced. Strength training (biceps curls, sit to stand, pull-ups and step-ups; 3 sets of eight repetitions) was performed three times a week (one supervised session). Hand weight started at 1kg and was increased as perceived exertion fell. The education component consisted of 1 hour each session and covered a range of topics including disease education, medication, diet, energy conservation, breathing control and relaxation.

*Statistical analysis:* The study was powered at 80% to detect a 15µmol/l within-group reduction in peak exercise blood ammonia with PR at 5% level of significance using a standard deviation of 16µmol/l, giving a sample size of 22 (STPLAN, Double Precision Study Planning Calculations software package, Department of Biostatistics & Applied Mathematics, University of Texas, USA). Subjects continued to be recruited to the study until at least 22 had completed. Subgroups were defined *a priori* by exercise-induced ammonia rise during cycling above (group 1) or below (group 2) 15µmol/l. Normality of data for outcome variables was confirmed. Intra- and inter-group differences were compared using the paired and unpaired Students t-tests respectively (SPSS package version 15.0, SPSS Inc Chicago, USA). Significance was assumed at p<0.05.

### **8.3 Results**

A total of thirty-five patients were recruited to the study and 25 patients (18 male) completed. This dropout rate is comparable to recent published PR data from the clinical service at Glenfield Hospital [Deacon SJ, 2008; Sewell L, 2006; Steiner MC *et al*, 2003]. No differences between subjects who dropped out and completed PR were identified in demographic variables, ISWT or ESWT performance, cycling and walking performance, or ammonia and lactate response to exercise. Completers had a mean (SD) age 67(8)years and FEV<sub>1</sub> 1.19(0.43)L, 47(18)%predicted. Mean (SD) BMI was 28(6)kg/m<sup>2</sup> and oxygen saturation at rest was 96(2)%. All patients reported breathlessness on exertion [Medical Research Council (MRC) breathlessness score 5 (n=3), 4 (n=7), 3 (n=8) or 2 (n=7)]. Baseline mean (SD) ISWT distance was 280 (131)metres and ESWT time was 172 (54)seconds.

#### ***Exercise response to PR***

Clinical outcome measures for PR indicated significant improvement following completion of the programme. ISWT distance increased by mean (95%CI) 93(62-124)m (p<0.001) and ESWT time by 502(346-658)seconds (p<0.001). The minimum clinically significant increase in ISWT has recently been defined as 48m [Singh SJ *et al*, 2008]. Health status measured by CRQ-SR increased in all domains; mean (95%CI) changes Dyspnoea 1.19 (0.68-1.70), Fatigue 0.89(0.36-1.43), Emotion 0.77(0.10-1.45), Mastery 0.86(0.18-1.53), p<0.001.

Data from the cycling and walking exercise tests are shown in **Table 8.1**. One subject did not exercise on the treadmill due to unsteadiness. Significant improvements were seen in exercise indices for both exercise modalities following PR.

Table 8.1: Exercise data from maximal cycling test (A; n=25) and walking test (B; n=24) in COPD subjects before PR and change following PR

A	Cycling			
	Pre-PR peak	Post-PR peak	Post-PR isotime <sup>1</sup>	Peak exercise change with PR
Peak workload (Watts)	56 (21)	69 (22)**	56 (21)	13 (9-17)**
VO <sub>2 peak</sub> (ml/kg/min)	15.9 (4.8)	17.2 (5.6)*	14.2 (4.8)**	1.34 (0.44-2.24)*
Peak V <sub>E</sub> (L/min)	34.9 (11.8)	38.4 (12.6)**	31.2 (8.8)*	3.5 (1.7-5.4)**
Peak V <sub>E</sub> (% MVV)	86 (19)	95 (19)**	78 (17) <sup>+</sup>	9 (5-13)**
Peak RER	0.96 (0.05)	0.96 (0.05)	0.93 (0.04)	0.00(0.00-0.01)
Peak BS <sup>‡</sup>	4 (4-5)	4 (3-5)	-	-
Peak PE <sup>‡</sup>	17 (15-18)	15 (14-17)	-	-
B	Walking			
	Pre-PR peak	Post-PR peak	Post-PR isotime <sup>1</sup>	Peak exercise change with PR
Distance (metres)	292 (184)	410 (218)**	292 (184)	119 (62-175)**
Treadmill time (secs)	315 (128)	398 (135)**	315 (128)	83 (46-120)**
VO <sub>2 peak</sub> (ml/kg/min)	17.1(4.5)	19.6 (4.9)**	16.5 (4.3)	2.52(1.28-3.77)**
Peak V <sub>E</sub> (L/min)	36.2 (10.2)	40.4 (11.5)**	32.3 (8.9)**	4.2 (2.1-6.2)**
Peak V <sub>E</sub> (% MVV)	89 (17)	99 (20)**	82 (16)**	10 (5-15) **
Peak RER	0.89 (0.07)	0.90 (0.07)	0.87 (0.07)	0.01(0.00-0.01)
Peak BS <sup>‡</sup>	4 (4-8)	4 (4-7)	-	-
Peak PE <sup>‡</sup>	16 (13-17)	16 (13-17)	-	-

Absolute values expressed as mean (SD). Changes expressed as mean (95% CI) <sup>‡</sup> Median (IQR)

Comparison between pre-PR and post-PR values: \*p<0.05, \*\*p<0.001, <sup>+</sup>p=0.005

VO<sub>2peak</sub>=peak oxygen uptake; Peak V<sub>E</sub>=peak ventilation; Peak RER= peak respiratory quotient; Peak BS=breathlessness measured by Borg score at peak exercise; Peak PE=perceived exertion at peak exercise; MVV=maximum voluntary ventilation (calculated as FEV<sub>1</sub> x 35)

<sup>1</sup> time in post-PR exercise test at which peak exercise was reached in pre-PR test

***Metabolic response to exercise (Table 8.2A & B)***

Exercise-induced ammonia accumulation [mean(SE)] at isotime (isowork) with pre-PR peak exercise was significantly attenuated following PR for cycling (pre-PR 29.0(4.5) $\mu$ mol/l, post-PR 10.6(1.7) $\mu$ mol/l;  $p < 0.001$ ) and walking (pre-PR 23.7(4.3) $\mu$ mol/l, post-PR 9.1(1.5) $\mu$ mol/l;  $p = 0.001$ ). Peak ammonia concentration post-PR was also significantly reduced in both exercise modalities ( $p < 0.05$ ), even though subjects achieved higher peak workrates. Similarly, lactate accumulation [mean(SE)] at isotime (isowork) with pre-PR peak exercise was significantly reduced in both cycling (pre-PR 1.16(0.14)mmol/l, post-PR 0.67(0.12)mmol/l;  $p < 0.001$ ) and walking (pre-PR 0.75(0.14)mmol/l, post-PR 0.27(0.05)mmol/l;  $p < 0.001$ ). However post-PR, peak exercise lactate rise was not significantly different compared with peak-exercise rise pre-PR. Ammonia and lactate accumulation over resting values are illustrated in **Figure 8.1 (A-D)**.

There were no differences in peak exercise indices, exercise-induced ammonia and lactate accumulation, or changes in these indices following PR between patients who did or did not reach ventilation limitation on pre-PR exercise.

Subgroup analysis was performed comparing rehabilitation responders ( $n = 16$ ) and non-responders ( $n = 9$ ) pre-defined as post-PR improvement in ISWT distance of  $\geq 48$ m or  $< 48$ m respectively. No differences in baseline demographics, medication and pre-PR ISWT, ESWT, cycle work, treadmill distance, exercise-induced lactate and ammonia rise and other exercise indices were found. Mean(SD) post-PR improvement was 30(10)m in non-responders and 131(68)m in responders. Mean improvement in cycle workload and treadmill distance with PR in responders was 17W and 128m and in non-responders was 8W and 103m respectively. Adaptation of the metabolic response following PR measured by lactate and ammonia was not different between the 2 groups.

Table 8.2A: Plasma ammonia and blood lactate concentrations at rest and response to incremental exercise in all COPD subjects for cycling

A Cycling (n=25)		Rest	1minute exercise	2minutes exercise	Isotime peak exercise <sup>1</sup>	Peak exercise
<b>Ammonia conc μmol/l</b>	Pre-PR	53.3 (13.4)	61.5 (16.4)	67.4 (16.4)		82.3 (21.0) <sup>++</sup>
	Post-PR	53.1 (12.4)	55.0 (13.1)*	58.1 (12.8)*	63.8(11.4) <sup>++***</sup>	73.3 (13.6) <sup>++*</sup>
<b>Lactate conc mmol/l</b>	Pre-PR	0.74 (0.16)	0.92 (0.25)	1.03 (0.30)		1.90 (0.71) <sup>++</sup>
	Post-PR	0.66 (0.13)*	0.73 (0.16)**	0.79 (0.16)**	1.33(0.64) <sup>++***</sup>	1.82 (0.88) <sup>++</sup>

A Cycling (n=25)		2minutes recovery	Exercise- induced change <sup>2</sup>
<b>Ammonia conc μmol/l</b>	Pre-PR	77.3 (25.0)	33.0 (4.7)
	Post-PR	73.0 (15.3)*	24.0 (2.8)*
<b>Lactate conc mmol/l</b>	Pre-PR	2.07 (0.82)	1.42 (0.14)
	Post-PR	2.10 (1.00)	1.46 (0.19)

Concentrations expressed as mean(SD) and change as mean(SEM)

<sup>+</sup>p<0.01, <sup>++</sup>p<0.001 Within group, compared with resting (values given for peak/isotime exercise)

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 Between pre-PR and post-PR group analysis

<sup>1</sup> time in post-PR exercise test at which peak exercise was reached in pre-PR test

<sup>2</sup>calculated as peak ammonia concentration measured minus resting concentration

Table 8.2B: Plasma ammonia and blood lactate concentrations at rest and response to incremental exercise in all COPD subjects for walking

B Walking (n=24)		Rest	1minute exercise	2minutes exercise	Isotime peak exercise <sup>1</sup>	Peak exercise
<b>Ammonia conc μmol/l</b>	Pre-PR	49.7 (17.4)	59.0 (23.2)	63.6 (22.7)		73.4 (26.5) <sup>++</sup>
	Post-PR	53.0 (13.5)	54.6 (14.3)	57.1 (14.7)	62.1(15.7) <sup>+++*</sup>	69.6 (16.5) <sup>+++*</sup>
<b>Lactate conc mmol/l</b>	Pre-PR	0.71 (0.20)	0.81 (0.28)	0.93 (0.35)		1.46 (0.73) <sup>++</sup>
	Post-PR	0.65 (0.15)	0.64 (0.15)*	0.71 (0.16)**	0.91(0.32) <sup>++++*</sup>	1.31 (0.66) <sup>++</sup>

B Walking (n=24)		2minutes recovery	Exercise- induced change <sup>2</sup>
<b>Ammonia conc μmol/l</b>	Pre-PR	66.2 (26.1)	26.1 (4.5)
	Post-PR	67.8 (14.4)	19.6 (2.4) <sup>+</sup>
<b>Lactate conc mmol/l</b>	Pre-PR	1.56 (0.88)	0.92 (0.16)
	Post-PR	1.51 (0.74)	0.86 (0.13)

Concentrations expressed as mean(SD) and change as mean(SEM)

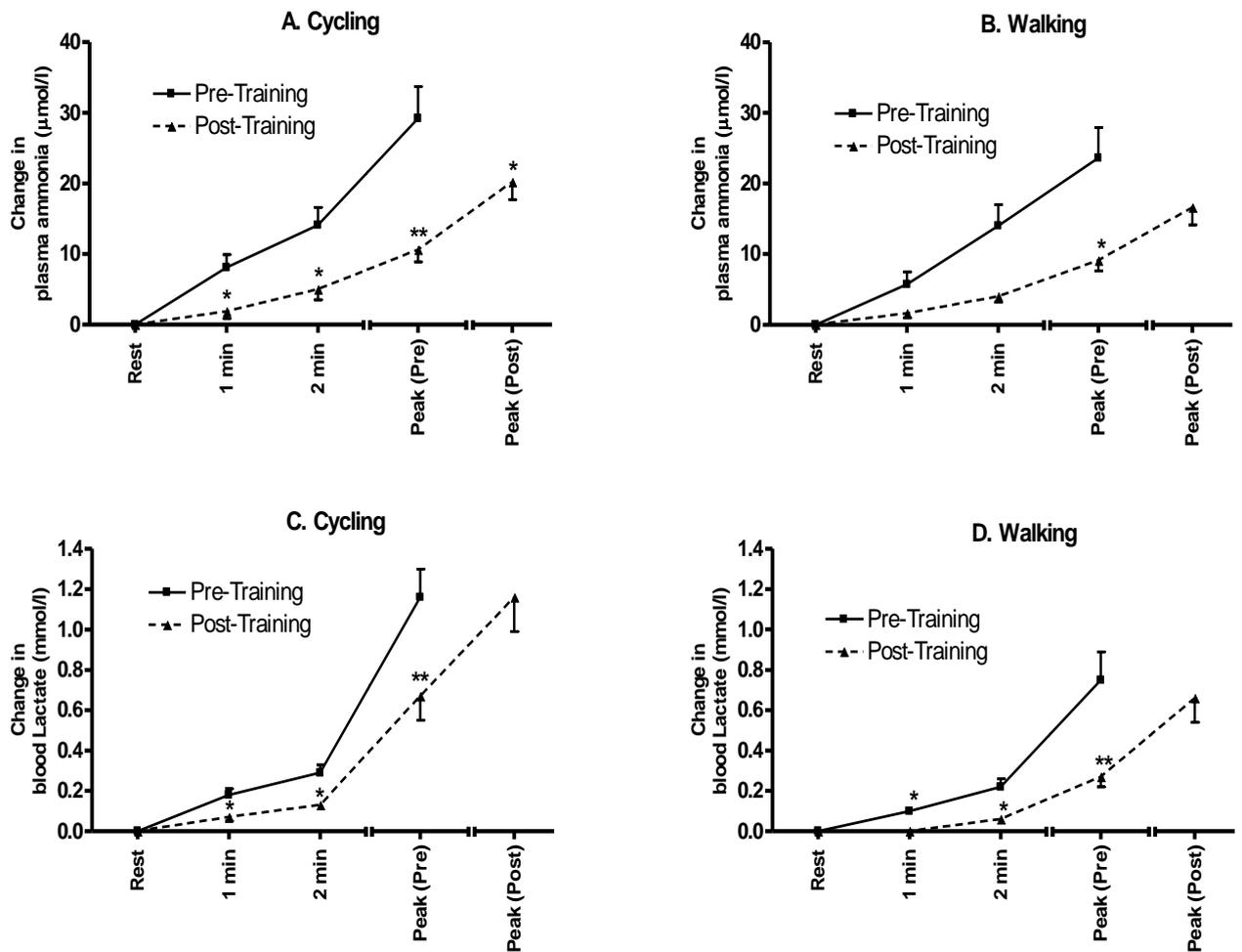
<sup>+</sup>p<0.01, <sup>++</sup>p<0.001 Within group, compared with resting (values given for peak/isotime exercise)

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, <sup>+</sup>p=0.09 Between pre-PR and post-PR group analysis

<sup>1</sup> time in post-PR exercise test at which peak exercise was reached in pre-PR test

<sup>2</sup>calculated as peak ammonia concentration measured minus resting concentration

Figure 8.1A-D: Plasma ammonia response to cycling exercise (A) and walking exercise (B). Blood lactate response to cycling exercise (C) and walking exercise (D). Pre-PR test is depicted in solid line and squares, post-PR test is depicted in dashed line and triangles



\*p<0.05, \*\*p<0.001 between pre-PR and post-PR analysis

***Subgroup analysis***

Subjects with (group 1; n=18) or without (group 2; n=7) an ammonia rise on pre-PR exercise tests (according to predetermined criteria for cycle exercise) were compared in subgroup analysis. There was no statistically significant difference in demographics, or in pre-PR ISWT, ESWT, cycle workload, treadmill distance and other exercise indices (**Table 8.3**). Resting concentrations and change with exercise pre-PR and post-PR for cycling are demonstrated in **Table 8.4**. Similar results on subgroup analysis were found with walking, and for simplicity the data have not been presented here.

The change in exercise-induced ammonia rise following PR is illustrated in **Figure 8.2**. The magnitude of the lactate response in group 2 was smaller but this was not statistically significant (**Table 8.4**). Lactate accumulation in both groups was attenuated by PR at all time points except when comparing peak exercise lactate concentrations (**Figure 8.2**). In group 1, plasma ammonia concentrations were lower following PR at all time points (**Figure 8.2**). In group 2 the lack of ammonia response to exercise was unchanged following PR, although there was a trend towards increased ammonia accumulation at peak exercise post-PR ( $p=0.079$ ). Two subjects in group 1 did not subsequently demonstrate an ammonia response following PR whereas one subject in group 2 did subsequently have a significant ammonia rise with exercise after PR.

Table 8.3: Baseline characteristics for COPD subjects for subgroup analysis (group1: n=18, subjects with an ammonia response to cycling, group2: n=7, subjects without an ammonia response to exercise)

	Group 1 (n=18)		Group 2 (n=7)	
	Pre-PR	Change with PR	Pre-PR	Change with PR
Age (years)	67 (7)	-	68 (10)	-
FEV <sub>1</sub> (% predicted)	48 (19)	5(2)	46 (15)	-2(3)
FFMI (kg/m <sup>2</sup> )	18 (2)	-	19 (3)	-
Oxygen sats at rest (%)	96 (2)	-	96 (1)	-
ISWT distance (meters)	309 (128)	97 (19)	203 (113)	82 (23)
ESWT time (seconds)	118 (57)	531 (89)	158 (46)	417 (143)
<i>Cycle test</i>				
VO <sub>2 peak</sub> (ml/kg/min)	16.4 (5.1)	1.36(0.50)	14.2 (3.2)	1.29(0.96)
Peak workload (watts)	61 (22)	14 (3)	43 (14)*	11 (3)
Peak V <sub>E</sub> (%MVV)	87 (19)	9.4(8.2)	84 (22)	7.3(14.8)
Heart rate (%predicted)	73 (12)	4.0(7.0)	77 (10)	-2.4(3.9)

Absolute values expressed as mean (SD). Change expressed as mean(SEM).

\*p=0.06 comparison between group 1 and group 2

FEV<sub>1</sub>= forced expiratory volume in 1 second; BMI= body mass index; FFMI= fat free mass index; ISWT= incremental shuttle walk test; ESWT= endurance shuttle walk test

Table 8.4: Exercise-induced change over resting concentration [mean (SEM)] in plasma ammonia and blood lactate in COPD subjects with (group 1: n=18) and without (group 2: n=7) an ammonia increase with exercise

		group 1		group 2	
		rest	Exercise-induced change <sup>1</sup>	rest	Exercise-induced change <sup>1</sup>
<b>Exercise-induced ammonia change</b> <b>µmol/l</b>	Pre-PR	51.4(12.7)	44.7 (3.9)	58.2(14.9)	3.0 (1.2) ††
	Post-PR	51.5(10.2)	28.7 (2.7)**	57.2(17.0)	11.9 (5.0) †
<b>Exercise-induced lactate change</b> <b>mmol/l</b>	Pre-PR	0.76(0.15)	1.54 (0.17)	0.68(0.18)	1.11 (0.21)
	Post-PR	0.65(0.13)*	1.65 (0.24)	0.68(0.14)	1.00 (0.22)

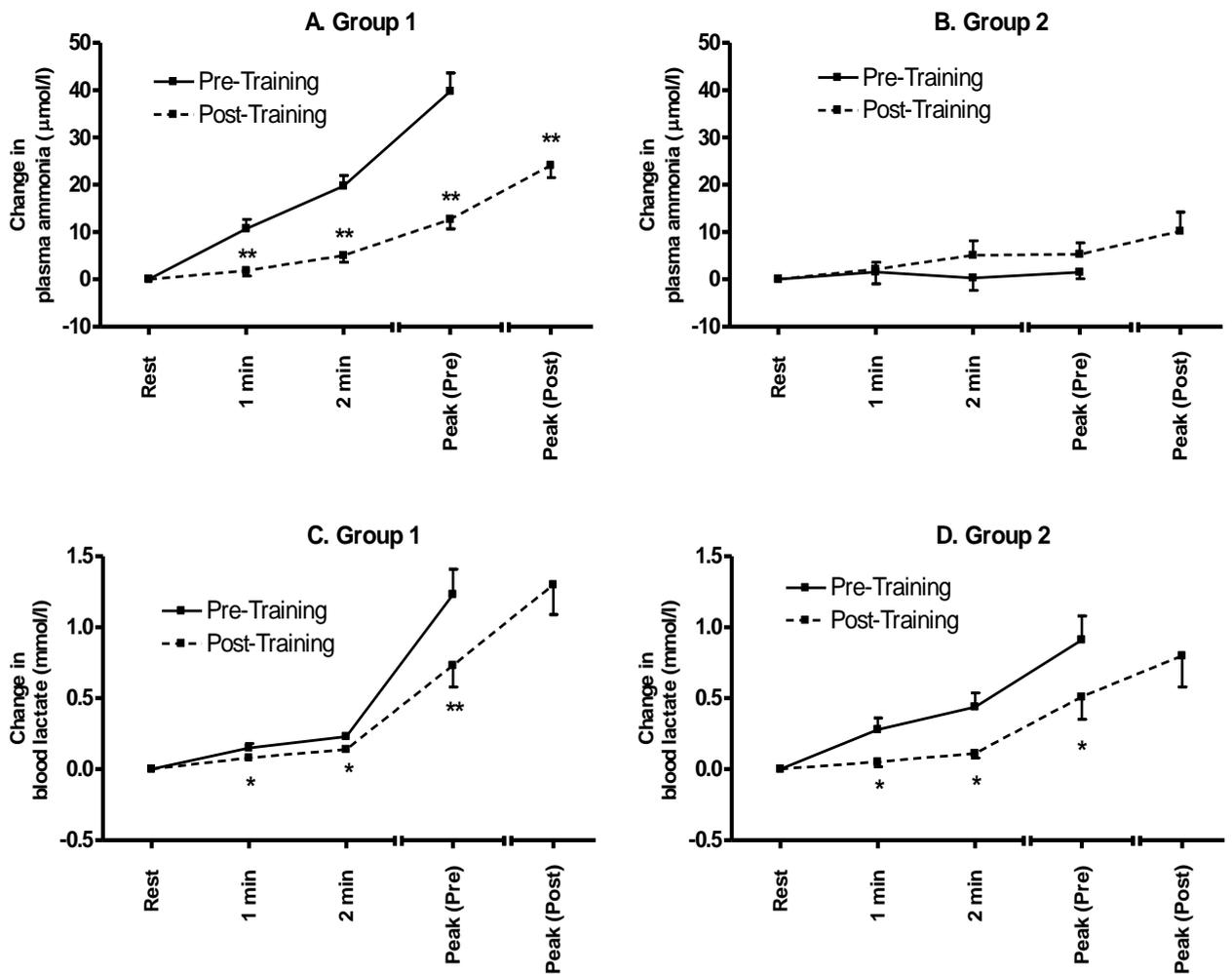
group 1= COPD subjects with ammonia rise during cycle exercise, group 2= COPD subjects without ammonia rise during cycle exercise

\*p=0.016, \*\*p=0.01 Between pre-PR and post-PR within group analysis

†p=0.005, ††p<0.001 Between group 1 and group 2 analysis

<sup>1</sup>calculated as peak ammonia concentration measured minus resting concentration

Figure 8.2A-D: Subgroup comparison of COPD subjects with (group1; n=18) and without (group 2; n=7) an ammonia rise on pre-PR cycling exercise. Plasma ammonia response to cycling exercise in group 1 (A) and group 2 (B). Blood lactate response to cycling exercise in group 1 (C) and group 2 (D). Pre-PR data is depicted in solid line and closed symbols circles, post-PR data is depicted in dashed line and open symbols triangles.



\*p< 0.05, \*\*p< 0.001

## **8.4 Discussion**

In this study I describe for the first time the adaptation of the plasma ammonia response to exercise in patients with COPD following exercise training during outpatient PR. PR significantly attenuated exercise-induced ammonia accumulation for both cycling and walking exercise. Moreover, my findings confirm that substantial improvements in skeletal muscle energy delivery can be brought about by a pragmatic UK outpatient PR programme comprising twice weekly supervised and daily unsupervised home exercise regimes which were not tightly regulated. However, no difference was seen in the functional response to PR in patients with or without an ammonia rise during pre-PR exercise.

I have previously shown in patients with COPD that plasma ammonia accumulation occurs during cycling exercise and that this is an indicator of inadequate skeletal muscle ATP supply in relation to demand. This imbalance in ATP turnover causes adenine nucleotide loss and may contribute to fatigue [Broberg S, 1989; Mutch BJ, 1983; Sahlin K, 1989a]. The ammonia response at high relative workrates in COPD is similar to healthy controls despite the low absolute workrates these individuals can achieve. In the current study ammonia accumulation was lower throughout exercise post-PR suggesting that the energy requirements of muscular work were more effectively met after exercise training. The likely explanation is an improvement in oxidative energy supply through mitochondrial (oxidative) adaptation. This is supported by my observation of a reduction in lactate accumulation during exercise post-PR in combination with reduced ammonia accumulation suggesting a shift from non-oxidative to oxidative sources of energy after training. My data are supported by findings in healthy subjects that reduced plasma ammonia accumulation following training corresponds to a reduction in muscle ATP

depletion [Lo PY *et al*, 1987; Stathis CG *et al*, 1994; Yuan Y, 2002]. The mechanisms underpinning these adaptations cannot be determined directly from my data but may be related to increased mitochondrial density, increased mitochondrial oxidative enzyme concentrations or a shift towards a greater proportion of type I (fatigue resistant) fibres. An alternative explanation for the reduction in ammonia accumulation is a reduction in the energy demands of exercise through an improvement in mechanical efficiency. However, I believe this explanation is less likely as the exercise testing procedure was carefully standardised and familiarisation tests were carried out beforehand.

My observation that there was little difference in ammonia and lactate responses between patients defined as ventilatory limited and non-ventilatory limited is interesting. This suggests that metabolic stress develops in the ambulatory muscles even in patients with an apparent ventilatory limit to exercise, and that similar improvements in energy delivery can be achieved with PR in these patients.

There is increasing recognition of the role of impaired skeletal muscle energy oxidative capacity in exercise limitation and disability in patients with COPD. Improving physical performance is an important therapeutic goal and impaired skeletal muscle function may be a remediable feature. Previous studies indicate that the capacity of oxidative energy production is reduced in the skeletal muscles of COPD patients with a consequent increased reliance on non-oxidative sources of energy [Gosker HR, 2002b; Maltais F, 2000b]. In keeping with this, significant muscle adenine nucleotide loss during exercise in subjects with COPD has previously been demonstrated [Steiner MC, 2005a]. This suggests that ATP production is unable to meet the energy demands of exercise even at the low absolute exercise intensities patients with COPD can achieve.

Previous studies have indicated that the contribution of oxidative energy production is improved by exercise training in COPD patients. Maltais and colleagues reported an increase in skeletal muscle oxidative enzymes after endurance training and observed an inverse correlation with the magnitude of exercise-induced lactataemia [Maltais F, 1996b]. Lactate production during exercise at a given workload has been shown to be lower following PR, and the magnitude of this reduction increased with training intensity [Casaburi R, 1991]. Further evidence from magnetic resonance (<sup>31</sup>P-MRS) studies suggests PCr degradation is lower and PCr resynthesis faster after training [Sala E, 1999]. My current data support these findings and extend our knowledge in this area by demonstrating directly through the measurement of ammonia that exercise training improves the mismatch between ATP supply and demand at maximum and isotime exercise. These findings were observed during both cycle and walking exercise. Moreover my findings confirm that substantial improvements in skeletal muscle energy delivery can be brought about by a pragmatic outpatient PR programme comprising twice weekly supervised and daily unsupervised home exercise.

In this study I have prospectively reproduced my previous post-hoc observation that there is a subgroup of patients with COPD who do not have a rise in plasma ammonia concentration with cycle exercise pre-PR despite having a blood lactate rise. Although exercise capacity was lower in patients without an ammonia rise, this was not statistically significant and no differences were seen in ventilatory response to exercise or in ventilatory limitation between the groups. This suggests that non-physiological causes such as motivation may be important limits to exercise in these subjects. PR had a similar effect on exercise performance and lactate accumulation in both groups, which suggests that the

subgroups cannot be characterised by a different functional response to training (although mechanisms involved might be different).

Some limitations to the study are acknowledged. Incremental exercise tests have been used in this study, but endurance tests may be more sensitive to changes in PR and therefore larger changes in the metabolic response might be observed during endurance exercise after training. I have not directly measured ATP turnover, but a relationship between adenine nucleotide loss and ammonia accumulation has previously been demonstrated in healthy subjects [Broberg S, 1989; Graham TE, 1990; MacLean DA, 1991; Mutch BJ, 1983] and by myself in COPD patients.

Muscle biopsy is a useful research technique but its invasiveness reduces its applicability in clinical practice. Blood markers of metabolic response might be more useful in this respect. Plasma ammonia, as a marker of skeletal muscle adenine nucleotide loss, could therefore be a useful outcome when assessing the impact of interventions that target skeletal muscles. The current study confirms plasma ammonia concentration is sensitive to exercise training in COPD subjects. I have already demonstrated the responsiveness of exercise-induced plasma ammonia accumulation to Dichloroacetate, a pharmacological agent that increases mitochondrial ATP production, in a cohort of patients with COPD.

In summary, exercise training during outpatient PR attenuates the exercise-induced plasma ammonia concentration with both cycling and walking in patients with COPD. My findings suggest that energy delivery better matched the demands of exercise following PR. The measurement of plasma ammonia accumulation during exercise may be a useful means of assessing skeletal muscle metabolic adaptation to exercise training in patients disabled by COPD.

## Chapter 9: Conclusions of Thesis

### **9.1 Summary of findings**

Work described in this Thesis provides an exploration of skeletal muscle energy metabolism during exercise in subjects with COPD. It is the first study to investigate in detail the plasma ammonia response during exercise in this population. Plasma ammonia has been used as a marker of energy delivery and this has enabled me to explore more closely the metabolic response to exercise. Comparison of the lactate and ammonia response has provided insight into the relative contributions of oxidative and non-oxidative ATP re-synthesis to energy delivery in COPD in relation to exercise performance. The results of the studies in this Thesis indicate that the skeletal muscles are subject to significant metabolic stress due to failure of energy delivery in relation to demand during exercise, and that this can be modified by interventions that target skeletal muscle performance. These studies also highlight the therapeutic potential of such interventions.

People with COPD become progressively disabled by their inability to carry out their activities of daily living because of exercise intolerance. Maximising exercise performance has therefore become an increasingly recognised goal in the clinical management of this population. Abnormal skeletal muscle function and impaired energy metabolism has been identified as an important contributor to exercise intolerance independent of lung function severity. In order to target performance-enhancing therapies appropriately, further understanding of skeletal muscle energy metabolism is required. A number of techniques have been developed in recent years which are useful research tools but impractical for clinical use. The aim of this Thesis firstly was to develop a new technique to measure metabolic functioning of skeletal muscle in patients with COPD.

This was then used to explore skeletal muscle energy delivery during exercise in this population.

The work described in **Chapters 5 and 6** introduced a technique for the study of the metabolic performance of peripheral muscles during exercise in COPD patients. Plasma ammonia response to cycle exercise was compared to healthy subjects. Ammonia accumulation at low absolute workrates was found in COPD subjects. A similar pattern of response was then confirmed with walking. Plasma ammonia change correlated to adenine nucleotide change within the skeletal muscle during cycling exercise, suggesting that plasma ammonia reflects muscle energy metabolism during exercise. However, importantly, my results indicate that the two exercise modalities cannot be used interchangeably when examining the adenine nucleotide response to exercise. Of interest, a clear ammonia response was absent in a subgroup of COPD subjects and this was consistent in both exercise modalities. This may suggest that in these patients energy supply was met by demand during exercise and that exercise was limited by other factors. Alternatively, exercise workrate may not have been sufficiently high to stress the muscles.

In **Chapters 7 and 8** plasma ammonia response as a marker of energy delivery following performance enhancement was examined. By comparing this with changes in the lactate response, further understanding of adaptation in the metabolic response to exercise has been gained. Improvement in exercise-induced plasma ammonia and lactate accumulation following PR (**Chapter 8**) indicates that oxidative ATP metabolism is improved with exercise training, resulting in reduced reliance on non-oxidative ATP resynthesis and reduced adenine nucleotide loss. In **Chapter 7** DCA infusion was used to activate PDC, a muscle enzyme involved in oxidative metabolism. DCA reduced ammonia and lactate accumulation during exercise and improved exercise performance compared

with placebo. This study confirms that skeletal muscle energy failure occurs in COPD and demonstrates that this is modifiable through activation of PDC. It also demonstrates that improving energy delivery has direct effects on exercise performance even in those subjects who appear to have overwhelming ventilatory limitation, emphasising the functional relevance of skeletal muscle impairment in this population. Furthermore, skeletal muscle PDC activation may be a target for pharmacological intervention in the management of exercise intolerance in COPD.

## **9.2 Techniques developed and problems encountered**

The collection and analysis techniques for measuring plasma ammonia concentration in COPD subjects were developed and validated as part of this Thesis. First, a safe environment for patients to exercise whilst using a handwarmer was established. Laboratory analysis was adapted from previous techniques used in healthy subjects after discussion with several different laboratories. Due to the sensitivity of the measurement it took me 6 months to ensure the final technique used was reliable and reproducible.

DCA has never been used in COPD subjects and is currently not used as licensed medication in clinical practice. Therefore careful ethical consideration and safety protocols were required prior to commencing the study using DCA.

## **9.3 Criticisms and limitations**

Some potential criticisms of the work presented in this Thesis are addressed in the discussion of each chapter. However, some more general issues are given particular attention here.

This Thesis establishes ammonia as a valid marker of energy metabolism. However, the technique used will need to be adapted for clinical practice and future work

using current clinical biochemistry laboratory analysis procedures for ammonia measurement may be helpful. As previously found in studies on COPD patients there were technical problems obtaining good quality biopsy specimens during exercise which highlights the need to find alternative markers of skeletal muscle metabolism. The metabolic responses to exercise have previously been found to be highly variable in this population both between patients and within patients over time. This probably reflects the variable impact of ventilatory and muscle factors limiting maximal exercise performance in COPD. In addition it may be difficult to achieve a true “resting” state in patients who regularly exercise near their maximal capacity during their normal activities of daily living. Patients also show wide variations in exercise performance and their response to training.

A healthy control group was not included in most of the studies described in this thesis. As the main purpose was to evaluate the effect of interventions on the metabolic response, it was decided that including a control group was not necessary. Furthermore, a comparison with COPD patients will be difficult to interpret because exercise in healthy subjects would be carried out at much higher absolute intensities.

#### **9.4 Future Work**

Therapeutic interventions directed to specific performance goals can ensure everyday exercise needs of individual patients are met. Such sophistication needs to be based on adequate assessment of the degree of local pulmonary and systemic involvement. Performance enhancement is likely to involve an integrated physiological approach combining exercise training, nutritional support, electrical stimulation and pharmacological agents to improve skeletal muscle function and hence exercise capacity. This will require the broader consequences of chronic lung disease to be considered in addition to

underlying pulmonary pathology in the context of drug development and a change in emphasis towards the use of combined treatment modalities in clinical trials.

There are a number of interesting developments in the field of muscle physiology which may lead to new treatments targeted at peripheral muscle performance in patients with COPD. It is becoming clear that impaired ATP production by oxidative phosphorylation is important in exercise intolerance in COPD and findings from this Thesis suggest that pharmacological approaches to improving oxidative energy metabolism during exercise are worth pursuing. The benefits of compounds such as DCA now need to be confirmed prospectively before such treatment can be recommended in routine clinical practice. In common with exercise training in healthy subjects, such treatment would probably provide most benefit if combined with an appropriate training programme and larger PR studies to look at this are indicated.

The differential ammonia response to exercise is interesting and worthy of further exploration, particularly in the context of response to performance-enhancing therapies such as DCA. A lack of ammonia response may identify patients who benefit from PR through improved confidence and capacity utilisation rather than physiological adaptation. Again, larger studies are required to identify whether different ammonia response is indicative of different clinical outcomes.

Current research suggests that skeletal muscles in patients with COPD are subject to significant oxidative stress during levels of exercise achieved performing daily activities. The purine nucleotide degradation pathway is a potential source of exercise-induced oxidative stress through the activity of xanthine oxidase. It is possible therefore that regular metabolic stress in the muscles is a trigger for free radical production, which may have a further impact on muscle performance. This relationship and its clinical

relevance is worthy of further exploration, particularly with respect to modification of the metabolic response.

Research over recent years, including work in this Thesis, has begun to answer important questions, and further advance our understanding of the basic mechanisms involved in the metabolic response seen within skeletal muscles of patients with COPD, and how this influences exercise performance. Work in this Thesis has demonstrated that there is an opportunity to develop new drug treatments targeted at improving skeletal muscle function. The similarity of muscle dysfunction in COPD to other chronic disabling conditions such as heart failure and peripheral vascular disease means that such therapy could have wide ranging applications and a significant impact on our clinical practice.

## Appendix 1: Reproducibility studies for ammonia analysis

**Table A1.1: Standard curve data for validation of ammonia analysis (n=7)**

High and low standard concentrations measured in duplicate (A and B) for each analysis performed are presented. Data has been analysed for reproducibility (Coefficient of Variation analysis)-see main text.

Standard curve	Ammonia concentration for low standard ( $\mu\text{mol/l}$ )		Ammonia concentration for high standard ( $\mu\text{mol/l}$ )	
	A	B	A	B
1	140	143	371	368
2	140	143	361	365
3	133	147	354	364
4	143	140	368	368
5	143	147	361	348
6	143	147	368	354
7	147	133	368	368

Low standard concentration:  $140\mu\text{mol/l}$

High standard concentration:  $350\mu\text{mol/l}$

## Appendix 2: Laboratory techniques- Muscle biopsy analysis

All muscle samples were analysed at the same time after completion of the study (Chapter 5) by John Fox under the supervision of Professor Paul Greenhaff at the Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences, University of Nottingham Medical School, Queens Medical Centre, Nottingham, UK.

Following freeze-drying, powdering and extraction (all visible connective tissue, blood and fat removed), samples were analysed for phosphocreatine (PCr) and creatine concentrations using the spectrophotometric method of Harris *et al* [Harris RC *et al*, 1974]. Adenine nucleotides (ATP, ADP and AMP) and their breakdown derivatives (IMP, inosine and xanthine) were measured using automated High Performance Liquid Chromatography (HPLC System Gold, Beckman Instruments, Bucks, U.K.) [Wynants J *et al*, 1985]. Total creatine concentration was calculated as the sum of PCr and creatine. All metabolites were corrected for the highest total creatine measured from the rest and exercising pair. By this means it is possible to compensate for contamination of samples with non-muscle tissue (connective tissue, blood and fat) [Hultman E *et al*, 1983].

## Appendix 3: Questionnaires

PHYSICAL ACTIVITY QUESTIONNAIRE**HOUSEHOLD ACTIVITIES**

1. Do you do light housework (dusting, washing dishes, ironing)?
 

Never	<input type="checkbox"/>
Less than once a month	<input type="checkbox"/>
Sometimes (only when partner or help is not available)	<input type="checkbox"/>
Mostly (sometimes assisted by partner or helper)	<input type="checkbox"/>
Always (alone or together with partner)	<input type="checkbox"/>
  
2. Do you do heavy housework (washing floors, washing windows, taking the rubbish out, Hoovering)?
 

Never	<input type="checkbox"/>
Less than once a month	<input type="checkbox"/>
Sometimes (only when partner or help is not available)	<input type="checkbox"/>
Mostly (sometimes assisted by partner or helper)	<input type="checkbox"/>
Always (alone or together with partner)	<input type="checkbox"/>
  
3. How many people live in the house including yourself? \_\_\_\_\_
  
4. How many rooms do you clean in your house?
 

None	<input type="checkbox"/>
1-6 rooms	<input type="checkbox"/>
7-9 rooms	<input type="checkbox"/>
10 or more rooms	<input type="checkbox"/>
  
5. What type of accommodation do you live in (eg flat, bungalow, house)? \_\_\_\_\_
  
6. Do you prepare or help prepare hot meals?
 

Never	<input type="checkbox"/>
Sometimes (1 or 2 times a week)	<input type="checkbox"/>
Mostly (3-5 times a week)	<input type="checkbox"/>
Always (more than 5 times a week)	<input type="checkbox"/>

7. How many times do you climb a flight of stairs (i.e. 10 steps) in an average day?

Never climb stairs

1-5 times

6-10 times

More than 10 times

8. If you go somewhere in your home town, what kind of transport do you use?

I never go out

Car

Public transport (eg bus)

Bicycle

Walking

9. How often do you go out to do the shopping?

Never

Less than once a week

Once a week

2-4 times a week

Every day

10. If you go out shopping, what type of transport do you use?

I never go out shopping

Car

Public transport (eg bus)

Bicycle

Walking

**SPORT ACTIVITY**

Do you play a sport? If yes- please list up to two that you play below

**Sport one**

Name of sport: \_\_\_\_\_

How many hours per week do you spend playing this sport? \_\_\_\_\_

How many months each year do you spend playing this sport? \_\_\_\_\_

Intensity of sport (fill in this question with help from researcher) \_\_\_\_\_

**Sport two**

Name of sport: \_\_\_\_\_

How many hours per week do you spend playing this sport?  
\_\_\_\_\_

How many months each year do you spend playing this sport?  
\_\_\_\_\_

Intensity of sport (fill in this question with help from researcher)  
\_\_\_\_\_

## LEISURE TIME ACTIVITIES

Do you have other hobbies or activities that are physically active? (This may include knitting, walking etc). If yes- please list up to 6 of them below.

### Activity 1

Name of activity: \_\_\_\_\_

How many hours per week do you spend doing this activity? \_\_\_\_\_

How many months each year do you spend doing this activity? \_\_\_\_\_

Intensity of activity (fill in this question with help from researcher) \_\_\_\_\_

### Activity 2

Name of activity: \_\_\_\_\_

How many hours per week do you spend doing this activity? \_\_\_\_\_

How many months each year do you spend doing this activity? \_\_\_\_\_

Intensity of activity (fill in this question with help from researcher) \_\_\_\_\_

### Activity 3

Name of activity: \_\_\_\_\_

How many hours per week do you spend doing this activity? \_\_\_\_\_

How many months each year do you spend doing this activity? \_\_\_\_\_

Intensity of activity (fill in this question with help from researcher) \_\_\_\_\_

### Activity 4

Name of activity: \_\_\_\_\_

How many hours per week do you spend doing this activity? \_\_\_\_\_

How many months each year do you spend doing this activity? \_\_\_\_\_

Intensity of activity (fill in this question with help from researcher) \_\_\_\_\_

**CHRONIC RESPIRATORY QUESTIONNAIRE (Self Reported)**

*This questionnaire is designed to find out how you have been feeling during the last two weeks. You will be asked how short of breath you have been, how tired you have been feeling and how your mood has been.*

**NAME**.....

**DATE**.....

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**ACTIVITIES**

We would like you to think of ways in which your shortness of breath limits your life. We are particularly interested in activities, which you still do, but which are limited by your shortness of breath.

Listed below are some activities, which can make people with lung problems feel short of breath.

**If you have felt short of breath doing any of the activities listed below during the last two weeks then please circle each relevant activity. If you have not done the activity during the last two weeks or it does not make you short of breath then leave it blank.**

THE ACTIVITIES ARE:

<b>1. BEING ANGRY OR UPSET</b>	<b>14. PLAYING SPORTS</b>
<b>2. HAVING A BATH OR SHOWER</b>	<b>15. REACHING OVER YOUR HEAD</b>
<b>3. BENDING</b>	<b>16. RUNNING - SUCH AS FOR A BUS</b>
<b>4. CARRYING - SUCH AS GROCERIES</b>	<b>17. SHOPPING</b>
<b>5. DRESSING</b>	<b>18. WHILE TRYING TO SLEEP</b>
<b>6. EATING</b>	<b>19. TALKING</b>
<b>7. GOING FOR A WALK</b>	<b>20. VACUUMING</b>
<b>8. DOING YOUR HOUSEWORK</b>	<b>21. WALKING AROUND YOUR OWN HOME</b>
<b>9. HURRYING</b>	<b>22. WALKING UPHILL</b>
<b>10. MAKING YOUR BED</b>	<b>23. WALKING UPSTAIRS</b>
<b>11. MOPPING OR SCRUBBING A FLOOR</b>	<b>24. WALKING WITH OTHERS ON LEVEL GROUND</b>
<b>12. MOVING FURNITURE</b>	<b>25. PREPARING MEALS</b>
<b>13. PLAYING WITH CHILDREN/GRANDCHILDREN</b>	

Please list any other activities you have done during the last two weeks that have made you feel short of breath. These should be activities which you do frequently and which are important in your day-to-day life.

.....

.....

.....

We would now like you to identify the **five most important activities** in which you have been limited by your shortness of breath.

Please write your **five most important activities** on the lines below and then tell us how short of breath you have been while performing each activity by ticking the box which best describes how you feel.

**HOW SHORT OF BREATH HAVE YOU BEEN DURING THE LAST 2 WEEKS WHILE PERFORMING THESE ACTIVITIES?**

	Extremely short of breath	Very short of breath	Quite short of breath	Moderate shortness of breath	Some shortness of breath	A little shortness of breath	Not at all short of breath
1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**PLEASE MAKE SURE YOU HAVE COMPLETED THE ABOVE TABLE BEFORE TURNING THE PAGE**

**Thank you**

6. In general, how much of the time during the last two weeks have you felt frustrated or impatient?

Please indicate how often during the last two weeks you have felt frustrated or impatient by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

7. How often during the past 2 weeks did you have a feeling of fear or panic when you had difficulty getting your breath?

Please indicate how often you had a feeling of fear or panic when you had difficulty getting your breath by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

8. What about fatigue? How tired have you felt over the last 2 weeks?

Please indicate how tired you have felt over the last 2 weeks by ticking one of the following options from the list below.

1. EXTREMELY TIRED
2. VERY TIRED
3. QUITE A BIT OF TIREDNESS
4. MODERATELY TIRED
5. SOMEWHAT TIRED
6. A LITTLE TIRED
7. NOT AT ALL TIRED

9. How often during the last 2 weeks have you felt embarrassed by your coughing or heavy breathing?

Please indicate how much of the time you felt embarrassed by your coughing or heavy breathing by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

10. In the last 2 weeks, how much of the time did you feel very confident and sure that you could deal with your illness?

Please indicate how much of the time you felt very confident and sure that you could deal with your illness by ticking one of the following options from the list below.

1. NONE OF THE TIME
2. A LITTLE OF THE TIME
3. SOME OF THE TIME
4. A GOOD BIT OF THE TIME
5. MOST OF THE TIME
6. ALMOST ALL OF THE TIME
7. ALL OF THE TIME

11. How much energy have you had in the last 2 weeks?

Please indicate how much energy you have had by ticking one of the following options from the list below.

1. NO ENERGY AT ALL
2. A LITTLE ENERGY
3. SOME ENERGY
4. MODERATELY ENERGETIC
5. QUITE A BIT OF ENERGY
6. VERY ENERGETIC
7. FULL OF ENERGY

- 12.** In general, how much of the time did you feel upset, worried or depressed during the past 2 weeks?

Please indicate how much of the time you felt upset, worried or depressed during the past 2 weeks by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

- 13.** How often during the last 2 weeks did you feel you had complete control of your breathing problems?

Please indicate how often you felt you had complete control of your breathing problems by ticking one of the following options from the list below.

1. NONE OF THE TIME
2. A LITTLE OF THE TIME
3. SOME OF THE TIME
4. A GOOD BIT OF THE TIME
5. MOST OF THE TIME
6. ALMOST ALL OF THE TIME
7. ALL OF THE TIME

- 14.** How much of the time during the last 2 weeks did you feel relaxed and free of tension?

Please indicate how much of the time you felt relaxed and free of tension by ticking one of the following options from the list below.

1. NONE OF THE TIME
2. A LITTLE OF THE TIME
3. SOME OF THE TIME
4. A GOOD BIT OF THE TIME
5. MOST OF THE TIME
6. ALMOST ALL OF THE TIME
7. ALL OF THE TIME

**15.** How often during the last 2 weeks have you felt low in energy?

Please indicate how often during the last 2 weeks you have felt low in energy by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

**16.** In general, how often during the last 2 weeks have you felt discouraged or down in the dumps?

Please indicate how often during the last 2 weeks you felt discouraged or down in the dumps by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

**17.** How often during the last 2 weeks have you felt worn out or sluggish?

Please indicate how much of the time you felt worn out or sluggish by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

- 18.** How happy, satisfied or pleased have you been with your personal life during the last 2 weeks?

Please indicate how happy, satisfied or pleased you have been by ticking one of the following options from the list below.

1. VERY DISSATISFIED, UNHAPPY MOST OF THE TIME
2. GENERALLY DISSATISFIED, UNHAPPY
3. SOMEWHAT DISSATISFIED, UNHAPPY
4. GENERALLY SATISFIED, PLEASED
5. HAPPY MOST OF THE TIME
6. VERY HAPPY MOST OF THE TIME
7. EXTREMELY HAPPY, COULD NOT HAVE BEEN MORE SATISFIED OR PLEASED

- 19.** How often during the last two weeks did you feel upset or scared when you had difficulty getting your breath?

Please indicate how often during the past 2 weeks you felt upset or scared when you had difficulty getting your breath by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

- 20.** In general how often during the last 2 weeks have you felt restless, tense or uptight?

Please indicate how often you have felt restless, tense or uptight by ticking one of the following options from the list below.

1. ALL OF THE TIME
2. MOST OF THE TIME
3. A GOOD BIT OF THE TIME
4. SOME OF THE TIME
5. A LITTLE OF THE TIME
6. HARDLY ANY OF THE TIME
7. NONE OF THE TIME

## Appendix 4: Publications

### Publications

**LD Calvert**, R Shelley, SJ Singh, PL Greenhaff, J Bankart, MD Morgan, MC Steiner. Enhanced exercise performance in COPD using Dichloroacetate: A pharmacological intervention targeting skeletal muscle *Eur Respir J*. 2008 Apr;31(4):751-8

**Calvert LD**, Singh SJ, Greenhaff PL, Morgan MD, Steiner MC. The plasma ammonia response to cycle exercise in chronic obstructive airways disease *Am J Respir Crit Care Med*. 2008 May 15;177(10):1090-4

**Calvert LD**, Singh SJ, Morgan MD, Steiner MC. Plasma Ammonia Response to Cycling and Walking in Chronic Obstructive Pulmonary Disease. [manuscript submitted]

**Calvert LD**, Singh SJ, Morgan MD, Steiner MC. Skeletal muscle energy delivery improves following pulmonary rehabilitation in COPD [manuscript submitted]

**Abstracts**

**LD Calvert** SJ Singh, PL Greenhaff, MD Morgan, MC Steiner. Blood ammonia accumulation during maximal incremental exercise in chronic obstructive pulmonary disease (COPD). [Poster] *Am J Respir Crit Care Med* 2006;3: A1279

**LD Calvert**, SJ Singh, PL Greenhaff, MD Morgan, MC Steiner. Characteristics of the blood ammonia response to exercise in chronic obstructive pulmonary disease (COPD). [Oral presentation]. *ERJ* 2006;28 (suppl 50): A3573

**LD Calvert**, RA Evans, SJ Deacon, PL Greenhaff, M Morgan, MC Steiner. Resting Quadriceps Muscle Energy Status and Exercise Performance in Chronic Obstructive Pulmonary Disease. [Oral presentation]. *Am J Respir Crit Care Med* 2007;175: A2763

**LD Calvert**, SJ Singh, PL Greenhaff, MD Morgan, MC Steiner. Plasma ammonia response to cycling and walking in chronic obstructive pulmonary disease [Poster discussion]. *ERJ* Sept 2007 A2368

**LD Calvert**, SJ Singh, MD Morgan, MC Steiner. Adaptation of the metabolic response to exercise following Pulmonary Rehabilitation in COPD [Poster discussion]. *ERJ* Oct 2008

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