The Nutritional Enhancement of Exercise Training in Chronic Obstructive Pulmonary Disease

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Thesis Submitted to the University of Leicester for the Degree of Doctor of Medicine

June ~~2002~~ 2003

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Declaration

I hereby declare that this thesis has been composed by myself and that the work, of which this is a record, has been performed by myself except where assistance has been acknowledged. I also received assistance from members of the Pulmonary Rehabilitation team who performed shuttle walk tests when I was unable to do so. Louise Sewell, Prakash Patel, Sue Revill and Rachel Barton provided assistance during the muscle biopsy procedure.

No part of this thesis has been submitted in any previous application for a higher degree.

I hereby give permission for this thesis to be made available for consultation, photocopying and use by other libraries directly or via the British Library.

Michael Charles Steiner

05 January 2003

Acknowledgements

I am indebted to my supervisor Dr. Mike Morgan for his friendship, support and encouragement during my time at Glenfield Hospital.

I would also like to thank Sally Singh and other members of the pulmonary rehabilitation team, Rachel Collier, Louise Sewell and Jo Williams for their support and for making me feel so welcome in the department.

My thanks also to Rachel Barton for all her help and enthusiasm during the nutritional supplementation trial.

I would like to thank Prof. Paul Greenhaff for his enthusiastic collaboration in the development of the muscle biopsy programme at Glenfield and for being such a patient sounding board for my endless questions on muscle physiology and metabolism. My thanks also to John Fox and Siun Campbell for performing the laboratory analysis of muscle samples.

I am grateful to Prof. Adrienne Hardman, Prof. Peter Jones and Prof. Henryk Lakomy from Loughborough University and to Dr. Peter Sheldon from Leicester University for their help and advice in the development of this project. My thanks also to Dr. Nick Taub at the Trent Institute for Health Services Research for providing statistical advice.

I am also grateful to Sue Revill, Prakash Patel and D.D. Vara for their advice and assistance in the development and application of the muscle biopsy procedure and for their expert tuition on laboratory exercise testing.

Finally, I would like to thank my wife Lisa for her unstinting support during this work and to my children Dominic and Hannah for providing such a delightful distraction from the writing of this thesis.

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Abbreviations

ADP	Adenosine di-phosphate
AMP	Adenosine mono-phosphate
ATP	Adenosine tri-phosphate
BIA	Bio-electrical impedance analysis
BMI	Body mass index
BS	Borg score
BTS	British Thoracic Society
CHO	Carbohydrate
COPD	Chronic Obstructive Pulmonary Disease
Cr	Creatine
CRQ-SR	Self reported chronic respiratory questionnaire
DEXA	Dual energy x-ray absorptiometry
ERS	European Respiratory Society
ESWT	Endurance shuttle walk test
FEV_1	Forced expiratory volume in one second
FFM	Fat free mass
FFMI	Fat free mass index
FM	Fat mass
FVC	Forced vital capacity
HGS	Handgrip strength
HR	Heart rate
IMP	Inosine monophosphate

ISWT	Incremental shuttle walk test
Kcal	Kilocalories
La	Lactate
LM	Lean mass
MRS	Magnetic resonance spectroscopy
PCr	Phosphocreatine
PDC	Pyruvate dehydrogenase complex
PE	Perceived exertion score
QS	Quadriceps strength
SaO_2	Oxygen saturation
SFA	Skinfold anthropometry
SLP	Substrate level phosphorylation
TAN Pool	Total adenine nucleotide pool
TCA Cycle	Tricarboxylic acid cycle
VO_{2peak}	Peak oxygen uptake
WR	Workrate

CHAPTER ONE

Introduction

CHRONIC Obstructive Pulmonary Disease (COPD) is a chronic condition characterised by progressive airflow obstruction leading to breathlessness, wheeze and sputum production. Mild disease may have minimal impact on function or quality of life but as the disease progresses, substantial physical disability may occur, mainly as a result of exercise limitation due to breathlessness. The physical limitations imposed by the disease may cause considerable handicap to the individual and has social consequences for the family and wider community.

There remains a significant unmet medical need for COPD patients. For many patients the pulmonary pathophysiology underlying their disease is irreversible and therapy aimed at improving lung function frequently fails to meet their needs. Broader strategies to address the secondary physical, psychological and social consequences of the disease may be more effective and warrant more attention. Pulmonary rehabilitation seeks to do this by restoring the patients to their maximum level of independence and functioning within the community. Improving physical fitness by progressive exercise training is central to successful rehabilitation programmes. In this thesis I will be exploring the potential for enhancing the physical benefits of exercise training within pulmonary rehabilitation.

Defining COPD

In 1834 Renee Laennec provided one of the first descriptions of the pathological changes of emphysema and the associated hyperinflation (Laennec, 1834) noting that: *"The lungs seem as if confined in their natural cavity, and, when explored, instead of collapsing as usual they rise in some degree and project beyond the borders of the thorax."* Importantly he also recognised the clinical consequences of the disease reporting that for sufferers: *"The difficulty of breathing is constant but is aggravated by paroxysms, which are irregular both in the period of their return and their duration. It is likewise increased by all the causes which usually increase dyspnoea such as the action of digestion, flatulence in the stomach or bowels, anxiety, living in elevated situations, strong exercise, running or ascending a height."*

With the increase in cigarette smoking in the twentieth century COPD is now the leading cause of respiratory disability in the United Kingdom (Herbst *et al.*, 2000). Approximately 600,000 people suffer with COPD in the UK most presenting between the ages of 50 and 80 (Calverley & Bellamy, 2000). Although prevalence rates are stable in this country, the burden of the disease worldwide is predicted to rise in line with the increase in tobacco consumption (Murray & Lopez, 1997).

When COPD was first defined, a distinction was made between Chronic Bronchitis which was defined by yearly recurrent periods of sputum expectoration and Emphysema, a pathological condition consisting of air space destruction, loss of elastic recoil of the lung parenchyma and progressive hyperinflation of the lungs (CIBA Guest Symposium, 1959). For practical purposes these conditions usually co-exist in the individual patient and represent the spectrum of disease known as COPD. The British Thoracic Society (BTS) defines COPD as a chronic, slowly progressive disorder

characterised by airflow obstruction, which does not change markedly over several months (British Thoracic Society, 1997). A smoking history of more than 20 pack years is usually obtained. The diagnosis requires objective evidence of airflow obstruction (an FEV_1 < 80 predicted and an FEV_1/FVC ratio < 70%) that does not return to normal with treatment.

Although the development of airflow obstruction is the initiating event in COPD the severity of lung function impairment is a poor predictor of symptoms and subsequent disability. Breathlessness and exercise limitation is the result of a chain of events that involves avoidance of physical activity, peripheral muscle deconditioning, loss of confidence and social isolation.

Disability and Handicap in COPD

The World Health Organisation recommends that the impact of a disease on an individual be considered in terms of impairment, disability and handicap (World Health Organisation, 1980). In the case of COPD, impairment is represented by the underlying pathophysiology such as a reduction in FEV_1 or peak oxygen uptake during exercise. Disability represents the loss of bodily function resulting from this impairment. In COPD this might be the inability to walk more than a few metres or climb a flight of stairs. Handicap is the social and psychological impact of this loss of function on quality of life.

Although disability and handicap ultimately result from impairment due to lung disease in COPD patients, the relationship between these entities is not straightforward. Many investigators have observed that there is a weak relationship only between spirometric impairment and exercise capacity in COPD patients (Killian *et al.*,

1992;Morgan *et al.*, 1983). In other words there are patients with severe lung disease whose exercise capacity is well preserved and conversely patients with relatively mild airflow obstruction who are severely disabled. Similarly disablement does not automatically lead to handicap both of which will be strongly influenced by social and psychological factors.

These observations have important implications for treatment of these patients. Much of the available pharmacological treatment is directed at restoring lung function. Although this may improve symptoms and health status in some patients the potential exists to target therapy at reducing disability and handicap independent of lung function. The value of this approach is illustrated by the clear benefits of pulmonary rehabilitation for patients with COPD. Other more straightforward interventions such as physical aids to mobility and ambulatory oxygen have also been shown to have a significant impact on disability in this population. These approaches are frequently overlooked.

Structure of Thesis

The broad aim of this thesis is to explore the potential for performance enhancement in COPD. The key hypothesis I have investigated is that nutritional support can improve the outcome of exercise training in the context of pulmonary rehabilitation. This hypothesis was tested by a randomised controlled trail of nutritional supplementation in COPD patients participating in pulmonary rehabilitation. This is described in **Chapter 8**.

In **Chapters 2** and **3** I give an overview of the current evidence to support the importance of peripheral muscle dysfunction in COPD and the available literature on therapeutic interventions to enhance physical performance in this population.

Chapter 4 describes the methodology used in the experimental work described in this thesis.

A number of additional research questions are also considered in this thesis. An investigation of the metabolic responses to exercise and the adaptations to training and nutritional support in the peripheral muscles was undertaken. To this end a technique for sampling the quadriceps muscle during exercise in COPD patients was developed as part of this thesis and a preliminary study to assess the viability of this technique is described in **Chapter 6**. The effects of exercise training and nutritional supplementation during the randomised controlled trial described above are examined using this technique and are detailed in **Chapter 9**. The laboratory analysis of muscle biopsies was not performed by the author and therefore does not form part of the experimental work presented in this thesis. Details of these analytical methods are given in brief in **Appendix I**.

A number of measures of physical performance and nutritional status were introduced for the assessment of patients participating in the nutritional intervention trial. An additional preliminary study exploring the relationship between these indices and their response to exercise training is described in **Chapter 5**.

An important aspect of the impact of nutritional support in this population is its effect on nutritional status. A study comparing methods of measurement of body composition in COPD patients is described in **Chapter 7**.

CHAPTER TWO

Peripheral Muscle Dysfunction in COPD

PATIENTS with Chronic Obstructive Pulmonary Disease (COPD) are frequently unable to carry out many activities of daily living because of exercise intolerance. This leads to increasing social isolation, depression and dependence. Impaired exercise performance is therefore an important clinical consequence of COPD and improving physical performance is an important therapeutic goal. In this chapter I will discuss the causes of exercise intolerance in COPD with particular reference to the role of the peripheral muscles. In **chapter 3** I will go on to consider current literature concerning performance enhancing therapy in this population.

Exercise Performance in COPD

It has long been assumed that exercise limitation in COPD is due to a ceiling on pulmonary ventilation and oxygen uptake during exercise. This is underlined by the finding of reduced oxygen uptake, low breathing reserve, expiratory flow limitation and dynamic hyperinflation in studies of exercise physiology in COPD (Gallagher, 1994;O'Donnell, 1994). However, exercise may also be limited because the load to the respiratory system is excessive. This load is represented by the metabolic demands of

exercising muscles. There is now accumulating evidence that these metabolic demands are high in COPD patients because of peripheral muscle dysfunction, indeed, in many patients impaired muscle functional capacity may be a limit to exercise performance in itself (American Thoracic Society, 1999).

Support for this notion comes from the observation in many studies that the relationship between impairment of lung function and exercise capacity is poor (Killian *et al.*, 1992;Morgan *et al.*, 1983). Moreover, patients who have their lung function restored to normal by transplantation may not achieve their predicted exercise capacity following the procedure (Levy *et al.*, 1993;Low *et al.*, 1992). Extra-pulmonary factors in exercise limitation are important because improving the performance of the respiratory system is difficult due to the irreversible nature of the underlying pathology whereas peripheral muscle dysfunction is potentially remediable. This is illustrated by the impressive improvements seen in exercise performance following exercise training despite it having no effect on lung function.

I will now discuss the nature and possible causes of peripheral muscle dysfunction in COPD and go on to discuss the potential for enhancing physical performance by improving the functional capacity and efficiency of the peripheral muscles. To place the importance of peripheral muscle dysfunction in its appropriate context, an understanding of healthy muscle physiology and energy metabolism is needed and I will briefly discuss this now.

Healthy Muscle Physiology

Before considering the nature and causes of peripheral muscle dysfunction in COPD, I will give a brief account of healthy muscle physiology and energy metabolism (for a detailed review see Maughan *et al*, 1997).

Skeletal Muscle Ultrastructure

Skeletal muscle cells are long multinucleated fibres, which may extend the entire length of the muscle. Muscle fibres are typically supplied by one motor endplate located near the centre of the fibre. The electrical impulse from this nerve ending is transmitted along the fibre by the sarcolemma, a thin membrane encasing the muscle fibre. This also serves to conduct fuels and waste products in and out of the muscle cell from surrounding capillaries.

The striated appearance of skeletal muscle is due to the specific arrangement of protein bands (myofibrils) in the muscle fibre. Thick filaments (myosin) are anchored to a protein sheet (the z line) and overlap with thin filaments (actin). It is the interaction of these filaments that leads to muscle contraction. The passage of an action potential from the sarcolemma results in calcium release into the sarcoplasm. This binds to troponin on the thin filament uncovering active myosin binding sites on the actin protein. The result is a sequence of crossbridge formations between the thick and thin filaments resulting in myofibril shortening and force development as the filaments slide over one another. The energy for this process is derived from the hydrolysis of adenosine triphosphate (ATP) to form adenosine diphosphate (ADP) and inorganic phosphate (P_i).

Muscle fibres can be subdivided into a number of types (usually Types I, IIa, and IIb) according to their contractile and metabolic properties (see **Table 2.1**). The fibre composition of a muscle will determine its ability to perform different tasks. For example the soleus muscle contains predominantly Type I fibres, as it is required to produce prolonged but low intensity contraction to maintain posture. By contrast muscles in the hand contain predominantly Type II fibres to allow fast but short-lived contraction. Muscle fibre composition varies widely between individuals and appears to be determined genetically. For example marathon runners have been shown to have a high proportion of Type I fibres in the vastus lateralis muscle whereas sprinters have a higher proportion of Type II fibres. Muscle fibre composition may also be altered by other factors such as training, ageing or disease.

Skeletal Muscle Energy Metabolism

Muscular contraction requires energy and this is provided by the breakdown of ATP. Intramuscular stores of ATP are sufficient to sustain contraction for only a few seconds and therefore if work is to continue ATP stores must be replenished. Much of the rest of the cellular architecture is devoted to maintaining energy supplies in the form of ATP.

There are three main pathways by which ATP can be replenished. These are summarized in **Fig 2.1**.

The Phosphagen System

ATP can be formed by the breakdown of Phosphocreatine (PCr) to creatine and phosphate. The resulting phosphate is transferred to ADP to form ATP. This system is able to replenish ATP at a very high rate but PCr stores are rapidly depleted and if no other sources of energy are available fatigue (in other words failure of contraction) will soon develop. This system provides energy for high intensity exercise and during the early stages of contraction. PCr, once depleted, must be later resynthesised, a process which itself requires energy in the form of ATP.

The Glycolytic System

This is a series of reaction by which intramuscular glycogen or blood borne glucose is converted to pyruvate. Pyruvate can then enter the tricarboxylic acid cycle in the mitochondria (see below). At higher exercise intensities pyruvate accumulation exceeds its uptake by the mitochondria and it must additionally be broken down to form lactate. Lactate is acidic and must be removed from the muscle cell and ultimately excreted by the lungs as carbon dioxide. Glycolysis produces energy more slowly than the phosphagen system but its capacity is greater. The formation of lactate from glycolysis and the breakdown of PCr is known as anaerobic metabolism because it occurs without the utilisation of oxygen.

	Type I	Type IIa	Type IIb
Speed	Slow	Fast	Fast
Endurance	High	Medium	Low
Power	Low	High	High
Metabolism	Oxidative	Oxidative, glycolytic	Glycolytic
Mitochondrial Density	High	Medium	Low
Capillary density	High	Medium	Low
Fatigability	Low	Low	High

Table 2.1. Fibre types in healthy human muscle.

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

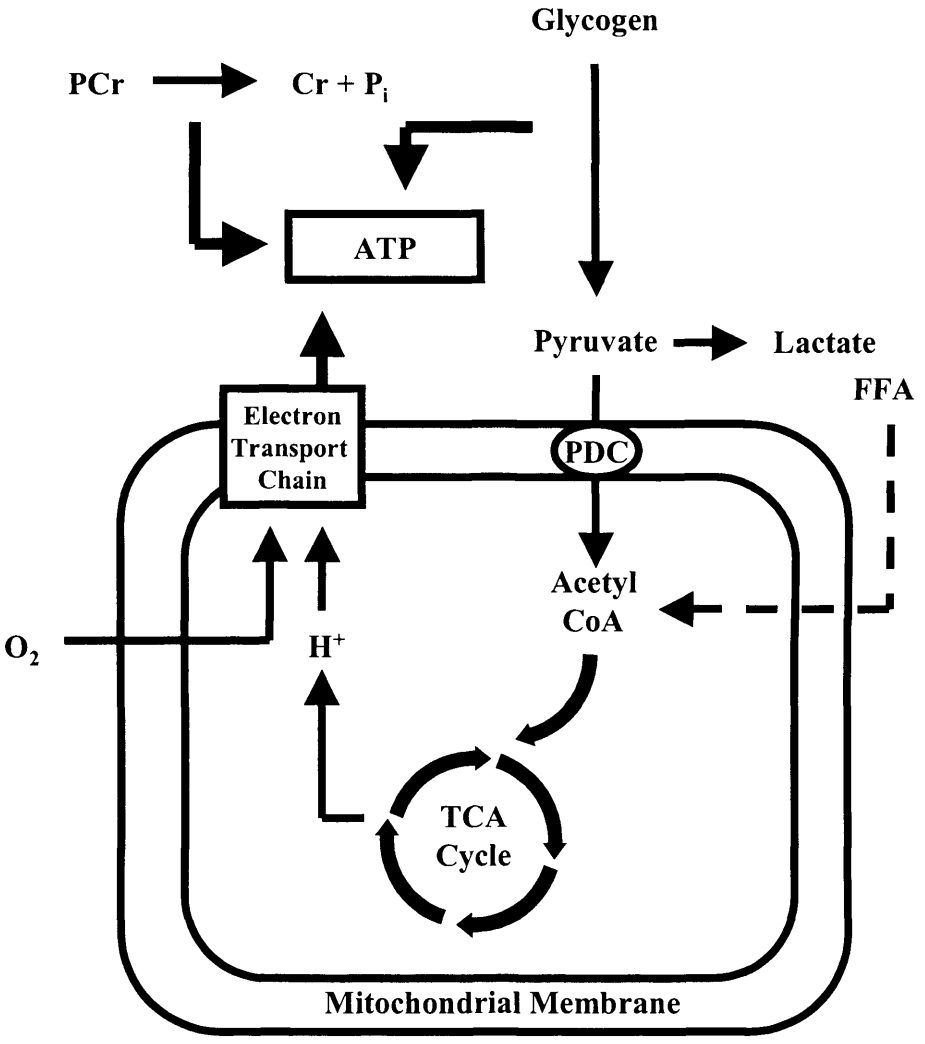


Fig 2.1. Cellular energy metabolism.

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

Abbreviations: PCr = Phosphocreatine; Cr = Creatine; P_i = Inorganic Phosphate; ATP = Adenosine Triphosphate; PDC = Pyruvate Dehydrogenase Complex; FFA = Free Fatty Acids; TCA = Tricarboxylic Acid.

Oxidative Phosphorylation

This is the process by which the products of carbohydrate, protein or fat metabolism enter the mitochondria and are metabolised to water and carbon dioxide. Pyruvate (from glycolysis) or the breakdown products of fat and protein metabolism enter the mitochondria and form acetyl coenzyme A. This then enters the tricarboxylic acid (TCA) cycle. This results in the production of carbon dioxide and hydrogen atoms in the form of nicotinamide and flavin dinucleotides (NADH and FADH). These are subsequently oxidised via the electron transport chain to allow the re-phosphorylation of ADP to ATP. This process requires oxygen to accept hydrogen atoms to form water and is therefore termed aerobic or oxidative metabolism.

The Regulation of Energy Metabolism

The energy required for muscular work is stored in the form of phosphorylated adenine nucleotides, principally ATP. This energy is released by the de-phosphorylation of ATP to ADP and AMP. This is termed the adenylate kinase reaction. Continuous resynthesis of ATP (fuelled from the pathways described above) is required if work is to be sustained. The energy for ATP resynthesis is regulated by the phosphorylation status of adenine nucleotides in the cell (Atkinson, 1968). Thus a rise in cellular ADP and AMP concentrations and a fall in the ATP:ADP ratio results in the activation of glycolysis and oxidative phosphorylation to provide energy for ATP resynthesis. However, if the phosphorylation status of adenine nucleotides continues to fall, adenylate kinase is inhibited and contraction will be unable to continue. During intense work, significant adenine nucleotide loss may occur due to the irreversible deamination of AMP to IMP and subsequently to inosine, xanthine and uric acid (**Fig 2.2**) (Sahlin *et*

al., 1989;Broberg & Sahlin, 1989). Although potentially detrimental to prolonged muscle contraction because of a reduction in the availability of adenine nucleotides for phosphorylation, in the short term this increases adenine nucleotide phosphorylation status, which allows ATP hydrolysis and thus contraction to continue. Reamination of IMP to form adenine nucleotides occurs more slowly (over hours or days) during periods of rest.

The Integration of Glycolytic and Oxidative Metabolism

For exercise to be sustained, intramuscular stores of carbohydrate must be mobilised. The breakdown of glycogen results in the formation of pyruvate and this is then either metabolised to lactate by glycolysis or enters the mitochondria to fuel oxidative phosphorylation. It has long been assumed that lactate formation is the result of inadequate mitochondrial oxygen concentrations. However, it can be demonstrated that significant lactate is produced despite the presence of adequate oxygen supplies and that other factors are crucial to the integration of oxidative and glycolytic metabolism (Timmons *et al.*, 1998). Lactate accumulation is likely to be determined by the balance of pyruvate production and oxidation in the mitochondria. In this respect, the role of the pyruvate dehydrogenase complex (PDC) appears to be pivotal (Greenhaff & Timmons, 1998). This enzyme is situated on the mitochondrial membrane and regulates the irreversible entry of pyruvate to the mitochondrion and the tricarboxylic acid cycle. The enzyme exists in an active and inactive form and the degree to which it is activated determines the rate of oxidation of pyruvate and hence the degree to which lactate accumulates (Heigenhauser & Parolin, 1999). PDC can be activated pharmacologically by infusing dichloroacetate (Putman *et al.*, 1995) and this has been shown to attenuate

lactate accumulation and increase maximal work rates in healthy subjects (Ludvik *et al.*, 1993). The expansion of intermediates of the tricarboxylic acid cycle (known as anapleurosis) is another potential stimulator of oxidative phosphorylation. However more recent evidence has shown that the pharmacological activation of PDC results in reduction of TCA intermediates whilst increasing mitochondrial pyruvate utilisation and reducing lactate accumulation. Furthermore, artificial expansion of the TCA cycle intermediate pool by the infusion of glutamine does not result in an increase in mitochondrial oxidative phosphorylation. These findings suggest that pyruvate availability through the activity of PDC is the principal regulator of mitochondrial oxidative metabolism rather than anapleurosis (Constantin-Teodosiu & Greenhaff, 1999).

Changes in Muscle Function in the Elderly

Patients with COPD are predominantly elderly and changes in muscle function from ageing may also be important in this population. There are well-documented changes in muscle morphology and function that occur with ageing. Whole body peak VO_2 declines with age along with muscle strength and bulk and these changes have been termed "sarcopaenia" (Evans & Campbell, 1993;Dutta *et al.*, 1997). Studies of muscle function in the elderly have indicated that elderly subjects have lower proportions of Type I fibres and reduced oxidative capacity than younger controls (Kemp *et al.*, 1996).

There is evidence however that these changes in performance and muscle function are not an inevitable consequence of ageing but are related to a decline in physical activity in the elderly. A number of studies have reported impressive

improvements in performance as a result of exercise training (Fiatarone *et al.*, 1990;Meredith *et al.*, 1989;Frontera *et al.*, 1988). Indeed there is some evidence that the magnitude of training adaptations may be greater in elderly subjects (Jubrias *et al.*, 2001).

Muscular Adaptations to Training

A number of profound changes occur in the skeletal muscles in response to training (for detailed reviews see (Holloszy & Coyle, 1984;Maughan *et al.*, 1997). It is a principle of training that these changes will only occur in circumstances of exercise overload. In other words, the muscle must be exercised at higher intensity than its habitual level of activity. The degree and character of muscle adaptations will depend on the frequency, intensity and mode of training. Improvements are restricted to the muscle being trained and will only occur in the mode of performance in which training is occurring.

Endurance training results in increases in oxidative enzyme concentration, glycogen and myoglobin concentrations, mitochondrial density and muscle capillarity. These changes result in improvements in aerobic endurance performance. Untrained muscle is highly reliant on carbohydrate as a source of fuel. Training increases the muscles ability to use lipid as a source of energy for contraction (Hurley *et al.*, 1986).

Strength training mainly induces changes in muscle size so that its force generating capabilities are increased. This change is reflected in the hypertrophy of Type II fibres. Muscle glycogen and PCr are also increased. The result of these changes is an increase in the ability of the muscle to sustain high intensity activity.

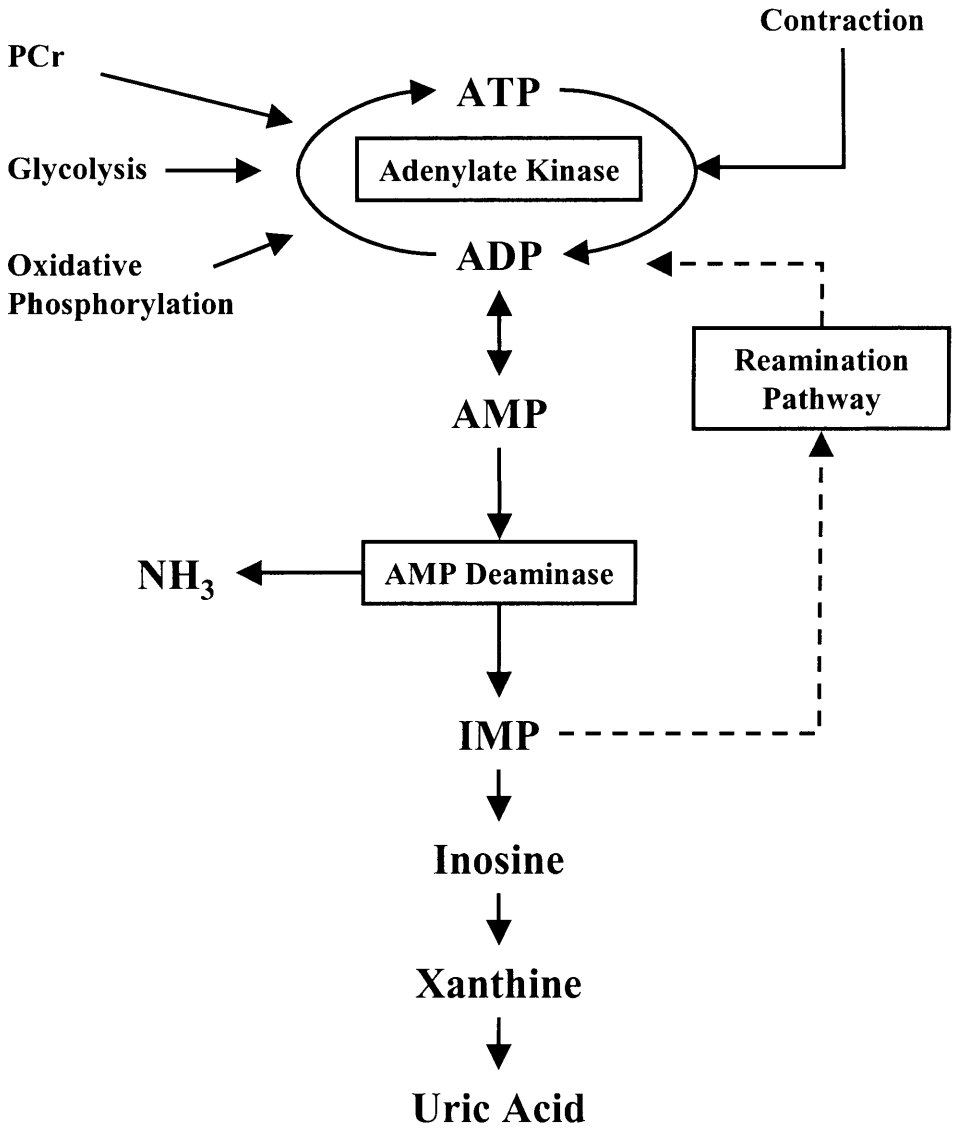


Fig 2.2. The Purine Nucleotide Cycle.

The irreversible deamination of AMP to form IMP allows the adenylate kinase reaction to continue by preventing the accumulation if ADP and AMP. The reamination of IMP occurs more slowly over a period of several hours or days.

The Nature of Peripheral Muscle Dysfunction in COPD

In recent years the peripheral musculature has become an important focus for the study of exercise limitation in COPD. Interest has been kindled because peripheral muscle dysfunction may be remediable and could be a target for new therapies for a disease where the primary pulmonary pathology is essentially irreversible.

A number of methodologies have been used to study the peripheral muscles in COPD and increase our understanding of the nature and causes of muscle dysfunction in this population.

Studies of Whole Body and Individual Muscle Performance

Most work in this area has concentrated on the lower limb muscles because difficulty walking is such a prominent symptom for patients with COPD. A number of studies have indicated that there is a reduction in leg muscle mass and strength compared with age matched healthy controls (Gosselink *et al.*, 1996;Bernard *et al.*, 1998;Schols *et al.*, 1991a;Clark *et al.*, 2000). These changes correlate with whole body exercise performance and may also be a factor in health-care utilisation (Decramer *et al.*, 1997).

Lactate release during laboratory exercise tests in COPD patients has also been extensively studied. Lactate accumulation is an important cause of muscle fatigue and results in metabolic acidosis during exercise. This is buffered in the blood by the production of CO_2 thereby imposing an additional load on the respiratory system already restricted by the underlying pathophysiology of COPD. Lactate accumulates within the muscle when pyruvate produced from glycolysis cannot be completely

oxidised in the mitochondria by oxidative phosphorylation. Mitochondrial oxidative metabolism requires oxygen and lactate is therefore considered a product of anaerobic metabolism. Lactate accumulation may however occur in the presence of oxygen when pyruvate production exceeds its uptake by the mitochondria (Greenhaff & Timmons, 1998). In other words its appearance indicates muscle glycolytic activity which is unmatched by mitochondrial oxidative phosphorylation. Recent studies have indicated that in COPD lactate accumulates in the blood earlier during exercise and at lower workloads than healthy controls (Maltais *et al.*, 1996a;Casaburi *et al.*, 1991). In a detailed study examining the limb responses to exercise in COPD and healthy, age matched controls, Maltais *et al* showed that this premature rise in blood lactate was not related to oxygen delivery (which was not lower than in healthy controls) suggesting that reduced oxygen extraction by the muscle was responsible (Maltais *et al.*, 1998). The importance of this effect was illustrated by the close correlation between venous pH and VO_{2peak} in both COPD patients and controls in the above study. These findings suggest that oxidative capacity is low in COPD, the result being increased glycolytic activity and accelerated lactate release. This may be an important cause of premature muscle fatigue in COPD patients and therefore a significant contributor to exercise limitation.

It is important to note that in many laboratory exercise studies in patients with moderate to severe COPD, a significant proportion of patients were not able to exercise at intensities sufficient to induce a blood lactate response. In these patients the contribution of impaired oxidative metabolism to exercise limitation is uncertain. However, lactate may accumulate and cause fatigue in the muscle before it appears in the blood and muscle mass in these patients may be very low. In these circumstances

individual muscles may experience high lactate levels although total lactate production is insufficient to raise blood levels.

Muscle Biopsy Studies

A number of studies have sought to characterise the nature and importance of peripheral muscle dysfunction through the examination of percutaneous muscle biopsies, most commonly taken from the quadriceps muscle of the thigh.

Initial studies described changes in resting energy metabolites particularly in patients with respiratory failure. Compared with healthy controls, COPD patients have been shown to have reductions in ATP, PCr and glycogen together with an increase in intramuscular lactate (Fiaccadori *et al.*, 1987;Jakobsson *et al.*, 1995). These findings were taken to indicate the impairment of muscle energy metabolism, although samples were taken at rest and did not give information about the dynamic response to exercise. In addition, many of the patients studied in these reports were hypoxic at rest and it is unclear how influential this feature was on the findings.

More recent biopsy studies have demonstrated reductions in mitochondrial enzyme concentrations in COPD patients compared with healthy controls whereas glycolytic enzymes are unaffected (Maltais *et al.*, 1996a). Changes in metabolic enzyme concentrations in muscle biopsy specimens may reflect changes in fibre type composition as well as differences in individual fibre enzyme activities. Histochemical analysis has shown altered fibre type profiles compared to healthy controls, with COPD patients showing a reduction in Type I ("slow twitch") fibres and a lower fibre to capillary ratio (Whittom *et al.*, 1998). Type I fibres are characterised by greater aerobic capacity and higher mitochondrial enzyme activities than Type II fibres. These

alterations in fibre composition lend support to the concept that oxidative capacity in the peripheral muscles in COPD is reduced.

Reductions in oxidative enzyme concentrations correlate with both maximal exercise capacity (Maltais *et al.*, 2000) and the magnitude of the lactate response (Maltais *et al.*, 1996a) during exercise, confirming the functional relevance of metabolic enzyme changes.

These biopsy studies support laboratory exercise data in suggesting that mitochondrial oxidative capacity is impaired, resulting in increased glycolytic activity during exercise and accelerated lactate release. Mitochondrial oxidative phosphorylation is the most efficient mechanism for ATP replenishment and its impairment in COPD may be one reason why ATP levels appear to be consistently lower at rest than in healthy, age-matched controls. In a recent study Pouw and colleagues demonstrated increased IMP concentrations in the calf muscles of COPD patients compared with healthy controls (Pouw *et al.*, 1998a). IMP is a degradation product of ADP and its accumulation may suggest impairment of the resynthesis of ATP during muscular contraction.

The specific nature of the impairment in mitochondrial function is uncertain but there is evidence that it is not due to generalised mitochondrial suppression. A recent study by Sauleda *et al* demonstrated an increase in the concentration of cytochrome oxidase (a key enzyme in the electron transport chain) in COPD patients, particularly in those with arterial hypoxaemia (Sauleda *et al.*, 1998). This suggests that mitochondrial dysfunction in COPD is complex with differential effects on the TCA cycle and the electron transport chain.

Magnetic Resonance Spectroscopy Studies

The study of muscle samples taken from COPD patients at rest has indicated changes in enzyme concentrations and histochemistry in these patients. From these findings, impairment in the metabolic response to exercise is inferred. Direct studies of peripheral muscle metabolism during exercise are scarce. Much of the available data comes from studies using Magnetic Resonance Spectroscopy (MRS). This technique measures the concentrations of inorganic phosphate, high-energy phosphate compounds (PCr, ATP and ADP) and intracellular pH (McCully *et al.*, 1999). The rate of PCr breakdown and re-synthesis is an indirect measure of oxidative phosphorylation (Mahler, 1985). MRS studies have indicated greater PCr breakdown and slower PCr re-synthesis in COPD patients compared with controls (Sala *et al.*, 1999;Kutsuzawa *et al.*, 1995). MRS has the advantage of being able to obtain data on muscle energy metabolism during exercise but is limited in only being able to measure high-energy phosphate compounds. The calculation of energy flux through other pathways is possible using MRS but there is evidence from a comparative study with muscle biopsies in healthy subjects that this may be inaccurate because of assumptions made about the pH buffering capacity of the intracellular milieu (Constantin-Teodosiu *et al.*, 1997). One further problem with MRS is that the modes of exercise that can be studied are limited. In particular whole body exercise is difficult to study within the confines of the equipment. This limits the applicability of MRS findings to the normal exercise needs of COPD patients.

Causes of Peripheral Muscle Dysfunction

Peripheral muscle dysfunction in COPD is multifactorial in origin and related to a range of secondary, extra-pulmonary consequences of COPD such as deconditioning, nutritional depletion, hypoxia and drug administration. These vary amongst patients and peripheral muscle dysfunction will therefore make a variable contribution to exercise limitation across the spectrum of COPD.

Deconditioning

Although a number of factors may be responsible for impaired peripheral muscle performance in COPD, deconditioning is likely to be a crucial component for most patients.

The fear of exertional symptoms results in a vicious circle where the avoidance of exercise and activity leads to progressive loss of fitness and worsening of symptoms. Over years, subsequent restrictions in lifestyle and social isolation may result in extreme disuse in the peripheral muscles, particularly in the lower limbs.

Alterations in muscle structure and function in COPD closely mirror those seen in detrained or immobilised healthy subjects (Booth & Gollnick, 1983;Appell, 1990) and in other chronic diseases where exercise is limited such as chronic heart failure (Gosker *et al.*, 2000).

The functional importance of these changes in lifestyle is illustrated by the improvements seen in physical performance and health status following exercise training. Evidence is now accumulating that these benefits are at least in part due to improvements in peripheral muscle performance.

Nutritional Depletion

Weight loss is an important clinical feature in COPD. Many patients are under-nourished and this is an adverse prognostic factor independent of lung function decline (Wilson *et al.*, 1989;Landbo *et al.*, 1999). Patients of normal weight may show relative reductions in muscle mass (Schols *et al.*, 1993;Schols *et al.*, 1991b) and this may have an influence on physical performance and health status (Engelen *et al.*, 1994;Mostert *et al.*, 2000). The assessment and treatment of nutritional depletion are considered in **Chapter 3**.

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 peripheral muscle dysfunction. Some patients show evidence of an exaggerated systemic inflammatory response and this may cause protein catabolism and contribute to muscle mass depletion (Eid *et al.*, 2001;Schols *et al.*, 1996). The importance of more general changes in energy balance to muscle performance remains unclear.

The observation of reduced mitochondrial oxidative capacity in COPD has lead to interest in the role of intermediates in TCA cycle. The TCA cycle is the means by which pyruvate is oxidised within the mitochondria. This results in the production of hydrogen atoms, which are subsequently combined with oxygen to form water in the electron transport chain. Many TCA cycle intermediates are formed by the deamination of amino acids and this may have a role in the maintenance of TCA cycle activity. Changes in TCA cycle intermediates have been found in COPD patients (Pouw *et al.*, 1998b;Engelen *et al.*, 2000a) and it has been hypothesised that inadequate replenishment of the TCA cycle intermediate pool may be a factor in impaired oxidative

metabolism and the accelerated lactate response. If this is the case, supplementation of the relevant amino acids such as glutamine, might be of therapeutic benefit. However, studies in healthy subjects suggest that changes in the TCA intermediate pool are secondary to alterations in mitochondrial pyruvate flux through oxidative phosphorylation rather than a primary cause of such alterations (Constantin-Teodosiu & Greenhaff, 1999).

Hypoxia

Arterial hypoxaemia is a common problem for patients with advanced COPD many of whom are severely disabled. Other patients who are normoxic at rest show significant haemoglobin desaturation during exercise. The importance of hypoxia in exercise limitation is illustrated by several studies, which have shown significant increases in exercise performance in these patients when supplemented with oxygen (Leach *et al.*, 1992;Leggett & Flenley, 1977).

The mechanism for these increases in performance is uncertain. A number of studies have suggested that oxygen reduces dyspnoea by a reduction in minute ventilation during exercise (O'Donnell *et al.*, 1997;Simon *et al.*, 1999). It would be expected that increasing the oxygen content of the blood would have a beneficial effect on oxidative metabolism in peripheral muscle leading to an increase in exercise capacity and there is support for this from MRS studies (Mannix *et al.*, 1995;Payen *et al.*, 1993). In addition VO_2 kinetics, which are slow in COPD may be improved by the oxygen supplementation (Palange *et al.*, 1995a).

However, there is also evidence that mitochondrial function is independent of oxygen delivery. Maltais and colleagues demonstrated in a detailed study of the

metabolic responses of the leg muscles to exercise that lactate output from the muscles showed no relationship to oxygen delivery (Maltais *et al.*, 1998). This implies that the failure of oxidative metabolism in peripheral muscle lies with its inability to extract oxygen rather than the inability of the lungs to provide oxygen to it.

The effects of acute hypoxia need to be distinguished from the impact of long-term hypoxia on muscle growth and function. Studies of muscle biopsies taken at rest suggest that intramuscular high-energy phosphates and glycogen are lower in hypoxic patients than normoxic controls (Jakobsson & Jorfeldt, 1995). Animal studies have suggested that prolonged hypoxia may influence muscle growth and attenuate the stimulus to growth of exercise in the skeletal muscles (Olfert *et al.*, 2001).

Drugs

Patients with COPD are frequently treated with systemic and inhaled drug therapy and these have the potential to adversely affect peripheral muscle function. Systemic corticosteroids are known to cause a myopathy and this may be an issue for some patients (Decramer *et al.*, 1994). Beta agonists have an anabolic effect on skeletal muscle (Martineau *et al.*, 1992;Revill & Morgan, 1998) and have been used illegally by athletes to increase muscle bulk. There is evidence from studies of performance in asthmatics that inhaled beta agonists do not have significant ergogenic effects (Meeuwisse *et al.*, 1992;Revill & Morgan, 1998). Some patients, however, do use large doses of these drugs by nebuliser and the impact of this on muscle function is unknown.

Summary

Although exercise limitation is a common and fundamental problem for COPD patients, it is impossible to generalise about the relative contributions of its possible causes. In some patients the effects of disordered pulmonary mechanics on the sensation of dyspnoea during exercise may predominate whereas in others muscle dysfunction may be more important.

The responses of the respiratory and circulatory systems to exercise are determined by the metabolic demands of exercising muscle. Impaired muscle function may therefore contribute to disability in patients with an apparent ventilatory limit to exercise. Moreover these factors may interact differently during different modes of exercise. For example the limits to performance may be different for peak and submaximal exercise.

In all patients the effects of motivation and mood are likely to have a crucial impact on the degree to which symptoms limit exercise.

There is now substantial evidence for peripheral muscle dysfunction in COPD patients. There are a number of secondary consequences of COPD, which have been suggested as its cause and the possibility of a specific "COPD myopathy" has been raised. However, there is no concrete evidence that peripheral muscle dysfunction in COPD differs significantly from other "deconditioned" states. This is illustrated by the relative preservation of structure and function in the respiratory muscles in COPD compared with the peripheral muscles. The respiratory muscles are subject to the same systemic consequences of COPD but, crucially, are not deconditioned but required to work under high workloads because of disordered pulmonary mechanics.

In conclusion, there is accumulating evidence that peripheral muscle dysfunction makes a contribution to exercise limitation in COPD. In **Chapter 3** I will review potential approaches to enhancing performance in COPD patients, an important therapeutic goal in this population.

CHAPTER THREE

Enhancing Physical Performance in COPD

IN **Chapter 2** I discussed the mechanisms underlying exercise limitation in patients with COPD. Although therapy aimed at improving airway function may improve symptoms in some patients, many remain incapacitated by disabling breathlessness during physical exertion. In this chapter I will outline therapies aimed at the improvement of physical performance in this group of patients. Apart from pulmonary rehabilitation, most of these therapies have been explored in research settings and have yet to be broadly applied to the management of the COPD patient in general clinical practice.

Exercise Training

COPD patients frequently enter a downward spiral of breathlessness, inactivity and deconditioning. This results in peripheral muscle dysfunction, which contributes to exercise limitation. Muscle reconditioning is an obvious approach to rectifying this problem and it is now clear that exercise training, as part of pulmonary rehabilitation is effective therapy for COPD patients.

Pulmonary rehabilitation is a multidisciplinary package of care that aims to reduce disability and handicap in people with lung disease (British Thoracic Society Standards of Care Subcommittee on Pulmonary Rehabilitation, 2001). This is encapsulated by a recent NIH workshop which defined pulmonary rehabilitation as "a continuum of services directed to persons with pulmonary disease and their families, usually by an interdisciplinary team of specialists, with the goal of achieving and maintaining the individual's maximum level of independence and functioning in the community" (American College of Chest Physicians. American Association of Cardiovascular and Pulmonary Rehabilitation, 1997). Although historically, pulmonary rehabilitation has been developed for patients with COPD, the benefits appear to apply equally to patients with other forms of chronic lung disease. Exercise is an essential requirement for the success of pulmonary rehabilitation but most programmes also provide a disease education package and may also include other services such as dietetic, occupational therapy and psychological support. Although these aspects are desirable, scientific evidence for their efficacy is less clear.

The recognition of the benefits of rehabilitation has its roots in the 1960's and 70's. Initial studies were commissioned in the hope that rehabilitation would improve survival. Although this proved not to be the case, important functional benefits were seen (Petty, 1993). The benefits of rehabilitation in improving exercise capacity and health-related quality of life have now been confirmed in large, well-conducted clinical trials (Griffiths *et al.*, 2000;Goldstein *et al.*, 1994). A recent meta-analysis has reiterated these findings (Lacasse *et al.*, 1996).

In this section I will focus on the physical benefits of exercise training in patients with COPD. There is now unequivocal evidence that properly prescribed

exercise results in improvements in exercise capacity despite having no effect on the primary pulmonary pathophysiology of the condition. Whilst there is increasing understanding of the physiological effects of training, it is important to emphasise that psychological and behavioural benefits also accrue. Regular exercise leads to increases in confidence and exercise efficiency and decreases in the fear of physical activity.

Although trials of pulmonary rehabilitation have demonstrated its effectiveness in improving both exercise performance and health status, the link between these two outcomes may be elusive. Changes in performance and health status are poorly correlated and the physical and lifestyle effects of rehabilitation may follow different time courses (Green *et al.*, 2001). The benefits of performance enhancing therapy to exercise tolerance may not always be translated into measurable increases in health status.

The Principles of Exercise Training

Considerable experience exists in the training of healthy subjects and from this a number of general principles to guide successful training can be identified. There is no reason to believe these principles do not apply in patients with respiratory insufficiency and so I will briefly outline them here.

For performance to increase, exercise overload is needed. In other words, exercise intensity must be above habitual levels for that individual. There is no evidence for an absolute intensity threshold for training, which means that benefits may accrue for individuals whose pre-training exercise capacity is very low. Indeed, severely deconditioned subjects may have the greatest potential for improvements in performance. In general, the higher the training intensity, the greater the performance

advantage but this will need to be balanced against the risk of injury. On a more practical note, very high intensity exercise may be too uncomfortable for subjects to reasonably perform on a regular basis.

In healthy individuals, a minimum exercise intensity of 55-60% VO_{2max} has been recommended by the American College of Sports Medicine (ACSM) to increase fitness (American College of Sports Medicine, 1998). The frequency and duration of training is also an important factor. Exercise at least three times per week has been recommended with a duration of at least 20 minutes per session. To a certain extent frequency and duration can be traded off so that shorter, more frequent exercises may be as effective. Exercise bouts should however not be shorter than 10 minutes. Again the more frequent and sustained the training, the greater the performance advantage.

An important principle of training is its selectivity. This applies both anatomically to the muscle groups trained and to the mode of training employed. There is ample evidence from studies of single limb training that adaptations are restricted to the trained limb (Saltin *et al.*, 1976) and is further illustrated in studies of canoeists who show similar mitochondrial activities in their legs to sedentary controls but much greater activities in their arms (Hardman, 1997).

Improvements in performance will only occur in the mode of training employed. In other words endurance training leads to increases in endurance but not strength and vice versa. This is of crucial importance when outcome measures of performance are chosen. Such measurements need to be appropriate to the mode of training used.

Although there may be intensity thresholds below which increases in performance will not be achieved, there may be other benefits of lower intensity

exercise that should not be ignored. Increases in physical activity can result in improvements in energy balance and flexibility. There may also be psychological benefits from greater physical activity at lower intensities.

Exercise Training in COPD

The principles of exercise training in healthy subjects appear to apply equally in patients with pulmonary disease. In other words training needs to be of adequate intensity, duration and frequency for improvements in performance to occur. In general the greater the intensity of exercise, the greater the performance benefits. COPD patients can exercise at a higher relative intensity than healthy subjects and it is feasible to ask patients to train at loads as high as 85% Peak VO_2. Potential adverse effects of over-training in COPD have received little attention but it should be remembered that exercise may be uncomfortable and frightening for patients. Asking patients to perform exercise at too high intensity or duration may be counterproductive and cause a decline in compliance. The risk of injury will also increase at higher intensities and this may be a factor in exercise prescription for the very frail or those with musculoskeletal problems.

The benefit of supervised over unsupervised exercise has been demonstrated in controlled studies (Puente-Maestu *et al.*, 2000a) but for logistic reasons many programmes combine weekly or twice weekly supervised sessions with home exercises.

The best mode of training in pulmonary rehabilitation has not been established; indeed this is likely to vary amongst patients. However, because patients frequently identify walking as their most pressing problem, lower limb endurance exercise has been most often used.

Studies have shown that lower limb training is effective in outpatient (Griffiths *et al.*, 2000;Singh *et al.*, 1998), inpatient (Goldstein *et al.*, 1994) and home settings (Strijbos *et al.*, 1996). Studies comparing high and low intensity training have confirmed the principle that high intensity training results in greater improvements in performance (Casaburi *et al.*, 1991;Gimenez *et al.*, 2000;Puente-Maestu *et al.*, 2000b). Specific trials comparing weekly exercise frequency have not been performed but programmes involving two to five training sessions weekly have been successful. Although patients may usefully perform exercises at home, this is not as effective as supervised exercise sessions where training intensity and duration is monitored and increased in a structured fashion (Puente-Maestu *et al.*, 2000a).

Progression of training load may occur by increasing exercise duration whilst keeping intensity constant (Singh *et al.*, 1998) or by increasing intensity once a target duration has been reached (Griffiths *et al.*, 2000). Both models are effective but direct comparisons of these approaches have not been made. Interval training also appears to be effective (Coppoolse *et al.*, 1999).

The optimum duration of training remains uncertain but physiological adaptations require at least two weeks to develop. Whilst training for less than four weeks is unlikely to be effective, there may be little additional benefit in prolonging supervised training beyond twelve weeks (Criner *et al.*, 1999). There is some evidence that within this range, longer training programmes may be more effective (Green *et al.*, 2001).

Most training programmes employ walking exercise, as this is both functionally relevant and practical. Setting absolute work intensities is more difficult for walking than cycling exercise but walking speed correlates closely with oxygen uptake

and this is frequently used for exercise prescription (Singh *et al.*, 1994). Stationary cycle aerobic training is also effective (Weiner *et al.*, 1992) and many programmes incorporate cycle exercise alongside walking.

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 oxygen uptake is conflicting with some studies showing an increase (Ries *et al.*, 1995) whereas others did not (Lake *et al.*, 1990;McGavin *et al.*, 1977). Submaximal endurance performance is generally more sensitive to training, perhaps unsurprisingly as endurance training is most frequently used in exercise training programmes (Revill *et al.*, 1999;Weiner *et al.*, 1992). Timed walk test (Goldstein *et al.*, 1994;Lake *et al.*, 1990) and incremental shuttle (Griffiths *et al.*, 2000;Singh *et al.*, 1998) performance 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. However, studies have shown that progressive weight training increases peripheral muscle strength (Clark *et al.*, 2000;Simpson *et al.*, 1992;Bernard *et al.*, 1999). In the studies by Clark *et al*, and Simpson *et al*, increases in whole body exercise performance were also seen although this did not occur in the study by Bernard *et al.* The impact of strength training on health status and domestic function remains uncertain. CRQ scores increased with strength training in the study by Simpson *et al* but did not in the study by Bernard *et al.* Given that some domestic tasks (such as stair climbing) may require a component of

muscle strength, this mode of training could be of significant benefit to selected patients.

Upper limb training is potentially of use for COPD patients because patients may experience difficulties with arm exercise and because the upper limb girdle muscles may help with ventilation in some patients. Studies of arm training have shown that performance in the arms increases after training (Martinez *et al.*, 1993;Ries *et al.*, 1988) and that the ventilatory requirements for arm exercise decrease (Couser Jr., *et al.*, 1993). Again, the importance of this type of training to functional performance remains uncertain and may be restricted to certain patients who have deficits in upper limb muscle function.

Adaptations to Training in COPD

An understanding of the metabolic adaptations to training is important because it will guide the design of rehabilitation programmes and allow greater individualisation of training schedules.

It was long thought that patients with COPD were unable to train at sufficient intensity to induce metabolic adaptations to training in the peripheral muscles. Early studies examining muscle biopsies before and after training appeared to support this (Belman & Kendregan, 1981). Improvements in performance were ascribed to improvements in exercise task efficiency, better tolerance of dyspnoea and reduced fear of exercise and activity. It is now clear, however, that COPD patients are able to exercise at higher relative intensities than initially thought and changes in muscle physiology do occur following training at higher workloads. More recent investigations have indicated that training results in lower ventilation and lactate production during

exercise at a given workload and that the magnitude of these adaptations increases with intensity of training (Casaburi *et al.*, 1991;Maltais *et al.*, 1997). Maltais and colleagues have also shown that endurance training results in increases in mitochondrial oxidative enzyme concentrations whilst glycolytic enzymes are unaffected (Maltais *et al.*, 1996b). This suggests that oxidative metabolism is improved by endurance training, a finding consistent with its effects in healthy adults. Moreover, in this study, changes in oxidative enzyme concentrations correlated inversely with changes in exercise induced lactataemia suggesting a greater contribution of oxidative metabolism to muscle energy metabolism after training.

A subsequent study by Sala and colleagues investigated the effect of training on muscle bioenergetics using magnetic resonance spectroscopy (Sala *et al.*, 1999). This study is important because, in contrast to studies of muscle biopsies taken at rest, it provides direct information about the effects of training on the metabolic response to exercise in the peripheral muscles. The results supported biopsy studies by demonstrating that PCr degradation was lower and PCr resynthesis was faster after training. The authors also measured the haemodynamic effects of training on leg blood flow and found that in contrast to healthy controls no improvement occurred in leg oxygen delivery suggesting that metabolic adaptations to training in COPD predominantly occur in the peripheral muscles.

Summary

There is now overwhelming evidence that exercise training is an effective means of improving physical performance in COPD. This is achieved despite having no effect on the primary pulmonary pathophysiology of airway disease. The precise mode

of training that should be used remains a matter of debate but it is clear that benefits will be greatest where the basic principles of exercise of exercise training are followed. Whichever mode is used, outcome measures should be matched to training method.

Our understanding of the mechanisms by which exercise training improves performance in COPD is at an early stage. Although the effects of regular exercise on mood, confidence and fear of activity undoubtedly make a substantial contribution to the benefits of rehabilitation, it is clear that metabolic changes in the exercising muscles do occur if training is performed at appropriate loads. If other performance enhancing therapies are to be successful they will probably need to be combined with an appropriate training programme. Developing our knowledge of the physiology of training in patients with respiratory insufficiency will also be crucial to progress in this area.

Nutrition

The importance of nutrition to performance in sports has received greater recognition in recent years. Sportsmen and women vary considerably in their dietary regimens depending on their discipline and although some of these regimens are not strictly evidence based, there is now a body of evidence to support the concept that attention to diet can enhance physical performance and training (Williams, 1995).

In this thesis I will be exploring the role that nutritional support might have in the rehabilitation of patients with COPD. Nutritional support has been widely studied in underweight patients with COPD but its role in enhancing the benefits of training has received little attention despite its clear benefit to athletic performance. In this section I will discuss current knowledge about the nature of weight loss, its treatment, and the

potential benefits that nutritional support might offer in the rehabilitation of patients with COPD.

Weight Loss in COPD

Weight loss is a common and important clinical feature in COPD. Population studies carried out in the last two to three decades have indicated that body weight is adverse prognostic indicator in COPD (Landbo *et al.*, 1999;Wilson *et al.*, 1989). The impact of body weight on mortality is independent of underlying lung function impairment. Underweight patients also have worse exercise capacity and health status than normal weight patients (Baarends *et al.*, 1997a;Schols *et al.*, 1991a).

The Nutritional Assessment of COPD patients

Body weight and body mass index (height normalised body weight) are the most straightforward and practical measures of nutritional status. However, changes in weight may be due to changes in fat, muscle or both. Simple measurements of weight and body mass index may underestimate the prevalence of nutritional depletion in because some patients show relative reductions in muscle mass despite being of normal overall weight (Schols *et al.*, 1993). For this reason, measurements of body composition are increasingly used for the nutritional assessment of COPD patients. These measurements subdivide the body into a number of compartments depending on the method of measurement used. Of importance is the fat free mass compartment because this contains functional muscle mass. Fat free mass depletion shows the closest relationship with impaired exercise performance (Engelen *et al.*, 2000b). The

measurement of body composition allows the identification of different patterns of fat mass and fat free mass depletion. This is important because these patterns may have different underlying causes and may respond differently to therapy.

The Causes of Weight Loss in COPD

Weight loss (particularly in the fat compartment) will occur if energy expenditure by the body exceeds energy intake in the diet. A number of studies have suggested that increases in energy expenditure and reductions in dietary intake may both contribute to energy imbalance. Several studies have demonstrated that resting energy expenditure is elevated in COPD (Creutzberg *et al.*, 1998a;Schols *et al.*, 1991c). This may be due to a number of factors such as the increased energy cost of breathing and drugs such as beta agonists (Creutzberg *et al.*, 1998b;Palange *et al.*, 1995b). In addition, evidence is now accumulating that some weight losing COPD patients have an exaggerated systemic inflammatory response, which makes a contribution to increased energy expenditure. Several studies have demonstrated elevated levels of circulating pro-inflammatory cytokines such as tumour necrosis factor alpha (TNFα) in these patients and these have been related to both weight loss and increased resting energy expenditure (Eid *et al.*, 2001;Schols *et al.*, 1996;de Godoy *et al.*, 1996).

It would also be predicted that the energy cost of physical activity would be increased in COPD. This might result from the greater ventilatory cost of exercise and decreases in work efficiency in the peripheral muscles resulting from impairment of oxidative metabolism. This is supported by the study of Baarends and colleagues, which suggested increases in the non-resting component of energy expenditure in COPD patients when compared to healthy controls (Baarends *et al.*, 1997b). In another study,

estimates of total daily energy expenditure were similar in COPD patients to healthy controls but at the expense of significantly lower levels of physical activity (Hugli *et al.*, 1996). These studies indicate that physical activity is costly in energy terms and may contribute substantially to energy imbalance in weight losing patients.

Under normal circumstances fluctuations in energy requirements are met by changes in energy intake. There is evidence however, that this adaptive mechanism is abnormal in weight losing COPD patients as undernourished patients show lower dietary intakes relative to energy expenditure than well-nourished controls (Schols *et al.*, 1991d). A number of factors may be involved in this. Meals may cause dyspnoea and can aggravate hypoxaemia in patients with respiratory failure. A full stomach may limit diaphragmatic movement in hyperinflated patients making large meals uncomfortable. More recently, evidence is emerging that appetite regulation may be altered particularly in patients with evidence for an exaggerated systemic inflammatory response. Leptin is an hormone acting on the hypothalamus regulating appetite in response to deposition of fat mass (Auwerx & Staels, 1998). In one study, plasma leptin levels were elevated when adjusted for fat mass in COPD patients compared to healthy controls and both leptin and TNFα correlated inversely to dietary intake (Schols *et al.*, 1999). Hormonal imbalance in the control of appetite may be one factor in energy imbalance in patients with COPD.

Imbalances between energy expenditure and intake are usually reflected in loss of fat mass but many patients with COPD may also show significant changes in muscle mass. These changes may be affected by alterations in protein metabolism as well as energy balance. Long term decreases in habitual physical activity are common in the elderly and lead to relative lean mass depletion known as sarcopaenia (Evans &

Campbell, 1993). In COPD this may be aggravated by other factors such as corticosteroid use, hypoxia and protein catabolism due to systemic inflammation. Such changes in muscle mass are unlikely to be alterable by energy supplementation alone but may require additional attention to these other factors.

Finally, it should not be forgotten also that for patients with COPD, physical disability may be a barrier to the purchase and preparation of food which may affect the size and nutritional balance of their diet. This may have an impact on the balance between energy expenditure and intake leading to progressive weight loss.

Nutritional Support in COPD

The adverse consequences of weight loss in COPD have lead to numerous trials of nutritional support in undernourished patients. These have been extensively reviewed elsewhere (Donahoe, 1997;Schols & Wouters, 2000;Stratton & Elia, 1999). These trials have shown inconsistent results and where weight gain was achieved this was small and of questionable clinical significance. A recent meta-analysis and systematic overview both concluded that there was no overall effect on anthropometric measures or functional performance (Ferreira *et al.*, 2001;Ferreira *et al.*, 2000). With regard to exercise performance the pooled effect sizes for changes in six minute walk test performance calculated in this meta-analysis were small and the confidence intervals included zero. Changes in 6 minute walk distance were below the minimal clinically important difference for this test (Ferreira *et al.*, 2000).

Studies in this area have frequently been small and are difficult to compare because of differences in the clinical setting (inpatient or outpatient), patient selection and the size and composition of the supplementation regime.

There may be a number of reasons for the failure of supplementation programmes. Patients who exhibit an exaggerated systemic inflammatory response may respond poorly to nutritional support (Creutzberg *et al.*, 2000). The inclusion of such patients in clinical trials might have obscured potential benefits for other patients. A proportion of patients may offset supplementation with a drop in normal dietary calorie intake (Knowles *et al.*, 1988). This and the gastro-intestinal side effects of supplementation may limit the size of supplementation that can be readily tolerated. Nutritional supplementation is more likely to be successful if combined with a stimulus to the appetite such as exercise. To date only one such study has been reported. In this trial, supplemented patients did gain weight, although this was predominantly in the fat compartment (Schols *et al.*, 1995). This trial is described in more detail in the next section.

Early regimes involved predominantly fat supplements because of concerns that carbohydrate, which produces more carbon dioxide when oxidised, might adversely affect ventilation (Efthimiou *et al.*, 1992;Goldstein *et al.*, 1989). More recently, data has emerged suggesting that the clinical relevance of this might have been overstated (Vermeeren *et al.*, 2001). Carbohydrate rich supplements may be better tolerated by patients because of more rapid gastric emptying. Moreover, carbohydrate supplementation may better meet the needs of patients if they are also participating in an exercise programme (see below).

Nutrition and Exercise Performance

The importance of nutrition to performance in sport is now well recognised (Williams, 1995). Emphasis is placed on maximising carbohydrate intake prior to and

during exercise. It is recommended that if regular physical activity is undertaken that 50 – 60% of dietary calorie intake should come from carbohydrate sources (Devlin & Williams, 1991). Carbohydrate is a crucial source of fuel for sustained exercise and fat sources are only able to exclusively fuel contraction at relatively low intensities. Carbohydrate stores are limited, however, and if these are depleted during prolonged exercise, fatigue results. Carbohydrate supplementation during heavy exercise can prolong endurance performance and feeding with a high carbohydrate diet for several days before exercise can increase muscle glycogen content and enhance performance (Ivy, 1999). Untrained individuals are particularly reliant on carbohydrate sources for exercise and an important training adaptation in the skeletal muscles is the ability to use fat as a fuel for energy metabolism (Maughan *et al.*, 1997). Although overall energy requirements decline with age, similar proportions of carbohydrate in the diet are recommended for exercise in the elderly (Sacheck & Roubenoff, 1999).

Several studies have also indicated that increasing carbohydrate intake during exercise training has beneficial effects on performance. Simonsen and colleagues demonstrated increases in muscle glycogen content and power output in rowers given a high carbohydrate compared to a moderate carbohydrate diet during training (Simonsen *et al.*, 1991). In a study of untrained men, Helge *et al* compared the effect of a high fat and high carbohydrate diet on endurance performance following an seven week training programme (Helge *et al.*, 1996). Subjects receiving the high carbohydrate diet increased their endurance time threefold whereas increases in the high fat group were only 1.8 fold. This was not due to the short-term benefit of carbohydrate intake because performance remained lower in the high fat group after a further week of training when both groups received the high CHO diet. These differences appeared to be due to

changes in substrate utilisation rather than due to alterations in glycogen stores suggesting that training adaptations had been modified.

The effect of nutritional support on the outcome of training in the elderly has also been investigated. These studies have focused on resistance training because of the benefits of increased strength in reducing falls and fractures in this population. In a study of elderly men, Meredith *et al* found that increases in strength and thigh muscle mass following resistance training were greater in subjects who received an additional calorie supplement (Meredith *et al.*, 1992). A similar study of frail nursing home residents showed impressive increases in strength from resistance training but no additional effect from nutritional supplementation (Fiatarone *et al.*, 1994). Although the increases in aerobic performance following training may be greater in the elderly compared with younger subjects (Evans, 1995), the impact of carbohydrate supplementation on the outcome of this mode of training has not been studied.

Although nutritional support has been extensively studied in COPD, only one has reported its effects in patients participating in exercise training (Schols *et al.*, 1995). In this study, patients undergoing pulmonary rehabilitation were randomised to receive a calorie supplement for the duration of rehabilitation or rehabilitation alone. An additional group also received anabolic steroids (see below). The supplement contained 420Kcal but was fat rich (fat 51%, CHO 35%, protein 14%). Patients in the supplemented group gained weight (principally fat mass) compared with those receiving rehabilitation alone but there was no significant difference between the groups in 12-minute walk distance and respiratory muscle strength. Whilst the supplementation regime in this study might have provided sufficient calories to induce weight gain, it may have not contained sufficient carbohydrate to optimise physical performance.

The effect of carbohydrate supplementation on performance during exercise training in COPD has not been explored. Indeed carbohydrate has been avoided in many studies because of concerns that the increased carbon dioxide production from carbohydrate oxidation might adversely load ventilation. However, COPD patients might benefit from carbohydrate supplementation because the muscles of deconditioned subjects are especially reliant on carbohydrate as a source of energy (Coyle *et al.*, 1986;Coggan & Williams, 1995). Moreover, most rehabilitation programmes involve exercise at a high relative intensity where carbohydrate energy sources might be particularly important.

Other Approaches

Hormones have been used illicitly by a number of athletes to increase muscle bulk and this has also been tried in COPD. In the study by Schols and colleagues described above, a further group of patients were randomised to receive anabolic hormones in addition to nutritional support and pulmonary rehabilitation (Schols *et al.*, 1995). In these patients there were increases in lean mass and respiratory muscle strength but no increases in whole body exercise capacity. In a six month trial of anabolic hormones during a respiratory muscle and whole body exercise programme, increases in lean mass were seen but there was no improvement in respiratory muscle strength or exercise performance over placebo (Ferreira *et al.*, 1998). Attempts have been made to augment rehabilitation programmes with growth hormone (Pape *et al.*, 1991;Burdet *et al.*, 1997). Exercise capacity did not increase but again these studies were primarily aiming to increase weight in nutritionally depleted COPD patients. These findings are in keeping with the anabolic effect of these interventions which may

be of value in increasing muscle size and strength but less so for endurance activities. These therapies may be of most use when combined with strength training programmes. At present their clinical role remains uncertain.

Many disabled patients with COPD are hypoxaemic and there is evidence to suggest that providing oxygen to these patients during exercise increases performance (Leach *et al.*, 1992;Leggett & Flenley, 1977). In the United States the use of portable liquid oxygen systems for these patients is widespread although this is not the case in Europe (Royal College of Physicians, 1999). The role of such systems for patients who are normoxic at rest but desaturate during exercise is less clear but it is likely that performance will also be improved in this group. Indeed, there may be benefits even for patients who do not desaturate during exercise from oxygen supplementation during exercise (Somfay *et al.*, 2001).

A distinction needs to be made between the immediate effect of oxygen on exercise performance and the role of supplemented oxygen in training programmes. The latter has received far less attention but is an issue of practical importance because most rehabilitation programmes provide oxygen for hypoxic patients during exercise. This has been driven by concerns about safety but there is little evidence to support this and many of these patients carry out home exercises safely without oxygen. It could be argued that oxygen might permit training at higher intensities thereby improving the outcome of rehabilitation. However, athletes frequently seek hypoxic conditions at altitude in which to train suggesting that supplementing oxygen to patients undergoing rehabilitation could be counterproductive.

Little is known about the effect of hypoxia and oxygen supplementation on training in COPD. Two recent studies have shown no benefit from supplemental oxygen

during rehabilitation for desaturating patients (Garrod *et al.*, 2000;Rooyackers *et al.*, 1997) but these have been small and may have lacked adequate power to detect significant benefits. Further studies in this area are needed particularly concerning the impact of training under hypoxic conditions on muscle metabolism.

Summary

The recognition that extra-pulmonary factors such as peripheral muscle dysfunction contribute to exercise limitation in COPD provides an opportunity to develop new therapies aimed at enhancing performance in COPD. The benefits of exercise training to COPD patients are now well established and, in common with athletes, these approaches are likely to be most effective when combined with an appropriate training regimen.

The importance of maximising carbohydrate intake prior to training and competition for athletes has been recognised for several decades but its impact in COPD has not been explored. In this thesis the hypothesis that carbohydrate supplementation can enhance the benefit of exercise training in COPD is tested and an understanding of the changes in muscle physiology brought about by these interventions is sought.

CHAPTER FOUR

Methods

THIS thesis explores the role of nutritional support in the enhancement of exercise training in COPD. The impact of this intervention was evaluated using several measurements of physical performance and nutritional status. In this chapter I will describe these outcome measurements. Methods sections in subsequent chapters will refer back to this chapter. In **Chapters 5** and **7** studies investigating the relationships between these outcomes are described.

An additional aim of this thesis was to study the metabolic responses to exercise in the peripheral muscles in COPD. In this chapter I will describe the development of a technique for sampling the quadriceps muscle during exercise in COPD patients. Studies using this technique are described in **Chapters 6** and **9**. The laboratory analysis of muscle samples obtained in this way was not performed by the author and is therefore described briefly in **Appendix I**.

Spirometry

This was measured in the seated position to BTS/ARTP standards (Vitalograph, model R, Bucks, UK) (BTS/ARTP, 1994). Values are expressed as a

percentage of predicted values calculated from ERS regression equations (Quanjer *et al.*, 1993).

Physical Performance

Physical performance is an important therapeutic goal in COPD and a core component of pulmonary rehabilitation. In this thesis a number of modes of performance are measured. The relationship between these measures and their responses to exercise training are considered in **Chapter 5**. In this thesis I have used both field and laboratory tests of performance.

Whilst laboratory exercise testing provides detailed information on the physiological response to exercise it is expensive and time consuming to perform and does not reflect the day-to-day activities of patients. The practical difficulties with laboratory exercise testing have lead to the development of simple exercise tests that can be used to measure performance without the need for specialised equipment and staff training. Concerns over the safety of field tests because of the lack of cardiorespiratory monitoring have not been substantiated.

The first field tests to be developed for respiratory patients were the timed walk tests. During these tests the distance patients can walk in a given time is recorded. Two, six and twelve-minute walk tests have been reported (Steele, 1996) although the latter two times have been most frequently used to measure performance in COPD. Patients are free to choose their own strategy for performing the test and are allowed to rest and restart walking during the test. This has the advantage of more closely reflecting the way patients perform day to day tasks but increases the between patient variability in exercise response. The disadvantages of the timed walk tests stem mainly from their

poor standardisation and reproducibility. As result they are subject to greater variation from verbal encouragement and require two to three practice tests (Guyatt *et al.*, 1984;Knox *et al.*, 1988;Steele, 1996). More recently the shuttle tests have been developed as reliable measures of performance for patients with cardiopulmonary insufficiency.

Shuttle Walk Tests

The shuttle tests are externally paced field walking tests. Patients walking speed is determined by beeps they hear on a cassette recorder. As a result, these tests are more reproducible, requiring only one practice walk. Moreover, patients cannot vary walking strategy during the tests imposing greater standardisation and easier comparison between patients and operators. The shuttle tests are easy to perform in conjunction and have the advantage of providing performance measurements in two modes of exercise. For these reasons the shuttle tests were used to measure exercise performance in this thesis.

Incremental Shuttle Walk Test

Maximum exercise capacity was measured using the Incremental Shuttle Walk Test (ISWT). . This is a symptom limited, maximal, field exercise test. Subjects are instructed to walk around a 10-metre course at a speed indicated by beeps played from a cassette recorder (Singh *et al.*, 1992). The speed increases incrementally until the patient is unable to continue or maintain the required speed. The total distance walked is recorded. Performance in the ISWT has been shown to be predictive of VO_{2peak} (Singh *et al.*, 1994) and the test is reproducible after a single practice walk. The incremental

shuttle walk test has been extensively used in pulmonary rehabilitation and is sensitive to this intervention (Griffiths *et al.*, 2000;Singh *et al.*, 1998).

Endurance Shuttle Walk Test

Endurance performance was measured using the Endurance Shuttle Walk Test (ESWT) (Revill *et al.*, 1999). This is a constant work rate, submaximal field exercise test. After a 2-minute warm up, patients are asked to walk around the 10-metre course at a speed set by beeps played from a cassette recorder. The speed is constant and is set at the equivalent of 85% of the predicted VO_{2peak} achieved during the ISWT. The total time achieved (excluding the warm-up time) is recorded.

Muscle Strength

Physical performance extends beyond whole body exercise performance. Changes in the strength of individual muscles or muscle groups may be of importance to physical functioning for patients. Muscle strength can be measured by a number of methods. The simplest are isometric measurements and this is how strength has been measured in this thesis. These measure the force generated by the muscle at a constant length.

Quadriceps Strength

Maximum quadriceps muscle strength (QS) was measured by isometric dynamometry. Patients were seated upright on a specially constructed chair with the knee flexed at 90°. A strap was placed around the lower shin and is connected by a chain to a pressure transducer (Sprint, Loughborough, UK). The position of the strap

relative to the lateral malleolus was recorded so that future measurements could be taken under identical conditions. Subjects were asked to maximally extend the knee against the strap. Two groups of three attempts were performed with 5 – 10 minutes rest between them. The highest value of the six attempts was recorded.

The dynamometer calibration was checked every three to four months. This was done by suspending known weights vertically from the pressure transducer. These measurements gave details of the zero value for force and the gain across a range of weights (0 to 100N). Over the period of use of this equipment there was no significant change in gain. Changes in the zero value greater than 10N were added to the measurements taken.

Handgrip Strength

Isometric Handgrip strength (HGS) was measured using a handgrip dynamometer (Takei Instruments, Japan). After a practice attempt two measurements were taken in each hand. The mean of the highest values in the right and left hand was recorded. Results were expressed as Kg Force (KgF).

Health Status

Health related quality of life (or health status) is established as an important therapeutic outcome particularly in the treatment of chronic diseases where symptom control rather than cure is frequently the target. Health status questionnaires have been designed both for use in patients with different diseases (generic measures) and for specific groups of conditions (disease specific measures). Generic measures have the advantage of allowing the comparison of treatment effects between different conditions

and may allow a health economic analysis to be performed. They are less able to reflect specific problems experienced by patients with particular conditions and therefore may be less sensitive to some therapies. Conversely, disease specific tools are more sensitive but cannot be applied across different diseases. In this thesis I have been concerned with the effect of therapy on the outcome of rehabilitation. For this reason a sensitive, disease-specific health status tool (the Chronic Respiratory Questionnaire) was used.

The Self-Reported Chronic Respiratory Questionnaire

The Self-Reported Chronic Respiratory Questionnaire (CRQ-SR) was used. This has been developed from the interviewer-led CRQ (Guyatt *et al.*, 1987) to allow patients to complete the questionnaire without the need for a time consuming interview from a member of staff (Williams *et al.*, 2001). This version of the CRQ has been shown to be reproducible for the measurement of disease specific health status in a rehabilitation population. The questionnaire scores four domains; dyspnoea, fatigue, emotion and mastery. The results are presented as mean scores per question in each dimension. The threshold for a clinically significant change for each dimension has been identified as 0.5 (Juniper *et al.*, 1994).

Nutritional Status

Anthropometry

Body weight was measured in light clothing using digital scales (Seca, UK) to the nearest 100g. Height was measured to the nearest centimetre using a wall-mounted scale. Body Mass index (BMI) was calculated as weight/height^2.

Body Composition

The measurement of body composition is an important aspect of the assessment of nutritional status. However, the measurement of body composition is difficult and no true "gold standard" method exists. More recently, Dual Energy X-ray Absorptiometry (DEXA) has been suggested as a suitable reference method for healthy subjects but experience of its use in COPD remains limited. Simpler bedside measures of body composition also exist but their accuracy in COPD remains uncertain. For these reasons three methods were used to measure body composition in this thesis: DEXA and two bedside methods, bioelectrical impedance analysis (BIA) and skinfold anthropometry (SFA). The agreement between these methods and their reproducibility are considered in **Chapter 7**.

Dual Energy X-ray Absorptiometry (DEXA)

Body composition was assessed by DEXA scanning. Different body tissue constituents are distinguished by measuring the attenuation of two X-ray beams of different energies. This provides a three compartment model of body composition, subdividing the body into fat mass, bone-free lean mass and bone mineral mass. Bone mass is measured directly from its x-ray absorption and soft tissue mass at each point is derived by subtracting bone mass from total mass. Lean and fat mass are distinguished by water content and their attenuation of x-ray beams is known from in vitro measurements. The method assumes constant intracellular hydration of bone free lean mass (Jebb, 1997). Validation studies of DEXA against laboratory methods have been performed and are discussed in more detail in **Chapter 7**.

Subjects were scanned using the Lunar Expert-XL Bone Densitometer (Lunar Radiation Corporation, Madison, USA). Subjects were scanned longitudinally in the supine position using X-rays at two different energies. The attenuation of these X-ray beams was measured as they pass through the body. Scanning took approximately 15 minutes. Body compartment sizes were calculated using software provided by the manufacturer. All DEXA scans were performed at Leicester Royal Infirmary by radiography staff under the supervision of Dr. Peter Sheldon, Senior Lecturer in Rheumatology.

Bio-electrical Impedance Analysis (BIA)

Whole body single frequency impedance was measured using the method described by Lukaski *et al* (Lukaski *et al.*, 1985). Self-adhesive electrodes were applied over the dorsal surfaces of the left hand and foot. Electrodes were sited over the second metatarsal and midway between the medial and lateral malleoli on the foot. Further electrodes were placed over the second metacarpal and medial to the distal process of the ulna on the hand. A current of 800microamps at 50Hz was passed between these electrodes and the impedance recorded. Measurements were taken in the morning after voiding of the bladder. Patients lay in the semi-supine position.

Whole body resistance measured by this method is related to total body water. This is based on the principle that the impedance of a conduction system is related to its length and its cross-sectional area.

From this the following relationship can be derived:

$$V = L^2 / R$$

where V is volume, L is length and R is resistance. Conduction in a biological system occurs predominantly through water and so V estimates to total body water. L can be represented by height. In biological systems, body water lies almost completely in fat free mass (FFM) rather than fat mass (FM) and so FFM can be estimated according to the following relationship:

$$FFM \propto Height^2/R$$

Regression equations for calculating FFM by this measurement have been derived for a number of different populations including patients with COPD (Schols *et al.*, 1991b;Kyle *et al.*, 1998) using a variety of reference methods for measuring body water. In this thesis an equation derived from a study of COPD patients using isotope dilution as a reference method was used (**See Appendix II**). Values for FM are calculated by subtracting FFM from total body weight. Fat free mass index (FFMI) is calculated as FFM/height^2.

Skinfold Anthropometry

Skinfold thickness was measured at four sites: biceps, triceps, subscapular and suprailiac (Harpenden, UK). Measurements were made twice to the nearest 0.5 mm and the average of the two values recorded. The sum of these skinfold measurements was calculated and percent body fat content recorded from published tables (Durnin & Womersley, 1974). From this fat and fat free mass in Kg was calculated.

Dietary Intake

The balance between energy intake and expenditure is an important factor in weight loss for many COPD patients. Dietary intake may be affected by a number of factors such as discomfort during eating from pulmonary hyperinflation, anorexia due to systemic inflammation and difficulties with access to a healthy diet because of physical disability and social circumstances. Moreover, during nutritional support programmes dietary intake may fall, reducing the effectiveness of supplementation. It is therefore important to make an assessment of dietary intake during such supplementation programmes. In this thesis dietary intake was recorded using a three-day food diary. Subjects were asked to record all food and drink eaten over a three-day period on a standardised diary card. This method has been extensively used to record dietary intake (Bingham, 1987) and has been shown to be reproducible (Toeller *et al.*, 1997). Food quantities were recorded in household measures. Food weights were then estimated from published reference values (Ministry of Agriculture, 1993). Queries about the type or quantity of food recorded were discussed with the patients during their attendance at rehabilitation. Mean daily calorie and macronutrient intake was calculated by entering food records into a computerised version of food composition tables (Microdiet version 9.1, University of Salford (Holland *et al.*, 1991). The collection and entering of food diary data was performed by a state registered dietician (Rachel Barton).

The Metabolic Response to Exercise

It is now clear that peripheral muscle dysfunction contributes to exercise limitation in COPD and that improvements in peripheral muscle function are, at least in

part, responsible for the improvements seen in performance in COPD following pulmonary rehabilitation. However, our knowledge of the nature of peripheral muscle dysfunction is derived from studies of muscle structure and chemistry at rest. More recently, data on the dynamic responses of the muscles to exercise in COPD has been obtained from MRS studies but this gives an incomplete picture of muscle energy metabolism and makes assumptions made about cellular pH buffering which may not be true for COPD patients.

The analysis of muscle samples taken during exercise is commonplace in studies of healthy muscle physiology but has not been reported in COPD patients. This would have the advantage of allowing a more direct analysis of the muscular responses to exercise in this population.

In this thesis I have developed a technique for sampling the quadriceps muscle during exercise in COPD patients. This technique was then applied to investigate the metabolic responses to exercise in COPD and the effect of exercise training and nutritional supplementation on these responses. In this section I will describe the development of this technique in detail. A pilot study to evaluate the feasibility and tolerability of this technique is described in **Chapter 6**. The use of this technique to determine the effects of the combination of exercise training and nutritional support is described in **Chapter 9**.

Because the aim of this investigation was to assess the effect of a specific therapeutic intervention, it was crucial that biopsies were taken under reproducible conditions. Samples were therefore taken before and immediately after a constant load exercise test. Biopsies were taken after a specified cumulative workload for each patient and an identical absolute cumulative workload was used when repeat assessments made.

Cycle exercise testing was used so that the workload could be accurately measured. The absolute workload for the constant load was determined by prior performance during a maximal incremental exercise test.

Incremental Exercise Test

Patients performed a maximal, symptom limited incremental exercise test on an electrically braked cycle ergometer to determine peak exercise work capacity. Patients were asked to cycle at a constant speed of 40 rpm. Following a three minute period of unloaded cycling, the workload was increased incrementally by 10 Watts every minute. Patients were encouraged to continue cycling at the required rate for as long as possible. The peak work achieved was recorded. During the test ventilation and gas exchange measurements were made using a breath by breath computerised system (Oxycon Beta, Erich, Jaeger, U.K. Ltd). Continuous ECG and saturation recording was also performed. Breathlessness was recorded at the end of the test using the modified Borg score.

Constant Load Exercise Test

This was performed at least 48 hours after the incremental test to allow sufficient time for muscle recovery. Patients were asked not to undertake exercise outside their normal daily activities for 24 hours before the test. Muscle biopsies were taken at rest and immediately after a constant load cycle exercise test performed for five minutes (**Fig. 4.1**). The workload for this test was set at 80% peak work achieved during the incremental test. The cycle cadence for all tests was constant at 40 revs/min. This

absolute workload was used for subsequent tests even if peak performance increased as a result of rehabilitation. In other words, the muscle responses to an identical exercise challenge were measured. Whilst this is a high relative workload, it was anticipated that the absolute workload would be low because of exercise limitation in this population. Furthermore, patients with COPD are able to exercise safely at a high proportion of their maximum capacity. This relative intensity and time was chosen to provide a significant exercise challenge whilst allowing the majority of patients to complete the test. Subjects who could not complete the five minutes had biopsies taken at the limit of exercise and the same time used for the post intervention exercise test. ECG and saturation monitoring was performed throughout the test but breath-by-breath measurements were not taken. This was because it was felt the equipment for this would make obtaining biopsies more difficult and would make the test too uncomfortable for patients. Breathlessness was assessed on the modified Borg scale at the end of the test.

Muscle Biopsies

Muscle samples were taken under aseptic conditions using the Bergstrom technique (Bergstrom, 1975). Samples were taken from the left Vastus Lateralis muscle midway between the patella and the greater trochanter. Local anaesthesia (Lignocaine 1%) was infiltrated in the skin, subcutaneous tissues and muscle fascial sheath. Two 5mm incisions were made through the skin and fascia. A muscle sample was taken with the patient at rest from one incision. The sample is taken by introducing the needle into the muscle, which is simultaneously compressed externally by the hand so that muscle tissue enters the needle. The inner trochar of the needle is then used to take the biopsy. A dressing was then applied and the patient asked to perform the constant load test. The

second sample was taken from the other incision immediately after the completion of the exercise test. Post exercising samples were obtained with the subject seated in the bike no longer than 10 seconds after the end of the exercise test. This is effectively an exercising sample (Bergstrom, 1975).

After each muscle biopsy pressure was applied to the biopsy site for at least five minutes. At the end of the procedure butterfly sutures were used to close the incisions and a compression bandage applied for 6 to 12 hours.

Muscle samples were frozen immediately and later removed from the needle to be stored in liquid nitrogen. They were later transported to the Dept of Biomedical Sciences, Nottingham University where biochemical analysis was performed by laboratory staff under the supervision of Prof. Paul Greenhaff. Details of the analytical techniques are given in **Appendix I**.

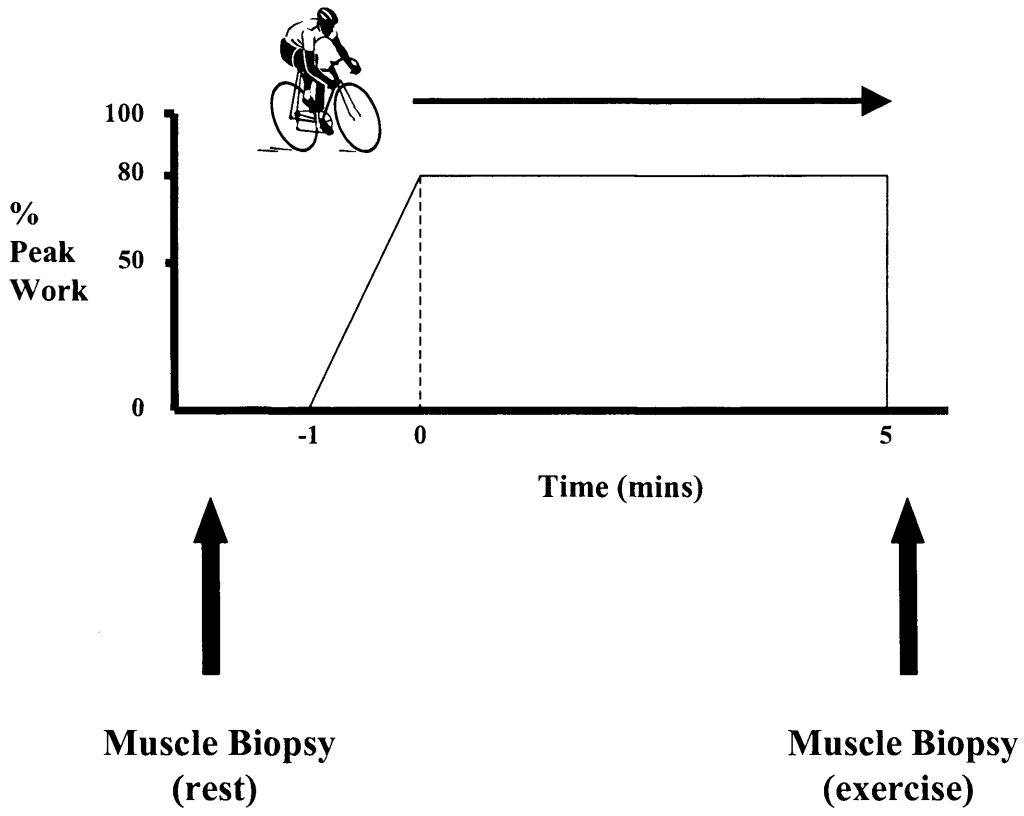


Fig 4.1. Protocol for the constant load test.

Muscle samples were taken at rest and again immediately after the completion of a five minute exercise test. The load was set at 80% Peak work achieved on the incremental cycle test. The workload was gradually increased from zero to this level over one minute before the start of the test. Post training samples were taken at an identical absolute time and workload.

CHAPTER FIVE

Measurements of Physical Performance and Nutritional Status in COPD

THE hypothesis I have investigated in this thesis is that nutritional support can enhance the physical benefits of pulmonary rehabilitation. To examine this hypothesis a number of outcome measures of nutritional status and physical performance were used. In this chapter I describe a preliminary study in which the relationship between these outcome variables and their responses to exercise training were investigated.

Introduction

Exercise intolerance is an important cause of disability for patients with COPD and physical performance is an important therapeutic outcome. Reliable measures of performance are needed because exercise capacity is only loosely predicted by measurements of lung function impairment and may be sensitive to interventions such as pulmonary rehabilitation, which have no effect on the underlying pulmonary pathophysiology of COPD.

A number of measures exist to evaluate the physical effects of exercise training. These may examine a range of physical characteristics such as peak or endurance whole body exercise capacity or muscle strength and mass. Frequently, little effort is made to match outcome measures of performance to the mode of exercise training used in the rehabilitation of COPD patients. This is important because it is a fundamental principle of exercise training that improvements in performance are confined to the mode in which the subject is trained (Maughan *et al.*, 1997).

The incremental (ISWT) and endurance (ESWT) shuttle walk tests have been used to evaluate exercise performance in patients attending pulmonary rehabilitation (Griffiths *et al.*, 2000;Revill *et al.*, 1999;Singh *et al.*, 1998). In this thesis a number of additional indices of physical performance and nutritional status were introduced as outcome measures. This chapter describes a preliminary study in which the relationships between shuttle walking performance and these indices were investigated. Like others, the training component of the rehabilitation programme at Glenfield Hospital consists almost exclusively of endurance walking exercise. A further aim of this study was to evaluate the responses of these measures to this endurance training programme.

Methods

Patients

This was a prospective, observational study of COPD patients undergoing pulmonary rehabilitation. Patients were recruited consecutively from those accepted for pulmonary rehabilitation at Glenfield Hospital. Patients with a clinical diagnosis of

COPD who met BTS spirometric criteria for COPD were included (The British Thoracic Society Standards of Care Committee, 1997).

Measurements

Measurements were taken at baseline and at the end of rehabilitation. A detailed description of these assessments is given in **Chapter 4**.

Spirometry

This was measured in the seated position to BTS/ARTP standards.

Nutritional Status

Body weight was measured in light clothing using standard scales. Height was measured using a wall-mounted stadiometer. Fat mass (FM) and Fat free mass (FFM) were measured by bio-electrical impedance analysis (BIA).

Physical Performance

Whole body exercise performance was measured using the incremental (ISWT) and endurance (ESWT) shuttle walk tests. Isometric handgrip (HGS) and quadriceps strength (QS) was also recorded.

Pulmonary Rehabilitation Programme

Patients participated in our standard rehabilitation programme. The programme is multidisciplinary and includes contributions from physiotherapy, occupational

therapy, dietetic, nursing and medical staff. After an initial assessment, patients attended twice weekly for a total of fourteen sessions, lasting at least seven weeks. Missed sessions were added to the end of the programme

The endurance training component of the programme comprised weekly sessions of endurance walking exercises. Patients were asked to walk at a speed equivalent to 85% of the predicted peak VO_2 achieved during the ISWT at their initial assessment. Walking times were increased progressively during the course of the programme. Patients were given a daily home walking programme and asked to record walking times and breathlessness on the Borg scale. At each training session adherence to the training programme was monitored, walking speeds checked and new targets set for walking times.

Patients also performed a weekly circuit of low impact conditioning exercises designed to increase suppleness and flexibility. The duration of these exercises was increased during the programme but there was no specific progressive weight training.

Each rehabilitation session included education sessions covering a range of topics including disease pathology, treatment, diet and relaxation.

Statistical Methods

Relationships between baseline measurements were analysed by Pearson correlation. Comparisons between before and after rehabilitation measurements for these data were made using paired student t-tests. Mean differences and 95% confidence intervals are quoted. Significance was tested at the 5% level.

The effect size for changes in outcome measures after rehabilitation was calculated by dividing the mean difference by the standard deviation of the pre

rehabilitation measurement. By calculating effect sizes, the magnitude of any changes can be judged according to the following criteria: small; 0.2 to 0.5, moderate; 0.5 to 0.8, large; > 0.8 (Cohen, 1988).

Post training ESWT showed a skewed distribution because there is a 20 minute ceiling on the measurement. Median and interquartile values are therefore quoted for this variable and therefore spearman coefficients and Wilcoxon rank tests were used for correlation and within group comparisons respectively. Effect sizes were calculated for ESWT to allow valid comparison between outcome variables because pre training ESWT and the change in ESWT were normally distributed.

Results

Baseline Data

Thirty-two patients (18 Male) took part in the study. All patients had airflow obstruction on spirometric testing. Baseline patient characteristics are shown in **Table 5.1**. FEV_1 did not correlate with body composition, muscle strength or exercise performance (**Fig 5.1**).

There was a significant correlation between FFM and both handgrip and quadriceps strength but FFM did not predict either ISWT or ESWT performance (**Fig 5.2**). There was a weak but statistically significant relationship between QS and ISWT. There was no correlation with ESWT (**Fig. 5.3**).

n = 32

Age (Years)	68.6 (5.7)
FEV_1 (L)	0.97 (0.38)
FEV_1 (% predicted)	38.8 (14)
Weight (Kg)	75.4 (15.5)
BMI (Kg/m^2)	27.0 (4.8)
FFM (Kg)	47.0 (10.0)
HGS (Kg)	26.8 (7.9)
QS (N)	314 (98.7)
ISWT (m)	201 (99.3)
ESWT (s)	150 (102) *

Table 5.1. Baseline patient characteristics.

Mean (SD) values are given apart from ESWT (*) where median (interquartile range) is given.

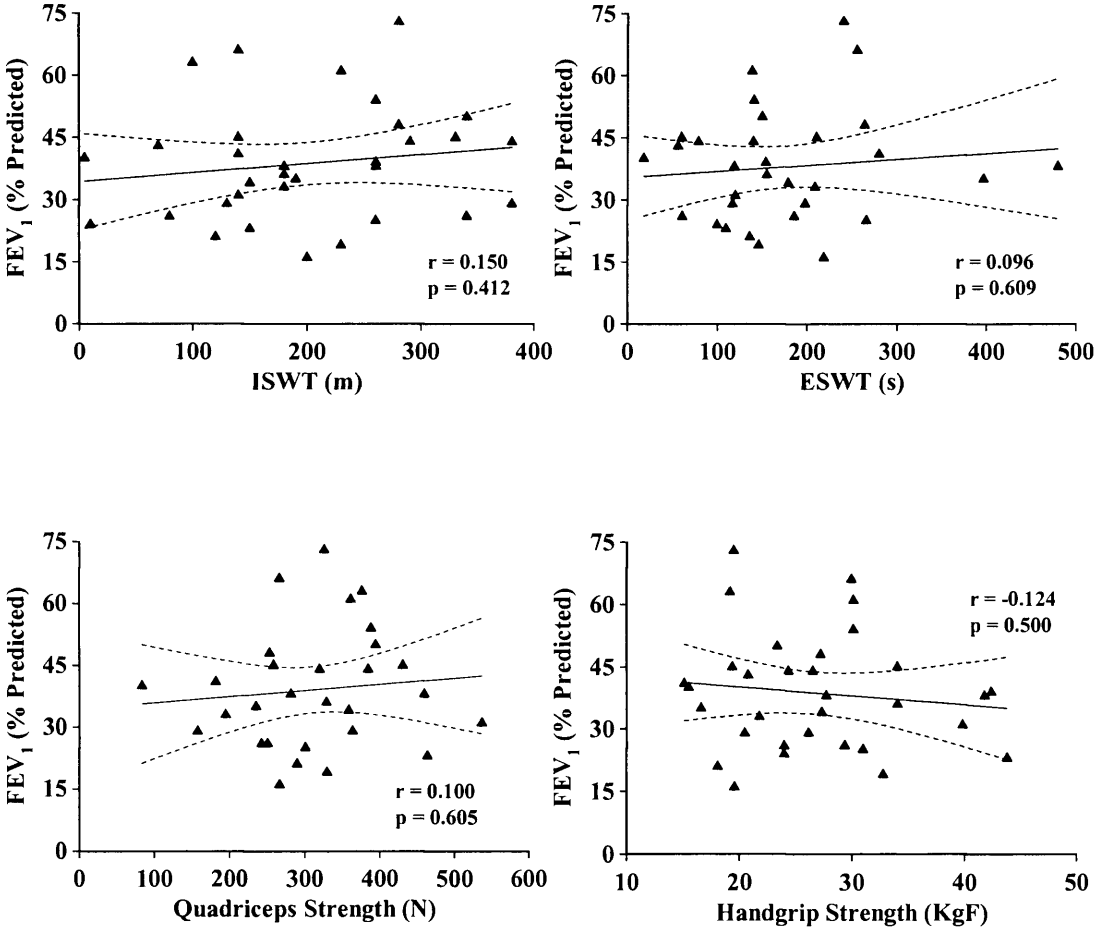


Fig. 5.1. Baseline lung function and physical performance.

Regression lines with 95% confidence intervals are plotted. Pearsons correlation was used to calculate r values for all variables except ESWT where Spearman correlation was used.

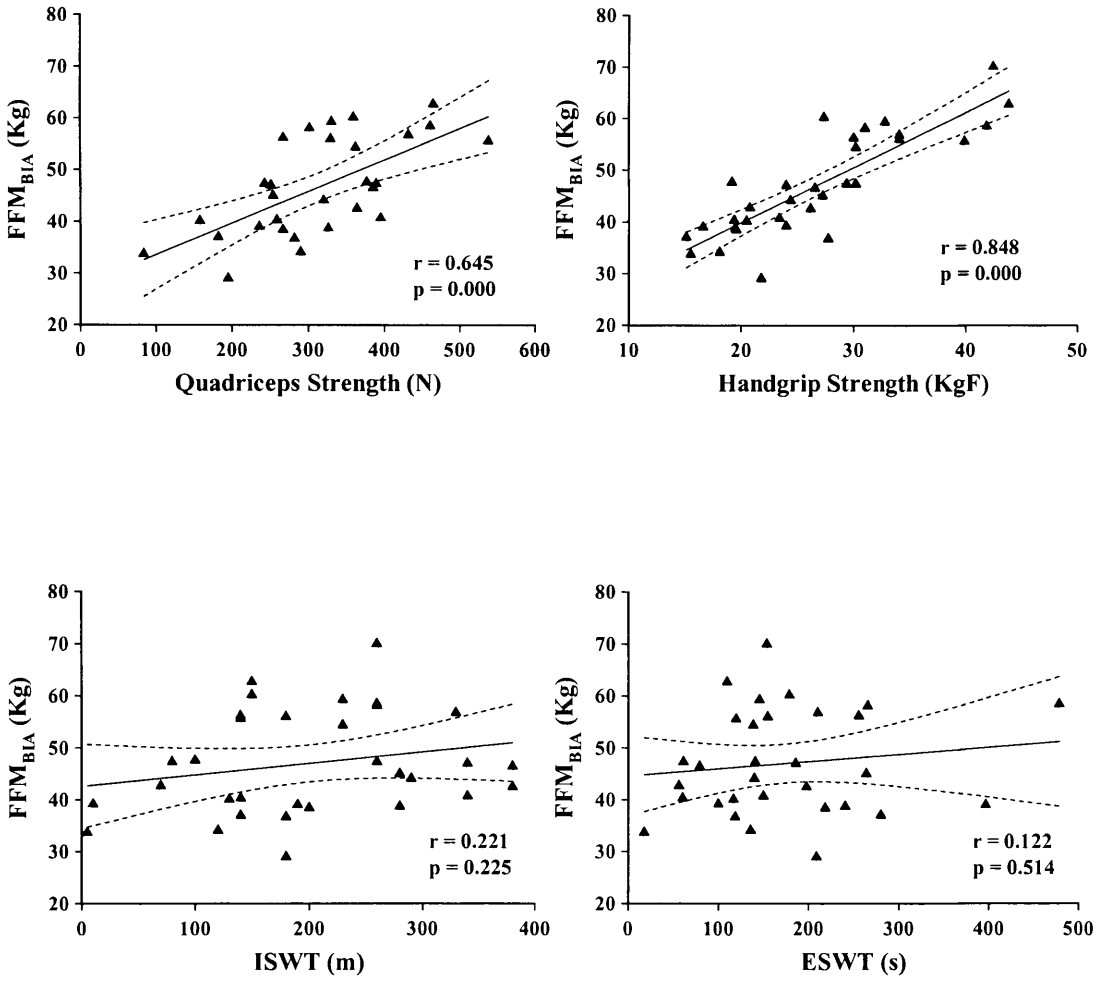


Fig. 5.2. Baseline FFM and physical performance.

Regression lines with 95% confidence intervals are plotted. Pearson correlation is used to calculate r values apart from ESWT where Spearman correlation was used.

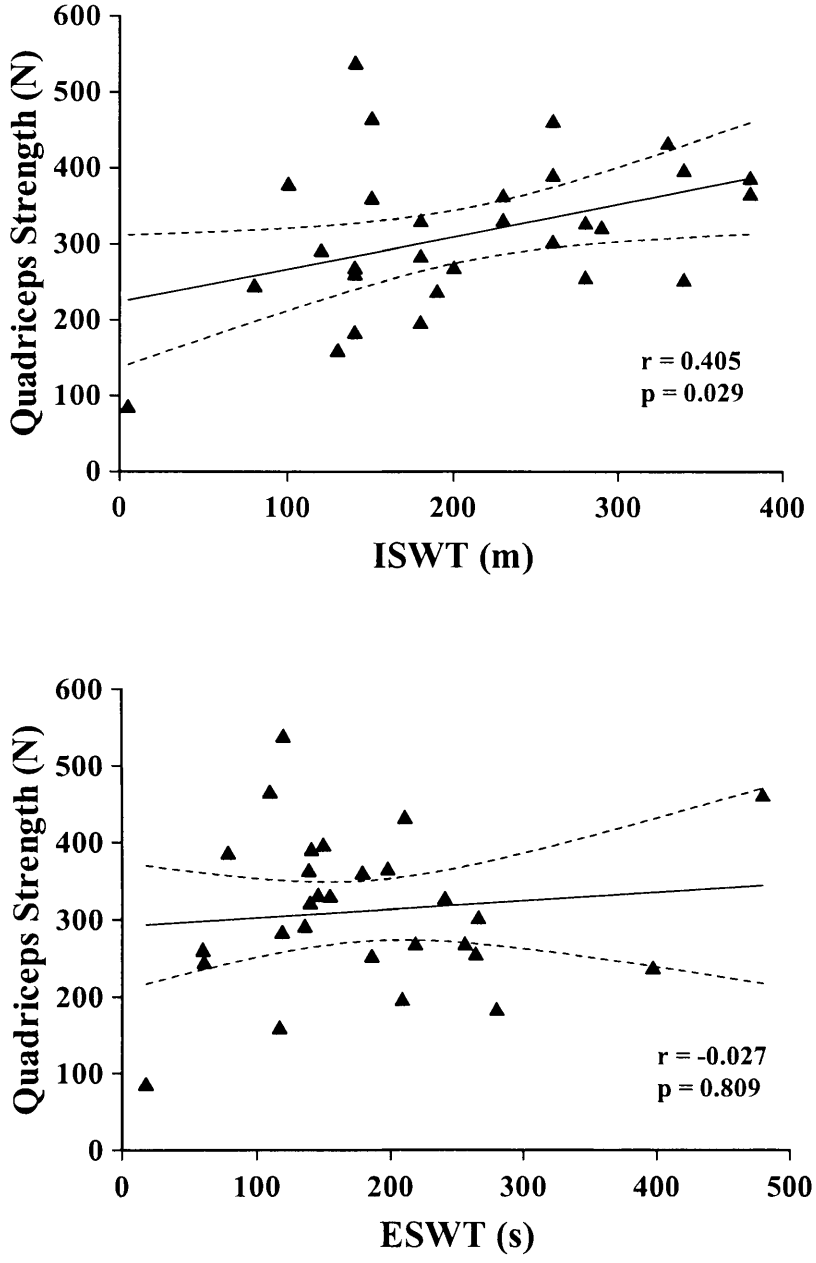


Fig. 5.3. Baseline quadriceps strength and shuttle walking performance.

Regression lines with 95% confidence intervals are plotted. Pearson correlation is used to calculate r values apart from ESWT where spearman correlation is used.

Outcome of Rehabilitation

26 patients completed rehabilitation. Of the six (19%) who did not, four developed prolonged exacerbations of their COPD and felt unable to resume rehabilitation, one suffered a myocardial infarction (unrelated to rehabilitation) and one developed gastro-intestinal problems and was unable to continue attending. Three patients did not have baseline quadriceps strength measurements because of equipment malfunction. Data analysis is based on the remainder and is shown in **Table 5.2**.

Mean differences between performance measurements before and after rehabilitation are shown in **Table 5.2** and **Fig 5.4**. Rehabilitation resulted in statistically significant increases in both ISWT and ESWT performance but the effect size was small for ISWT (0.44) whilst it was large for ESWT (1.04). There was no statistically significant change in FFM, HGS or QS following rehabilitation.

n = 26	Mean Change	95% CI	Effect Size	p value
FEV_1 (L)	0.01	-0.06, 0.05	0.03	0.827
FFM_{BIA} (Kg)	-0.1	-0.66, 0.47	-0.01	0.731
HGS (Kg)	0.51	-0.59, 1.6	0.06	0.349
QS (N)	10	-20, 41	0.1	0.498
ISWT (m)	45	26, 64	0.45	0.000
ESWT (s)*	365	230, 573	4.35	0.000

Table 5.2. Effect of pulmonary rehabilitation on outcome measures.

Mean (SD) changes in outcome measures for patients completing rehabilitation. 95% confidence intervals for the differences are quoted. p values are calculated using paired students t-tests.

* Median difference given. Wilcoxon Rank Test used to calculate p value.

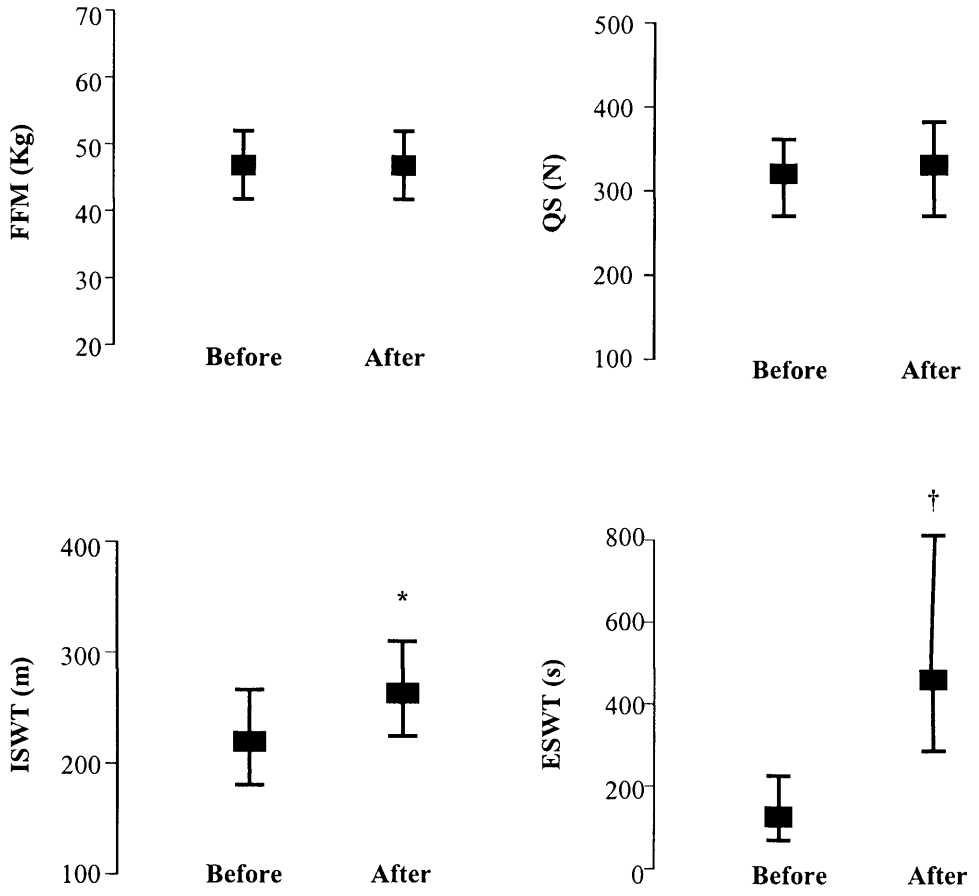


Fig. 5.4. Effects of pulmonary rehabilitation on outcome measures.
Squares represent mean values (Median values for ESWT). Error bars represent 95% confidence intervals.

* significant change after rehabilitation (paired t-test, p = 0.000).

† significant change after rehabilitation (Wilcoxon Rank Test, p = 0.000)

Discussion

This study investigates the relationship between different measurements of physical performance and other indices of physiological function in a cohort of COPD patients. At baseline, quadriceps muscle strength showed a weak but statistically significant correlation with maximal exercise but not endurance performance. Fat free mass and peripheral muscle strength were strongly correlated but there was no correlation between FFM and shuttle walking performance. There was no relationship between lung function impairment and any measure of physical performance.

Endurance training had a selective effect on different measures of physical performance in COPD patients. The increases in endurance performance after rehabilitation were of significantly greater magnitude compared with maximal exercise capacity. There were no changes in peripheral muscle strength or fat free mass.

These results indicate that in COPD measures of individual muscle strength and field tests of endurance and peak exercise capacity represent distinct modes of physical performance, which do not necessarily overlap. There is a marked difference in the responses of these measures to endurance training. Furthermore, ISWT performance appears to have a strength component whilst the ESWT performance does not. This confirms that adaptations to training occur in COPD patients in the same way as healthy subjects where improvements principally occur in the mode of performance in which subjects are trained.

The benefits of pulmonary rehabilitation in improving exercise capacity and health status are now well established (Lacasse *et al.*, 1996). Exercise training is an essential component of such programmes for these benefits to be realised (Ries *et al.*, 1995). Several studies in this area have observed greater effects on endurance than

maximal exercise performance (Goldstein *et al.*, 1994;Niederman *et al.*, 1991). These programmes included a significant endurance training component in their regimes. Field tests of exercise capacity are increasingly being used to evaluate rehabilitation programmes. These have the advantage of reflecting more closely the day-to-day exercise activities of patients. They are also easier to perform and require no specialised equipment or staff training. Both the six-minute (6MWT) and the twelve-minute (12MWT) walk test have been extensively used and found to be responsive to rehabilitation (Weiner *et al.*, 1992;Cockcroft *et al.*, 1981;Goldstein *et al.*, 1994). These tests probably comprise elements of maximal and endurance exercise capacity. However, this may vary between patients as subjects are allowed to choose the strategy with which the test is performed. The shuttle tests have the advantage of being externally paced and therefore not subject to such variation. Data is now available confirming the responsiveness of the shuttle tests to rehabilitation (Griffiths *et al.*, 2000;Revill *et al.*, 1999).

The lack of improvement in muscle mass and strength in the current study is not surprising, as the rehabilitation programme did not include specific muscle strength training. Patients did perform low impact upper and lower limb conditioning exercises but these were non-progressive with no increase in training load. Similarly, the aerobic training component focussed on increasing walking times with no graded increases in speed. Given the apparent relationship between strength and ISWT performance it remains possible that the magnitude of the changes in the latter could be increased if progressive strength training was included in the training regime.

Several studies have examined the impact of strength training on exercise capacity (Clark *et al.*, 2000;Simpson *et al.*, 1992;Bernard *et al.*, 1999). These have

shown impressive improvements in peripheral muscle strength after training. Interestingly, some have shown improvements in endurance although the addition of strength training to aerobic exercise appears not to have resulted in extra improvements in health status (Bernard *et al.*, 1999). To my knowledge, the impact of strength training on shuttle walking performance has not been studied.

A relationship between muscle strength and exercise performance has previously been reported (Gosselink *et al.*, 1996;Bernard *et al.*, 1998;Hamilton *et al.*, 1995) and appears to apply to both maximal exercise performance and six minute walking distance. This contrasts with my finding of only a weak correlation of quadriceps strength with ISWT and none with ESWT. This is likely to be due to the different characteristics of the performance measures used and the degree to which they involve a strength component. Several of these studies used isokinetic rather than the isometric strength measurements used in this study. These are more dynamic tests of muscle strength and therefore may more closely reflect muscle movement during whole body exercise (Hunter *et al.*, 1998).

There was no relationship between fat free mass and exercise performance although FFM and peripheral muscle strength were closely correlated. Several studies, however, have shown a relationship between nutritional depletion and exercise capacity (Schols *et al.*, 1991a;Gray-Donald *et al.*, 1989;Palange *et al.*, 1998). Most of these studies used incremental cycle ergometry to measure capacity, which may require greater strength than walking exercise (Palange *et al.*, 1998;Baarends *et al.*, 1997a;Gray-Donald *et al.*, 1989). In one study a strong correlation between FFM measured by BIA and SFA and twelve-minute walking distance was found (Schols *et al.*, 1991a). The reasons for the disparity with the current study are unclear. Given the

correlation between QS and ISWT it is possible that a study of greater size might have shown that FFM predicted shuttle-walking performance. It might also be explained by differences in the performance requirements of the shuttle and the 12 minute walk tests. Accurate measurements of body composition are technically difficult and bedside measurements potentially subject to error. However, this is unlikely to be the explanation for the lack of correlation with exercise capacity because these results were reproduced when different measures of body composition were compared (**Chapter 7**).

It is probable that the improvement in performance seen after rehabilitation is at least in part due to improved peripheral muscle function. There is increasing evidence of peripheral skeletal muscle dysfunction in COPD and that this is a significant factor in exercise intolerance in these patients (American Thoracic Society, 1999). Several studies have indicated that there is impaired oxidative capacity in the peripheral muscles of COPD patients (Whittom *et al.*, 1998;Maltais *et al.*, 1996a;Sala *et al.*, 1999). Adequate replenishment of ATP supplies by oxidative metabolism is a prerequisite for sustained endurance exercise. Training in healthy subjects results in significant increases in mitochondrial oxidative enzyme concentrations and this has also been shown after training in COPD patients (Maltais *et al.*, 1996b).

The finding of no relationship between lung function impairment and physical performance has been noted in other studies (Morgan *et al.*, 1983;Killian *et al.*, 1992). This emphasises that for many patients exercise intolerance is not simply due to a limit to pulmonary ventilation. Other factors such as peripheral muscle dysfunction are also likely to play a role in the disability experienced by COPD sufferers. Identifying the limits to exercise in patients might allow better prediction of those who will respond to rehabilitation and permit the individualised prescription of exercise.

It is recognised that psychological factors such as reduced fear of breathlessness and greater confidence are likely to have made a significant contribution to the outcome of rehabilitation. These remain difficult to quantify but are important benefits for patients.

In summary, this chapter describes data confirming that physical performance in COPD as in health can be measured in a number of modes. These measures are distinct from one another in their characteristics and responses to training. For performance enhancing therapy to be effectively evaluated, performance measures need to be carefully chosen. The ability to selectively test modes of performance may allow tailoring of training and other performance enhancement therapies to meet the specific exercise needs of patients.

CHAPTER SIX

The Sampling of the Quadriceps Muscle During Exercise in COPD patients. A Pilot Study

THE aim of this thesis was to explore the potential for the enhancement of the physical benefits of training in patients with COPD and to investigate the physiological mechanisms underlying these benefits. In this chapter I will describe the development of a technique for sampling the quadriceps muscle of COPD patients during exercise. The purpose of this approach was to develop a tool that could be used to measure the metabolic response to exercise in COPD. This technique was later applied to evaluate the effects of exercise training and nutritional support in this population (see **Chapter 9**).

Introduction

It is now clear that peripheral skeletal muscle dysfunction makes an important contribution to exercise limitation in COPD (American Thoracic Society, 1999). Several studies have demonstrated a reduction in the size and strength of the lower limb muscles of COPD patients compared to healthy controls (Gosselink & Decramer, 1998;Clark *et*

al., 1996;Schols *et al.*, 1993). Others have provided evidence that energy metabolism is altered in COPD with a reduction in oxidative metabolism and a consequent increase in glycolytic activity and lactate release (Whittom *et al.*, 1998;Maltais *et al.*, 1996a). There is also evidence that exercise training in this population can improve peripheral muscle performance and bioenergetics (Maltais *et al.*, 1996b).

Much of this evidence is drawn from the analysis of muscle samples taken at rest. Direct information on the skeletal muscle responses to exercise in COPD, however, remains scanty. More recently, magnetic resonance spectroscopy studies (MRS) have allowed dynamic studies of exercising muscle (Sala *et al.*, 1999;Kutsuzawa *et al.*, 1995) but this gives an incomplete picture of skeletal muscle energy metabolism and imposes limits on the modes of whole body exercise that can be studied (Constantin-Teodosiu *et al.*, 1997).

The sampling of the peripheral muscles during exercise is commonplace in the study of exercise metabolism (usually involving healthy young subjects) but has not been reported in patients with COPD. This technique would allow the muscular responses to exercise to be measured directly. Such information would be useful as the peripheral muscles could be a target for novel therapies aimed at reducing disability in patients with chronic lung disease.

The aim of the study described in this chapter was to determine the feasibility and tolerability of obtaining samples of the quadriceps muscle during exercise in COPD patients attending rehabilitation. During this procedure muscle samples were obtained before and after a specified exercise challenge that could be accurately reproduced. The metabolic responses to exercise were measured in a small cohort of patients before and after the completion of rehabilitation.

Methods

Patients

Participants were recruited from those accepted for pulmonary rehabilitation at Glenfield Hospital. Patients with a clinical diagnosis of COPD who met BTS spirometric criteria for COPD were included (British Thoracic Society, 1997). Approval for the study was obtained from the Leicestershire Research Ethics Committee.

Patients were excluded if they were unable to perform a cycle ergometry test or were receiving anticoagulant therapy.

Measurements

Tests were performed at baseline and after a seven-week pulmonary rehabilitation programme (see below). A detailed description of measurement methods is provided in **Chapter 4**.

Physical Performance

Maximum quadriceps muscle strength (QS) and handgrip strength (HGS) were measured by isometric dynamometry.

Maximal and submaximal field exercise capacity was measured using the incremental (ISWT) and endurance (ESWT) shuttle walk tests.

Patients performed a maximal, incremental exercise test on an electrically braked cycle ergometer to determine peak exercise work capacity. Ventilation and gas exchange measurements were made using a breath-by-breath computerized system.

Muscle Biopsies

A detailed description of the muscle biopsy technique is given in **Chapter 4**. A brief outline is given here. Patients attended at least 48 hours after performing the maximal incremental cycle test. Patients were asked to undertake a five minute constant load cycle exercise test performed at 80% peak work achieved during the incremental cycle test. Post training biopsies were taken at an identical absolute cumulative workload.

Muscle biopsies were obtained from the Vastus Lateralis muscle using the Bergstrom technique at rest and immediately after the completion of the constant load test. Post exercising samples were obtained (with the subject seated on the bike) no longer than 10 seconds after the end of the exercise test. Samples were frozen immediately and stored in liquid nitrogen.

Biopsy Analysis

Muscle samples were analysed at the Dept of Biomedical Sciences, The University of Nottingham under the supervision of Prof. Paul Greenhaff. Samples were analysed for Adenosine Triphosphate [ATP], Phosphocreatine [PCr], Creatine [Cr] and lactate [La]. Analytical techniques are described in **Appendix I**.

To allow for contamination of the samples with non-muscle tissue (Fat, blood and connective tissue) all values apart from lactate were corrected for total creatine content.

Energy for ATP resynthesis can be generated from oxidative or non-oxidative sources. Substrate level phosphorylation represents total ATP energy derived from non-oxidative sources. As oxidative metabolism is the only other source of ATP energy, its

contribution to energy metabolism can be measured by calculating SLP (Spriet *et al.*, 1987).

Substrate level phosphorylation (SLP) was calculated as follows:

$$SLP = \Delta[PCr] + \{\Delta[La] \times 1.5\} + \{\Delta[ATP] \times 2\}$$

Where Δ refers to the exercise induced change in each metabolite.

Rehabilitation programme

Patients participated in the standard outpatient rehabilitation programme at Glenfield Hospital. The programme is multidisciplinary and includes contributions from physiotherapy, occupational therapy, dietetic, nursing and medical staff. After an initial assessment, patients attended twice weekly for a total of fourteen sessions, lasting at least seven weeks. Missed sessions were added to the end of the programme

The endurance training component of the programme comprised weekly sessions of endurance walking exercises. Patients were asked to walk at a speed equivalent to 85% of the predicted peak VO_2 achieved during the ISWT at their initial assessment. Walking times were increased progressively during the course of the programme. Patients were given a daily home walking programme and asked to record walking times and breathlessness on the Borg scale. At each training session adherence to the training programme was monitored, walking speeds checked and new targets set for walking times.

Patients also performed a weekly circuit of low impact conditioning exercises designed to increase suppleness and flexibility. The duration of these exercises was increased during the programme but there was no specific progressive weight training.

Each rehabilitation session included education sessions covering a range of topics including disease pathology, treatment, diet and relaxation.

Data Analysis

Differences between the analysis of resting and exercising biopsies were analysed using paired students t tests. Relationships between variables were analysed using Pearson correlation. Paired t-tests were used to compare exercise induced changes in muscle metabolites before and after training.

Results

Baseline Data

Twelve patients (8 Male) took part in the study. Baseline characteristics and physical performance data are shown in **Tables 6.1** and **6.2**. In three patients insufficient muscle was obtained for a paired rest and exercise comparison to be made at baseline. There was significant contamination of the majority of the remaining samples particularly those taken during after exercise but sufficient muscle was present for analysis. The biopsies were well tolerated by patients. A few patients experienced local discomfort and bruising but this was infrequent and self limiting.

There were significant exercise induced changes in muscle [ATP], [PCr], and [La] (**Table 6.3; Fig. 6.1**). Lactate accumulation and PCr degradation were strongly

correlated ($r = 0.8$, $p < 0.001$)(**Fig. 6.2**). The changes in muscle [PCr] and [La] showed a relationship with absolute exercise intensity although these were not statistically significant. Muscle [ATP] declined during exercise but this was not related to exercise intensity (**Fig. 6.3**).

n = 12	
Age (years)	69.5 (5.8)
Weight (Kg)	81.9 (15.5)
BMI (Kg/m^2)	27.6 (4.7)
FEV_1 (L)	1.04 (0.3)
FEV_1 (% predicted)	39.1 (12.0)

Table 6.1. Baseline patient characteristics.

Figures refer to mean (SD) values.

	Before Training	After Training
	n = 9	n = 9
Physical Performance		
QS (N)	334 (65)	320 (143)
ISWT (m)	204 (75)	251 (78) *
ESWT (m) †	141 (125)	388 (240) *
Peak Cycle WR (W)	67 (23)	71 (21)
Peak VO_2 (mls/min)	1185 (365)	1163 (364)
Constant Load Test		
Workload (W)	53 (18)	---
End Exercise HR (bpm)	117 (19)	116 (17.6)
End Exercise SaO_2 (%)	89 (5.3)	90.7 (6.1)
End Exercise BS †	4 (1.0)	4 (2.5)
End Exercise PE †	15 (4)	13 (3)

Table 6.2. Physical performance before and after rehabilitation.

Identical workloads were used for post training tests. Except where stated figures are mean (SD) and within group comparisons made using paired students t test. Data for patients completing rehabilitation is shown.

† median (interquartile range) values given, Wilcoxon rank test used to calculate p values.

* significant change from before rehabilitation on paired analysis: $n = 6$, $p < 0.05$.

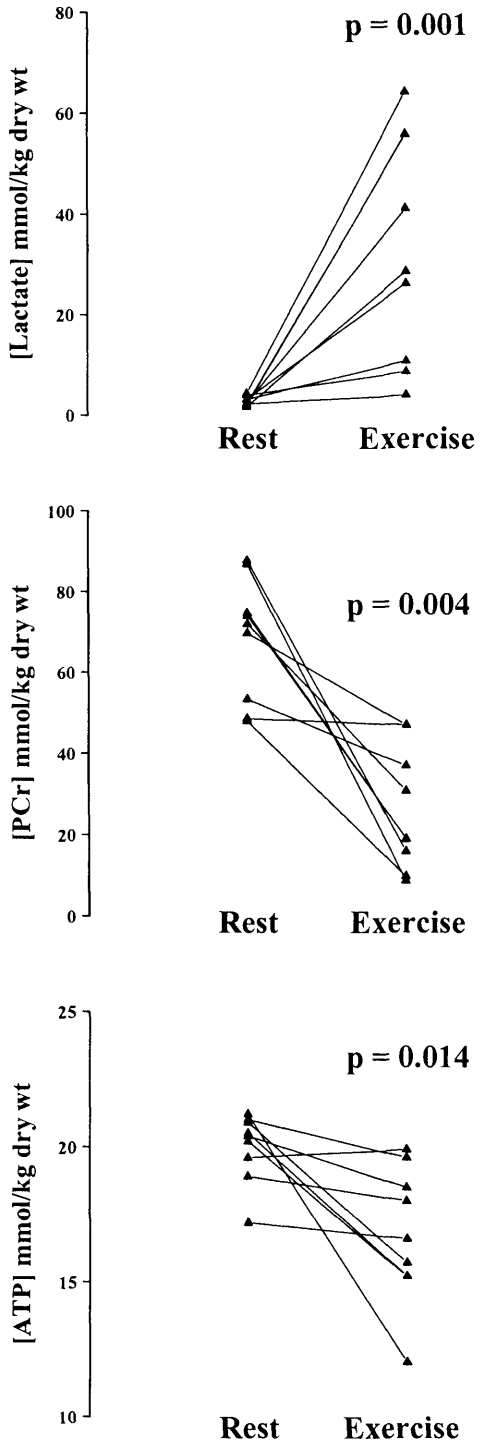


Fig. 6.1. Exercise induced changes in muscle metabolites at baseline.

Paired students t-tests used to calculate p values.

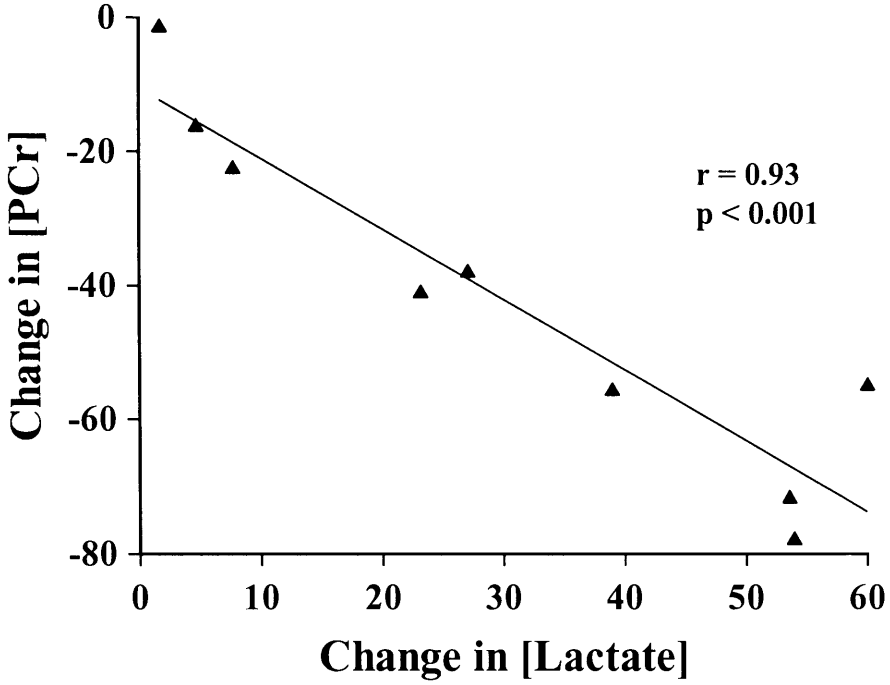


Fig. 6.2. Lactate accumulation and PCr degradation during exercise.

Units are mmol/Kg dry weight.

Data from biopsies taken at baseline (before training). Pearson correlation used to calculate r values.

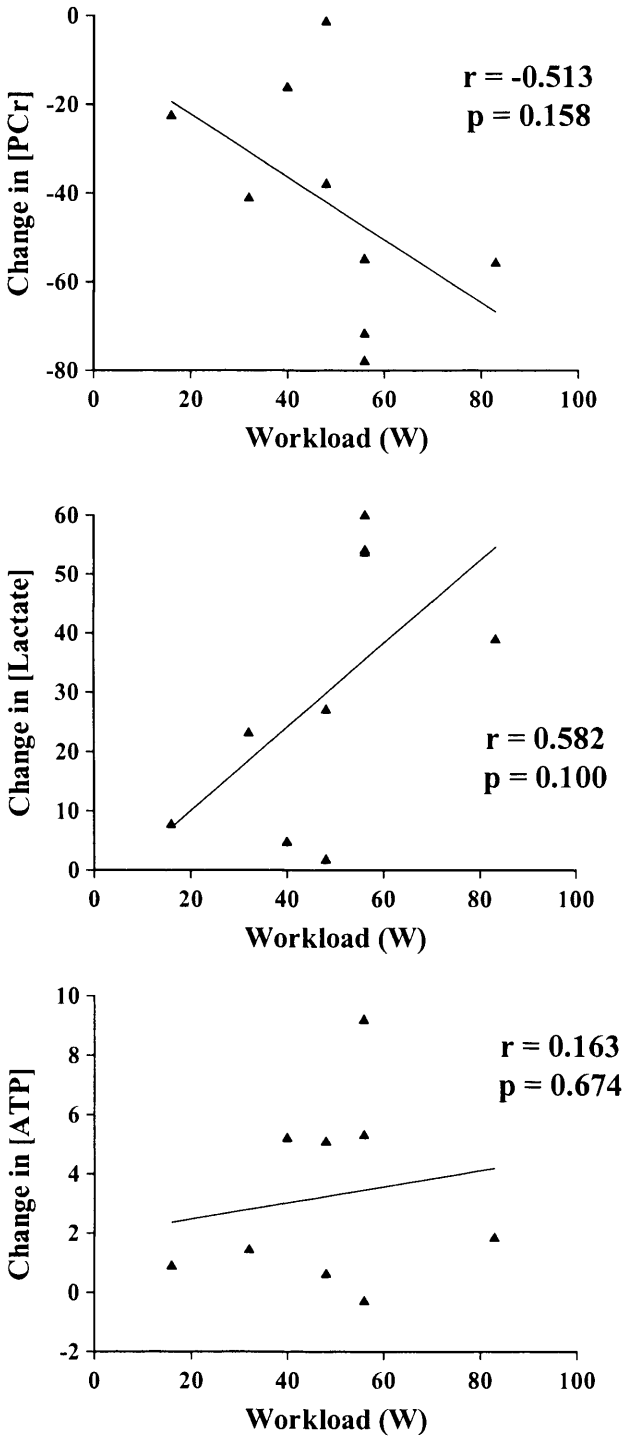


Fig. 6.3. Muscle metabolites and exercise workload at baseline.
Baseline (pre training) data are shown. All units for muscle metabolites are mmol/kg dry wt. Workloads refer to work performed during the constant load test. Pearson correlation used to calculate r values.

	Before Training (n = 9)			After Training (n = 9)		
	Rest	Exercise	Mean Change	Rest	Exercise	Mean Change
PCr	69 (15)	26 (15)	-42 (-62, -23)*	64 (11)	33 (16)	-31 (-44, -17)*
ATP	20 (1.3)	16.7 (2.5)	-3.2 (0.6, 5.6)†	18.3 (2.6)	18.6 (2.1)	0.3 (-2.2, 1.6)
Lactate	2.7 (0.9)	32.7 (22.5)	30 (12.6, 47.4)*	4.0 (2.3)	28.5 (22.3)	24.5 (6.8, 42.3)†

Table 6.3. Muscle metabolites before and after training.

Mean (SD) muscle metabolites at rest and exercise before and after endurance training. All units are mmol/Kg dry wt. Mean (95% CI) changes during exercise are calculated by subtracting resting from exercising values. Paired t-tests used to calculate p values.

* significant change from rest to exercise $p < 0.01$.

† significant change from rest to exercise $p < 0.05$.

Effect of Exercise Training

Nine patients completed the rehabilitation programme. Of the three who did not, one declined to have further muscle biopsies, one developed a prolonged exacerbation and felt unable to resume rehabilitation and one suffered a myocardial infarction unrelated to the study. All post training samples contained sufficient muscle for analysis. There were therefore six paired pre- and post-training biopsy samples available for comparison.

There were significant improvements in ISWT and ESWT performance but not in HGS, QS or incremental cycle performance following rehabilitation (**Table 6.2**). Exercise induced changes in muscle metabolites following training are shown in **Table 6.3** and **Fig. 6.4**. After training ATP loss during exercise did not occur. This difference was just outside the limits of statistical significance on paired analysis ($p = 0.057$).

Mean lactate accumulation and PCr degradation were lower after training but this was not statistically significant. Mean (SD) substrate level phosphorylation during exercise declined substantially after training although this change was not statistically significant (Pre training: 93.8 (61.1) vs Post training: 71.4 (47.6) mmol ATP/Kg dry wt, $p = 0.170$).

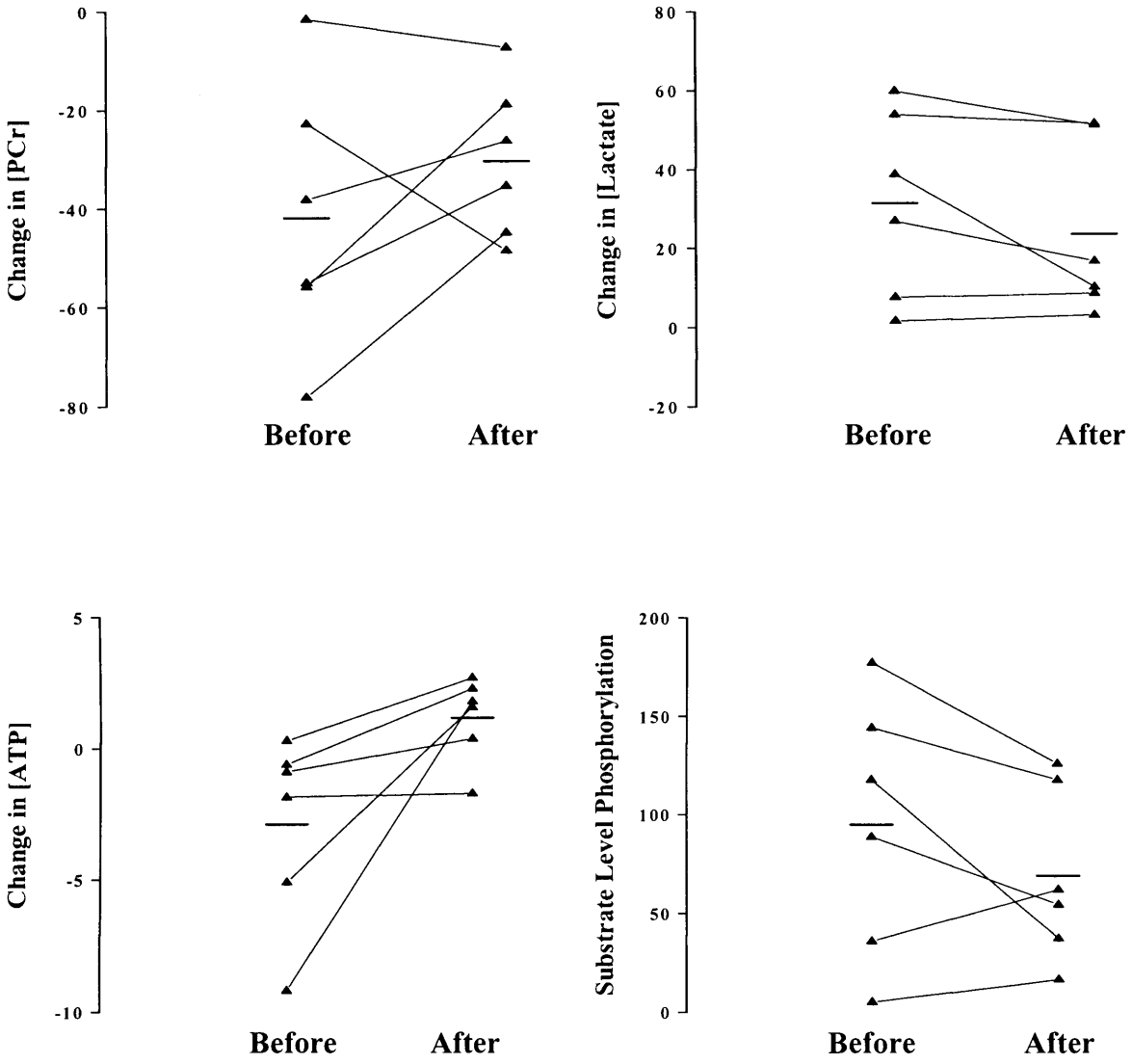


Fig. 6.4. The muscle metabolic response to exercise before and after training.

Bars refer to mean values. See **Table 6.4** for p values and 95% confidence intervals.

All units mmol/kg dry wt apart from Substrate Level Phosphorylation (mmol ATP/kg dry wt).

Discussion

This study demonstrates that sampling the quadriceps muscle during exercise in elderly COPD patients is both feasible and tolerable. There were significant exercise induced changes in muscle [ATP], [lactate] and [PCr]. There was a trend towards the abolition of the decline in [ATP] during exercise after training.

Biopsy Technique and Baseline Data

The examination of muscle biopsies taken in during exercise is commonplace in the investigation of muscle metabolism in young, healthy subjects but has not been reported in patients with COPD. Samples in these studies are taken and frozen within a few seconds of cessation of exercise. This accurately reflects the muscle in its exercising state (Soderlund & Hultman, 1986). This was achieved in all patients in the current study.

A number of problems arise when applying this technique to COPD patients. The smaller size of the peripheral muscles in this population means that obtaining adequate samples quickly after exercise is difficult. Contamination of samples with fat, connective tissue and blood can be a universal problem but is greater for elderly COPD patients because muscle quality is poorer. The effect of this variation can be substantially reduced by correcting the results for total creatine content, as this is a constituent of muscle only. The strong relationship between PCr degradation and Lactate accumulation in both pre and post rehabilitation biopsies suggests that these changes reflect genuine responses to exercise and were not significantly affected by variations in the quality of the biopsies.

The use of this technique allows the skeletal muscle metabolic response to exercise to be investigated directly in COPD patients. This is important because it is now clear that peripheral muscle dysfunction has a role in physical disability in COPD (American Thoracic Society, 1999). Samples were taken after a fixed cumulative workload so that the exercise challenge could be exactly reproduced after an intervention such as training. Furthermore, this form of exercise most closely reflects the type of training undertaken during the rehabilitation programme. The exercise time and intensity was chosen to be feasible for most patients to achieve prior to rehabilitation and all patients managed to do this. The significant changes in muscle metabolites seen during exercise indicate that this exercise test posed a substantial challenge to the muscles.

The decline in [ATP] during exercise seen in our patients suggests that the muscles were under substantial metabolic stress during this exercise challenge. Stores of ATP in muscle are limited and only sufficient to sustain a few seconds of exercise. A decline in muscle [ATP] will be due to an imbalance in the rate of ATP hydrolysis to yield energy and its resynthesis from anaerobic and oxidative energy metabolism. The decline in [ATP] was not restricted to those with severe exercise intolerance as ATP decline was unrelated to absolute exercise intensity.

These results are consistent with a similar muscle biopsy study of 10 patients with congestive heart failure (CHF) compared with healthy controls (Naveri *et al.*, 1997). In this study there was a significant drop in muscle [ATP] at peak exercise in CHF patients but not controls. Changes in lactate and PCr were similar in the two groups but these changes occurred at lower exercise workload in CHF patients. This study differed from previous muscle biopsy studies in CHF where ATP turnover rates

appeared normal but the magnitude of the changes in lactate and PCr were lower in the CHF group (Schaufelberger *et al.*, 1996;Sullivan *et al.*, 1991). The reasons for these differences are unclear but direct comparisons between studies are difficult because different exercise protocols were used.

Direct data on the peripheral muscle responses to exercise in COPD are scarce. MRS studies have shown increased PCr degradation, slower PCr resynthesis following exercise in COPD compared with healthy controls (Payen *et al.*, 1993;Sala *et al.*, 1999). These findings are supported by studies of muscle samples taken at rest from COPD patients, which have suggested impairment of mitochondrial oxidative capacity with a consequent increase in glycolytic activity and lactate release (Maltais *et al.*, 1996a;Whittom *et al.*, 1998). MRS is a valuable technique because it allows dynamic studies of exercising muscle but is limited in only being able to measure high-energy phosphate compounds. Furthermore, the modes of exercise that can be studied with MRS are limited (Constantin-Teodosiu *et al.*, 1997).

Effect of Exercise Training

The decline in [ATP] during exercise did not occur after training, although this difference was just outside limits of statistical significance. This suggests that training may improve muscle energy metabolism, allowing maintenance of ATP turnover during exercise.

There was also a decline in substrate level phosphorylation after training. Although this was not statistically significant in this small pilot study, the magnitude of this change was large (around 30%). This suggests that anaerobic metabolism may

make a smaller contribution to ATP resynthesis following training. This can only occur if the contribution of oxidative metabolism increases.

These findings are consistent with evidence from previous studies of endurance training in healthy subjects and COPD indicating that oxidative metabolism is improved (Maltais *et al.*, 1996b;Wibom *et al.*, 1992). Improvements in oxidative metabolism would be expected to result in a reduction in lactate accumulation and PCr degradation during exercise. Changes in these metabolites did occur following training but these were small and not statistically significant. The reasons for this are unclear but given the variability of the metabolic responses in our patients, our study may have been too small to pick up such changes. Samples were obtained during cycling exercise whereas the endurance training programme was primarily walking exercise. In general, improvements in performance are specific to the mode of training employed and this may have reduced the magnitude of any adaptations to training.

Rehabilitation resulted in significant improvements in both incremental and endurance shuttle walking performance but no changes in peripheral muscle strength or peak cycle performance. Given the nature of the training programme this is not surprising, as no specific progressive strength training was included. Changes in VO_{2peak} measured by cardiopulmonary exercise testing after rehabilitation are frequently modest (in the region of 5%).

Conclusion

This pilot study demonstrates that sampling of the quadriceps muscle during exercise is tolerable and feasible in patients with COPD. Although the quality of the samples obtained after exercise was variable, adequate samples were obtained for the

analysis of the metabolic responses to exercise in this group. This technique may prove a valuable tool for the further study of peripheral muscle function in COPD.

Interpretation of the biopsy analysis presented in this chapter requires caution because of the small number of patients studied. However, the improvement in ATP turnover following training suggests that improvements in performance may be at least in part due to adaptations to energy metabolism in the peripheral muscles. The sampling of the peripheral muscles during exercise may be a sensitive means of evaluating the physiological effect of training or other performance enhancing interventions. This is important because peripheral muscle dysfunction is a potential target for new treatments for a condition where current pharmacological therapy is largely ineffective. In **Chapter 9** I will describe a larger study using this technique to evaluate to effects of endurance training and nutritional support in COPD patients.

CHAPTER SEVEN

A Comparison of Measurement Methods for the Nutritional Assessment of COPD Patients

ALTHOUGH the primary aim of this thesis was to explore the role of nutritional support in enhancing performance during exercise training, important secondary outcomes were changes in body weight and composition. Body composition is difficult to determine accurately. In this chapter I will describe a study comparing methods for its measurement.

Introduction

Weight loss is an important clinical feature in patients with COPD. Reduced body weight and muscle mass is an independent predictor of mortality (Wilson *et al.*, 1989;Landbo *et al.*, 1999) and correlates with physical performance (Schols *et al.*, 1991a) and health status (Mostert *et al.*, 2000) in this population.

The prevalence of nutritional depletion in COPD patients may be underestimated by simple measurements of body weight and body mass index because

patients may show relative reductions in muscle mass despite being of normal overall weight (Schols *et al.*, 1993). For this reason, measurements of body composition are increasingly used to assess the nutritional status of COPD patients. These measurements subdivide the body into a number of compartments depending on the method of measurement used but of most interest is the fat free mass (FFM) compartment, which contains functional muscle mass. Increasing muscle mass is an important therapeutic goal for rehabilitation and nutritional support programmes, emphasising the importance of the measurement of FFM.

There is no gold standard method for the measurement of FFM. The choice of measurement method for body composition balances accuracy with practicality and cost. Furthermore, age specific normal ranges for FFM have not been established making the identification of nutritionally depleted patients difficult.

Recently, Dual Energy X-ray Absorptiometry (DEXA) has been suggested as a suitable clinical reference method for the measurement of body composition (Fuller *et al.*, 1992;Van Loan, 1998). Experience of its use in COPD patients, however, remains limited and although it is safe and easy to perform, it may be inconvenient for patients and is costly. By contrast, Bioelectrical Impedance Analysis (BIA) and Skinfold anthropometry (SFA) are simple bedside measurements of body composition. However, they may be subject to greater inaccuracy in the elderly and in disease because of inherent assumptions about cellular hydration and the distribution of body fat.

In this study fat free mass was measured in a cohort of COPD patients using these three methods. Our aim was to define the limits of agreement between these methods and determine how interchangeable they are for the measurement of FFM in COPD.

Methods

Subjects recruited for the nutritional intervention trial described in **Chapter 8** were studied. All patients met BTS clinical and spirometric criteria for COPD and were aged between 40 and 80 years (British Thoracic Society, 1997). Patients with a BMI greater than 30 Kg/m^2 were excluded.

A detailed description of the methods used in this study is given in **Chapter 4**.

Body Composition

Subjects had Bioelectrical Impedance Analysis (BIA), Skinfold Anthropometry (SFA) and Dual Energy X-ray Absorptiometry (DEXA) performed within a seven-day period. All measurements were taken at baseline before patients commenced rehabilitation and nutritional supplementation. Body weight was measured in light clothing to the nearest 0.1Kg (SECA, UK). Body height was measured using a wall-mounted stadiometer to the nearest 1cm.

Fat Free Mass was measured using DEXA, BIA and SFA (see **Chapter 4**). Fat free mass index (FFMI) was calculated as $FFM/Height^2$ (VanItallie *et al.*, 1990). Patients were considered to be nutritionally depleted if they had a BMI ≤ 21 or a FFMI ≤ 15 (in females)/ FFMI ≤ 16 (in males).

Statistical Analysis

All data was normally distributed. The limits of agreement between measurement methods were determined by plotting the mean inter-method measurement difference (the *bias* of the measurement) $\pm$ 2SD (the *error* of the measurement) as

described by Bland and Altman (Bland & Altman, 1986). FFM and FFMI derived from BIA and SFA were compared in turn with DEXA, as the latter would be considered by most authorities to be the most accurate. The mean inter-test differences were compared in males and females using independent samples t –tests. The sensitivity and specificity of BIA and SFA relative to DEXA for identifying nutritionally depleted patients were also calculated (Altman, 1991).

Results

Eighty-five patients were recruited to the study. Baseline characteristics of the patients are shown in **Table 7.1**. Mean FFM and FFMI measurements using each method are shown in **Table 7.2**.

The limits of agreement between the measures of FFM are shown in **Fig 7.1**. Measurements of FFM by BIA and SFA are compared with FFM_{DXA}. For the whole group, BIA underestimated FFM relative to DEXA (mean difference (FFM_{DXA} – FFM_{BIA}): 0.72 Kg, limits of agreement: -5.68, +7.20 Kg). By contrast SFA overestimated FFM relative to DEXA (Mean difference (FFM_{DXA} – FFM_{SFA}: -1.70 Kg, limits of agreement: -8.20, +4.80 Kg). There was a systematic increase in bias with mean FFM for both FFM_{DXA} vs. FFM_{BIA} ($r = 0.51$, $p < 0.01$) and FFM_{DXA} vs. FFM_{SFA} ($r = 0.27$, $p < 0.05$.)(**Fig. 7.1**). However, when FFM Index was plotted rather than FFM, these correlations were considerably weakened or eliminated ($FFMI_{DXA}$ vs. $FFMI_{BIA}$: $r = 0.23$, $p < 0.05$, $FFMI_{DXA}$ vs. $FFMI_{SFA}$: $r = 0.18$, $p = 0.1$) (**Fig. 7.2**).

Within this cohort, there were significant gender differences in the bias of FFM measurements for these three methods (**Fig. 7.3**). In males, BIA underestimated FFM whereas in females it was overestimated. SFA overestimated FFM relative to DEXA in

both males and females but this bias was significantly larger in females than in males. These differences were statistically significant. These inter-method differences were also seen when FFM Index was used.

The overall prevalence of nutritional depletion for DEXA, BIA and SFA was 49%, 48% and 38% respectively. Differences in the identification of depletion between genders are shown in **Table 5.2**. Using DEXA as the reference method, the sensitivity for BIA and SFA for detecting nutritional depletion was 86% and 74% respectively and the specificity 88% and 98%.

	Male	Female
	n = 53	n = 32
Age (Years)	67.7 (8.4)	65.6 (8.7)
Height (m)	1.72 (0.07)	1.59 (0.06)
Weight (Kg)	70.2 (13.0)	60.2 (10.5)
BMI (Kg/M^2)	23.7 (3.9)	23.7 (3.3)
FEV_1 (L)	0.91 (0.38)	0.84 (0.36)
FVC (L)	2.56 (0.79)	1.92 (0.53)
FEV_1 (% Predicted)	30.9 (12.8)	40.6 (13.7)

Table 7.1. Patient characteristics

Mean (SD) baseline characteristics of patients recruited to the study.

	Males	Females
FFM_{DXA} (Kg)	50.6 (7.6)	36.4 (5.0)
$FFMI_{DXA}$ (Kg/m^2)	17.0 (1.9)	14.4 (1.4)
% Depleted by DEXA	36	72
FFM_{BIA} K(g)	48.8 (6.4)	37.5 (4.6)
$FFMI_{BIA}$ (Kg/m^2)	16.5 (2.0)	14.8 (1.3)
% Depleted by BIA	42	59
FFM_{SFA} (Kg)	51.7 (6.9)	39.1 (5.0)
$FFMI_{SFA}$ (Kg/m^2)	17.4 (1.8)	15.4 (1.4)
% Depleted by SFA	28	53

Table 7.2. FFM and FFM Index measured by DEXA, BIA and SFA.

Mean (SD) values are given. Nutritional Depletion defined by BMI ≤ 21 or a FFMI ≤ 15 (in females)/ FFMI ≤ 16 (in males).

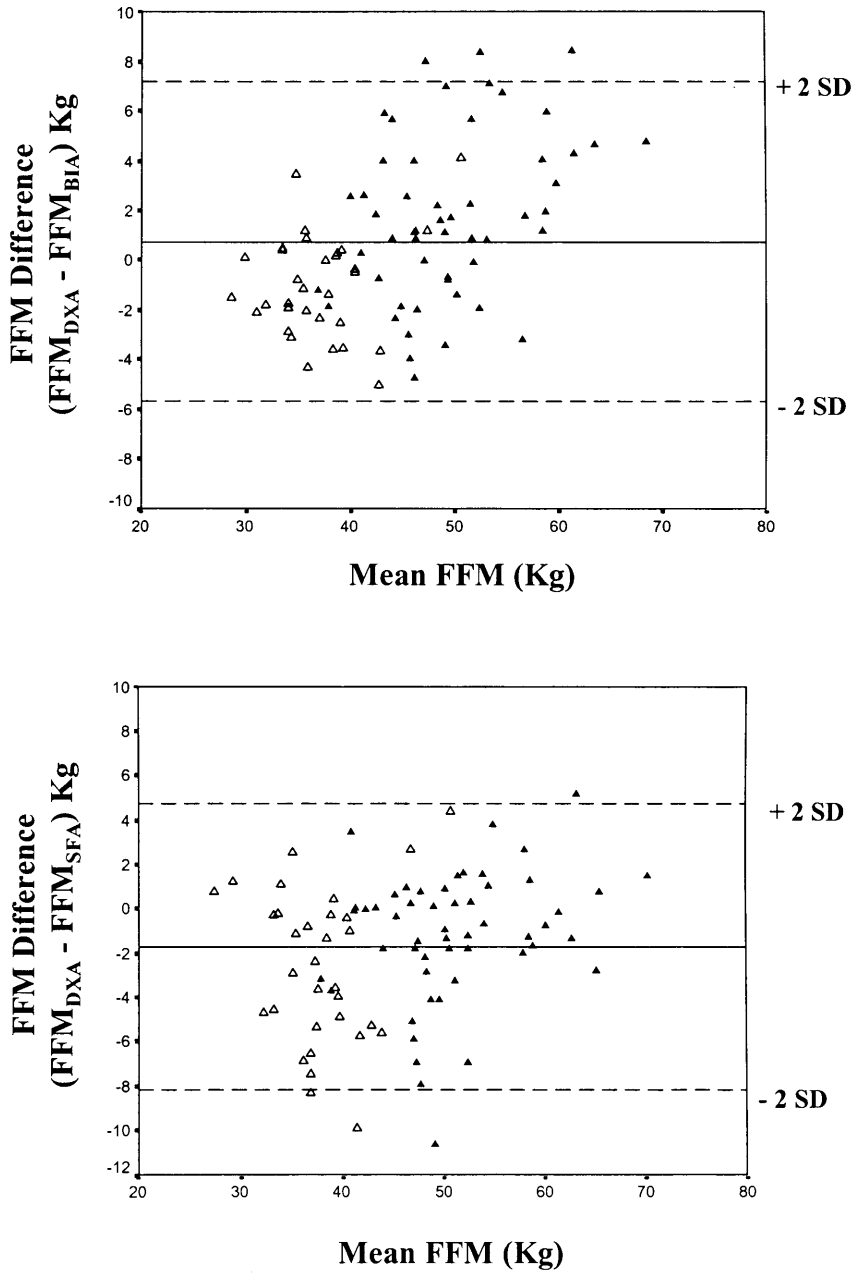


Fig. 7.1. Inter-method agreement for measurements of fat free mass. Bland and Altman Plots of the differences between measurement methods of FFM. Open symbols (Δ) represent females, solid symbols (♦) represent males. Mean Differences (solid lines) and limits of agreement (broken lines) for the whole population are shown. (See Text for figures)**Upper Panel:** FFM measured by DEXA and BIA. **Lower Panel:** FFM measured by DEXA and SFA.

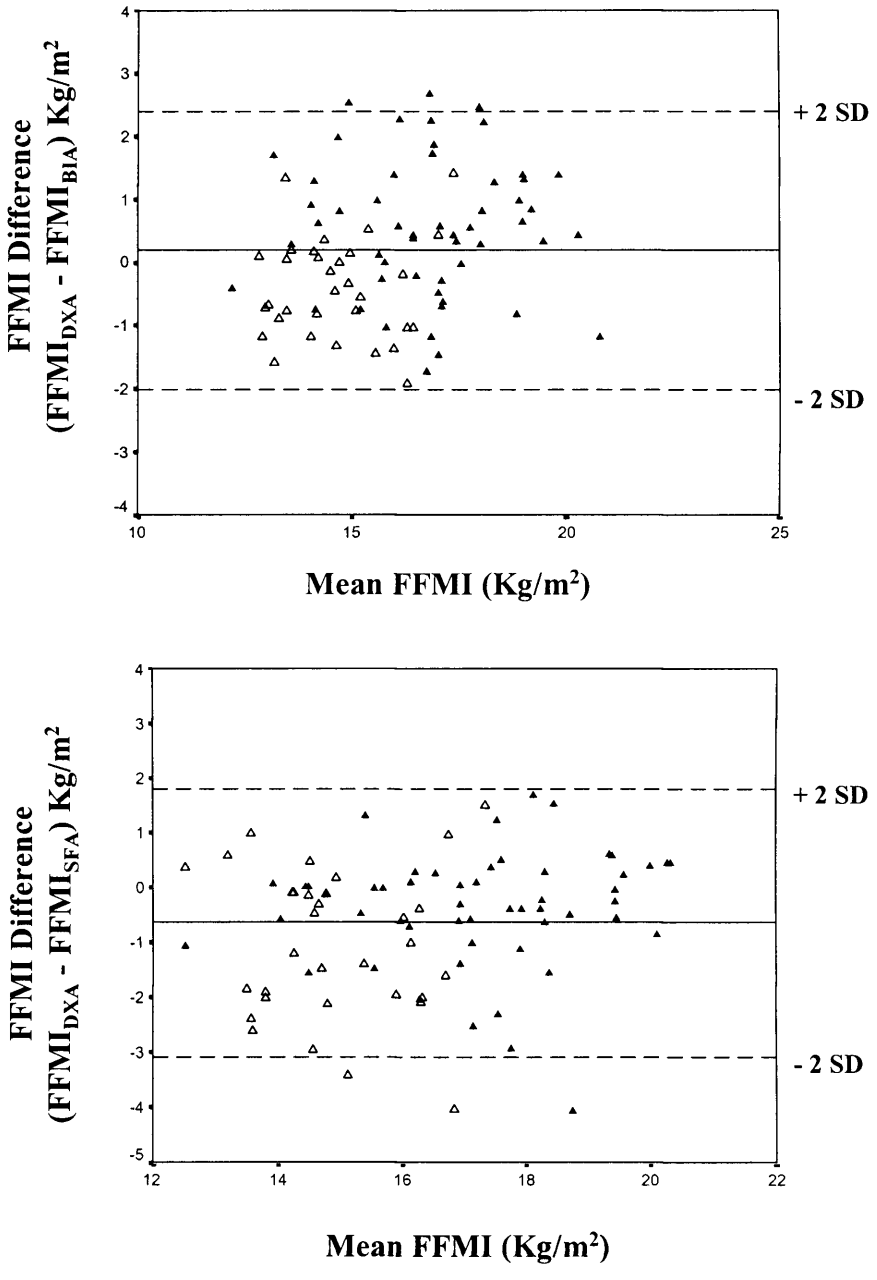


Fig. 7.2. Inter-method agreement for measurements of fat free mass index. Bland and Altman Plots of the differences between measurement methods of FFM Index. Open symbols (Δ) represent females, solid symbols (♦) represent males. Mean Differences (solid lines) and limits of agreement (broken lines) for the whole population are shown. (See Text for figures)**Upper Panel:** FFM Index measured by DEXA and BIA. **Lower Panel:** FFM Index measured by DEXA and SFA.

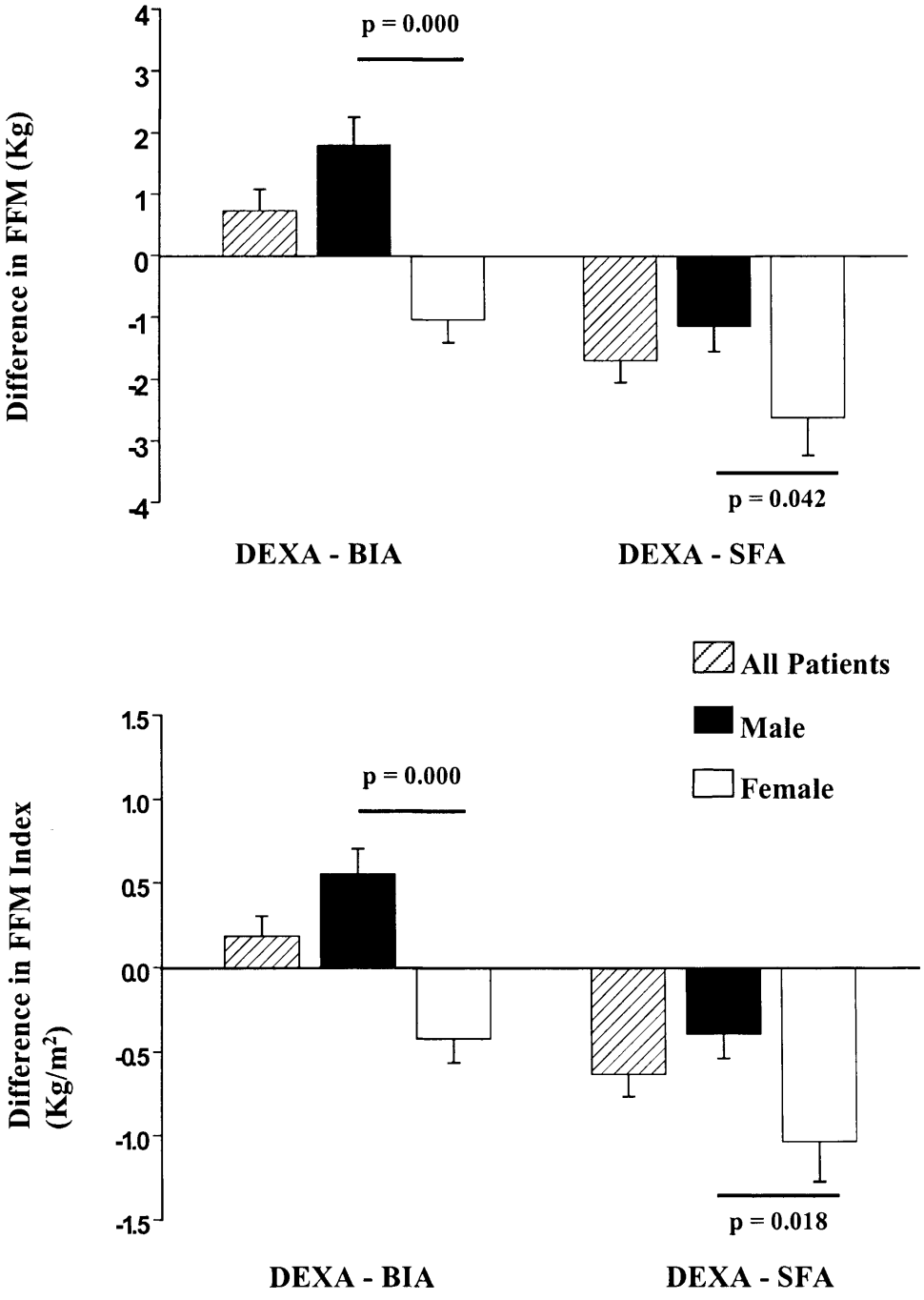


Fig. 7.3. Gender differences in body composition measurements. Mean (SE) inter-method differences in FFM (upper panel) and FFM Index (lower panel) measurements for all patients and between genders.

Discussion

This study defines the limits of agreement for three different methods of measuring body composition in a cohort of COPD patients presenting for rehabilitation. Overall, BIA underestimated FFM relative to DXA whilst SFA overestimated it. The mean differences between methods (the *bias*) were small but the limits of agreement (the *error*) were relatively large. The bias was greater for SFA relative to DEXA than BIA. There were significant gender differences in bias for both BIA and SFA relative to DEXA.

This study highlights the importance of the choice of method for measuring body composition in COPD patients. The inter-method differences demonstrated in this study are reflected in the lower sensitivity of BIA and SFA for detecting nutritional depletion relative to DEXA. The systematic increase in bias for FFM from both BIA and SFA with mean FFM was almost eliminated when FFMI was used suggesting that height was a crucial factor. The effect of height on the accuracy of FFM measurements appears to apply to each method as the same effect was observed when BIA and SFA were compared independently from DEXA. The gender difference in bias between measurements was not explained by the lower height and weight in females because when FFMI was substituted the gender effect persisted whereas the systematic bias was lost.

DEXA was used as the reference method for measuring FFM in this study. It should be recognised that this is not a measurement of true FFM; indeed, a gold standard method for measuring true FFM does not exist. However, DEXA has been proposed as a suitable reference method for the measurement of body and has been validated in animals, whose chemical composition is known in detail, and in humans

using hydrodensitometry as a reference method (Kohrt, 1995). DEXA also has the advantage of providing a three compartment model of body composition and allows the quantification of bone free lean mass. Commonly used reference methods for measuring body composition such as hydrodensitometry or isotope dilution techniques measure total body water and then calculate FFM by making the assumption that intracellular hydration is constant. This may not be true in the elderly or in disease. Whilst the calculation of soft tissue compartments from DEXA also requires the assumption of constant intracellular hydration, there is evidence from studies where the hydration factor is manipulated, that this method may be less prone to such errors (Kohrt, 1995). The true precision of DEXA remains uncertain, however and there are significant differences when soft tissue mass is compared using machines from different manufacturers (Tothill *et al.*, 1994).

Defining nutritional depletion is difficult because there is no range of normality for FFM in this population. The definition of nutritional depletion used in this study (BMI $\leq$ 21 or a FFMI $\leq$ 15 (females)/ FFMI $\leq$ 16 (males)) is arbitrary but widely used and corresponds with earlier definitions for COPD patients using percent ideal body weight (Baarends *et al.*, 1997a;Schols *et al.*, 1993). Nutritional depletion by this definition has been shown to have significant consequences for health status and physical functioning in COPD patients (Baarends *et al.*, 1997a;Mostert *et al.*, 2000).

There are a number of possible reasons for the differences between measurement methods for body composition seen in this study. BIA relies on the estimation of total body water from measurements of whole body impedance. FFM is calculated from total body water using a prediction equation derived from comparison with a reference method. Errors may arise from incorrect assumptions about the

hydration of the lean tissue compartments in the population studied or from the population and reference method used to derive the prediction equation. In a study of patients with respiratory insufficiency, Pichard and colleagues demonstrated that the choice of prediction equation is critical to the accuracy of FFM measurements using BIA (Pichard *et al.*, 1997). In their study the agreement of DEXA with BIA using a reference equation derived from COPD patients (Schols *et al.*, 1991b) was particularly poor. This study uses a more recent equation from the same institution derived from a larger group of group of COPD patients using deuterium dilution as a reference method (A.M. Schols; Personal Communication; see **Appendix II**). In contrast to their original equation, this provides gender specific equations for COPD patients and may explain the agreement between BIA and DEXA is better in this study than in that of Pichard *et al* (Pichard *et al.*, 1997). More recently, a prediction equation for COPD patients using DEXA as a reference method has been published (Kyle *et al.*, 1998). Perhaps not surprisingly (as the equation used was derived from data within the study), the limits of agreement between DEXA and BIA reported in this latter study were narrower than those reported here.

The gender differences in bias between BIA, SFA and may relate to differences in regional fat distribution in men and women. Impedance is inversely proportional to the circumference of the conduction system and therefore BIA may be subject to errors resulting from changes in the distribution of fat between the limbs and the trunk (Gray *et al.*, 1989). It is important to recognise, however, that DEXA may underestimate the effect of central fat redistribution in the elderly resulting in errors of FFM measurements when these populations are studied (Snead *et al.*, 1993) and may result in gender differences in FFM measurements from DEXA. In the study by Kyle and

colleagues (Kyle *et al.*, 1998), no gender differences were detected but such differences were seen in the study by Engelen et al who compared FFM from DEXA with deuterium dilution (Engelen *et al.*, 1998). The results reported here suggest that the effect of gender applies across each method as similar differences were found when BIA was compared with SFA independently from DEXA.

The finding of greater error for FFM from SFA relative to DEXA contrasts with those of Fuller et al who found SFA to be the most accurate bedside method for the measurement of body composition when compared to a range of reference methods (Fuller *et al.*, 1992). This is likely to be due to differences in the study population, which was considerably younger in the study by Fuller and colleagues. Significant errors between SFA and reference methods for FFM have been found in COPD patients and other elderly groups (Schols *et al.*, 1991b). Errors for this method in the elderly have been ascribed to changes in fat distribution with age, which may not be reflected in the depth of subcutaneous fat (Weits *et al.*, 1988;Seidell *et al.*, 1987). Although skinfold measurements do not directly measure total body water, the prediction of FFM is derived from a comparison with hydrodensitometry (Durnin & Womersley, 1974) and may therefore be subject to similar errors in hydration status as other methods. Some patients in this study were taking low dose diuretic therapy. Although this could affect intracellular hydration, it is unlikely to explain the inter-method differences seen in the measurement of body composition.

The choice of method for the measurement of body composition should be determined by the purpose for which the measurement is intended. In clinical practice this is likely to be the identification of nutritionally depleted patients as there is evidence that simple measurements of body weight are inadequate in COPD patients.

This study demonstrates that the identification of depleted patients is crucially dependent on the method of measurement of body composition. In practice, the choice of measurement method is likely to be determined by availability of resources and equipment. Whilst DEXA may be the most accurate method for measuring FFM, it may impose logistic difficulties for patients with limited mobility (in our institution, for example, it requires travel to a different hospital in the city) and has a cost implication. For these reasons, it is appealing to use a bedside method for the measurement of body composition. These results would support the use of BIA rather than SFA if this option were chosen. Furthermore, this data provides support for the use of FFMI to express body composition data.

In conclusion, this study demonstrates significant inter-method differences in the measurement of body composition in COPD patients indicating that they are not interchangeable in this population. These differences need to be borne in mind when choosing a method for the assessment of nutritional status in clinical practice or research studies.

CHAPTER EIGHT

The Nutritional Enhancement of Exercise Training in COPD

THE purpose of this thesis was to explore the potential for the enhancement of exercise training by nutritional support. In this chapter I will describe the key body of work in this thesis, a randomised, controlled trial of carbohydrate rich nutritional supplementation in patients undergoing pulmonary rehabilitation.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is an important cause of disability and handicap in the developed and developing world (Murray & Lopez, 1997). Patients with COPD are frequently unable to carry out many activities of daily living because of poor exercise performance. This leads to increasing social isolation, depression and dependence. Improving physical performance is therefore an important therapeutic goal.

The efficacy of pulmonary rehabilitation in improving exercise performance and health status in COPD is now established (Lacasse *et al.*, 1996;Griffiths *et al.*,

2000). These benefits are large when compared with other therapies currently available to improve symptoms. Exercise training is a core component of pulmonary rehabilitation and improvements in physical performance are an important clinical outcome (British Thoracic Society Standards of Care Subcommittee on Pulmonary Rehabilitation, 2001). Any intervention that enhances the physical effects of training might therefore be of significant benefit.

The importance of nutrition in improving performance in sport is now recognised. Emphasis is placed on maximising carbohydrate intake prior to training and competition (Devlin & Williams, 1991). Carbohydrate balance may be of particular importance in physically deconditioned individuals including those with COPD because they are more reliant on carbohydrate sources as fuel for muscular contraction (Hurley *et al.*, 1986). Moreover, exercise may impose greater energy costs in patients with COPD because of increases in the work of breathing and impaired oxidative metabolism in the peripheral muscles (Maltais *et al.*, 1996a;Palange *et al.*, 1995b).

Weight loss is a common clinical problem in COPD. Nutritional depletion is both an independent prognostic factor (Landbo *et al.*, 1999) and a correlate of exercise limitation and health status (Mostert *et al.*, 2000;Schols *et al.*, 1991a). Unfortunately, trials of nutritional supplementation in underweight patients have proved disappointing (Ferreira *et al.*, 2000). This might in part be due to the inclusion of patients with progressive weight loss due to an exaggerated systemic inflammatory response who respond particularly poorly to nutritional support (Creutzberg *et al.*, 2000). In addition, there are difficulties achieving adequate calorie intake in elderly subjects who may offset supplementation with a reduction in normal food intake (Lewis *et al.*, 1987). For appetite to be maintained, supplementation may need to be combined with an anabolic

stimulus such as exercise. However, few studies have done this and none have explored the effect of carbohydrate supplementation in enhancing the benefits of training. Importantly, the benefits of carbohydrate provision for training and performance in sport are not restricted to underweight individuals. This may also be true for patients with COPD who are not overtly malnourished.

In this chapter I describe a double blind randomised controlled trial of nutritional supplementation in COPD patients participating in a pulmonary rehabilitation programme. Because the aim of this study was to augment exercise training, patients randomised to the treatment arm were supplied with a carbohydrate rich supplement. The study hypothesis was that this intervention would enhance the physical outcome of pulmonary rehabilitation and that these benefits would not be confined to underweight patients. A secondary aim was to measure the changes in health status, body weight and composition resulting from this therapeutic combination.

Methods

Patients

Patients referred to the pulmonary rehabilitation programme who met clinical and spirometric criteria for COPD (British Thoracic Society, 1997) were assessed for inclusion in the study. Patients were excluded if they were considered unsuitable for the exercise component of the programme when assessed by the pulmonary rehabilitation co-ordinators. Reasons for this were limited mobility due to musculoskeletal disorders, neuro-psychiatric disorders such that compliance with exercise would not be possible or other significant medical conditions such as symptomatic ischaemic heart disease.

Additional exclusion criteria were a diagnosis of diabetes or glucose intolerance and a body mass index (BMI) of greater than 30. These exclusion criteria were applied because it was felt that nutritional supplementation was inappropriate in these situations. All patients gave written informed consent for participation in the study. Ethical approval for the study was granted by the Leicestershire Research Ethics Committee.

Study Design

All patients participated in the standard outpatient pulmonary rehabilitation programme at Glenfield Hospital. After baseline measurements had been taken, patients were randomly allocated to receive a carbohydrate rich nutritional supplement three times a day for the duration of the rehabilitation programme or a non-nutritive placebo. Randomisation was performed consecutively in blocks of four from a pre-prepared list. Treatment was allocated and dispensed independently by a member of staff in the pharmacy department who was not involved with the conduct of the study. Both the investigators and the patients were blinded to the treatment allocation. Unblinding of the study did not occur until the last patient had completed their final assessment.

Outcome Measurements

Study assessments were made before randomisation and immediately after the completion of the rehabilitation programme apart from dietary intake, which was repeated during the course of the rehabilitation and supplementation programmes. A detailed description of these measurements is given in **Chapter 4**.

Spirometry was measured in the seated position to BTS/ARTP standards.

Walking performance was measured using the incremental (ISWT) and endurance (ESWT) shuttle walk tests. Isometric handgrip and quadriceps strength was also measured.

Disease specific health status was measured using the Self Reported Chronic Respiratory Diseases Questionnaire (CRQ-SR).

Body weight was measured in light clothing using digital scales (Seca, UK) to the nearest 100g. Height was measured to the nearest centimetre using a wall-mounted stadiometer. Body Mass index was calculated as weight/height^2 (Kg/m^2).

Body composition was measured using Dual Energy X-ray Absorptiometry (DEXA). Values for Fat mass (FM) and bone free lean mass (LM) were calculated.

Dietary Intake

The effect of the intervention on normal dietary intake was assessed using a three-day food diary. This was performed prior to randomisation and again during the second half of the rehabilitation programme (weeks four to seven).

Pulmonary Rehabilitation Programme

Patients participated in the standard rehabilitation programme at Glenfield Hospital. The programme is multidisciplinary and includes contributions from physiotherapy, occupational therapy, dietetic, nursing and medical staff. After an initial assessment, patients attended twice weekly for a total of fourteen sessions, lasting at least seven weeks. Missed sessions were added to the end of the programme

The endurance training component of the programme comprised weekly sessions of endurance walking exercises. Patients were asked to walk at a speed equivalent to 85% of the predicted peak VO_2 achieved during the ISWT at their initial assessment. Walking times were increased progressively during the course of the programme. Patients were given a daily home walking programme and asked to record walking times and breathlessness on the Borg scale (Borg, 1982). At each training session adherence to the training programme was monitored, walking speeds checked and new targets set for walking times.

Patients also performed a weekly circuit of low impact conditioning exercises designed to increase suppleness and flexibility. The duration of these exercises was increased during the programme but there was no specific progressive weight training.

Each rehabilitation session included education sessions covering a range of topics including disease pathology, treatment, diet and relaxation.

Nutritional Supplementation

Patients allocated to the treatment arm of the study were asked to drink a 125ml supplement (Respifor, Nutricia, Netherlands) three times per day for the duration of their attendance at rehabilitation. The supplement provided 570Kcal daily in the following macronutrient composition: Carbohydrate 60%, Fat 20%, Protein 20%. This supplement was chosen because its macronutrient profile and low volume was thought particularly suitable for the needs of exercising patients. Patients had a choice of vanilla or strawberry flavour. Patients in the placebo group received an identically packaged and flavoured, non-nutritive placebo.

Patients were supplied with cartons each week when attending the rehabilitation sessions. At each visit each patient was interviewed by a state registered dietician (RLB). During these interviews self reported compliance, adverse events and changes to concomitant therapy were recorded.

Because the aim was to maintain the combination of exercise training and supplementation, patients missing more than one week of rehabilitation due to ill health were asked to stop taking the cartons until they restarted rehabilitation. If sessions were missed because of holiday, patients were asked to continue taking the cartons if it was practical to do so and they were planning to continue daily home exercises during their absence.

Data Analysis

Mean increases in ISWT performance from our rehabilitation programme are around 50m. It was judged that an additional benefit of 35m in ISWT performance resulting from adjunctive nutritional supplementation would be clinically significant. To detect this difference with 80% power we required 56 patients to complete the study. Allowing for the dropout rate from rehabilitation at our centre (around 25%) we planned to recruit 85 patients to the study.

It was hypothesised that severely wasted patients might respond differently to the intervention. A post-hoc subgroup analysis of the main study outcomes was made therefore in patients with a BMI above and below 19Kg/m^2. This is an accepted lower limit of normal for BMI.

The purpose of the study was to determine if the trial intervention (nutritional supplementation) could enhance the outcome of rehabilitation. The analysis was

therefore confined to those who completed the course of rehabilitation. An intention to treat analysis was planned for patients in patients who completed rehabilitation but dropped out from supplementation. However, no patients fell into this category.

Outcome variables were assessed for normality of distribution. Within group changes were compared using paired students t-test (Wilcoxon signed rank tests for non-normally distributed data). Between group changes in for normally distributed variables were compared with analysis of covariance (ANCOVA) using the corresponding baseline value as a covariate. Between group changes in non-parametric data were analysed with the Mann Whitney U test. Because health status data is ordinal in character, non-parametric tests were also applied to this data. Advice on the statistical analysis of this study was provided by the Trent Institute for Health Services Research.

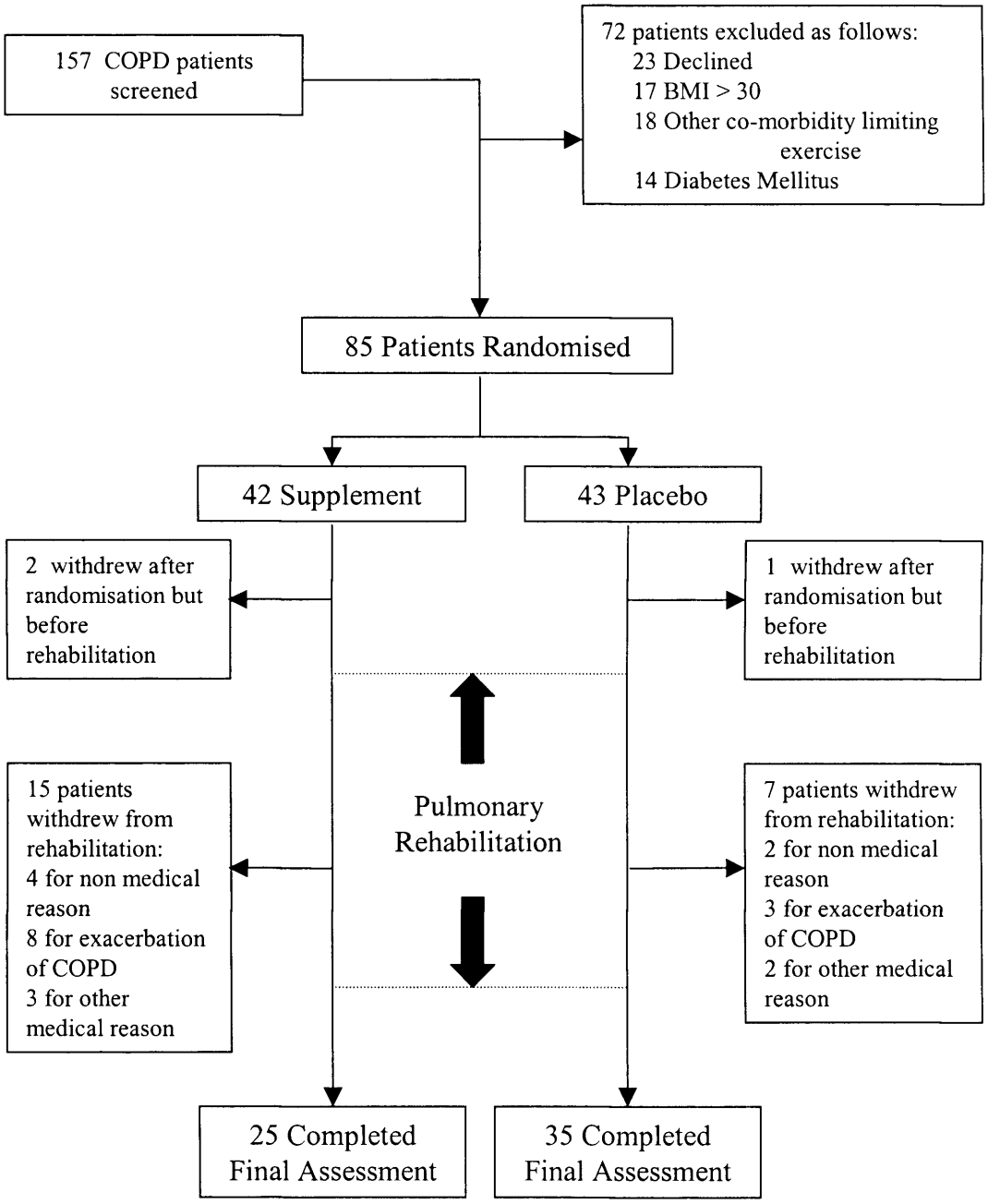


Fig. 8.1. Trial outline.

Based on Completion of Shuttle walk tests.

Results

Eighty-five patients were recruited to the study. Baseline characteristics are shown in **Table 8.1**. There were no significant differences between treatment groups prior to randomisation. The trial outline is shown in **Fig. 8.1**. Sixty patients completed the trial. More patients dropped out from the supplement (17 patients) than the placebo (8 patients) group (Chi squared test: $p = 0.027$). Dropouts were due to inability or reluctance to complete rehabilitation rather than refusal to consume supplement or placebo. Reasons for withdrawal are detailed in the trial outline. There was a trend to a greater dropout rate due to exacerbations of COPD in the supplement group but this was not statistically significant (Supplement group: 8 patients, Placebo group: 3 patients. Chi squared test: $p = 0.058$). One patient withdrew because he was unable to tolerate the cartons of drink but he subsequently also withdrew from rehabilitation. For those completing the trial the median number of rehabilitation sessions missed was 1 in both groups (supplement group: range 0 to 5, placebo group: range 0 to 8). One patient (supplement group) completed the final performance and health status assessments but suffered a ruptured abdominal aortic aneurysm and died before his final DEXA scan.

Self reported compliance with the supplement (% cartons taken/ cartons prescribed) was excellent in both groups (supplement group: 97.6%, placebo group 98.8%). Overall macronutrient intake increased significantly in the supplement group (**Table 8.2a**) but the effect of supplementation was attenuated by a reduction in food intake. As a result, the mean increase in calorie and macronutrient intake in the supplemented group was equivalent to about 70% of the prescribed supplement.

The effect of rehabilitation within both treatment groups in shown in **Table 8.3**. There were significant increases in shuttle walking performance and health status in

both groups. Although there was a statistically significant increase in quadriceps strength in the supplement group this was probably too small to be of clinical relevance. Patients in the supplement group gained weight whilst those in the placebo group lost weight. These changes were principally due to changes in fat mass.

The differences between treatment groups in changes in outcome variables are shown in **Table 8.5**. Because there was an uneven dropout rate between the groups a further analysis of covariance was carried out to determine if this explained any differences between treatment groups. When patients dropping out were compared with those completing the study, they were found to have generally lower baseline values for age, BMI, FEV_1 (% predicted) and quadriceps strength. These variables were therefore used as covariates in this analysis. This did not substantially affect the between group differences or levels of significance in the study as a whole or the post-hoc analysis.

Both incremental and endurance shuttle walking performance increased more in the supplement group but these differences were not statistically significant. There were no significant differences in the change in health status between the groups. Weight changes between the supplement and placebo groups were significantly different (**Table 8.5**). These changes were predominantly in the fat compartment. Changes in ISWT and fat mass correlated with increases in carbohydrate intake (**Fig. 8.2**.).

The results of a post-hoc subgroup analysis of patients with a BMI greater than 19Kg/m^2 are also shown. **Table 8.4** shows the within group changes resulting from the intervention and **Table 8.5** the between group changes in this subgroup. The effect of the intervention on dietary macronutrient intake in this well nourished group is shown in **Table 8.2b**. Fifty-two patients fell into this group (22 supplement, 30 placebo). Data on

patients with a BMI below 19 Kg/m^2 are not presented, as their numbers were too small to allow a meaningful analysis. Baseline characteristics in the well-nourished subgroup were not different between treatment groups. In this population there was a significantly greater increase in ISWT in supplemented patients compared to those receiving the placebo. The differences in change in ESWT, weight and fat mass were also of greater magnitude. In addition, the relationship between changes in carbohydrate intake and changes in fat mass ($r = 0.42$, $p = 0.003$) and ISWT ($r = 0.46$, $p = 0.001$) were stronger (**Fig 8.3**). Normal food intake was better maintained in this subgroup resulting in greater increases in carbohydrate intake in the supplemented group compared with placebo group (**Table 8.2b**).

	Supplement n = 42	Placebo n = 43
Females	16	16
Males	26	27
Age	66 (9.0)	68 (8.0)
FEV_1 (L)	0.91 (0.42)	0.84 (0.31)
FEV_1 (% Predicted)	34.7 (13.6)	34.4 (14.3)
Weight (Kg)	67.4 (14.2)	65.4 (11.9)
BMI (Kg/m^2)	23.9 (3.5)	23.5 (3.8)
Lean Mass (Kg)	43.3 (9.4)	42.4 (9.0)
Fat Mass (Kg)	20.8 (8.4)	19.4 (8.9)
ISWT (m)	210 (113)	220 (123)
ESWT (s) *	163 (132)	219 (159)
HGS (KgF)	27.6 (7.2)	27.1 (8.6)
QS (N)	305 (105)	305 (113)
Total Calorie Intake (Kcal)	1832 (536)	1719 (494)
CHO Intake (g)	210 (60)	208 (71)
Protein Intake (g)	73 (20)	68 (21)
Fat Intake (g)	75 (30)	70 (23)

Table 8.1. Baseline characteristics of patients entering the study.

Mean (SD) values are given. Dietary macronutrient values are mean daily intakes.

* Median (IQR) values.

	Supplement			Placebo		
	Change	95% CI	p value*	Change	95% CI	p value*
Total Calories (Kcal)	394	248, 541	0.000	100	-37, 238	0.147
CHO (g)	63.7	41.7, 85.7	0.000	4.5	-11.3, 20.3	0.566
Fat (g)	6.3	-3.4, 15.9	0.194	6.1	-2.3, 14.5	0.147
Protein (g)	25.2	19.3, 31.1	0.000	6.1	0.3, 11.8	0.039

Table 8.2a. Changes in daily macronutrient intake for the whole study population.

	Supplement			Placebo		
	Change	95% CI	p value*	Change	95% CI	p value*
Total Calories (Kcal)	437	323, 551	0.000	34.8	-103, 172	0.608
CHO (g)	72.6	51.8, 93.4	0.000	-5.3	-20.6, 10.1	0.489
Fat (g)	8.4	1.4, 15.4	0.021	4.2	-4.8, 13.1	0.349
Protein (g)	25.7	19.1, 32.3	0.000	4.0	-1.8, 9.9	0.168

Table 8.2b. Changes in daily macronutrient intake for well nourished patients.

Figures calculated by subtracting baseline values from those measured during the intervention. Well nourished patients are defined as those with a BMI > 19Kg/m^2. Figures include contribution from supplement cartons (additional daily intake: Total energy: 570 Kcal, Carbohydrate: 84g, Fat: 12.5g, Protein: 28g). Negative values denote a decrease from baseline to rehabilitation.

* Paired students t-test.

	Supplement			Placebo		
	Change	95% CI	p value	Change	95% CI	p value
Physical Performance						
ISWT (m)	60.0	39.1, 80.9	0.000	42.6	25.0, 60.1	0.000
ESWT (s) †*	328	223, 554	0.000	191	131, 304	0.000
HGS (KgF)	0.64	-0.03, 1.31	0.059	-0.05	-0.78, 0.68	0.893
QS (N)	17.4	3.3, 31.6	0.018	3.6	-8.1, 15.3	0.536
Health Status †						
CRQ: Dyspnoea	0.7	0.2, 1.1	0.005	1.0	0.6, 1.4	0.000
CRQ: Fatigue	0.6	0.1, 1.1	0.028	0.7	0.4, 1.1	0.000
CRQ: Emotion	0.6	0.3, 1.0	0.002	0.5	0.2, 0.8	0.005
CRQ: Mastery	0.4	0.01, 0.8	0.038	0.9	0.5, 1.3	0.000
Body Composition						
Weight (Kg)	0.63	0.03, 1.23	0.040	-0.58	-1.11, -0.05	0.034
BMI (Kg/m^2)	0.24	0.03, 0.44	0.028	-0.22	-0.42, -0.02	0.029
Lean Mass (Kg)	0.13	-0.43, 0.70	0.631	0.63	0.18, 1.08	0.007
Fat Mass (Kg)	0.67	0.03, 1.30	0.041	-0.76	-1.27, -0.25	0.004

Table 8.3. Within group changes after pulmonary rehabilitation in the whole study population.

Mean differences calculated by subtracting baseline from post rehabilitation measurements. Unless specified, within group comparisons are made using the paired students t-test. Negative values denote a decrease over the rehabilitation period.

* Median values are given.

† Within group changes compared using Wilcoxon Signed Ranks Test.

	Supplement			Placebo		
	Change	95% CI	p value	Change	95% CI	p value
Physical Performance						
ISWT (m)	65.0	47.2, 82.8	0.000	38.0	19.1, 56.9	0.000
ESWT (s) †*	370	269, 550	0.000	176	146, 449	0.000
HGS (KgF)	0.68	-0.07, 1.43	0.075	-0.18	-0.98, 0.63	0.656
QS (N)	17.8	2.3, 33.3	0.026	1.3	-11.6, 14.2	0.839
Health Status †						
CRQ: Dyspnoea	0.72	0.19, 1.25	0.011	1.01	0.56, 1.48	0.000
CRQ: Fatigue	0.64	0.07, 1.20	0.026	0.81	0.45, 1.17	0.000
CRQ: Emotion	0.62	0.22, 1.01	0.005	0.41	0.08, 0.74	0.026
CRQ: Mastery	0.41	0.01, 0.83	0.072	0.95	0.48, 1.42	0.000
Body Composition						
Weight (Kg)	0.78	0.15, 1.41	0.017	-0.62	-1.14, -0.10	0.022
BMI (Kg/m^2)	0.29	0.07, 0.51	0.013	-0.24	-0.43, -0.04	0.020
Lean Mass (Kg)	0.26	-0.36, 0.89	0.391	0.71	0.20, 1.22	0.008
Fat Mass (Kg)	0.80	0.09, 1.51	0.029	-0.87	-1.39, -0.36	0.002

Table 8.4. Within group changes after pulmonary rehabilitation in well nourished patients.

Mean differences calculated by subtracting baseline from post rehabilitation measurements. Well nourished patients are defined as those with a BMI > $19Kg/m^2$. Unless specified, within group comparisons are made using the paired students t-test. Negative values denote a decrease over the rehabilitation period.

* Median values are given.

† Within group changes compared using Wilcoxon Signed Ranks Test.

	All Patients (n = 60)			BMI > 19 Kg/m^2 (n = 52)		
	Difference	95% CI	p value	Difference	95% CI	p value
Physical Performance						
ISWT (m)	18	-8, 45	0.174	27	1, 53	0.041
ESWT (s)*	103	-55, 255	0.182 †	121	-44, 286	0.129 †
HGS (KgF)	0.93	-0.04, 1.89	0.060	0.85	-0.26, 1.97	0.129
QS (N)	16.5	-1.2, 34.2	0.068	16.5	-3.1, 36.1	0.097
Health Status						
CRQ: Dyspnoea	-0.19	-1.0, 0.2	0.307 †	-0.17	-1, 0.4	0.435 †
CRQ: Fatigue	-0.07	-0.75, 0.25	0.502 †	-0.14	-0.75, 0.5	0.583 †
CRQ: Emotion	0.16	-0.29,0.57	0.657 †	0.15	-0.29, 0.71	0.486 †
CRQ: mastery	-0.24	-1.0, 0.0	0.119 †	-0.29	-1.25, 0.0	0.093 †
Body composition						
Weight (Kg)	1.23	0.42, 2.05	0.004	1.50	0.68, 2.33	0.001
BMI (Kg/m^2)	0.46	0.17, 0.75	0.002	0.55	0.26, 0.84	0.000
Lean Mass (Kg)	-0.51	-1.23, 0.20	0.156	-0.42	-1.23, 0.39	0.299
Fat Mass (Kg)	1.46	0.65, 2.27	0.001	1.74	0.88, 2.60	0.000

Table 8.5. Mean differences between supplement and placebo groups after rehabilitation.

Data for the whole group and a subgroup excluding patients who were underweight (BMI < 19 Kg/m^2) are shown. Mean differences are adjusted for baseline using ANCOVA. Negative values indicate higher values in the placebo group.

* Median values are given.

† Between group comparisons made using the Mann Whitney U test.

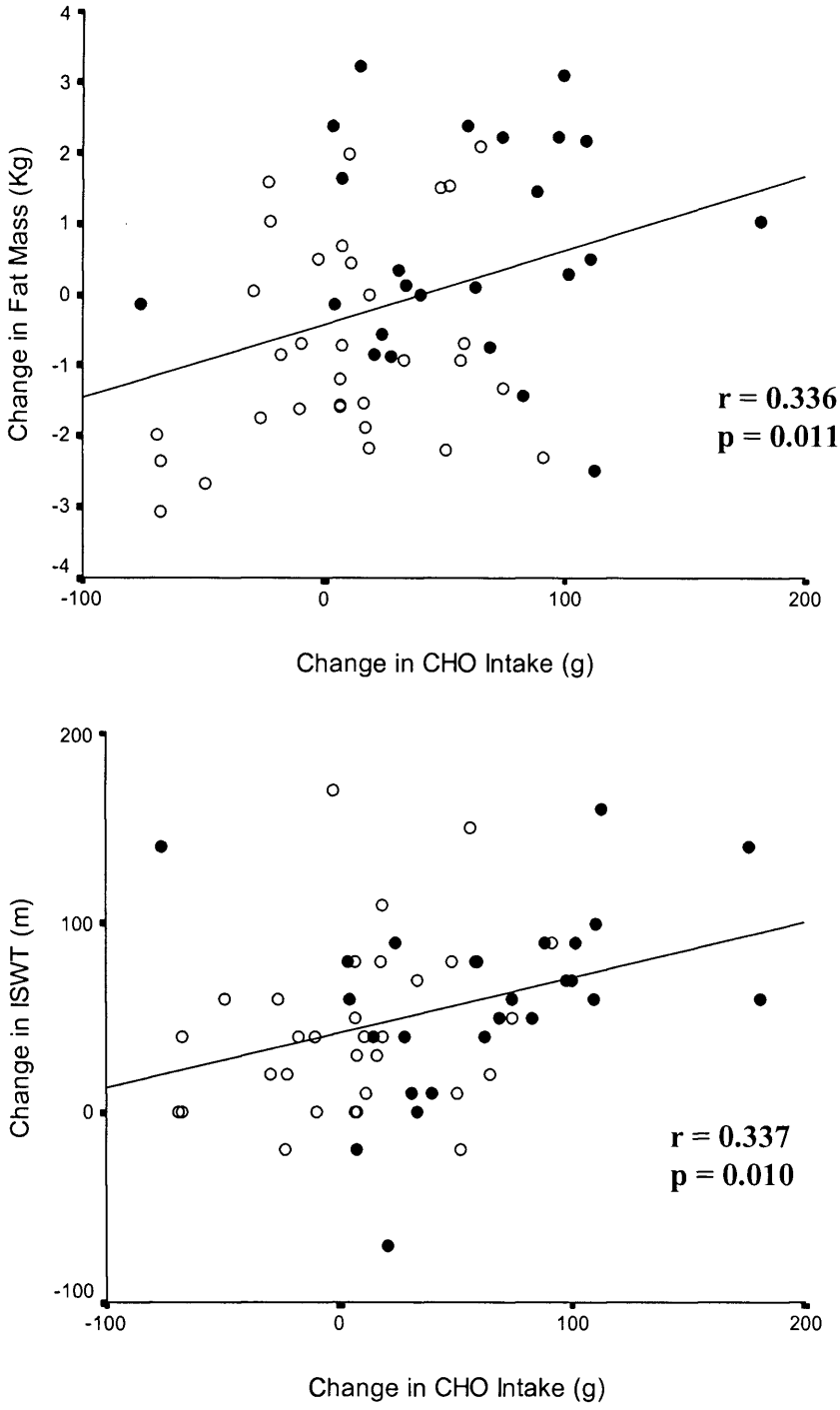


Fig 8.2. Changes in carbohydrate intake,ISWT performance and fat mass in whole study population.

Open circles = Placebo Group; Closed circles = Supplement group.

Changes calculated by subtracting baseline from post rehabilitation measurements.

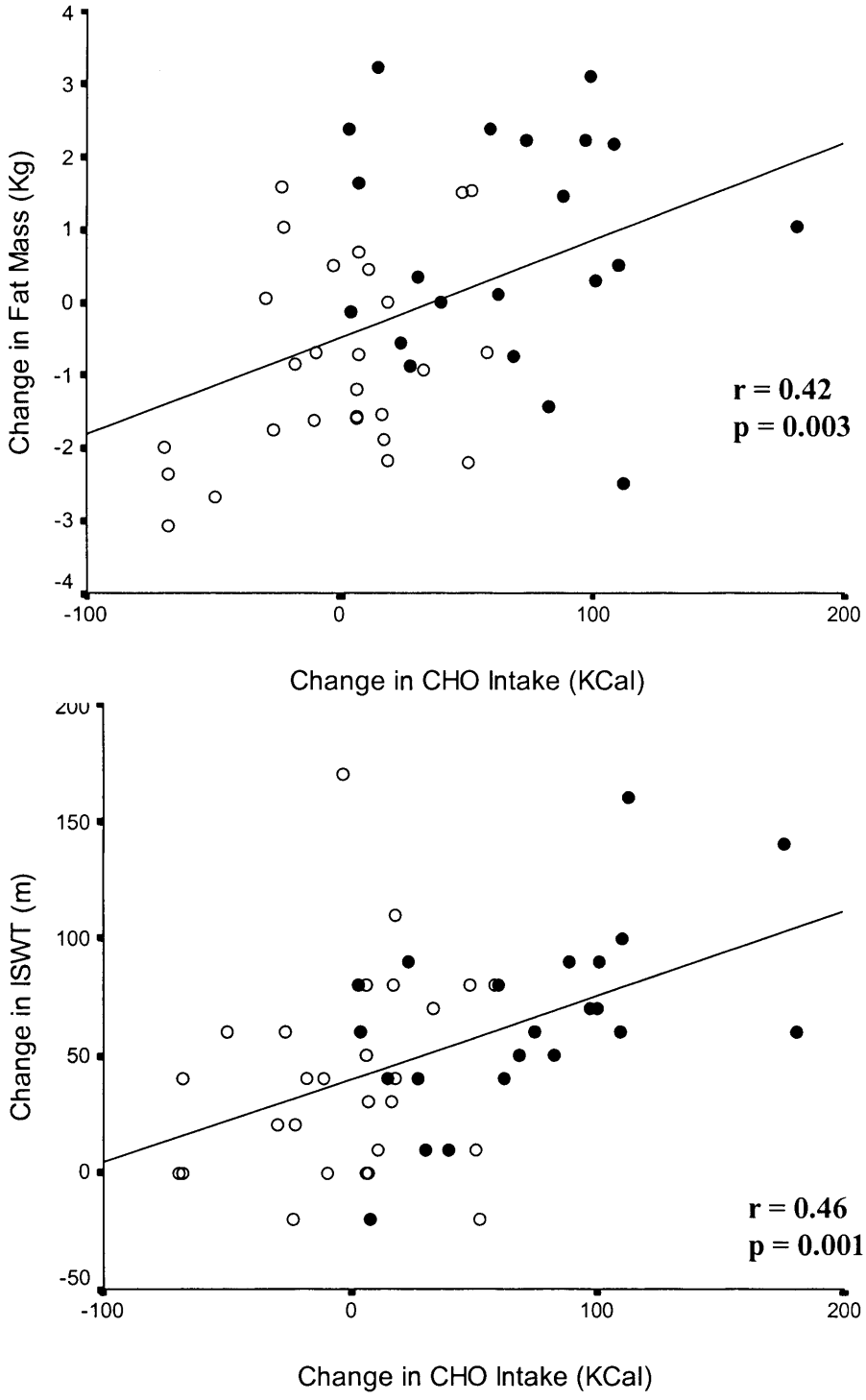


Fig 8.3. Changes in carbohydrate intake, ISWT performance and fat mass in well nourished patients.

Open circles = Placebo Group; Closed circles = Supplement group.

Changes calculated by subtracting baseline from post rehabilitation measurements. Well nourished patients defined as those with a BMI > 19Kg/m^2.

Discussion

In this chapter I have described the results of a randomised, controlled trial of nutritional support in COPD patients undergoing pulmonary rehabilitation. This trial differs from previous investigations because improving physical performance, rather than nutritional status, was the principal aim of the study. The combination of nutritional supplementation and exercise training was successful in increasing weight and energy intake in our patients. In unselected patients this intervention did not significantly enhance the physical benefits of exercise training but in well-nourished patients (with a BMI > 19 Kg/m^2) the increase in ISWT was significantly greater in the supplemented group. Moreover, the magnitude of changes in ESWT and weight were also greater in well-nourished patients. This suggests there may be a role for nutritional support in enhancing physical performance in this group of patients.

The improvements in performance in the supplemented group are likely to be meaningful for many patients. The increase in ISWT in well-nourished patients was 70% greater in the supplemented group than those receiving rehabilitation alone. Furthermore, in this subgroup the increase in ESWT was two minutes greater in the supplement group although this difference was not statistically significant. The ESWT is considerably more responsive to endurance training but shows greater biological variability than ISWT performance. The current study was powered to detect changes in ISWT and may therefore have been too small to detect clinically important changes in ESWT. Major changes in muscle strength were not expected because strength training was not part of our rehabilitation programme. However, the small improvement in strength seen in the supplemented group suggests that nutritional support could also be beneficial where this mode of training is used.

The results indicate marked differences in the pattern of weight changes in the supplement and placebo groups. Rehabilitation resulted in weight loss in the placebo group, whereas those in the supplement group gained weight. Notably, these changes in weight were due to alterations in fat rather than lean mass. Lean mass appeared to increase significantly in the placebo group whilst it was unchanged in the supplement group. The explanation for this is unclear. However, the importance of an isolated within group change is questionable and, in contrast to the changes in weight and fat mass, there were no significant between group changes in lean mass.

Whilst loss of lean or fat free mass may be considered to be more relevant to function in COPD, lean mass depletion may not simply be the result of energy imbalance but reflects disordered protein metabolism due to other factors such as muscle disuse, hypoxia or drug therapy. Many of these factors will not have been addressed by rehabilitation and supplementation. Whilst muscle mass can be readily increased by strength training, this would not be expected from endurance training. Although normal dietary intake fell in the supplement group, supplementation successfully increased calorie and macronutrient intake by around 70% of the prescribed supplement. This is in line with previous outpatient supplementation programmes in COPD (Stratton & Elia, 1999). Fat mass changes were related to changes in carbohydrate intake across both groups suggesting that maintenance of normal dietary intake during supplementation was an important factor in its efficacy.

The study findings suggest that exercise training results in negative energy balance in many patients, which was overcome by supplementation. Physical activity imposes a high energy cost for patients with COPD (Baarends *et al.*, 1997b). The increase in physical activity for many patients who attend rehabilitation is substantial

and dietary calorie intake may be insufficient to meet this new metabolic demand. I would speculate that this might also impose a limit on the amount of exercise patients can do. Nutritional supplementation might therefore confer a performance advantage by allowing greater adherence to an exercise training programme. This is supported by the finding that increases in walking performance were related to increases in carbohydrate ingestion.

An improvement in performance from carbohydrate supplementation is biologically plausible. Carbohydrate is an important source of energy for endurance exercise but intramuscular stores are limited (Maughan *et al.*, 1997). Carbohydrate feeding can prolong endurance in healthy subjects (Coyle *et al.*, 1986) and there is evidence that a high carbohydrate diet can enhance the effects of endurance training (Helge *et al.*, 1996). Muscle glycogen stores may be lower in COPD patients (Jakobsson & Jorfeldt, 1995) and, like other deconditioned individuals, they are likely to be highly reliant on carbohydrate as a source of fuel for muscular contraction (Hurley *et al.*, 1986). If physical activity increases, carbohydrate availability may become an important factor in sustaining exercise.

This is the first study to investigate the performance benefits of nutritional supplementation when combined with exercise training in COPD. A recent meta-analysis of clinical trials of nutritional supplementation failed to identify significant improvements in weight or exercise capacity (Ferreira *et al.*, 2000). There are a variety of possible reasons for this. In many trials supplementation was predominantly fat based because of concerns about the adverse effect of carbohydrate oxidation on ventilatory load. Some studies were unable to successfully supplement patients because of a fall in normal food intake (Lewis *et al.*, 1987). To date, only one trial combining nutritional

support and rehabilitation has been reported (Schols *et al.*, 1995). In this study similar changes in weight and fat mass were seen but there was no performance advantage above that of rehabilitation alone. One major difference between the study by Schols *et al* and the current study was the composition of the supplement, which was fat rather than carbohydrate rich. It is possible that fat supplementation provides sufficient energy to allow weight gain but does not meet the metabolic demands of exercising muscles. Direct comparison between studies is also complicated by the use of different measures of physical performance. These involve varying degrees of strength and endurance and will therefore respond differently to therapeutic or training interventions.

The finding of greater benefits from supplementation in well-nourished patients contrasts with the traditional aims of nutritional support in COPD. Well-nourished patients also showed larger increases in weight and fat mass and better maintenance of dietary calorie intake suggesting that in these patients the suppression of appetite by supplementation was not as great. Some underweight COPD patients show signs of an exaggerated systemic inflammatory response leading to an increase in resting energy expenditure, appetite suppression and progressive cachexia (Schols *et al.*, 1996). These patients appear to respond poorly to nutritional support (Creutzberg *et al.*, 2000) and show appetite suppression in the face of increased energy requirements (Schols *et al.*, 1999). Some of our patients with a BMI under 19Kg/m^2 may have fallen into this "non-responder" category and therefore attenuated the effect of the supplementation in the study population as a whole. This might be because the energy imbalance in these patients is too large to be bridged by calorie supplementation in the amounts that can be readily tolerated. The inclusion of this group of "non-responders" may be one reason for the disappointing results of previous trials of nutritional support.

The results do suggest, however, that nutritional support combined with exercise may be beneficial for patients of normal nutritional status.

The dropout rate for the study is in line with the experience of pulmonary rehabilitation at Glenfield Hospital but the rate was greater in the supplemented group. There was also a higher dropout rate due to disease exacerbations although this difference was just outside statistical significance. This may have occurred by chance but it is possible that carbohydrate had an adverse effect on patients suffering an exacerbation because of an increase in carbon dioxide production resulting from its oxidation. This effect has been demonstrated experimentally but its clinical relevance is unclear (Efthimiou *et al.*, 1992). Moreover, the small volume supplement used was designed to reduce any impact this might have and be easy for patients to tolerate. Studies using this supplement have indicated there are no short-term adverse effects on ventilation or respiratory quotient in COPD patients (Vermeeren *et al.*, 2001).

Weight gain for some patients in our study might be considered disadvantageous. However, the small increases in weight seen would be outweighed by a significant increase in exercise capacity. Improvements in health status were not greater in the supplemented group despite benefits for the physical outcome of rehabilitation. However, the relationship between changes in performance and health status is not straightforward and the time course of changes in performance and adaptations in lifestyle due to rehabilitation may be different (Green *et al.*, 2001).

Pulmonary Rehabilitation is established as effective therapy for enhancing performance in COPD. There is now accumulating evidence that peripheral muscle dysfunction is an important determinant of exercise limitation in COPD (American Thoracic Society, 1999) and that the physical benefits of rehabilitation are at least in

part due to metabolic adaptations to training in the peripheral muscles (Maltais *et al.*, 1996b). Any intervention that maximises these adaptations has the potential to be of great benefit for patients with COPD. This study indicates that this approach is feasible and that combining exercise training with other therapeutic modalities may be a promising avenue for future study.

In conclusion, this trial indicates that, when universally prescribed, carbohydrate rich nutritional support does not enhance the rehabilitation of patients with COPD. Our data suggests that exercise training results in negative energy balance that can be overcome by supplementation and that in selected patients this improves the outcome of training. The finding of benefit in well-nourished patients highlights a potential role for nutritional supplementation beyond the treatment of weight loss in COPD.

CHAPTER NINE

The Skeletal Muscle Metabolic Response to Exercise in COPD

IN the previous chapter of this thesis I described a clinical trial investigating the potential of nutritional supplementation to enhance the physical benefits of exercise training in COPD. This chapter describes a study exploring the mechanisms underlying improvements in physical performance seen following training and nutritional support.

Introduction

The clinical benefits of exercise training to patients with COPD are now well established but the mechanisms underlying these benefits are less well understood. This is important because an understanding of metabolic adaptations to training in this population might allow refinement and individualisation of training and performance enhancement therapies so maximising functional benefits.

Early studies suggesting that training adaptations did not occur in COPD probably trained patients at insufficient intensity (Belman & Kendregan, 1981). More recently Casaburi *et al* showed that higher intensity training results in lower lactate

release during exercise (Casaburi *et al.*, 1991) and Maltais and colleagues reported a rise in skeletal muscle oxidative enzymes after exercise training in a small group of COPD patients (Maltais *et al.*, 1996b). In a further study the same group showed an increase in the cross-sectional area of Type I and Type IIa fibres following training (Whittom *et al.*, 1998). There is also evidence from MRS studies that PCr utilisation and resynthesis is improved by training in COPD (Sala *et al.*, 1999).

These findings are all consistent with an improvement in mitochondrial oxidative metabolism following training in COPD. However, studies directly examining the detailed metabolic response to exercise in the skeletal muscles in this population have not been performed.

Chapter 6 described a pilot study demonstrating the feasibility and tolerability of the sampling of the quadriceps muscle during a controlled exercise challenge in COPD patients. In this small sample there was a trend towards an improvement in ATP turnover following endurance training. The aim of the study described in the current chapter was to examine the skeletal muscle metabolic responses to exercise in COPD and explore the effects of exercise training and nutritional support on these responses. To this end, a subgroup of patients participating in the nutritional intervention study described in **Chapter 8** underwent quadriceps muscle sampling during exercise before and after rehabilitation. Patients were randomised to receive a carbohydrate rich nutritional supplement or placebo for the duration of the training programme as described in **Chapter 8**.

In addition, the variability of the metabolic response to exercise over a seven week control period before the start of rehabilitation was studied in a further subgroup of stable COPD patients.

Methods

Patients and Study Design

All patients participating in the muscle biopsy study were also participants in the nutrition and training intervention study described in **Chapter 8**. Patients were recruited to the muscle biopsy study if they were able to perform and cycle ergometry test and consented to the procedure. Patients were not recruited if significant desaturation (SaO_2 < 85%) occurred during incremental walk testing at baseline. Additional exclusion criteria for the muscle biopsy study were concomitant anticoagulation therapy and maintenance oral steroid therapy. Patients were randomised to receive a carbohydrate rich nutritional supplement or placebo for the duration of a seven week endurance walking programme as described in **Chapter 8**.

Within the muscle biopsy group, a further subgroup was recruited to assess the biological variability of the measurements over a seven week period (the standard length of rehabilitation at Glenfield Hospital). These patients underwent study assessments before and after a seven week control period during which no rehabilitation or change to maintenance therapy occurred. These patients then entered rehabilitation and were randomised in the same way as other patients participating in the nutritional intervention study. Measurements taken after the seven week control period were used as baseline measurements for the intervention study.

Study Assessments

All patients had functional and nutritional outcome measurements before and after the intervention as described in **Chapter 8**. The following additional measurements were performed.

Incremental Exercise Testing

Patients performed a symptom limited maximal cycle ergometry test as described in **Chapter 4**. Ventilation and gas exchange measurements were made using a breath by breath computerized system.

Muscle Biopsies

At least 48 hours after performing the incremental test, patients undertook a constant load cycle test performed at 80% peak workrate achieved during the incremental test. The time for this test was set at five minutes. Two patients at the start of the study exercised for six minutes because it was felt after the pilot work (**Chapter 6**) that patients might be able to sustain exercise for longer, thus increasing the magnitude of the metabolic changes. However, it became clear that this would be difficult for many patients and five minutes was subsequently used. Muscle biopsies were obtained from the Vastus Lateralis muscle using the Bergstrom technique at rest and immediately after the completion of the constant load test. Post exercising samples were obtained (with the subject seated on the bike) no longer than 10 seconds after the end of the exercise test. Samples were frozen immediately and stored in liquid nitrogen. Patients who could not complete five minutes of exercise at this intensity were asked to continue for as long as possible at which point the exercising muscle sample was taken

and the time recorded. Post training samples were taken at an identical workload and time. Details of the constant load exercise test and the muscle biopsy technique are given in **Chapter 4**.

Muscle Biopsy Analysis

Once all samples had been collected they were transported to the Dept of Biomedical Sciences, The University of Nottingham and analysed under the supervision of Prof. Paul Greenhaff.

Samples were freeze dried, powdered and muscle tissue separated from blood, connective tissue and fat. They were then analysed for [Total Creatine], [Creatine], [PCr], [Lactate], [Glycogen], [ATP] and purine nucleotide derivatives (ADP, AMP, IMP, Inosine, Xanthine and Uric Acid). Metabolites measured in biopsy pairs taken during a single exercise test (apart from lactate and glycogen) were corrected for total creatine. This allows for differences in biopsy contamination by non muscle tissue. A detailed description of analytical techniques is given in **Appendix I**.

Energy for ATP resynthesis can be generated from oxidative or non-oxidative sources. Substrate level phosphorylation represents total ATP energy derived from non-oxidative sources. As oxidative metabolism is the only other source of ATP energy, its contribution to energy metabolism can be measured by calculating SLP. The glycolytic metabolism of glycogen to lactate yields 3 ATP molecules from each glycosyl unit. This yields two molecules of lactate (1.5 molecules of ATP per lactate molecule produced). Due to the rise in IMP concentration when muscle ATP falls the net yield of 'ATP equivalents' is the equivalent of 2 ATP molecules for each molecule change in its concentration (Spriet *et al.*, 1987).

Substrate level phosphorylation (SLP) is therefore calculated as follows:

$$SLP = \Delta[PCr] + \{\Delta[La] \times 1.5\} + \{\Delta[ATP] \times 2\}$$

Where Δ refers to the exercise induced change in each metabolite.

The potential for energy delivery for muscular work depends is determined by the phosphorylation of the total adenine nucleotide (TAN) pool. This can be expressed by calculating the energy charge potential. The energy charge gives a measure of the extent to which the TAN pool is phosphorylated and therefore available to release free energy (Atkinson, 1968). The Total Adenine Nucleotide (TAN) Pool was calculated as [ATP] + [ADP] + [AMP]. The energy charge potential was calculated by the following formula:

$$\text{Energy Charge} = \frac{[ATP] + (0.5 \times [ADP])}{[ATP] + [ADP] + [AMP]}$$

Data Analysis

A clinically important change in metabolic response to exercise is not known for COPD patients and so a power calculation could not be performed. For logistic reasons only a limited number of patients could be included in the muscle biopsy part of the study and it was anticipated that approximately a third of patients participating in the nutritional intervention study would be recruited for this study. It was expected that a combination of patient dropouts and non-viable muscle samples would result in significant missing data. Analysis was therefore based on patients who completed the study assessments from whom viable muscle samples were obtained. Data is missing for some measurements because some samples were too small to run all the laboratory

assays. Numbers of viable samples for each measurement are given in the relevant tables. Analysis was performed on a total of 20 pre and post training resting samples (9 supplement, 11 placebo) and 15 pre and post training rest and exercise pairs (9 supplement, 6 placebo).

Within and between group changes were analysed using paired and unpaired student t-tests. Relationships between variables were assessed using Pearson Correlation Coefficients. For the purposes of relating changes in muscle metabolites during exercise, results from biopsy pairs taken at baseline, during the control period and after training were pooled to increase the power of the analysis. Because of the large number of variables measured, p values of less than 0.01 were considered statistically significant for Pearson correlation.

The reproducibility of these measurements over the seven week control period was expressed by the within subject standard deviation. This is calculated as the standard deviation of the measurement differences divided by the square root of the number of measurements (in this case 2) (Chinn, 1991).

Results

Training and Nutritional Supplementation Study

Twenty-eight patients participating in the nutritional intervention study were recruited to the muscle biopsy part of the study. Baseline demographic, nutritional and field exercise performance data for this group are shown in **Table 9.1**. There were no significant differences between treatment groups at baseline.

Data from incremental tests and constant load tests (during which biopsies were taken) are shown in **Table 9.2**.

Five patients dropped out of the muscle biopsy study (one from placebo group, 4 from supplement group). Baseline samples from these patients were not analysed to reduce laboratory workload. One patient was too disabled to perform a cycle test but provided a resting biopsy before and after training. Other missing data was due to insufficient muscle tissue for analysis to be performed.

	Supplement	Placebo
	n = 14	n = 14
Females	11	8
Males	3	6
Age	64 (9.8)	64 (8.9)
FEV_1 (L)	1.14 (0.43)	1.07 (0.39)
FEV_1 (% Predicted)	38.3 (14.1)	41.3 (17.0)
BMI (Kg/m^2)	24.5(3.4)	24.3 (4.5)
Lean Mass (Kg)	48.4 (7.7)	44.3 (9.4)
Fat Mass (Kg)	22.2 (8.1)	20.9 (10.9)
ISWT (m)	243 (107)	262 (123)
ESWT (s) *	172 (89)	197 (190)
HGS (KgF)	30.9 (6.2)	27.9 (6.7)
QS (N)	355 (112)	355 (102)

Table 9.1. Baseline patient characteristics.

Mean (SD) values are given.

* Median (IQR) values

	Supplement	Placebo
Incremental Cycle Test		
Peak WR (W)	72.5 (19.1)	70.0 (16.5)
VO_{2peak} (mls/kg/min)	15.9 (4.39)	17.2 (4.61)
VCO_{2peak} (mls/kg/min)	15.8 (4.26)	17.8 (4.85)
Peak V_E (L)	38.7 (12.7)	41.2 (8.5)
Peak RQ	0.99 (0.07)	1.04 (0.10)
Peak SaO_2 (%)	94.8 (2.8)	94.3 (2.5)
Peak HR (BPM)	120 (19)	129 (17)
Constant Load Test		
WR (W)	56.9 (15.1)	55.3 (14.5)
End Exercise HR (BPM)	123 (20)	131 (17)
End Exercise SaO_2 (%)	91.6 (4.3)	92.2 (4.7)
End Exercise BS	5.1 (1.8)	4.5 (2.0)
End Exercise PE	14.4 (2.2)	14.4 (2.5)

Table 9.2. Exercise test variables at baseline.

Data from maximal incremental exercise tests and constant load tests (during which biopsies were taken) are shown (mean (SD) values). [Abbreviations: WR = Workrate; VO_{2peak} = Peak Oxygen Uptake; VCO_{2peak} = Peak Carbon Dioxide Release; Peak V_E = Peak Ventilation; RQ = Respiratory Quotient; BS = Borg Score; PE = Perceived Exertion Score].

The Metabolic Response to Exercise at Baseline

Resting muscle biopsies from 21 patients and rest/exercise biopsy pairs from 19 patients were available for analysis at baseline. Mean (SD) corrected total creatine concentration at baseline was 122 (18.3) mmol/kg dry weight. Rest and exercise metabolite concentrations for the whole study cohort are shown in **Table 9.3**. There were significant exercise induced changes in [PCr], [Lactate], [Glycogen], [ATP], [IMP] and [Uric acid] (**Fig. 9.1**). There were also statistically significant reductions in Energy Charge and TAN Pool at baseline.

Across all biopsy pairs there were statistically significant ($p < 0.01$) correlations between exercise induced changes in [PCr] and [Lactate], [ATP] and Energy Charge (**Fig. 9.2**). The accumulation in [IMP] during exercise correlated with changes in Energy Charge, TAN Pool and [ATP] (**Fig. 9.3**). IMP accumulation also correlated with changes in [PCr] ($r = -0.673$, $p = 0.000$) and [Lactate] ($r = 0.792$, $p = 0.000$). Exercise induced changes in [PCr] and [ATP] correlated with their respective resting values (**Fig. 9.4**).

There were no significant correlations between resting or exercise induced changes in muscle metabolites and field or laboratory exercise performance or with pulmonary mechanics or gas exchange during exercise.

	n	Rest	Exercise	Mean Change (95% CI)	p
PCr	19	67.3 (16.8)	28.9 (16.2)	-38.4 (-48.9, -28.8)	0.000
Lactate	19	2.5 (1.6)	27.6 (25.6)	25.1 (12.5, 37.7)	0.000
Glycogen	9	252 (55)	218 (69)	-33 (-64, -3)	0.001
ATP	18	19.5 (4.5)	15.2 (3.5)	-4.3 (-7.0, -1.6)	0.004
ADP	18	2.67 (0.87)	3.20 (0.71)	0.53 (-0.03, 1.09)	0.063
AMP	18	0.03 (0.03)	0.06 (0.09)	0.03 (-0.02, 0.08)	0.212
TAN	18	22.2 (5.15)	18.5 (3.5)	-3.7 (-6.8, -0.7)	0.019
EC	18	0.94 (0.01)	0.90 (0.03)	-0.03 (-0.05, -0.01)	0.001
IMP	18	0.14 (0.06)	2.17 (2.77)	2.03 (0.64, 3.42)	0.007
Inosine	18	0.73 (1.05)	0.47 (0.70)	-0.26 (-0.75, 0.24)	0.285
Xanthine	18	0.07 (0.03)	0.06 (0.03)	-0.01 (-0.03, 0.01)	0.375
Uric Acid	13	0.25 (0.13)	0.40 (0.22)	0.16 (0.03, 0.28)	0.018

Table 9.3. Rest and exercise muscle metabolites at baseline.

All units are mmol/kg dry weight. Paired t-test used to calculate p values. Negative values denote a decrease. Mean (SD) values from viable pairs of rest and exercise biopsies are shown. Lower sample numbers for some metabolites are due to insufficient muscle available for analysis. TAN = Total Adenine Nucleotide Pool, EC = Energy Charge.

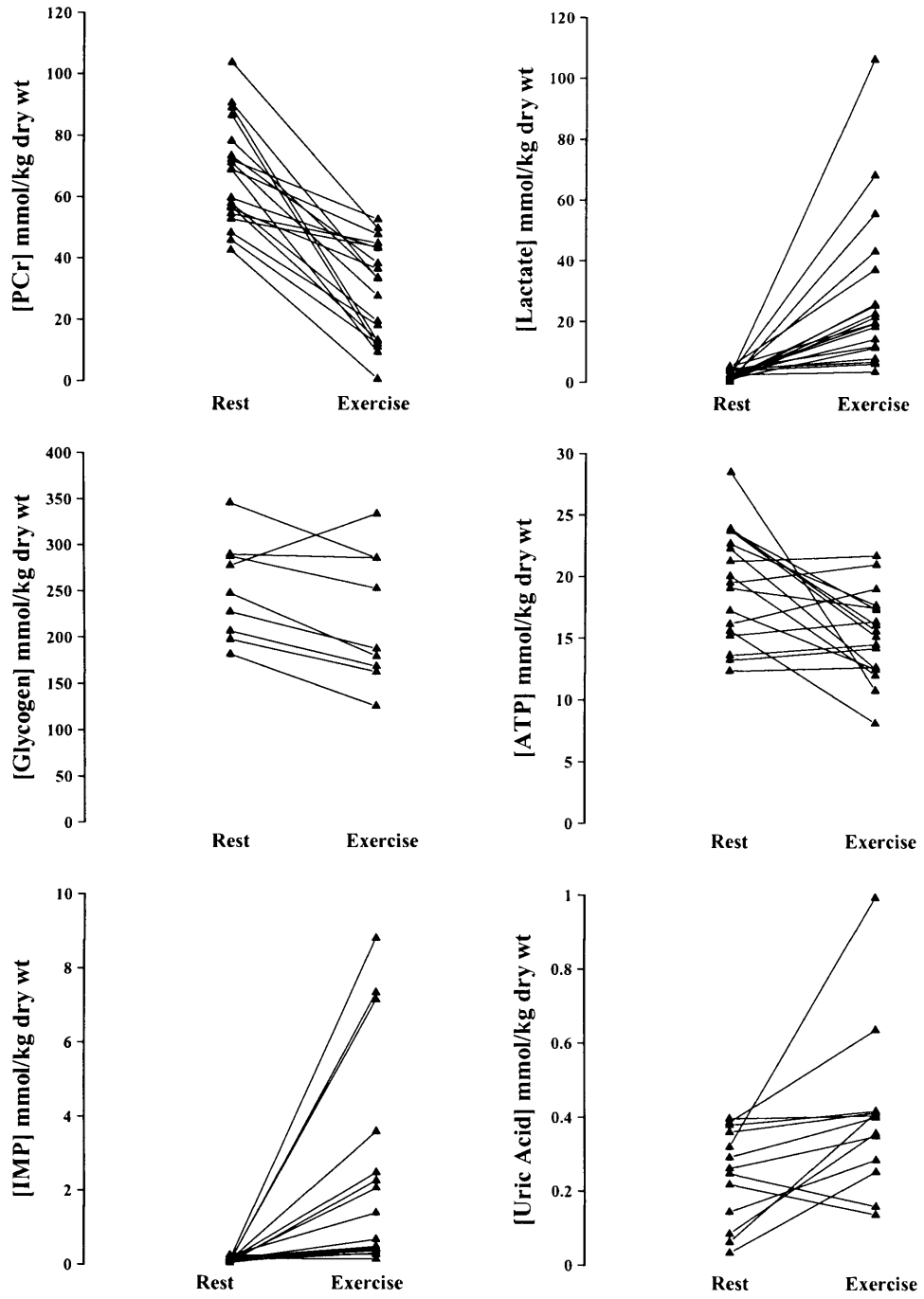


Fig. 9.1. Muscle metabolites at rest and exercise at baseline.

See **Table 9.3** for mean differences and p values.

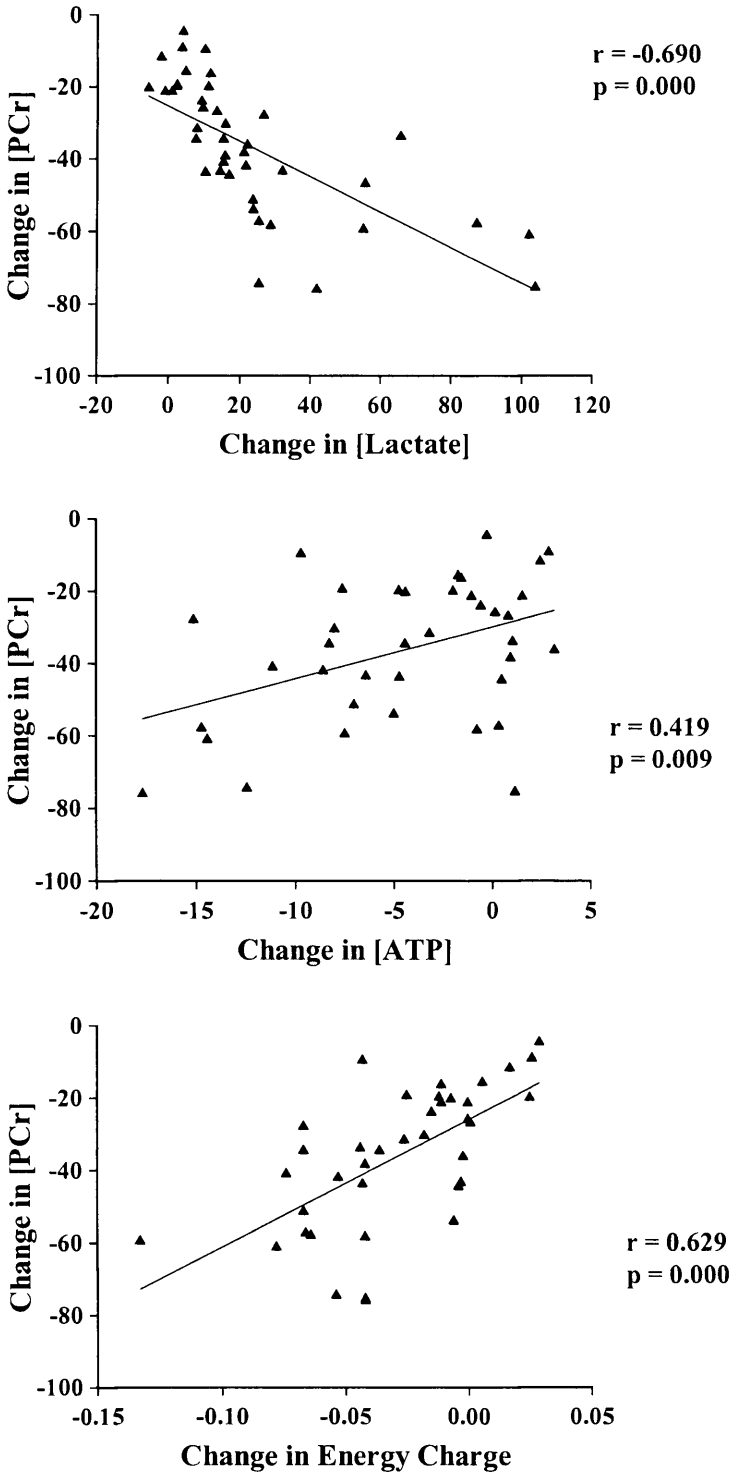


Fig. 9.2. Relationship between exercise induced changes in muscle [PCr] with [ATP], [Lactate] and cellular energy charge.

Negative values denote a decrease during exercise. Pearson Correlation used to calculate r and p values. All units (apart from energy charge) in mmol/kg dry weight.

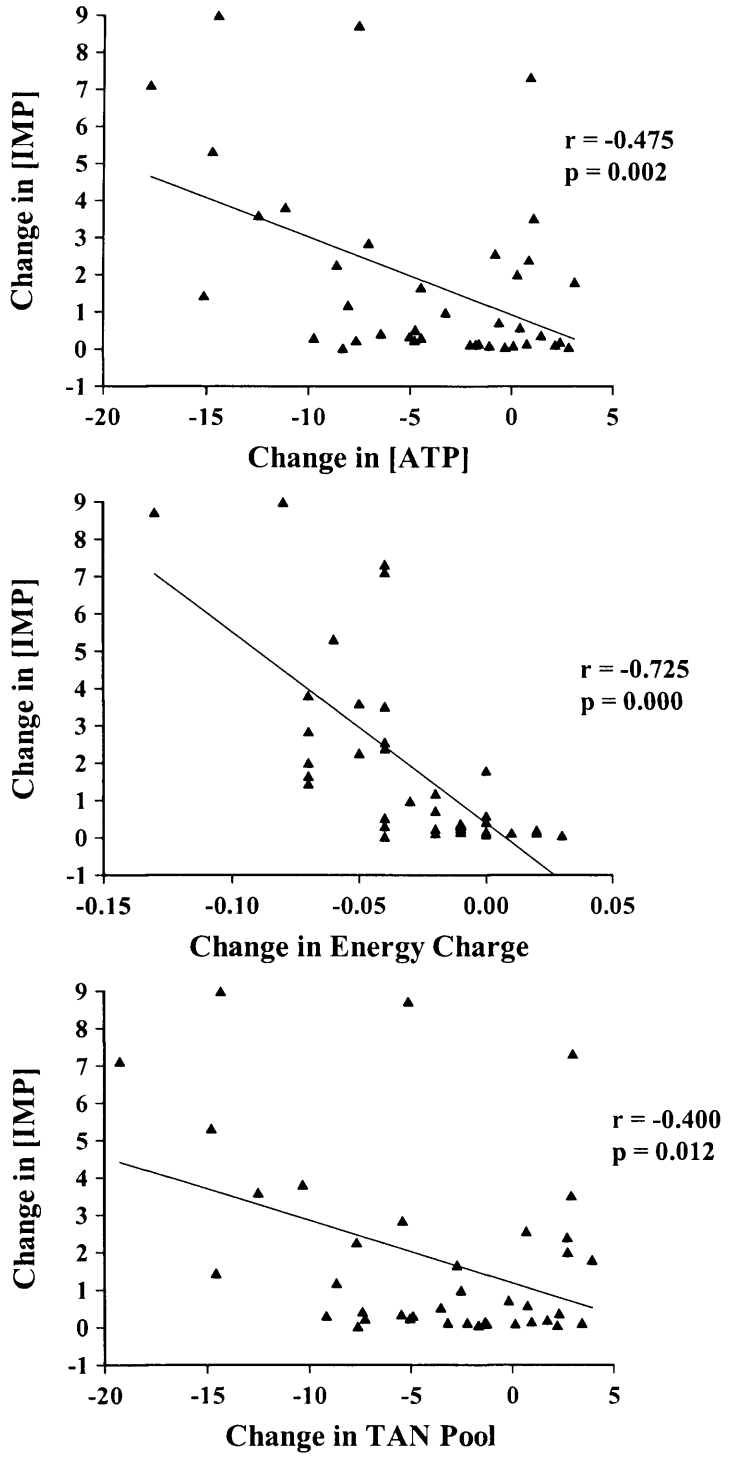


Fig. 9.3. Relationship between changes in IMP and ATP, TAN Pool and Energy Charge during exercise.

TAN = Total Adeine Nucleotide Pool. All units mmol/kg dry weight (apart from energy charge). Negative values denote a decrease during exercise. Pearson correlation used to calculate r and p values.

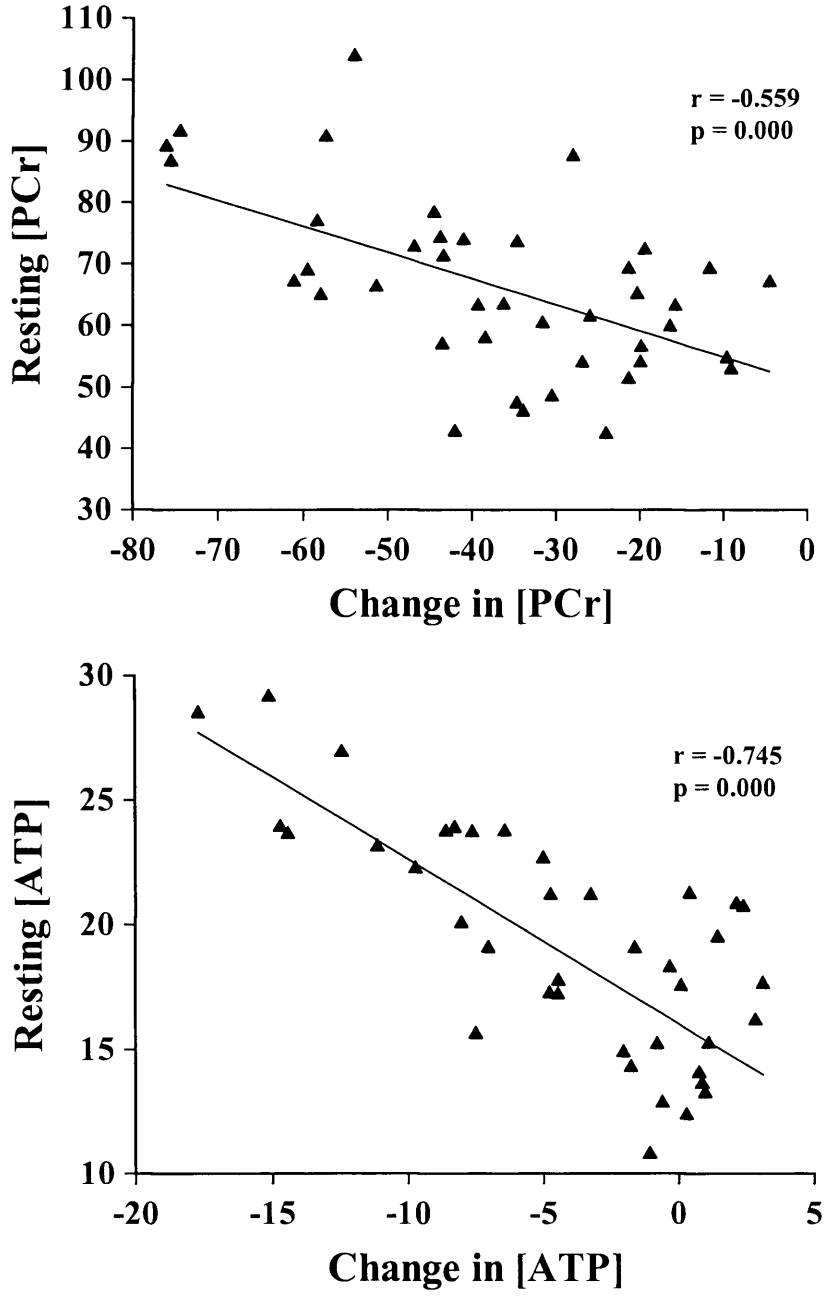


Fig. 9.4. Relationship between changes in [PCr] and [ATP] and their respective resting values.

Pearson correlation used to calculate r and p values. All units mmol/kg dry weight. Negative values denote a decrease during exercise.

Effects of training and Nutritional supplementation

There were significant changes in ISWT and ESWT after training across both treatment groups (All Patients: Mean (95% CI) change in ISWT: 61 (34, 88)m, p = 0.000; Median (95%CI) change in ESWT: 417 (289, 583)s, p = 0.000 (Wilcoxon Rank Test)). There were no statistically significant differences in field test performance between the supplement and placebo groups.

Changes in incremental and constant load cycle test variables after training for the whole group are shown in **Table 9.4**. Absolute workload during the constant load test was identical to the pre training value. There were no statistically significant changes in peak workrate, peak oxygen uptake or other gas exchange variables following training. There were small decreases in Borg breathlessness score and Perceived exertion score during the constant load test after training. The changes in saturation and heart rate after training were small and of questionable clinical relevance. There were no differences between treatment groups in incremental test or constant load test variables after training.

Changes in resting muscle metabolites after training are shown in **Table 9.5**. Exercise induced changes in muscle metabolites before and after training are shown in **Table 9.6** and **Fig. 9.5**. Training did not result in statistically significant changes in resting muscle metabolites or the metabolic response to exercise. Mean substrate level phosphorylation decreased after training but this was not statistically significant.

There were no significant differences in resting or exercise induced muscle metabolites between treatment groups. Glycogen concentrations after training did not differ between treatment groups. Insufficient muscle was present in post-training biopsy

pairs to allow the effect of training on exercise induced changes in glycogen to be measured.

Changes in resting and exercise induced muscle metabolites after training were not related to changes in performance or pulmonary mechanics and gas exchange variables during exercise.

Reproducibility Study

Nine patients participated in this part of the study (7 male, Mean (SD) age: 64.6 (7.3) years; FEV_1: 39.5 (16.9) % predicted). None dropped out of the control period but biopsies from one patient were of insufficient quality for metabolite analysis. Resting and exercise induced changes in muscle metabolites over the control period are shown in **Table 9.7**.

	Mean Change	95% CI	p value
Incremental Cycle Test			
Peak WR (W)	3.2	0.5, 6.8	0.083
VO_{2peak} (mls/kg/min)	-0.12	-1.14, 0.90	0.802
VCO_{2peak} (mls/kg/min)	-0.11	-1.25, 1.04	0.846
Peak V_E (L)	-1.05	-3.67, 1.57	0.409
Peak RQ	-0.003	-0.03, 0.04	0.782
Peak SaO_2 (%)	-1.0	-1.96, 0.04	0.043
Peak HR (BPM)	-4.2	-8.6, 0.1	0.056
Constant Load Test			
End Exercise HR (BPM)	-7.8	-13.5, -2.0	0.011
End Exercise SaO_2 (%)	1.1	-0.4, 2.6	0.149
End Exercise BS*	-1.0	-2.0, 0.0	0.055
End Exercise PE*	-0.9	-1.8, -0.1	0.041

Table 9.4. Exercise test variables after training.

Mean changes were calculated by subtracting baseline from post-training measurements. Figures refer to the whole study cohort. Negative values denote a decrease.

* p values calculated using Mann Whitney U test.

	n	Pre Training	Post Training	Mean Change (95% CI)	p
PCr	20	65.5	57.1	-8.4 (-20.9, 4.0)	0.173
TCr	20	119.8	113.0	-6.8 (-19.1, 5.6)	0.267
Lactate	20	2.4	2.9	0.4 (-0.6, 1.4)	0.386
Glycogen	14	250	266	16 (-13, 45)	0.254
ATP	19	18.7	17.5	-1.2 (-4.2, 1.8)	0.414
ADP	19	2.52	2.64	0.12 (-0.52, 0.75)	0.707
AMP	19	0.04	0.04	0.01 (-0.02, 0.03)	0.613
IMP	19	0.14	0.14	-0.01 (-0.04, 0.26)	0.613
Inosine	19	0.70	0.90	0.20 (-0.48, 0.88)	0.542
Xanthine	19	0.07	0.07	0.00 (-0.02, 0.02)	0.995
Uric Acid	13	0.24	0.23	-0.00 (-0.08, 0.08)	0.937

Table 9.5. Resting muscle metabolites before and after training.

Negative values denote a decrease after training. All units mmol/kg dry weight. Results are from pooled supplement and placebo groups.

	n	Pre-Training*	Post-Training*	Mean Change (95% CI)	p
PCr	14	-37.5	-34.8	2.7 (-7.5, 12.8)	0.582
Lactate	15	26.2	16.7	-9.5 (-24.6, 5.6)	0.197
ATP	12	-1.8	-3.9	2.2 (-1.9, 6.1)	0.266
SLP	12	80.6	63.7	-16.9 (-54.3, 20.5)	0.337
ADP	12	0.64	0.37	-0.28 (-1.09, 0.54)	0.471
AMP	12	0.02	0.05	0.02 (-0.03, 0.08)	0.337
IMP	12	1.67	1.11	-0.56 (-1.93, 0.80)	0.383
Inosine	10	-0.42	-0.53	-0.11 (-1.14, 0.91)	0.816
Xanthine	12	0.00	0.01	0.00 (-0.04, 0.04)	0.889
Uric Acid	9	0.21	0.09	-0.13 (-0.35, 0.09)	0.214

Table 9.6. Exercise induced changes in muscle metabolites before and after training.

Data from subjects with viable pre- and post-training rest and exercise biopsy pairs are shown. Negative values denote a decrease. All units are mmol/kg dry weight except SLP (Substrate Level Phosphorylation), which is mmol ATP/kg dry weight.

* Pre and Post training values refer to exercise induced changes calculated by subtracting resting from exercising muscle metabolites.

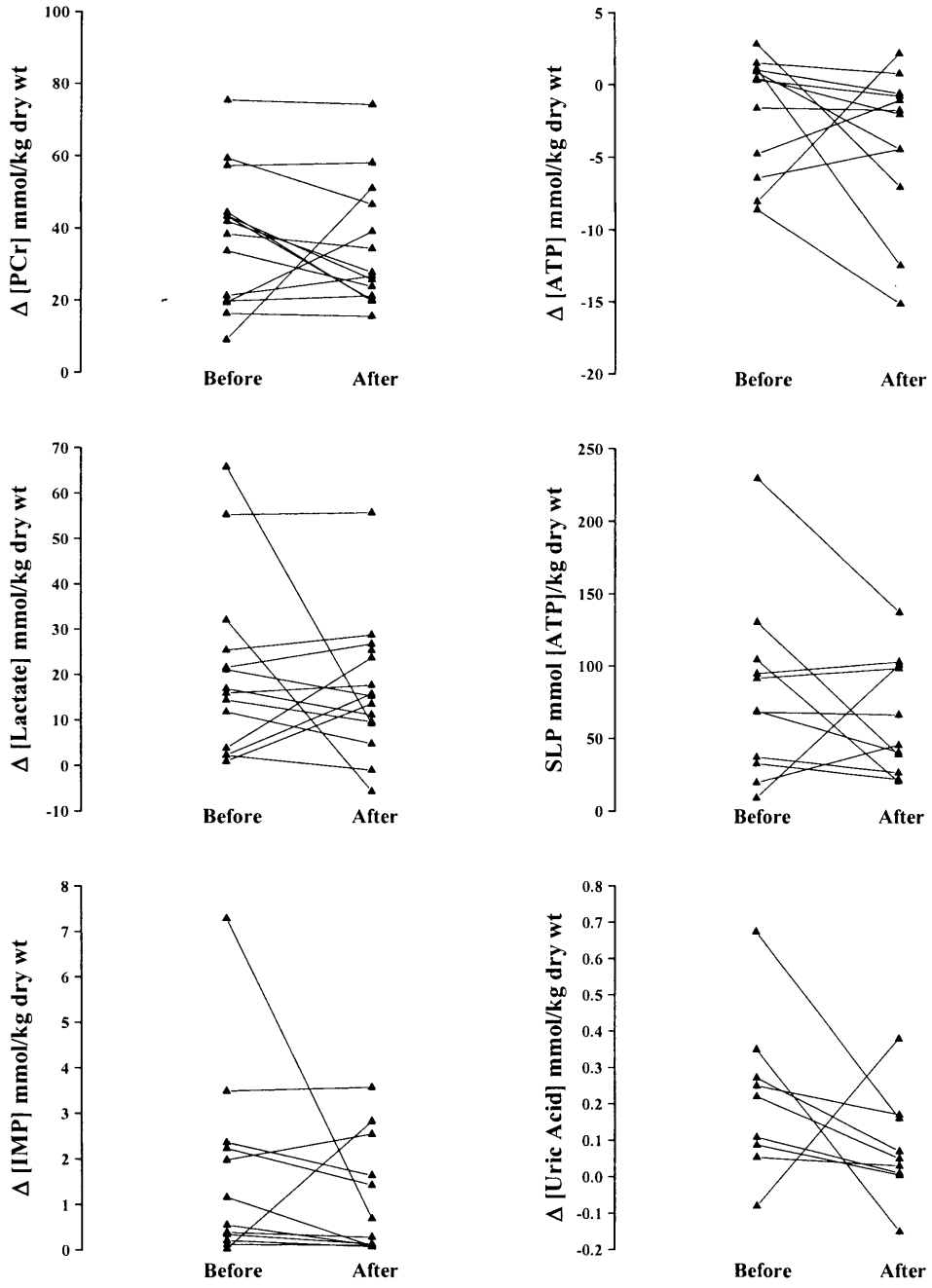


Fig 9.5. The metabolic response to exercise before and after training.

Δ Refers to exercise induced changes in metabolites measured during a the constant load test, calculated by subtracting rest from exercise measurements. Metabolites which show a significant exercise induced change are shown. For other metabolites see **Table 9.6**.

	Resting Values			Exercise Induced Changes		
	Baseline	Mean Difference	Within Subject SD	Baseline Change	Mean Difference	Within Subject SD
TCr	132 (9.5)	9.2 (13.8)	9.8	- -	- -	- -
PCr	67.5 (4.8)	1.8 (15.0)	10.6	-36.0 (20.0)	4.2 (19.2)	13.6
Lactate	2.8 (1.9)	0.9 (2.8)	2.0	31.0 (40.4)	-0.9 (35.2)	24.9
Glycogen	288 (68)	52 (69)	49	*	*	*
ATP	21.2 (2.3)	1.6 (5.9)	4.2	-5.4 (7.2)	-0.3 (8.1)	5.7
ADP	3.38 (0.34)	0.58 (1.07)	0.76	0.15 (0.80)	-0.42 (1.08)	0.76
AMP	0.06 (0.06)	0.04 (0.06)	0.04	0.04 (0.10)	0.00 (0.17)	0.12
IMP	0.16 (0.11)	0.04 (0.11)	0.08	2.67 (3.15)	-0.67 (2.95)	2.09
Inosine	1.03 (1.65)	0.61 (2.01)	1.42	-0.43 (1.38)	-0.43 (1.38)	0.98
Xanthine	0.08 (0.02)	0.01 (0.04)	0.03	0.00 (0.18)	0.01 (0.05)	0.04
Uric Acid	0.21 (0.11)	-0.07 (0.13)	0.09	0.11 (0.18)	-0.05 (0.31)	0.22

Table 9.7. Variability of muscle metabolites.

Left hand panel: Resting muscle metabolites. Right hand panel: Exercise induced change in muscle metabolites. Baseline values with the between subject standard deviations are shown in the first column. Mean Differences calculated by subtracting the first measurement from the second. Figures in parenthesis refer to standard deviations. Within subject standard deviations calculated by dividing the standard deviation of the differences by the square root of the number of measurements. No exercise induced change in total creatine is given as this is used to correct other measurements.

* insufficient muscle to measure exercise induced change in glycogen in 7 out of 8 subjects.

Discussion

In this study I have described the metabolic responses of the peripheral muscles to a standardised exercise challenge in a population of COPD patients at baseline, after a period of exercise training and nutritional support and during a seven week control period.

The Metabolic Response to Exercise in COPD

The analysis of rest and exercise muscle biopsy pairs demonstrated marked metabolic changes in response to the constant load exercise test. There was substantial utilisation of PCr and accumulation of lactate, and as demonstrated in **Chapter 6**, these changes were closely correlated confirming the metabolic integrity of the biopsy samples (**Fig 9.2**).

There was a significant decline in muscle [ATP] during exercise with an increase in [ADP] and a reduction in energy charge. ADP and AMP accumulation activates glycolysis, PCr breakdown and mitochondrial oxidative metabolism providing the energy needed for the adenylate kinase reaction and ATP resynthesis. This is supported in the current study by the correlation between ATP decline and both PCr degradation and lactate accumulation during exercise. The decline in muscle [ATP] and the TAN pool also suggests that ATP resynthesis was unable to match its utilisation during exercise.

The energy charge is an indicator of the energy status of the cell. Significant decreases in energy charge during exercise are detrimental to cellular function and inhibit the adenylate kinase reaction. This is an important cause of fatigue and failure of

muscle contraction because of reduced ATP availability (Atkinson, 1968). Accumulation of ADP and AMP is known to stimulate the irreversible deamination of AMP to IMP (Sahlin, 1992). Although this may appear counterproductive for sustained contraction due to depletion of the TAN pool, in the short term the result is to remove AMP and increase the energy charge. This allows the adenylate kinase reaction to continue thus maintaining ATP availability. The formation of IMP is thought to occur only at low energy charge conditions when the muscle is under considerable metabolic stress. In healthy subjects this has been shown to occur during high intensity or prolonged, fatiguing exercise (Broberg & Sahlin, 1989;Sahlin *et al.*, 1989). Significant IMP accumulation has also been demonstrated during exercise under hypoxic conditions in healthy subjects (Sahlin & Katz, 1989).

In the current study there was a substantial increase in [IMP] in many patients and this was correlated with the decline in energy charge and the TAN pool (**Fig. 9.3**). These results suggest that there is considerable metabolic stress during exercise in COPD. Moreover, the metabolic responses to exercise in COPD patients appear to be unrelated to the absolute intensity of exercise. Whilst the relative workloads at which subjects were moderately high (80% peak WR), the absolute workloads were low and it is likely that skeletal muscles are under regular metabolic stress during normal day-to-day activities in many patients.

A limitation of this study is that COPD patients were not compared with healthy age matched controls using this protocol. It cannot therefore be determined if the muscular response to exercise in COPD is substantially different to the healthy elderly. However, the comparison with healthy subjects is problematic because the latter are able to exercise at considerably higher intensities. Using the protocol developed in

this thesis, healthy subjects would be exercised at 80% peak exercise capacity. This would in all probability represent a substantially higher absolute intensity than COPD patients and plainly could explain differences in the magnitude of the metabolic response.

Detailed studies of energy metabolism during exercise have not previously been performed in COPD patients. Several studies have reported muscle metabolite concentrations from muscle samples taken at rest in COPD patients both with and without respiratory failure compared with healthy controls (Fiaccadori *et al.*, 1987;Jakobsson *et al.*, 1990;Moller *et al.*, 1982). These studies showed lower muscle PCr and ATP concentrations than age matched healthy controls. Muscle [ATP] and [PCr] were similar to healthy controls in a more recent study but values across both groups in this paper were lower than controls in previous studies (Pouw *et al.*, 1998a). Although healthy subjects were not studied in the current study, metabolite concentrations at rest were broadly in agreement with these previous reports. The functional consequences of these alterations in muscle metabolites at rest were not investigated in these studies but it is notable that in the current study exercise induced changes in [PCr] and [ATP] were related to their respective resting values (**Fig. 9.4**). The reasons for this are unclear but indicate that the resting condition of the muscle is intimately linked to its response to exercise.

The metabolic response of the skeletal muscles during exercise has been studied in COPD patients using Magnetic Resonance Spectroscopy (MRS) (Sala *et al.*, 1999;Kutsuzawa *et al.*, 1995). Whilst this is a non-invasive technique, the information that is obtained is limited. Data on purine nucleotide metabolism, for example, such as that described in this chapter could not be obtained. Moreover, whole body exercise

cannot be readily studied in the confines of the scanner. This mode of exercise challenge is of greater functional relevance to patients with disability due to COPD.

Changes in IMP and other products of adenine nucleotide metabolism during exercise have not been reported in COPD. Pouw *et al* measured IMP in the anterior tibialis muscle of COPD patients at rest and found measurable concentrations whereas it was undetectable in healthy controls (Pouw *et al.*, 1998a). The authors suggested this was due to an imbalance in ATP utilisation and resynthesis due to impaired energy metabolism. The findings of significant IMP accumulation coupled with loss of ATP during exercise in the current study support this hypothesis.

There was considerable between patient variability in both resting metabolite concentrations and the metabolic responses to exercise. This is unlikely to be due to variations in biopsy quality across rest and exercise pairs because these were corrected for total creatine content and there were consistent correlations between changes in metabolites during exercise.

There are a number of possible factors contributing to this variability. Most important was probably differences in the limitations to peak exercise across a heterogeneous population of COPD patients. All exercised at 80% of their peak work rate but some were unable to complete five minutes whilst others appeared to do so with relative ease. If peak incremental exercise was limited by ventilation, the load to the muscles during an exercise test at 80% peak work may have been less than in those patients whose peak exercise was limited by muscle fatigue.

Variations in muscle fibre composition could also account for between patient variations in metabolic responses. Type II fibres show greater adenine nucleotide loss and IMP accumulation that Type I fibres during exercise (Sahlin *et al.*, 1989). Changes

in fibre composition have been reported in COPD patients (Whittom *et al.*, 1998) and variations in the extent of this change would have a significant impact on measured changes in muscle metabolites during exercise.

It was anticipated that a component of between patient variability would be related to the severity of lung function impairment, exercise limitation and nutritional depletion but no relationship between these indices and muscle metabolites was found.

The Reproducibility of Measurements of Muscle Metabolites

In this part of the study the within subject variability of rest and exercise induced changes in muscle metabolites was investigated. This was performed over a seven week control period equivalent to the standard length of pulmonary rehabilitation at Glenfield Hospital. It can be seen from **Table 9.7** that within subject standard deviations were frequently comparable to the magnitude of the measurement and its standard deviation (the between subject variation) at baseline. This indicates that in addition to between subject variability in the metabolic responses to exercise there was considerable within subject variation in both resting and exercise induced changes in muscle metabolites.

There are a number of possible reasons for this variability. Whilst rest and exercise biopsy pairs were corrected for total creatine content because it can be assumed that total creatine will not vary over a period of a few minutes, this is not valid for biopsies taken seven weeks apart. Some of the variability over the control period may therefore have been due to differences in biopsy contamination with non-muscle tissue. However, the between subject SD for total creatine was small relative to the baseline values suggesting that this was a minor factor.

It appears that there was also considerable biological variability in the metabolic response to exercise over time. The reasons for this are unclear as the exercise challenge was carefully controlled and repeated at an identical cumulative workload. It was also evident that there were variations in resting metabolites over the seven week control period. Patients were asked not to undertake heavy exercise for 48 hours prior to attendance to ensure the muscle was in a rested condition before samples were taken. However, it can be seen from the results at baseline that considerable metabolic stress occurs in the muscles of some patients at low absolute workloads and the performance of routine activities of daily living may have prevented the muscle returning to its resting state. The deamination of AMP to form IMP is irreversible and it may take some hours or even days for replenishment of the TAN pool to occur by other pathways (Maughan *et al.*, 1997). It is also clear from the results at baseline that the resting concentrations of ATP and PCr are implicated in the responses of these metabolites to exercise. Changes in the condition of the muscles at rest may therefore be an important factor in the biological variability of the metabolic response to exercise.

The Effects of Training and Nutritional Supplementation

There were no significant changes in resting or exercise induced muscle metabolites after training. There were reductions in PCr utilisation, Lactate accumulation and substrate level phosphorylation after training but these were not statistically significant. ATP loss increased after training, contrasting with the findings of the pilot study described in **Chapter 6**, which suggested a trend towards amelioration of ATP loss after training. It is likely that the lack of training effect seen in this study is due to the considerable between subject and within subject variability in muscle

responses which I have previously alluded to. The study may therefore have had insufficient power to detect significant changes. Previous MRS and muscle biopsy studies have indicated that metabolic adaptations to training do occur in COPD patients and there is no reason to believe that this did not occur in this study. Subjects were trained at intensities that have previously been shown to induce such adaptations (Casaburi *et al.*, 1991;Maltais *et al.*, 1996b). There were no correlations between changes in performance and changes in muscle metabolites suggesting that variations in the outcome of rehabilitation did not contribute to these negative findings.

An important factor may have been that different modes of exercise were used during training (walking) and during testing (cycling). Training effects in the muscles are restricted to the muscle groups that are trained and to the mode of training. It is possible that walking exercise did not induce adaptations in quadriceps muscle performance that could be detected during a cycle exercise test. This is supported by the finding of only small changes in incremental cycling performance despite substantial improvements in incremental shuttle performance.

Carbohydrate supplementation appeared also to have no effect on muscle metabolism but the number of subjects available for analysis of the effect of supplementation was probably too small for any differences to be detected.

Summary

This study has demonstrated that during exercise COPD patients show substantial metabolic changes in the peripheral muscles. In many patients there is also evidence of metabolic stress during exercise with adenine nucleotide loss and

accumulation of IMP. These stresses occur at workloads that are probably reached regularly during day-to-day activities in many patients.

The results also indicate that there is considerable biological variability in these responses both between patients and within patients over time. The reasons for this diversity of response are unclear but it suggests that the contribution of peripheral muscle dysfunction to exercise limitation varies widely between patients. Measurements of muscle metabolism during exercise may allow the identification of patients for whom impaired muscle performance is clinically relevant. This would be useful as these patients might particularly benefit from performance enhancing therapy targeted at the skeletal muscles.

CHAPTER TEN

Conclusions

THE aim of this thesis was to explore the potential for the enhancement of exercise performance in COPD patients. Exercise intolerance is a central component of the symptoms and disability experienced by COPD sufferers so this is an important therapeutic goal. Whilst exercise training has an established role in this area, the role of other therapies adjunctive to training has received little attention. This contrasts to the sphere of athletic competition where considerable attention is paid to maximising performance, not infrequently by the illicit use of performance enhancing drugs to enhance training regimens.

The hypothesis tested in this thesis was that carbohydrate rich nutritional supplementation would enhance the physical benefits of exercise training in an unselected population of COPD patients attending pulmonary rehabilitation. In addition, studies were described which examine a number of other questions relating to the measurement of physical performance and nutritional status and the functioning of the peripheral muscles in this population.

Main Findings

The study described in **Chapter 8** demonstrated that whilst nutritional supplementation appears to be unhelpful when prescribed universally to COPD patients, participating in pulmonary rehabilitation, it may have a role in the enhancement of training in better nourished patients. On first examination this may seem paradoxical but it is clear that the benefit of nutrition to sports performance does not reside in the improvement of nutritional status but in improved energy balance and substrate availability for the exercising muscles. It appears that this also holds true for individuals with COPD. This is supported by the finding in **Chapter 8** that improvements in exercise performance correlated with increases in carbohydrate intake during the study period.

Examination of dietary macronutrient intake during the rehabilitation and supplementation programme demonstrated striking differences in energy balance between supplemented patients and those allocated to rehabilitation alone. The decrease in fat mass seen in patients receiving rehabilitation without supplementation suggests that patients do not increase dietary energy intake sufficiently to overcome the energy costs of the increase in physical activity during rehabilitation. These changes in weight and fat mass were relatively small. However, if, as is hoped, rehabilitation results in a change to a more active lifestyle, this negative energy balance could, over time result in significant changes in nutritional status. Conversely, energy imbalance might be a barrier to the maintenance of physical activity after rehabilitation.

Chapter 5 demonstrated that physical performance can be measured in a number of different modes, which do not necessarily overlap and will respond differently to specific modes of training. **Chapter 7** demonstrated that the measurement

method used for the nutritional assessment of patients has an important impact on the identification of patients who are nutritionally depleted. These results increase our understanding of the limitations of such measurement tools and highlight the importance of choosing appropriate outcome measures when therapeutic trials are designed.

The work described in **Chapters 6** and **9** introduced a novel technique for the study of the performance of the peripheral muscles during exercise in COPD patients. **Chapter 9** demonstrated that many patients with COPD show considerable metabolic stress in the muscles during exercise at relatively low absolute intensities. In these patients peripheral muscle dysfunction may make a significant contribution to exercise intolerance. These chapters also highlighted the limitations of this technique. Firstly, the metabolic responses to exercise were highly variable both between patients and within patients over time. This is probably a biological phenomenon relating to the variable impact of ventilatory and muscular 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. Furthermore, there were technical problems obtaining good quality biopsy specimens during exercise in this population and although the procedure was well tolerated, it is invasive and time consuming to perform. Whilst this technique may increase our understanding of the mechanisms of muscle dysfunction in COPD, these difficulties will limit its use in clinical trails.

Future Work

The use of nutritional support during rehabilitation is worthy of future study. The benefits to well nourished patients suggested by this thesis were based on a subgroup analysis and will need to be confirmed prospectively before such treatment can be recommended in routine clinical practice. The results from **Chapter 8** imply that the benefits of carbohydrate supplementation were related to the improvements in energy balance during training. An understanding of the causes of such energy imbalance and its relationship to the frequency, duration and intensity of exercise prescribed during rehabilitation would be of great interest and might allow the identification of patients who will benefit most from nutritional support.

The selective benefit of supplementation to well nourished patients in **Chapter 8** suggests that nutritional supplementation will be less helpful in the severely undernourished. Although at first glance this appears counterintuitive, this finding is supported by the failure of previous nutritional support programmes to benefit nutritionally depleted patients. These patients may have an exaggerated systemic inflammatory response with an increase in circulating pro-inflammatory cytokines. This may lead to both a large increase in energy expenditure and abnormal protein metabolism with resulting severe depletion of lean and fat mass. The treatment of severely undernourished patients is a challenging clinical problem, which is unlikely to be solved by calorie supplementation alone.

The data presented in this thesis confirm the effectiveness of pulmonary rehabilitation but there remain a number of unanswered questions about the mode, duration and intensity of such programmes. In COPD, the role of aerobic training has received greatest attention whereas strength training is used less frequently. Common to

most rehabilitation programmes is prescription of a generic training regimen to all participants. Whilst the intensity of exercise will be individualised, the mode of training is not. The results in **Chapters 5** and **8** confirm that, as in healthy subjects, training in COPD is mode selective. Patients with poor muscle strength may therefore be less well served by exercise programmes that concentrate on endurance performance. The use of a wide range of performance outcome measures may allow individual performance deficits to be identified and rectified.

There is now accumulating evidence to support a role for peripheral muscle dysfunction in exercise intolerance in COPD patients and this is supported by the results in **Chapter 9**. The decline in muscle [ATP] and the accumulation of purine nucleotide derivatives is an indicator of metabolic stress in the muscle but it remains unclear if purine metabolism in COPD is disordered or whether this is merely a result of deconditioning. A healthy control group was not included in the studies described in this thesis, as the main purpose was to evaluate the effect of training and nutritional support. Including a healthy control group would be useful because few studies have documented the metabolic responses to exercise in the elderly. However, a comparison with COPD patients will be difficult to interpret because exercise in these subjects would be carried out at much higher absolute intensities.

I have already alluded to the variability in the metabolic response to exercise between patients and this is and important theme throughout this thesis. Patients also show wide variations in exercise performance, nutritional status and their response to training. Patients with COPD are defined by the severity of lung function impairment but this is a poor predictor of the secondary consequences of COPD such as muscle dysfunction and nutritional depletion that are the focus of this thesis. The

characterisation of patients beyond the measurement of lung function will be needed if the treatment of these consequences is to be successful.

Concluding Remarks

Exercise intolerance is the hallmark of the disability suffered by COPD patients. Treatment strategies have mostly been directed at increasing the capacity of the respiratory system to perform during exercise by reducing airflow obstruction. Less attention has been paid to reducing the load imposed by the muscles during exercise. The success of pulmonary rehabilitation suggests this will be a fruitful avenue to pursue but the potential for improving muscle function and therefore performance by pharmacological means has not been explored. This thesis suggests there is a future for such an approach. For such therapy to be realised a change in philosophy is needed in drug development so that the broader consequences of chronic lung disease are considered in addition to the underlying pulmonary pathology. 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. However, the lesson from sports and athletic competition is that performance-enhancing drugs are most effective when combined with an appropriate training regimen. This will require a change in emphasis towards the use of combined treatment modalities in clinical trials.

The use of performance enhancing drugs may be considered unfair for those who engage in competitive sport. A different view is required for patients with chronic lung disease who wish to enhance their ability to carry out basic activities of living by improving their exercise performance.

Appendices

Appendix I. Laboratory Methods

In this appendix, I will briefly describe the laboratory methods used in the analysis of muscle biopsies taken in the course of studies described in **Chapters 6** and **9**. This analysis was performed in the Department of Biomedical Sciences, University of Nottingham under the supervision of Prof. Paul Greenhaff. Whilst the analysis and interpretation of muscle biopsy data was performed by myself, I did not perform the laboratory work and the methodology is therefore presented here rather than in the main body of the thesis.

All muscle samples were frozen immediately and stored in liquid nitrogen. They were later transported to the University of Nottingham for analysis. Muscle samples were freeze dried and subsequently stored at -80°C. They were then powdered using a mortar and pestle and all visible connective tissue, blood and fat removed.

Samples were analysed in random order. Samples were labelled numerically and the staff performing the analysis were unaware which were pre or post training samples.

Muscle PCr, Creatine, Lactate and Glycogen Determination

These metabolites were extracted from powdered muscle by the addition of 0.5M perchloric acid. Extracts were then centrifuged and neutralised with potassium

bicarbonate. Metabolite concentrations were then determined spectrophotometrically (Harris *et al.*, 1974).

Muscle glycogen was extracted using buffered HCl from a separate portion of muscle after heating in NAOH. The mixture was then incubated at room temperature in amyloglucosidase and its concentration subsequently determined spectrophotometrically (Harris *et al.*, 1974).

Muscle ATP and Purine Nucleotide Derivative Determination

These metabolites were determined by High Performance Liquid Chromatography (HPLC). Muscle samples were freeze dried, powdered and metabolites extracted as described for lactate and PCr. Concentrations of ATP, ADP, AMP, IMP, Inosine, Xanthine and Uric acid were then determined by automated HPLC (HPLC System Gold, Beckman Instruments, Bucks., U.K.) (Wynants & Van Belle, 1985).

Metabolite Correction for Total Creatine

All metabolites other than those that can move freely out of the muscle (lactate and glycogen) were corrected for the highest total creatine measured from that 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 & Sjoholm, 1983).

Appendix II. BIA Equations

Fat Free Mass (FFM_{BIA}) was estimated from impedance measurements using sex specific regression equations as follows:

Males: FFM (kg) = 8.383 + (0.465*ht^2 (cm)/ resistance (Ohm)) + (0.213*wt (kg))

Females: FFM (kg) = 7.610 +(0.474*ht^2 (cm)/ resistance (Ohm)) + (0.184*Wt (kg))

These equations were derived from a validation study using a population of COPD patients with deuterium dilution as a validation method (A.M. Schols; Personal Communication). (See **Fig. II**.)

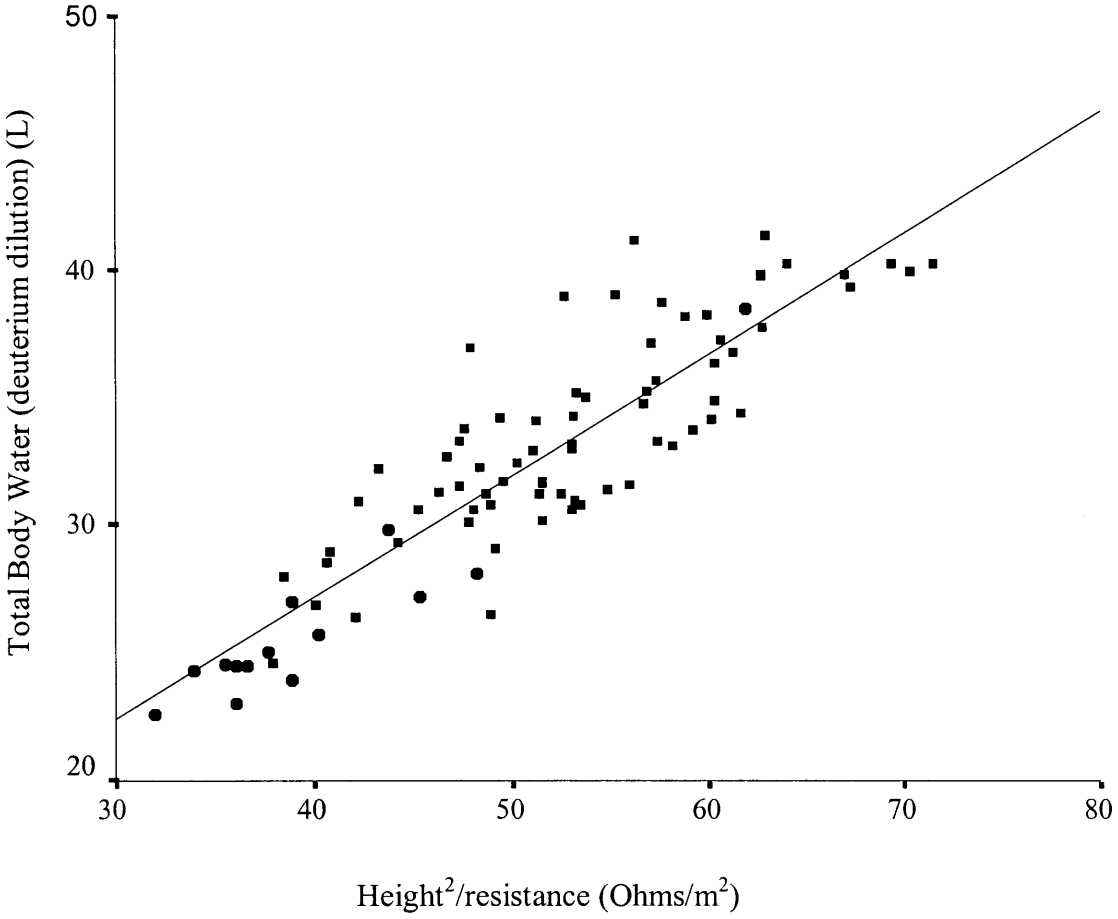


Fig. II. Validation graph for BIA against deuterium dilution.

Data was obtained from a study of 117 COPD patients. Sex specific regression equations for the calculation of FFM from impedance measurements were derived from this study.

Reproduced with permission from unpublished data by A.M.W. Schols.

Squares: Male patients, Circles: Female patients.

Appendix III. Reproducibility Studies

The measures of physical performance and nutritional assessment used in this thesis were assessed for reproducibility over the study period (seven weeks). A subgroup of subjects recruited to the nutritional intervention trial (See **Chapter 8**) underwent assessment before and after a seven week control period prior to starting pulmonary rehabilitation. The between patient standard deviations for these variables are shown in **Table III** together with the mean differences between repeat measurements and the within patient standard deviations. The latter was calculated by dividing the standard deviation of the differences by the square root of the number of measurements (in this case 2) (Chinn, 1991). Intraclass correlation coefficients (ICC) are also given. This 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. Patients did not reach the ceiling of 20 minutes for the ESWT during the control period. This variable was approximately normally distributed and parametric analysis was therefore applied. Reproducibility data for the muscle biopsy analysis are documented in **Chapter 9**.

The repeated measure differences are plotted against the mean of the two measurements in **Figs. IIIa** and **IIIb** for physical performance and body composition respectively.

The stability over the equivalent of the study period was acceptable for all measurements. Variability was greater for ESWT. This was mainly due to variability at higher values (See **Fig. IIIa**).

	Baseline Mean	Between Subj. SD	Mean Difference	Within Subj. SD	ICC
Physical Performance					
ISWT (m)	240	107	-0.4	24.3	0.97
ESWT (s)	239	132	-39.1	80.5	0.84
QS (N)	324	117	1.0	30.4	0.97
HGS (KgF)	27.3	8.2	-0.2	1.5	0.98
Nutritional Assessment					
Weight (Kg)	70.7	12.8	-0.4	0.69	0.99
Impedance (Ohms)	585	110	-6.6	24.0	0.98
FFM_{DXA} (Kg)	47.4	10.4	0.2	0.99	0.99
SFT (mm)	57.2	21.6	-1.9	5.4	0.97

Table III. Reproducibility Data

Mean differences are calculated by subtracting the second measurement from the first. Within subject standard deviations calculated by dividing the standard deviation of the differences by the square root of the number of measurements. ICC = Intraclass Correlation Coefficient.

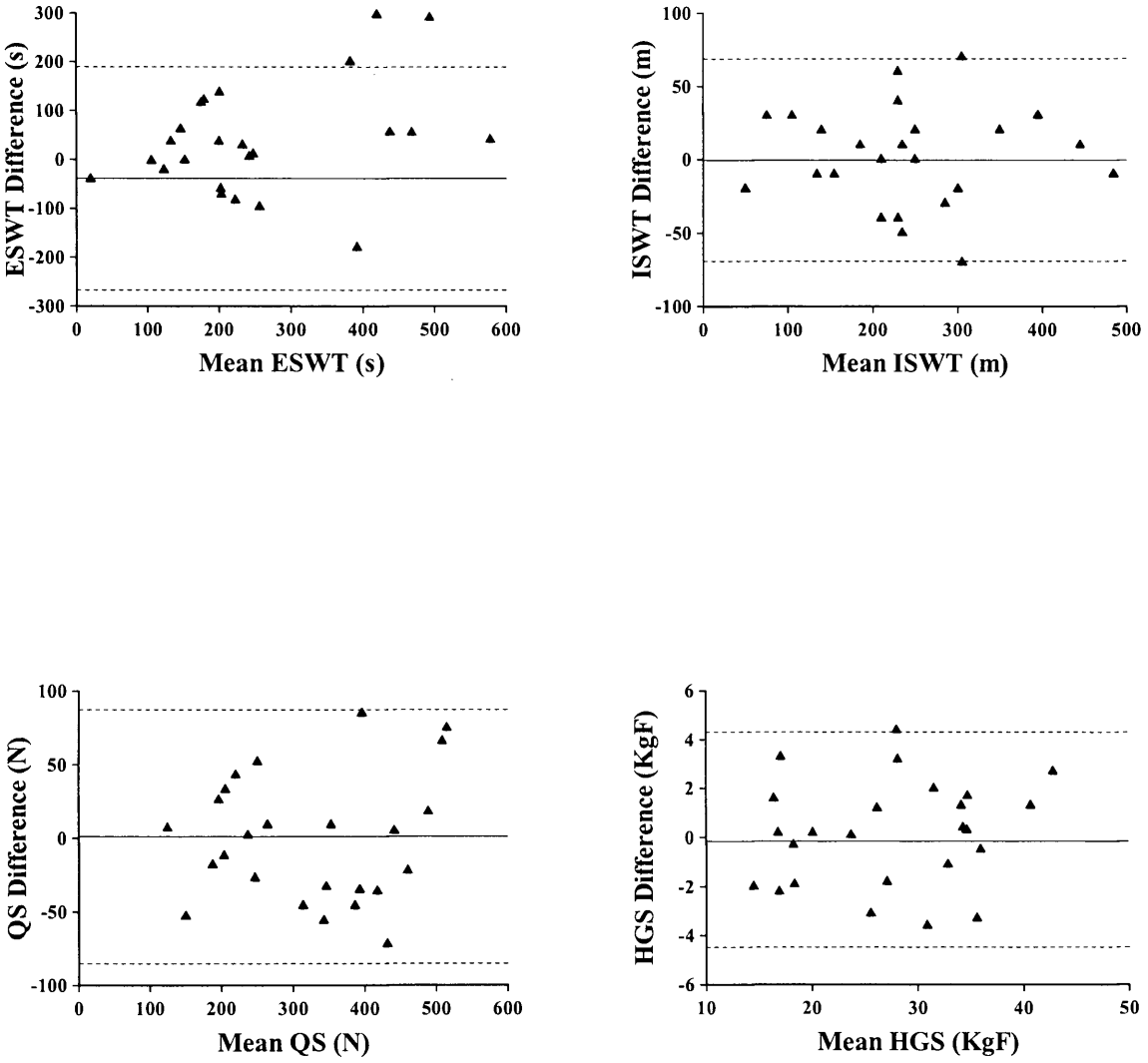


Fig. IIIa. Variability of measures of physical performance.

The difference between repeat measurements are plotted against the mean of the two measurements. The mean of the differences (solid lines) plus or minus two standard deviations (Dashed lines) are also shown.

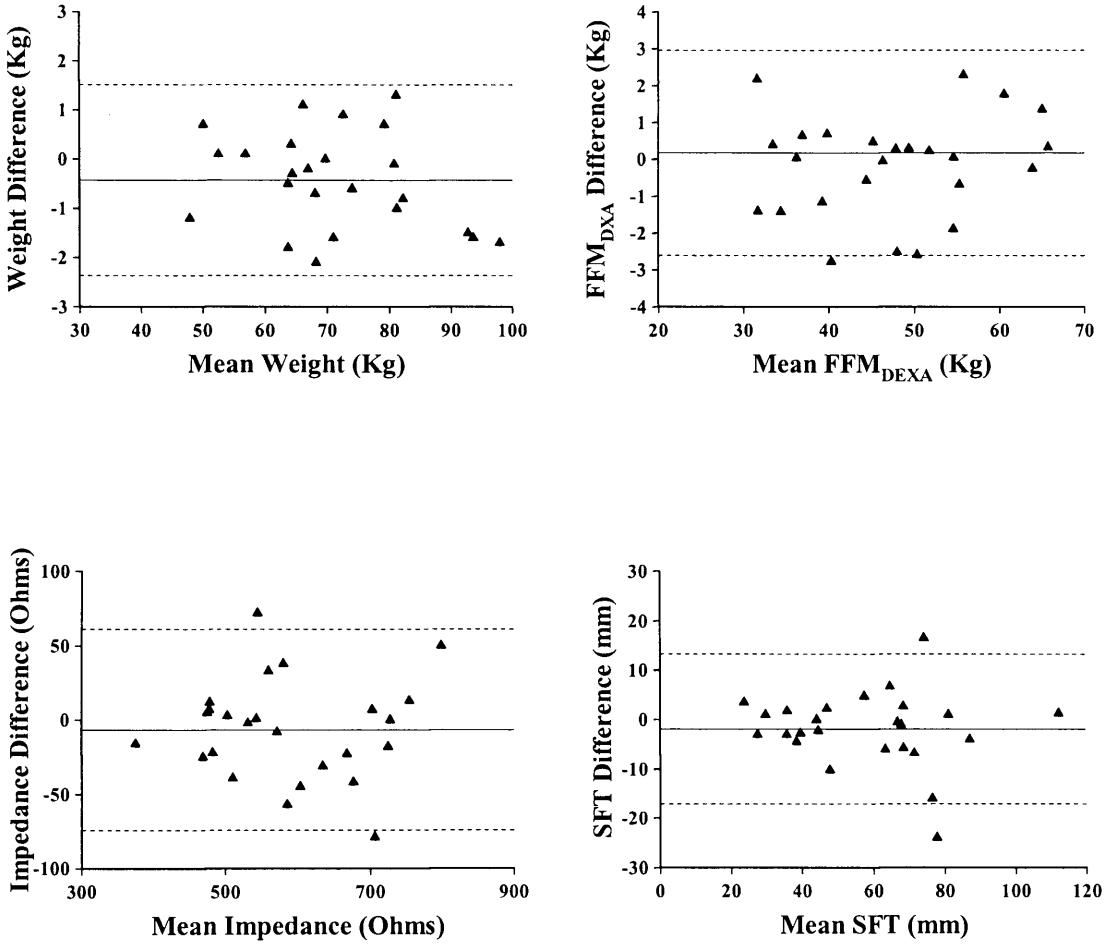


Fig. IIIb. Variability of measures of nutritional assessment.

The difference between repeat measurements are plotted against the mean of the two measurements. The mean of the differences (solid lines) plus or minus two standard deviations (Dashed lines) are also shown. SFT = sum of skinfold thickness at four sites.

Appendix IV. Questionnaires

Food Record Booklet

Details of the contents of the food record booklet given to patients to record dietary intake are shown here including the instructions given to patients (see below). An example to guide patients was included (**Fig. IVa**) and a blank record for a single day is shown in **Fig. IVb**. Food record data was collected and entered into computerised food composition tables by a state registered dietician (R.L. Barton).

Instructions

It would help the Dietitian if you could be as accurate as possible by following the instructions below:

1. State **type** of food, (e.g. *wholemeal* bread, white rice)

2. State **method of cooking,** (e.g. boiled, grilled, fried)

3. Please state the **quantity** of food eaten, using household measures, e.g:

   Wholemeal Bread - 1 slice medium loaf

   Cornflakes - 4 *tablespoons*

   Please state weight of packets / tins used. Use ounces or grammes

4. Please state brand of food where possible, for example:

   McVites Rich Tea Biscuits

   Heinz Cream of Mushroom Soup

5. If two items are eaten together, state individual amounts, for example:

   Apple & Custard

   - stewed apple - 2 tablespoons
   - custard - 4 tablespoons

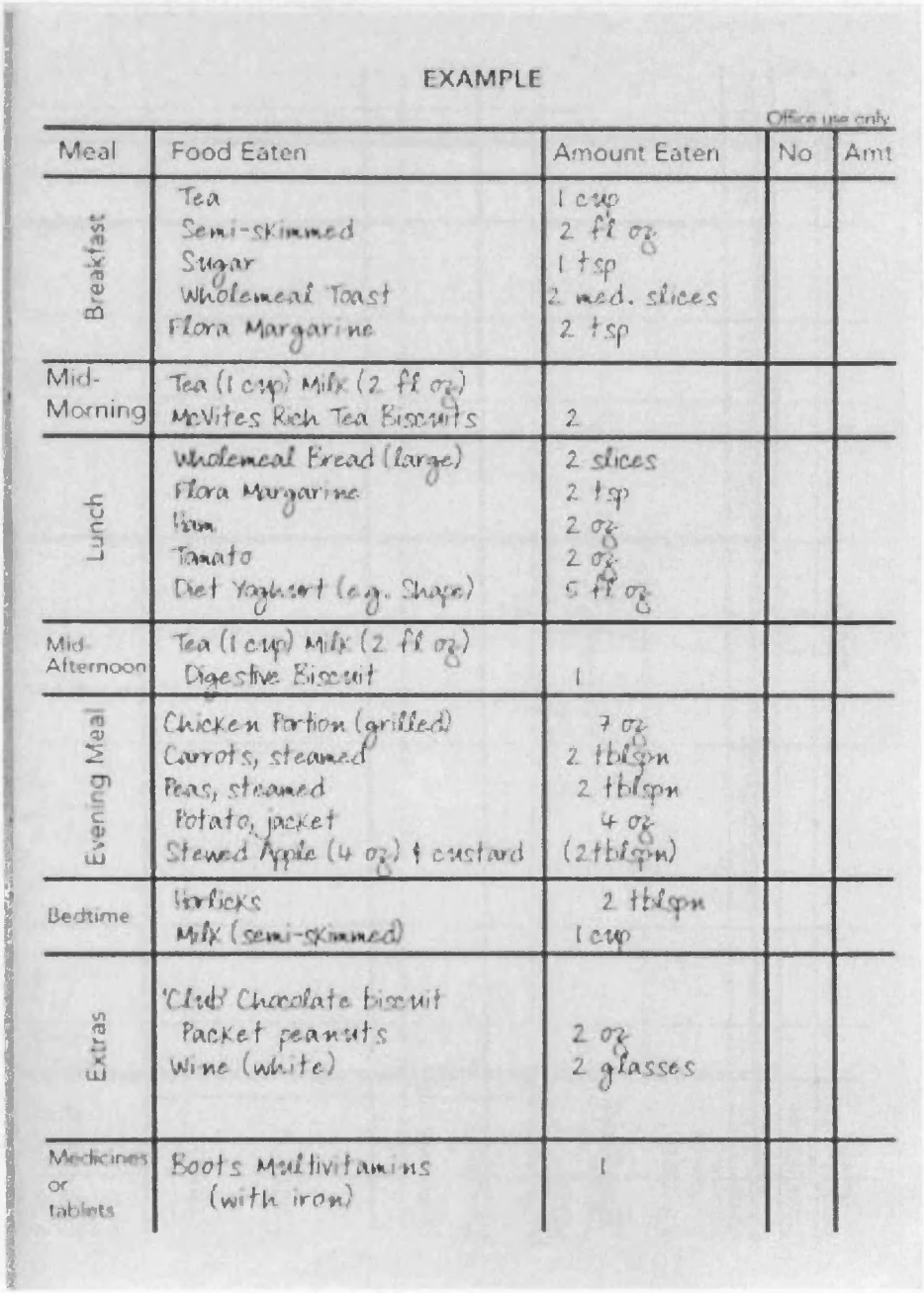
   Cottage Pie

   - beef mince - 2 tablespoons
   - potato - 2 tablespoons

6. Remember to record all snacks and drinks

7. Remember to record all medicines and/or supplements taken

8. Please attach the 'nutrition information' panel from any ready meals eaten



EXAMPLE

Office use only

Meal	Food Eaten	Amount Eaten	No	Amt
Breakfast	Tea Semi-skimmed Sugar Wholemeal Toast Flora Margarine	1 cup 2 fl oz 1 tsp 2 med. slices 2 tsp		
Mid-Morning	Tea (1 cup) Milk (2 fl oz) McVities Rich Tea Biscuits	2		
Lunch	Wholemeal Bread (large) Flora Margarine Ham Tomato Diet Yoghurt (e.g. Shape)	2 slices 2 tsp 2 oz 2 oz 5 fl oz		
Mid-Afternoon	Tea (1 cup) Milk (2 fl oz) Digestive Biscuit	1		
Evening Meal	Chicken Portion (grilled) Carrots, steamed Peas, steamed Potato, jacket Stewed Apple (4 oz) + custard	7 oz 2 tblspn 2 tblspn 4 oz (2 tblspn)		
Bedtime	Horlicks Milk (semi-skimmed)	2 tblspn 1 cup		
Extras	'Club' Chocolate biscuit Packet peanuts Wine (white)	 2 oz 2 glasses		
Medicines or tablets	Boots Multivitamins (with iron)	1		

Fig. IVa. Example included food record booklet instructions.

DAY ..... DATE ..... Office use only

Meal	Food & Drink	Amount Eaten	No	Amt
Breakfast				
Mid-morning				
Lunch				
Mid Afternoon				
Evening				
Bedtime				
Daily Totals	Milk Margarine Sugar			
Extras e.g. Sweets Biscuits Drinks				
Medicine or Tablets				

Fig. IVb. Sample page from food record booklet.

Patients were asked to complete pages for three separate days using household measures to estimate quantities.

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.....

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:

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

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	_____	☐	☐	☐	☐	☐	☐	☐
2	_____	☐	☐	☐	☐	☐	☐	☐
3	_____	☐	☐	☐	☐	☐	☐	☐
4	_____	☐	☐	☐	☐	☐	☐	☐
5	_____	☐	☐	☐	☐	☐	☐	☐

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

Thank you

5. 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

6. 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

7. 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

8. 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

9. 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

10. 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

11. 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

12. 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

13. 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

14. 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

15. 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

16. 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

17. 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

18. 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

19. 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 V. Patient information and consent forms.

DIET AND EXERCISE TRAINING IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE. A RANDOMISED CONTROLLED TRIAL

PATIENT INFORMATION SHEET

Principal Investigator: Dr. M. Morgan
Investigator: Dr. M. Steiner
Dr. S. Singh

You May Contact: Dr. M. Steiner
Dr. M. Morgan
Dr. S. Singh

Department of Respiratory Medicine
Glenfield Hospital
Leicester
Tel No: 0116 256 3663
0116 287 1471

What is the purpose of the study?

You have a condition called Chronic Obstructive Pulmonary Disease (COPD) and are about to enter the pulmonary rehabilitation programme at Glenfield Hospital. Patients who suffer with COPD usually complain of being unable to carry out some activities because of breathlessness and muscle fatigue. We know that exercise training as part of a pulmonary rehabilitation programme can improve this but we don't fully understand how. In addition many patients are under-nourished and even those who are of normal weight have reduced muscle bulk because of the restrictions in activity caused by the disease.

We wish to study the impact of exercise training and dietary supplementation on symptoms and muscle performance in patients with COPD. We hope that providing extra energy whilst participating in an exercise programme will provide extra benefits for patients with COPD.

The results will allow us to provide a more effective rehabilitation programme and increase the benefits of rehabilitation to patients.

What will be involved if I take part in the study?

Tests to be Carried Out

You will undergo a number of tests before entering the programme in addition to those, which all patients have.

We will measure the strength of the leg muscles and the strength of your handgrip.

We will measure the ratio of fat and muscle in your body by taking an electrical recording from your skin. This is completely harmless and painless.

We will measure the thickness of your skin at four different sites on your body. This gives us a measure of the proportion of fat in your body.

We will ask you to weigh and keep a record of what you eat and drink for three days. We will provide weighing scales to do this.

We will ask you to have a special x-ray, which measures the proportion of fat and muscle in your body called a DEXA scan. This takes about five minutes and is painless. The x-ray will be done at Leicester Royal Infirmary.

We will ask you to fill out a questionnaire on how your breathing problems affect your daily life.

Those patients who are suitable will also have the following tests:

We will ask you to exercise on an exercise bike for as long as you can. The effort you will need to put in to cycling gradually increases during the test. During this period you will be breathing through a tube, which allows us to measure what is in the air you breathe in and out.

After a gap of at least 48 hours we will again ask you to exercise on the bicycle but this time the effort you will need to put in will not change. We will ask you to exercise for five minutes but if you cannot carry on that long that doesn't matter. Before starting we will take a muscle biopsy from the thigh (the details of this are described below). Within a few seconds of you finishing we will take another biopsy.

Muscle Biopsies

These are taken from the thigh. The area is anaesthetised with a small injection and a small incision is made in the skin. The biopsy (a small sample of muscle) is taken through this incision with a special needle. Afterwards the cut is closed using adhesive strips and a compression bandage is placed around the thigh. This can be removed after 12 hours.

You may experience a small amount of discomfort when the skin incision is made and when the biopsy is taken. Your thigh may ache for a day or two after the biopsy (similar to as if you had banged it against a piece of furniture).

Dietary Supplementation and the Rehabilitation Programme

The study aims to compare the effect of dietary supplementation and rehabilitation with no treatment (apart from your usual medication). There is a one in three chance that you would be allocated to the no treatment group. If this happens, your course of rehabilitation will be delayed for seven weeks. At the end of this period you will undergo the above tests again immediately before starting on the rehabilitation programme.

At the start of the rehabilitation programme you will be randomly allocated to one of two groups:

Group A - will receive cartons of nutrients to drink every day for the duration of the seven week rehabilitation programme in addition to your normal diet. You will not need to alter your normal diet in any way.

Group B - will receive similar cartons during the rehabilitation programme but containing no nutrients.

Neither you nor we will know which group you are in until after the study is finished. You will need to drink three cartons a day throughout the pulmonary rehabilitation programme.

Visits to Hospital

The tests will involve three extra visits two hospital both before and after the pulmonary rehabilitation programme. One of these visits is to the Leicester Royal Infirmary for the DEXA scan.

Patients allocated to the "No Treatment" group will need three sets of tests requiring an extra three visits.

If necessary we will provide transport to and from the hospital for these visits.

Benefits and risks

Currently, dietary supplementation is not provided for patients during the rehabilitation programme because we don't know if it helps or not. You may therefore gain the benefit of this new treatment by entering the study. You will be helping future patients by helping us understand how to maximise the benefits of pulmonary rehabilitation.

There is a small risk of injury during the exercise test. The muscle biopsies involve a small amount of discomfort but the risks are negligible.

Will information obtained in the study be confidential?

The treatment you receive in the study will be recorded in your medical records and is confidential under the data protection act. You will not be identified in any documents relating to the study.

Your GP will be notified of your participation in the study.

What if I am harmed by the study?

Medical research is covered for mishaps in the same way as for patients undergoing medical treatment in the NHS i.e. compensation is only available if negligence occurs.

What happens if I do not wish to participate in this study or wish to withdraw from the study?

We are extremely grateful for your help with this research. If you would like to help but feel unhappy about having the muscle biopsies it will still be possible to take part whilst missing this part of the study.

If you do not wish to participate at all in the study or if you wish to withdraw from the study you may do so without justifying your decision and your future treatment will not be affected.

DIET AND EXERCISE TRAINING IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE. A RANDOMISED CONTROLLED TRIAL

PATIENT CONSENT FORM

Principal Investigator: Dr. M. Morgan

Investigator: Dr. M. Steiner

This form should be read in conjunction with the Patient Information Leaflet

I agree to take part in the above study as described in the Patient Information Sheet.

I understand that I may withdraw from the study at any time without justifying my decision and without affecting my normal care and medical management.

I understand that members of the research team may wish to view relevant sections of my medical records, but that all the information will be treated as confidential.

Medical research is covered for mishaps in the same way as for patients undergoing medical treatment in the NHS i.e. compensation is only available if negligence occurs.

I have read the patient information leaflet on the above study and have had the opportunity to discuss the details with .....and ask any questions. The nature and the purpose of the tests to be undertaken have been explained to me and I understand what will be required if I take part in the study.

I agree to participate in: The main study only / The main study plus the muscle biopsy study*

* delete as applicable

Signature of patient .....

Date .....

(Name in BLOCK LETTERS)

.....

I confirm I have explained the nature of the Trial, as detailed in the Patient Information Sheet, in terms which in my judgement are suited to the understanding of the patient.

Signature of Investigator .....

Date .....

(Name in BLOCK LETTERS)

.....

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