EXERCISE INTOLERANCE IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: NOVEL MEANS OF ASSESSMENT AND INTERVENTION

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ABSTRACT

Exercise limitation is a hallmark of chronic obstructive pulmonary disease (COPD) and countering this is a major therapeutic goal. Chapter 1 considers the factors contributing to exercise intolerance with particular reference to the respective roles of ventilatory limitation and skeletal muscle dysfunction. A review of the strategies studied or currently used to counter exercise limitation then follows. Chapter 2 describes the methodology of the assessments of exercise limitation used in this thesis, along with an evaluation of other currently available techniques.

Chapter 3 describes how different means of body composition evaluation can effect the functional conclusions made from the data obtained. Assessment of peak exercise capacity related to muscle mass was found to be underestimated by anthropometry in comparison to air displacement plethysmography or bioelectrical impedance. It is concluded that inter-method differences in estimation of fat-free mass are large enough to require that a single methodology of assessment is used for comparative evaluations.

Chapter 4 describes a trial of creatine supplementation, both in isolation and in combination with exercise training. Muscle mass, strength and endurance, and health status were all enhanced by creatine, with or without exercise training. Whole body exercise capacity, when examined by field and laboratory tests, was not affected. It is concluded that creatine may be a potentially useful ergogenic agent for use in COPD patients.

Chapter 5 describes a crossover study of formoterol, a long acting bronchodilator, in advanced COPD patients with poor spirometric response to bronchodilation. Formoterol is shown to decrease exercise induced dynamic hyperinflation and breathlessness during activities. An equivocal effect on exercise tolerance is demonstrated. This study supports the rational for the use of long acting bronchodilators in symptomatically limited COPD patients, largely irrespective of individuals lung function response to bronchodilator.

Chapter 6 considers the implications of the work performed and suggests lines of investigation for future research.

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LIST OF SYMBOLS AND ABBREVIATIONS

The following outline defines the key symbols and abbreviations used throughout the thesis.

Exercise and Gas exchange

VO_2 *Oxygen (O_2) consumption*; expressed in $ml.min^{-1}$

$VO_{2\ PEAK}$ *Oxygen consumption at maximal symptom limited exercise* expressed as $L.min^{-1}$ or body weight corrected as $mls.min^{-1}kg^{-1}$.

VO_2 AT *Oxygen consumption at the anaerobic threshold*; expressed as %predicted maximal oxygen uptake

VCO_2 *Carbon dioxide (CO_2) production*; expressed in $ml.min^{-1}$.

V_E Expiratory minute ventilation ($L.min^{-1}$.).

V_E/VCO_2 *Ventilatory equivalent for CO_2* - also called the ventilatory response expressed as $L.L^{-1}$ carbon dioxide production

MVV *Maximum voluntary ventilation;* a measure of a patients ventilatory capacity which can be measured directly or estimated by multiplying the FEV_1 by 40; expressed in litres

HR/VO_2 *Heart rate reponse on exertion*; expressed as beats L^{-1} oxygen uptake

O_2 Pulse *The amount of oxygen delivered for each heart beat* (surrogate marker of stroke volume) expressed as mls per beat

A-aO_2 *Alveolar-arterial oxygen gradient (*a measure of physiological shunting i.e.indication of lung units with low ventilation for their perfusion) expressed in kPa.

V_D/V_T *Dead space to tidal volume ratio* (also known as the physiological dead space i.e. indication of lung units with a high ventilation for their perfusion)

Lung Volumes and Forced Expiration

FVC *Forced vital capacity*: the volume of gas which is exhaled during forced expiration starting from a position of full inspiration and ending at full expiration

FEV_t *Forced expiratory volume in one second*: the volume of gas exhaled in the first second of the FVC manoeuvre.

FRC *Functional residual capacity*: the volume of gas present in the lung and airways at the average end-expiratory level.

RV *Residual volume*: the volume of gas remaining in the lung at the end of a full expiration.

TLC *Total lung capacity*: the volume of gas in the lung at the end of full inspiration.

Diffusion

TL_{CO} *Pulmonary transfer factor for carbon monoxide (CO) - also called pulmonary diffusing capacity*: the rate of carbon monoxide uptake from alveolar gas to pulmonary capillary blood; expressed in $mmol.min^{-1}.kPa^{-1}$.

K_{CO} *Transfer coefficient - also referred to as* T_L/V_A: TL_{CO} per unit alveolar volume (V_A); expressed in $mmol.min^{-1}.kPa^{-1}L^{-1}$.

Body Composition

ADP	Air displacement plethysmography
BIE	Bioelectrical impedance
ANTHRO	Anthropometry
FFM	Fat-free mass

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DECLARATION

The work described in this thesis was performed during a three year period of research in the Department of Respiratory Medicine, Glasgow Royal Infirmary and the Centre for Exercise Sciences, University of Glasgow. Except where specifically stated in the acknowledgement and in the text of the thesis, I have carried out all the experimental work, including the overall planning, design and coordination of the studies, all cardio-pulmonary exercise tests, some of the resting lung function tests, and all data collection and analysis. The conclusions and concepts advanced throughout the various parts of the thesis represent my interpretation of the results in the light of previous knowledge in the field.

CHAPTER 1

INTRODUCTION

This thesis concerns itself with the assessment and treatment of factors relating to exercise limitation in chronic obstructive airways disease (COPD). In this introduction, an overview of the disease COPD will be presented. Current and historical evidence for the features relating to exercise intolerance will be addressed. Since pulmonary rehabilitation is the current cornerstone of intervention for exercise intolerance its applications and effects will be discussed. In addition, a range of other interventions aimed at improving exercise tolerance are considered; namely dietary, hormonal and pharmacological means.

1.1 COPD overview

1.1.1 Definition

Chronic respiratory diseases became important to the UK medical fraternity following the disastrous smog pollutions affecting London in the 1950's. These caused increased sulphur dioxide levels that, when coupled with cigarette smoking, led to a high prevalence of chest problems. Following improved environmental conditions a decline in the prevalence of chronic bronchitis occurred. However it was during this time that there became an increased awareness of the significance of chronic airflow limitation. It was established in 1983 that mortality in patients with chronic respiratory disease was related to the degree of airflow obstruction rather than symptoms of mucus production, (Peto *et al.*, 1983).

Diseases that led to chronic airflow limitation became collectively referred to as "Chronic Obstructive Pulmonary Disease" (Fletcher & Pride, 1984). COPD is a chronic disease characterised by largely irreversible airways obstruction. It is a progressive and debilitating condition, with one of its hallmarks being exercise intolerance. Previously, COPD was commonly subdivided into emphysema or chronic bronchitis, whereas more recently efforts have been made to standardise classification of this disease (Hurd, 2000b). Publication of a series of international consensus statements makes it likely that a universal approach to the stratification and clinical management of COPD will be adopted (BTS COPD Guidelines Group, 1997a; Celli & MacNee, 2004; American Thoracic Society Standards, 1987; Pauwels *et al.*, 2001).

1.1.2 Epidemiology

COPD is at present the only major cause of mortality in the world to have a rising prevalence and by 2020 it is expected to become the fourth most common cause of death worldwide (Lopez & Murray, 1998). The health economic costs of this disease are placing ever-

increasing burdens upon national health services (Hurd, 2000a). Moreover, exacerbations of COPD account for at least 10% of wintertime admissions during periods of already great strain upon hospital services (Guest, 1999). COPD is usually secondary to tobacco smoking; the direct relationship between number of cigarettes consumed and degree of lung damage having been established over 30 years ago (Auerbach *et al.*, 1972). Tobacco clearly accelerates the annual decline in lung function associated with ageing in susceptible individuals (Fletcher & Peto, 1977). Cigarette use has increased in the western world since the Second World War and more recently increasing consumption of tobacco by women, particularly in the developing world is predicted to result in further rises in the incidence of chronic lung disease (Varkey, 2004). It has been estimated that 20% of those presently living in developed countries will be killed by tobacco consumption (Peto *et al.*, 1992). This clearly devastating impact upon society and individuals as a consequence of COPD has made treatment of the symptoms and attempted alteration of the prognosis an international focus of research (Hurd, 2000b).

1.1.3 Clinical overview

The disease is in part due to a chronic inflammatory reaction in response to the environmental antigen presented within the lungs. The inflammation is largely neutrophil mediated, with elastolytic enzymes such as elastase, contributing to the structural damage occurring (Stockley, 1994; Barnes, 2000). More recently lymphocyte driven chemokines have been implicated in the causation of airway inflammation (Cosio *et al.*, 2002). The impact of the inflammation is differing degrees of mucosal gland hypertrophy (chronic bronchitis) and alveolar tissue damage (emphysema). A consequence of these pathological processes is airflow limitation, caused by the loss of elastic tissue of the lungs, resulting in increased compliance and subsequently increased resistance of the airways (Heard, 1969). Although not mirroring factors such as exercise tolerance, declining forced expiratory volume (FEV_1) is a correlate of mortality (Peto *et al.*, 1983). With the inevitable decline of lung function with age, there is often an inexorable march towards disability and associated features of severe COPD in those with established disease, irrespective of smoking status.

COPD is a chronic condition with the cardinal feature of lung impairment. However it also displays features of multi-systemic disease in many cases, which contributes to the morbidity seen (Wouters *et al.*, 2002). Not uncommon are findings such as sarcopaenia, cardiac dysfunction and systemic inflammation. It is these very primary and ancillary features of the

disease that must be addressed in order to improve an individual's well-being or prognosis through intervention.

The pharmacological interventions utilised in COPD are costly and only partially efficacious, particularly in comparison to the impact these drugs have in asthma. Although the use of inhaled bronchodilators can alter spirometric measurements of lung function, their overall benefit is thought to derive more from the reduction both in dynamic hyperinflation and "work of breathing" that results from their administration (O'Donnell *et al.*, 2001a). Both long and short acting bronchodilators have proven benefits upon exercise tolerance (O'Donnell *et al.*, 1999; Ayers *et al.*, 2001). Long acting beta agonists may exert some benefit through improved ciliary motility (Johnson & Rennard, 2001). Recent large and well-performed studies have provided a rationale for the use of inhaled steroids in severe symptomatic COPD (Burge *et al.*, 2000; Calverley, 2000a). These have been followed by similarly designed multi-centre studies which demonstrate the additive benefit of combination treatments of long-acting beta agonist and inhaled steroid (Szafranski *et al.*, 2003; Calverley *et al.*, 2003a). There is also evidence for the administration of oral prednisolone and antibiotics during exacerbation (Davies *et al.*, 1999; Stockley, 1994). Even with acceptance that newer drugs such as long acting anticholinergics will improve on present pharmacological interventions, a certain pessimism is widespread (Calverley, 2000b; Barnes, 2001). All our medical treatments in COPD have failed to demonstrate benefit with regard to the cornerstone of intervention; namely alteration of prognosis or rate of decline of lung function. The only interventions that have been shown to impact upon mortality are long-term oxygen therapy and smoking cessation (Medical Research Council Working Party, 1981; Nocturnal Oxygen Therapy Trial Group, 1980). It is in this context of ineffective treatment for the established lung problems in COPD that rehabilitation and exercise strategies have become of immense interest to health care professionals and scientists (Lacasse *et al.*, 1996).

1.2 Pathophysiology of exercise intolerance

1.2.1 Introduction

The traditional view of the exercise limitation seen in COPD has considered ventilatory impairments to be the overwhelming and largely irreversible barrier (Leaver & Pride, 1971; Macklem, 1973). It is now known that through the physiological adaptations that take place with exercise training, beneficial impacts on exercise capacity can be achieved (Belman & Kendregan, 1982; Casaburi *et al.*, 1991). This implies that not all the factors leading to exercise intolerance are fixed and irreversible. It is evident that flow limitation and dynamic hyperinflation impair lung function, increase "work of breathing" and cause a ventilatory ceiling to be reached during exercise (O'Donnell, 2001). Although loss of a "breathing reserve" is evident in almost all exertions to limitation in COPD, there is now a greater understanding of the contribution of other impairments present in the disease.

The acceptance of a multifactorial cause for exercise limitation has furthered our understanding of the benefits that derive from interventions such as exercise training. As an example, subjects may have different total ventilatory requirements for a specific work rate due to variations in their lactate threshold, whereas their absolute oxygen uptake could be very similar. With endurance training, skeletal muscle adaptations can result in a decreased ventilatory requirement at sub-maximal work rates (Casaburi *et al.*, 1991; Puente-Maestu *et al.*, 2000) and improve the whole body physiological response to exercise.

1.2.2 Ventilatory system

1.2.2.1 Lung mechanics

In health, predictable and beneficial alterations to lung mechanics and breathing timing occur in response to increased ventilatory requirements. Effective minute ventilation (V_E, L/min) is a product of tidal volume (V_T) and breathing frequency (BF, breaths/min). During the transition of rest to moderate exercise the rise in ventilation is first achieved by an increase in tidal volume, to approximately 50% VC (Cotes, 1975). Following that there is a commensurate rise in V_T and BF. More latterly only BF is able to increase as higher ventilation is required (Spiro *et al.*, 1975). The initial increase in VT seen in health is a result of the increasing ratio of end inspiratory lung volume (EILV) to expiratory reserve volume (ERV), which has the important ancillary benefit of optimising the ratio of respiratory muscle power/length (Henke *et al.*, 1988). Furthermore there are alterations in the proportion of time

spent in the respiratory cycle on inspiration and expiration as work rates change. Increasing work leads to a predictable and progressively declining expiratory time (t_E) and increasing inspiratory time (t_I) as breathing frequencies increase to 50-60 breaths per minute (Neder *et al.*, 2003). The final major beneficial change affecting lung mechanics in health is exercise induced bronchodilatation, which decreases airway resistance and therefore resistive work of the lung is lowered (Warren *et al.*, 1984; Johnson *et al.*, 1999c; Johnson *et al.*, 1995). Despite these changes some increase in the elastic work of breathing is inevitable as the tidal volumes rise.

In COPD there are a number of factors present at rest that worsen on exercise and produce ventilatory impairment during exertion. Most simply there is flow limitation, such that as the ventilatory requirements rise the flow volume loop encroaches upon its maximum, particularly during expiration. This is the basis for the crudest, but most effective, tool for screening for ventilatory limits to exercise; calculation of the maximum voluntary ventilation (MVV).

MVV is calculated by multiplication of the FEV_1 by a fixed amount (either 35 or 40) or by measurement of the maximum ventilation achieved during a fixed period (15 seconds) multiplied by a factor of 4. The resulting difference in the calculated MVV and maximum ventilation measured during an exercise test will give a value for the "breathing reserve" (BR). This may be minimal in COPD but would be expected to be between 30 to 50% in health.

In COPD there is a correspondingly lower VT and higher BF for a given V_E than in health. The flow limitation seen in COPD prolongs T_E; the direct mechanism for the development of dynamic hyperinflation (DH) during exercise (O'Donnell *et al.*, 2001b). This directly places the respiratory muscles at a mechanical disadvantage with them being forced to work at a high proportion of their maximal capacity; through increased loading, physical lengthening and reduced strength at the larger lung volumes. A vicious cycle becomes evident as the required increased tachypnoea necessary to alter ventilation further worsens DH.

The changes described above clearly increase the elastic work of breathing during exercise in COPD patients. Furthermore the increased blood flow demands of the ventilatory muscles and the impaired venous return may compromise the oxygen delivery to the exercising muscles (Aliverti & Macklem, 2001). In particular, it is known in COPD that individual leg VO_2 can increase in response to improved oxygen delivery, and therefore that oxygen delivery to the lower limb muscles, perhaps altered by "respiratory steal", does play an important limiting role (Simon *et al.*, 2001).

Illustrated below are the lung volume changes seen in health and COPD during exercise.

Figure 1.1 Changes in operational lung volumes are shown as ventilation increases with exercise in patients with COPD and in normal subjects.

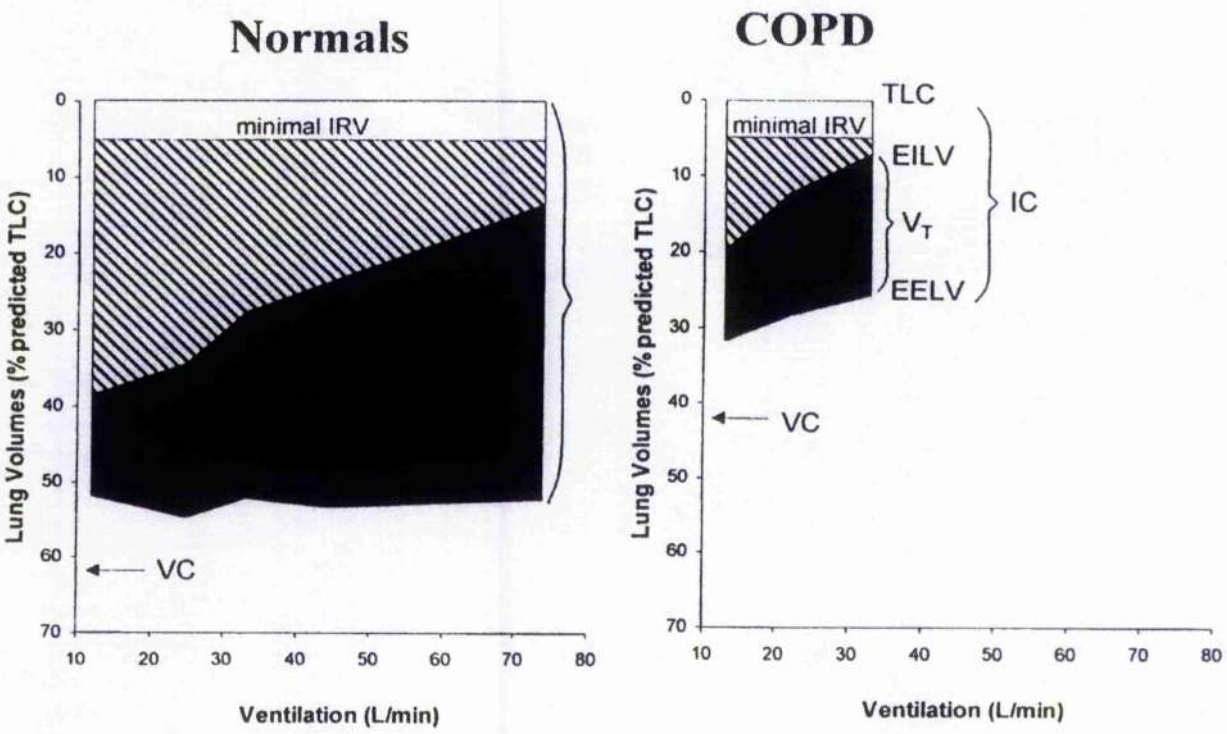


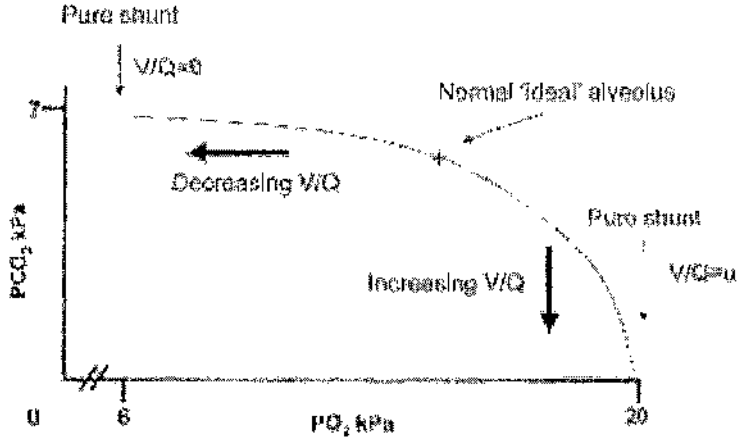
Figure 1.1 Changes in operational lung volumes are shown as ventilation increases with exercise in patients with COPD and in normal subjects. "Restrictive" constraints on tidal volume (VT, *solid area*) expansion during exercise are significantly greater in the COPD group from both below (reduced IC) and above (minimal IRV, *open area*). Taken from Dynamic Hyperinflation and Exercise Intolerance in Chronic Obstructive Pulmonary Disease (O'Donnell *et al.*, 2001b).

1.2.2.2. Ventilation-perfusion inequality

The ventilatory requirements for exercise in COPD are increased because of high ventilation/perfusion ratios and low tidal volumes that increase V_D/V_T, this promotes dyspnoea by compromising ventilatory capacity and preventing adequate alveolar ventilation. Moreover in COPD, and emphysema particularly, the pulmonary vascular bed is damaged which leads to decreased perfusion in certain areas of the lung and subsequently causes high V_D/V_T values especially on exertion.

The ventilation and perfusion (V/Q) inequalities within the lung lead to increasing alveolar-arterial pO_2 difference, assessed by concurrent arterial blood gas measurement with the knowledge of the inspired fraction of oxygen. Other measures of VQ mismatch approximate the degree of physiological shunting and alveolar dead space. Physiological shunting refers to the quantity of capillary blood that passes through the lungs without taking part in gas exchange. In figure 1.2 below (adapted from Rahn) this effect is illustrated by the horizontal left arrow (Rahn & Fenn, 1955). In quantifying alveolar dead space, one is considering that portion of lung that is ventilated without significant perfusion (vertical downwards arrow). This portion of lung is functionally the same as the anatomical dead space, and will consequently add to the volume of the physiologic dead space. In health the physiologic dead space is 30% (or less on exercise) of the tidal volume, whereas in COPD this volume can rise to 50% or more due to VQ mismatch.

Figure 1.2 A graphical representation of physiological shunting.



Taken from "A graphical analysis of the respiratory gas exchange". (Rahn & Fenn, 1955)

Related to the concept of VQ mismatch is the consideration of shunting. This represents blood in the systemic arterial system that has not passed through ventilated portions of lung. In health this occurs to a small degree because of the blood supply to the coronary and bronchial arteries. In COPD this can be seen due to extremes of abnormality in VQ, particularly in emphysema phenotypes. A particular feature of shunts is that they induce a hypoxia that is refractory to oxygen therapy, allowing their detection when subjects are given 100% oxygen to breathe.

1.2.2.3 Arterial hypoxaemia

An additional stimulus to ventilation and dyspnoea is the development of arterial hypoxemia during exertion. This results from an increased A-aO2 gradient and provides the best indication of abnormal pulmonary gas exchange. In normal subjects there is little rise in the A-aO2 gradient on mild to moderate exercise, indeed it typically falls over the lower work rates but has been demonstrated to increase during moderate and maximal exercise (Jones *et al.*, 1966; Wasserman *et al.*, 1967); while in health values of around 10 mmHg at rest are expected, at maximal exertion this rises to 15 mmHg in healthy young men (Wasserman *et al.*, 1999). However the A-aO2 gradient is characteristically larger at rest in COPD due to the perfusion of the relatively poorly ventilated airspaces, and can be somewhat unpredictable in its response to exercise, not infrequently rising to values around 50 mmHg (Wasserman *et al.*, 1999).

1.2.2.4 Dyspnoeagenesis

It is generally accepted that the subjective sensations of dyspnoea largely relate to corresponding increases in ventilation (Killian & Jones, 1994; Mahler *et al.*, 2001; Ward, 2000). This has largely been based on quantifying the rate of increase of dyspnoeic sensation through the use of visual analogue or Borg scales (Aitken, 1969; Borg, 1982). Although it is likely that the mechanisms contributing to the sensation of dyspnoea are multifactorial, it appears that afferent information from the lung and chest wall musculature is central. There is evidence that the resistive and elastic loads placed upon the chest wall directly influence dyspnoeagenesis through length-tension relationships or through absolute work loads of the respiratory musculature (Killian *et al.*, 1979; Killian & Jones, 1984). The carotid bodies and other peripheral chemoreceptors, such as ergoreceptors, are also likely to contribute to the sensation of dyspnoea (Ward & Whipp, 1989; Grieve *et al.*, 1999). Thereby, in COPD, the combination of increased work of breathing, hyperinflation and possibly skeletal muscle dysfunction could be seen to contribute to the uncomfortable, and often limiting, sensations of dyspnoea at rest and during exertion.

1.2.3 Skeletal muscle

Although the precise mechanisms contributing to skeletal muscle dysfunction in COPD remain elusive, the consensus of opinion is that skeletal muscle plays a pivotal role in the aetiology of exercise intolerance in COPD.

Several lines of investigation have produced evidence to clarify the existence of skeletal muscle problems in COPD, elucidating some of the potential causes and illuminating the impact of interventions on these changes. Whereas for many years the benefits of pulmonary rehabilitation have been recognised, without an explanation of how improvements occur, we are now in a position to attempt to isolate the meaningful alterations that take place. This is one focus of international research: to increase our understanding of muscle dysfunction in chronic disease (ATS/ERS Statement, 1999). The second widespread aim is to translate these advances into novel treatments (ATS/ERS Statement, 1999). In the sections below the structural and functional alterations seen in COPD muscle are discussed, with relation to the interventions utilised and postulated for this patient group.

1.2.3.1 Muscle structure

Muscle fibres tend to be arranged within separately functional units, each one comprising of a specific muscle type, innervated by a single motor neuron. Such fibres within one unit tend to be of the same muscle fibre type, either, slow twitch type (I) or fast twitch (type IIa or IId(x) – often called type IIb) fibres in humans. Fibre type distribution varies in health and such differences have been most closely observed from examinations of the vastus lateralis muscle. For, example endurance athletes have a high proportion (>90%) of type I (predominantly oxidative enzyme activity) whereas sprint runners may have only 20 to 30% type I, thereby having a greater percentage of glycolytic enzyme based fibres (Wasserman *et al.*, 1999).

Structural changes in the skeletal muscle of patients with COPD have been established through case-controlled investigations. A degree of atrophy of individual fibres has been noted, with suggestion that the severity of these changes correlates with loss of fat-free mass and FEV1 (Hughes *et al.*, 1983;Sato *et al.*, 1997). The correlations seen are used as evidence to demonstrate that muscle fibre atrophy underlies the loss of bulk and function seen in COPD. However, the role of atrophy in determining loss of specific function, for example, endurance activity is controversial. This has been shown to more likely relate to proportion of specific fibre types (Allaire *et al.*, 2004). Analysis of fibre type proportions has produced differing results from separate studies. From the quadriceps femoris, histological examination revealed no alteration in muscle fibre proportions, but atrophy of type II fibres (Hughes *et al.*, 1983). In contrast studies examining the *vastus lateralis* muscle have reported conflicting results. Initial investigations suggested a decreased proportion of type I fibres and associated increases in the proportion of type II fibres were present in COPD (Jakobsson *et al.*, 1990;

Whittom *et al.*, 1998). In support of this is a finding of increased proportion of myosin heavy chain type IIb isoform from the *vastus lateralis* in patients with COPD (Satta *et al.*, 1997). These features are in keeping with the notion of such changes representing poor aerobic function of the skeletal muscle. It is type I fibres (slow-twitch fatigue resistant) that are associated with prolonged aerobic activity and conversely type IIb fibres (fast twitch fatigable) are more suited to short bursts of anaerobic activity (Burke *et al.*, 1973). These findings have been more recently refuted in a well conducted investigation that took care to use appropriately matched subjects and utilised muscle fibre CSA rather than fibre diameter (Gosker *et al.*, 2002). The main finding was that atrophy occurs mainly in type IIa and IIb fibres. As additional evidence of alterations that are reasonably specific to these fibres, a selective loss of stainable activity of the oxidative enzymes was demonstrated. In association with atrophy there is a decreased capillarity of skeletal muscle; these changes have been postulated to represent poor aerobic ability resulting from a hypoxic environment (Jobin *et al.*, 1998; ATS/ERS Statement, 1999).

While atrophy is an accepted finding in COPD muscles it appears that the individual muscle fibre type proportions seen are variable and more closely relate to endurance activity of the muscle groups (Allaire *et al.*, 2004).

1.2.3.2 Muscle metabolism

The terminal phosphate bond of adenosine triphosphate (ATP) is the essential source of energy for skeletal muscle contraction. The energy provided by breakage of this bond is directly used by the myosin/actin cross bridges to enact the functional changes resulting of shortening or increasing tension of muscle. ATP can be regenerated predominantly through aerobic (oxidation of carbohydrates and transfer of energy utilising a proton shuffle) and anaerobic (breakdown of pyruvate to lactate without use of oxygen) glycolysis. The former is much more efficient; however the latter becomes a necessary component as energy demands rise. Phosphocreatine (PCr) breakdown within the skeletal muscle allows a ready source of ATP as the "oxygen deficit" incurred by the rapid energy demand is met. PCr exists in a state of flux with creatine and inorganic phosphate, the Lohmann reaction, catalysed by creatine kinase.

Along with the structural changes noted, there are alterations in metabolic profiles within the skeletal muscle of patients with COPD. Muscle homogenate from the *vastus lateralis*, as well as the staining methods already mentioned, compared to aged matched normal subjects, demonstrated reduction in levels of enzymes associated with aerobic glycolysis. These

included decreases in citrate synthase (CS), succinate dehydrogenase and 3-hydroxy-CoA dehydrogenase (HADH) (Jakobsson *et al.*, 1995; Maltais *et al.*, 1996c). More recently it has been demonstrated that CS levels correlate with functional status, including VO_{2PEAK} and peripheral isometric endurance muscle function, in patients with COPD (Allaire *et al.*, 2004;Maltais *et al.*, 2000). In examination of anaerobic enzyme levels, there has been no decrease demonstrated, in comparison to normal subjects, (Maltais *et al.*, 1996c). Lactate dehydrogenase and hexokinase amounts were normal, phosphofructokinase levels were increased possibly suggesting augmented anaerobic activity (Jakobsson *et al.*, 1995).

As far back as 1977 it was determined that patients with COPD had lower resting levels of phosphocreatine than normal subjects (Gertz *et al.*, 1977). More recently, through work utilising the technique of phoshorous-31 magnetic resonance spectroscopy, the characteristics of phosphocreatine utilisation in COPD have been clarified. This involves the performance of exercise within a magnetic resonance scanner, allowing determination of tissue pH, adenosine triphosphate, phosphocreatine and inorganic phosphate. From the values determined it is possible to quantify rates of PCr level alteration. Consistently it has been demonstrated that patients with COPD more rapidly deplete PCr than age matched normal controls at the onset of exercise (Thompson *et al.*, 1993; Payen *et al.*, 1993; Kutsuzawa *et al.*, 1992). These investigations and one other study also showed slowing of recovery of PCr levels following exercise (Mannix *et al.*, 1995). Latterly there has been evidence produced to suggest that phosphocreatine utilisation and resynthesis, as determined by magnetic resonance spectroscopy, mirrors oxygen uptake kinetics in normal subjects (Rossiter *et al.*, 1999). This finding rests comfortably with concepts such as the "oxygen deficit" which relate to that amount of work performed utilising anaerobic energy sources. However in subjects with COPD the evidence suggests that there are significant differences in oxygen kinetics as compared to normal subjects. Although "on and off symmetry" is maintained during moderate exercise the "time constant" (τ) is markedly prolonged (Nery *et al.*, 1982a; Puente-Maestu *et al.*, 2003; Sala *et al.*, 1999a). These findings may relate to alterations in oxidative metabolism within the muscle and there is considerable evidence of this occurring (Maltais *et al.*, 2000; Maltais *et al.*, 1996c).

1.2.3.3 Muscle mass and function

Weight loss is a recognised feature of COPD. It is well established that compared to normal controls, body weight and body mass index are decreased (Schols *et al.*, 1993a). The importance of this manifestation is established through studies that have correlated body composition with overall prognosis (Engelen *et al.*, 1994; Wilson *et al.*, 1989a; Marquis *et al.*, 2002). There is a large body of evidence concluding that muscle mass in COPD is decreased compared to aged matched subjects. Anthropometrical data suggests a high incidence of loss of fat free mass in subjects with COPD (Schols *et al.*, 1991b; Schols *et al.*, 1993b; Engelen *et al.*, 1994). As part of a study utilising MR spectroscopy, the investigators were able to demonstrate significantly smaller calf muscle cross sectional area compared to age matched subjects (Wuyam *et al.*, 1992).

In particular, the relationship between body mass and peak exercise capacity is clear and positive (Wilson *et al.*, 1989b; Gray-Donald *et al.*, 1989; Palange *et al.*, 1995). The degree by which body composition directly impacts upon exercise capacity has therefore been investigated. Fat free mass, a surrogate of muscle mass, can be estimated through techniques such as 4 site skin fold thickness, dual-energy X-ray absorptiometry and bioelectrical impedance. All these methods have been validated for use in COPD (Engelen *et al.*, 1998). Initial investigations demonstrated that FFM correlated better with exercise capacity, represented by 12 minute walking distance to VO_{2PEAK} on a ramp incremental test, than body weight (Schols *et al.*, 1991a; Baarends *et al.*, 1997a). Moreover there is evidence to suggest that FFM depletion contributes to reduced aerobic capacity, blunted tidal volume responses to exertion and lower peak oxygen pulse (Baarends *et al.*, 1997a). Further evidence that sarcopenia is involved in inducing early onset of anaerobic glycolysis is formed from the negative correlation between nutritional status and the speed of oxygen uptake kinetics, as assessed by mean response times (Palange *et al.*, 1998).

Functional studies have demonstrated impairment of skeletal muscle strength and endurance in patients with COPD. There is consistent evidence of lower limb weakness (predominantly quadriceps), usually in combination with loss of muscle mass (Gosselink *et al.*, 1996; Engelen *et al.*, 2000). After matched exercise protocols involving cycle ergometry or twitch stimulus, with healthy controls, subjects with COPD have been demonstrated to have increased quadriceps fatigability (Mador *et al.*, 2003b; Mador *et al.*, 2003a). Moreover, recently it has been demonstrated that impaired quadriceps endurance was present in a COPD

cohort and that the degree of impairment was unrelated to disease severity or strength of the same muscle group (Coronell *et al.*, 2004).

There is substantial consensus now that skeletal muscle dysfunction is a major contributor to exercise intolerance as evident through the residual impairment seen following lung transplant and the established improvements in exercise capacity following training (Casaburi, 2000; Gosker *et al.*, 2000). There are physiological alterations (raised lactate threshold, increased maximum oxygen uptake, speeded oxygen kinetics) seen following exercise training that can predominantly be attributed to changes within the skeletal muscle (Puente-Maestu *et al.*, 2000; Casaburi *et al.*, 1997). These markers have implications about fibre type changes or metabolism and therefore have direct relationships to skeletal muscle function. Otherwise the only means of determining whether changes have taken place at the level of the skeletal muscle would be through invasive means such as biopsy. Lactate rises indicate a reliance on anaerobic means of energy production and decreases following interventions such as exercise training tell us that alterations in the function of the skeletal muscle have taken place (Casaburi *et al.*, 1991).

1.3 Interventions in COPD for exercise intolerance

1.3.1 Exercise training- strength and endurance

1.3.1.1 Introduction

The role of rehabilitation in the care of patients is established, and coupled with an ageing population, is growing in importance. Exercise based interventions performed in subjects with pulmonary, cerebrovascular disease, ischaemic heart disease and cardiac failure have proven efficacy and are integral to current standards of care (Knekt *et al.*, 1988; Cuzick *et al.*, 1988). Rehabilitation involves the restoration of, or towards, "normality"; in this context from a diseased state. Pulmonary rehabilitation is an established method of successfully addressing some of the debilitating features of COPD. Over 20 years ago the improvements in exercise tolerance and associated reduction in perception of dyspnoea were accepted to be benefits of pulmonary rehabilitation(MacDonell, Jr., 1981; Fergus & Cordasco, 1977). Through recently published consensus guidelines, the importance of rehabilitation in appropriately caring for

those with chronic lung disease has been firmly established(Celli, 1997; ACCP/AACVPR Guidelines, 1997c; Weise, 2000). A standard pulmonary rehabilitation programme would now be expected to produce enhancements in endurance ability, perception of breathlessness at rest and in response to exercise, and health status. These benefits have also been demonstrated in chronic lung diseases other than COPD such as bronchiectasis and fibrosing alveolitis (Foster & Thomas, III, 1990). Although there has long been acceptance of some role for rehabilitation in COPD and other diseases, the scientific basis on which to justify providing this service was lacking. However there is now a clear understanding that certain components of rehabilitation, particularly exercise training, lead to discernable physiological benefit (Casaburi *et al.*, 1997; Maltais *et al.*, 1996b; Sala *et al.*, 1999b). Also rehabilitation induces fatigue resistance within the exercised muscles (Mador *et al.*, 2001). This overview considers current understanding of the effects, particularly physiological, induced by rehabilitation, the most effective means of attaining these changes and the prospects for improved rehabilitative strategies.

1.3.1.2 Exercise components

Rehabilitation can take many different forms, with some common features deemed essential for success. At the core of those beneficial programmes, as determined by randomised controlled trials, are structured exercise training components (Lacasse *et al.*, 1996). This training is efficacious when provided during inpatient stays, through home based programmes or, most commonly, as part of an out patient developed programme (Strijbos *et al.*, 1996; Singh *et al.*, 1998a). There is uncertainty concerning the optimal duration of a course of rehabilitation. There is evidence to suggest that a successful inpatient programme may last just a few weeks, whereas some outpatient courses last 3 months (Casaburi *et al.*, 1997; Cambach *et al.*, 1997). It seems logical that benefits are more likely to be sustained if lifestyle adaptations, allowed by the increased exercise capacity, occur.

A core principle of exercise training, in health and disease, is that training effects are task specific, and therefore the greatest benefits are usually to be found if one uses the same instrument for assessment as used for training (Sale & MacDougall, 1981; Jones & Rutherford, 1987). It is essential to direct the training tasks clearly toward the desired goals. In the context of chronic disease, the main emphasis of studied interventions has been toward improving endurance activities, for example, walking ability. It is through these simple

evaluations that overall benefit from rehabilitation programmes have been firmly established (Lacasse *et al.*, 1996). This almost universal focus in combination with obvious convenience has meant that supervised walking, perhaps using a treadmill, and cycle ergometry are invariably included in the design of rehabilitation interventions. There is evidence to support the training specificity of upper or lower limb endurance activities in COPD, with crossover benefit being demonstrated between differing lower limb endurance training modalities (Cambach *et al.*, 1997).

1.3.1.3 Endurance training

Intensity of training is a highly contentious issue as it can relate to a number of parameters, such as degree of work performed, physiological responses to such loads or perceived exertions. The appropriate intensity of exercise training has been examined by assessing which levels of work result in physiological adaptations. It may be more beneficial to train patients at a level above the lactate threshold (Casaburi *et al.*, 1989; Casaburi *et al.*, 1991). Such training enables physiological changes to occur: shortening of oxygen uptake kinetics times, increase in the lactate threshold (θ_L), and decrease lactate values and ventilation demands for a given supra-θ_L work rate (Casaburi *et al.*, 1991; Maltais *et al.*, 1996a). However there is clear evidence that a variety of exercise interventions, at differing training intensities also result in physiological benefit (Clark *et al.*, 1996). Even with low intensity training exercise benefits are seen, probably relating to tolerance of dyspnoea and factors such as improved confidence (Gimenez *et al.*, 2000). Therefore in practice there is support for a number of strategies for the setting of work performed; through intensity or duration, usually set by work rate, treadmill speed or heart rate. Dyspnoea closely mirrors intensity ratings and is a practical alternative (Stulbarg *et al.*, 1999; Horowitz *et al.*, 1996). In one randomised study the use of a fixed exercise duration with gradually increasing work rates towards a target over the course of training was shown to have benefit (Troosters *et al.*, 2000). Conversely there is proven benefit for setting high work intensities and targeting towards increased work duration (Punzal *et al.*, 1991; Strijbos *et al.*, 1996). Although high intensity training (80% peak work rate) is regarded as the present gold standard for exercise prescription, more severe COPD patients cannot sustain this workload through a rehabilitation programme(Maltais *et al.*, 1997; Casaburi *et al.*, 1997). An alternative strategy involves short burst high intensity work rates, which have recently been demonstrated to provide similar benefit (endurance exercise time, health status) to continuous training at

identical total work (Vogiatzis *et al.*, 2002). Also the chronic application of functional electrical stimulation of the quadriceps muscle has been shown to cause improvements in symptom-limited oxygen uptake in a severely debilitated group (Neder *et al.*, 2001a). In particular, the active treatment group were then able to enter the standard pulmonary rehabilitation programme following the electrical stimulation.

1.3.1.4 Strength Training

Strength training is feasible and safe in patients with COPD (Kaelin *et al.*, 1999). Isolated strength training of *quadriceps femoris* has been demonstrated to improve endurance exercise time, muscle endurance and health status (Clark *et al.*, 2000; Simpson *et al.*, 1992). Unfortunately these studies were not able to demonstrate benefit in maximal exercise capacity. However the relative importance of lower limb strength intervention, in combination with endurance training has not yet been clearly established. Although increased muscle bulk and strength are achievable there is no clear additional benefit gained in the standard outcome measures of pulmonary rehabilitation (Bernard *et al.*, 1999a).

1.3.1.5 Respiratory muscle training

Lower limb, skeletal muscle training is an essential component of a successful pulmonary rehabilitation programme, however there is no established requirement for respiratory muscle training. Consensus statements from the American College of Chest Physicians and the American Thoracic Society have concluded that further evidence is required before it is appropriate to recommend this approach (1997c;Weise, 2000).

In conjunction with disabling leg effort, dyspnoea is the main limitation to exercise in patients with COPD (Hamilton *et al.*, 1996). In contrast to the skeletal muscles, the respiratory musculature is continually working, often under stress. Such an environment has been demonstrated to alter the relative fibre type composition in COPD patients: the diaphragm has a higher proportion of type I (slow twitch, endurance) fibres (Levine *et al.*, 1997). However the factors that predispose to systemic muscle dysfunction, such as malnutrition and hypoxia, remain present. Respiratory muscle function in COPD patients is thereby frequently found to be subnormal. This is manifested through decreased maximal expiratory and inspiratory pressures and reduced endurance as measured by 2 minute threshold tests (Morrison *et al.*, 1989; Gosselink *et al.*, 2000; Wijkstra *et al.*, 1995). There is

also a clear mechanical disadvantage, at rest and exercise, for the respiratory muscles; hyperinflation flattens the diaphragm and impairs peak force generation of the muscles, particularly for inspiratory manoeuvres (O'Donnell, 2001). With bronchodilation and cumbersome therapies such as heliox inhalation or the use of ventilatory support, the main means available to relieve the mechanical impairment, any approach which may be favourable to breathing patterns or expiratory flow capability could potentially provide benefit. Patients with COPD have established airflow obstruction, resulting in prolongation of the total expiratory period (T_E) in comparison to the inspiratory time (T_I) (O'Donnell *et al.*, 2001b). The increased T_E/T_I and rising ventilatory demands during exercise result in dynamic hyperinflation and the associated factors that are so detrimental to exercise performance.

It is established in health that directed respiratory muscle training can result in improvements in strength or endurance (Leith & Bradley, 1976). Similar benefits, usually targeting strength training, have been demonstrated in COPD (Larson *et al.*, 1988; Harver *et al.*, 1989; Lisboa *et al.*, 1995; Dekhuijzen *et al.*, 1991). This has largely been through the use of handheld resistive devices, whereby repeated manoeuvres are performed against a closed shutter on inspiration or through inspiratory flow limitation. The former provides only short bursts of training and has been largely replaced by the more efficacious flow-limiting devices. With respiratory muscle training as a sole intervention, unfortunately often without controls, there have been favourable, but inconsistent beneficial outcomes demonstrated for impact upon exercise capacity (Dekhuijzen *et al.*, 1990; Larson *et al.*, 1988; Belman & Mittman, 1980) and dyspnoea ratings (Harver *et al.*, 1989; Lisboa *et al.*, 1995) in patients with COPD. The impact upon health status has been poorly addressed, however one study of resistive inspiratory loading has been able to demonstrate benefits on maximal inspiratory pressures, SWT distance at study completion and also dyspnoea and health status at 6 month follow up (Sanchez *et al.*, 2001). Unfortunately, any demonstration of improved respiratory muscle function has consistently failed to provide additional benefit in conjunction with exercise training (1997c). The positive studies that exist represent a small cohort of studied patients (Wanke *et al.*, 1994; Weiner *et al.*, 1992), and larger investigations are needed before this treatment can be routinely recommended.

1.3.1.6 Supplementary oxygen

Exercise induced hypoxemia is a common feature of patients with severe COPD, occurring during many activities of day-to-day life (Soguel *et al.*, 1996). Long-term oxygen therapy in hypoxaemic COPD patients has proven physiological benefit; including normalisation of polycythaemia, improved exercise performance, and reduced pulmonary hypertension (1980;1981; Weitzenblum *et al.*, 1991). There are a number of good reasons why supplementary oxygen would have an immediate beneficial effect upon exercise capacity. Oxygen alters the sensations of dyspnoea, probably through a combination of central means (eg silencing of ongoing drive from carotid body chemoreceptor with increased PaO_2) (Ward, 2000; Whipp & Ward, 1992) and the consequent decreased ventilatory requirement (Swinburn *et al.*, 1991). The lowered pulmonary artery and intra thoracic pressures, subsequently decreasing both right and left ventricular afterloads, may improve cardiac output. In addition myocardial oxygen delivery is enhanced. It directly increases oxygen delivery to the stressed muscles through raised arterial pO_2 and indirectly through increased cardiac output. However increasing fraction of inspired oxygen (and increased pO_2) can cause increased vascular resistance in tissues such as skeletal muscle. This may partially offset the effect of increased pO_2 on oxygen delivery. Interestingly an examination of lactate and oxygen uptake kinetics during transitions to constant work rate tests of moderate intensity in COPD patients failed to demonstrate any alteration of these during supplemental oxygen delivery (Somfay *et al.*, 2002). This was despite significant reductions in levels of ventilation. However it is established that below the anaerobic threshold VO_2 kinetics in normal subjects are not affected by alterations in levels of ventilation (Diamond *et al.*, 1977). The authors concluded that the decreased ventilatory stress was more related to the impact of oxygen upon dyspnoea sensation than a later onset of anaerobiosis and lactate formation. Placebo controlled studies of oxygen supplementation have demonstrated improved endurance exercise capacity irrespective of the presence of exercise induced hypoxaemia (Revill *et al.*, 2000; Somfay *et al.*, 2002). In particular, a dose-response relationship was evident up to 50% FiO_2 in non hypoxemic patients upon endurance capacity, degree of dynamic hyperinflation and associated dyspnoea ratings (Somfay *et al.*, 2002). In attempting to elucidate the primary mechanism by which oxygen supplementation aids exercise capacity, it has been demonstrated that increased FiO_2 leads to an associated increase in leg blood flow (measured a thermodilution catheter) and leg oxygen uptake (by arterial and venous blood gases coupled with leg blood flow), thereby facilitating the improved exercise capacity(Maltais *et al.*, 2001).

This signifies that the lower limb musculature is operating in COPD patients with a performance reserve in situations without oxygen supplementation. It also may be postulated that the decreased ventilatory requirements for a given exercise, in the presence of oxygen, allows blood flow redistribution to the oxygen requiring skeletal muscles (Simon *et al.*, 2001).

Although the benefit in the short term is established for improved exercise capacity with oxygen supplementation, there is no consensus as to whether it is appropriate in an exercise-training situation. A controlled trial of oxygen vs. air supplementation in severe COPD subjects, with exercise hypoxemia, undergoing pulmonary rehabilitation demonstrated benefit solely in dyspnoea ratings (Garrod *et al.*, 2000). There was no improved outcome in the oxygen supplementation group with regard to health status or exercise capacity. This may relate to the reduction of muscle hypoxia, thereby possibly alleviating an important training stimulus (Sundberg *et al.*, 1993). However one may have expected the higher training intensities achievable to be beneficial to the rehabilitation outcomes (Casaburi *et al.*, 1991). This has been tested more latterly in a double blind placebo controlled trial of oxygen therapy in non hypoxemic patients undergoing exercise training (Emtner *et al.*, 2003). Significant increases in the training intensities achieved were demonstrable in the oxygen administered group. Subsequent improvements in exercise capacity and breathing rate were evident.

1.3.1.7 Comprehensive rehabilitation

It is usual for additional components to be incorporated into the rehabilitation programme structure for COPD. These often include disease education, psychological counselling, dietary advice and social support. Although these aspects of rehabilitation are standard, the accepted benefits in dyspnoea and quality of life have not been shown to occur with programmes lacking in exercise training (Benzo *et al.*, 2000; Devine & Pearcy, 1996; Ketelaars *et al.*, 1996; Sassi-Dambron *et al.*, 1995). This leaves an uncertain role for these ancillary aspects of rehabilitation programmes.

1.3.1.8 Conclusion

Standard pulmonary rehabilitation programmes aimed at patients limited symptomatically by their COPD have been shown to have benefits that will last at least one year following rehabilitation (Troosters *et al.*, 2000; Guell *et al.*, 2000; Griffiths *et al.*, 2000). These long-

term benefits include significantly increased walking endurance, sustained improvements in health status and decreased total hospital inpatient days. Whether there may be a demonstrable survival benefit has yet to be established.

The evidence base for this approach to chronic lung disease has demonstrated pulmonary rehabilitation to be most effective when compared to programmes of exercise training employed in other chronic diseases. The type of patients enrolled in pulmonary rehabilitation are typically debilitated and more severely affected by their illness than patients in comparative cardiac schemes (Reardon *et al.*, 1995). And while there is evidence that pulmonary rehabilitation programmes are cost effective, pulmonary rehabilitation is unfortunately significantly less well resourced than equivalent schemes in other disease states (Scherer & Schmieder, 1998; Reina-Rosenbaum *et al.*, 1997; Parish *et al.*, 1995).

1.3.2 Dietary

It is an appealing proposition to combine rehabilitation with other strategies to improve well-being in COPD. The structured nature of the standard pulmonary rehabilitation enables the compliance and efficacy of any additional intervention to be assessed more easily. A number of investigators have utilised such approaches when trying to optimise rehabilitation outcomes.

As previously described, anorexia, cachexia, sarcopaenia and skeletal muscle dysfunction are crucial problems associated with severe COPD. A number of studies have attempted to counter these problems with nutritional supplementation. A recent meta-analysis of these approaches concluded that it was not easy to demonstrate success in gaining weight or muscle mass in these patients (Ferreira *et al.*, 2000a). Attempts to increase body weight through outpatient nutritional intervention proved difficult to demonstrate increases in FFM and did not impact upon exercise tolerance (Efthimiou *et al.*, 1988; Sridhar *et al.*, 1994). Although dietary changes may be able to alter morbidity in COPD patients, it is generally thought that the difficulty in achieving such positive outcomes will preclude recommendation of such approaches (Braun *et al.*, 1984). Nevertheless, there are data suggesting some benefit in these approaches. Nutritional supplementation is proven in aiding weaning of patients with respiratory failure from mechanical ventilation (Bassili & Deitel, 1981). An inpatient programme has proven to be more successful; leading to increased muscle mass, strength and exercise tolerance, albeit requiring a three month intervention period (Rogers *et al.*, 1992).

1.3.2 Hormonal

There is demonstrated deficiency of certain hormones in patients with COPD (Casaburi, 1998). Some of these, such as insulin like growth factor-1 (IGF-1) and testosterone, have established anabolic influences in healthy subjects. These hormones, when administered to healthy people, lead to skeletal muscle changes; randomised controlled trials of testosterone injections have demonstrated increased lean body mass and improved muscle strength (Giorgi *et al.*, 1999; Bhasin *et al.*, 1996). In COPD, the effects of rehabilitation on skeletal muscle are wide ranging, but without strength training appreciable muscle fibre hypertrophy does not occur. There is therefore a rationale for using anabolic steroids in chronic lung disease (Creutzberg & Schols, 1999). In placebo controlled, randomised trials, nandrolone and stanozolol, have been demonstrated to increase fat free mass (Schols *et al.*, 1995a; Creutzberg *et al.*, 2003; Ferreira *et al.*, 1998a). Nandrolone led to a comparatively higher fat free mass compared to the control group, with both groups being administered dietary intervention. Respiratory muscle strength was significantly improved in the group receiving the anabolic steroids. Unfortunately there is no clear benefit with respect to endurance capacity or health status. As with anabolic steroid administration in athletes, there is concern as to the safety of such interventions (Bagatell & Bremner, 1996).

Growth hormone administration is a strategy that has been tried in health, cachexial diseases such as AIDS and in COPD patients. In the only placebo controlled study and in combination with exercise training, growth hormone has been shown to lead to comparatively higher increases in fat free mass and resting energy expenditure, with no demonstrable translation into improved muscle strength or exercise capacity (Burdet *et al.*, 1997).

1.3.4 Pharmacological

Several authors have shown that increased exercise capacity after bronchodilator correlates closely with less dynamic hyperinflation (DH) and breathlessness, especially during endurance exercise: these studies used salbutamol (Belman *et al.*, 1996), salmeterol (O'Donnell *et al.*, 2004b) and anticholinergics (O'Donnell *et al.*, 1999; O'Donnell *et al.*, 2004a). However the more fundamental question of whether these inhaled medications are able to alter exercise capacity remains largely unanswered. A meta-analysis of bronchodilator therapies in relation to exercise capacity concluded that there was insufficient evidence to show benefit from these strategies (Liesker *et al.*, 2002b). However they did specifically

indicate that there is a lack of published data utilising more sensitive indices of exercise tolerance, such as constant load cycle ergometry. Despite this, there is evidence through randomised controlled trial that in comparison to placebo, agents such as ipratropium can impact upon exercise capacity (Liesker *et al.*, 2002a).

1.4 Summary

COPD is a common condition with a major global impact upon health. Exercise intolerance is one of the most important manifestations of this disease. The aetiology of this is multifactorial and relates to lung function abnormalities, which can be static and dynamic in nature. The systemic effects of COPD also play an important role in the causation of exercise impairment. Strategies to improve exercise tolerance have targeted either the skeletal muscle through hormonal, dietary and physical means or by altering lung function through the use of pharmacological agents.

The first study in this thesis addresses evaluation of fat free mass estimation and its subsequent clinical relevance. The second study investigates the utility of creatine supplementation in COPD. The third study examines the effects seen of a long acting bronchodilator upon dynamic hyperinflation and exercise capacity.

This work represents investigations of novel assessments and interventions in COPD patients in relation to the diminished exercise capacity that they present.

CHAPTER 2

GENERAL METHODS

2.1 Subjects

All patients taking part in the investigations detailed in this thesis had an established clinical and functional diagnosis of moderate-to-severe COPD (FEV_1 < 60% predicted and FEV_1/FVC ratio < 70%) (1995). Patients were recruited from the Pulmonary Rehabilitation Assessment Clinic at the Department of Respiratory Medicine, Glasgow Royal Infirmary. Inclusion criteria were absence of locomotor or neurological diseases, and no change in medication dosage or exacerbation of symptoms requiring oral prednisolone or antibiotics in the preceding 4 weeks. All patients were optimised in terms of standard medical therapy: maintenance medication included β_2-agonists, anticholinergics, theophylline or inhaled steroids.

Fully informed written consent was obtained in all patients for each study. The Research Ethics committee of the Glasgow Royal Infirmary approved all the studies.
The following details the standard protocols of the procedures performed during the investigations described in chapters 3 to 5.

2.2 Procedures

2.2.1 Pulmonary function

2.2.1.1 Spirometry

Spirometry usually involves the performance of maximal inspiratory, then exhalatory manoeuvres through a pneumotach. The volume exhaled in the first second is known as the forced expiratory volume (FEV_1), with the total volume of air exhaled known as the forced vital capacity (FVC). The FEV_1 and ratio of FEV_1/FVC are reduced in COPD. These procedures are essential for the diagnosis of COPD, and FEV_1 remains a strong independent predictor of mortality, but they have less clear longitudinal value due to their weak relationship to most disease outcome measures (Fletcher & Peto, 1977; Hodgkin, 1990; Anthonisen *et al.*, 1986; Corris, 1995). Flow volume curves have a characteristic shape in COPD, representing the reduced maximal flow rates in comparison to lung volume and a "scooped-out" appearance due to airways collapse following the peak flow rates.

Standard spirometry was measured in our studies using the flow-volume module of a constant volume body plethysmograph (V6200 Autobox, SensorMedics Corporation, California USA). Flow measurements were carried out using a calibrated pneumotach. Patients

completed at least three acceptable maximal forced and "slow" expiratory manoeuvres. The following spirometric variables were recorded and expressed as BTPS values: Forced Vital Capacity (FVC), Forced Expiratory Volume in the 1st second (FEV_1) (*l*), Inspiratory Capacity (IC). Spirometric tests were performed before and 20 min after 400 μg of inhaled salbutamol: an FEV_1 increase equal to or higher than 12% or 200 ml of control considered a positive response to the bronchodilator.

2.2.1.2 Plethysmography

Body plethysmography enables total gas volume within the lung (including gas trapped behind closed airways) to be quantified. It utilises Boyle's law which states that volume*pressure is constant for a gas at constant temperature. Subjects performing respiratory efforts against closed shutters, in a sealed container, enable total lung volume inferences to be made from small lung volume and pressure changes.

2.2.1.3 Lung Diffusion Capacity for Carbon Monoxide (DL_{CO})

Lung diffusing capacity (DLCO) represents a measure of the combined properties of area, thickness and diffusion of the lung and relevant gas (Krogh, 1914; Wagner, 1977). Carbon monoxide is used most commonly as its transfer across the alveolar membrane is limited solely by diffusion, with blood flow having no influence. As there is a negligible amount of carbon monoxide in capillary blood, the DLCO can be derived solely from the volume of carbon monoxide transferred.

DL_{CO} was measured by the modified Krogh technique (single-breath, SB) using a computer-based automated system (Vmax29 System™ , SensorMedics Corp.) (Gardner *et al.*, 1988). The procedure was explained to the patients and demonstrated by a designated technician prior to testing. The patients did wear noseclips and were in the seated position. After exhaling to RV, patients inspired a vital capacity breath of a pre-mixed gas mixture (0.3% carbon monoxide, 10% helium, 21% O_2, balance N_2) from the system to TLC, and then held their breath for 10 seconds. Patients then exhaled a fixed washout volume (750 ml), and a sample of alveolar gas (500 ml) was taken for analysis using a multiple-gas chromatographic analyzer. DL_{CO} was then calculated as:

DL_{CO} (*ml CO/min/mmHg STPD*) = VCO/PACO-PcCO

where VCO is the pulmonary uptake of carbon monoxide (CO) (*ml STPD*), PACO is the mean alveolar CO partial pressure (*mmHg*), and PcCO is the mean pulmonary capillary CO partial pressure (*mmHg*) - assumed to be negligible (i.e. the affinity of Hb for CO is high, and carboxyhaemoglobin levels are very low). Computation of VCO and PACO were obtained from measurements taken across the breath-hold and an independent measurement of alveolar volume (VA) by the single-breath He dilution technique (Gardner *et al.*, 1988; Cotes *et al.*, 1993).

The following additional technical aspects were standardised (Gardner *et al.*, 1988; Cotes *et al.*, 1993): inspired volume higher than 90% of VC and attained in less than 2.5 seconds; timing from the beginning of inspiration to the beginning of alveolar sample, and both Mueller and Valsalva manoeuvres were avoided by instructing the patients to perform a relaxed breath-holding manoeuvre. At least two tests were performed, with the results being within 10% or 3 ml CO/min/mmHg STPD, whichever is greater.

2.2.1.4 Maximal Respiratory Pressures

Maximal inspiratory pressure (MIP) followed by maximal expiratory pressure (MEP) were obtained from RV and TLC, with the patients seated with a nose clip and a rigid, plastic flanged mouthpiece in place. The patients were connected to an electronic shutter apparatus (Sensor Medics Corp.; measurement range: ± 300 cm H_2O). A small leak was introduced between the occlusion and the mouth in order to prevent glottic closure; in addition, the patients supported their cheeks with one hand during the manoeuvre. Inspiratory or expiratory effort was sustained for at least one second. The measurements were undertaken by two designated technicians who explained and demonstrated the correct manoeuvre. The patients performed three to five acceptable and reproducible maximal manoeuvres (i.e. differences between values within 10%): the recorded value being the highest unless this is obtained from the last effort (Celli, 1989). An interval of about one minute will be interposed between efforts.

2.2.2 Body composition

Background

Weight loss and particularly sarcopenia are independent predictors of mortality in COPD patients (Schols *et al.*, 1998a; Marquis *et al.*, 2002). A most simple and essential longitudinal measurement in COPD is that of body mass. BMI represents a useful indicator of performance status. However measurements that quantify muscle bulk are more accurate predictors and when quantifying nutritional status it is essential to take into account loss of body tissue other than body fat (Baarends *et al.*, 1997b). This is because subjects may be overweight yet have skeletal muscle loss in association with preserved body fat (Schols *et al.*, 1993a). Also FFM has been demonstrated to be a better predictor of exercise capacity than body mass (Schols *et al.*, 1991a). The relationship between tissue fat and fat free mass loss is weak enough to mean that a measure that solely examines fat loss will miss some patients with fat free mass decline (Schols *et al.*, 1993a). Such loss is poorly represented by measures, which normally are utilised in the representation of obesity, such as waist/hip ratio or body mass index (Laaban *et al.*, 1993). Therefore one should incorporate assessments that quantify percentage body fat-free mass, allowing a relationship to body weight to be determined. One such measure is the fat free mass index; a directly relevant measure of body composition in those with cachexia.

Underwater weighing

The gold standard method of body composition evaluation is by underwater weighing, where the difference between weights in and out of water is used to estimate the proportions of lean and fat mass. The method uses the Archimedes principle and is reliant upon accurate estimation, or preferably measurement of the residual volume of the lungs.

DEXA scanning

DEXA scanning utilises the principle of alteration of an X-ray beam by the tissue as it passes through, more specifically, the mass attenuation coefficient of the absorber. Homogenous absorbers such as water or bone have specific mass attenuation coefficient which in conjunction with known X-ray emissions, allow mass of substance per unit area to be calculated. DEXA scanning is designed to evaluate body composition using three components: fat mass, lean body tissue and bone mineral mass. Its dual emission method at any one time can differentiate proportions of lean and fat mass in soft tissue, and proportions

of soft tissue and bone in areas where both are present. This is calculated from the known R-values for the differing tissues and allows estimation of whole body proportion by extrapolating one region's behaviour to another's.

Bio Electrical Impedance

Bio Electrical Impedance (BIE) is based on the use of the differing resistances of body tissue to electrical current. In particular, tissue such as fat is known to have high resistance (or low impedance) due to its low water content, and vice versa for muscle. Factors which can alter the body's impedance include hydration status, tissue orientation and water distribution. It has been demonstrated that patients with COPD can have a selective loss of intracellular water compartments(Telfer *et al.*, 1968; Baarends *et al.*, 1997c). Despite this there are validated prediction equations for the use of BIE in COPD (Schols *et al.*, 1991b). Therefore this technique requires standardisation of hydration status and positioning within and between subjects. It allows derivation of level of total body water using prediction equations and resistance values, from which fat free mass may be estimated.

It has recently been demonstrated that thigh muscle cross sectional area is a stronger independent predictor of mortality than BMI or even FEV_1 (Marquis *et al.*, 2002). This highlights the appropriateness of evaluating body composition over and above weight, the exact method which is most appropriate being of considerable controversy (Schols *et al.*, 1991b; Steiner *et al.*, 2002; Kilduff *et al.*, 2002a).

Procedures

All body composition measurements (ANTHRO, BIE, ADP) were performed on a single day by the same investigator. Height was measured (to the nearest 1 cm) using a stadiometer, with subjects standing barefoot. Body mass was assessed (to the nearest 0.1 kg) with subjects wearing only a swimsuit. Skinfold thickness was measured on the right side at appropriately marked sites (to the nearest 0.1 mm) using a Harpenden calliper (British Indicators Ltd, St Albans, UK).

2.2.2.1 Anthropometry

Skinfold thickness was assessed at the triceps, biceps, iliac crest and subscapular sites, according to the standardised anatomic locations and methods reported by Durnin and

Wormersley (Durnin & Womersley, 1969). A minimum of three skinfold measurements were performed at each location, with a difference of no greater than 2 mm allowed between acceptable measures: the recorded values were the average of these measurements. The appropriate anthropometric and demographic data (sum of four skinfolds, age, sex) were entered into Durnin and Womersley's regression equation to determine body density (Db) (Durnin & Womersley, 1969). Total body fat and FFMANTHRO were calculated from Db, using Siri's equation (Siri, 1961).

2.2.2.2 Bioelectrical Impedance

Bioelectrical Impedance (BIE). Measurement of FFM by bioelectrical impedance (FFMBIE) (Bodystat-5000, Bodystat Ltd, Douglas, UK) was performed on the right side, with subject's supine, and with their limbs slightly apart from the trunk (Figure 2.1).

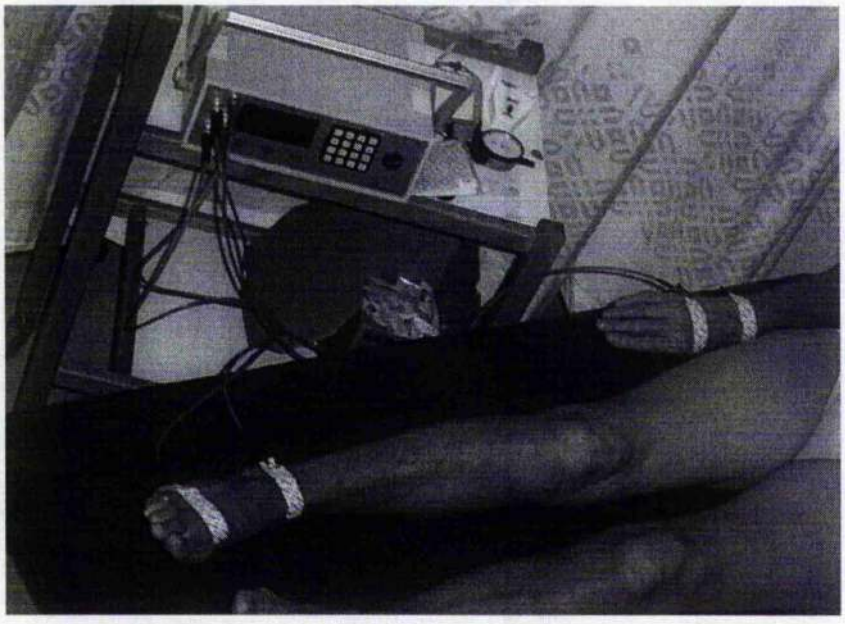


Figure 2.1 Positioning of patient for measurement of BIA.

After the skin had been cleaned with 70% alcohol, two injector electrodes were placed on the dorsal surface of the right hand and foot, and two detector electrodes were placed between the radius and ulna and on the ankle between the medial and lateral malleoli. The impedance to current flow (50 kHz) between the injector and detector electrodes was determined. Two methods for estimating FFM were initially used (see Results):

(i) a patient-specific prediction equation based on resistance (R), body mass (BM), height (H) and sex (S, males=1 and females= 0) (Kyle *et al.*, 1998):

FFM (kg)= -6.06 + (H x 0.283) + (BM x 0.207) – (R x 0.024) + (S x 4.036) (eq. 1) and

(ii) a general population prediction equation based on the same covariates and age (A) (Deurenberg *et al.*, 1991):

FFM (kg)= -12.44 + (H2/R x 0.34) + (H x 0.1534 + (BM x 0.186) – (A x 0.127) + (S x 4.56)

(eq. 2)

This patient specific formulation has been shown to provide the best estimate of FFM in COPD subjects amongst the general prediction equations available for FFM estimation (Pichard *et al.*, 1997). The patient specific equation examined 75 adult patients with stable COPD and was developed to match DEXA body composition evaluations.

2.2.2.3 Air displacement plethysmography

The principles of measurement of FFM by air displacement plethysmography (ADP) have been explained in detail elsewhere (Dempster & Aitkens, 1995). Briefly, when a subject sits inside an enclosed chamber of fixed volume, a volume of air equal to his or her body volume is displaced. Since, under adiabatic conditions, changes in gas volume are inversely related to pressure raised to the power 1.4 (Poisson's law) (Daniels & Alberty, 1967), the magnitude of pressure variations relative to those for a reference chamber provide the actual air volume displaced by the body.

Initially, a standard two-point volume calibration (0 L, 50 L) of the air-displacement plethysmograph (BOD POD®, Life Measurement, Inc, Concord, CA, USA) was performed, according to the manufacturer's instructions. The patients (wearing minimal clothing and a swim cap) then entered the chamber (volume = 450 l) and sat quietly with an erect posture, breathing normally with hands folded in their laps and feet placed on the floor (illustrated in Figure 2.2).

Figure 2.2 Subject sitting prior to ADP measurement.

Their uncorrected body volume (Vbuncorr) was then measured, over a 50-second period. A minimum of two tests were conducted: when two consecutive measurements of Vbuncorr were within 0.2% or 150 ml (whichever was the larger), the results were averaged. The corrected Vb (Vbcorr) was then calculated, taking into account the confounding effect of isothermal air near the skin surface (surface area artefact, SAA) and the intrathoracic gas volume (VTG) (Dempster & Aitkens, 1995):

Vbcorr (l) = Vbuncorr (l) - SAA (l) + 40% VTG (l)

Two methods for estimating VTG were used: (i) employing the standard values provided in the system software, and (ii) employing the individual subject values measured by body plethysmography (see Results). Db was then calculated as body mass/Vb, and % FFM was estimated from Siri's equation (Siri, 1961).

2.2.3 Skeletal muscle function

All tests of maximal exercise capacity were carried out on separate testing days, with at least 48 hours rest in-between.

Background

Measures of skeletal muscle function are usually performed as part of research programmes. These could include examination of the histological, biochemical and metabolic features of the COPD skeletal muscle by muscle biopsies or magnetic resonance spectroscopy scanning. These techniques are cumbersome, expensive and require considerable expertise. Examination of form or function of skeletal muscle has been generally thought to be non

essential in clinical practice. However the GOLD guidelines do suggest that a measure of peripheral muscle function is incorporated into the assessment of patients with COPD who undergo rehabilitation (Pauwels *et al.*, 2001). This is due to the realisation that rehabilitation impacts significantly upon peripheral muscle strength, and particularly can do so even in severely affected patients through innovative strategies (Bernard *et al.*, 1999a; Neder *et al.*, 2001a). This is usually through examination of the lower limb, particularly the quadriceps muscle. A measure of isometric force is the easiest to obtain, either electronically or mechanically, through dynamometry. In the mechanical forms of testing there is usually a steel spring that is compressed, thereby moving a pointer. These mechanical means of measurement are reliable and can be related to reference values (Mathiowetz *et al.*, 1985; Aniansson *et al.*, 1980). Although there is an established relationship between lower and upper skeletal muscle strength there is known to be a relative preservation of upper body limb musculature (Ringbaek, 2001). Therefore it is not generally accepted that singular upper limb evaluation is appropriate, despite it being a much more easily evaluated entity.

Peripheral muscle performance is reproducible and may be compared to reference values; however it is reliant on adequate effort from the subject. Testing through the use of magnetic or electrically superimposed twitch contractions removes this variable, but is less practical in a routine setting (Allen *et al.*, 1995).

Procedures

2.2.3.1 Lower limb

Peripheral muscle testing occurred on the same day and followed body composition measurement. Lower-limb muscle performance was measured using a Kin-Com II isokinetic dynamometer (Chattecx Corporation, Chattanooga, USA). Each patient's position on the dynamometer was standardised by having (a) the anatomical axis of the knee joint aligned with the rotational axis of the dynamometer (by adjusting the seat position and the lever head of the dynamometer) and (b) seat length and height individualised for each patient; these settings were retained for all subsequent tests. Patients were maintained in the seated position by Velcro belts around the waist, thigh and lower leg proximal to the ankle; this also allowed for complete isolation of the leg being tested (i.e. the dominant leg). During the measurements, patients kept their arms crossed over the chest, with the non-involved leg and upper body being kept stationary. The same investigator conducted all tests.

Following a standardised warm-up on the dynamometer, quadriceps muscle strength was measured as the isokinetic peak torque. Patients were instructed to exert maximal effort throughout the full range of motion during each repetition. Verbal encouragement was given to maximise performance. Five consecutive maximal isokinetic concentric contractions were performed at a speed of 70 °·sec^{-1}, with a 30-second rest period between repetition. After a 5-minute recovery period, quadriceps muscle endurance was measured as the cumulative work over five sets of 15 repetitions at a speed of 150 °·sec^{-1}. Patients were given a two-minute rest period between sets (Greenhaff *et al.*, 1994a).

2.2.3.2 Upper limb

Following a further 10-minute rest, upper-limb strength and endurance were measured on the dominant and non-dominant hands (the dominant hand was always tested first), using a handgrip dynamometer (Grip-A, Takei Scientific Instruments Co., Niigta, Japan). The same investigator conducted all tests. Following a handgrip-specific warm-up, handgrip strength was measured from five consecutive maximal voluntary isometric contractions, with a 30-second rest between each contraction. Handgrip endurance was then measured as the number of contractions that a patient could complete to the point of fatigue in each of three consecutive sets, with two-minutes rest between each set; intensity was set at 70% of the individual pre-determined one-repetition maximum. Fatigue was defined as the failure to exert the required intensity for three consecutive contractions. Verbal encouragement was given throughout to maximise performance and to counteract potential boredom.

2.2.4 Exercise capacity

2.2.4.1 Field tests

Background

Evaluation of exercise capabilities most commonly involves lower limb exercise. This is seen to be the most appropriate form due to its direct relevance to day-to-day activities such as walking. Also use of the lower limb musculature usually sufficiently stresses the cardiovascular and respiratory systems for evaluation of their function.

Walking tests were developed as a means of performing evaluations of exercise capacity in a simplified inexpensive setting, while remaining reproducible and meaningful. In 1970 a twelve minute test of exercise capacity in healthy young men was described by Cooper. It was demonstrated that distance ran correlated to maximum oxygen uptake on a treadmill. This was first extrapolated to assessment in COPD in 1976 by McGavin and co-workers (McGavin *et al.*, 1976). Subjects (35 for reproducibility testing, 29 in conjunction with incremental cycle ergometry) attempted to cover as much ground as possible over 12 minutes on a flat corridor. It was established that there was a significant learning effect between 1st and 2nd bouts but not subsequently. Weak correlations between peak oxygen uptake and walking distance were found. In 1985 Guyatt et al demonstrated the feasibility of a shorter 6-minute walking test (Guyatt *et al.*, 1985). However the same limitations of learning effect between 1st and 2nd bout, and importance of standardising motivational factors was evident. A close relationship between the 6 and 12 minute walking tests was later established (Butland *et al.*, 1982). Reference values have been published for the six minute walking distance and are predicted on the basis of age, weight, sex and height (Enright & Sherrill, 1998; Gibbons *et al.*, 2001). In order to address the problem of variability of factors such as motivation and judgement of walking speed, to performance on a walking test, Singh et al. developed the incremental shuttle walking test (ISWT) (Singh *et al.*, 1992). Subjects are asked to walk consecutive 10 metre distances, with the pace increasing gradually each minute. Thereby the test becomes an evaluation of peak ability rather than that of endurance. As with other walking tests there is a published correlation of distance walked to peak oxygen uptake and the same awareness of learning effects are necessary. Recently, through direct comparison using portable exhaled gas analysis, it has been demonstrated that the ISWT represents a more close approximation of maximal oxygen uptake than the 6 minute walking test (Onorati *et al.*, 2003). This ISWT has been modified to test endurance capacity (the endurance shuttle walk test/ESWT) in order that it may more sensitively detect improvements following intervention (Revill *et al.*, 1999). This test requires the subject to perform repeated shuttles (in an identical fashion to the ISWT) at a pace set at 85% of the maximal speed achieved on the ISWT. This is utilising the likelihood that this value will be close to, but above, the critical power for that activity (Neder *et al.*, 2000). Therefore the walking pace will be non sustainable at baseline, but may be potentially completed (stopped in the case of the ESWT at 20 minutes) after an intervention which affects exercise capacity or critical power.

The walking tests described all share the same disadvantages; a marked learning effect and the influence of external factors such as corridor traffic! Also these tests do not include detailed physiological measurements.

It is on that basis that laboratory based cardio pulmonary evaluations of exercise capacity remain the gold standard.

Procedure

Incremental and endurance shuttle walking tests (ISWT and ESWT respectively) were performed according to standardised protocols (Singh *et al.*, 1992; Revill *et al.*, 1999). This included the use of practice shuttle walking tests. This was to familiarise the patient with the technique, and prevent any learning effect by the time it came to performing the second incremental walk. All shuttle walking tests were carried out under the supervision of the Glasgow Royal Infirmary physiotherapy department. The shuttle walking tests involve the patient walking for 10 metres around two markers, 9.5 m apart. Each 10 m walked represents one shuttle. In the incremental shuttle walking tests, each patient started off at three shuttles per minute. This was increased by a further shuttle every minute, indicated by a bleep on an audio cassette, until the exercise became symptom limited. In the endurance shuttle walk test, which also takes place over the 10 m course, the speed was determined at 85% of the maximum in the incremental test, using figure 2.3 below. This level of work was calculated from the predicted maximum oxygen uptake requirement judged by the ISWT. The regression equation used to calculate this value, predicted maximum VO_2 in ml/kg/min was 4.19 + (0.025 * distance) (Singh *et al.*, 1994). In this test the patient carried on walking at the determined level until they could no longer continue.

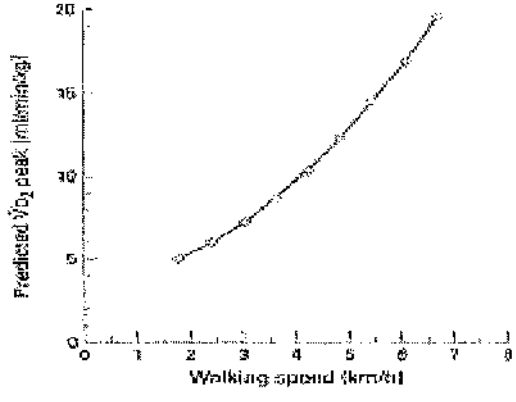


Figure 2.3 Oxygen uptake prediction during the ISWT and ESWT walking speed.

2.2.4.2 Incremental CPET

Background

Exercise testing in conjunction with breath-by-breath analysis of respired gases has allowed non-invasive determination of a number of physiological parameters. Ramp incremental exercise evaluation was developed as a rapid means of assessing a number of clinically meaningful exercise parameters in a single standardised test (Whipp *et al.*, 1981). In a ramp test there is a continuous alteration in the work rate, whereas an incremental test has fixed increases at set timepoints. The four aerobic parameters of interest that may be determined are: maximum oxygen uptake, lactate threshold, work efficiency and time constant of oxygen uptake. However further interpretation of information obtained during the ramp test can help with diagnosis and evaluation of patient groups. For example, there are relationships of interest such as those of heart rate or carbon dioxide output with oxygen uptake.

A ramp test involves asking subjects to exercise to the limit of their tolerance. This represents a "peak" achieved work rate (or oxygen uptake), but not necessarily the absolute maximum, as that is influenced by a number of subject (dyspnoea, leg discomfort) and protocol (ramp increment rate, exercise modality) related factors. Investigators commonly use either cycle ergometry or treadmill exercise modalities. Although treadmill exercise utilises a higher muscle mass, and therefore can produce higher oxygen uptake values, there are a number of reasons why cycle ergometry is a more commonly preferred modality (Hansen, 1984). The unloaded pedalling work rate relates largely to leg mass, and remains an essentially constant factor throughout a changing test (i.e. leg VO_2 = leg mass * rpm). The seated subject is at less risk of self-injury. The electromechanically braked versions allow a very accurate determination of the actual work rate performed, as the workload is independent of pedalling frequency.

A standardised exercise test usually takes the following form: rest, 3 or more minutes of unloaded pedalling, incremental exercise and recovery. The incremental exercise portion should be gauged to last between 8 and 12 minutes and may take the form of a stepwise work rate progression to a linear ramp increase. This length of time gives the most appropriate data density for interpretation of the findings (Buchfuhrer *et al.*, 1983). Appropriate work rate increments range from 30 watts per minute in athletes to 5 watts per minute in many patients with chronic lung disease. Too high slopes of increment can cause the appearance of a "pseudo threshold" for lactic acid (Ozcelik *et al.*, 1999), too low and subjects may lose volition to complete the exercise. A "pseudo threshold" can also arise through

hyperventilation, which prior to a test directly reduces the body's stored CO_2 content, and then results in an initially lower slope of theVCO_2-VO_2 relationship. The CO_2 stores are then refilled and when the deficit in stored CO_2 is overcome there is subsequently an acceleration in VCO_2. This acceleration in VCO_2 appears similar to θ_L although at a lower VO_2 than normally expected.

Appropriate output of the variables allows inferences of the responses of the whole body to exercise. Some major parameters have an established role in evaluation and diagnosis. These are briefly summarised below and are described in detail in the established texts of exercise testing performance, normality and interpretation (Wasserman *et al.*, 1999; Jones, 1975; Gallagher, 1990; Roca & Whipp, 1997). More latterly it has been proposed to examine the normalcy of relationships over exercise increments, rather than solely examining the peak values (Neder *et al.*, 2001c).

VO_2 –WR relationship

This is largely a consistent value, whether relating to step increments of work rate or continuously changing ramp increments and it is normally 9-11 ml/min/Watt in health during cycle ergometry (Hansen *et al.*, 1984; Riley *et al.*, 1996). It is generally lower in cardio respiratory disease, which may be due to different rates of change of oxygen uptake with different work rates or a higher reliance on anaerobic metabolism (Koike *et al.*, 1992). Also the slope of oxygen uptake vs. work rate becomes shallower above the lactate threshold due to the development of the slow phase of oxygen uptake kinetics, but this is not thought to have clinical significance due to the small changes seen (Hansen *et al.*, 1988).

Maximum VO_2

This is apparent when oxygen uptake plateaus despite increasing work rate. This is rarely achieved in clinical setting as subjects tend to stop exercising before this true maximum. Therefore a "peak" oxygen uptake is only defined as the maximum one if stringent criteria are met. However, despite this distinction, the reproducibility of peak oxygen uptake has consistently been found to be within 5% (Brown *et al.*, 1985; Cox *et al.*, 1989; Killian *et al.*, 1992; Marciniuk *et al.*, 1993).

VCO_2-VO_2 relationship

This slope is influenced at baseline by substrate use and its gradient is altered during exercise by the occurrence of the lactate threshold. The lactate threshold occurs at the time of lactate production from the exercising muscles and consequent hydrogen ion release, which causes CO_2 production due to the bicarbonate buffering of these hydrogen ions. This point has been

validated as representative of the lactate threshold within COPD patients (Griffiths *et al.*, 1996;Sue *et al.*, 1988).

V_E-VCO_2 relationship

Tight regulation of acid-base balance is achieved through precise control of ventilation (Whipp & Ward, 1998; Ward, 2000; Jones, 1975). Therefore there is close coupling of V_E to VCO_2. The expected linear response of VCO_2 is modified by the varying influence of lung dead space (V_D) during differing levels of ventilation. Physiological dead space may be increased through altered patterns of breathing (e.g. shallow rapid breaths) as well as lung pathology (particularly the dynamic hyperinflation of COPD). The slope of the V_E-$V'CO_2$ ratio is thereby influenced solely by $PaCO_2$ and V_D/V_T. Therefore increases in the values of V_E/VCO_2 at a given VCO_2 point toward, for example, impaired gas exchange, metabolic derangements or increased dead space ventilation. This slope remains linear through a ramp test until which time a respiratory compensation point is reached, where V_E rises to provide buffering of the acidosis occurring. The relationship is linear through the passing of the anabolic threshold.

Peak exercise variables

In their most simplistic form peak exercise variables can be compared to the expected maximal values to allow identification of which major systems are being stressed. Maximal predicted HR is crudely calculated by 220 minus age in years, with the actual value being rather variable in individuals. Maximum achievable ventilation is commonly measured in one of 2 ways – either forced voluntary maximal respiration over one minute through a pneumotachograph, or by multiplying the FEV_1 by a constant (usually 35 or 40). These approaches allow an estimation of whether subjects stop exercise with a reduced "breathing reserve" or cardiovascular capacity, thereby indicating potential system disease.

Procedure

Exercise testing was performed using an electomagnetically braked cycle ergometer (Corival) with gas exchange variables analysed breath-by-breath using a software based system (MGC-CPX System, Medgraphics Copr. (MGC), St Paul, MN, USA) following calibration of flow and gas level detection. The equipment used is illustrated below in Figure 2.4.

Figure 2.4 Cardiopulmonary exercise testing equipment.

Calibration is with a precision three-litre syringe where the flow ranges extend beyond those seen during exercise. Patients had a nose clip and customised mouthpiece (MGC) in place. Respired flow is measured continuously by a rapid response flow module (MGC) enabling ventilation to be measured. A sampling line continuously draws a small amount of respired gases from the mouthpiece to be analysed rapidly for O_2 and CO_2 concentrations. Cardiac monitoring by means of a 12 lead ECG was also performed.

To begin each test, subjects sat quietly at rest on the ergometer for a period of ~3 minutes. This allowed them to become accustomed to breathing through the mouthpiece as, despite thorough familiarisation, there can be a tendency to hyperventilate on insertion of the mouthpiece. This period of rest was essential to monitor the subject's breathing to ensure that they were not hyperventilating, or had not been in the period immediately prior to monitoring. For non-invasive estimation of the lactate threshold, it is critical that the subject does not hyperventilate prior to the test commencing, as this can predispose to a false positive or "pseudo" threshold as described previously (Ozcelik et al, 1999; Whipp et al, 1987; Ward and Whipp, 1992).

The incremental exercise test consisted of: (i) 3 minutes at rest; (ii) 3 minutes at unloaded pedalling (approximately equivalent to 20 W); (iii) the incremental phase; and (iv) a 5 minute recovery period. The power (W) was increased each minute of the incremental phase by between 5 and 10 W, dependent on the performance of the patient during the field tests: the aim being to have an incremental phase of more than 8 and less than 12 minutes.

The modified Borg category scale was used to rate the perceived intensity of breathlessness and leg fatigue during rest, unloaded pedling, at the end of exercise and at 2 and 5 minutes recovery (Illustrated in Table 2.1) (Borg, 1982). Simple verbal expressions were linked to numbers from 0 to 10, zero being no appreciable breathlessness or leg fatigue and 10 being the maximum. Full explanations were given prior to the test and the scale was shown to the patient at the end of exercise testing. Care was taken to instruct the patients to rate only the breathing effort or leg effort and no other sensation during exercise.

The scale runs from 0 to 10, 0 being equivalent to "Nothing at all" with 10 being equivalent to "Maximal". The word anchors for the scale are reproduced below:

0	Nothing at all	
0.5	very, very weak	(just noticeable)
1	very weak	
2	Weak	(light)
3	Moderate	
4	Somewhat Strong	
5	Strong	(heavy)
6		
7	Very strong	
8		
9	Very, very strong	(almost max)
10	Maximal	

Table 2.1 The modified Borg scale

The modified Borg scale is a ratio scale i.e. a doubling of the numeric value indicates a doubling of the perceived breathlessness or leg effort. The scale is designed to be easy to be used by a lay population.

The computation of ventilation and pulmonary gas exchange was performed online, breath-by-breath, using the algorithms of Beaver et al (Beaver *et al.*, 1973). These algorithms calculate pulmonary gas exchange over the duration of a single breath. The basic concept is the same as for gas collection methods (e.g. Douglas bags), whereby the continuously-measured expired flow is divided into consecutive temporal samples at the same frequency the gas concentrations are being analysed (Wasserman *et al.*, 1999). Therefore, in the limit, the volume of gas expired over a given period (T) is given by:

$$V_E = \int_{t=0}^{T} Vdotexp(t)dt$$

where Vexp is the expired flow during an infinitesimally short time interval dt, and the corresponding increase in expired volume is given by the product Vdotexp(t) x dt. However, in practice dt is replaced by a constant Δt and the mean flow across the time interval $(t + \Delta t)$ replaces the instantaneous flow at (t):

$$V_E = \sum_{t=0}^{T} Vdotexp(t + \Delta t) * \Delta t$$

where Vexp is the mean flow rate during the time interval $t + \Delta t$. Minute ventilation ($Vdot_E$) is obtained by summing V_E across the duration of an expiration (T_E) and that sum divided by T_E. To calculate VO_2 and VCO_2 the same process is applied to the product of the gas concentration and the expired flow for each small sampling period, such that in the limit:

$$VO_2 = \int Vexp(t)dt * [(\Delta FO_2)true]$$

and, assuming F_ICO_2 to be quantitatively negligible,

$$VCO_2 = \int Vexp(t)dt * F_ECO_2$$

However, in practice dt is substituted for the sampling time interval Δt:

$$VO_2 = \sum Vexp(t + \Delta t) * \Delta t * [(\Delta FO_2)true]$$

and:

$$VCO_2 = \sum Vexp(t + \Delta t) * \Delta t * F_ECO_2$$

where the true O_2 difference $[(\Delta FO_2)true] = \dfrac{(F_IO_2 - F_EO_2 - F_IO_2 * F_ECO_2)}{(1-F_IO_2)}$

Thus $VdotO_2$ and $VdotCO_2$ are the summed VO_2 and VCO_2's across the duration of an expiration (T_E) and subsequently divided by T_E.

Through the principles of these equations and correction to account for the small dead space of the turbine assembly, it is possible to measure accurately on-line the pulmonary exchange of oxygen and carbon dioxide on a breath-by-breath basis. A note of caution which must be remembered when dealing with respiratory variables is the convention to express V_E as body temperature, pressure and saturated with water vapour (BTPS), i.e. its natural conditions, but to standardise VO_2 and VCO_2 to standard temperature and pressure dry (STPD). Therefore, the computer applied the appropriate correction factors to take account of the atmospheric conditions during each experiment.

Therefore using these "expiratory-only" algorithms the following variables were calculated breath by breath: oxygen uptake (VO_2, ml/min), carbon dioxide output (VCO_2, ml/min), respiratory exchange ratio (R), minute ventilation (V_E, l/min), breathing frequency (f, /min), ventilatory equivalent for O_2 and CO_2 (V_E/ VO_2 and V_E/VCO_2), end tidal pressures of O_2 and CO_2 ($PETO_2$ and $PETCO_2$, mmHg) and oxygen pulse (ml O_2/beat). Heart rate was determined from the R-R interval of the 12 lead electrocardiogram.

2.2.4.3 Constant Load CPET

Background

Constant work load tests can provide quite distinct information to that gained during incremental testing. This is further demonstrated by the differing roles of low and high intensity protocols in both the estimation of physiological parameters and the evaluations of responses to intervention.

Definition of intensity of work rates performed largely relate to whether the exercise is sustainable with attainment of steady state values (low and moderate intensity) or is non sustainable with consequent fatigue (severe). The severe intensity work rates will be set importantly above the level of the critical power for a particular activity, in order that they are fatiguing (Neder *et al.*, 2000; Wasserman *et al.*, 1999). Heavy work rates are such that they are above the lactate threshold (with consequent rise in lactate and hydrogen levels) but at or below critical power, allowing a steady state for VO_2.

Constant work rate tests below the level of the critical power (moderate or heavy) allow determination of effort independent parameters that may be indicative of performance status

and response to training. Oxygen uptake kinetics reflect the interplay of gas exchange at the level of the muscle mitochondria, muscle capillarity and cardiovascular oxygen transportation and are demonstrated to be lengthened in patients with COPD (Nery *et al.*, 1982b). However following exercise training in COPD the findings are partially ameliorated (with shortening time of the constant) (Casaburi *et al.*, 1997; Puente-Maestu *et al.*, 2000). This measure is feasibly obtained from one transition and is reproducible in COPD patients with established statistically meaningful differences for the time constant established (Puente-Maestu *et al.*, 2001). Tests which achieve steady state oxygen uptake are able to quantify accurately the WR/VO_2 relationship.

Severe intensity work rate tests are to the limit of exhaustion. In COPD subjects they provide values for VO_{2PEAK} similar to those obtained on ramp testing (Neder *et al.*, 2000). They are most widely used as a measure of non sustainable exercise tolerance in relation to an intervention. The proximity of the assigned constant load work rate to the critical power makes the test more sensitive to intervention than incremental protocols. Whereas one commonly expects a rise in the order of 5-10% for VO_{2PEAK} (and thereby absolute WR) after exercise training, previously non sustainable constant work rate can become at or below the subjects critical power, leading to striking improvements in the time to limitation (Casaburi *et al.*, 1997). Constant load tests have been recently shown to be reproducible at 75% of peak work on an incremental and also valid i.e. the work performed related to VO_{2PEAK} and 12 minute walking distance in a COPD population (Van 't *et al.*, 2003).

Procedure

The endurance CPET consisted of: a three minute rest period, three minutes of unloaded pedalling and a constant work load test set at 80% of the maximum work rate achieved on the incremental test. The time to tolerance (Tlim) is taken as the period, in seconds, between the start of the constant high intensity work rate and the point at which the patient can no longer sustain a satisfactory pedalling frequency (greater than 40 rev per minute) despite encouragement. Following that time the patient continued cycling for 5 minutes, at unloaded peddling during the recovery period. Identical variables as to those identified during the incremental exercise test are measured breath by breath, along with a 12 lead electrocardiogram. Symptom questioning also occurred at rest, during unloaded peddling, at maximal exercise and at 2 and 5 minutes recovery time.

Where appropriate, in order to evaluate the presence and degree of respiratory-mechanical limitation during exercise, tidal and maximal inspiratory flow-volume loops will be obtained each minute and compared to resting maximal flow-volume curves obtained before and after exercise (Martinez *et al.*, 1996; Yan *et al.*, 1997; Johnson *et al.*, 1999a; Johnson *et al.*, 1999b). This will allow better estimation of the available flow and volume reserves during exercise; i.e. by measuring the differences between exercise flow-volume loops and the maximal available limits for mechanical generation of both flow and volume (Martinez *et al.*, 1996; Yan *et al.*, 1997; Johnson *et al.*, 1999a; Johnson *et al.*, 1999b). This will therefore be a measure of the degree of exercise related dynamic hyperinflation.

2.2.5 Health status and breathlessness

Background

Health status defines the impact an individual's illness has upon his/her well being (Wilson & Cleary, 1995). Health related quality of life is becoming to be seen as the most important indicator of the benefit of certain treatments, particularly when considering the health economic value of recommending interventions. The scope of the assessments involved in these measures tends to cover such areas as physical, emotional, social and cognitive functioning: the aim being to gain a broad perspective on an individual's quality of life. These measures have an established role in populations when looking cross-sectionally at health status, but the specificity of these tools is less suited to the individual patient, unless longitudinal data is being obtained. Once longitudinal evaluations are made then appropriate judgments as to whether "minimally clinically significant" changes, i.e. useful ones, have been achieved. These tools have allowed us to not only define which interventions improve health status, but also establish the natural history of health status decline in COPD patients (Spencer *et al.*, 2001).

Health related quality of life can be assessed by generic and disease specific forms. Generic measures include the 36-item Short Form (SF-36) and the Nottingham Health Profile Questionnaire (Ware, Jr. & Sherbourne, 1992;Hunt *et al.*, 1980). These questionnaires were designed for healthier populations, such that the difference in scores between COPD and other less ill patient groups can be in the order of 30 points for the SF-36, whereas the questionnaire is looking for changes of 5 points to detect minimally clinically significant differences in COPD (Spencer *et al.*, 2001).

Valid, reliable and moreover, disease specific questionnaires have been developed for COPD patients. There are 2 in widespread use: The St George's Respiratory Questionnaire (SGRQ) and The Chronic Disease Respiratory Questionnaire (CRQ) (Jones *et al.*, 1992;Guyatt *et al.*, 1987). They both assess specific areas of functioning (e.g. for SGRQ symptoms, activity and Impacts) and allow a total score to be calculated. The mean SGRQ for a subject with 50% FEV_1 is 50 (out of 100). Worsening health status is signified by increasing scores and a 4 point difference in the total or impacts score indicates a clinically significant health status change, for example, the ability to be free of breathlessness walking with others, bending and while washing (Jones, 2002). The CRQ has a minimum clinically significant change of 0.5 points for all its assessment areas (Jaeschke *et al.*, 1989). Patients with COPD have been demonstrated to have a fall in health status equivalent to the clinically meaningful change, every 14 months (Spencer *et al.*, 2001). Although health status is lower in COPD patients with increased mortality, it has been demonstrated that CRQ is not an independent predictor of mortality in COPD, unlike age, FEV_1 and body composition (Oga *et al.*, 2002).

Procedure

Quality-of-life and symptoms were evaluated using the St George's Respiratory Questionnaire (Jones *et al.*, 1992). The St George's Respiratory Questionnaire (SGRQ) scores the following parameters: "Symptoms", "Activities" and "Impacts". A total score, out of 100, is also calculated, with a change of 4 points representing the minimum clinically significant difference.

The modified Borg scale was used during exercise testing to quantify breathlessness (Borg, 1982). Mahler's baseline and transitional dyspnoea measurement questionnaires were administered to quantify breathlessness during day to day activities (Mahler *et al.*, 1984).

2.3 Pulmonary rehabilitation programme

The programme consisted of 2 weekly sessions of 1 hr for a total of 16 sessions. Subject may have been permitted to continue the programme if they only missed up to a few sessions. This was at the discretion of the physiotherapist, in accordance with standard practice in the department. The exercise was conducted by a physiotherapist and took place in the physiotherapy gym of Glasgow Royal Infirmary. Prior to the start of each training session, all

patients underwent a standardised warm-up which comprised light intensity exercise for 5 - 10 min, followed by a series of stretches with an emphasis on stretching the musculature associated with the exercises that were to follow. Then dynamic strength training of the upper (shoulder abduction and elbow flexion weight lifting, wall presses, bench press) and lower extremities (seated leg press, seated leg curl, standing calf raise, sit to stand, step exercises) were performed. Each of these components had 3 sets of 10 repetitions completed at intensities (where variation possible) set to match perceived exertion scores 2 values less than that reached during maximal incremental exercise. Then endurance training on a cycle ergometer and repeat stretching were performed. The cycle ergometer work rate was gauged as to provide pervieved breathlessness scores 2 below the maximum reached on the incremental exercise protocol. Patients were expected to have increasing training work rates during the course of the programme. Patients were also given a copy of alternative exercises, so they could train at home.

The training intensity was progressively increased as patients successfully completed the required number of sets and repetitions (initially 3 sets of 10 repetitions for each exercise). Patients kept training logs throughout the duration of the study detailing rating of dyspnoea during exercise sessions and the weight, sets and repetitions lifted during strength training of the upper and lower extremities. Training sessions lasted on average 60 min (including the 10 min warm-up and 10 min cool-down).

2.4 Analysis

2.4.1 Data editing

Recorded breaths in the CPET output have to be edited to ensure that all are appropriate for inclusion in analysis. Either the subject voluntarily takes a breath out of synch of the current breathing pattern, such as a particularly large or short breath, or the on-line software 'mis-triggers' a breath. This can occur if the subject coughs or swallows during a breath, which the computer registers as a change in the direction of the volume signal. A balance between choosing higher volumes for accurate breath detection results in less 'mis-triggering' of breaths, but conversly leads to underestimation of exhaled O_2 and CO_2 volumes and to more breaths with small tidal volumes (e.g. at rest) being ignored.

To deal with 'mis-triggered' breaths, the raw signals of respired O_2, CO_2 and volume recorded on a chart were analysed in conjunction with numerical print-outs of the constituent parts of the breath, i.e. inspired time and expired time, tidal volume and end tidal pCO_2 and pO_2. From this, breaths that were clearly atypical of the surrounding breathing pattern were removed from the data set. Breaths where there was any degree of uncertainty over the classification of a 'mis-trigger' were left in the data set.

The 'noise' typically associated with breath-by-breath gas exchange has been extensively classified as an uncorrelated Gaussian distribution (Lamarra *et al.*, 1987; Rossiter *et al.*, 2000; Puente-Maestu *et al.*, 2001). Therefore, it is reasonable to conclude that any breaths lying outwith prediction bands enveloping 4 standard deviations from the mean are unlikely to be part of the underlying physiological response to the imposed work-rate forcing (Lamarra *et al.*, 1987; Rossiter *et al.*, 2000; Puente-Maestu *et al.*, 2001). Based on this criterion, breaths that were not part of the underlying response, either through 'mis-triggering' or the subject voluntarily taking an uncharacteristic breath, were removed from the data set.

2.4.2 Calculation of VO_{2PEAK}

VO_{2PEAK} was calculated as the mean VO_2 during the final 15s of an incremental test. It is often reported in the literature that two of the three following criteria should be met before the test can be classified as having being maximal (e.g. Hale et al, 1998):

1. a plateau in VO_2 was achieved.
2. the peak heart-rate was within +/-10 bpm of the age-predicted maximum heart-rate (i.e. max. heart-rate = 220 – age).
3. RER was greater than 1.15 at exhaustion.

However, for a number of reasons, particularly in patient populations, these criteria can not be fulfilled. In practice, rapid-incremental tests are unlikely to produce a plateau in VO_2. Thus it is conventional to refer to this 'maximal' value as the VO_{2PEAK}. In cases where the subject provides a full volitional effort and is not limited by symptoms such as dyspnoea, angina or leg pain, then VO_{2PEAK} and maximal VO_2 are closely related (Cooper *et al.*, 1984). Furthermore, the variability in maximum heart rate is too high to reliably exclude subjects on

the basis of a 'low' peak heart rate at the end of an incremental test. Additionally, the final RER achieved will depend critically on the rate at which stored CO_2 is unloaded as a result of HCO_3^- buffering of metabolic acid and also because of respiratory compensation, both of which are highly dependent on the work-rate incrementation rate (Whipp, 1987). Therefore all values reported are VO_{2PEAK} rather than VO_{2MAX}.

2.4.3 Lactate threshold estimation

The lactate threshold (θ_L) was estimated non-invasively from the pulmonary gas exchange and ventilatory consequences of the proton produced in association with the lactate anion. For rapid incremental exercise tests, these changes can be detected using a cluster of indices that include the V-slope and a series of ventilatory-based variables (Beaver *et al.*, 1986; Reinhard *et al.*, 1979; Wasserman *et al.*, 1999; Reinhard *et al.*, 1979; Wasserman *et al.*, 1999). The underpinning physiology is the production of extra non-metabolic CO_2 during the bicarbonate buffering reaction:

$$La^- + H^+ + HCO_3^- \longleftrightarrow H_2CO_3 \longleftrightarrow CO_2 + H_2O$$

where La^- is the lactate anion and x is Na in the muscle or K in the blood. The result of this is an acceleration from θ_L onwards of VCO_2 relative to VO_2 as the work rate continues to increase.

The increased rate of CO_2 clearance above θ_L is associated with a proportional increase in V_E, such that V_E immediately increases out of proportion to VO_2. This change can be observed as the VO_2 at which V_E/VO_2 and $P_{ET}O_2$ begin to increase while there is no concominant rise in V_E/VCO_2 or $P_{ET}CO_2$, i.e. hyperventilation relative to O_2 but not to CO_2, which has been termed isocapnic buffering (Wasserman *et al.*, 1977). The increase in V_E/VO_2 without change in V_E/VCO_2 is important, as it is atypical of hyperventilation caused by non-specific factors unassociated with the increasing work-rate (e.g. anxiety or hypoxia). Typically, 2-3 minutes after θ_L a further increase in V_E, out of proportion to VCO_2, is observed. This is known as the 'respiratory compensation point' (RCP) (Wasserman *et al.*, 1999)and has been argued to reflect H^+ stimulation of the carotid bodies although the reason for the delay is as yet not fully understood (Rausch *et al.*, 1991; Whipp *et al.*, 1981; Whipp *et al.*, 1981). While the RCP does not directly impinge on the determination of θ_L, it is used to set the range of data over

which the determination of θ_L will be made (Beaver *et al.*, 1986). That is, the data from: resting, unloaded pedalling, the early kinetic lag phase, and above the RCP is excluded, leaving only the two 'regions of interest' (Beaver *et al.*, 1986).

The V-slope method estimates the lactate threshold as the intersection of best-fit linear regression to the upper and lower regions of interest of the VO_2-VCO_2 plot. The effects of θ_L on ventilatory-based variables can be seen by viewing the changes in V_E/VO_2 and $P_{ET}O_2$ with an absence of change, detailed above, in V_E/VCO_2 and $P_{ET}CO_2$ from plots of V_E/VO_2 and $P_{ET}O_2$ vs VO_2 with V_E/VCO_2 and $P_{ET}CO_2$ vs VO_2. The lactate threshold determined is therefore expressed as a VO_2 value.

2.5 Statistics

All data were sampled and analysed using the Statview statistical package (Statview for Windows, Statview corp. USA). Details of the different statistical methods are presented in the respective chapters. The following statistical methods were applied throughout the process of analysis as appropriate:

1. **Checks for normality**: All relevant data were checked for normality of distribution before applying any statistical test based on this assumption.
2. **Comparison of means**: For normally distributed data, a two independent sample Student's t-test was used for comparison between two means for separate groups and a paired Student's t-test was used for comparison between two means from the same group.
3. **Assessment of the relationship between various factors:** The relationship between 2 or more variables was assessed using the Pearson's correlation coefficient, stepwise multiple linear regression analysis and Bland and Altman analysis.
4. **Statistical significance:** A level of $p<0.05$ was considered significant

CHAPTER 3

CLINICAL RELEVANCE OF INTER-METHOD DIFFERENCES IN FAT-FREE MASS ESTIMATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Abstract

Background: Evaluation of fat-free mass (FFM) is becoming recognised as an important component in the assessment of clinical status and prognosis in patients with established chronic obstructive pulmonary disease (COPD). The aim of this study was to determine whether potential differences in FFM estimation performed by differing methods; air-displacement plethysmography (ADP), bioelectrical impedance (BIE) and anthropometry (ANTHRO), would have clinical significance.

Methods: Twenty-eight patients with moderate-to-severe COPD were submitted to FFM estimation by ADP, BIE and ANTHRO. FFM was then allometrically related to peak oxygen uptake (VO_{2PEAK}) as determined by symptom-limited incremental cycle ergometry.

Results: We found that ANTHRO classified fewer patients as "FFM-depleted" than the other two techniques ($P<0.05$). Although mean biases of the BIE-ADP differences were close to zero, their 95% confidence limits extended as high as 5.9 kg (16 %). The ANTHRO-based allometric exponents for VO_{2PEAK} correction of FFM, therefore, were typically higher than those obtained by the other two methods in both depleted and non-depleted patients (ANTHRO: 1.45-1.41, BIE: 0.97-1.18; ADP: 1.08-1.14, respectively).

Conclusion: We conclude that between-method differences in FFM estimation can be sufficiently large to have practical implications in relation to modes of measurement utilised and determinants of exercise tolerance in patients with moderate-to-severe COPD. A single method of body composition assessment, therefore, should be used for FFM estimation in these patients.

3.1 Introduction

Exercise intolerance is a major feature of chronic obstructive pulmonary disease (COPD) (Pauwels *et al.*, 2001), which is predominantly due to largely irreversible airflow obstruction and particularly dynamic hyperinflation which develops during exertion. Abnormalities of skeletal muscle structure and function have more recently become recognised as also playing a significant role in such exercise limitation (Wouters *et al.*, 2002). Importantly, for example, loss of fat-free mass (FFM), a widely-used surrogate of skeletal muscle mass, has been associated with progressive disability, increased utilisation of health-care resources, and increased mortality (Mostert *et al.*, 2000; Decramer *et al.*, 1997; Gray-Donald *et al.*, 1996).

Estimation of FFM has traditionally been performed by a wide range of methods, each differing with respect to assumptions, level of expertise needed and cost (Ellis, 2000). In large part, previous reports of FFM status in patients with COPD have been concerned with validation of techniques; comparing relatively inexpensive testing procedures (e.g. anthropometry (ANTHRO), bioelectrical impedance (BIE)), with more complex "criterion" methods (e.g. hydrostatic weighing and deuterium dilution) (Schols *et al.*, 1991b; Pichard *et al.*, 1997; Kyle *et al.*, 1998; Engelen *et al.*, 1998).

Whether these differences found between the methodologies used for FFM estimation have a clinical significance is a fundamental question. Steiner et al have demonstrated that the body composition testing modality used in COPD patients can influence the categorisation of the patient as to whether or not they are depleted of fat free mass. Such differences in FFM estimation between dual energy X-ray absorption (DEXA) and anthropometry (ANTHRO) have been shown to be of practical importance in normal subjects (Neder *et al.*, 2001b). In this particular study, the between-method discrepancies were sufficiently large to significantly affect the values of the exercise capacity, corrected for the fat free mass component. Since the exponent used to eliminate the functional inequalities (VO_{2PEAK}) which could be ascribed to differences in 'size' (FFM) depended on the method chosen to measure FFM, these discrepancies became of clinical importance (Welsman *et al.*, 1996; Nevill & Holder, 1995). Whether between-method discrepancies in FFM estimation in patients with COPD also yield results that differ to a degree great enough to have clinical significance is not known.

We were therefore interested to determine the clinical implications of between-method differences of FFM estimation and how they related to the assessment of exercise limitation in patients with COPD. We wished to establish the limits of agreement between three methods of body composition assessment, ANTHRO, BIE and the relatively new technique of air-displacement plethysmography (ADP). We also wished to evaluate whether any inter-method differences would be sufficiently large to influence the analysis of maximum aerobic capacity (as estimated by VO_{2PEAK}) when allometrically corrected for FFM.

3.2 Methods

3.2.1 Subjects

Twenty-eight patients (11 females and 17 males) with established clinical and functional diagnosis of moderate-to-severe COPD (FEV1 < 60% predicted and FEV1/FVC ratio < 70%) comprised the study group (Table 1) (1987). Inclusion criteria were absence of locomotor or neurological diseases, and no change in medication dosage or exacerbation of symptoms in the preceding 4 weeks. All patients were optimised in terms of standard medical therapy: maintenance medication included β2-agonists, theophylline, oral and/or inhaled steroids.

Before the tests, the procedures, including the known risks, were described in detail and informed consent was obtained from all subjects. The study was approved by the North Glasgow University Hospitals NHS Trust Medical Ethics Committee.

3.2.2 Measurements

3.2.2.1 Pulmonary Function Testing

Spirometric tests and static lung volume measurements were performed using methods detailed in chapter 2 (Section 2.2.1). Measured variables included forced vital capacity (FVC, l), forced expiratory volume in one second (FEV_1, l), FEV_1/FVC ratio, and total lung capacity (TLC, l).

3.2.2.2 Body Composition Estimation

All body composition measurements (ANTHRO, BIE, ADP) were performed on a single day by the same investigator. These methodologies are detailed in Chapter 2 (Section 2.2.2).

3.2.2.3 Cardiopulmonary Exercise Testing

Exercise tests were performed on an electromagnetically-braked cycle ergometer with gas exchange and cardiovascular variables being analysed breath-by-breath using a calibrated computerised exercise system as detailed in Chapter 2 (Section 2.2.4.2).

The exercise test consisted of: (a) 2-3 minutes at rest; (b) 3 minutes of unloaded pedalling, and (c) an incremental phase performed to the limit of tolerance, during which the work rate was progressively increased at a fixed rate (5-10 W every min) to provide a duration of the incremental phase that lay between 8 and 12-minutes (Buchfuhrer *et al.*, 1983). Peak oxygen

uptake (VO_2 $ml.min^{-1}$) was calculated as the average of the last 15 seconds of the test. Subjects subsequently completed 5 minutes of unloaded pedalling representing a recovery period.

3.2.3 Data Analysis

Between-group differences in patients separated by presence or absence of FFM depletion (see Results) were analysed by a non-paired Student's t test. Between-methods concordance in classifying the patients as depleted or non-depleted was assessed by McNemar's test. The probability of a Type I error was established at 0.05 for the hypothesis tests.

The limits of agreement between ANTHRO, BIE and ADP were investigated by plotting the individual between-method differences against their respective means (Bland-Altman plot) (Bland & Altman, 1986). Heterocedasticity was examined by plotting the absolute (positive) differences against the individual means and calculating the Spearman's correlation coefficient (Bland & Altman, 1996). If the heterocedasticity correlation was close to zero and the differences were normally distributed (Shapiro-Wilk's test), the mean bias and the 95% limits of agreement were calculated as mean ± 1.96 SD of the between-method differences (Bland & Altman, 1986). In addition, in order to provide a relative (%) difference between any two of the three methods, data were transformed by taking natural logarithms of the means for each of the two methods before the calculation of the limits of agreement (Nevill, 1997). After taking the antilogarithms of these values, the mean bias and the 95% limits were expressed on a ratio scale.

The allometric relationships between VO_{2PEAK} (y) and FFMANTHRO, FFMBIE and FFMADP (x) were analysed by applying a linear regression to the logarithmic transformation of both x and y:

$$\ln y = \ln a + b \ln x \qquad \text{(eq. 4)}$$

where a is the y-intercept, and the slope b is equal to the exponent of the power function $y = axb$.

3.3 Results

3.3.1 Technical considerations

As discussed in the methods (Section 2.2.2.2), we firstly investigated the effect of using a general population equation (Deurenberg *et al.*, 1991) versus a patient-specific equation (Kyle *et al.*, 1998) for FFM estimation using BIE. We found substantial differences in these estimates, with the Kyle *et al.* equation providing generally lower values than the Deurenberg et al. equation: as illustrated in Figure 3.1A, these differences increased as a function of FFM ($P<0.01$). The patient-specific equation of Kyle *et al.* was therefore subsequently used for the BIE-based inter-method comparisons.

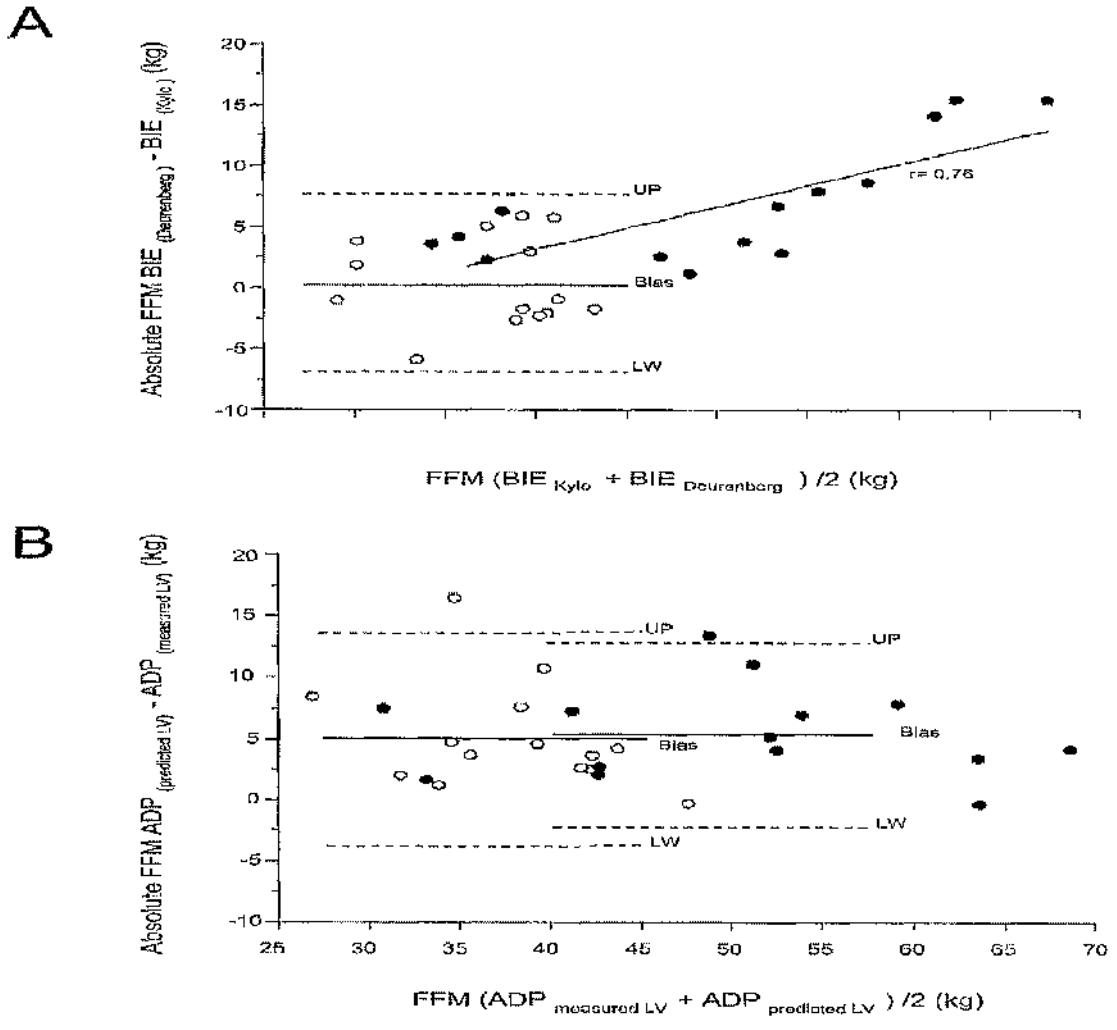
We also sought to investigate whether the use of measured rather than predicted lung volumes would be of practical relevance for FFM estimation by ADP. Systematically higher values for FFMADP resulted when the theoretical values were used instead those actually measured (Figure 3.1B). This is to be expected, given the increased lung volumes that are characteristic of this patient population (e.g., Table 3.1). We therefore chose to use the individually-measured VTG values to calculate FFMADP for the ADP-based inter-method comparisons.

Table 3.1 Characteristics of patients separated by presence or absence of fat-free mass (FFM) depletion

	FFM-Depleted Patients (n=14, 7 male)	FFM-Non-depleted Patients (n=14, 10 male)
Age (yr)	62 ± 9	66 ± 8
Height (m)	1.6 ± 0.1	1.7 ± 0.1
Body mass (kg)	50.4 ± 5.6	76.4 ± 16.5*
BMI ($kg.m^{-2}$)	16 ± 1	19 ± 3*
$\dot{V}_{O_2\,PEAK}$ ($ml.min^{-1}$)	666 ± 207	974 + 356*
FVC (l)	2.7 ± 0.6	3.0 ± 0.8
FEV_1 (l)	1.0 ± 0.2	1.2 ± 0.4
FEV_1 (% pred)	41.8 ± 12.9	45.9 ± 15.8
FEV_1/FVC (%)	37.4 ± 9.6	38.4 ± 10.4
TLC (% pred)	126.3 ± 15.7	125.1 ± 21.0

* $P<0.01$. Values are presented as mean ± standard deviation (SD). Definition of abbreviations: BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; TLC: total lung capacity; pred: predicted value.

Figure 3.1 Limits of agreement of fat free mass estimation.



A. Mean bias and the upper (UP) and lower (LW) 95% limits of agreement between a patient-specific (Kyle et al.) and a general (Deurenberg et al.) equation for fat-free mass (FFM) estimation according to bioelectrical impedance (BIE) in FFM-depleted (open circles) and non-depleted (solid circles) patients with COPD. Note that a significant heterocedastic correlation was found in non-depleted patients ($P<0.01$).

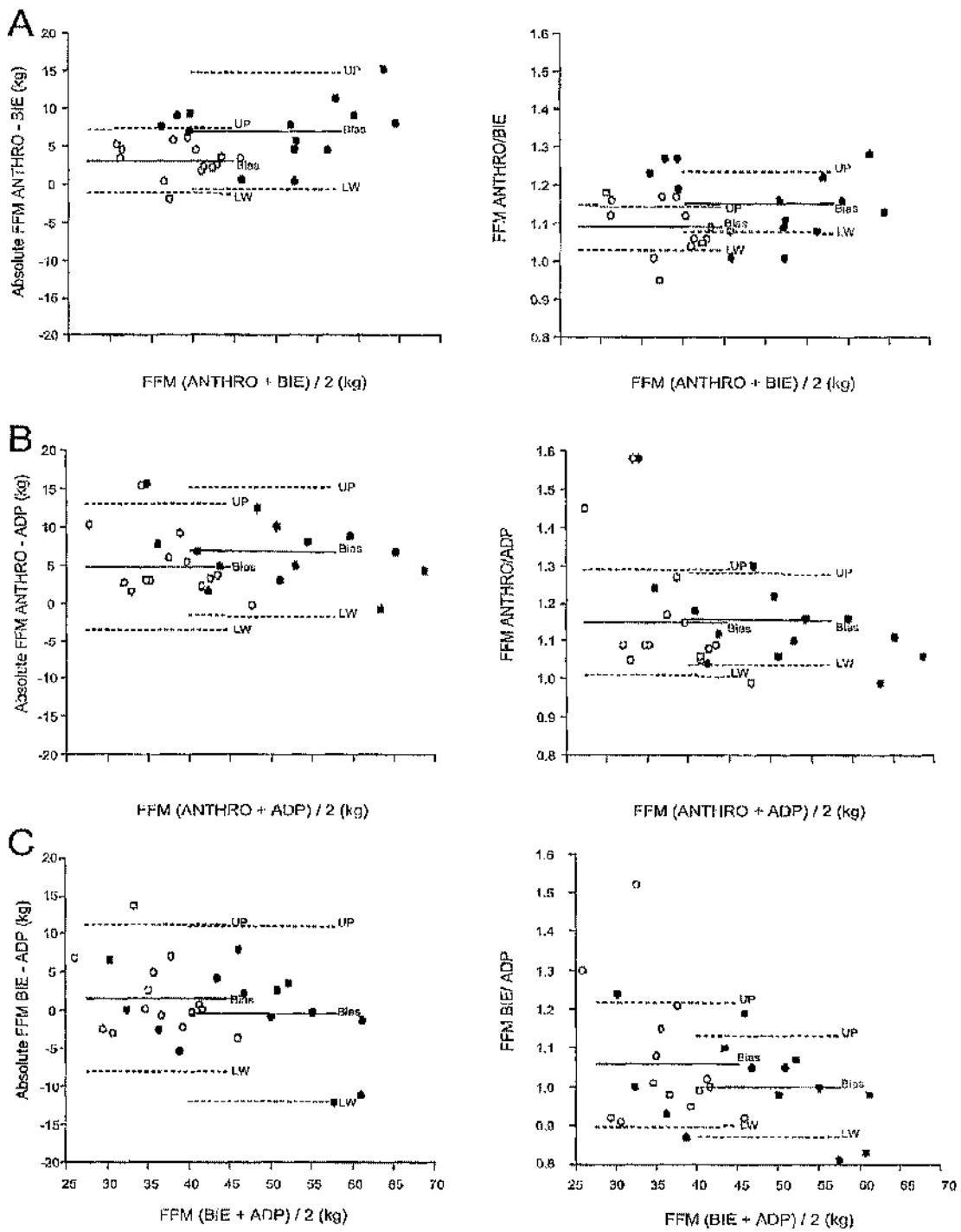
B. Mean bias ± 95% limits of agreement between FFM values estimated by air displacement plethysmography (ADP) using estimated or measured lung volumes (LV) in the same patients.

3.3.2 FFM Depletion and the Limits of Agreement

Nutritional depletion was established according with the criteria proposed by VanItallie et al.: body mass index (BMI) ≤ 21 $kg.m^{-2}$, or fat-free mass index ($FFM.height^{-2}$) ≤ 16 and ≤ 15 $kg.m^{-2}$ in males and females, respectively (VanItallie *et al.*, 1990). If ADP is categorised as a criterion method, 14 patients were classified as FFM-depleted and 14 patients as FFM-non-depleted (Table 3.1): similar results were found when the BIE readings were used. In contrast, there was a significant association between ANTHRO and FFM-depletion; fewer patients were classified as 'depleted' by ANTHRO than the other two techniques (21 patients, $P<0.05$).

Consistent with these results, we found that ANTHRO FFM readings were systematically higher than those obtained by either BIE or ADP, independent of the degree of FFM depletion. The mean bias ± 95% confidence limits of these differences for the FFM-depleted and non-depleted groups, respectively, were: ANTHRO-BIE = 3.1 ± 4.3 kg (9 ± 6 %) and 7.1 ± 7.8 kg (16 ± 8 %) and ANTHRO-ADP = 4.8 ± 6.2 kg (15 ± 14 %) and 6.7 ± 8.5 kg (16 ± 12 %) (Figures 3.2A and 3.2B). In contrast, the mean bias of the BIE-ADP differences were close to zero, although their 95% limits of agreement were as wide as those found for the other comparisons: 1.7 ± 9.6 kg (6 ± 16 %) in the FFM-depleted group and –0.4 ± 11.6 kg (0 ± 13 %) in the non-depleted group, respectively (Figure 3.2C).

Figure 3.2 Limits of agreement according to fat-free mass depletion.



Absolute (left) and relative (right) limits of agreement between anthropometry (ANTHRO), bioelectrical impedance (BIE) and air displacement plethysmography (ADP) in FFM-depleted (open circles) and non-depleted (solid circles) patients with COPD. Definition of abbreviations: UP: upper 95% limit of agreement; LW: lower 95% limit of agreement.

3.3.3 VO_{2PEAK} Correction

As expected, our patients demonstrated substantial exercise limitation, as judged by the lower than normal VO_{2PEAK} (Table 3.1). Consistent with earlier observations, VO_{2PEAK} in the FFM-depleted group was significantly lower than in the non-depleted group (Table 3.1).

In both FFM-depleted and non-depleted groups, allometric correction of VO_{2PEAK} by FFMANTHRO provided higher exponents than either FFMBIE or FFMADP: 1.45-1.41 vs. 0.97-1.18 and 1.08-1.14, respectively (Figure 3.3). The consequence of this being that for both FFM depleted and non depleted patients, the VO_{2PEAK} values corrected by FFMANTHRO were substantially lower than those normalised by FFMBIE or FFMADP (Table 3.2).

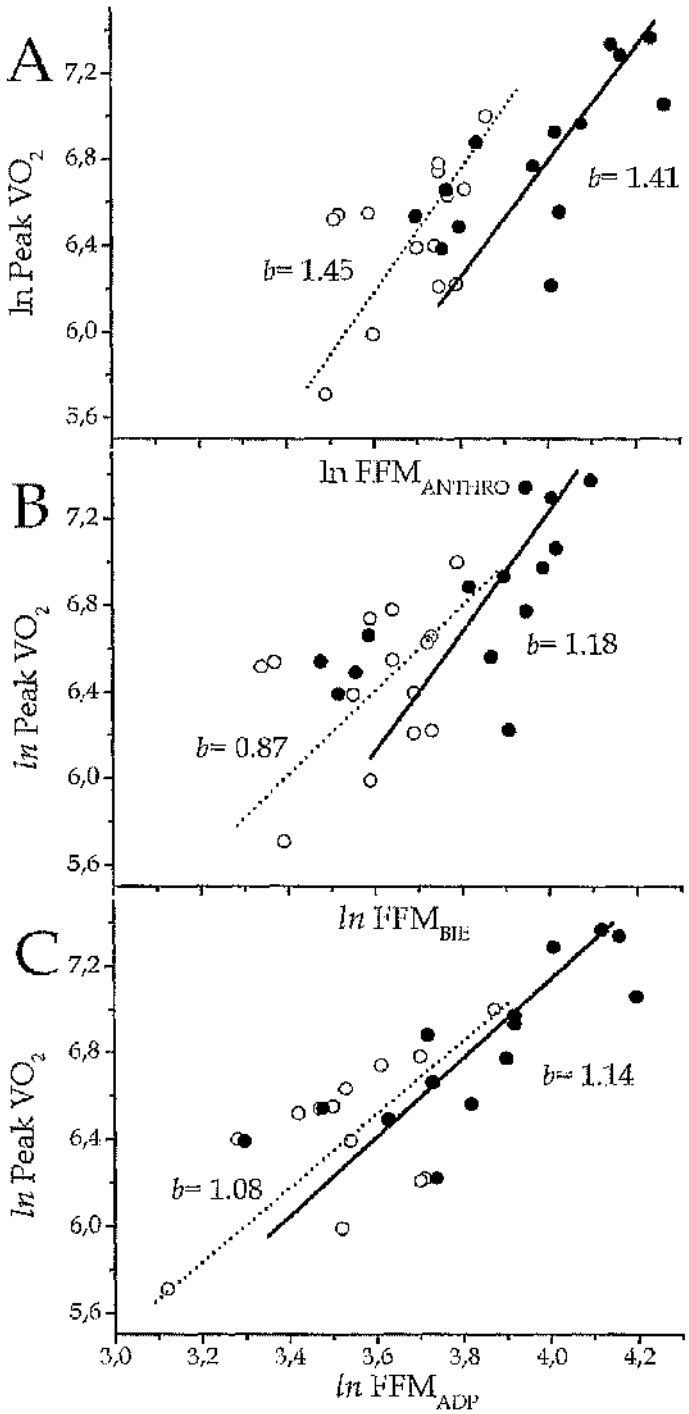
Table 3.2 Absolute and VO_{2PEAK} corrected for fat-free mass (FFM) estimation.

Absolute and vo_{2PEAK} corrected (allometry) fat-free mass (FFM) values according to anthropometry (*ANTHRO*), bioelectrical impedance (BIE) and air displacement plethysmography (*ADP*) in FFM depleted and non-depleted patients with COPD.

	ANTHRO		BIE		ADP	
	FFM (kg)	$Vo_{2PEAK}.FFM^{-x}$ ($ml.min^{-1}.kg^{-x}$)*	FFM (kg)	$Vo_{2PEAK}.FFM^{-x}$ ($ml.min^{-1}.kg^{-x}$)*	FFM (kg)	$Vo_{2PEAK}.FFM^{-x}$ ($ml.min^{-1}.kg^{-x}$)*
Depleted	40.2 ± 4.8	3.62 ± 0.97	37.1 ± 5.1	28.7 ± 8.3	35.4 ± 6.5	14.1 ± 3.3
Non-depleted	54.2 ± 10.0	2.94 ± 0.65	47.1 ± 9.1	10.2 ± 2.4	47.5 ± 11.6	11.8 ± 2.3

*x= FFM_{ANTHRO}: 1.45-1.41 ; FFM_{BIE}: 0.97-1.18 and FFM_{ADP}: 1.08-1.14, in FFM-depleted and non-depleted patients, respectively.

Figure 3.3 Procedures for extraction of the allometric exponents for vo_{2PEAK} correction by FFM according to body composition evaluation method.



A. Procedures for extraction of the allometric exponents for vo_{2PEAK} correction by FFM according to anthropometry (FFMANTHRO), bioelectrical impedance (FFMBIE) and air displacement plethysmography (FFMADP) in FFM-depleted (open circles) and non-depleted

(solid circles) patients with COPD. Definition of abbreviations: ln = natural logarithm; b = regression slope; UP: upper 95% limit of agreement; LW: lower 95% limit of agreement.

3.4 Discussion

The present study demonstrated that the differences in values for FFM estimation based on two widely-used (ANTHRO and BIE) methods and an additional new (ADP) method have clinical relevance in patients with moderate or severe COPD. In relation to this we found that: (i) the between-method limits of agreement were larger than the changes in FFM usually ascribed as consequences of interventions in these patients, (ii) the diagnosis of FFM depletion was less frequent when ANTHRO, instead of BIA or ADP, was used for measurement of body composition and (iii) these discrepancies were sufficiently large to provide different exponents for the allometric correction of $\dot{V}O_{2PEAK}$. From our results we conclude that a single method of FFM estimation should be used, either in cross-sectional or longitudinal evaluations of patients with COPD, and that anthropometry, in particular, tends to underestimate the prevalence of FFM depletion.

The practical importance of body composition determination in patients with COPD is now well-established. Several authors have demonstrated that loss of FFM is associated with reduced exercise tolerance and a number of negative prognostic indicators (Mostert *et al.*, 2000; Decramer *et al.*, 1997; Gray-Donald *et al.*, 1996; Baarends *et al.*, 1997a; Yoshikawa *et al.*, 1999). With this in mind, we have analysed our data taking into consideration the confounding aspects of FFM-depletion. In addition, others have already shown that there are some discrepancies in the estimation of body compartments in COPD patients, depending on the specific method used for body composition assessment (Schols *et al.*, 1991b; Pichard *et al.*, 1997; Kyle *et al.*, 1998; Engelen *et al.*, 1998). A novel aspect of the present study is that we addressed the clinical significance of potential inter-method differences. We wished to determine to what extent these differences would affect clinical aspects that more directly relate to body composition measurements in patients with COPD. We have demonstrated that these differences are large enough to cast doubt on the validity with which results derived from multiple methods can be compared (Figure 3.2). Also, importantly, such differing results are also likely to distort the analysis of FFM-corrected $\dot{V}O_{2PEAK}$ values (Figure 3.3 and Table 3.2). These results are consistent with those recently described by Steiner et al. who also concluded that ANTHRO tended to overestimate FFM in patients with COPD (Steiner *et al.*, 2002). In the present study, we have extended their findings by showing that these discrepancies are wide enough to influence the analysis of symptom-limited $\dot{V}O_{2PEAK}$ in this patient population (Figure 3.3 and Table 3.2).

In this regard, vo_{2PEAK} has been 'normalised' for body mass, or more properly, for FFM in order to relate the metabolic activity to its structural determinants. We analysed this relationship in patients separated by presence or not of FFM-depletion since large differences in distribution of the independent variable (here FFM) are known to influence the describing parameters. However, the traditional procedure of simply dividing a physiological variable (y) by an anthropometric or performance attribute (x) is prone to misinterpretation. It is important to emphasise that this approach can provide valid results only when: (i) the coefficient of variation for x divided by the coefficient of variation for y equals the Pearson product moment correlation between the two variables, (ii) there is no heterocedasticity or skewness in the residuals and (iii) the relationship goes through the origin (Nevill & Holder, 1995). These shortcomings of the so-called 'ratio standard' analysis are even more relevant in subjects with disproportionately lower x values, such as is the case for patients with COPD: in these circumstances, there is a progressive bias which tends to give an advantage to individuals with the lowest x values. Conversely, the allometric analysis (power function) assumes a multiplicative error around the regression line and is able to scale correctly since the relationship passes through the origin (Nevill & Holder, 1995).

In the present study, we found that the inter-method differences were sufficiently wide to result in different power function exponents: differences in FFM-corrected vo_{2PEAK} therefore increased exponentially after allometric adjustment (Table 3.2). These exponents, however, differed both from the values based on the theory of geometric similarity (i.e., vo_2 in geometrically similar bodies varies with the cross-sectional area which, in turn, relates to body mass to the power 0.67 (Astrand & Rodahl, 1986)) and those estimated with consideration of the elasticity of tissues.

A number of hypotheses could be considered to explain this finding. Firstly a substantial portion of the measured vo_2 may not have been related to the functional activity of the peripheral muscles, e.g., the vo_2 associated with increased respiratory muscle work. Secondly, although it has been previously demonstrated that the metabolic cost of cycle ergometry is closely related to leg mass, (Neder *et al.*, 2000b) in the present study we have determined total body mass rather than leg FFM. Lastly, the exponents we derived presented relatively wide confidence intervals, indicating that they should be viewed with some caution. Regardless of these limitations, however, it should be pointed out that we used allometry solely to investigate whether the choice of a specific method for body composition assessment would present practical consequences. Therefore, more comprehensive studies are

warranted to determine the precise allometric relationship between vo_{2PEAK} and FFM in patients with COPD.

We believe that our study is the first to use ADP for body composition determination in patients with COPD. This method utilises the inverse relationship between pressure and volume (Boyle's law) to determine body volume (Vb). The basic principles of densitometry are subsequently used to determine body composition (body density = body mass/Vb) (Dempster & Aitkens, 1995). ADP has been validated against more traditional methods, such as hydrostatic weighing (McCrory *et al.*, 1995; Biaggi *et al.*, 1999). The main advantage of ADP is that it is based on air displacement rather than water immersion; i.e., it is simpler, more comfortable and might well present wider clinical applications. However, the ADP method also requires that lung volumes be taken into account: Vb determined by Boyle's law is underestimated by 40%, since the isothermal nature of the thoracic gas means that this air is 40% more compressible than the chamber air which temperature is free to change (adiabatic conditions) (Dempster & Aitkens, 1995). While the ADP system allows the operator to use predicted lung volumes, we have demonstrated that this strategy should not be used in airflow-obstructed patients with COPD: as shown in Figure 3.1B, it grossly overestimated FFM in our sample.

A finding of particular note in our study was the systematic overestimation of FFM by ANTHRO (Figure 3.2). As mentioned, these results are consistent with those described by Schols et al. (Schols *et al.*, 1991b) and Steiner et al. (Steiner *et al.*, 2002): the latter authors, for example, found that the upper 95% limit of agreement between ANTHRO and DEXA reached +8.2 kg in favour of ANTHRO. In fact, skinfold measurements are notoriously imprecise in older subjects, particularly in those who have more fat mass. Furthermore, the measurement outcomes are sensitive to the investigator's experience with the method (Ellis, 2000). In our study, as all measurements were performed in triplicate by the same experienced investigator, we are confident that this was not a relevant issue to explain our results. It is more likely, therefore, that the combined effects of COPD and age influenced the pattern of fat distribution (e.g., a more 'central' pattern) and also the degree of the hydration of the lean tissues (Ellis, 2000;Schols *et al.*, 1991b; Pichard *et al.*, 1997; Kyle *et al.*, 1998). Moreover, it has been demonstrated that some patients with COPD might present relatively preserved upper body strength (Gosselink *et al.*, 2000). Since our skinfold measurements were obtained in these body regions, we might speculate that this led to an overly optimistic

view of the total body FFM. Nonetheless, our results, and those of others,(Schols *et al.*, 1991b; Steiner *et al.*, 2002) suggest that anthropometric readings should be viewed with extreme caution in patients with COPD.

A major limitation of our study relates to the relatively small sample size. The Bland-Altman analysis is sensitive to the number of observations, since a few discrepant values can affect the mean bias and its confidence limits when small samples are used. However, the limits of agreement found in the present study are remarkably similar to those reported between DEXA and ANTHRO by Steiner et al. (Steiner *et al.*, 2002), for example. It should also be acknowledged that the ADP and ANTHRO methods are based on the same densitometry principles: this might have led to an expectation for improved intrinsic comparability between ADP and ANTHRO, to the detriment of BIE.

In conclusion, we have demonstrated that the between-method differences in FFM estimation are likely to be sufficiently wide to present clinical consequences in-patients with moderate-to-severe COPD. Therefore, it is advisable that a single method be used for FFM estimation both in cross-sectional and longitudinal evaluations of this patient population. The degrees of exercise limitation and the factors which determine them become more recognised and examined in COPD patients yearly. Therefore other aspects, such as means of measurement of values, taken to quantify the disability must be examined carefully. The same rigour that is applied to the standards of measures such as those of pulmonary function should be introduced for practices including FFM estimation, as these methodologies become more widely introduced into clinical, as well as research, practice.

CHAPTER 4

CREATINE SUPPLEMENTATION AS AN INTERVENTION FOR EXERCISE INTOLERANCE

Abstract

Background: Skeletal muscle wasting and dysfunction are strong independent predictors of mortality in patients with chronic obstructive lung disease. Creatine nutritional supplementation increases muscle mass and exercise performance in health. We therefore performed a controlled study to examine for similar effects in COPD patients.

Methods: Thirty-eight patients with COPD (FEV_1= 46±15% predicted) were randomised between placebo (glucose polymer 40.7g) or creatine (creatine monohydrate 5.7g, glucose 35g) supplements in a double blind trial. After 2-weeks loading (1 dose three times daily), patients participated in an out-patient pulmonary rehabilitation programme combined with maintenance (once daily) supplementation. Pulmonary function, body composition, and exercise performance (peripheral muscle strength and endurance, shuttle walking, cycle-ergometry) took place at baseline (n=38), post loading (PL) (n=36) and post rehabilitation (PR) (n=25).

Results: Reporting between group differences as mean (95%CI), we found no difference in whole body exercise performance e.g. incremental shuttle walk distance, -23.1 m (-71.7 to 25.5) PL and -21.5 (-90.6 to 47.7) PR. Creatine increased fat-free mass by 1.09 kg (0.43 to 1.74) PL and 1.62 kg (0.47 to 2.77) PR. Peripheral muscle performance improved; knee-extensor strength by 4.2 (1.4 to 7.1) N.m, and endurance by 411.1 (129.9 to 692.4) J PL, knee-extensor strength 7.3 (0.69 to 13.92) N.m and endurance 854.3 (131.3 to 1577.4) J PR. Between baseline and post rehabilitation creatine improved health status, St. George's Respiratory Questionnaire total score -7.7 (-14.9 to -0.5).

Conclusions: Creatine supplementation led to increases in fat free mass, peripheral muscle strength and endurance, health status, but not exercise capacity. Creatine may constitute a novel ergogenic therapy in COPD.

4.1 Introduction

Chronic obstructive pulmonary disease (COPD) is a disabling condition, predicted to become the fourth most common cause of death world-wide by 2020 (Lopez & Murray, 1998). There is a growing recognition that the muscle wasting and compromised muscle strength often associated with COPD are predictors of handicap, disability and increased mortality (Marquis *et al.*, 2002). Wide-ranging strategies have been employed in an attempt to restore muscle mass, such as nutritional manipulations (Steiner *et al.*, 2003), the administration of recombinant human growth hormone (Burdet *et al.*, 1997), anabolic steroids (Creutzberg *et al.*, 2003) and appetite stimulants (Weisberg *et al.*, 2002). However, despite the majority of these strategies leading to an increase in muscle mass, muscle strength and measures of whole-body exercise performance were typically unaffected.

Creatine monohydrate is widely used by healthy individuals as an aid to exercise performance. In skeletal muscle, creatine undergoes rapid and reversible phosphorylation catalysed by the enzyme creatine kinase, a reaction that forms Phosphocreatine (PCr). PCr provides an immediate source of high-energy phosphate which is crucial for maintaining the rate of ATP resynthesis during the initial stages of exercise, especially when energy demands are high. Uptake and incorporation of exogenous creatine into skeletal muscle is evident in increased resting intramuscular phosphocreatine (PCr) levels (Harris *et al.*, 1992; Greenhaff *et al.*, 1994a). In addition, in healthy young and older subjects, increases in body mass, fat-free mass, muscle strength and muscle endurance have been demonstrated (Gotshalk *et al.*, 2002; Kilduff *et al.*, 2002b).

These observations raise the interesting premise that creatine supplementation might elicit similar ergogenic effects in patients with COPD, especially those who manifest muscle wasting. However, there have been few studies of creatine supplementation in disease states, particularly those characterised by impaired exercise tolerance secondary to systemic dysfunction. Thus, while muscle performance has been reported to be improved following creatine administration in chronic heart failure (Andrews *et al.*, 1998a), in mitochondrial myopathies (Tarnopolsky *et al.*, 1997) and following rehabilitation from disuse atrophy (Hespel *et al.*, 2001), there have to date been no formal evaluations of creatine supplementation in patients with COPD.

Using a randomised, double-blind and placebo-controlled design, the objective of the present investigation was therefore to evaluate the effects of oral creatine supplementation in patients with moderate-to-severe COPD, administered both in isolation and in combination with an exercise-training programme.

4.2 Methods

4.2.1 Subjects

Patients referred for pulmonary rehabilitation with an established diagnosis of COPD were assessed for inclusion in the study ((1987). Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma.). All were clinically stable, as defined by no change in medication dosage or exacerbation of symptoms in the preceding 4 weeks. No patient presented cardiovascular, locomotor or neurological conditions which precluded exercise training; in addition, oral corticosteroids were not allowed in the preceding month. All patients were optimised in terms of standard medical therapy: maintenance medication included β_2-agonists, anticholinergics, theophylline, and inhaled steroids. No patient was receiving chronic domiciliary oxygen therapy.

Before the tests, the procedures, including the known risks, were described in detail and written, informed consent (as approved by the Glasgow Royal Infirmary Ethics Committee) was obtained from all patients.

4.2.2 Study design

This was a double-blind placebo-controlled trial. Patients were randomly assigned to receive: (i) creatine monohydrate with glucose polymer (5.7 g of creatine and 35 g of glucose per dose) or (ii) glucose polymer only (40.7 g per dose). The Cr group ingested 17.1 $g \cdot d^{-1}$ $Cr \cdot H_2O$ (equivalent to 5 g Cr × 3 times daily) for the first 14 days (loading). From day 15, patients consumed 5.7 $g \cdot d^{-1}$ $Cr \cdot H_2O$ (equivalent to 5 g Cr daily) for the remainder of the study (maintenance). This maintenance dose was selected on the basis of work demonstrating that 2 $g \cdot d^{-1}$ $Cr \cdot H_2O$ was adequate in maintaining elevated muscle PCr stores in patients not involved in strenuous exercise (Harris *et al.*, 1992). As the patients in the present study were training twice a week at high intensities, it was decided to increase the maintenance dose to 5 $g \cdot d^{-1}$ $Cr \cdot H_2O$ in an attempt to maintain muscle PCr stores. Each supplement consisted of 5.7 g of $Cr \cdot H_2O$ and 35 g of glucose polymer made up in approximately 500 mls of warm to hot water. Dissolving Cr in warm to hot water prevented any detectable formation of creatinine and no parts of the supplement remained undissolved. The addition of glucose to the Cr has been shown to significantly enhance the uptake of Cr (Green *et al.*, 1996; Steenge *et al.*,

1998). Patients were instructed to ingest the supplements at equal intervals throughout the day. The placebo group consumed 120 $g \cdot d^{-1}$ of glucose polymer (40 g × 3 times daily) for the first 14 days, followed by 40 g a day for the subsequent duration of the study. The placebo group followed the same procedure as the Cr group with regard to the preparation of the supplements. These preparations were prepared by personnel not involved in the study and in sites separate from the investigators. The two supplements were identical in weight, appearance and taste. Randomisation sequences, labelling and quality control were all performed independently by the Glasgow Western Infirmary pharmacy production unit. After the initial loading period, all patients entered the hospital, out-patient based pulmonary rehabilitation programme. The study design is represented below in figure 4.1.

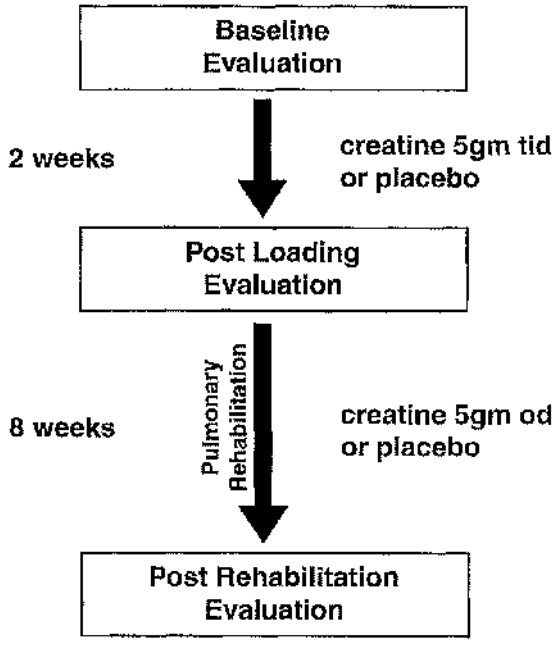


Figure 4.1 Study design

The study evaluations were performed on separate days, with at least 2 days rest in-between. Randomisation sequences were centrally generated at the Western Infirmary production unit and block randomised in groups of four. Patients underwent evaluation on three occasions: baseline, post creatine/placebo loading and following exercise training. The study visit schedule is detailed in table 4.1 below.

STUDY PERIOD	BASELINE	POST LOADING	POST REHAB
FULL PFT, BODY COMPOSITION and STRENGTH	A	A	A
INCREMENTAL CPET, HEALTH STATUS and BLOODS	A		A
ENDURANCE CPET, ISWT and ESWT	B	B	B

Table 4.1 Visit Schedules

Comprehensive evaluations were performed at baseline and after exercise training. Some investigations were not performed post loading, as detailed above. Weeks A and B represent distinct periods of evaluation, as most exercise tests had to be performed on separate days.

Both supplements had similar taste, texture and appearance and were placed in generic containers to ensure double-blind administration. Patients were also requested to eliminate caffeine and caffeine containing foods from their diet over the loading phase to minimize the possible inhibitory effects of caffeine on the ergogenic effect of Cr (Vandenberghe, *et al.*, 1996). At the end of the study all patients gave verbal assurance that they had complied with all instructions.

Considering that the amount of metabolically active tissue (muscle) could affect response to the interventions, patients were previously stratified according to muscle mass depletion: reduced body mass index (< 21 $kg.m^{-2}$) and/or reduced fat-free mass index: ($(FFM.height^{-2})$ < 15 $kg.m^{-2}$ in females or < 16 $kg.m^{-2}$ in males) (Schols *et al.*, 1993; VanItallie *et al.*, 1990). Therefore, care was taken that a similar number of FFM-depleted patients were presented in each group. Data were pooled for analysis only after certification that there was no effect of FFM depletion on the responses to the intervention.

4.2.3 Procedures

The methodologies employed during the study evaluations are detailed in chapter 2. Full pulmonary function testing (Section 2.2.1) and health status assessment (Section 2.2.5) were performed. Comprehensive evaluations of body composition using air displacement plethysmography (Section 2.2.2.3), lower (Section 2.2.3.1) and upper (Section 2.2.3.2) body peripheral muscle function, and whole body exercise capacity (Section 2.2.4) we undertaken. The pulmonary rehabilitation exercise programme is described in Section 2.3. The approach to statistical analysis is detailed in Section 2.5.

4.2.4 Power Calculation

The primary outcome measure of this trial was distance walked on the incremental shuttle walk test. The study was powered to detect equivalent effects to those reported for pulmonary rehabilitation (mean improvement of 56 metres (SD 58) on ISWT recalculated from Singh et al (Singh *et al.*, 1998b)). We aimed to recruit 60 patients, with an expected 33% drop out over the rehabilitation period.

4.3 Results

4.3.1 Study compliance

The supplementation was well tolerated in most subjects without reported side effects. Two patients, both allocated to placebo, were unable to take their supplements. These patients attributed this to the sweet taste of the drinks. The pattern of recruitment and drop out from the study is represented in figure 4.4.

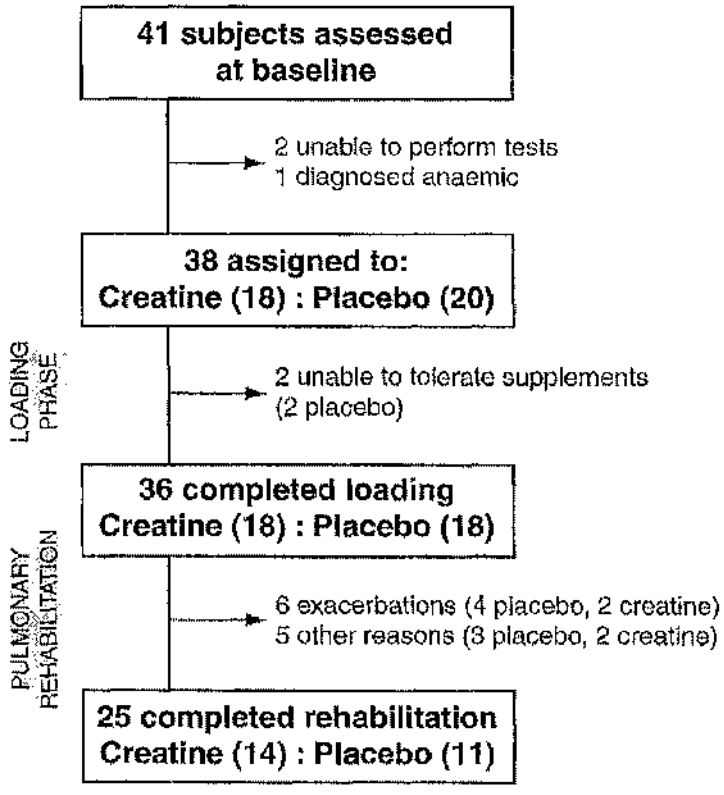


Figure 4.2 Study recruitment and drop out.

The number of patients unable to complete the course was in keeping with the number of drop-outs expected during our standard pulmonary rehabilitation programme. Twenty-five patients successfully completed the exercise-training programme. The reasons for study discontinuation were as follows (Figure 2): severe exacerbations (6 subjects, 4 placebo, 2 creatine), poor motivation/non compliance to exercise programme (3 subjects, 2 placebo, 1 creatine) backache (1 subject, creatine) and vestibular disease (1 subject, placebo). A reassuring aspect is that there was no difference in the number of patients unable to complete the rehabilitation programme between the creatine and placebo groups (7 subjects placebo vs. 4 subjects creatine, non significant difference).

4.3.2 Body composition stratification

Stratification of the patients into groups according to fat free mass depletion was vital to try to match subjects as closely as possible with regard to functional parameters. The impact of fat-free mass depletion on exercise performance and health related quality of life is illustrated below in table 4.2. It is clearly demonstrated that, despite adequate matching for pulmonary function, other aspects of health, fundamentally important to COPD patients can be significantly different if body composition is not taken into account.

	Depleted (n=18, 10 male)	Non-depleted (n=18, 13 male)
FEV1 % predicted	42.89 ± 11.70	46.72 ± 16.52
Body mass, kg	52.66 ± 8.62*	74.71 ± 15.64
Fat-free mass, kg	38.52 ± 8.72*	46.96 ± 11.14
Fat-free mass index, kg/m^2	14.7 ± 2.8*	17.1 ± 3.3
Body mass index, kg/m^2	20.0 ± 3.2**	27.3 ± 4.4
Peak torque, N.m	71.27 ± 26.35*	96.66 ± 29.48
Shuttle walk distance, m	293.88 ± 105.94	330.56 ± 145.62
VO_{2PEAK}, l/min	0.71 ± 0.24*	1.02 ± 0.36
SGRQ total	72.06 ± 10.92*	61.28 ± 12.45
SGRQ symptoms	76.06 ± 17.36	80.94 ± 16.24
SGRQ activities	85.51 ± 9.95*	75.40 ± 15.45
SGRQ impacts	59.92 ± 13.35*	46.60 ± 14.10

Table 4.2 Impact of fat free mass upon functional characteristics of our COPD cohort. Values are mean ± SD.

*p<0.05, **p<0.01 for unpaired t test, between group difference

The table demonstrates that the extent of airflow obstruction was comparable in both groups. As expected, mean body weight and fat-free mass was significantly higher ($p<0.05$) in the non-depleted group, and interestingly, mean peak torque and exercise capacities were significantly greater ($p<0.05$) in non-depleted patients. In keeping with the definition of tissue depletion, significantly lower values for BMI and FFMI were seen in the depleted group of patients. Although the mean shuttle walk distance in the non-depleted group was greater than in the depleted group, this difference was not significant. The most salient

finding when considering our patients as nutritionally depleted and non-depleted, was the significantly greater health-related quality of life ($p<0.05$) in the non-depleted group, as measured by the St. George's Hospital Respiratory Questionnaire.

4.3.3 Baseline results

We found that there were no significant differences in most baseline characteristics between the placebo and creatine groups, although the heterogeneity of the group inevitably makes it difficult to match subjects for all of the variables relating to pulmonary function, body composition and health related quality of life. In the table below (Table 4.3) pulmonary function characteristics for creatine and placebo groups are represented. Vital capacity was the only measurement to show a significant between group difference.

Table 4.3 Baseline characteristics; pulmonary function.

	Creatine (n= 18, 10 male)	Placebo (n= 18, 13 male)
Age, years	61.7 ± 8	63.9 ± 10
CP year	52.3 ± 32	51.8 ± 30
Pulmonary Function		
FEV_1, litres	1.12 ± 0.3	1.12 ± 0.5
FEV_1 % predicted	45.4 ± 14	44.2 ± 15
FEV_1/FVC	35.9 ± 10.6	40.0 ± 8.9
VC, litres	3.21 ± 0.7	2.76 ± 0.9
VC % predicted	103.2 ± 22.0 **	83.4 ± 16.7
TLC, litres	6.93 ± 1.2	7.10 ± 1.4
TLC, % predicted	125.3 ± 17.2	127.6 ± 16.8
RV, litres	3.72 ± 1.1	4.40 ± 1.09
RV, % predicted	179.2 ± 55.5	210.6 ± 58.2
DLCO, units	46.4 ± 23	44.7 ± 27
MIP, cm H_20	62.3 ± 25	61.4 ± 30
MEP, cm H_20	81.4 ± 33.4	77.2 ± 29.5

** Significant between group difference. $P < 0.01$

Table 4.4 Baseline body composition and muscle function characteristics.

	Creatine (n= 18, 10 male)	Placebo (n= 18, 13 male)
Age, years	61.7 ± 8	63.9 ± 10
Body Composition		
Fat-free mass, Kg	44.3 ± 8.9	42.2 ± 12.6
Fat-free mass, %	71.3 ± 11.3	65.7 ± 11.7
BMI, Kg/m^2	23.2 ± 3.6	24.3 ± 6.6
Upper Limb Muscle Function		
Peak force, N	28.4 ± 8.3	25.0 ± 7.7
Total repititions	48.9 ± 14.5	50.6 ± 17.8
Lower Limb Muscle Function		
Peak Torque, N.m	85.7 ± 26.6	82.2 ± 34.5
Total Work, J	1832.7 ± 740.1	1648.1 ± 881.0

There were no demonstrable differences in baseline measurements between the creatine and placebo groups with regard to body composition and muscle function. The 'normal' mean BMI represents the successful stratification by body composition classification, but it should be noted that within each group there remains a wide spread of individual muscle bulks.

Table 4.5 Baseline exercise performance indices.

	Creatine (n= 18, 10 male)	Placebo (n= 18, 13 male)
Field tests		
Distance walked ISWT, m	331 ± 111	293 ± 142
Maximum Borg ISWT., Breathlessness	7.22 ± 1.80	6.50 ± 1.47
Time walked ESWT, s	340 ± 209	392 ± 297
Incremental Cycle ergometry		
Peak WR, Watts	60.8 ± 27.1	56.4 ± 30.5
VO_{2PEAK}, l/min	0.858 ± 0.393	0.887 ± 0.288
Peak Borg, Breathlessness	6.82 ± 1.88	6.77 ± 1.68
Peak Borg, Legs	7.00 ± 2.29	7.06 ± 2.11
Lactate threshold l/min	0.525 ± 0.15	0.523 ± 0.25
Peak Ventilation, l/min	41.79 ± 10.2	38.3 ± 9.6
Breathing Reserve, l/min	3.3 ± 8.2	8.2 ± 12.4
Peak Heart Rate, Beats/min	126.1 ± 17.6	127.1 ± 10.3
Endurance cycle ergometry		
Peak Borg Breathlessness	7.79 ± 1.9	6.50 ± 2.2
Tlim, s	164 ± 66	126 ± 44

Data are presented as group-mean values, ± one standard deviation.

There were no statistically significant differences in baseline exercise capacity for either group. The mean distances walked on the ISWT (331m in the creatine and 293m in the placebo groups) give predicted oxygen uptakes significantly below that expected of adults, where 450m walked would signify an oxygen uptake of greater than 14 ml.kg-1.min^{-1} (Lewis *et al.*, 2001). These findings, coupled with a mean peak oxygen uptake of under 1 l/minute represents severe exercise impairment in this patient group. From the mean FEV_1's, a predicted maximum voluntary ventilation of between 39l and 45l (depending on the multiplier used i.e. 35 or 40(Wasserman *et al.*, 1999)) is found. This determines the likely ceiling of maximum ventilation during exertion. From the values obtained at peak exercise

during the ramp incremental test it can be seen that both groups approached (38.3 $l.min^{-1}$ for placebo) or were within (41.8 $l.min^{-1}$ in the creatine group) this domain, representing the fundamental ventilatory impairment present. Subjects stopped exercise with slightly higher ratings for leg effort than dyspnoea at maximal exercise during the incremental test; a finding previously recognised in this patient group (Killian *et al.*, 1992). The mean ages of the patients give a predicted maximum heart rate of approximately 160 beats per minute. Both groups had demonstrable heart rate reserve at peak exercise. These findings are typical of patients with established disability secondary to COPD (Wasserman *et al.*, 1999).

The table below (Table 4.6) displays health related quality of life for the 2 groups at baseline.

Table 4.6 Health related quality of life at baseline for the placebo and creatine groups.

	Creatine (n= 18, 10 male)	Placebo (n= 18, 13 male)
HEALTH STATUS		
St George's Respiratory Questionnaire		
SGRQ total score	65.6 ± 14.0	67.8 ± 11.7
SGRQ symptoms	80.4 ± 18.5	76.6 ± 15.1
SGRQ activity	82.5 ± 12.9	78.4 ± 14.7
SGRQ impact	50.5 ± 60.7	56.0 ± 13.3
Chronic Respiratory Disease Questionnaire		
CRQ Dyspnoea	2.35 ± 0.8	2.46 ± 0.7
CRQ Fatigue	2.90 ± 1.5	2.93 ± 0.9
CRQ Emotional Function	3.86 ± 1.5	4.03 ± 1.5
CRQ Mastery	4.00 ± 1.7	3.66 ± 1.23

Data are presented as group-mean values, ± one standard deviation.

For all the above baseline characteristics there were no statistically significant differences between the placebo and creatine groups. The baseline health status scores in our patient group are typical of the deleterious effect COPD has on quality of life. Age matched healthy subjects have SGRQ scores of 7 ± 7, 12 ± 15, 11 ± 13, and 3 ± 5 for total, symptoms, activity

and impact respectively. In fact our patient group displayed worse baseline health status than of similar COPD cohorts. In the ISOLDE study, where the mean FEV_1 was 1.23 l (somewhat higher than our values), mean SGRQ scores were 49, 66, 62 and 37 for total, symptoms, activity and impact respectively (Burge *et al.*, 2000). These values are all about 20 points worse in our cohort of studied patients. This is likely to represent social deprivation in the East end of Glasgow (the main area of subject recruitment).

4.3.4 Effect of creatine supplementation

4.3.4.1 Post-Loading Evaluation

The supplementation was generally well tolerated without reported side effects. However, two patients from the placebo group did not complete the loading phase, because of a dislike of the sweet taste of the glucose. Therefore, 36 subjects successfully completed this phase (Figure 4.4).

For the creatine group, the initial loading intervention was associated with significant increases from the pre-intervention baseline (Δ) in several muscle-performance measures, compared to the corresponding changes for the placebo group (Table 4.7). Thus, body weight in the creatine group was increased by 1.1 kg or 1.8% *vs.* 0.1 kg and 0.2% in placebo ($p<0.05$, between-group difference); predominantly in the fat-free mass component (Δ = 0.9 kg or 1.3% *vs.* -0.2 kg or 0.5%; $p<0.05$, between-group difference) (Figures 4.3 and 4.4).

Similarly, lower-limb muscle (knee-extensor) strength (peak torque) was increased in the creatine group compared to placebo (Δ = 3.5 N.m or 4.1% *vs.* -0.7 N.m or -0.9%; $p<0.05$, between-group difference) (Figure 4.3), as was endurance (knee-extensor total work) (Δ = 424 J or 23.1% *vs.* 13 J or 0.8%; $p<0.01$, between-group difference). Upper-limb muscle (handgrip) endurance in the creatine group was also increased significantly, relative to placebo (Δ = 3.9 repetitions or 8.0% *vs.* 1.1 repetitions or 2.2%; $p<0.05$, between-group difference). Upper-limb strength (peak handgrip force) was unaffected in either group.

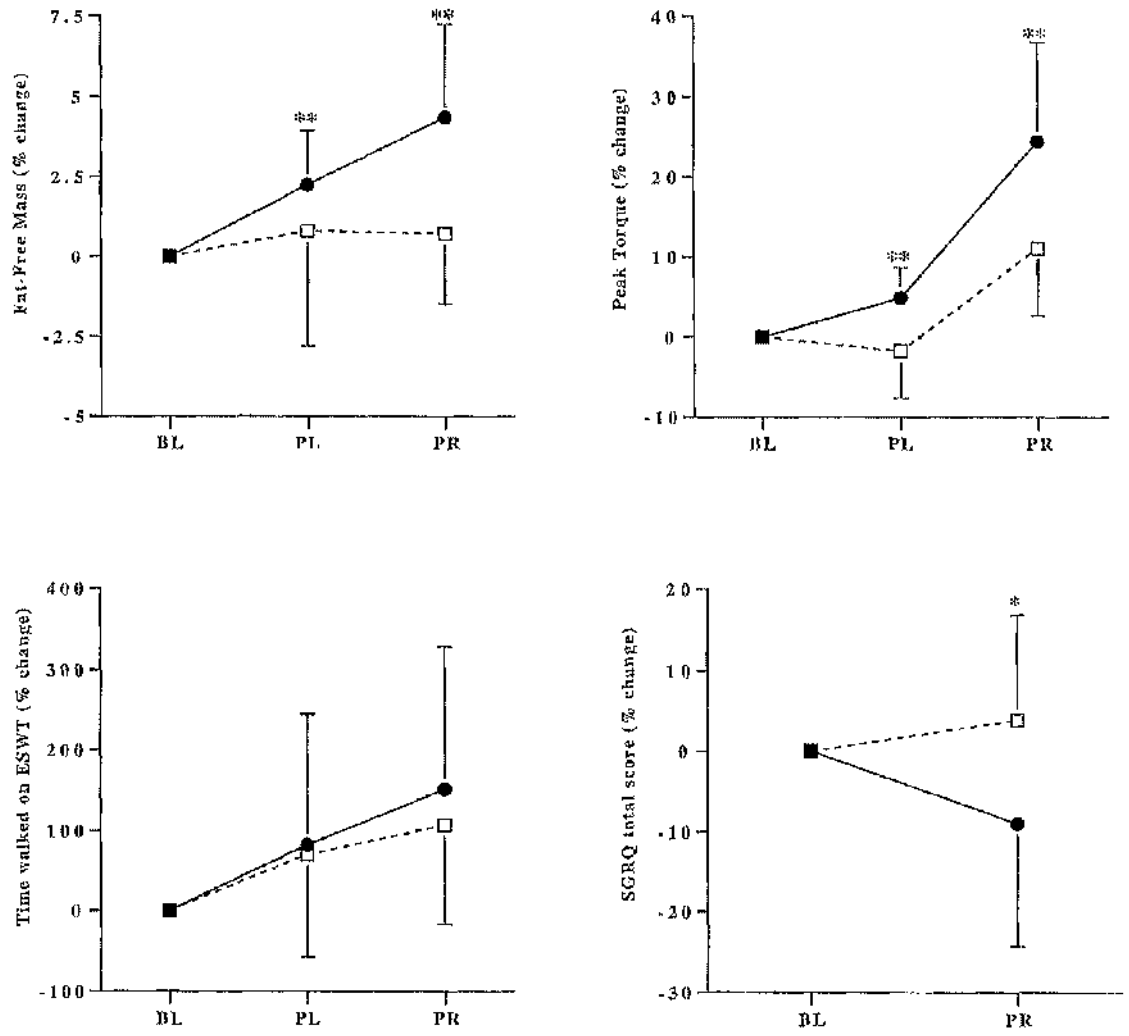
Table 4.7 Responses to the initial loading phase for creatine and placebo groups.

	Creatine (n= 18)	Placebo (n= 18)	Creatine versus Placebo difference
Pulmonary Function			
FEV_1, L	-0.01 (-0.1 to 0.1)	-0.05 (-1.79 to 0.07)	0.05 (-0.11 to 0.19)
MIP, cmH_2O	7.13 (-0.3 to 14.5)	3.69 (-2.2 to 9.6)	3.44 (-5.62 to 12.49)
Body Composition			
Total body mass, kg	1.1 (0.7 to 1.4) §§	0.1 (-0.3 to 0.5)	0.95 (0.43 to 1.8) *
Fat-free mass, kg	0.9 (0.6 to 1.3) §§	-0.2 (-0.7 to 0.4)	1.09 (0.43 to 1.74) *
Fat mass, kg	0.1 (-0.1 to 0.3)	0.2 (-0.3 to 0.8)	-0.13 (-0.71 to 0.44)
Upper-limb Muscle Function			
Peak Force, N	0.6 (-1.4 to 2.6)	-0.12 (-1.92 to 1.68)	0.72 (-0.58 to 2.02)
Total Repetitions	3.9 (1.9 to 6) §§	1.1 (-2.4 to 4.6)	2.89 (0.26 to 5.52) *
Lower-limb Muscle Function			
Peak Torque, N.m	3.5 (2.3 to 4.7) §§	-0.7 (-3.4 to 2.0)	4.2 (1.4 to 7.1) *
Total Work, J	424 (137 to 710) §§	13 (-44 to 69)	411.1 (129.9 to 692.4) **

Exercise Capacity			
Time to intolerance (CWR), s	15 (-30 to 60)	54 (18 to 90) §	-40.0 (-94.3 to 16.3)
End-ex Dyspnoea (CWR)	-0.3 (-1.9 to 1.3)	0.2 (-0.7 to 1.1)	-0.5 (-2.0 to 1.0)
End-ex RPE (CWR)	0.2 (-1 to 1.4)	-1.0 (-2 to 0.0)	1.3 (0.4 to 2.9)
Distance walked (ISWT), m	-8 (-44 to 28)	15 (-19 to 50)	-23.1 (-71.7 to 25.5)
Time walked (ESWT), s	132 (61 to 203) §§	92 (-59 to 242)	40.5 (-115.7 to 196.7)

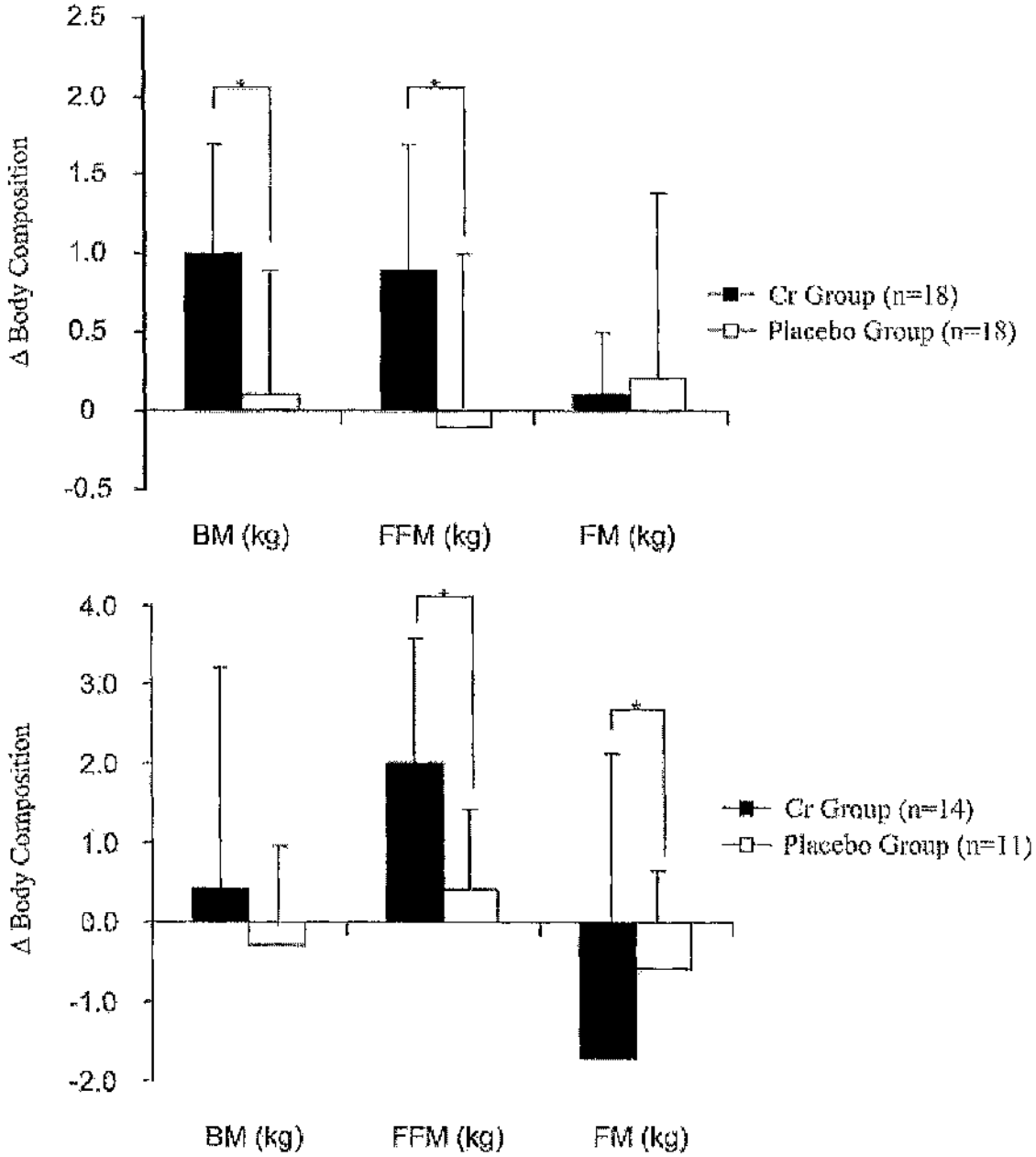
Data are presented as group-mean values (± 95% confidence intervals) for the absolute changes from baseline and between group difference. Abbreviations: IET: incremental exercise test; CWR: constant work-rate exercise test; ISWT: incremental shuttle-walk test; ESWT: endurance shuttle-walk test; RPE: Rating of perceived exertion. Statistically-significant differences: § $p<0.05$, §§ $p<0.01$ (within-group difference from baseline); * $p<0.05$, ** $p<0.01$ (between-group differences).

Figure 4.3 Group mean outcomes for major measures.



Group-mean responses (± one standard deviation) of fat-free mass, knee-extensor peak torque, time walked on the endurance shuttle-walk test (ESWT) and the total score on the St. George's Respiratory questionnaire (SGRQ) to the initial loading phase (post-loading: PL) and the subsequent training phase with supplementation maintained (post-rehabilitation: PR). *Creatine group*: solid circles; *Placebo group*: open squares. Responses are expressed as percentage changes from baseline (BL). Statistically significant within-group differences from BL: * $p<0.05$, ** $p<0.01$.

Figure 4.4 Group body composition outcomes.



Group-mean responses (± one standard deviation) of body mass (BM), fat-free mass (FFM) and fat mass (FM) to the initial loading phase (*upper panel*) and the subsequent training phase with supplementation maintained (*lower panel*) for the *Creatine group* (solid bars) and the *Placebo group* (open bars). Responses are expressed as absolute changes from baseline (Δ); bars represent ± one standard deviation.

The effects of the initial creatine loading on measures of whole-body endurance exercise capacity were more variable, however (Table 4.6). In the creatine group, there was a

significant increase in time walked on the endurance shuttle-walk test, compared to baseline (Δ = 132 s or 38.8%; $p<0.01$, within-group difference) which was greater than that for the placebo group (Δ = 92 s or 23.5%; $p=0.21$, within-group difference) (Figure 4.3); however the between-group difference did not reach statistical significance ($p=0.8$). The distance walked on the incremental shuttle-walk test was unaffected in either group, compared to baseline. For the symptom-limited constant work-rate exercise test, there was a non-significant increase in exercise time for the creatine group (15 s or 9.1%; $p=0.49$, within-group difference) and, surprisingly, a significant increase for the placebo group (Δ = 54 s or 42.8%; $p<0.05$); but with no between-group difference ($p=0.17$). The associated degrees of breathlessness and perceived exertion at end-exercise for the constant work-rate exercise test were unaffected.

4.3.4.2 Post-Training Evaluation

Twenty-five patients successfully completed the exercise training programme (Figure 4.4). The reasons for study discontinuation were: severe exacerbations (n=2 creatine; n=4 placebo), poor motivation and/or non compliance with the exercise programme (n=1 creatine group; n=2 placebo group), back ache (n=1 creatine group) and vestibular disease (n=1 placebo group).

4.3.4.2.1 Exercise Training

Changes seen in response to exercise training, with or without creatine, are calculated from baseline entry into the study for both groups. The effects of the exercise training itself are demonstrated by responses of the placebo group (Table 4.7). Lower-limb (knee extensor) muscle strength was increased, relative to baseline (Δ = 12.2 N.m or 14.8%; $p<0.05$, within-group difference) (Figure 4.3), as was lower-limb muscle endurance (Δ = 362 J or 22%; $p<0.01$, within-group difference). Upper-limb muscle endurance (total handgrip repetitions) was also increased (Δ = 8.4 or 16.6%; $p<0.05$, within-group difference), although strength (peak handgrip force) was unaffected.

Whole-body exercise capacity was improved, as judged by the results of the incremental shuttle-walk test (Δ = 76m or 25.9%; $p<0.05$, within-group difference) and the endurance

shuttle-walk test (Δ = 275 s or 70.1%; $p<0.01$, within-group difference) (Figure 4.3). In addition, exercise time on the symptom-limited constant work-rate test was increased (although with considerable individual variability), which was on the borderline of statistical significance (Δ = 243 s or 193%; $p=0.05$, within-group difference). The associated breathlessness and perceived exertion at end-exercise on the constant work-rate test were unaffected. $\dot{V}o_2$ peak and the lactate threshold on the incremental exercise test were also unaffected

Table 4.8 Responses after the training phase (with supplementation maintained) compared to baseline for creatine and placebo groups

	Creatine (n= 14)	Placebo (n= 11)	Creatine versus Placebo difference
Pulmonary Function			
FEV_1, l	-0.036 (-0.1 to 0.07)	-0.06 (-0.8 to 0.06)	0.02 (-0.13 to 0.18)
MIP, cmH_2O	1.15 (-8.1 to 3.9)	-0.27 (-11.6 to 6.3)	1.43 (-8.8 to 11.7)
Body Composition			
Total body mass, kg	0.4 (-1.3 to 2.0)	-0.3 (-0.9 to 0.4)	0.66 (-1.19 to 2.52)
Fat-free mass, kg	2.0 (1.1 to 3.0) §§	0.4 (-0.2 to 1.0)	1.62 (0.47 to 2.77) *
Fat mass, kg	-1.7 (-2.9 to -0.4)	-0.6 (-1.0 to -0.1)	-1.10 (-2.47 to 0.28)
Upper Body Muscle Function			
Peak Force (dominant), N	2.9 (0.3 to 5.5) §	0.6 (-1.1 to 2.3)	2.27 (0.33 to 4.21) *
Total Repetitions (dominant)	15.6 (8.4 to 22.8) §§	8.4 (3.9 to 12.9) §	7.28 (2.16 to 12.40) *
Lower Body Muscle Function			
Peak Torque, N.m	19.5 (14.9 to 23.3) §§	12.2 (4.4 to 14.4) §	7.30 (0.69 to 13.92) *
Total Work, J	1216 (634 to 1798) §§	362 (48 to 710) §§	854.3 (131.3 to 1577.4) *
Exercise Capacity			
Peak work rate (W)	-0.71 (-13.2 to 11.8)	-5.0 (-14.0 to 4.0)	4.29 (-12.5 to 21.1)
$\dot{V}o_{2\ PEAK}$ (IET), L/min	0.061 (-0.06 to 0.20)	-0.074 (-0.2 to 0.02)	0.14 (-0.02 to 0.29)

$\hat{\theta}_L$ (IET) , L/min	0.11 (0.03 to 0.19) §	0.04 (-0.07 to 0.15)	0.07 (-0.07 to 0.21)
En d-ex Dyspnoea (IET)	-0.69 (-1.94 to 0.55)	0.00 (-1.44 to 1.44)	-0.69 (-2.44 to 1.05)
End-ex RPE (IET)	-1.15 (-2.84 to 0.54)	0.00 (-1.38 to 1.38)	-1.15 (-3.30 to 0.99)
Exercise Time (CWR), s	214 (20 to 409) §	243 (-1 to 488)	-29.0 (-317.8 to 259.8)
End-ex Dyspnoea (CWR)	-1.3 (-2.3 to -0.3) §	-0.4 (-1.4 to 0.6)	0.9 (-2.4 to 0.6)
End-ex RPE (CWR)	-1.1 (-2.4 to 0.2)	-0.4 (-1.7 to 0.9)	0.7 (-2.7 to 1.2)
Distance walked (ISWT), m	55 (5 to 104) §	76 (21 to131) §	-21.5 (-90.6 to 47.7)
Time walked (ESWT), s	365 (181 to 550) §§	275 (90 to 460) §§	90.5 (-157.6 to 339)
SGRQ			
Total score	-5.9 (-1.1 to -10.8) §	1.8 (-4.1 to 7.7)	-7.7 (-14.9 to -0.5) *
Activity domain	-5.3 (-1.7 to -8.9) §§	11.0 (1.8 to 20.1) §	-16.3 (-24.8 to -7.9) **
Impact domain	-3.46 (-10.2 to 3.3)	-3.45 (-10.3 to3.4)	-0.01 (-9.2 to 9.2)
Symptoms domain	-4.3 (-15.1 to 6.4)	0.2 ± (-10.0 to 10.5)	-4.5 (-18.9 to 9.8)

Data are presented as group-mean values (± 95% confidence intervals) for the absolute changes from baseline and between group difference. Abbreviations: IET: incremental exercise test; CWR: constant work-rate exercise test; ISWT: incremental shuttle-walk test; ESWT: endurance shuttle-walk test; RPE: Rating of perceived exertion; SGRQ: St George's Respiratory Questionnaire. Statistically-significant differences: § $p<0.05$, §§ $p<0.01$ (within-group difference from baseline); * $p<0.05$, ** $p<0.01$ (between-group differences).

There was no significant change in the total score of the St George's Respiratory Questionnaire. However, there was a surprising and significant worsening of the activity domain for the placebo group over the study duration (Δ = 11.0 or 14.0%; $p<0.05$, within-group difference).

4.3.4.2.2 Exercise Training with Creatine

The effects of the exercise training being performed in conjunction with ongoing creatine supplementation, relative to those to training alone, are provided by comparing the training responses of the creatine group with those of the placebo group (Table 4.8).

Fat-free mass following combined training and creatine was substantially greater than for the training alone (Δ = 2.0 kg or 2.8% *vs.* 0.4 kg or 0.6%; $p<0.05$, between-group difference: Table 4.7) (Figures 4.3 and 4.4).

The gains in lower-limb muscle performance with combined training and creatine were enhanced, relative to the training alone, both with regard to peak torque (Δ = 19.5 N.m or 22.8% *vs.* 12.2 N.m or 14.8%; $p<0.05$, between-group difference: Table 4.8) (Figure 4.3) and total work (Δ = 1216 J or 66% *vs.* 362 J or 22.0%; $p<0.05$, between-group difference: Table 4.8).

In addition, the improvements in upper-limb performance with combined training and creatine were greater than for training alone, both for peak handgrip force (Δ = 2.9 N or 10.9% *vs.* 0.6 N or 2.3%; $p<0.05$, between-group difference: Table 4.8) and for total handgrip repetitions (Δ = 15.6 or 31.9% *vs.* 8.4 or 16.6%; $p<0.05$, between-group difference: Table 4.8).

As judged by shuttle-walk or cycle-ergometer performance, creatine supplementation combined with training provided no further statistically significant improvement in whole-body exercise capacity than with training alone (Table 4.8). For example, the improvement in the tolerable duration of the symptom-limited constant work-rate test was not different between placebo and creatine groups, although the change from baseline was significant in the creatine group (Table

4.8). Again, $\dot{V}o_{2\,PEAK}$ was unaffected, although the lactate threshold significantly increased from baseline in the creatine group (Δ = 0.11 l/min or 20%; $p<0.05$, within-group difference: Table 4.8). There were no significant differences between or within groups in exercise-related symptoms at peak exercise for the incremental exercise test (Table 4.8). While there were no differences between groups in exercise-related symptoms at peak exercise for the incremental exercise test, there was a significant decline in breathlessness for the creatine group at end-exercise for the constant work-rate test, compared to baseline (Δ = -1.3 or -16.7%; $p<0.05$, within-group difference: Table 4.8).

The magnitude of improvement in quality-of-life (SGRQ total and activity domain scores) was significantly enhanced by the combined creatine supplementation and training: SQRQ total score was decreased by 5.9 or 9.0% *vs.* an increase of 1.8 or 2.7% for placebo ($p<0.05$; between-group difference: Table 4.7, Figure 4.3) and SGRQ activity domain score was decreased by 5.3 or 6.4% *vs.* an increase of 11.0 or 14% for placebo ($p<0.01$; between-group difference: Table 4.8).

There were no significant relationships between baseline functional indices (i.e. pulmonary function, body composition, exercise tolerance) and the subsequent benefit from creatine supplementation, without or with exercise training.

Importantly, the average improvements seen post-training in both exercise capacity and health-related quality-of-life represent clinically-significant improvements. Thus, there was an increase in the incremental shuttle-walk test of over 50m for both the creatine and the placebo groups, with 6 patients in each group exceeding this value(2001). Likewise, the St. George's Respiratory questionnaire total score decrease of more than 4 points in the creatine group should be noted, with 6 patients in this group exceeding this value and one in the placebo group (Jones *et al.*, 1991).

4.3.4.2.3.1 Influence of Fat-Free Mass

We were unable to discern any statistically significant effects of the loading intervention or the subsequent training regimen relative to the presence or absence of fat-free mass depletion. Indeed, it should be pointed out that as the study was powered primarily to examine the effect of creatine administration per se, it was therefore likely underpowered for fat-free mass comparisons. Also we did not find any correlation between change in fat free mass and peak torque ($r= 0.08$, $p= 0.8$ for post loading and $r= 0.24$, $p= 0.4$ for post rehabilitation) over the study period.

4.3.4.4.4 Pulmonary function

There were no significant changes in pulmonary function measures, either following Cr loading or rehabilitation. We did not anticipate any alteration of static lung volumes over the course of the intervention. An initial trend of increased maximal inspiratory respiratory muscle pressure over creatine loading did not persist following rehabilitation. No specific respiratory muscle training was being performed.

4.4 Discussion

This study has demonstrated that 2 weeks of oral creatine supplementation (15 g/day) was effective in increasing fat-free mass, lower-limb muscle strength and endurance in patients with moderate to severe COPD. Furthermore, compared to pulmonary rehabilitation alone, subsequently incorporating a 10-week pulmonary rehabilitation programme with maintained creatine administration (5 g/day) proved to be more effective in increasing fat-free mass and lower-body muscle performance, as well as inducing gains in upper-body muscle performance and quality of life. While there were clear training-induced effects on whole-body exercise performance, these were not systematically enhanced in the presence of creatine.

This study demonstrates that oral creatine monohydrate supplementation is associated with beneficial effects on skeletal muscle mass and function in patients with moderate-to-severe COPD. These effects can be further enhanced with whole-body exercise training. This study, therefore, suggests that creatine supplementation, either in isolation or in combination with physical exercise, constitutes a novel ergogenic aid for patients with COPD.

4.4.1 Creatine supplementation in COPD

Energy production (via ATP genesis) for skeletal muscle work is derived from one of three sources: anaerobic breakdown of phosphocreatine via the Lohmann reaction, also anaerobic and aerobic glycolysis. During moderate intensity exercise, as oxygen uptake increases to reach a steady state sufficient to meet the energy demands solely by aerobic glycolysis, the shortfall (or 'oxygen debt') is met by phosphocreatine breakdown. This Lohmann reaction can be represented by the equation:

$$\text{Phosphocreatine} + \text{ADP} \longrightarrow \text{ATP} + \text{Creatine}$$

$$\text{ATP} \longrightarrow \text{ADP} + \text{Pi}$$

The enzyme creatine kinase enables the Lohmann reaction to proceed in either direction. Creatine, or methyl guanidine acetic acid (Cr) is a substance found naturally in foodstuffs such as

meat and fish. At present there is much interest in creatine supplementation in health and this is reflected in a number of reviews on the subject recently published (Terjung *et al.*, 2000b; Jacobs, 1999; Kraemer & Volek, 1999). Creatine monohydrate is widely used by healthy individuals worldwide as an aid to exercise performance (Terjung *et al.*, 2000a). In the skeletal muscle, creatine undergoes suffers rapid and reversible phosphorylation catalysed by the enzyme creatine kinase, a reaction that forms phosphocreatine (PCr). PCr provides an immediate quick source of high-energy phosphate which is crucial for maintaining the rate of ATP resynthesis and generation during the initial stages of exercise, especially when demands are high (Kilduff *et al.*, 2003). Studies have shown that oral supplementation with creatine monohydrate increases total and free intra-muscular creatine (Harris *et al.*, 1992), in addition to increasing intra-muscular PCr stores during exercise and recovery (Greenhaff *et al.*, 1994b). An increased availability of PCr may allow for a 'proton sink' to be formed as initially energy release from the breakdown of PCr to ATP and Cr consumes a hydrogen ion. This has potential for beneficial effects in the muscle, through the delay of the development of acidosis. This also has particular relevance to COPD patients because of the role of systemic acidosis in determining ventilatory demands (Wasserman *et al.*, 1999). It has a proven benefit upon physical performance, most specifically in those activities that involve repeated short bouts of high intensity exercise (Terjung *et al.*, 2000b). In healthy subject creatine has an impact on short bursts of high intensity exercise, such as sprinting, leading to an increased maximum and total power produced. These effects have been demonstrated in placebo controlled double blind experiments. Repeated maximal voluntary contraction on an isokinetic dynamometer, after creatine supplementation, led to significantly increased peak torque production (Greenhaff *et al.*, 1993). Repeated episodes of maximal power output during cycling were increased following creatine supplementation (Birch *et al.*, 1994). This is thought to result from increased rate of phosphocreatine resynthesis resulting from raised muscle creatine levels. Creatine is considered to benefit subjects taking it to different degrees; so called 'responders' and 'non-responders'. These groups are characterised by the extent to which they uptake creatine. Determining those who will become responders is a focus of research and cannot presently be predicted. The higher the resting stores of creatine are, the less exogenous creatine is likely to be taken up. Thereby one may expect increased uptake in groups such as vegetarians. So far there is no evidence published on this matter. However there are data to suggest that the degree of benefit derived from creatine supplementation is relative to the amount

taken up (Casey *et al.*, 1996). In normal subjects, resting phosphocreatine levels are between 70-90 mmol.kg^{-1}.s^{-1}, which does not allow for sustained energy production from this source. Creatine supplementation may be expected to increase this by approximately 20% (to between 85-105 mmol.kg^{-1}.s^{-1}) (Febbraio *et al.*, 1995; Hultman *et al.*, 1996).

However many published creatine studies are hampered by small sample sizes, poor control groups and the short-term nature of the investigations. There is evidence that creatine can have anabolic benefits in the context of muscle rehabilitation (Hespel *et al.*, 2001). The benefits described, in body composition and performance, have also been demonstrated in elderly subjects (Gotshalk *et al.*, 2002). More recently there has been evidence that creatine can exert an ergogenic effect in elder healthy persons (Gotshalk *et al.*, 2002). Disease groups have been studied, albeit in small numbers and most usually in uncontrolled trials. In a well conducted investigation Cr was demonstrated to improve lower limb muscle performance in patients with chronic heart failure (Gordon *et al.*, 1995).

There are no established common side effects of creatine administration. A meta-analysis has concluded that the supplement is safe (Graham & Hatton, 1999). In particular a long-term study found no evidence of impact upon renal function (Poortmans & Francaux, 1999). This is supported by our investigation where the dropout rate, both due to intolerance of the supplement and total, was higher in the placebo group than in the Cr group.

4.4.2 Body Mass and Fat-free Mass

Consistent with earlier reports in healthy young (Birch *et al.*, 1994; Balsom *et al.*, 1995; Kreider *et al.*, 1998;Kilduff *et al.*, 2002b) and older (Gotshalk *et al.*, 2002; Chrusch *et al.*, 2001; Jakobi *et al.*, 2001) subjects, in healthy young subject following rehabilitation from disuse atrophy (Hespel *et al.*, 2001) and in patients with myaesthenias gravis (Stout *et al.*, 2001), the initial two-week loading phase of creatine supplementation in our cohort of COPD patients led to an increased body mass that was effectively accounted for by an increase in fat-free mass, compared to the placebo group (Table 4.7; Figures 4.3 and 4.4). The subsequent phase of maintenance

supplementation and training was associated with a further increase in fat-free mass of similar magnitude (2.0 kg, on average: Table 4.8) to those reported for healthy subjects (Kilduff *et al.*, 2002b; Gotshalk *et al.*, 2002), but no further increase in body mass, consistent with our observation of a reduced fat mass (Table 4.8 Figures 4.3 and 4.4).

The exact mechanism underlying this creatine-induced increase in fat-free mass is unclear. Creatine ingestion has been shown to cause water retention that is presumed to reflect local gains within skeletal muscle rather than more-generalised water retention. However, the technique used to measure FFM in this study (air-displacement plethysmography) is known to be insensitive to variations on tissue water content. Therefore, we cannot exclude body water retention as an explanation for the gain in fat-free mass. Whether other body composition methodologies that account for tissue water content, such as BIA, or regional body composition, such as DEXA, would be more enlightening in relation to mechanistic changes is of interest. It has been suggested that creatine-induced intramuscular water retention can, through myocyte swelling, induce an increase in protein synthesis (Haussinger *et al.*, 1993). Also protein synthesis in cultured myocytes has been shown to be stimulated with increased creatine availability (Ingwall *et al.*, 1974), which may involve up-regulation of myogenic transcription factors (e.g. MRF4 and myogenin) (Hespel *et al.*, 2001). There is evidence to suggest that the changes found after exercise training relate to appropriate increases in intracellular water accompanying anabolism (Francaux & Poortmans, 1999).

The potential importance of this finding should be viewed in the recognition that peripheral muscle wasting is a strong independent predictor of mortality in COPD, irrespective of disease subtype (Schols *et al.*, 1998b; Marquis *et al.*, 2002). There is increasing realisation of the importance of skeletal muscle, both in the causation, and amelioration of exercise intolerance (Palange *et al.*, 1995; Gosselink *et al.*, 1996). Skeletal muscle dysfunction is likely to be multi factorial in nature and is distinct from corticosteroid myopathy. Disuse atrophy, cellular hypoxia, circulating inflammatory cytokines and malnutrition are almost certain to be major components (Wouters *et al.*, 2002). It manifests itself most obviously through reduced muscle bulk and strength of COPD patients compared to healthy controls (Bernard *et al.*, 1998b). Within the

skeletal muscle there is seen a selective loss of type IIx fibres, (Gosker *et al.*, 2002) decreased resting levels of PCr (Jakobsson *et al.*, 1990) and oxidative enzymes (Jakobsson *et al.*, 1995) and evidence of increased levels of oxidative stress. The muscle groups themselves are found to have relatively well preserved function in more the proximal muscle groups (Gosselink *et al.*, 2000). Patients with muscle bulk loss are found to have worse health status, increased exacerbation frequency and increased mortality; moreover these impacts may be reversible (Schols *et al.*, 1998a). Also patients who gained weight following either a nutritional intervention (Schols *et al.*, 1998b) or treatment with an anabolic steroid (Schols *et al.*, 1995b) had decreased mortality compared to those who did not. Consequently, considerable attention has been given to identifying tolerable intervention strategies that might reverse muscle wasting in patients with COPD. For example, Burdet et al (Burdet *et al.*, 1997), Yeh et al (Yeh *et al.*, 2002) and Creutzberg et al (Creutzberg *et al.*, 2003) have demonstrated that recombinant growth hormone and anabolic agents (Oxandrolone, Nandrolone), respectively, increase fat-free mass in this patient group. We have demonstrated in the present study that creatine supplementation, over a continuous period of up to 12 weeks, also induced gain of fat free mass and was well tolerated (with no patients reporting any associated side-effects).

4.4.3 Muscle Strength and Endurance

The initial creatine loading in the present study was associated with gains in lower-limb muscle strength and endurance and upper-limb endurance (Table 4.7; Figure 4.3). The subsequent maintenance period of creatine supplementation combined with exercise training elicited further improvements in both lower and upper limb strength and endurance (Table 4.8; Figure 4.3). It has been shown that lower-limb muscle strength is compromised to a greater degree than that of the upper limbs in patients with COPD (Hamilton *et al.*, 1995; Gosselink *et al.*, 1996), likely reflecting the reduced habitual use of the locomotor muscles expected in this patient group. This might predispose the lower-limb musculature to be more sensitive to the effects of the supplementation, consistent with a supposition that muscle performance gains with creatine are greater for muscle groups which are relatively detrained.

There have been a number of interventions performed that have attempted to counter the progressive skeletal muscle dysfunction seen in COPD. The most successful, and only one with proven good efficacy, is exercise training, particularly if in combination with strength training (Bernard *et al.*, 1999b). To our knowledge, this is the first additional intervention study in COPD to show an increase in muscle function as measured by strength or endurance in association with an increased fat-free mass. Previously-reported interventions in COPD patients such as administration of anabolic steroids (Ferreira *et al.*, 1998a; Yeh *et al.*, 2002; Schols *et al.*, 1995b; Creutzberg *et al.*, 2003), recombinant growth hormone (Burdet *et al.*, 1997), appetite stimulants (Weisberg *et al.*, 2002), hypercaloric protein-rich diets (Slinde *et al.*, 2002) and high-carbohydrate diets (Steiner *et al.*, 2003) while successfully effecting some restoration of fat-free mass, were without effect on peripheral muscle performance. Control groups are often not present, and have not been on placebo. There is a difficulty in blinding subjects and the investigators. Also study numbers have often been very small. These factors are liable to have been instrumental to the findings of the recently published Cochrane review on this subject; namely that outpatient nutritional intervention is ineffective in COPD with regard to anthropometry or muscle function (Ferreira *et al.*, 2000b). A 6-month study of testosterone and stenozalol was able to demonstrate increases in body weight and fat-free mass but not functional capacity (Ferreira *et al.*, 1998b). Growth hormone administered over 2 months during a pulmonary rehabilitation programme induced increases in fat free mass, but did not influence muscle performance and seemed to worsen exercise capacity (Burdet *et al.*, 1997). A recently published study examining the effects of anabolic stroids in a cohort of male COD patents with low serum testosterone, was suggestive of an effect upon muscle strength (Casaburi *et al.*, 2004). However the changes seen in one repetition maximum quadriceps strength did not have significantly different changes between placebo and testosterone groups in those who received exercise training. Therefore the benefits upon muscle function, exercise capacity and health status from interventions other that exercise training are unproven at present.

COPD patients can also manifest reduced lower-limb muscle endurance, with Serres et al (Serres *et al.*, 1998) reporting that COPD patients achieved fewer dynamic contractions of the quadriceps muscles than controls, when matched for maximal strength. It is of interest, therefore, our patients demonstrated a significant increase in quadriceps and forearm endurance following

creatine loading (as measured by total work and total repetitions respectively), compared to the placebo group. This finding is in agreement with previously-published data in healthy resistance-trained young adults (Kilduff *et al.*, 2002b), healthy older subjects (Rawson *et al.*, 1999; Gotshalk *et al.*, 2002) and heart-failure patients (Gordon *et al.*, 1995; Andrews *et al.*, 1998b).

The exact mechanisms responsible for these observed creatine-induced increases in muscle strength and endurance are uncertain. As we did not make any direct measurement of creatine uptake in our patients, we can only speculate on the potential mechanisms. Interestingly, peripheral muscle abnormalities in COPD include low resting ATP and PCr concentrations (Gertz *et al.*, 1977;Jakobsson *et al.*, 1990) and lower intracellular pH and slower PCr resynthesis during recovery from exercise (Payen *et al.*, 1993; Sala *et al.*, 1999a). Both an increase in resting PCr levels and an accelerated rate of post-exercise PCr resynthesis may be involved in the mediation of the ergogenic effect of creatine supplementation, certainly in healthy subjects (Greenhaff *et al.*, 1994a; Balsom *et al.*, 1995). It is tempting to speculate, therefore, that the muscle performance gains our COPD patients demonstrated following creatine supplementation derive from an amelioration of PCr-related abnormalities in muscle bioenergetics.

The subsequent inclusion of exercise training with continuing creatine supplementation amplified the muscle performance gains in our patients. Compared to the effects of the training alone, both quadriceps and handgrip strength and endurance were increased (Table 4.8; Figure 4.3) This raises the interesting possibility that the supplementary creatine may have acted as an extra stimulus during training, thus allowing patients in the creatine group to train at a greater exercise intensity and therefore leading to a greater improvement in muscle performance (Earnest *et al.*, 1995; Volek *et al.*, 1999). And while we did not comprehensively document work completed in each training session for each patient, the significantly greater loss of fat mass observed in the Cr group could support this notion (Table 4.8). The enhanced muscle performance seen in the present study when combined with exercise training has also been observed in both healthy young subjects (Vandenberghe *et al.*, 1997; Kreider *et al.*, 1998; Volek *et al.*, 1999) and healthy older subjects (Chrusch *et al.*, 2001) but not before in patient groups. However, the fact that functional improvements were obtained without training suggests that

creatine supplementation in isolation may be beneficial for patients with advanced COPD who, not uncommonly, are unable to undertake formal whole-body exercise training sessions.

4.4.4 Whole-Body Exercise Performance

The effects of creatine supplementation on whole-body exercise performance in healthy subjects are generally acknowledged to be more variable. The consensus is that performance for high-intensity sprint exercise (e.g. intermittent exercise) can be improved (Greenhaff *et al.*, 1993; Terjung *et al.*, 2000b). However, for graded exercise and step tests (and for which reliance on PCr is less marked), the effects are more variable (Stroud *et al.*, 1994; Smith *et al.*, 1998; Balsom *et al.*, 1993). In this context, therefore, the more variable results in our COPD patients for shuttle-walking and with symptom-limited cycle-ergometry are perhaps not entirely surprising. The improvements in endurance shuttle-walk test performance that resulted from creatine supplementation were clinically significant when compared to baseline, but not when compared with placebo (Table 4.7: Figure 4.3). Similarly, exercise time on the symptom-limited step test was increased in some of the supplemented patients, but not in others, with the overall prolongation of the exercise time not being statistically significant. Similarly, there was a lack of effect on $\dot{V}O_{2\,PEAK}$ and the lactate threshold (Table 4.7).

Training per se induced some performance gains for whole-body exercise, notably for shuttle-walking, consistent with expected gains (Lacasse *et al.*, 1996). The lack of effect on VO_2 max and lactate threshold is not without precedent, especially in patients who are substantially impaired in terms of respiratory limitation (Reardon *et al.*, 1994). There was considerable variability in the changes seen in exercise endurance time following intervention. This is consistent with the influence of learning effects and the high variability of these tests (Revill *et al.*, 1999; Noseda *et al.*, 1989). We were unable to incorporate additional familiarisation sessions into our protocol.

There is established evidence that training responses are specific to training modalities. A strength training programme will have less of an impact on endurance ability than an endurance

based programme. Anabolic steroids have been demonstrated to induce weight gain (largely lean mass) in COPD patents. Improvements in strength have been seen, however the failure of impact upon endurance activity or whole body exercise has been attributed to the nature of the muscle adaptation seen following anabolic agents. The potential for strength training/muscle bulk intervention to increase whole body exercise capacity is highlighted by the demonstration that a severely disabled group of COPD patients had significant increases in peak O_2 uptake following neuromuscular electrical stimulation administration (Neder *et al.*, 2002). More practically they were able to then partake in a standard pulmonary rehabilitation programme. This may indicate that anabolic agents, which we believe includes creatine, have a clear potential to impact upon whole body exercise.

4.4.5 Quality of Life

We also found clinically meaningful improvements in health related quality of life when combined creatine and training was compared with the training alone (Table 4.8; Figure 4.3). The extent of this is striking and was seen largely to arise from the activity domain of the SGRQ. Peripheral muscle weakness is thought to contribute to the "dyspnoea spiral" through inactivity and disuse atrophy. An intervention which benefits skeletal muscle therefore has the theoretic advantage of favourably influencing activities of daily living. A short-term study such as ours was not powered to detect differences in exacerbation frequency, which is clearly one of the main factors that influence health status. We are not aware of any interventions that when combined with exercise training lead to significantly greater changes in health related quality of life.

4.4.6 Study Limitations

To our knowledge this is the first study of creatine supplementation in patients with COPD. We were well aware of the weakness of previous investigations of nutritional and specifically creatine supplementation that have been performed in health and disease. Therefore we aimed to conduct a double-blind, placebo-controlled trial, that was sufficient in power and duration. Although it was our initial primary outcome measure, it is likely that we were underpowered to

detect any between group differences in exercise capacity as the variability of these measures is so high. Specifically, whereas we can report a coefficient of variation for the shuttle walking distance, once learning effect has been accounted for, of 13.9%, this compares unfavourably to the repeatability of the strength measurements which over the first 2 peak torque bouts of quadriceps was 3.7% for the same subjects. The published studies that demonstrate improvements in exercise capacity with treatments, out with those involving exercise training, have utilised cross over designs. The incorporation of exercise training meant that such a design was not feasible. Also the beneficial effects of creatine in healthy subjects seem largely to relate to short-term high intensity exercise. There is no established means of assessing this aspect of whole body exercise in patient groups and our evaluations therefore may not have been the most appropriate. It is also possible that the magnitude of effect was insufficient, perhaps made less likely by the positive health status findings.

We have demonstrated convincing beneficial changes in the skeletal muscle in both bulk and function when compared with simple carbohydrate administration. These changes were persistent over at least 12 weeks. These effects on muscle strength and endurance have not been previously demonstrated with any previously investigated putative ergogenic aid.

We were not able to measure creatine uptake, either by repeated muscle biopsy or through urinary excretion of creatine. Neither were we able to assess dietary behaviour and dietary intake of creatine in our patient group. Therefore we are not able to examine subgroups of "responders" or "non responders" to creatine. We attempted to minimise this potential confounder by including carbohydrate in the supplementation mixture which has been shown to enhance the uptake of Cr by 60-100% (Green *et al.*, 1996).

The design of our study also does not allow us to resolve the relative potencies of creatine supplementation and the training regimen on body composition and muscle performance benefits, nor the longer term impact of creatine administration and separate studies would be required to clarify this.

We are also unable to provide any insight on the relative effect of creatine supplementation in COPD patients who demonstrate appreciable muscle wasting, compared to those who do not.

That is, while we classified our patients in this way, the small numbers of patients constrained the analysis because of underpowering. This is clearly an issue of considerable importance that will, therefore, require a substantially larger study; the primary purpose of our study was to explore the effects of creatine alone and with exercise training in a largely unselected patient group. However the main study limitation remains that of insufficient sample size, though we believe that our investigation does represent the largest examination of Cr supplementation published to date in health or disease. The cohort of patients from inner city Glasgow represents a very severely diseased and socially deprived group. This explains the high rehabilitation drop out rate; one that is expected with our local programme. Although we did see improvements in strength and exercise capacity in both groups following rehabilitation, the placebo group did not have any significant change in health status. This may relate partly to the reasonably small number of placebo patients that finished the exercise training. However a more intensive rehabilitation service would have been preferable, as our twice-weekly 8-week programme was the minimum recommended for successful outpatient exercise training (ATS statement, 1987). Our placebo group were administered carbohydrate, as well as the Cr cohort. Although this means that we did not provide a true placebo, this regime has successfully removed carbohydrate as a confounding factor, establishing that the effect seen were solely due to creatine.

It is likely that our study was underpowered to detect differences in exercise capacity. As expected, approximately a third of our patient group dropped out of the study during exercise training, albeit more in the placebo arm. However our data do not suggest that creatine was leading towards benefits in whole body exercise capacity, though it is possible that trends in perceived breathlessness and exertion could have become significant with increased numbers of subjects.

Finally, we cannot comment on issues of mortality and morbidity in the context of our findings to date. A much larger study would be required to determine whether the observed improvement in fat-free mass proved to be beneficial in this regard.

4.4.7 Conclusions

In summary, this study demonstrates that oral creatine monohydrate supplementation, both in isolation and in combination with exercise training, improved fat-free mass, skeletal muscle function and health-related quality of life in a group of patients with advanced COPD. These data therefore suggest that creatine may constitute a novel ergogenic aid for clinical use in this patient population.

CHAPTER 5

THE EFFECT OF FORMOTEROL 12 μG TWICE DAILY ON EXERCISE TOLERANCE AND DYNAMIC HYPERINFLATION IN STABLE MODERATE-TO-SEVERE COPD PATIENTS

ABSTRACT

Introduction: Exercise intolerance is a hallmark of COPD. Long acting bronchodilators have established benefits upon lung function, health status and exacerbation frequency. Formoterol is a well tolerated and effective bronchodilator in COPD patients. However its impact upon dynamic hyperinflation, and therefore potentially exercise tolerance, is not known. We wished to determine whether this long acting beta agonist significantly enhances exercise tolerance and reduces the associated dynamic hyperinflation in COPD patients.

Methods: Patients with established COPD were enrolled: history of smoking (>20 pack-years) and diagnosis of COPD according to American Thoracic Society criteria, with pre-bronchodilator FEV1 <60% and FEV1/FVC ≤70% of predicted normal value, and an increase in FEV1 <12% from predicted normal value following inhalation of 400 µg salbutamol. This was a randomized, double-blind, placebo controlled, crossover study consisting of two 2-week treatment periods separated by a 2 day washout phase of formoterol 12µg b.i.d. versus placebo. The primary efficacy parameter was the time to limit of tolerance (Tlim), taken as the interval between onset of constant work rate and the point at which the patient could no longer maintain 60 revolutions per minute on a cycle ergometer. Secondary endpoints included Mahler's questionnaire of Chronic Activity-Related Breathlessness, Borg dyspnea scores during exercise and measures of dynamic hyperinflation.

Results: Twenty one patients (14 male, age 60 +- 9 years, FEV_1 1.00 +- 0.34 l) were enrolled, giving eighteen evaluable subjects. From the results of the ANCOVA, the estimated difference between treatments was 130 seconds (CI -1.2 to 261.8 seconds), to the advantage of formoterol (formoterol 479 seconds, placebo 349 seconds) and was very close to achieving statistical significance (p=0.052). The secondary parameters showed advantages to formoterol, those of end expiratory lung volume (-0.13 l, -0.27 to –0.00 at Tlim, p = 0.045), inspiratory reserve volume (0.2 l, 0.09 to 0.3 at isotime, p = 0.0019) and Mahler's questionnaire of chronic activity-related breathlessness ($p < 0.05$ for all three indices) being statistically significant.

Conclusions: Formoterol led to significant amelioration of dynamic hyperinflation without a significant effect upon absolute exercise tolerance (p=0.052). However this study suggests that formoterol may improve the time to limit of exercise tolerance by approximately 2 minutes.

5.1 Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by chronic, and only partially reversible, airways obstruction (ATS Standards, 1995). COPD therefore presents with symptoms of breathlessness; the result of irreversible changes in the lung parenchyma and airways attributed in most cases to cumulative exposure to tobacco smoke. The multi-factorial functional impairment in COPD frequently reduces exercise tolerance within this group, thereby decreasing activity levels. This usually progressive deterioration of symptoms and health is often referred to as the "dyspnoea spiral". Exercise intolerance is therefore a hallmark of the disease and commonly associated with reduced quality-of-life, increased morbidity and mortality (Ries *et al.*, 1995; Gerardi *et al.*, 1996).

Exercise limitation in COPD has traditionally been ascribed to flow limitation and gas exchange disturbances. Patients usually stop exercise when their level of ventilatation reaches a severely-reduced maximum capacity (Neder *et al.*, 2000). This ventilatory driven exercise ceiling is further exacerbated by dynamic hyperinflation, whereby lung volumes progressively increase with consequent impact upon associated work of breathing. Any intervention, therefore, which leads to diminished ventilatory demand (e.g. physical training) (Casaburi *et al.*, 1997), an improved ventilatory capacity (e.g. bronchodilation) (Belman *et al.*, 1996) or reduced work of breathing (eg reduced dynamic hyperinflation, ventilatory support, heliox administration) for a given ventilation has the potential to improve exercise tolerance in patients with COPD. Both short acting (atrovent, salbutamol) and, more latterly, long acting (salmeterol, tiotropium) bronchodilators have been demonstrated to impact upon dynamic hyperinflation in COPD patients (O'Donnell *et al.*, 2004b; O'Donnell *et al.*, 2004a; O'Donnell *et al.*, 1999).

Formoterol fumarate (Foradil®) is a bronchodilator drug of the class of long-acting β2-adrenoceptor agonists. It is a formylamino-substituted catecholamine derivative: 2-hydroxy-5-[(1RS)-1-hydroxy-2-[[(1RS)-2-(p-methoxyphenyl)-1-methyl]ethyl amino] ethyl] formanilide fumarate dihydrate. In vitro and in vivo formoterol exerts a preferential effect on β2-adrenergic receptors of bronchial smooth muscle. Bronchodilator activity can be demonstrated at very low doses following oral administration and at even lower doses after inhalation (Anderson, 1993).

Foradil delivered via the Aerolizer has been shown to have beneficial effects on pulmonary function and quality of life measures in patients with COPD (Friedman *et al.*, 2002). Dose

titration studies demonstrated a duration of action of about 12 hours after inhalation of 12 and 24μg of formoterol (Wallin *et al.*, 1993). In asthma, formoterol has been shown to be therapeutically effective and well tolerated for periods of up to 12 months without significant changes in bronchoprotective and bronchodilating activities (Hekking *et al.*, 1990). In single-dose studies in asthmatic adults, 12 and 24μg of formoterol aerosol and dry powder provided a rapid onset of action within one to three minutes, similar to that of the short-acting β2-adrenoceptor agonist salbutamol (Wegener *et al.*, 1992). In COPD regular β2-agonist treatment is intended to provide "round the clock" relief of symptoms already in existence and in conjunction therefore has more striking benefits upon health status, breathlessness and exacerbation frequency (Jarvis & Markham, 2001; Friedman *et al.*, 2002; Mahler, 2002).

Exercise intolerance results when a patient is unable to sustain a designated physical task sufficiently long for its successful completion. Most patients with COPD therefore confine their activities of daily living to tolerable sub-maximal exercise intensities. Exercise capacity has been traditionally assessed by rapid-incremental exercise tests performed to a symptom-limited maximum, which provides relatively little information about a patient's actual ability to carry on activities of daily living. More recent test formats that focus on endurance capacity in COPD, provide a better frame of reference as to whether a patient is able to carry on activities of daily living – an important goal of any intervention in patients with COPD. The sub-maximal constant-load test performed to the limit of tolerance is one such test (Oga *et al.*, 2000;Wasserman *et al.*, 1999). When performed in conjunction with direct measurements of flow-volume reserves and systematic symptom assessment this strategy should provide a more reproducible and sensitive approach for quantifying exercise tolerance (Van 't *et al.*, 2003).

Therefore the primary objective of this study was to demonstrate that treatment with formoterol fumarate, delivered for 14 days significantly enhances exercise tolerance in COPD patients with moderate-to-severe stable airflow obstruction, compared to placebo. Formoterol has a faster onset of action than other long acting bronchodilators and therefore may have particular pertinence to use in relation to exercise in COPD patients. Secondary objectives were to evaluate whether formoterol reduces effort-related breathlessness during everyday activities and whether formoterol reduces dynamic hyperinflation during exercise.

5.2 Methods

5.2.1 Subjects

Twenty one patients (14 male) were enrolled to the study, which had a randomized, double-blind, placebo-controlled, cross-over design. Key inclusion criteria were: 1) male and (non-pregnant/non-lactating) female patients aged ≥40 years with a history of smoking (>20 pack-years) and a diagnosis of COPD according to the American Thoracic Society criteria, 2) pre-bronchodilator FEV_1 <60% and FEV_1/FVC ≤70% of predicted normal value and 3) at screening, 30 minutes after inhalation of 400μg salbutamol, an increase in FEV_1 of <12% of the patient's predicted normal value.

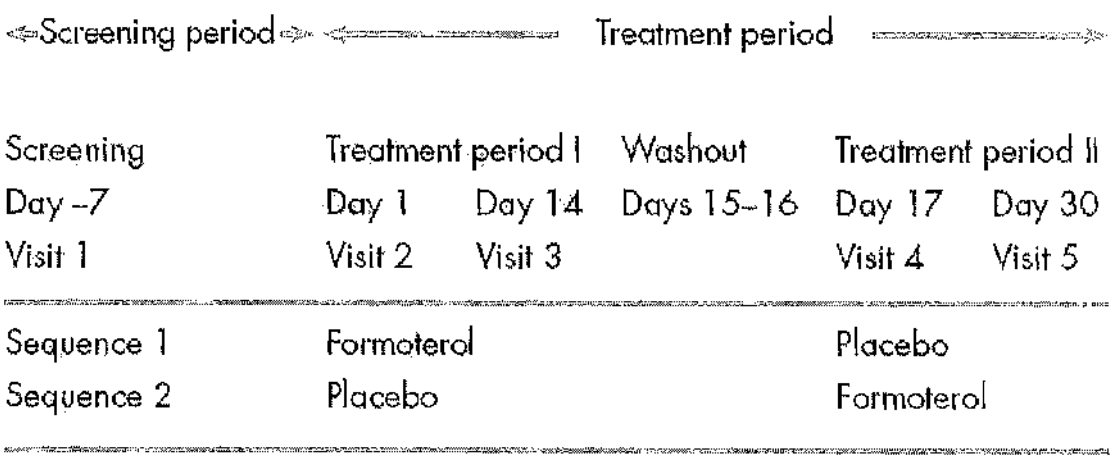
Exclusion criteria were 1) left ventricular heart failure, myocardial ischemia, QTc from a resting ECG >0.46 s, or history or presence of significant cardiovascular disease including coronary artery disease, arrhythmia and uncontrolled hypertension 2) either hospitalization for a COPD exacerbation or presence of a respiratory tract infection within 1 month prior to screening, current or childhood asthma, a history of allergic rhinitis or other atopic disease, or a total blood eosinophil count over 400 mm^3, 3) inability to interrupt treatment with usual bronchodilator medication prior to testing, need for long-term oxygen therapy, or arterial oxygen saturation <85% at rest, or history of untoward reactions to sympathomimetic amines or inhaled medication, 4) anaemia, hypo- and hyperthyroidism, hyperadrenergic state, uncontrolled insulin dependent diabetes mellitus, malignancy, or any disease or condition which limits exercise performance, other than COPD, 5) History of alcohol or drug abuse, history of non-compliance to medical regimens, or treatment with any investigational drug within 1 month prior to screening. 6) Change in the use of inhaled or nasal corticosteroids, change in the use of oral modified-release theophylline, or treatment with oral corticosteroids, within 1 month prior to screening. 7) Use of short acting β2-agonists within 6 hours, long acting β2-agonists within 24 hours, or inhaled anticholinergics within 8 hours, prior to Visit 1. 8) The use of the following after Visit 1 (screening): non-potassium sparing diuretics, beta-blocking agents, quinidine or quinidine-like medications (anti-arrhythmics), tricyclic antidepressants, fluoxetine and other selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, or antihistamines.

Before the tests, the procedures, including the known risks, were described in detail and written, informed consent (as approved by the Glasgow Royal Infirmary Ethics Committee) was obtained from all patients.

5.2.2 Study design

The study consisted of a one week screening phase and two 2-week treatment periods separated by a 2 day washout phase. Eligible patients who completed the screening phase were randomized to one of two treatment sequences: formoterol 12μg b.i.d followed by matching placebo, or vice versa. The study design is illustrated below in Figure 5-1.

Figure 5-1 **Study design**

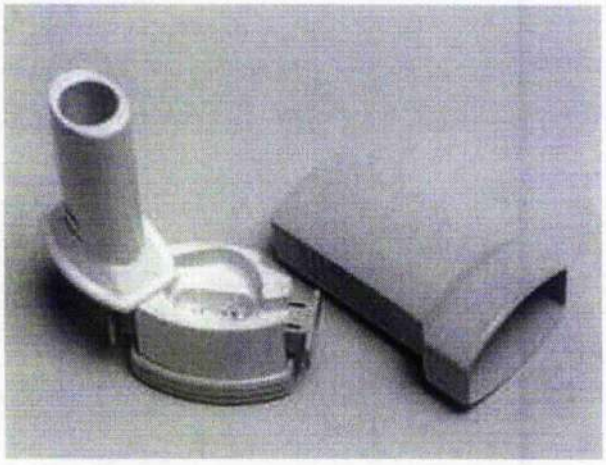


5.2.3 Treatment administration

During the active treatment periods of the study (Periods I and II) patients took study medication twice daily: one dose in the morning (6.00-9.00 hrs), and one in the evening (18:00-21:00 hrs) about 12 hours later. On visit days patients took their morning dose at the study centre.

Study medication was supplied as dry powder capsules containing 12μg formoterol fumarate and inhaled using the Acrolizer™ device (illustrated below in Figure 5.2).

Figure 5.2 The Aeroliser™ inhaler device.



Study medication capsules were supplied by the Novartis Corporation in blisters of 8 capsules, packed in boxes and supplied with the Aerolizer™ device. Study medication was supplied to Glasgow Royal Infirmary as individual randomized patient packs containing two period packs - one for each treatment period. Study medication was then dispensed to each patient at the start of the two treatment periods (Visits 2 and 4). Patients were randomly assigned to one or other treatment sequence (formoterol/placebo or placebo/formoterol). Blinding was maintained by the use of identical active or placebo capsules. Randomization was performed by Novartis Drug Supply Management using a validated system that automates the random assignment of treatment groups to randomization numbers. Only when the study had been completed, the data file verified, and the protocol violations determined were the drug codes broken and made available for data analysis.

Patients were permitted to take rescue medication for worsening COPD symptoms. Two puffs of Combivent were permitted as rescue medication delivered via a metered dose inhaler (100μg salbutamol/ 20μg ipratropium bromide per actuation) not to exceed 8 inhalations per 24 hour period. Patients requiring 8 inhalations on two consecutive days were required to contact the Department of Respiratory Medicine, Glasgow Royal Infirmary. Taking rescue medication within 8 hours prior to a study visit required postponement of that visit. Visits 2 and 4 could be rescheduled at the investigator's discretion whilst visits 3 and 5 could be rescheduled such that the total period treatment duration did not exceed 17 days.

Drug compliance was checked during study visits and at completion. Patients were requested to return all unused medication including rescue medication as well as inhalers, empty blisters and containers.

5.2.4 Measurements

Details of the evaluations performed at scheduled visits are presented in Table 5-1.

Patients with mild ailments at screening or evidence of sub-optimal exercise performance were re-evaluated after one week. If they had not resolved by then, the patient was excluded from the study.

Details of the methodology of pulmonary function testing (spirometry, static lung volumes, CO diffusing capacity, respiratory muscle pressures) is contained in the general methods chapter (chapter 2, Section 2.2.1). Incremental and cardiopulmonary exercise testing methodologies are also explained in Chapter 2, Sections 2.2.4.2 and 2.2.4.3 respectively.

Table 5-1 Evaluation and visit schedule.

Examination	Screening	Visit 2	Visit 3	Visit 4	Visit 5
	Day -7	Day 1	Day 14	Day 17	Day 30
Informed consent	X				
Eligibility	X	X			
Randomization		X			
Resting spirometry	X	X	X	X	X
Full PFT	X				
Inc CPET	X				
CW CPET		X	X	X	X
Concomitant Medications, ECG	X	X	X	X	X
Chest X-Ray[1]	X				
Dyspnoea questionnaires	X		X		X
Adverse Experiences		X	X	X	X

[1] where a recent chest X-Ray (within 6 months) was not available.

5.2.5 Outcome Variables

5.2.5.1 Primary outcome variable

The primary efficacy variable was the time to limit of tolerance (Tlim), being the interval between the onset of the imposed work rate and the point at which the patient could no longer consistently maintain the required cycling rate.

5.2.5.2 Secondary efficacy variables

Secondary efficacy measurements consisted of various lung mechanical and respiratory parameters, perceived leg effort and dyspnea recorded during the CW CPET and the Mahler Transitional Dyspnea index which was administered at the start and end of each treatment period. Each parameter recorded during the CW CPET was measured at four time points these being:

- at rest – with the patient sitting on the cycle ergometer, but not pedalling
- unloaded cycling – cycling at the required rate of revolutions with no resistance imposed .
- isotime – this was defined as the shortest duration of exercise challenge an individual patient completed during either treatment period.
- Tlim – defined as the point the patient could no longer consistently maintain the required cycling rate

The secondary efficacy assessments were oxygen uptake (V_{O_2} absolute), carbon dioxide output, leg effort, dyspnea, ventilation, end expiratory lung volume, inspiratory reserve volume, flow limitation, Mahler's Questionnaire of Chronic Activity-Related Breathlessness, heart rate, ventilation, respiratory frequency, tidal volume, arterial oxygen saturation and inspiratory capacity.

In addition, patients recorded the number of inhalations of rescue medication taken during the night and the day in a Patient Diary. Patients completed the diary twice daily, before the morning and evening doses of study medication.

3.2.6 Safety assessments

Safety assessments consisted of monitoring and recording all adverse events, serious adverse events (with their severity and relationship to study drug), a routine hematology and blood chemistry screen at Visit 1 (with additional monitoring of potassium 3 days after Visits 3 and 5 for patients receiving theophylline), the regular measurement of vital signs and performance of physical examinations. Pre-dose ECGs were recorded at each visit and continuous ECG monitoring took place during exercise tests. Laboratory measurements were performed by the Departments of Haematology and Biochemistry at Glasgow Royal Infirmary.

5.2.7 Statistical methods

All statistical analysis took place using version 6.12 of the SAS statistical package. For continuous variables the following summary statistics were calculated: mean, standard deviation, median, inter-quartile range, and minimum and maximum values. For discrete variables the following summary statistics were calculated: number of values in each category, and percentage in each category of the total number of non-missing values for that variable.

The intent-to-treat population was defined as being all patients randomized to study medication and who received at least one dose of randomized medication. Patients were not excluded from the intent-to-treat population even if they did not fulfil all inclusion and exclusion criteria or violated the protocol.

Only patients who had measurements of Tlim available for both treatment periods, including the measurements made during the CW CPET before the start of trial treatment at Visits 2 and 4, were included in the statistical analyses. These patients will be referred to as the efficacy population. This population was used for the efficacy analyses of all primary and secondary parameters.

The safety population was defined as being all randomized patients who received at least one dose of study medication, the same as the intent-to-treat population.

The primary efficacy variable was the time to limit of tolerance (Tlim), being the interval between the onset of the imposed work rate and the point at which the patient could no longer consistently maintain the required cycling rate.

The null hypothesis tested was that there was no difference in Tlim following 14 days of treatment with formoterol 12μg compared with the Tlim following 14 days of treatment with placebo.

Analysis of covariance (ANCOVA) was used to test the null hypothesis, with terms for sequence group, patient within sequence (fitted as a random effect), period, treatment and baseline Tlim as a covariate, where baseline Tlim is the value measured before treatment for that period. The treatment difference between formoterol and placebo was presented together with the 95% confidence intervals. The treatment effect was tested at the 5% significance level.

Most secondary efficacy parameters were analyzed using the same ANCOVA model as for the primary efficacy parameter, except Mahler's Questionnaire (dyspnea index) and Borg dyspnea, was analyzed using Koch's non-parametric method for a two-period cross-over design, based upon the Wilcoxon Rank Sum Test (Koch, 1972). The estimate of the median difference between treatments (Hodges-Lehmann estimator) and the associated 95% confidence interval have been calculated (Conover & Iman, 1982).

Sample size and power considerations.

No suitable data was available from other studies using the same exercise technique. Therefore no information was obtainable concerning the variability of the primary efficacy variable, the Tlim. Consequently no formal sample size calculations were possible. The number of patients chosen (26, with the aim of having 20 evaluable patients) was considered appropriate based upon our current understanding of the patient population being studied and the exercise test being used.

Twenty-one patients were randomized into the study, with 18 evaluable patients. Recruitment was stopped before the planned sample size was enrolled because of recruitment difficulties and an approaching expiry date of the study drug. The study was therefore potentially underpowered for its primary outcome measure.

5.3 Results

5.3.1 Study participants

Thirty (30) patients were screened (at Visit 1) and 21 patients were randomized into the study at Visit 2: 10 to the formoterol/placebo treatment sequence group and 11 to the placebo/formoterol sequence group. Of the 9 patients who were not randomized, 3 were not because of test procedure results excluding continuation, 4 because of unacceptable use of excluded medications/therapies prior to study commencement and 2 patients withdrew consent.

Eighteen patients completed treatment as planned. Three patients discontinued prematurely, because of increased breathlessness and rescue medication use (two during placebo period, one during formoterol). These three patients could not be included in the efficacy population as they did not complete both treatment periods.

The mean age of enrolled subjects was 60 years. The mean duration of smoking was 39 years, with all patients having smoked for at least 25 years. The mean FEV_1 was 1.0 l, the mean FEV_1/FVC was 38%, the mean FEV_1 as a percentage of predicted was 39% and the mean reversibility as a percentage of predicted normal was 6%. All these reflect the severe nature of the COPD in the patients included in the study. Table 5.2 below summarizes the main results concerning demography and disease status.

Table 5.2 Demographic and disease characteristics.

Total (N=21, 14 male)	**Mean**	**SD**	**Range**
Age (yr)	60.0	9.0	42.0 - 75.0
Number of years smoking	38.5	10.0	25.0 – 60.0
Duration of illness (yr)	4.0	2.3	1.0 – 11.0
FEV_1 (l)	1.00	0.34	0.49 – 1.73
FEV_1 percentage predicted	38.8	11.7	21.0 – 58.3
Reversibility as percentage of predicted normal	5.5	3.3	-0.9 – 10.7
Reversibility (%)	14.3	8.9	-2.7 – 35.2
FEV_1/FVC (%)	37.5	7.0	25.9 – 52.5

5.3.2 Primary efficacy results

Table 5.3 shows the results from the ANCOVA analysis of the primary efficacy variable, time to limit of tolerance (Tlim): both the estimated treatment means and the estimated treatment difference are presented as least squares (LS) means.

Table 5.3 Time to limit of tolerance (secs).

Treatment/contrast (n=18)	Least square mean	95% confidence	p-value
Formoterol	478.9		
Placebo	348.5		
Formoterol vs placebo	130.3	–1.2, 261.8	0.0518

All statistics are based on the ANCOVA model.
Both estimated treatment means and the estimated treatment difference are presented as least square means.

The treatment difference in favour of formoterol, estimated as 130 seconds (over 2 minutes) was very close to achieving statistical significance at the 5% level (p=0.052). It is possible that the sample size was not large enough as it was chosen for practical purposes on empirical grounds, given there was no information available on the variability of Tlim. This is supported by an exploratory analysis of the percentage change from baseline in Tlim, which was carried out after the trial was unblinded, in which the treatment difference in favour of formoterol was statistically significant. The same ANCOVA model was used as for the primary efficacy variable. The mean percentage increase from baseline in Tlim was 29.7% with formoterol with a mean decrease of 8.2% with placebo. The treatment difference in favour of formoterol was 37.8%, with a 95% confidence interval of 9.4% to 66.2% (p=0.012).

5.3.3 Secondary efficacy results

The secondary efficacy parameters identified prior to study enrolment (change in dyspnoea per time, change in the end expiratory lung volume per time, end expiratory lung volume, inspiratory reserve volume per time, Borg dyspnoea index and Mahler's questionnaire) are reported in this results section. Other exploratory analyses are referred to, and are represented in more detail, where statistical significance was achieved.

Rate of the Δ dyspnoea per second

A reduction in the rate of the Δ dyspnoea per seconds would indicate that the rate of increase in breathlessness with time was attenuated.

Table 5.4 shows the results from the ANCOVA analysis. The least square means (at isotime from unloaded cycling and from at rest) show lower values when the patients were under formoterol than when the patients were under placebo (as seen in the negative estimated difference).

The differences (from unloaded and from at rest) between treatments were not statistically significant. The between treatment difference at rest was larger than the difference during unloaded cycling.

Table 5.4 Rate of the Δ Dyspnoea per second.

	Treatment / contrast	LS mean	95% confidence interval	p-value
Isotime - unloaded	Formoterol	0.0226		
(n=17)	Placebo	0.0265		
	Formoterol vs placebo	-0.0039	-0.016, 0.008	0.5072
Isotime – at rest	Formoterol	0.0307		
(n=18)	Placebo	0.0472		
	Formoterol vs placebo	-0.0165	-0.036, 0.003	0.0857
Tlim - unloaded	Formoterol	0.0202		
(n=17)	Placebo	0.0226		
	Formoterol vs placebo	-0.0024	-0.011, 0.006	0.5515
Tlim – at rest	Formoterol	0.0304		
(n=18)	Placebo	0.0346		
	Formoterol vs placebo	-0.0042	-0.031, 0.023	0.7434

All statistics based on the ANCOVA model: Δ Dyspnea/Time = sequence group+ patient + treatment + period + Baseline Δ Dyspnea/Time where Baseline is the value measured before treatment for that period

Rate of the Δ end expiratory lung volume per second

A reduction in the rate of the Δ end expiratory lung volume per second would indicate that the rate of increase in lung hyperinflation with time was attenuated.

Table 5.5 shows the result from the ANCOVA analysis. The least square means (from unloaded cycling and from at rest) show lower values when the patients were under placebo than when the patients were under formoterol.

The differences (from unloaded and from at rest) between the treatments were not statistically significant.

Table 5.5 Rate of the Δ End expiratory lung volume (l) per second.

	Treatment / contrast	**LS mean**	**95% confidence interval**	**p-value**
Isotime - unloaded	Formoterol	0.0010		
(n=16)	Placebo	0.0007		
	Formoterol vs placebo	-0.0003	-0.000, 0.001	0.3545
Isotime – at rest	Formoterol	0.0022		
(n=14)	Placebo	0.0019		
	Formoterol vs placebo	0.0003	-0.001, 0.001	0.4173
Tlim - unloaded	Formoterol	0.0010		
(n=17)	Placebo	0.0008		
	Formoterol vs placebo	0.0002	-0.000, 0.001	0.2842
Tlim – at rest	Formoterol	0.0025		
(n=16)	Placebo	0.0028		
	Formoterol vs placebo	-0.0003	-0.002, 0.001	0.6011

All statistics based on the ANCOVA model: Δ End expiratory lung volume/Time = sequence group+ patient + treatment + period + Baseline Δ end expiratory lung volume/Time where Baseline is the value measured before treatment for that period

End expiratory lung volume (l)

A reduction in the end expiratory lung volume would indicate a reduction in the absolute degree of lung hyperinflation.

Table 5.6 shows the result from the ANCOVA analysis. The least square means show lower values when the patients were under formoterol than when the patients were under placebo.

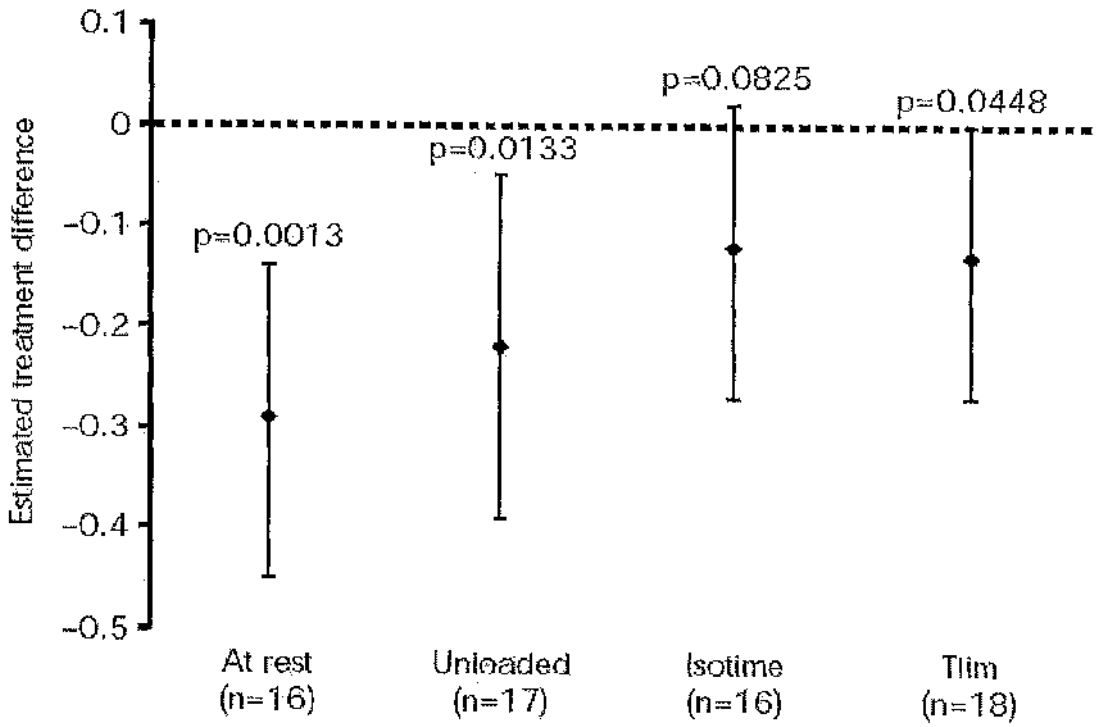
The differences between the treatments were statistically significant at the 5% level of significance at rest, at unloaded cycling and at Tlim, and was close to statistical significance at isotime. These data are illustrated in Figure 5.3.

Table 5.6 End expiratory lung volume (l).

	Treatment / contrast	**LS mean**	**95% confidence interval**	**p-value**
At rest	Formoterol	4.84		
(n=16)	Placebo	5.13		
	Formoterol vs placebo	-0.29	-0.45 , -0.14	0.0013
Unloaded	Formoterol	5.18		
(n=17)	Placebo	5.40		
	Formoterol vs placebo	-0.22	-0.39 , -0.05	0.0133
Isotime	Formoterol	5.17		
(n=16)	Placebo	5.30		
	Formoterol vs placebo	-0.12	-0.27 , 0.02	0.0825
Tlim	Formoterol	5.49		
(n=18)	Placebo	5.62		
	Formoterol vs placebo	-0.13	-0.27 , -0.00	0.0448

All statistics based on the ANCOVA model: End expiratory lung volume = sequence group + patient + treatment + period + Baseline end expiratory lung volume where Baseline is the value measured before treatment for that period

Figure 5.3 End expiratory lung volume (l), estimated treatment differences (formoterol-placebo) and 95% confidence intervals.



Rate of change of inspiratory reserve volume with time

An increase in Δ Inspiratory Reserve Volume / Time would indicate that more volume was available for inspiration, but not used, for a given change in time.

Table 5.7 shows the results from the ANCOVA analysis. The least square means show higher values when the patients were under formoterol than when the patients were under placebo at each time point except for the difference at isotime from at rest.

The differences between the two treatments were not statistically significant at any time point.

Table 5.7 Δ Inspiratory Reserve Volume (l) per second.

	Treatment / contrast	LS mean	95% confidence interval	p-value
Isotime – unloaded	Formoterol	-0.0018		
(n=16)	Placebo	-0.0020		
	Formoterol vs placebo	0.0002	-0.001, 0.001	0.7382
Isotime – at rest	Formoterol	-0.0040		
(n=14)	Placebo	-0.0036		
	Formoterol vs placebo	-0.0003	-0.002, 0.001	0.6977
Tlim – unloaded	Formoterol	-0.0013		
(n=17)	Placebo	-0.0017		
	Formoterol vs placebo	0.0004	-0.000, 0.001	0.3198
Tlim – at rest	Formoterol	-0.0033		
(n=16)	Placebo	-0.0044		
	Formoterol vs placebo	0.0011	-0.001, 0.003	0.2031

All statistics based on the ANCOVA model: Δ Inspiratory reserve volume/Time = sequence group+ patient + treatment + period + Baseline Δ Inspiratory reserve volume where Baseline is the value measured before treatment for that period.

Mahler's questionnaire of chronic activity-related breathlessness

A positive value indicates an improvement compared with the baseline values. Table 5.8 shows the median for the transition values of the dyspnoea index. All three parameters showed an increase when the patients were under formoterol and no change when the patients were under placebo (Figure 5.4). The minimal clinically important difference for each focal score is 1 unit.

Table 5.8 Mahler's Questionnaire of chronic activity-related breathlessness, transition dyspnoea index, efficacy population.

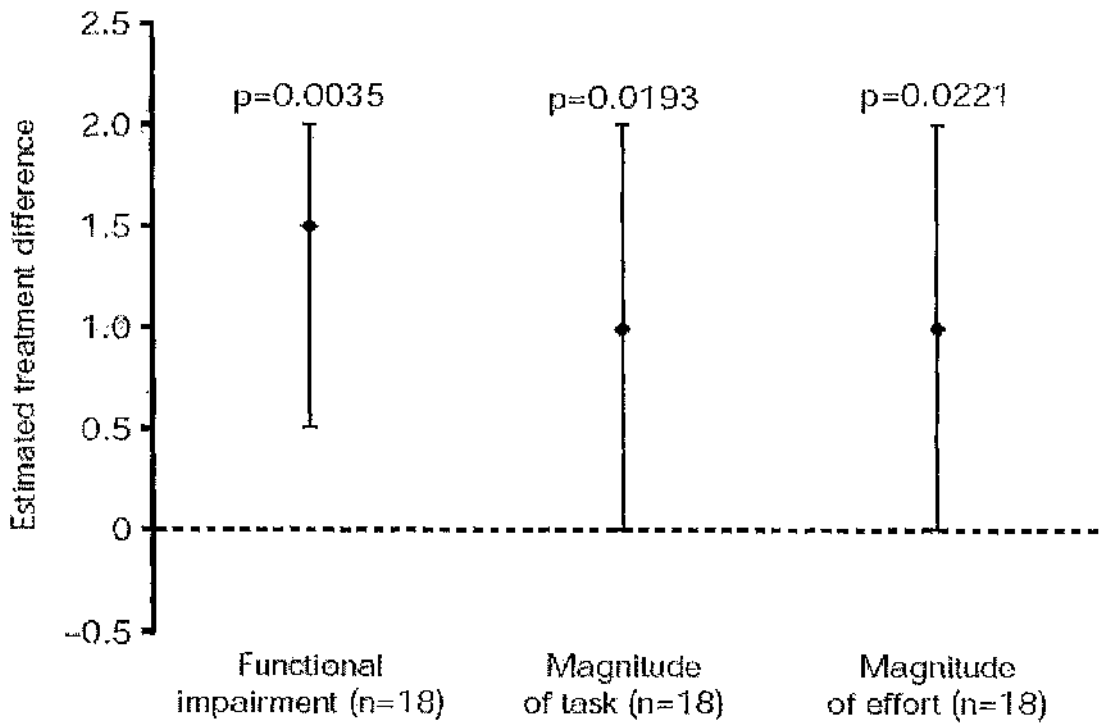
Change in (n=18)	**Time point**	**Formoterol** (median score)	**Placebo** (median score)
Functional Impairment	After treatment	1.5	0.0
Magnitude of Task	After treatment	1.0	0.0
Magnitude of Effort	After treatment	1.0	0.0

Table 5.9 shows the results from Koch's non parametric method. Statistically significant treatment differences in favour of formoterol were seen in all 3 parameters.

Table 5.9 Mahler's Questionnaire of chronic activity-related breathlessness, transition dyspnoea index – Analyses.

Treatment contrast (n = 18)	**Change in**	**Hodges-Lehman estimator**	**95% confidence interval**	**p-value**
Formoterol vs. Placebo	Functional Impairment	1.50	0.50 , 2.00	0.0035
	Magnitude of Task	1.00	0.00 , 2.00	0.0193
	Magnitude of Effort	1.00	0.00 , 2.00	0.0221
Based on Koch's non parametric method				

Figure 5.4 Mahler's questionnaire activity-related breathlessness, transition dyspnea index score, estimated treatment differences (formoterol – placebo) and 95% confidence interval.



Borg dyspnoea index

The Borg index rates a patient's degree of breathlessness on a scale from 0 (none) to 10 (maximal). Lower scores indicate a lower level of breathlessness.

After treatment, the median of the Borg dyspnoea index at isotime was lower when the patients were under formoterol than when the patients were under placebo (Table 5.10).

Table 5.10 Borg dyspnoea index.

	Time point	**Formoterol** (median score)	**Placebo** (median score)
At rest	Before treatment	0.0	0.0
(n=18)	After treatment	0.0	0.5
Unloaded	Before treatment	1.0 [a]	1.0
(n=18)	After treatment	1.0	1.0
Isotime	Before treatment	7.0	6.0
(n=18)	After treatment	4.5	7.0
Tlim	Before treatment	8.5	7.0
(n=18)	After treatment	9.0	7.0

[a]n=17 due to missing data. The analysis is based on the after treatment data and does not use the before treatment data.

Table 5.11 shows the results from Koch's non parametric method. The estimated difference between the treatments was in favour of formoterol at unloaded cycling and at isotime but was not statistically significant.

Table 5.11 Borg dyspnoea index – Analyses.

Treatment contrast (n = 18)		**Hodges-Lehman estimator**	**95% confidence interval**	**p-value**
Formoterol vs. Placebo	At rest	0.00	-0.05 , 0.00	0.2312
	Unloading	-0.50	-1.00 , 0.00	0.1742
	Isotime	-1.50	-2.50 , 0.50	0.0833
	Tlim	0.00	-1.00 , 1.00	0.9375

Based on Koch's non parametric method

Inspiratory Reserve Volume (l)

An increase in this parameter would indicate that the elastic load of inspiration was being reduced at these time points. Table 5.12 shows that the inspiratory reserve volume was higher when the patients were treated with formoterol than when the patients were treated with placebo. These data are also illustrated in Figure 5.5.

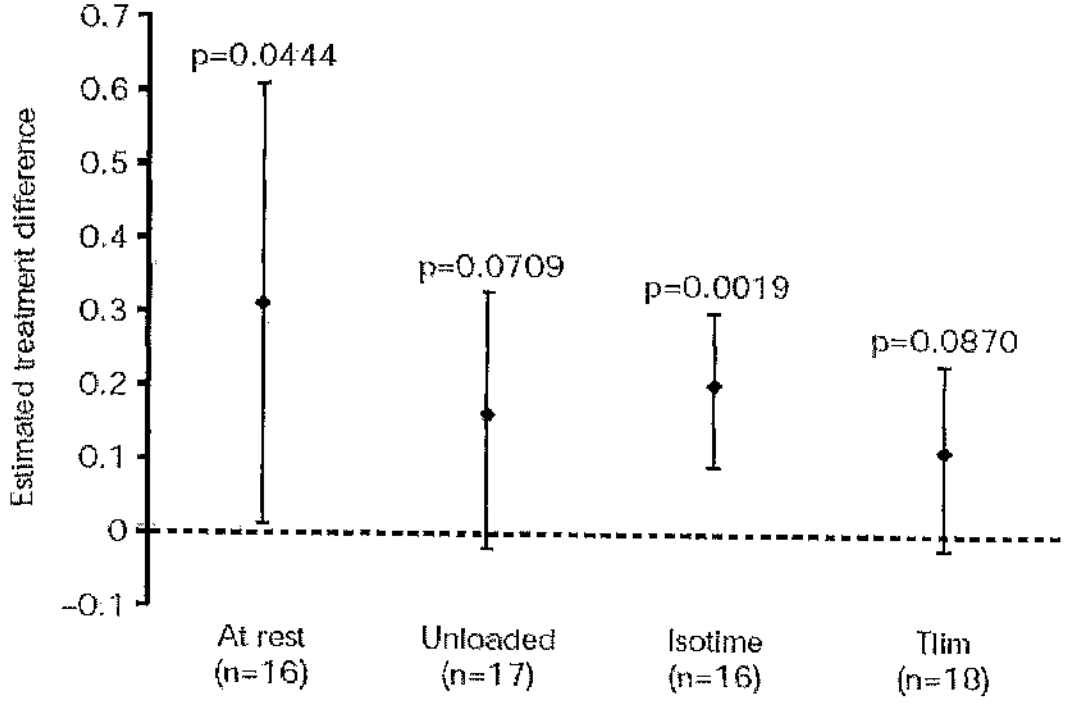
The differences between treatments at rest and at isotime were statistically significant.

Table 5.12 Inspiratory reserve volume (L).

	Treatment / contrast	**LS mean (L)**	**95% confidence interval**	**p-value**
At rest	Formoterol	1.47		
(n=16)	Placebo	1.16		
	Formoterol vs placebo	0.31	0.01 , 0.61	0.0444
Unloaded	Formoterol	0.98		
(n=17)	Placebo	0.82		
	Formoterol vs placebo	0.16	-0.02 , 0.33	0.0709
Isotime	Formoterol	0.59		
(n=16)	Placebo	0.40		
	Formoterol vs placebo	0.20	0.09 , 0.30	0.0019
Tlim	Formoterol	0.47		
(n=18)	Placebo	0.36		
	Formoterol vs placebo	0.11	-0.02 , 0.23	0.0870

All statistics based on the ANCOVA model: Inspiratory reverse volume = sequence group+ patient + treatment + period + Baseline inspiratory reverse volume where Baseline is the value measured before treatment for that period

Figure 5.5 Inspiratory reserve volume (l), estimated treatment differences (formoterol – placebo) and 95% confidence intervals.

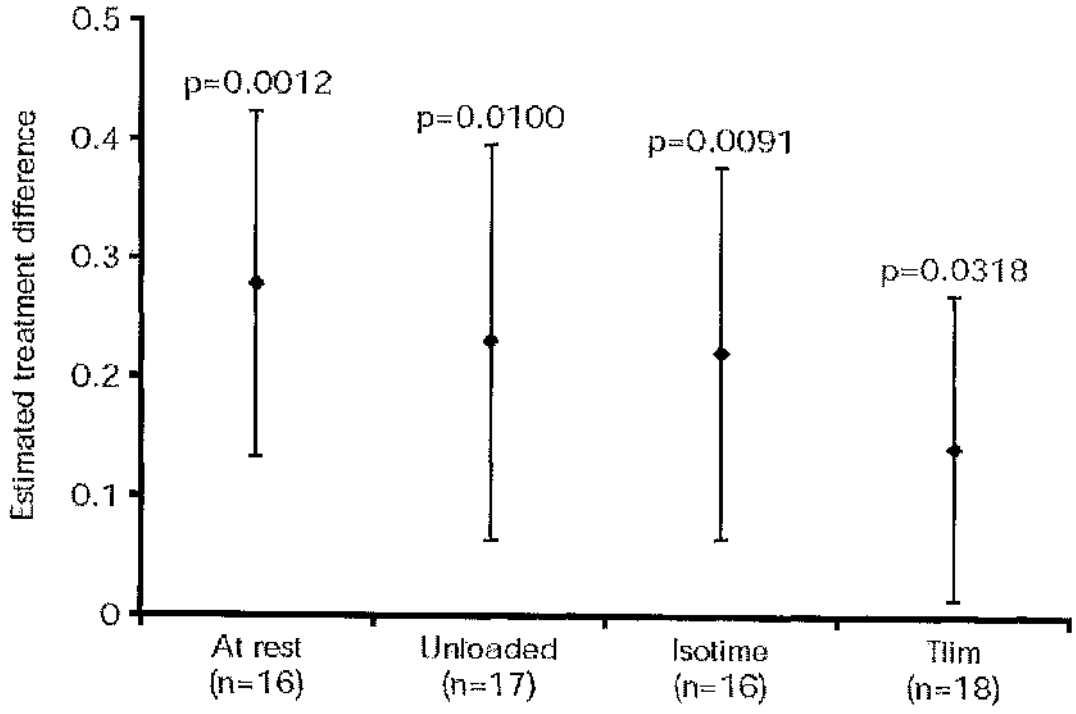


Additional exploratory parameters

There were no significant differences between formoterol and placebo groups for oxygen uptake (mL/min), Δ Leg effort / Δ Oxygen uptake, Δ Dyspnoea / Δ Oxygen uptake, Δ Dyspnoea / Δ ventilation, Δ Leg effort / Time, Δ End Expiratory Lung Volume / Δ Ventilation, Δ Inspiratory Reserve volume / Δ Ventilation and flow limitation in relation to time or ventilation.

IC measurement allows measurements to be calculated which allow us to infer conclusions regarding the degree of dynamic hyperinflation. As is illustrated below in Figure 5.6, it was IC that had the most consistent change with formoterol, in comparison to the other parameters described in detail above.

Figure 5.6 Inspiratory capacity (l), estimated treatment differences (formoterol – placebo) and 95% confidence intervals.



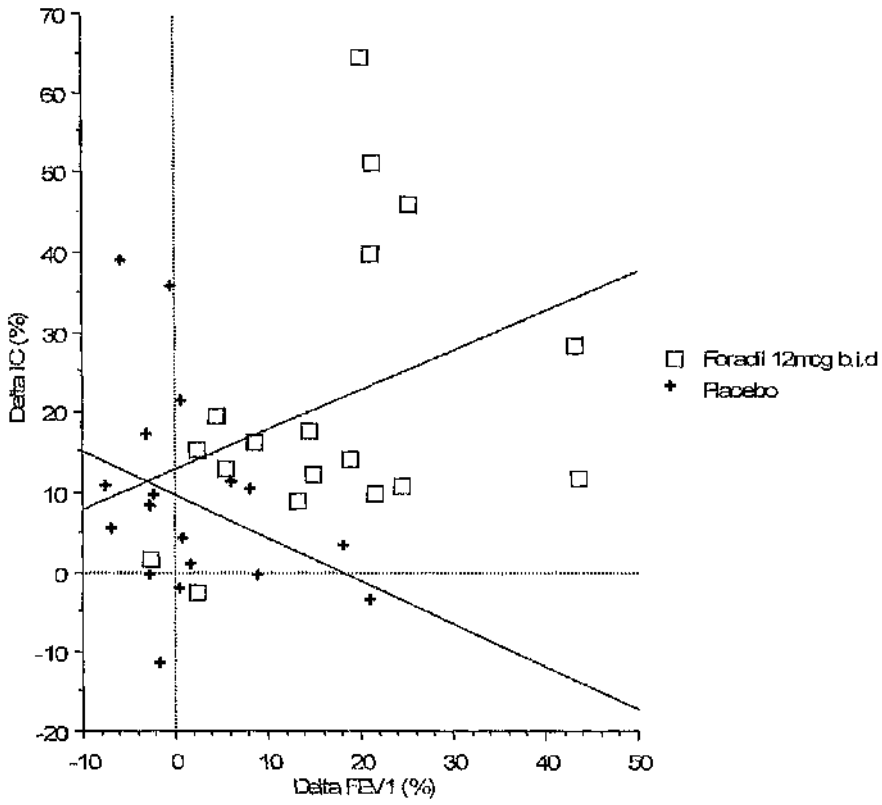
We were interested as to whether resting IC or FEV1 would relate to the exercise or dynamic hyperinflation changes seen after the bronchodilator administration. Both IC and FEV1 differed after Foradil, compared to placebo at rest. The between group differences were significant as illustrated in table 5.13 below.

Table 5.13 Between group differences for resting post treatment lung function.

	Between group difference	95% lower CI	95% upper CI	p-value
IC (%)	12.6	2.0	23.2	0.02
FEV1 (%)	14.5	7.3	21.7	0.0003

However, although there was a positive correlation between these two measures as seen following Foradil administration, this was not statistically significant (R= 0.35, p= 0.15). The percentage change of FEV1 and IC following placebo and Foradil administration is illustrated in the scattergram below (Figure 5.3).

Figure 5.7 Bivariate scattergram for percentage responses of IC and FEV1 with placebo and Foradil treatments.



5.3.4 Safety results

The safety information collected included adverse events, baseline routine blood tests (haematology, biochemistry), pre exercise ECG's and data on vital signs. There were no incidences of findings that indicated lack of safety of the trial design. Apart from the episodes of increased breathlessness, leading to study discontinuation, there were no severe adverse events or deaths during the study. The profile of adverse experiences is detailed below (Table 5.13),

however these findings would be in keeping with that expected for subjects of this age and disease severity.

5.4 Discussion

This study demonstrates that the long acting bronchodilator, formoterol, has a beneficial effect upon exercise induced dynamic hyperinflation and resting breathlessness. This is despite selecting a group of severe patients that have poorly reversible expiratory flow in response to inhaled bronchodilator. The impact of the drug upon exercise capacity was less clear, with only subgroup analyses of percentage change of Tlim achieving unambiguous statistical significance. We believe that this is the first study of the effects of fomoterol, which is a rapidly acting but long duration bronchodilator, on exercise tolerance and dynamic hyperinflation in COPD.

The primary efficacy endpoint was very close to, but did not, achieve statistical significance (p=0.0518). The estimated difference between the treatments was large (130 seconds) and to the advantage of formoterol. Whilst there is no generally recognized formal definition of a clinically meaningful increase in Tlim during a CW CPET, it is highly probable that the increase in Tlim observed in this study would equate to benefits in patient's ability to perform their normal day-to-day physical tasks. In a recently published study of the effects of salmeterol on endurance exercise, with 23 patients completing the study, an increase of 96 seconds or 58% was seen, representing similar findings to ours (O'Donnell *et al.*, 2004b). As we were unable to reach our target recruitment then it is likely that the study was underpowered and this is discussed in more detail later.

A large number of secondary efficacy variables were analyzed in this study. The majority of these variables have not previously been examined in the context of a long acting bronchodilator clinical trial. The effect of formoterol on such parameters was therefore very difficult to predict at the time this study was designed.

There was a statistically significant decrease in end-expiratory lung volume (EELV) at rest, during unloaded cycling and at Tlim. In addition at isotime the decrease almost achieved significance (p=0.083). Similarly, formoterol significantly increased inspiratory reserve volume at rest and at isotime (almost achieving significance during unloaded cycling, p=0.071), and at Tlim, p=0.087). Both of these measures are indicators of dynamic hyperinflation, which is known to be a crucial component of the factors which limit exercise capacity in COPD patients (O'Donnell, 2001). In COPD the slower rate of lung emptying leads to the expiratory time being insufficient to exhale what has been previously inspired. The subsequent increase in operating

lung volumes, although it may allow increased flow-generating capacity, leads to static tidal volumes despite a greater work of breathing. This is largely due to the respiratory system working on the flat portion of the pressure/volume relationship. It seems likely that the dynamic hyperinflation induced positive end expiratory pressure is crucial in elevating the work of breathing in these patients, a factor likely altered by bronchodilatation (Sliwinski *et al.*, 1998). The respiratory muscles are placed at a mechanical disadvantage as they are required to work at near maximal lengths and loads. The subsequent increased work of breathing increases the oxygen demands of the exercising muscles, which may have deleterious effects upon oxygen delivery elsewhere (Aliverti & Macklem, 2001). The decrease in EELV and concurrent increase in IRV demonstrated in this study may therefore have been instrumental in allowing the patients to continue the CW CPET for longer due to the beneficial effects of decreased work of breathing. Reproducibility of inspiratory capacity measurements during exercise is high in COPD, irrespective of the disease severity, and more so, may be more repeatable during exercise compared to rest (Dolmage & Goldstein, 2002; Taube *et al.*, 2000).

The three parameters evaluated by Mahler's questionnaire showed an improvement when the patients were taking formoterol. Differences between treatments in all the 3 parameters 'functional impairment', 'magnitude of task' and 'magnitude of effort', were statistically significant at the 5% level of significance, indicating that formoterol decreased patients' dyspnea during their two weeks of treatment. The results from the Borg dyspnea scale measured during the exercise challenge whilst being in favour of formoterol were not statistically significant. Effects of long acting bronchodilators on breathlessness at rest are well established and these findings offer additional support for this (Di Marco *et al.*, 2003; Ramirez-Venegas *et al.*, 1997). Long acting bronchodilators are being seen to have a role in symptomatic COPD patients, irrespective of bronchodilator response. This is principally for two reasons. Firstly, traditional measures of reversibility, such as FEV1 response, may remain largely unaltered, whereas other indices, perhaps inspiratory capacity or lung volumes, show changes (Newton *et al.*, 2002; Duranti *et al.*, 2002; O'Donnell *et al.*, 2004b). Secondly, such bronchodilator responses, either through steroid or beta agonist administration, do not predict longer term benefit in the form of symptoms or exacerbations for these drugs (Calverley *et al.*, 2003b; Tashkin & Kesten, 2003). Our data support the use of bronchodilators in severe groups of patients without significant bronchodilator reversibility.

A cross-over design was chosen to allow for a within-patient placebo-controlled comparison thereby limiting the number of patients required. The fourteen day duration for each treatment period was considered sufficient to allow for the patients' respiratory status to be stable whilst allowing a total study duration for each patient of not more than one month. During a one month period the patients' lung function was not expected to decline significantly and the relatively short evaluation period was not expected to hinder patient compliance. A formoterol dose of 12μg b.i.d is commonly used in the treatment of COPD and is the lower of two formoterol doses submitted for licensing approval in COPD.

The primary outcome of time to limit of tolerance (Tlim) was based on a constant work-load challenge. This type of challenge was chosen because it is more representative of the type of activity level and exertion with which COPD patients need to cope during their daily routine activities. Endurance type tests are more sensitive than incremental exercise test to intervention as they are likely gauged to be near to the "critical power" of any activity (Neder *et al.*, 2000a). This is also applies to "field" based exercise tests such as incremental and endurance shuttle walking (Revill *et al.*, 1999). For this study we chose to utilise a more formal laboratory based assessment of exercise endurance as that setting allowed the concurrent evaluation of dynamic hyperinflation. A CW CPET was performed at the start of each study treatment period prior to receiving study medication and at the end of each treatment period 2 hours after study medication inhalation, this time point being chosen because this was when a clinically significant effect was expected to be seen for most patients. O'Donnell has, in a comprehensive review on the subject, concluded that measures of dynamic hyperinflation during exercise, in conjunction with dyspnoea and quality of life, are more sensitive than spirometric measures to the effects of bronchodilators (O'Donnell *et al.*, 1998).

FEV_1 reversibility testing following inhalation of 400μg of salbutamol during screening provided evidence of a non-reactive obstructive defect. It was hoped that by selecting the more severe end of the COPD spectrum, any resulting improvement in exercise capacity would be seen to have potential for a wide applicability.

This study has a clear limitation, in that the projected number of patients required to show benefit in exercise capacity were not recruited. The reasons for this shortcoming were mainly due to pressures relating to expiry time of the study drug provided. However it must also be said

that a significant majority of the patients attending Glasgow Royal Infirmary were already established on long acting bronchodilators, and in that setting a placebo – formoterol crossover study did not seem appropriate in the context of the established benefits of these therapies in COPD (Pauwels *et al.*, 2001). We do believe that a larger sample size would have led to a significant impact upon exercise capacity being demonstrated. Such a supposition is supported by our exploratory analysis of percentage changes in exercise capacity, which demonstrated clear benefit to formoterol. In general, the study had a high completion rate, with only three subjects discontinuing prematurely (Two due to due to increased breathlessness and one because of excess rescue medication use). Otherwise only minor adverse occurrences were documented as expected with this well tolerated therapy (Rabe, 2003).

This study suggests that formoterol may improve the time to limit of exercise tolerance in patients with COPD. We did not achieve statistical significance for the primary outcome of absolute endurance exercise time ($p=0.052$), however percentage improvements in exercise capacity were significantly greater in the formoterol group ($p=0.012$). In addition, and likely in relation, dynamic hyperinflation was significantly lessened with formoterol.

CHAPTER 6

CONCLUSIONS AND FUTURE RESEARCH

6.1 Thesis summary

This thesis contains studies that have examined the assessment and management of disability in chronic obstructive lung disease. Whereas there was until relatively recently little focus of attention on this disease, there is now a much-improved understanding of the pathogenesis, clinical course and multi-system manifestations of COPD. Diagnosis is a simple matter of a compatible history, environmental antigen exposure and spirometry (Pauwels *et al.*, 2001). Evaluation of the impact of the disease is much more complex. More sophisticated respiratory function is commonly undertaken, with current controversy as to which resting lung volume measurement is most meaningful (Newton *et al.*, 2002). To further classify the impairment produced by the disease, one can examine body composition or exercise capacity, utilising either simple or complex means. These methodologies have themselves largely been used in the context of pulmonary rehabilitation, but with more recent clarification of the important prognostic indices in COPD, these are likely to enter routine outpatient clinical practice (Marquis *et al.*, 2002;Celli *et al.*, 2004). It is undoubtedly the hope of researchers and clinicians that the assessments made in the COPD patient will not only provide prognostic information, but also be useful as guides for the most appropriate treatment approaches to take.

In chapter 3 we have considered to what extent utilising a complex method of body composition assessment adds meaningful information over that provided by body mass index or anthropometry. We have concluded that the inaccuracies of anthropometry, compared to a more complex measurement, are such to lead to significant discrepancies in estimates of exercise capacity. Therefore more detailed assessment clearly can improve accuracy of impairment evaluation. Systematic bias can exist with some measures, meaning that consistency of measurement methodology is of particular importance in individual centres when longitudinal changes are being looked for in body composition. This valid observation should not, however, discourage clinicians from addressing body composition through other means. This is because an understanding of whether a patient is depleted or not of muscle bulk, and to what extent, is crucial to the understanding of their clinical phenotype, required intervention and prognosis. The impact of fat-free mass depletion on strength, exercise capacity, and health-related quality of life, independent of lung function is clearly demonstrated from the cohort of patients taking part in the creatine intervention study.

In chapter 4, the effect of creatine supplementation was examined. This included assessment after two weeks loading and following a period of outpatient exercise training. The supplement was well-tolerated and lead to demonstrable beneficial effects. Unequivocal improvements in muscle bulk, muscle strength and endurance, and health status were seen. For reasons that may relate to the test involved (insensitivity or lack of reproducibility) or efficacy of the intervention, exercise capacity was not seen to be affected. This finding may highlight the gulf between those aspects of a disease that we can measure and the elements that relate to the patients well being. For health status to be altered, particularly in activity domains, suggest that there may well be an effect of an intervention such as creatine on aspects not measurable clinical in the laboratory. The investigation of the utility of alternative measures such as activity monitors is underway in a number of centres. Creatine has been shown to be efficacious and safe. Its applicability to the wider population of COPD patients, particularly which form, duration or timing of administration is most appropriate requires further evaluation. Our study would suggest that creatine benefits are present for a wide range of severe COPD patients, irrespective of body habitus, and that these benefits are of a meaningful magnitude.

In chapter 5 the impact of a long acting bronchodilator, formoterol, upon dynamic hyperinflation, exercise tolerance and breathlessness was assessed. Once again a severe cohort was examined, this one with features of poorly reversible lung disease. The partial fallacy of this statement is evident as the flow limitation remains largely fixed while inspiratory manoeuvres neatly demonstrate the benefits that can be derived from bronchodilation. We found a borderline, and likely so because of underpowering, benefit in regard to endurance exercise capacity. Significant effects upon dynamic hyperinflation were clearer, along with the expected changes in resting measures of breathlessness. Bronchodilators evidently have benefits to patients, irrespective of their FEV_1 response to β_2-agonists.

In all of the studies presented, complex methods of assessment have demonstrated benefits of intervention in patients selected through very simple criteria. We may in the future require in-depth evaluations outside of research settings, but at present the utility of novel treatments seems

wide and interventions such as inhaled bronchodilators or nutritional supplements can be targeted to appropriate patients through straightforward means.

6.2 Future research

A large body of research is addressing the causes of skeletal muscle dysfunction in COPD. This becomes of particular importance as its role in relation to morbidity and mortality in this patient group becomes clearer. My research has addressed measures of disease status in COPD and has evaluated two specific treatments.

We would consider the following areas to be a natural progression of the research undertaken in this thesis.

6.2.1 Assessment of disease

Bio-electrical impedance is likely to emerge as the most convenient measure of body composition, without compromise of the findings. There is no evidence to help clarify to what extent performing these measures in a general respiratory setting can alter outcome through targeted means. Although some groups have enthusiastically addressed this area in the setting of pulmonary rehabilitation, the transition from prognostic indicator to management guide has not yet been shown to be appropriate.

Bronchodilator reversibility remains the standard means of diagnosing COPD. Its utility as a method of identifying asthmatic tendencies seems accepted by clinicians. However if measures of inspiratory reserve are more sensitive to change, then further research is clearly required to guide clinicians as to what determines meaningful bronchodilator response, if any response is meaningful at all.

Measures of endurance exercise are effective in showing response to interventions that directly target that exercise testing modality. They are currently relatively crude means of identifying benefit from muscle or lung treatments that are not exercise based. The applicability of activity monitors or questionnaires, or other laboratory exercise tests warrant investigation.

6.2.2 Treatment

Creatine has been shown to be beneficial to a small cohort of stable patients entering a rehabilitation programme. Specific research is required to address aspects of this treatment to include; optimal duration, optimal dose, requirements of a loading regime, optimal setting, effect on morbidity and mortality.

Long acting bronchodilators and more latterly combination therapy with inhaled corticosteroids are established therapies for patients with symptomatic COPD. An investigation that helped unravel the relative importance of symptom relief, exercise improvement and exacerbation frequency in COPD patients would help direct the direction of further research.

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