Exercise tolerance and skeletal muscle structure and function in patients with chronic obstructive pulmonary disease

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Submitted for the degree of MSc (Med) Exercise Science

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DEDICATION

To my parents, for your unfailing support, your love, your encouragement and your belief in me.
You truly are "the wind beneath my wings".

To Emma and Tracey, both of whom I miss dearly, I have picked your stars and think of you often.

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"The trite observation that familiarity breeds contempt is essentially true with regard to the outlook of chronic bronchitis; those afflicted are inclined to accept the complaint as inevitable..... those called upon to treat it do not find it sufficiently interesting to study it closely" Collis. Lancet 1923

DECLARATION

I, Kirsty Lee Coleman, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work or any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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ABSTRACT

Exercise intolerance is well documented in patients with chronic obstructive pulmonary disease (COPD). Historically, this exercise intolerance has been attributed to the central factors of lung damage and subsequent heart failure. However, recent evidence suggests that (i) patients with cardiac and renal failure suffer from skeletal muscle (SM) abnormalities that impair exercise tolerance and (ii) patients with chronic obstructive pulmonary disease (COPD) may have metabolic and functional abnormalities of SM. However, no studies have conducted a detailed investigation of SM structure and function and their relation to exercise tolerance in patients with COPD.

In the first study of this dissertation, exercise tolerance and SM function was examined in 13 patients with moderate COPD (FEV_1/FVC = 37 ±9%) and compared to age matched controls. Patients and subjects underwent i) tests of lung function ii) anthropometry iii) six minute walk test (SMWT) iv) graded cycle ergometry to exhaustion for determination of peak $V0_2$, peak workload (WL) and peak ventilation (V_E) and v) isokinetic tests of SM strength and endurance.

Patients achieved a significantly lower Peak $V0_2$ (17 ±3 vs 25 ±5 $ml0_2$/kg/min; $p<0.01$), WL (72 ±37 vs 211 ±69 W; $p<0.01$) and V_E (41 ±13 vs 87 ±22 l/min; $p<0.01$) during the cycle test and distance walked during the SMWT (494 ±150 vs 718 ±75 m; $p<0.01$) compared to controls. Similarly, concentric quadriceps strength was reduced in patients compared to controls before (169 ±90 vs 257 ±99 Nm; $p<0.01$), but not after correction for lean thigh volume (LTV) (62 ±28 vs 69 ±28 Nm/l; NS; COPD vs C). Eccentric quadriceps strength was lower in patients before (135 ±43 vs 198 ±51 Nm; $p<0.05$) and after correction for LTV (0.9 ±1.2 vs 2.6 ±0.7 Nm/l; $p<0.05$). Quadriceps total power (92 ±45 vs 161 ±54 W; $p<0.01$) and work done (2110 ±998 vs 3936 ±1386 Nm; $p<0.01$) during the isokinetic endurance test were lower in patients compared to controls before and after correction for LTV. In addition, correlations were found between variables of skeletal muscle function and measures of exercise capacity in patients with COPD. These data support the hypothesis that SM function may play a role in exercise limitation in patients with COPD.

In the second study of this dissertation, 11 patients and 11 control subjects underwent a SM biopsy

for analysis of histology and ultrastructure. All patients with COPD demonstrated pathological abnormalities including Type II fibre atrophy, variation in fibre size, fibre splitting, moth-eaten fibres, subsarcolemmal aggregations and abnormalities of nuclei including; nuclear knots, nuclear bags, nuclear chains, internal and pyknotic nuclei. A blinded impartially scored method for grading SM pathology showed significant pathology in patients compared to age matched counterparts (9 $\pm$3 vs 3 $\pm$6; COPD vs C; $p<0.01$). A relationship was shown between SM structure and; parameters of exercise capacity, SM function and distance walked during the SMWT ($r= -0.63$; $p=0.05$). These data suggest that SM structure may play a role in the exercise intolerance experienced by patients with COPD.

In the third study of this dissertation, predictors of exercise capacity in patients with COPD were evaluated. FEV_1/FVC was related to peak $V0_2$ during the incremental cycle test ($r=0.72$; $p<0.01$) and distance walked during the SMWT ($r=0.81$; $p<0.01$). The biopsy pathology score correlated with peak $V0_2$ ($r=-0.63$; $p=0.05$), peak WL ($r=-0.67$; $p<0.05$) and distance walked during the SMWT ($r=-0.65$; $p<0.05$). Duration of disease correlated with peak WL ($r=-0.60$; $p<0.05$) and distance walked during the SMWT ($r=-0.65$; $p<0.05$). Various measures of SM function correlated with peak WL and distance walked during the SMWT. These data suggest that measures of lung function and measures of SM structure and function are useful predictors of exercise capacity in patients with COPD. However, patients with COPD who undergo exercise training improve their exercise capacity with no alterations in lung function. This implies that factors other than improved lung function are responsible for the improved exercise capacity in patients with COPD and renders the FEV_1/FVC a less accurate predictor of exercise capacity in trained patients.

Therefore the last study in this dissertation was a case study that examined changes in SM structure and function in relation to exercise tolerance in a single patient with COPD after exercise training. The patient showed improved exercise tolerance and improved SM structure and function after training with no corresponding change in lung function. This preliminary finding suggests that improvements in SM structure and function may be responsible for improved exercise capacity in trained patients with COPD.

The data in this dissertation therefore suggest that (i) SM structural and functional abnormalities

exist in patients with COPD (ii) these abnormalities may play a role in the exercise intolerance experienced by these patients, (iii) these abnormalities may be a useful predictor of exercise capacity in patients with COPD and (iv) that improvements in SM structure and function may account, at least in part, for the improved exercise tolerance experienced by patients with COPD.

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GLOSSARY OF ABBREVIATIONS

In the study of respiratory, muscle and exercise physiology frequent use is made of abbreviations. To simplify the text in this dissertation abbreviations are used where appropriate. Therefore, a glossary of abbreviations is provided.

ADP = Adenosine diphosphate

ATP = Adenosine triphosphate

ATS = American thoracic society

C = Celsius

C0= Carbon monoxide

$C0_2$ = Carbon dioxide

CS = Citrate synthase

FEV_1 = Forced expiratory volume in the first second of maximal forced expiration

FEV_1/FVC = FEV_1 as a % of the total forced expiratory volume

FEF_{50} = Expiratory flow at 50% of the total forced expiratory flow volume

FRC = Functional residual capacity

FVC = Forced vital capacity

HAD = 3-hydroxyacyl-CoA dehyrdogenase

He = Helium

HK = Hexokinase

HR = Heart rate

J = Joules

Kg = kilogram

kPa = Kilopascals

kp.m = kiloponds per metre

K_{10} = Transfer coefficient

l = litres

LDH = Lactate dehydrogenase

LTV = Lean thigh volume

ml = Millilitres

MSVC = Maximum sustained ventilatory capacity

MVV = Maximum voluntary ventilation

NADH = β- nicotinamide adenine dinucleotide reduced

Nm = Newton metres

$PaC0_2$ = Partial pressure of carbon dioxide in arterial/arterialised blood

$Pa0_2$ = Partial pressure of oxygen in arterial/arterialised blood

PAS = Periodic acid shiff's glycogen

PCr = Phosphocreatine

PEFR = Peak expiratory flow rate

PFK = Phosphofructokinase

Pi = Inorganic phosphate

PIFR = Peak inspiratory flow rate

PK = Pyruvate kinase

RER = Respiratory exchange ratio

rpm = Revolutions per minute

RV = Residual volume

$Sa0_2$ = Saturation of oxygen in arterial blood

SD = Standard deviation

SDH = Succinate Dehydrogenase

SMWT = Six minute walk test

TDP = Transdiaphragmatic pressure

μm = Micrometres

VC = Vital capacity

$VC0_2$ = Output of carbon dioxide during breathing

VD = Physiological deadspace

V_E = Minute ventilation

$V0_2$ = Oxygen uptake during breathing

VT = Tidal volume

VT/VC = Tidal volume/Vital capacity

W = Watts

WOB = Work of breathing

CHAPTER ONE

A REVIEW OF THE LITERATURE

1. Introduction

(a) Definition of COPD

COPD is the medical term applied to a disease that is generally a result of emphysema, chronic bronchitis or a mixture of these two pathophysiological processes (West 1992). Emphysema is a result of enlarged air spaces distal to the terminal bronchioles and the destruction of their walls. Chronic bronchitis is a result of excessive mucus production and inflammation in the bronchial tree with resultant narrowing of the airways (Guyton 1976; Wiles et al., 1984; West 1992). It is often difficult to determine the extent to which patients with COPD suffer from either emphysema or chronic bronchitis because a combination of the two pathophysiological processes is common (Guyton 1976; Anderson 1980; Wiles et al., 1984; Belman 1990; West 1992; Weaver et al.,1997). COPD is therefore characterized by gradual, largely irreversible chronic airflow limitation, a chronic cough, overinflated lungs and impaired gas exchange. Despite ample documentation of the pathophysiology of COPD, (Guyton 1976; Anderson 1980; Wiles et al., 1984; Belman 1990; West 1992; Weaver et al., 1997), the epidemiology of the disease is not well understood (MacNee 1997).

(b) Incidence of COPD

In the USA COPD is reported to be the fourth highest cause of death, similarly in the European Union as the third highest cause of mortality (Siafakis 1995; Weaver et al., 1997). In South Africa, the incidence of COPD is rapidly rising as demonstrated by the Guardian National Insurance Company statistics (1996) showing that COPD is the third highest cause of disability in South Africa. Additionally, COPD is the only one of the top five causes of disability in South Africa that is increasing (Guardian National Insurance Company Statistics 1996). In the Medical Research Council Technical Statistical report for the period 1985-1990, COPD accounted for 4% of total deaths in South Africa and for the first time exceeded deaths from acute respiratory incidents. In addition, cigarette smoking is cited as a major cause of chronic lung disease (McNee 1997; O'Donnel 1997) and whilst the incidence of people smoking cigarettes is decreasing in the USA and Europe, it is rapidly increasing in most African countries, including South Africa (Tobacco Action Group statistics 1996).

(c) Treatment of COPD

COPD is regarded as a largely irreversible disease. However, treatment of patients suffering from COPD focuses primarily on improvements in, or maintenance of, functional capacity. This is an attempt to allow continued participation in activities of daily living by these patients (Weaver et al., 1997; MacNee 1997).

(d) Functional capacity in patients with COPD

Functional capacity is often clinically measured by exercise tolerance, particularly in patients with chronic disease (Wiles et al., 1984). Patients with COPD demonstrate marked exercise intolerance compared to healthy individuals (Gallagher 1990; Carter et al., 1991; Belman 1992, Cooper et al., 1995; Clark et al., 1996) and typically present with exaggerated breathlessness, muscle wasting and often malnutrition (Karlsson et al., 1992).

Much research in the last three decades has focused on the possible factors causing exercise limitation in patients with COPD. Because COPD is primarily a disease of the lungs, in the period between the 1960's and the 1980's most of the research examining exercise intolerance in patients with COPD focused on central factors relating directly to the lungs and heart, such as ventilation mechanics, impaired gaseous exchange and cardiovascular function. However during the 1990's the concept that peripheral factors may contribute to exercise tolerance in patients with COPD has emerged.

Therefore, for the sake of simplicity this review of the literature examining possible causes of exercise intolerance in patients with COPD will be discussed under the following sections; Ventilation mechanics as a limiting factor during exercise in patients with COPD, impaired gas exchange as a limiting factor during exercise in patients with COPD, peripheral skeletal muscle as a limiting factor during exercise in patients with COPD and other non respiratory variables that may play a role in exercise intolerance in patients with COPD. Lastly, a section discussing factors predicting exercise capacity in patients with COPD will be reviewed.

2. Ventilation Mechanics as a Limiting Factor During Exercise in Patients with COPD

(a) Dynamic hyperinflation

The inflammation and mucus obstruction typically found within the respiratory bronchioles of patients with chronic bronchitis causes narrowing of the airways. Similarly, alveolar wall destruction, typically seen in patients with emphysema, results in a loss of elastic tissue in the alveoli leading to a loss of radial traction which causes narrowing of the respiratory airways. Therefore expiration becomes slower and more difficult in patients with COPD compared to normal healthy individuals (Gallagher 1990; West 1992). The extent of this obstruction to expiration is traditionally measured by the FEV_1 (Guyton 1976; Wiles et al., 1984; West 1992), which is the amount of air expired in the first second of a maximal expiratory breath. In a healthy individual this would be 80% of the maximal forced vital capacity. As expiration is slower in patients with COPD, the FEV_1 may be markedly reduced to 10-60% of a maximal expiratory breath (West 1992) and therefore less air is expired. This results in an increased residual lung volume (Anderson 1980; Belman 1992). This excess residual volume "inflates" the lungs and is termed "dynamic hyperinflation" (Gallagher 1990; Wiles et al 1984; Anderson 1980). The more severe the reduction in FEV_1, the more likely the residual volume is to approach the TLC (De Troyer et al., 1995).

This dynamic hyperinflation facilitates **expiratory** airflow in patients with COPD by two mechanisms. Firstly, a positive relationship exists between lung volume and expiratory air flow such that the higher the lung volume, the faster the expiratory airflow due to the elastic recoil of the lungs, this is termed the "expiratory flow volume curve" (West 1995). Therefore, dynamic hyperinflation allows expiration to function along a higher portion of the expiratory flow volume curve, which increases the speed of expiratory airflow (Belman 1993). The second mechanism by which dynamic hyperinflation aids expiratory air flow is by increasing muscular force during expiration. In healthy individuals, the internal intercostal muscles act as expiratory muscles whilst the external intercostals act as inspiratory muscles (West 1995). In patients with COPD, dynamic hyperinflation shifts the external intercostal muscles to a mechanical position that results in their behaving as an expiratory muscle. In studies on dogs, De Troyer et al., (1995) reported that as RV approaches TLC both the internal and the external intercostal muscles act as expiratory muscles, aiding expiration with increased muscular force.

However, whilst aiding expiration, dynamic hyperinflation hinders inspiration (Derenne et al., 1978). Firstly, hyperinflation shifts the ribs from the normal oblique position to a more horizontal position, making it difficult for the inspiratory intercostal muscles to lift the ribs and expand the rib cage, movements necessary for inspiration (Belman 1993). Secondly, in healthy individuals, the diaphragm functions as the major inspiratory muscle. In order to generate transdiaphragmatic pressure and aid inspiration the diaphragm needs to be in a curved position with an upward convexity (West 1992). However, in patients with COPD, during dynamic hyperinflation the diaphragm is moved to a shortened position with a flattened shape. In this shortened, flattened position, the diaphragm acts as an expiratory muscle rather than an inspiratory muscle (Tobin 1988). Accordingly, the contribution of muscular force to inspiration is reduced. In addition, inspiration becomes more difficult as it occurs on a less compliant portion of the pressure volume curve with a resultant decrease in the elastic recoil by the lungs and increased WOB (Belman 1992). As a result of this impaired ability to inspire, the 0_2 cost of breathing increases. Healthy subjects have an 0_2 cost of breathing of 2.5ml/min at rest, this increases to 30ml/min in patients with COPD. This translates to 15% vs 1-2% of total body 0_2 consumption relative to healthy control subjects (Tobin 1988).

Thus dynamic hyperinflation aids expiration but hinders inspiration in these patients. It has been hypothesized that this impaired inspiration may play a role in exercise intolerance in patients with COPD (Belman 1985; O'Donnell et al., 1988; Gallagher 1990).

(b) Abnormal breathing patterns in patients with COPD

As these patients experience slow and difficult expiration, inspiration often begins before FRC is reached. As a result, during inspiration the respiratory muscles are forced to generate additional pressure to overcome the elastic recoil of the lungs that occurs during expiration (Belman 1992). This further increases the inspiratory WOB. In addition, patients with COPD reportedly recruit their respiratory muscles in an inefficient manner during ventilation, further increasing the work of breathing (WOB) (Derenne et al., 1978).

An abnormal breathing pattern of increased VT/VC is present at rest and is exacerbated during exercise in patients with COPD and may therefore contribute to exercise intolerance (Marin et al., 1993). It would appear that those patients with the greatest impairment of resting lung mechanics showed the greatest abnormalities in breathing patterns at rest and during exercise (Marin et al., 1993).

Therefore, both dynamic hyperinflation and abnormal breathing patterns result in an increased WOB and an increased ventilatory demand which may limit exercise capacity in patients with COPD.

(c) Measurement of ventilatory demand during exercise in patients with COPD

To examine the hypothesis that increased ventilatory demand may limit exercise tolerance in patients with COPD, researchers have made use of comparisons between an MVV achieved at rest and the maximum V_E achieved during exercise in these patients. The MVV is a measure of the maximum ventilation in subjects when asked to breathe maximally for 12 seconds. The result of this test is then extrapolated to one minute to calculate a rate in l/min. The maximum V_E achieved during exercise is then compared to the MVV to assess whether the maximum ventilatory capacity was reached during exercise. Many authors have reported that V_E during exercise approaches or exceeds an MVV measured at rest in some patients with COPD (Clark et al., 1969; Spiro et al., 1977; Mahler et al., 1988; Mathews et al., 1989). In contrast, in a more recent study, Marin et al., (1993) reported an average V_E/MVV of 76% in 29 patients with COPD. This finding implies that a factor other than ventilatory mechanics may limit exercise tolerance in these patients.

(d) The relationship between changes in ventilatory mechanics and changes in exercise tolerance after exercise training in patients with COPD

An important consideration when reviewing the role of ventilatory mechanics in exercise intolerance in patients with COPD are exercise training studies. Patients with COPD demonstrate an improved functional capacity after exercise training (McGavin et al., 1977; Belman 1982; Gallagher 1990). If ventilation mechanics were limiting exercise ability in these patients, then an improvement in ventilation mechanics would be expected after exercise training to explain the

improved functional ability. However, many authors have shown improved exercise performance in patients with COPD after training, without changes in dynamic hyperinflation, breathing patterns or V_E/MVV ratio (McGavin et al., 1977; O'Donnell et al.,1993, Olopade et al., 1992; Carter et al.,1991). This finding suggests that factors other than ventilatory mechanics are responsible for the improved exercise tolerance reported in patients with COPD after exercise training and therefore that ventilatory mechanics are unlikely to have been the sole cause of exercise intolerance prior to training.

(e) The role of dyspnea in exercise intolerance in patients with COPD

The increased muscle effort required for ventilation during exercise in patients with COPD, as discussed previously, results in a parallel increase in the sensation of dyspnea (El-Manshawi et al., 1986; Leblanc et al., 1986; McParland et al., 1986). Some authors argue that the increased sensory perception of dyspnea and the anxiety and distress accompanying dyspnea, cause early termination of exercise in patients with COPD (Jones 1984; Kilian et al., 1986; Woodcock et al., 1982; Olopade et al., 1992). Woodcock et al., (1982) tested this theory by administering a mild central nervous system depressant (dihydrocodeine) to patients with COPD suffering from dyspnea. Dihyrdocodeine would decrease the stress and anxiety that accompany the sensation of dyspnea. Woodcock et al., (1982) reported decreased exertional dyspnea and increased exercise tolerance in patients after ingesting dihydrocodeine. This finding suggests that the anxiety and stress accompanying the sensation of dyspnea contribute to exercise intolerance in patients with COPD. This theory is further supported by studies demonstrating decreased dyspnea and subsequent increased exercise tolerance after exercise training in patients with COPD (McGavin et al., 1977; O'Donnel et al., 1993). This finding suggests that these patients may experience decreased anxiety due to familiarization with the sensation of dyspnea during exercise training and thereby improve their exercise capacity (O'Donnel et al., 1993).

In contrast, Carrieri-Kohlman et al., (1996) recently demonstrated that it was not the stress and anxiety accompanying dyspnea that prompted early exercise termination in 54 patients with COPD, but the sensation of dyspnea itself. Subjects were asked to differentiate between four components of dyspnea during exercise. The four components included : shortness of breath,

perceived work of breathing, distress associated with dyspnea and anxiety associated with dyspnea. The authors assumed that both work of breathing and shortness of breath were a reflection of the sensation of dyspnea itself. The distress and anxiety associated with the dyspnea were regarded as the affective components that accompany dyspnea in the patients. The 54 patients with COPD rated work of breathing and shortness of breath significantly higher compared to the affective components of dyspnea during exercise. Therefore, the authors suggest that (i) patients with COPD are able to distinguish between the physical sensation of dyspnea and the affective components of dyspnea and (ii) that the physical sensation of dyspnea and not it's affective components limit exercise tolerance.

The role of dyspnea in exercise intolerance in patients with COPD is therefore unclear. Furthermore, the psychological components that accompany the sensation of dyspnea make it difficult to effectively investigate dyspnea in a well controlled trial.

(f) The role of the respiratory muscles in exercise intolerance in patients with COPD

(i) Respiratory muscle weakness and fatigue in patients with COPD

Conflicting evidence is presented in the literature concerning the role that respiratory muscle weakness and fatigue may play in limiting exercise tolerance in patients with COPD. As explained previously, it is the inspiratory muscles of these patients that are overworked and therefore prone to fatigue (Derenne et al., 1978; Dodd et al., 1983). The inspiratory muscles in humans are the external intercostal muscles and the diaphragm (West 1985). The diaphragm is the most important inspiratory muscle in the body (West 1985). In a healthy individual the diaphragm is stronger and more resistant to fatigue than other skeletal muscles (Derenne et al., 1978). However, as explained in the section on dynamic hyperinflation, in patients with COPD the diaphragm is moved to a shortened, flattened position that is mechanically disadvantageous (Rochester 1991; Tobin 1988). This decreased mechanical efficiency may lead to functional weakness and an increased susceptibility to fatigue during exercise (Rochester 1991).

Various authors argue that respiratory muscle fatigue may limit exercise in patients with COPD (Pardy et al., 1980; Bellemare et al., 1983; Dodd et al., 1983; Polkey et al., 1995). In contrast,

Gallagher (1986) argues that respiratory muscle fatigue does not occur during exercise in these patients. Table 1.1 presents a summary of the literature reviewing inspiratory muscle fatigue during exercise in patients with COPD.

Table 1.1- Summary of studies examining inspiratory muscle fatigue during exercise in patients with COPD

Authors	Sample size	Diaphragm fatigue	Inspiratory intercostal fatigue
Pardy et al.,1980	n= 12	n=6	n=6 (same 6 as for DF)
Bellamare et al., 1983	n= 20	n=20	NE
Dodd et al., 1983	n=7	n=4	n=7
Gallagher 1986	n=7	n=0	n=0
Polkey et al., 1995	n=6	n=0	n=6

Abbreviations: NE, not examined; DF, diaphragm fatigue; n, sample size

Pardy et al., (1981) demonstrated diaphragm fatigue and external intercostal muscle fatigue in six of 12 patients with COPD using EMG analysis during an incremental cycle test to exhaustion. Similarly, Bellemare et al., (1983) demonstrated diaphragm fatigue using EMG analysis in 20 patients with COPD during maximum breathing. As mentioned previously, the diaphragm functions by creating transdiaphragmatic pressure (TDP) to assist inspiration. Therefore, the amount of TDP generated is used to assess the effectiveness of the diaphragm during inspiration. If the TDP generated during exercise is close to the maximum TDP possible, then the diaphragm would be functioning close to its maximum ability for a prolonged period of time which is likely to result in fatigue (Bellemare, 1983). As a result of this fatigued diaphragm, early exercise termination is possible. Bellamare (1983) demonstrated a TDP close to maximum TDP during maximal breathing in the 20 patients with COPD and therefore concluded that diaphragm fatigue is likely to cause exercise intolerance in these patients.

Dodd et al., (1983) reported that the diaphragm and the inspiratory intercostal muscles in patients with COPD who demonstrated exercise intolerance generated a higher proportion of peak inspiratory pleural pressure and peak TDP compared to those patients who were able to exercise longer. Dodd et al., (1983) concluded that the inspiratory muscle are required to function close to their maximum during exercise in patients with COPD and therefore inspiratory muscle fatigue is likely to limit exercise tolerance.

As previously mentioned, various authors have concluded that if patients with COPD produce a TDP close to their maximum during exercise, then the inspiratory muscles are fatigued and exercise is likely to be terminated early. If this were the case then a drop off in TDP at end exercise would be expected. However, Polkey et al., (1995) reported that six patients with COPD failed to demonstrate reduced TDP at termination of exhaustive exercise. The same six patients did however show a decline in maximal relaxation rate in the inspiratory intercostal muscles at the termination of exhaustive exercise. This finding would suggest that the inspiratory intercostal muscles fatigue during exercise in patients with COPD. The authors therefore suggest that the inspiratory muscle fatigue during exercise in patients with COPD results in dyspnea, which in turn causes early exercise termination prior to diaphragm fatigue. The authors suggest that this is a protective mechanism to preserve the diaphragm in patients with COPD.

In contrast to the studies previously discussed, Gallagher (1983) argued that neither the inspiratory intercostal muscles nor the diaphragm fatigue during exercise in patients with COPD and therefore do not play a role in their exercise intolerance. Gallagher (1983) argued that inspiratory muscle fatigue is known to cause rapid shallow breathing and as he failed to demonstrate rapid shallow breathing in seven patients with COPD immediately after maximal exercise, he concluded that respiratory muscle fatigue did not cause early exercise termination in these patients.

Therefore, the role of respiratory muscle fatigue in exercise intolerance in patients with COPD is unclear although it does appear that functional abnormalities exist in the respiratory muscles of patients with COPD.

(ii) The effect of inspiratory muscle training on exercise tolerance in patients with COPD

Various authors have demonstrated that exercise tolerance improves in patients with COPD after participation in an inspiratory muscle training programme (Belman et al., 1980; Pardy et al., 1980; Wanke et al., 1994).

Belman et al., (1980) demonstrated improvements in maximum sustained ventilatory capacity

(MSVC), in ten patients with COPD after inspiratory muscle training. Oxygen consumption during the MSVC, arm and leg ergometer submaximal exercise time and maximum distance walked in a 12 minute walk test all improved in ten patients with COPD after six weeks of inspiratory muscle training. Additionally, Pardy et al., (1980) demonstrated improvements in peak $V0_2$ during cycle ergometry, peak endurance time during cycle ergometry and distance walked in six of 12 patients with COPD after an eight week inspiratory muscle training programme. Upon entry to the study, the extent of inspiratory muscle fatigue during exercise was measured in the patients using EMG analysis. The six patients who initially demonstrated inspiratory muscle fatigue showed improved exercise tolerance after inspiratory muscle training. In contrast, the six patients who did not initially demonstrate inspiratory muscle fatigue during exercise did not benefit from inspiratory muscle training. Similarly, Wanke et al., (1994) reported improved maximum power output and 0_2 uptake in 42 patients with COPD following an eight week cycle ergometer training programme. However, in the 21 patients in this group who included inspiratory muscle training in addition to the cycle ergometer training in their rehabilitation programmes, the improvements in maximal power output and 0_2 uptake were, on average, significantly greater.

Thus it would appear that in some patients with COPD, inspiratory muscle fatigue may play a role in exercise intolerance. Accordingly, training of the inspiratory muscles may therefore improve exercise tolerance in those patients with impaired inspiratory muscle mechanics.

(iii) Histology of respiratory muscle in patients with COPD

The studies discussed previously suggest that functional abnormalities exist in the respiratory skeletal muscle of patients with COPD. Investigation into the structure or histology of the respiratory skeletal muscles in patients with COPD is therefore warranted.

Patients with COPD demonstrate an altered respiratory muscle fibre composition, respiratory pattern/function and neuromotor innervation compared to healthy subjects (Mizuno 1991). Mizuno (1991) noted a decreased fibre size in both Type I and Type II fibres in the thoracic respiratory muscles in patients with COPD. The author was uncertain as to whether the decreased fibre size was due to overuse as a result of increased ventilatory WOB, or disuse due to an increased

involvement of the extra thoracic muscles. Mizuno (1991) also reported a reduced percentage of slow twitch fibres (49%) in the inspiratory and expiratory intercostal muscles in some patients with COPD, whilst in other patients the percentage of slow twitch fibres was the same as in normal human respiratory muscle (62%).

Campbell et al.,(1980) biopsied the internal intercostal muscles, the external intercostal muscles and the latissimus dorsi muscles of 22 patients suspected of pulmonary neoplasm and suffering from clinical airways obstruction (COPD). Morphological changes were observed in the respiratory muscles of 17 of the 22 patients. The changes included: fibre splitting, variation in fibre size, fibre atrophy and centralized nuclei. None of these changes were evident in the latissimus dorsi muscle. A greater percentage of Type II fibres was reported in the intercostal muscles compared to the latissimus dorsi muscle in the patients with COPD. A similar distribution of Types I and II fibres was found in both intercostal muscles whilst atrophy was only evident in the Type II (fast twitch) fibres. The expiratory intercostal muscles demonstrated greater atrophy of Type II fibres than the inspiratory intercostal muscles in the patients with COPD. In addition, the authors reported a relationship between FEV_1/FVC and extent of Type II atrophy in the expiratory intercostal muscles ($r=0.78$, $p<0.001$). No hypertrophy was found in any of the biopsies. Biochemical analysis of the skeletal muscle biopsies revealed decreased concentrations of ATP and PCr in both the inspiratory and the expiratory intercostal muscles and in the lattisimus dorsi muscles. Thus, morphological and biochemical abnormalities were clearly evident in the respiratory muscles of the patients.

In the opinion of Campbell et al., (1980), the morphological changes seen in the respiratory muscles of the patients were specific to the respiratory muscles and not a general effect in the skeletal muscle of the body. The author argued this conclusion as only one of the eight latissimus dorsi muscle biopsies showed morphological abnormalities in the patients with COPD. Additionally, Campbell et al., (1980) concluded that the morphological changes seen in the respiratory muscles of the patients were due to disuse rather than overuse for two reasons. Firstly, the expiratory intercostals had greater morphological abnormalities than the inspiratory intercostals, whilst, as previously discussed the inspiratory muscles in patients with COPD are

overused to a greater extent than the expiratory muscles. Therefore, if overuse was the cause of the morphological abnormalities seen in the respiratory muscles of the patients with COPD, a greater abnormality would have been expected in the inspiratory intercostal muscles which was not the case. Secondly, there was an absence of fibre hypertrophy, which is likely to be found in muscle subjected to overuse.

In contrast to the morphological abnormalities, the authors suggested that the biochemical changes measured in the respiratory muscle of the patients with COPD were a more generalized abnormality, as these same changes have been reported by two other authors in the quadriceps muscles in patients with COPD (Bergstrom et al., 1976, Gertz et al., 1977). Unfortunately, Bergstrom et al., (1976) and Gertz et al., (1977) did not examine the morphological status of the quadriceps muscles in the patients with COPD.

In summary, Campbell et al., (1980) reported biochemical and morphological abnormalities in the respiratory muscles of patients with COPD, which in their opinion were due to disuse of these respiratory muscles. They concluded that the morphological abnormalities were specific to the respiratory muscles whilst the biochemical abnormalities reported may have been a more general abnormality in the skeletal muscle of these patients.

In contrast to Campbell et al., (1980) who reported a greater percentage of Type II fibres in the respiratory muscles of patients with COPD, Karlsson et al., (1992) reported a higher than normal percentage of Type I (slow twitch) fibres in the respiratory muscles of patients with COPD. However, these results need to be interpreted with caution as (i) only four patients underwent skeletal muscle biopsy and (ii) to negate a genetic effect, the relative predominance of fibre types in the respiratory muscles were calculated by dividing the percentage of fibre types in the respiratory muscles relative to the percentage fibre types in the same subject's vastus lateralis muscle. This would effectively negate a generalized fibre type shifting which might be the case in patients with COPD. In fact, when the results are scrutinized, the percentage fast twitch fibres in the internal and external intercostal muscles of the four patients biopsied averages 52%, which is within the "normal" range for fast twitch percentage in normal adult males (Dubowitz 1985). The

percentage fast twitch fibres in the vastus lateralis muscles of the patients was not presented.

Lastly, a recent case report demonstrated histological abnormalities in the mitochondria of the diaphragm muscle, yet no abnormalities in the latissimus dorsi and intercostal muscles of a patient with COPD (Lloreta et al., 1996a) were found. The histological abnormalities described in the diaphragm of the patients with COPD included large aggregates of mitochondria with mitochondrial paracrystalline rectangular inclusions. In contrast, the latissimus dorsi and intercostal muscles showed only a moderately increased number of mitochondria and no mitochondrial paracrystalline rectangular inclusions. This is the first known report of paracrystalline deposits in the respiratory muscles of a patient with COPD. As these paracrystalline deposits are more commonly found in the myopathy of hyperthyroidism (Lloreta et al., 1996), the authors concluded that selective overuse of the diaphragm may be responsible for these mitochondrial changes. It is interesting that mitochondrial paracrystalline deposits have been reported in the vastus lateralis muscle of patients with end stage renal failure (Diesel et al., 1993).

In summary, it would appear that functional, biochemical and structural abnormalities exist in the respiratory muscles of patients with COPD. It is therefore possible that these abnormalities may contribute to exercise intolerance in these patients.

(iv) Enzyme activity in the respiratory muscles of patients with COPD

As mentioned, biochemical abnormalities have been reported in the respiratory muscles of patients with COPD. Similarly, Sanchez et al., (1988) measured activities of the glycolytic enzyme lactate dehydrogenase (LDH), and the oxidative enzymes; hexokinase (HK), citrate synthase (CS) and 3-hydroxyacyl –CoA dehydrogenase (HAD) in the expiratory and inspiratory intercostal muscles, the serratus anterior muscle (an accessory inspiratory muscle) and the latissimus dorsi (a non-respiratory muscle), in nine subjects with normal lung function and 11 patients with moderate COPD.

The metabolic activities of HK, CS and HAD were higher in the internal and external intercostal muscles when compared with the latissimus dorsi muscle in the patients with COPD. Additionally,

the metabolic activities of HK, CS and HAD in the internal and external intercostal muscles of the patients was significantly higher compared to the control group. No differences were found in activity levels of LDH (an enzyme necessary for energy production using the non-oxidative pathway in skeletal muscle) in the patients compared to the control group. These metabolic changes are normally seen in skeletal muscle that has undergone endurance training (Jones 1990). Therefore, the authors concluded that because of diaphragm fatigue the intercostal muscles were subjected to overuse and consequently demonstrated the metabolic effects of endurance training. Accordingly, this finding suggests that the intercostal muscles are unlikely to cause early exercise termination in patients with COPD. However, the author's suggest that the diaphragm does fatigue during exercise in these patients.

In summary, the respiratory muscles of patients with COPD demonstrate functional, structural, biochemical and metabolic abnormalities and these abnormalities may play a role in exercise intolerance. Furthermore, although various hypotheses have been suggested, the mechanism of these abnormalities in the respiratory muscle of patients with COPD is not known.

3. Impaired Gas Exchange as a Limiting Factor During Exercise in Patients With COPD

As discussed earlier, patients diagnosed with COPD may have a larger degree of either bronchial obstruction and inflammation (chronic bronchitis) or alveoli destruction (emphysema), depending on the primary component of their disease. Those patients with a large degree of alveolar destruction suffer greater impaired gaseous exchange than those with bronchiolar obstruction and inflammation.

Patients with emphysema have an increased physiological dead space due to the destruction of the alveoli (West 1992). In a healthy individual, during exercise, physiological dead space decreases relative to the increasing tidal volume (VD/VT) to compensate for the increased ventilatory demand (West 1992). However, in patients with COPD, the VD/VT ratio increases during exercise due to increased physiological dead space. This results in an exacerbation of ventilation/perfusion mismatch (Olopade et al., 1992). An increased ventilation/perfusion mismatch results in less blood being effectively oxygenated with resultant systemic hypoxaemia (low $Pa0_2$)

(West 1992). Whilst some patients with COPD respond to the hypoxaemia by increasing V_E, others do not (Jones 1988). This systemic hypoxaemia may limit exercise tolerance in two ways. Firstly, in the patients who respond to the hypoxaemia by increasing V_E, the resultant increased ventilatory demand and the mechanical factors limiting their ability to respond to this increased ventilatory demand (as previously discussed in the section on ventilation) may limit exercise capacity (Belman 1993). Secondly, the subsequent rise in $PaC0_2$ as a result of hypoventilation may limit exercise tolerance (Jones 1988).

However, blood gas concentrations vary between patients with COPD. Patients may be hypoxaemic at rest, hypoxaemic during exercise or maintain normal levels of $Pa0_2$ at all times (West 1992). Some patients display transient oxygen desaturation during daily living activities such as walking, washing and eating even in the absence of resting hypoxaemia (Soguel Schenkel et al.,1996). This variable hypoxaemic state results in conflicting literature when considering exercise intolerance in patients with COPD.

If hypoxaemia was the factor limiting exercise capacity in patients with COPD, then oxygen supplementation should improve exercise capacity in these patients. Palange et al., (1995) demonstrated decreased V_E and $V0_2$ at the same submaximal work load during cycling in nine patients with COPD whilst taking supplemental oxygen. Similarly, Woodcock et al., (1981) demonstrated significant improvement in walking distance in only six of ten patients who used supplemental oxygen during a walking test. These finding suggest that only some patients with COPD demonstrate improved exercise tolerance when using supplemental oxygen and that impaired gas exchange was initially limiting exercise capacity in some patients with COPD. However, not all the patients demonstrated improved exercise tolerance with supplemental oxygen. Thus factors other than hypoxaemia may have been responsible for early exercise termination in other patients with COPD.

Accordingly, other authors argue against hypoxaemia as a major limiting factor during exercise in patients with COPD. Chester et al., (1970); Nicholas et al., (1977) and Patessio et al., (1992) demonstrated that although exercise tolerance in patients with COPD improved with exercise

training, no parallel improvements in either resting or exercising levels of $Pa0_2$ were evident. Therefore, it is unlikely that impaired gas exchange was responsible for the exercise intolerance in these patients with COPD before training. In addition, Spiro et al., (1975) reported that changes in blood gas tensions in a group of patients with moderate COPD were small (less than 2mmHg) and in the authors opinion unlikely to have contributed to exercise intolerance in these patients. Spiro et al., (1975) did however report a significant decrease in blood gas tensions during exercise in a group of patients with severe COPD.

In summary, it seems unlikely that hypoxaemia is the major limiting factor during exercise in patients with COPD not in chronic respiratory failure. However, in patients suffering from severe emphysema, chronic respiratory failure and consequent severe hypoxaemia at rest ($Pa0_2$ < 60mmhg), exercise may be limited by insufficient gas exchange.

3. The Role of Skeletal Muscle in Exercise Intolerance in Patients with COPD

Most research investigating exercise tolerance in patients with COPD has focused on central factors, including cardiorespiratory factors. Far less research has investigated the role of skeletal muscular factors in exercise intolerance in these patients.

(a) Skeletal muscle strength and endurance in patients with COPD

Skeletal muscle weakness may contribute to exercise intolerance in patients suffering from other chronic diseases. Patients suffering from renal failure (Kempeneers et al., 1990; Diesel et al., 1990) and patients in cardiac failure (Derman et al., 1993), demonstrate a skeletal muscle weakness which contributes to exercise intolerance. In end stage renal dialysis patients, Diesel et al., (1990) reported peak isokinetic muscle strength to be a much better predictor of exercise capacity than variables determining blood oxygen carrying capacity. Similarly, Lipkin et al., (1988) found a significant correlation between isokinetic muscle power and peak oxygen consumption in patients with congestive heart failure. It therefore appears that skeletal muscle function may be an important component of exercise intolerance in patients with chronic diseases. Additionally, a significant number of patients with COPD terminate exercise because of muscular fatigue (Kilian et al., 1992). Accordingly, in the 1990's, the hypothesis that skeletal muscle may contribute to

exercise intolerance in patients with COPD is being researched.

Most of the central variables discussed previously, including ventilation mechanics and gas exchange, do not improve in patients with COPD after exercise training. This is despite improved exercise tolerance in these patients. In contrast, exercise training studies in patients with COPD demonstrate improvement in skeletal muscle strength and improved exercise tolerance (Simpson et al., 1992; Clark et al., 1996). Clark et al., (1996) reported improved whole body endurance, measured by treadmill walking time, in 32 patients with COPD after 12 weeks of low intensity skeletal muscle conditioning. Additionally, various measures of upper and lower body skeletal muscle isotonic endurance in the 32 patients with COPD improved significantly after training. The same group of patients demonstrated no improvements in maximum $V0_2$ or in peak isokinetic quadriceps strength after exercise training, but did show lower $V0_2$ and ventilatory requirements at the same submaximal work load during progressive cycle ergometry. The findings of this study suggest that isotonic skeletal muscle endurance contributes to exercise capacity in patients with COPD and improves with training.

Simpson et al., (1992) reported similar results in 14 patients with COPD who underwent a controlled weightlifting training programme for eight weeks. They demonstrated improvements of isotonic and isometric skeletal muscle strength, exercise endurance and subjective responses to some of the demands of daily living compared to an untrained control group (n=14). Improvements in isotonic strength were measured as a 1 repetition max (1RM). Improvements of 33% in arm curl 1RM, 44% in leg extension 1RM and 16% in leg press 1RM ($p<0.01$ for all) in the trained patients were reported. The control group showed no change in isotonic muscle strength after an eight week period. Similarly the mean isometric force in the quadriceps muscle of the patients with COPD who participated in the exercise training programme improved by 18%, whilst the control group showed no improvement. Exercise endurance was measured by a submaximal cycle ergometry test at 80% of maximal power output until self termination. The trained group of patients demonstrated a 73% improvement in endurance time during this submaximal exercise test (518 seconds vs 898 seconds; $p<0.01$) whilst the control group showed no change in exercise endurance time. Peak $V0_2$ recorded during an incremental cycle test to exhaustion and distance

walked during the SMWT did not improve in either the trained patients or the control group. The results of this study suggest that isotonic and isometric skeletal muscle strength may contribute to submaximal exercise endurance in patients with COPD.

A third study investigating skeletal muscle weakness in patients with COPD was conducted by Gosselink et al., (1996) who measured skeletal muscle isometric strength (peak quadriceps force and peak hand grip force) and exercise capacity (peak $V0_2$ achieved during an incremental cycle test to exhaustion and distance walked during a SMWT) in 41 patients with COPD. Peak isometric quadriceps strength (QF) and peak isometric hand grip strength (HF) correlated to distance walked during the SMWT ($r=0.63$, $p<0.05$; $r=0.61$, $p<0.05$; QF;HF) and to peak $V0_2$ achieved during the incremental cycle test ($r=0.55$, $p<0.05$; $r=0.53$, $p<0.05$; QF;HF) in the patients with COPD. Additional correlations were found between peak $V0_2$ achieved during the incremental cycle test and TLCOsb ($r=0.68$;$p<0.05$). Significant, but lower correlations were found between TLCOsb and distance walked during the SMWT ($r=0.38$; $p<0.05$) and between inspiratory muscle strength (PI max) and distance walked during the SMWT ($r=0.49$; $p<0.05$). The findings of this study suggest that isometric skeletal muscle strength contributes significantly to exercise tolerance in patients with COPD.

In summary, it would appear that isometric and isotonic skeletal muscle strength as well as isotonic skeletal muscle endurance may all contribute to exercise intolerance in patients with COPD. In contrast, isokinetic muscle strength may not be an important contributing factor. The results of the above mentioned studies can be interpreted in a different way. The measures of isotonic skeletal muscle endurance improved in response to isotonic muscle endurance training (Clark et al., 1996) whilst in a second study isotonic and isometric skeletal muscle strength improved in response to isotonic and isometric skeletal muscle strength training (Simpson et al., 1992). In contrast isokinetic skeletal muscle peak strength did not improve in response to isotonic muscle endurance training (Clark et al., 1996). Thus it would appear that if the muscle testing conducted in the studies was specific to 1) the type of muscle training conducted (strength vs endurance) and 2) the method of muscle training (isotonic vs isokinetic vs isometric), improvements were noted in the functional ability of the skeletal muscle of the patients with COPD. To our knowledge, no study has

examined the role of isokinetic or isometric skeletal muscle endurance in determining exercise capacity in patients with COPD. Additionally, the mechanism causing this skeletal muscle weakness and fatigue is not known. Further research is therefore warranted.

(b) Skeletal muscle and health care utilization in patients with COPD

Decramer et al., (1997) studied factors predicting the frequency of health care utilization in 57 patients with COPD. Patients were divided into two groups: those admitted to hospital twice, or more than twice in the previous year and those that were not admitted at all. The only significant difference reported between the groups was isokinetic peak muscle strength (quadriceps force 63±20 vs 82 ±26 % of predicted respectively; $p< 0.05$) and, to a lesser degree, respiratory muscle force. No differences were found between the two groups for pulmonary function tests, serum electrolyte concentrations, blood gas concentrations, ASA score (a scoring system used to categorize patients with COPD in terms of severity of disease) or distance walked during the SMWT. Additionally a significant inverse relationship was found between the number of days spent in hospital and the peak quadriceps force ($r=-0.35$; $p<0.05$). The authors concluded that frequency of health care utilization was related to skeletal muscle weakness in patients with COPD. Thus it would seem that skeletal muscle weakness as measured by isokinetic peak quadriceps force may contribute, at least in part, to the health care utilization of patients with COPD.

(c) Skeletal muscle metabolism in patients with COPD

With the advent of nuclear magnetic resonance imaging it became possible to study the metabolism of skeletal muscle in patients with COPD. Abnormalities exist in oxidative skeletal muscle metabolism of patients with COPD (Jacobbsen et al., 1990; Tada et al., 1992; Wuyam et al., 1992;Thompson et al., 1992). These abnormalities will be discussed firstly at rest and secondly during exercise.

(i) Skeletal muscle metabolism at rest in patients with COPD

A summary of research papers analyzing skeletal muscle metabolism in patients with COPD at rest is presented in Table 1.2

Table 1.2 Summary of studies examining skeletal muscle metabolism in patients with COPD at rest

Authors	Sample size	Muscle Biopsied / NMR	ATP	PCr	Gly	Pi	pH	Pi/PCr
Gertz et al., 1977	n=12 (ARF)	Quadriceps Femoris	↓	↓				
Fiaccordi et al., 1987	n=10 (ARF)	Quadriceps Femoris	↓	↓				
Jacobbson et al., 1990	n=10 (NRF)	Quadriceps Femoris	↔	↔	↔			
Jacobbson et al., 1990	n=8 (CRF)	Quadriceps femoris	↓	↓	↓			
Tada et al., 1992	n=11 (NRF)	Flexor Digitorum Superficialis (NMR)				↔	↔	↔
Wuyam et al., 1992	n=8 (CRF)	Gastrocnemius (NMR)					↔	↔

Abbreviations: **ATP**, adenosine triphosphate; **PCr**, phosphocreatine; **Pi**, inorganic phosphate; **pH**, intracellular pH; **Pi/PCr**, inorganic phosphate:phosphocreatinine; **ARF**, acute respiratory failure; **CRF**, chronic respiratory failure; **NRF**, patients with COPD not in respiratory failure; ↓, significantly decreased relative to controls; ↑ , significantly increased relative to controls; ↔ no significant difference between patients with COPD and controls; **NMR**, Nuclear magnetic resonance (in these studies no biopsy was performed); **Gly**,Glycogen.

Gertz et al., (1977) reported reduced ATP and PCr concentrations in 12 patients with COPD admitted to hospital for acute respiratory failure compared to patients admitted to hospital for reasons other than respiratory disease. These finding suggest impaired skeletal muscle oxidative metabolism in patients with COPD who are in acute respiratory failure. In 1987 Fiaccadori et al., biopsied the quadriceps femoris muscle of ten hypoxic-hypercapnic (acute respiratory failure) patients with COPD. These patients demonstrated a 30-35% reduction in ATP and PCr concentrations and in the ATP/ADP ratio.

Similarly, Jacobbson et al., (1990) reported reduced ATP , PCr and glycogen concentrations in the quadriceps femoris muscles of eight patients with COPD in respiratory failure compared with ten patients with COPD not in respiratory failure. This finding suggests that the oxidative metabolism in the skeletal muscle of patients with COPD who are in acute respiratory failure is impaired. In contrast, those patients with COPD not in acute respiratory failure maintain normal skeletal muscle oxidative metabolism at rest. In support of this hypothesis, Tada et al., (1992) reported that resting concentrations of PCr and Pi in the calf muscles of 11 patients with chronic lung disease, not in respiratory failure, where not different from healthy controls. However, Wuyam et al., (1992) reported no significant difference in pH and no difference in the Pi/PCr ratio at rest in the calf muscles of eight patients with COPD who were in respiratory failure, compared to nine healthy control subjects.

In summary, it therefore appears that at rest, impaired oxidative skeletal muscle metabolism is present in the skeletal muscle of patients with COPD who are in acute respiratory failure. In contrast, the oxidative skeletal muscle metabolism at rest appears to be maintained in patients with COPD who are not in acute respiratory failure. It is interesting that Campbell et al., (1980) reported decreased ATP and PCr in the intercostal muscles of patients with COPD.

(ii) Skeletal muscle metabolism during exercise in patients with COPD

A summary of studies investigating skeletal muscle metabolism during exercise in patients with COPD is presented in Table 1.3

Table 1.3 Summary of studies examining skeletal muscle metabolism during exercise in patients with COPD.

		Oxidative Enzymes			Glycolytic Enzymes			
Authors	**Sample size**	**CS**	**HAD**	**HK**	**PFK**	**LDH**	**pH**	**Pi/PCr**
Tada et al., 1992	n=11(NRF)						↓	↑
Wuyam et al., 1992	n=8 (CRF)						↓	↑
Thompson et al., 1992	n=5 (CRF)						↓	↑
Maltais et al., 1997	n=9 (NRF)	↓	↓	↔	↔	↔		

Abbreviations: ATP, adenosine triphosphate; PCr, phosphocreatine; Pi , inorganic phosphate; pH, intracellular pH; Pi/PCr, inorganic phosphate:phosphocreatine ratio; ARF, acute respiratory failure; CRF, chronic respiratory failure; NRF, patients with COPD not in respiratory failure; ↓, significantly decreased relative to controls; ↑ , significantly increased relative to controls; ↔ no significant difference between patients with COPD and controls.

Metabolic abnormalities in the skeletal muscles of patients with COPD during exercise were reported by Tada et al., (1992). Eleven patients with COPD, who were not in respiratory failure, produced a higher Pi/PCr ratio in the third and fourth minute of four minutes of repeated isotonic muscular contractions of the forearm compared to control subjects. An elevated Pi/PCr ratio reflects a reduction in oxidative capacity of skeletal muscle (Tada et al., 1992). The authors measured blood flow to the area during the exercise and found no significant difference between the groups. Additionally, nutritional parameters assessed by means of anthropometry and serum chemistry were not different between groups. The results of this study therefore suggest that metabolic abnormalities, independent of nutritional status or blood flow to the working muscles, exist in the skeletal muscle of patients with COPD. Similar results were reported by Wuyam et al., (1992) who demonstrated an elevated Pi/PCr ratio during repeated contractions of the calf musculature and a slower resynthesis of PCr during recovery in eight patients with COPD who were in chronic respiratory failure compared to eight healthy control subjects. Additionally,

Thompson et al., (1992) investigated skeletal muscle metabolism during repeated contractions of the calf musculature in five patients with COPD who were in respiratory failure. They reported faster depletion of PCr and more rapid onset of intracellular acidosis compared to control subjects. However, no difference in PCr depletion and intracellular acidosis was reported between groups in recovery. The findings from these studies suggest that the oxidative ability of peripheral skeletal muscle during exercise in patients with COPD is impaired.

Further evidence of reduced skeletal muscle oxidative capacity in patients with COPD was provided by Maltais et al., (1996) who demonstrated significantly lower activity of the oxidative enzymes CS and HAD in the vastus lateralis muscle of nine patients with COPD compared to controls. In contrast, no differences were reported in activity levels of the glycolytic enzymes PFK, HK and LDH between groups. This finding implies that the oxidative metabolism in the skeletal muscles of patients with COPD is impaired, whilst the non oxidative, glycolytic metabolism remains intact.

Various mechanisms for impaired oxidative capacity in the skeletal muscles of patients with COPD have been suggested. These include: reduced arterial oxygen content, physical detraining (Tada et al., 1992) and hypoxaemia in patients in chronic respiratory failure (Wuyam et al., 1992; Thompson et al., 1993). However, at present, the mechanism causing the peripheral skeletal muscle abnormalities in patients with COPD is unknown.

In summary, therefore, a review of the available literature suggests impaired oxidative metabolism in the skeletal muscles of patients with COPD during exercise. In conflict to this, as discussed earlier, the intercostal muscles in patients with COPD demonstrate an increase in the skeletal muscle oxidative enzymes CS, HAD and NADH (Sanchez et al., 1988).

Tada et al., (1992) found identical abnormalities in the forearm musculature of nine patients with chronic heart failure. This finding suggests that the metabolic abnormalities seen in the peripheral skeletal muscle of patients with COPD may be common to other chronic diseases.

(iii) The effect of training on skeletal muscle metabolites in patients with COPD

Maltais et al., (1996) exercise trained the 11 COPD patients who had demonstrated abnormalities in skeletal muscle oxidative metabolism during exercise. The training programme included 30 minutes of cycle ergometry, upper body resistance exercises, walking and stretching for three sessions per week for a period of 12 weeks. After exercise training, the patients demonstrated a significant increase in the activity levels of the oxidative enzymes CS and HAD in the vastus lateralis muscle. However, no improvements in the glycolytic enzymes PFK, HK and LDH were reported. The peak $V0_2$ achieved during an incremental ergometer cycle test improved by 14%. Additionally, V_E and blood lactate accumulation were significantly reduced at a set submaximal work load and a significant inverse relationship was found between blood lactate accumulation during exercise and the activity levels of CS ($r=0.83$; $p<0.01$) and HAD ($r=0.81$; $p<0.01$). These findings imply that exercise training in patients with COPD increases the activity of CS and HAD in the working skeletal muscle, as would be the case in control subjects.

In contrast, Belman et al., (1981) failed to demonstrate an increase in the activity of the oxidative enzymes CS, HAD and PK after six weeks of training in 15 patients with COPD despite improved endurance time during an incremental cycle test to exhaustion. Additionally, the patients with COPD failed to demonstrate a typical cardiovascular training response, as measured by heart rate and blood pressure during the incremental cycle test. The training programme involved two, 20 minute sessions of ergometry four times a week. The exercise training load was initially set at one third of the maximum reached during the incremental cycle test to exhaustion and increased as rapidly as possible to a load that could be tolerated for the 20 minute period. The authors concluded that the patients were unable to train at an intensity high enough to generate a cardiovascular or oxidative skeletal muscle training effect and that some other factor must be responsible for the improved submaximal endurance in these patients with COPD. It is possible that the six week period was not long enough, or the training intensity and/or frequency not high enough to induce changes in skeletal muscle oxidative enzymes in these patients.

In summary, the literature presents evidence of abnormal skeletal muscle oxidative metabolism during exercise in patients with COPD, the mechanism of which is unknown. Additionally, the

response of skeletal muscle oxidative metabolism in patients with COPD to exercise training remains unclear and further research is therefore required.

(d) The effect of age on skeletal muscle metabolites

Due to the progressive nature of COPD, most patients are middle aged or elderly. As various physiological changes occur with increasing age, it is important that these changes are considered when the metabolic profile of skeletal muscle in patients with COPD is discussed. Taylor et al., (1984) found no significant differences in ATP, PCr, Pi, Pi/PCr or pH in the exercising skeletal muscle of subjects aged between 20-45 years compared to subjects aged between 70-80 years. In contrast, Moller et al., (1980) reported intramuscular concentrations of total adenine nucleotides and PCr to be 5% lower in the elderly (age range 52-79yrs) compared to subjects aged between 18 to 36 years. The total ATP and ATP/ADP ratio was, however, unchanged compared to 40 year old individuals. These findings suggest that age alone is unlikely to cause the changes in phosphagen concentrations seen in the skeletal muscles of patients with COPD.

(e) Skeletal muscle morphometry and histology in patients with COPD

(i) Morphometry of skeletal muscle in patients with COPD

Very few studies have examined the morphometry of the skeletal muscle in patients with COPD. Jacobsson et al., (1990) reported a decrease in the percentage of Type I (slow twitch) fibres in the quadriceps femoris muscle of eight patients with COPD in respiratory failure (17% decrease) and 11 patients with COPD not in respiratory failure (22% decrease) relative to control subjects. These findings suggest morphometric abnormalities in the skeletal muscle of patients with COPD, independent of the degree of respiratory failure.

Thus, very little is known about skeletal muscle morphometry in patients with COPD. Considering the hypothesis that skeletal muscle may play a role in the exercise intolerance experienced by patients with COPD, research into skeletal muscle morphometry of these patients is indeed warranted.

(ii) Skeletal muscle histology in patients with COPD

Abnormal skeletal muscle histology has been reported in patients with chronic heart failure (Derman et al., 1993) and patients with end stage renal disease (Diesel et al., 1990). Evidence that both skeletal muscle function and skeletal muscle oxidative metabolism in patients with COPD is impaired has been provided. Despite this, to the best of our knowledge no studies have provided a detailed histological analysis of the skeletal muscle of patients with COPD.

4. Non Respiratory Variables that may Play a Role in Exercise Limitation in Patients with COPD

(a) Cardiovascular function in patients with COPD

Patients with COPD develop increased pulmonary vascular resistance as a consequence of the destruction of the vascular bed by emphysema, increased alveolar pressure and increased haematocrit (Wilkinson 1988). Increased pulmonary vascular resistance results in decreased stroke volume and a subsequent rise in heart rate relative to $V0_2$ in patients with COPD at rest and during exercise. Furthermore, a higher pulmonary artery pressure is generated at rest and during exercise in patients with COPD (Matthay et al., 1992). The increased heart rate and higher pulmonary artery pressure increases the afterload on the right ventricle and can lead to right ventricular dysfunction in patients with COPD (Wilkinson et al., 1988; Gallagher 1990; Fujii et al., 1996). Fujii et al., (1996) demonstrated an inverse relationship between $V0_2$ max and P-Q slope (an indicator of the severity of pulmonary vasculature dysfunction) during exercise in 21 patients with COPD. Fuji et al., (1996) therefore suggested that right ventricular failure and subsequent left ventricular dysfunction, may play a role in exercise intolerance in patients with COPD by means of impaired 0_2 delivery to the working muscles.

Several studies present evidence opposing this hypothesis. Chester et al., (1977) exercise trained 21 patients with COPD for four weeks on a daily basis. The exercise training involved daily walking on a treadmill at an average heart rate of approximately 125b/min. After training, the patients with COPD showed no changes in heart rate, pulmonary vascular resistance and cardiac index at the same submaximal work loads during incremental exercise, yet total work performed on the treadmill and minute ventilation improved significantly. Similarly, Degre et al., (1974) trained 11

patients with COPD. The programme involved 25 minutes of cycling three times a week for a six week period. The patients showed a ten percent improvement in peak $V0_2$ achieved during maximal cycle ergometry, despite no improvement in cardiac output or stroke volume. These findings suggest that factors other than cardiovascular limitations are responsible for the exercise intolerance experienced by patients with COPD.

(b) Altered blood lactate accumulation during exercise in patients with COPD

Patients with COPD experience increased blood lactate concentrations compared to healthy individuals of the same age and gender at the same absolute submaximal work load (Sue et al., 1988; Gallagher 1990; Patessio et al., 1993). The mechanism for this increased blood lactate accumulation during exercise is not known. Various hypothesis including hypoxaemia resulting in dependence on "anaerobic pathways" (Degre et al., 1974), physical deconditioning, cardiovascular dysfunction and lactate production by overactive inspiratory muscles have been suggested (Gallagher 1990).

It has been argued that early onset of "lactic acidosis" may play a role in exercise intolerance in patients with COPD (Degre et al., 1974; Sue et al., 1988; Patessio et al., 1993;). However, patients with COPD terminate exercise at much lower concentrations of blood lactate compared to healthy individuals (Spiro et al., 1975; Belman 1973; Gallagher 1990) and it therefore seems unlikely that excessive concentrations of blood lactate are limiting exercise tolerance in these patients.

(c) Nutritional status as a limiting factor during exercise in patients with COPD

Malnutrition has a well documented adverse effect on exercise tolerance (McArdle et al., 1991; Burke et al., 1994). Although the overall incidence of malnutrition in patients with COPD is unknown (Driver et al., 1982), it is a documented side effect of COPD (West 1992). As many as 50% of patients with COPD hospitalized for acute respiratory failure may suffer from malnutrition (Ferguson et al., 1993).

Various factors may cause malnutrition in patients with COPD. These include: interference with gastrointestinal function by medications including theophylline and corticosteroids, vascular

congestion of the gut due to heart failure, the catabolic effects of corticosteroids, physical inactivity, a high metabolic rate due to increased ventilatory demand (Olopade et al., 1992), and a deficient dietary intake caused by dyspnea or hypoxaemia during eating (Brown et al., 1983). It is therefore possible that poor nutritional status may play a role in impaired exercise tolerance in patients with COPD.

(d) Physical deconditioning as a cause of exercise intolerance in patients with COPD

As patients with chronic diseases are often physically inactive and/or bedridden, their exercise intolerance has often been attributed to physical deconditioning (Sinoway et al., 1988; Drexler et al., 1992). Patients with COPD are often extremely sedentary and the physiological changes associated with bed rest and inactivity are likely to contribute to their exercise intolerance (Olopade et al.,1992). It is well established that bed rest and physical inactivity decrease maximal oxygen uptake and stroke volume, increase heart rate and blood pressure response to exercise and cause a reduction in the size of the left ventricle (Mcardle et al., 1991; Olopade et al., 1992). Additionally, deconditioning causes skeletal muscle atrophy (Dubowitz 1985). Gallagher (1990) suggests that decreased limb strength in patients with COPD may be due to physical inactivity.

Various authors attribute the exercise intolerance experienced by patients with COPD, at least in part, to physical deconditioning (Gallagher 1990; Olopade et al., 1992; Belman 1993; Tade et al., 1992). However, to the best of our knowledge no well controlled studies examining the effect of physical inactivity on the exercise intolerance experienced by patients with COPD are reported in the literature.

(e) Psychological contribution to exercise intolerance in patients with COPD

The psychological implications of the sensation of dyspnea and the effect this may have on exercise intolerance in patients with COPD has been discussed. However, in addition, depression, anxiety and inappropriate fear of exercise are present in patients with COPD (Lustig et al.,1972) and the role that these psychological factors may play in limiting exercise capacity in patients with COPD should not be overlooked (Olopade et al., 1992).

In summary therefore, these findings suggest that a variety of factors may play a role in the exercise intolerance experienced by patients with COPD.

5. Predictors of Exercise Capacity in Patients with COPD

In an attempt to determine what factors limit exercise capacity in patients with COPD, various researchers have performed correlation analyses to assess the role of: lung function, respiratory muscle strength, blood lactate accumulation, cardiovascular factors and peripheral skeletal muscle function in the exercise intolerance experienced by these patients. Although many of these studies have been mentioned through the review of literature, a concise summary of some of the studies is presented in Table 1.4.

Table 1.4 Summary of research studies investigating factors predicting exercise capacity in patients with COPD.

		Measures of exercise capacity		
Authors	**Variables**	**Peak $V0_2$**	**Endu time**	**Distance walked during a SMWT**
Pulmonary Function				
Spiro et al., 1975 (n=40)	FEV_1 (l)	r=0.70 (p<0.001)		
Sue et al., 1988 (n=43)	FEV_1 (l)	NSC		
Bauerle et al., 1995 (n=53)	FEV_1 (% of predicted)	r=0.49 (p<0.05)		
Montes et al., 1996 (n= 25)	FEV_1 (l) and FEV_1/FVC	NS	NS	
Spiro et al., 1975 (n=40)	V_E	r=0.85 (p<0.001)		
Bauerle et al., 1995 (n=53)	IC	r=0.46 (p<0.05)		
Gosselink et al., 1996 (n=42)	TLCOsb	r=0.68 (p<0.05)		r=0.38 (p<0.05)
Miscellaneous factors				
Sue et al., 1988 (n =22)	[Lactate]	r=0.75 (p<0.05)		
Fujii et al., 1996 (n=21)	PQ slope	r=-0.67 (p<0.001)		
Respiratory muscle strength				
Montes et al., 1996 (n= 25)	Pdi Max	r=0.68 (p<0.05)	r=0.54 (p<0.05)	
Gosselink et al., 1996 (n=42)	Pi Max			r=0.49 (p<0.005)
Peripheral SM factors				
Gosselink et al., 1996 (n=42)	QF			r=0.63 (p<0.005)
Gosselink et al., 1996 (n=42)	HGF			r=0.61 (p<0.005)
Maltais et al., 1997 (n=9)	CS	r=0.78 (p<0.01)		
Maltais et al., 1997 (n=9)	HADH	r=0.61 (p=0.08)		

Abbreviations: **Peak $V0_2$**, maximal 0_2 consumption measured during an incremental maximal exercise test; **Endurance time**, time to exhaustion during an incremental exercise test; **SMWT**, six minute walk test; **NS**, not statistically significant; **V_E**, minute ventilation at peak exercise; **IC**, inspiratory capacity; **TLCOsb**, transfer factor (a measure of diffusing capacity in the alveoli); **PQ - slope**, an indicator of the severity of pulmonary vascular disorder; **[Lactate]**, blood lactate concentration at peak exercise; **Pdi Max**, measure of transdiaphragmatic twitch pressure; **Pi max**, inspiratory muscle strength; **SM**, skeletal muscle; **QF**, peak isometric quadriceps force; **HGF**, peak isometric hand grip force; **CS**, citrate synthase; **HADH**, 3-hydroxyacyl CoA dehydrogenase

In Table 1.4 measures of exercise capacity are listed horizontally across the top and include: Peak $V0_2$ and endurance time achieved during an incremental exercise test to exhaustion and distance walked during a SMWT. Variables correlated to the measures of exercise are listed in column two of Table 1.4.

It is clear that conflicting results are presented in the literature regarding the role that FEV_1 plays in predicting exercise capacity in patients with COPD. This is further confounded by authors expressing FEV_1 in different ways. Therefore, some authors have shown a strong correlation between FEV_1 and measures of exercise ability (Spiro et al., 1975), others have shown a weaker but significant correlation (Bauerle et al., 1995), and yet others have reported no significant correlation between these two variables (Sue et al., 1988; Montes et al, 1996).

Various other parameters, including blood lactate concentrations at peak exercise (Sue et al., 1988), cardiovascular function (Fujii et al., 1996), respiratory muscle strength (Montes et al., 1996; Gosselink et al., 1996) and various indices of peripheral skeletal muscle function (Gosselink et al., 1996; Maltais et al., 1996) have been shown to correlate with exercise tolerance in patients with COPD.

In summary, therefore, it appears that multiple factors may account for the exercise intolerance experienced by patients with COPD.

6. A Summary of the Review of Literature

A review of the literature investigating exercise limitation in patients with COPD over the last four decades demonstrates a focus on those factors related to the heart and lungs. However, despite three decades of research, none of these factors appear to be the major limiting factor during exercise in patients with COPD. In contrast, preliminary research in the last decade suggests that skeletal muscle may play a major role in this exercise intolerance. This finding is suggested by reports of functional, metabolic and morphometric abnormalities in the skeletal muscle of patients with COPD. In addition preliminary research supports the hypothesis that after exercise training

the improved skeletal muscle metabolism and skeletal muscle function experienced by these patients is related to their improved exercise tolerance.

However, no study, to our knowledge, has investigated the histology and ultrastructure of the peripheral skeletal muscle, or investigated both isokinetic skeletal muscle strength and endurance in the skeletal muscle of patients with COPD. Additionally, no study has examined the relationship between skeletal muscle structure and skeletal muscle function in patients with COPD or the relationship between skeletal muscle structure and general exercise performance. Therefore, further research investigating the role of peripheral skeletal muscle in exercise intolerance in patients with COPD is clearly necessary.

CHAPTER TWO

GENERAL METHODS

1. General Considerations

To be included in the studies described in this thesis, patients had to be free of any serious chronic illness other than COPD; and be able to exercise. Patients had to present with an FEV_1 of less than 50% of predicted and were excluded from the studies if they had experienced an acute exacerbation of their disease or an episode of chest infection in the four weeks immediately prior to entry into the study. Similarly, control subjects had to be free of any chronic disease.

The study protocols were approved by the Ethics and Research Committee of the Faculty of Medicine at the University of Cape Town. Prior to commencement of the study all participants provided written informed consent and the patients with COPD obtained the full consent of their attending physician. Patients were instructed to remain on their medication throughout the testing period and both patients and controls were instructed to avoid any strenuous physical exercise for 36 hours preceding each laboratory test.

On entry to the study all patients underwent a forced maximal expiratory manoeuvre before and after bronchodilation for measurement of FEV_1 and FVC using a vitalograph ™ (Wurzburg, Germany). Those with a greater than 15% reversibility in FEV_1 after bronchodilation were excluded from the study due to a presumed asthmatic component to their disease.

2. Physical Activity Questionnaire

The Yale physical activity questionnaire for older adults (Dipietro et al., 1992) was administered to all patients with COPD and control subjects. Participants were asked to report the number of hours in a "normal" week spent completing various pre-determined activities. Categories of activities included work, yardwork, caretaking, exercise and recreational activities (appendices, Chapter nine). The examiner recorded the participants' responses on the data sheet supplied with the questionnaire. Accordingly, weekly caloric expenditure for each participant was calculated (Dipietro et al., 1992).

The second part of the questionnaire included questions regarding the frequency of certain activities in a "normal" month. Subjects were questioned about the frequency of their participation

in various activities. In retrospect, this section of the questionnaire was not analysed, as the frequency of different activities in patients with COPD was not being investigated in this dissertation.

3. Medical Questionnaire

A brief medical questionnaire was conducted on entry to the study by all participants. This served to highlight risk factors for chronic disease, chronic medical complications and/or contra-indications for participation in maximal exercise or any of the tests included in the studies conducted in this dissertation.

4. Pulmonary Function Tests

All pulmonary function tests were conducted in the Groote Schuur Hospital Respiratory Clinic by trained respiratory technologists, using the Masterlab (Erich Jaeger Vers 3.4,Jaeger, Wurzburg, Germany 1992) computerised system utilising a Jaeger pneumotach with rubber mouthpiece (Jaeger, Wurzburg, Germany 1992) (Fig 2.1). Tests of pulmonary function included tests of (a) forced spirometry, (b) static lung volumes and (c) transfer factor.

(a) Forced Spirometry

Patients and control subjects were asked to expire and inspire normally for three breaths followed by a forced maximum inspiratory and expiratory manoeuvre. The manoeuvre was performed according to ATS criteria (American Thoracic Society, 1987). During normal breathing TV was calculated (Fig 2.2). During the forced manoeuvre, FVC, FEV_1 (Fig 2.3), FEF_{50}, PIFR and PEFR were measured from the flow volume loop (Fig 2.4).

Fig 2.1 Masterlab Erich Jaeger lung function analyser Vers 3.4

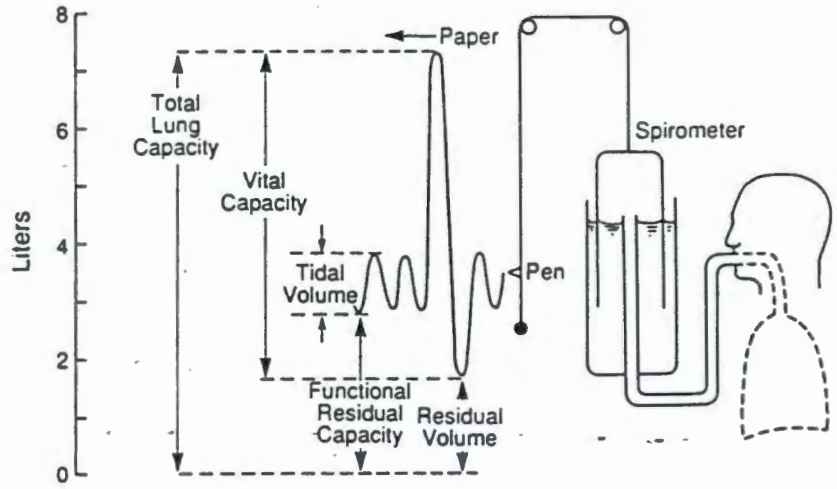

Fig 2.2 Lung volumes calculated from spirometry. Note that the FRC and RV cannot be measured with spirometry. VC is also known as IC (West 1995)

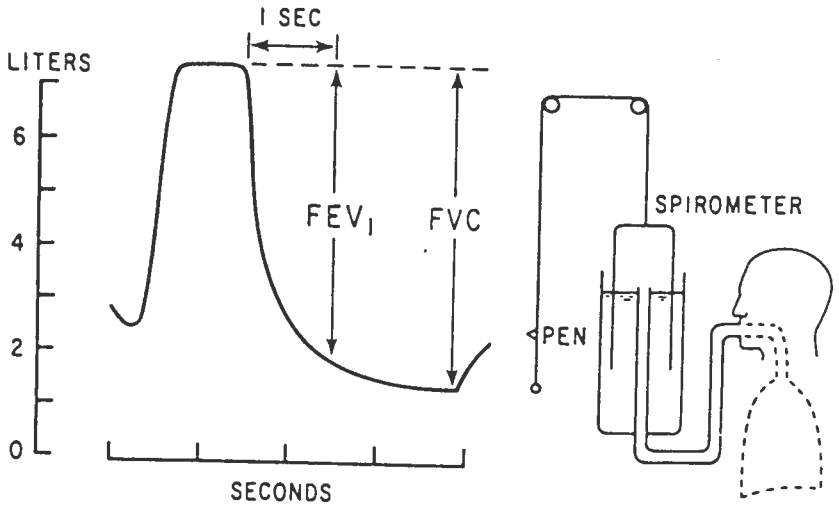

Fig. 2.3 Diagrammatic representation of measures of FEV_1 and FVC (West 1995)

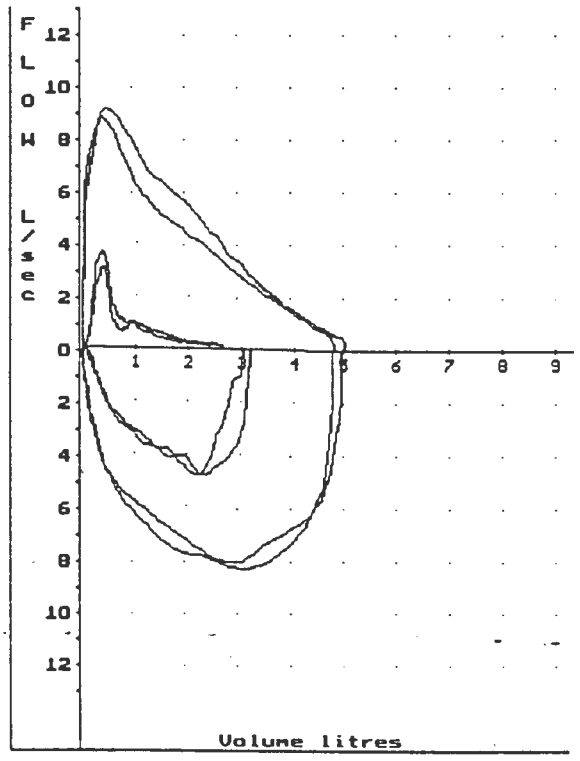

Fig 2.4 Inspiratory and expiratory flow volume curves in normal subjects and in patients with COPD (West 1992). The COPD flow volumes are represented by the smaller of the two curves

The manoeuvre was repeated at least three times and the highest values recorded. FEV_1/FVC was calculated from these measures. During a test of MVV, subjects were asked to inspire and expire as deeply and as quickly as possible for a period of 12 seconds whilst receiving verbal encouragement. Gas volumes were measured during the 12 second period. The subjects repeated this manoeuvre twice and the highest value obtained was multiplied by five to calculate MVV (ATS, 1987).

Fig 2.5 Airtight Erich Jaeger Masterlab body plethysmography box used to measure static lung volumes

(b) Static Lung Volumes

Body plethysmography was used for calculation of FRC and RV in this study. This method is more accurate than the helium dilution technique in patients with COPD due to the likelihood of gas trapping in these patients. Patients were seated in a large airtight box and asked to breathe normally into a mouthpiece (Fig 2.5).

At the end of normal expiration, a shutter sealed off the mouthpiece whilst the subject was asked to continue breathing normally. As the subjects attempted to inhale, lung gas volume increased and the box pressure increased as gas volume decreased (Fig 2.6). Boyle's law states that pressure x volume is constant at constant temperature. Thus FRC and RV were calculated using the following equations:

RV:

Pressure in box before inspiratory effort = P1

Pressure in box after respiratory effort = P2

Pre-inspiratory box volume = V1

Change in volume of the box = ΔV.

$(P1)(V1) = P2 (V1 - \Delta V)$

Therefore ΔV can be calculated

FRC:

P3 = mouth pressure before inspiratory effort

P4 = mouth pressure after inspiratory effort

V2 = FRC

$(P3)(V2) = P4 (V2 = \Delta V)$

Therefore V2 can be calculated

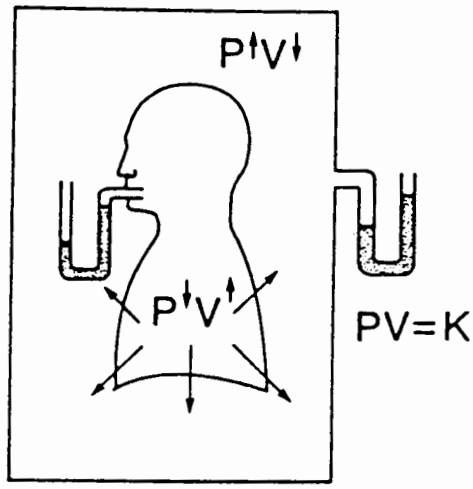

Fig 2.6 Diagrammatic representation of measurement of FRC with a body plethysmograph. When the subject makes an inspiratory effort against a closed airway, lung volume is slightly increased causing airway pressure to decrease and box pressure to increase

(c) Transfer factor

The single breath carbon monoxide diffusion method was used to determine transfer factor in the studies conducted in this dissertation. Participants were asked to take a single VC breath of a gas containing 0.3% C0, 10% He, 18% 0_2 and 71.7% N_2. The rate of diffusion of the C0 from the alveolar gas during a ten second breath hold was then measured. This rate of diffusion of the C0 was expressed relative to lung volume to determine the transfer coefficient (K_{10}).

5. Anthropometrical Analysis of Body Composition

Standing height without shoes was measured to the nearest 0.1 cm. Total body mass with clothes on and shoes off was measured to the nearest kg. For determination of body composition a Holtain skinfold caliper (Crymych, UK) was used for measurements of subcutaneous fat at seven sites. A Holtain bone caliper (Crymych, UK) was used for measurement of bone diameters at six sites and a Rabone Chesterman Silverflex metal tape measure (Crymych, UK) was used for the measurement of 12 girths. Body composition was assessed using an anthropometric technique that fractionated the body composition into muscle mass (Martin et al., 1990), fat and lean body

mass (Durnin and Womersley 1974).

Distance from the sub-gluteal fold to one centimetre above the knee was recorded. Using this distance, the measures of midthigh girth, sub gluteal girth, above the knee girth (1cm) and midthigh skinfold, LTV was calculated (Noakes et al., 1980).

6. Graded Exercise Test to Exhaustion

All subjects performed a maximal progressive cycle ergometry exercise test to exhaustion using an electro-mechanically braked cycle ergometer with manual adjustment of resistance in Kp.m (Siemens, Elena, Erlangen, Germany). Prior to testing, the height of the saddle was adjusted to the preferred height for the subject. During exercise, subjects cycled with a mouthpiece connected to a CPX/D pneumotach (Medgraphics, Boston, USA). A nasal clip prevented nasal breathing.

Patients commenced pedalling at no resistance for the first minute. As the electromechanically braked bicycle was linked to a manual resistance adjustment system measured in Kp.m, the workload was increased by 50 Kp.m (8.3 W) every minute until the patient voluntarily terminated the test. For the first ten minutes of the exercise trial control subjects followed an identical protocol to the patients with COPD. However, to ensure that the control subjects attained true maximal exercise, their workload was increased by 200 Kp.m (33.3 W) every minute, as opposed to 50 Kp.m every minute, once the first ten minutes of the initial protocol were completed. Patients and control subjects were asked to maintain a fixed pedalling speed (60 rpm). Failure to maintain the fixed rpm resulted in test termination.

During exercise, breath by breath 0_2, $C0_2$ and V_E analysis was performed using the Medgraphics Cardiopulmonary Exercise CPX/D System and an on-line micro computer (Boston, USA). Analysers were calibrated before and after each test using gases of known composition. Accordingly, $V0_2$, $VC0_2$ and RER were calculated. The average value for each minute for each parameter was stored for later printing. The VO_2 max was taken as the highest rate of oxygen consumption measured during any 60 second period (Noakes 1988).

Peak workload was taken as the highest workload maintained for a complete minute during the test. When a subject was unable to complete the full minute at a particular workload, the workload of the immediately preceding, completed workload was recorded as the peak workload. The same method was used to calculate the exercise time to exhaustion.

Patients and control subjects were asked to report their level of perceived exertion from the Borg scale after each minute of the exercise test. Most studies have used the 20-point Borg scale. This method causes inaccurate scaling of variables such as blood lactate concentrations and ventilation, which increase according to non-linear power functions (Noble et al.,1982). The 10-point category scale with ratio properties (Borg 1980;1982) was therefore used in this study. The ratio scale has verbal expressions that are simple to understand and more accurately describe sensations of perceived peripheral effort.

Indications for halting the exercise test before exhaustion included adverse symptoms such as light headedness, confusion and chest pain. Adverse clinical signs such as facial pallor, decline in heart rate or blood pressure, failure of blood pressure or heart rate to rise with increasing effort, systolic blood pressure exceeding 280 mmHg, or diastolic blood pressure exceeding 140 mmHg were additional indicators for early test termination.

Prior to the bicycle test, the resting heart rate and blood pressure (Korotkoff Phase I and IV) were measured and recorded. Blood pressure at rest and every two minutes during exercise was measured by means of audible sphygmomanometry using a calibrated mercury column sphygmomanometer with an appropriately sized cuff. The cuff was placed on the arm that did not have an intravenous cannula placed in the forearm vein.

Although, the measurement of diastolic blood pressure during exercise can be difficult, the results are quite reproducible if Phase IV of the Korotkoff sound is taken. Heart rate and rhythm were determined at rest and each minute during exercise, using a Sirccust 403-2P Siemens monitor (Erlangen, Germany). The monitor transferred information directly into the Medgraphics

Cardiopulmonary Exercise CPX/D System for later printing. Self-adhering electrodes were placed in the CM5 position.

Arterial oxygen saturation was measured noninvasively at rest and continuously during exercise using a fibreoptic ear-pulse oximeter (Ohmeda Biox 3700; Boulder; USA). The saturation values were transferred directly into the Medgraphics Cardiopulmonary Exercise System (CPX/D) and stored for later printing.

7. Collection of Blood for Analysis of Blood Lactate Concentration, Partial Pressure of Carbon Dioxide and pH Measurements in Arterialised Blood During the Graded Exercise Test to Exhaustion

(a) Blood Sample Collection

Resting arterial blood samples were drawn from the radial artery of the COPD patients using a winged needle infusion set (Venisystems™, 4721 Butterfly®, 21 INT, Sligo, Ireland). Resting arterial samples were not drawn from the control group due to the invasive nature of the procedure and the fact that normal values are well established amongst healthy subjects (West 1995).

Prior to the start of the cycle test, an 18-gauge flexible cannula (Criticon, Tampa, Florida, USA) was inserted into a subcutaneous forearm vein of each subject. A 3-way stopcock (Discofix-3, Belgium) was attached for multiple blood sampling. A custom made heating blanket was placed around the arm immediately proximal to the catheter and was set to maintain a temperature of 27^{o}C for the duration of the exercise test.

Arterialised venous blood samples were drawn at rest and subsequently at one minute intervals during the exercise bout. Five millilitres of blood was drawn from the stopcock directly into a five millilitre syringe. Three millilitres was decanted into a sodium oxalate lined glass test tube and immediately placed on ice for later analysis of plasma lactate concentrations. The two millilitres remaining in the syringe were capped and immediately placed on ice for determination of arterialised $PaCO_2$, pH and $Sa0_2$. Additional blood samples were drawn at exhaustion and every

minute for five minutes in the post exercise recovery period and an identical collection procedure was followed.

(b) Blood Sample Analysis

The resting arterial blood was analysed immediately for $Pa0_2$, $PaC0_2$, pH and $Sa0_2$ by means of a pH/blood gas analyser (Radiometer ABL3, Copenhagen, Denmark).

Measurement of $Pa0_2$ is not considered accurate from arterialised blood (AST, 1987). Accordingly, the two millilitres of arterialised blood in the capped syringe was analysed immediately upon termination of the exercise test for $PaC0_2$, pH and $Sa0_2$ by means of a pH/blood gas analyser (Radiometer ABL3, Copenhagen, Denmark).

At termination of the exercise test, the three millilitre blood samples in the sodium oxalate test tubes were centrifuged using a Sigma 302-K centrifuge (Munich, Germany) at 3000 rpm for 12 minutes at 4^oC. Lactic acid concentrations were measured on plasma by means of an enzymatic kit technique (bioMerieux sa, lactate PAP 69280, Marcy l" Etoille, France).

8. Six Minute Walking Test

The SMWT was administered by having patients cover as much ground as possible whilst walking repeated lengths of a 30m corridor in the six minute period. Subjects were encouraged to pace themselves to avoid requiring a rest during the six minute period. Verbal encouragement was given throughout the test. The number of lengths walked was recorded and used for calculation of distance covered to the nearest metre. Heart rate was measured continuously during the six minute period by means of a Polar heart rate monitor (Polar Company, Kempele, Finland). Additionally, subjects reported a rating of fatigue from the 10 point Borg RPE scale (Borg 1980, 1982, described in section five of this Chapter) in the last ten seconds of every minute.

9. Tests of Skeletal Muscle Function

(a) Isokinetic Concentric Skeletal Muscle Function

Isokinetic muscle strength was measured using an Akron Isokinetic Dynamometer (Ipswitch, Suffolk, UK) and an Akron Data Reduction computer (Vers. 2.0) (Fig 2.7). This system is designed to maintain angular velocity at a constant pre-set level regardless of the torque generated by the subject. The subjects were seated on the Akron apparatus with the head of the femur fully supported and the knee flexed to 90^{o}. The fulcrum of the lever was positioned in line with the axis of rotation corresponding to the transverse line through the femoral condyles. The lever arm was secured to the subject via a shin strap placed just proximal to the malleoli. In an attempt to isolate movement to the knee joint, the thigh, waist and shin straps were fastened as tight as was tolerable for the patient. During the testing, subjects were instructed to keep their arms folded across their chests.

Each patient was given a practice bout at each testing speed. This warm-up consisted of five familiarisation repetitions at 60^{o}/sec and at 125^{o}/sec. After the familiarisation period, patients rested for two minutes before starting the test. Any necessary adjustments to the equipment were made during this time. The strength test measured maximum isokinetic torque of the quadriceps and the hamstring muscle groups of the dominant limb, recording data during four maximal contractions through a full range of motion at a limb contraction speed of 60^{o}/sec. After a five minute rest, an endurance test of the dominant leg was performed. The subject performed 30 repeated full knee extensions at a rate of 125^{o}/sec. Total power generated, work done and peak torque generated by the quadriceps and hamstrings at this speed were recorded. Verbal encouragement was given throughout the tests.

Fig 2.7 An Akron isokinetic dynamometer used for measurement of skeletal muscle strength and endurance

(b) Eccentric Skeletal Muscle Strength

Isokinetic eccentric muscle strength was measured using a Kin-Com Isokinetic Dynamometer (Chatanooga, USA) and Kin-Com data reduction computer (Vers. 5.13) (Fig 2.8) on six patients with COPD and six controls. The small sample number was the result of equipment unavailability until midway through the data collection phase of the study. Equipment alignment and subject positioning was as for the Akron skeletal muscle function tests. Each subject was instructed to complete a warm up including five minute of cycling and two hamstring stretches prior to being positioned on the machine. For familiarisation, a practice bout of two concentric and two eccentric contractions was given. For testing purposes, two repetitions of concentric contractions were performed, followed by two repetitions of eccentric contractions for both the quadriceps and the hamstring muscle groups at a speed of 60^{o}/sec.

Fig 2.8 A Kin-Com isokinetic dynamometer used for measures of skeletal muscle strength and endurance

10. Skeletal Muscle Biopsy Procedure

A sample of skeletal muscle was removed from the vastus lateralis muscle under local anaesthesia according to the needle biopsy technique of Bergstrom et al., (1962), as modified by Evans et al., (1982).

The skeletal muscle sample was divided into two pieces. One piece of the muscle was snap-frozen in isopentane and cooled in liquid nitrogen. Transverse sections (5-7 μm) were stained for haemotoxylin and eosin, Gomoris trichrome, NADH, SDH, PAS, Cytochrome C Oxidase, Oil Red O and ATPase at pH 4.3, 4.6, and 9.4 (appendices, Chapter nine). The fibre type proportions and the minimum diameter of approximately 200 Type I and Type II muscle fibres differentiated by ATPase staining were measured using an IBAS Kontron image analysis system (Munich, Germany).

The second portion of the skeletal muscle was desensitised in 3% phosphate buffered

glutaraldehyde for ten minutes. The tissue was sliced into smaller sections and fixed overnight. The slivers of transverse and longitudinal muscle sections were post-fixed in 1% buffered osmium tetroxide (pH 7.4) and processed by standard methods into Spurrs epoxy resin. Ultra-thin transverse and longitudinal sections were stained with uranyl acetate and lead citrate and examined using a Phillips 201 electron microscope (Einthoven, The Netherlands).

11. Statistical Analysis

Statistical differences between the control group and the patients with COPD were determined using independent t tests for continuos and ordinal variables by means of Statgraphics computer software (Vers. 6.1; Statistical Graphical Corporation Inc., USA). Correlation coefficients within the patient population were calculated by means of the Spearmans rank correlation coefficient in the case of rank ordered data and the Pearsons product moment correlation coefficient in the case of continuous data using the Statgraphics computer software (Vers. 6.1). Levels of significance were calculated at the 0.01 and 0.05 confidence levels (Glanz 1980) and data was presented as the mean ± the SD. In the case of the correlations, correlation coefficients were denoted as r and the sample size as n.

CHAPTER THREE

EXERCISE TOLERANCE AND SKELETAL MUSCLE FUNCTION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

1. Introduction

In Chapter One, it was documented that the past three decades have witnessed an increased interest in the factors limiting exercise performance in patients with COPD (Gallagher 1990; Kilian et al., 1992; Karlsson et al., 1992; Olopade et al., 1992; Belman 1993). To date, the exercise intolerance reported in patients with COPD has been attributed to central limiting factors directly related to pathological changes in the heart and lungs. These factors include ventilation mechanics (Derenne et al., 1978; O'Donnell et al., 1988; Marin et al., 1993; Belman 1993), impaired gaseous exchange (Woodcock et al., 1981; Jones 1988; Olopade et al., 1992; Palange et al., 1995; Soguel Schenkel et al., 1996), respiratory muscle weakness (Derenne et al., 1978; Rochester et al., 1979; Bellemare et al., 1983; Dodd et al., 1983; Tobin 1988) and central cardiovascular limitations secondary to pulmonary disease and pulmonary hypertension (Wilkinson 1988; Matthay et al., 1992; Fujii 1996). Despite contradictory research regarding the contribution of central factors to the exercise intolerance in patients with COPD, until recently, the possibility that peripheral factors in skeletal muscle might play a role in this exercise intolerance has not been considered.

Recent research has however demonstrated that peripheral skeletal muscle plays a pivotal role in exercise intolerance in patients with renal failure and in patients with cardiac failure (Derman et al., 1992; Diesel et al., 1993). It is possible, for a variety of reasons, that this could be the case in patients with COPD. Firstly, traditional cardiovascular exercise training programmes for patients with COPD result in improved functional capacity. However, no parallel improvements in traditionally measured central factors of lung function (McGavin et al., 1977; Carter et al., 1991; Olopade et al., 1992; O'Donnell et al., 1993), $Pa0_2$ at rest (an indicator of gas exchange proficiency) (Patessio et al., 1992) or central cardiovascular factors (Degre et al., 1974; Chester et al., 1977) have been reported with training in patients with COPD. This suggests that factors other than the central factors of lung function, gas exchange or cardiovascular capacity may be responsible for the improved functional ability in trained patients with COPD. It is possible that peripheral factors adapt to the training stimulus and are responsible for the improved functional ability in the patients with COPD.

Secondly, low intensity skeletal muscle circuit weight training programmes have also induced improvements in skeletal muscle strength (Simpson et al., 1992) and endurance (Clark et al., 1996) with a corresponding improvement in general functional ability and endurance capability in patients with COPD (Simpson et al., 1992; Clark et al., 1996). These findings suggest a relationship between peripheral skeletal muscle function and exercise tolerance in these patients.

Thirdly, Gosselink and co-workers (1996) reported a relationship between quadriceps muscle force and distance walked during the SMWT in patients with COPD. This research further supports the suggestion of a relationship between peripheral skeletal muscle function and exercise tolerance and/or functional ability.

Finally, Decramer et al., (1997) demonstrated a relationship between skeletal muscle strength and frequency of health care facility use. Those patients who demonstrated greater skeletal muscle weakness required a greater degree of health care. This suggests a possible relationship between skeletal muscle function and acute exacerbations of the disease requiring health care utilisation in patients with COPD. This preliminary study quite obviously has important implications for health care economy and suggests that further research investigating skeletal muscle strength in patients with COPD is warranted. The importance of investigating the relationship between peripheral skeletal muscle functional ability and exercise tolerance in patients with COPD has therefore clearly been established.

Accordingly, the aims of the study reported in Chapter Three were threefold. Firstly, to document the exercise tolerance of 13 patients with COPD and compare their exercise capacity to an age and gender matched healthy control group. Secondly, to document skeletal muscle functional ability in these patients relative to the control group. Finally, to determine the relationship between skeletal muscle function and exercise tolerance in the 13 patients with COPD.

2. Methods

Ten male patients and three female patients (mean age 57 ± 7 years; average mass 65 ± 14 kg) with an FEV_1 of below 50% of predicted were recruited for participation in this study. The patients

were recruited from either the Respiratory Clinic at Groote Schuur Hospital or from private pulmonologists in Cape Town. One patient, whilst on a three week vacation to Cape Town, was recruited from a pulmonologist in Johannesburg.

Patients were excluded from the study if they had any signs, symptoms or history of a second chronic illness or if they had suffered an acute exacerbation of their disease or an episode of chest infection in the four weeks immediately prior to the start of the study. Initially, patients were excluded if they were on steroid treatment. However, it proved too difficult to recruit patients with moderate to severe COPD who were not ingesting oral steroid medication. Therefore five patients were ingesting low doses of prednisone (5-10mg daily) during the course of this study. Patients ingesting medications at the start of the trial were instructed to continue with their medication during the period of exercise testing.

Thirteen sedentary age and gender matched individuals free of any chronic disease (mean age 55 ± 7 years; average mass 83 ± 10 kg) were recruited to form a control group.

(a) Exercise testing

Patients and controls were tested on three separate occasions with a 48 hour rest between testing sessions. On the first occasion, subjects performed a SMWT, an isokinetic muscle function test to determine concentric skeletal muscle strength and endurance and full anthropometrical analysis for determination of body composition. Additionally subjects completed the Yale physical activity questionnaire for older adults to determine activity levels and a precautionary summarised medical questionnaire for exclusion of high risk subjects on this testing occasion.

On the second occasion, subjects performed tests of eccentric isokinetic skeletal muscle strength. This was to allow a 48 hour rest between tests of skeletal muscle concentric strength and skeletal muscle eccentric strength.

During the third testing occasion, pulmonary function was measured in both groups and a resting arterial blood sample was drawn from the patients with COPD. Arterial blood samples were not

drawn from control subjects as the procedure is painful and invasive and norms are well established (West 1995). Additionally, an incremental cycle ergometer test to exhaustion was conducted to determine peak workload achieved and time to completion of the exercise bout. In addition, the following variables were measured at regular intervals throughout the exercise bout and at peak exercise: $V0_2$, $VC0_2$, RPE, RER, HR, BP, V_E, arterialised blood lactate concentration, pH and $PC0_2$. All tests were conducted according to the methods detailed in Chapter Two.

(b) Statistical analysis

All data are expressed as mean ± SD. Statistical analysis was performed according to the general methods detailed in Chapter Two.

3. Results

(a) Subject characteristics on entry to the study

Subject characteristics on entry to the study are detailed in Table 3.1.

Table 3.1 Characteristics of patients with COPD and the control group on entry to the study

Patient Nº	Gender	Age (yr)	Mass (kg)	Ht (cm)	DOD (months)	WPAE (kCal/wk)
1	F	50	50	153	84	1785
2	M	54	44	163	84	4305
3	M	58	69	168	180	2145
4	M	55	48	155	120	7350
5	M	58	57	164	120	3986
6	M	54	66	181	12	2700
7	M	55	78	164	UK	NC
8	M	56	83	171	12	4320
9	M	56	61	162	36	6945
10	M	72	84	180	12	4005
11	F	55	63	173	130	4800
12	F	54	61	177	108	5955
13	M	65	82	172	48	8505
Mean		**57**	**65****	**167**	**78**	**4733**
SD		**5**	**14**	**8**	**55**	**2098**
Controls						
Mean	as above	**55**	**83**	**174**	**n/a**	**6669**
SD		**7**	**10**	**7**	**n/a**	**2985**

Abbreviations: M, male; **F**, female; **m**, metres; **kg**, kilograms; **DOD**, duration of disease, **WPAE**, weekly physical activity energy expenditure; **cm**; centimetres; **Yr**, years; **SD**, standard deviation; ******, $p<0.01$ COPD vs Control; **UK**, unknown; **NC**, not completed.

The average time since diagnosis of COPD was 78 ± 55 months. One patient did not known the date of diagnosis and it could not be established from the patient file. The mean weekly energy expenditure was not different between groups although the control subjects tended to be more active (4733 ± 2098 vs 6669 ± 2985 kCal/wk; COPD vs control; $p=0.07$). The results from one patient's physical activity questionnaire were uninterpretable. Average mass was significantly different between groups (65 ±14 vs 83 ±10; COPD vs Control; $p<0.01$).

(b) Subject resting lung function and blood gas variables on entry to the study

Resting lung function results for the patients and the control group are presented in Table 3.2

Table 3.2 Resting lung function variables of patients with COPD and the control group on entry into the study.

Patient	Gender	FEV_1 (l)	FEV_1 / FVC (%)	MVV (l/min)	PEFR (l/min)	TLCOsb (ml/mmHg/min)	PIFR (l/min)	IC (l)	$Pa0_2$ (kPa)	$Sa0_2$ (%)	$PaC0_2$ (kPa)
1	F	0.7	25	31	150	7	150	1.6	9.86	93	4.96
2	M	1.2	36	67	300	20	270	2.4	11.88	99	5.61
3	M	0.5	24	31	220	14	190	1.4	NSO	95	NSO
4	M	1.7	49	66	292	20	225	2.3	10.68	94	5.01
5	M	1.7	47	53	170	21	160	2.2	10.24	91	5.97
6	M	1.6	39	71	307	17	278	2.3	10.32	98	5
7	M	1.4	54	89	380	25	270	2.6	9.17	100	5.34
8	M	1.9	43	160	290	32	271	3.6	9.99	NMR	5.65
9	M	1.6	43	78	356	24	308	2.0	NSO	96	NSO
10	M	1.8	33.9	80	311	23	375	3.3	NSO	98	NSO
11	F	1.6	25	23	152	14	295	1.2	8.70	93	4.77
12	F	0.6	32	26	173	7	191	1.6	8.25	93	4.62
13	M	1.1	29	55	271	14	391	2.7	8.71	91	5.78
Mean		***1.3*** **	***37*** **	***64*** **	***259*** **	***18*** **	***260*** **	***2.2*** **	***9.78***	***95.08***	***5.27***
SD		***0.5***	***9***	***36***	***78***	***7***	***75***	***0.7***	***1.1***	***3.09***	***0.46***
Controls											
Mean		***3.4***	***76***	***141***	***547***	***30***	***355***	***3.6***	***11.9-13.2 (norm)***	***99-100 (norm)***	***4.8-6.3 (norm)***
SD		***0.7***	***7***	***29***	***92***	***6***	***102***	***0.7***	-	-	-

Abbreviations: **FEV_1**, forced expiratory volume in 1 second; **FEV_1 (%)**, forced expiratory volume in 1 second/forced vital capacity; **MVV**, maximum voluntary ventilation; **PEFR**, peak expiratory flow rate; **TLCOsb**, transfer factor; **PIFR**, peak inspiratory flow rate; **IC**, inspiratory capacity; **NMR**, no measurement recorded; **NSO**, no sample obtained; **$Pa0_2$**, partial pressure of oxygen in arterial blood; **$Sa0_2$**, arterial oxygen saturation levels; **$PaC0_2$**, partial pressure of carbon dioxide in arterialised blood; **kPa**, kilopascals; **, $p < 0.01$ COPD vs control subjects; **SD**, standard deviation, norm; norms (West; 1992).

FEV_1, FEV_1/FVC, MVV, PEFR, TLCOsb, PIFR and IC were all significantly lower in patients compared to control subjects. None of the 13 patients presented with chronic respiratory failure [criteria for respiratory failure = $Pa0_2$ < 8.0 kPa and/or $PaC0_2$ > 6.7 kPa (Wiles et al., 1984)] at the time of testing. However, five of the 13 patients were hypoxic at rest (criteria for hypoxia $Sa0_2$ < 94%, Gimenez et al., 1992) and two patients used supplemental oxygen when sleeping.

(c) Medications ingested by the patients with COPD

Medications ingested by the patients with COPD during the study are presented in Table 3.3

Table 3.3 List of medications ingested by patients with COPD during the course of the study

Pt No.	*Medication (Generic name)*
1.	Ipatropium Bromide; Prednisone; Theophylline; Salbutamol.
2.	Theophylline; Salbutamol.
3.	Ipatropium Bromide; Prednisone; Budesonide; Theophylline
4.	Fenoterol; Prednisone; Salbutamol
5.	Ipatropium Bromide
6.	Beclometasone; Ipatropium Bromide; Budesonide; Theophylline/Etofylline; Salbutamol
7.	Beclometasone; Theophylline; Ipatropium Bromide
8.	Fenoterol; Prednisone; Theophylline
9.	Prednisone; Ipatropium Bromide
10.	nil
11.	Beclometasone; Salmeterol; Theophylline; Ipatropium Bromide
12.	Ipatropium Bromide; Budesonide; Formoterol; Theophylline
13.	Ipatropium Bromide; Theophylline

Categories of medications included $ß_2$ agonists, anticholinergics, theophyllines and corticosteroids. Most patients in this study were ingesting bronchodilators, anticholinergic agents and in the case of five patients corticosteroids. Individual doses of corticosteroids ranged from 10mg daily to 10mg on alternate days with a mean daily ingestion of 6mg.

(d) Anthropometrical analysis

Anthropometrical data are detailed in Table 3.4.

Table 3.4 Anthropometrical data for patients with COPD and control subjects on entry to the study

Patient	*Ht (cm)*	*Body Mass (kg)*	*BF (%)*	*LTV (l)*
Mean	168	65**	21*	2.76**
SD	9	14	8	0.55
Control				
Mean	174	83	26	3.73
SD	7	10	5	0.66

Abbreviations: cm, centimetres; **kg,** kilograms; **BF,** percentage body fat; **LTV,** lean thigh volume; **l,** litres; **SD,** standard deviation; *, p<0.05 COPD vs control subjects; **, p<0.01 COPD vs control subjects

Body mass, percentage body fat and LTV were significantly lower in the patients with COPD compared to the control group. Height did not differ between the two groups.

(e) The six minute walk test: Exercise performance, cardiovascular and fatigue measurements

All patients and control subjects completed the SMWT.

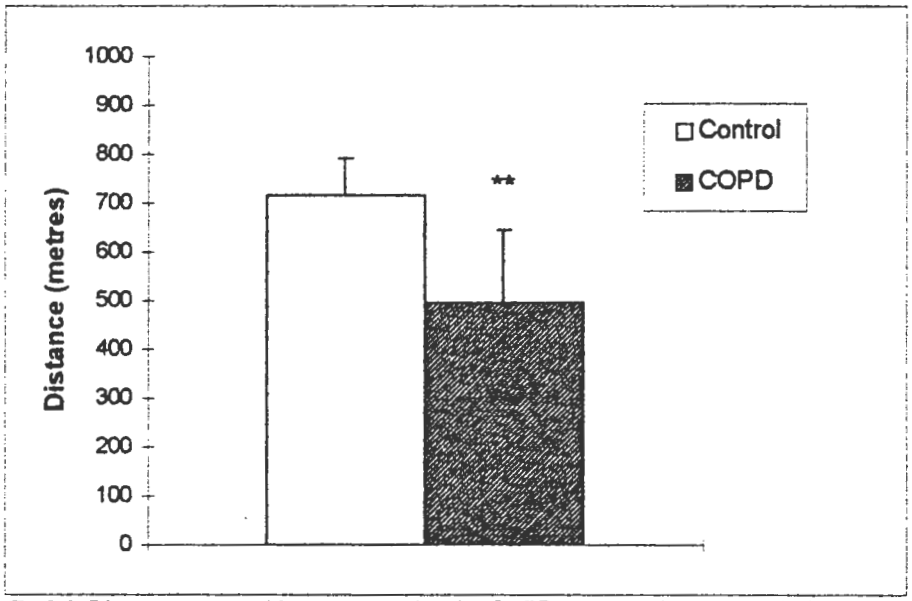

Fig 3.1 Distance covered by the patients with COPD compared to the control group during the six minute walk test (= p<0.01). Data are presented as mean ± SD.**

The distance covered during the SMWT was significantly lower in the patients with COPD compared to the control group (494± 150 vs 718± 75 m, p<0.01) (Fig 3.1). Peak RPE was higher (3.5 ± 2.1 vs 1.3 ± 1.5 Borg units, p<0.01) whilst peak HR tended to be lower in the patients with

COPD compared to the control group (127 ± 21 vs 138 ± 15 b/min; p=0.07) at termination of the SMWT.

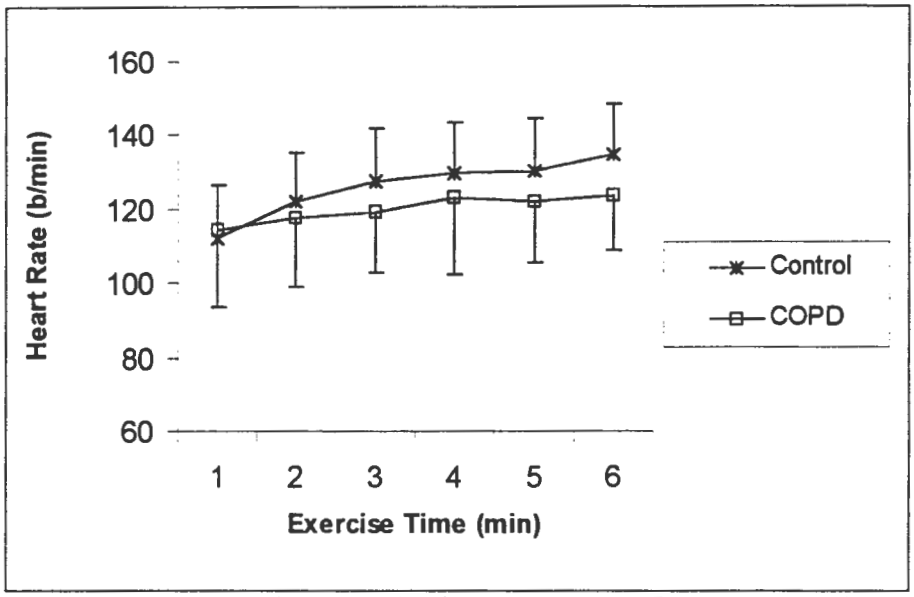

Fig 3.2 Heart rate for the duration of the six minute walk test. Data are presented as mean ± SD.

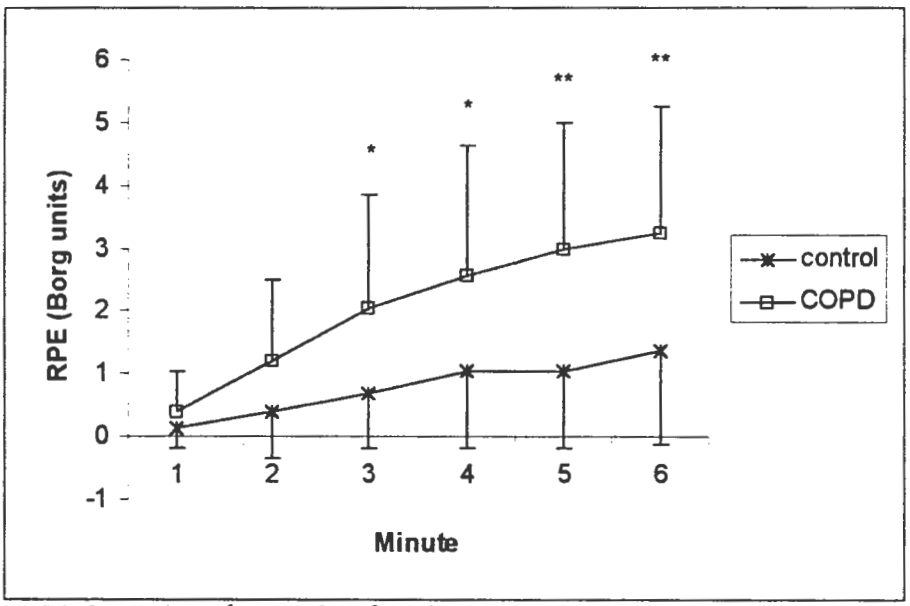

Fig 3.3 RPE for the duration of the six minute walk test (* p<0.05) (p<= 0.01). Data are presented as mean ± SD.**

Heart rate was not different between patients with COPD and control subjects for the duration of the SMWT (Fig 3.2). In contrast, RPE was higher in the patients with COPD compared to controls at minutes three, four, five and six (Fig 3.3) of the SMWT. Three of the 13 patients with COPD reported greater leg fatigue than shortness of breath during the SMWT. All 13 patients reported "tired legs" associated with shortness of breath at termination of the SMWT.

(f) Heart rate, oxygen consumption, minute ventilation and blood lactate concentration at submaximal workloads during the incremental cycle test to exhaustion

Twelve patients and 13 control subjects completed the maximal exercise tests to exhaustion according to the protocol detailed in Chapter Two. One patient did not complete the maximal cycle ergometer test due to equipment malfunction on the day of testing. This patient resided in Johannesburg and was returning that afternoon, thus repeat testing was not possible.

Peak $V0_2$ (17 ±3 vs 25 ±5 $ml0_2$/kg/min; $p<0.01$), V_E (41 ±13 vs 87 ±22l; $P<0.01$) and workload (72 ±37 vs 211 ±69W; $p<0.01$) achieved during the cycle test were significantly lower in patients compared to controls. Comparisons of HR, V_E, $V0_2$ and arterialised blood lactate concentrations were made at the same absolute workloads between groups. Additionally, relative comparisons of V_E, HR and arterialised blood lactate concentrations were made at 25, 50, 75 and 100% $V0_2$ max.

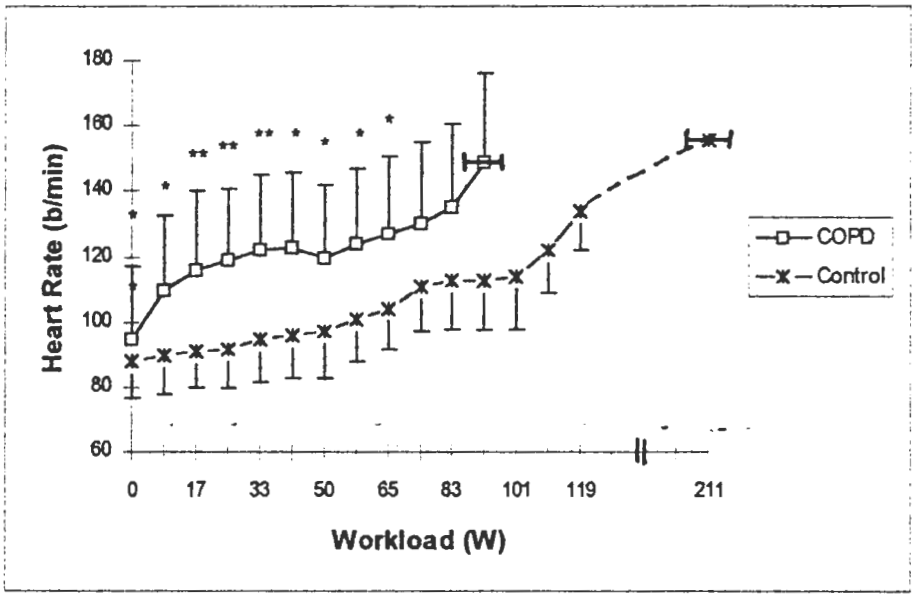

Fig 3.4 Heart rate during the incremental cycle test to exhaustion (p=<0.01; * p= <0.05).**

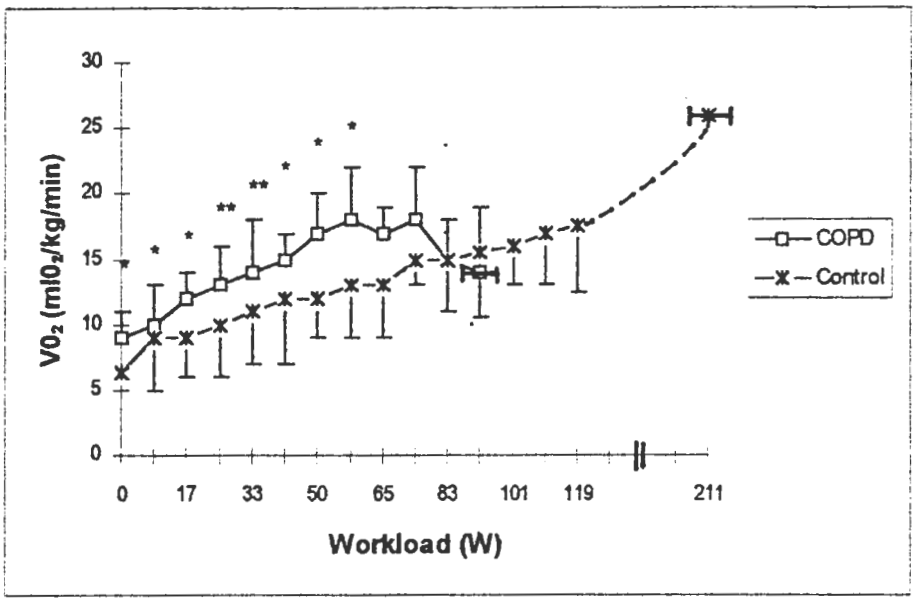

Fig 3.5 $V0_2$ during the incremental cycle test to exhaustion (p=<0.01; * p= <0.05). Data are presented as mean ± SD.**

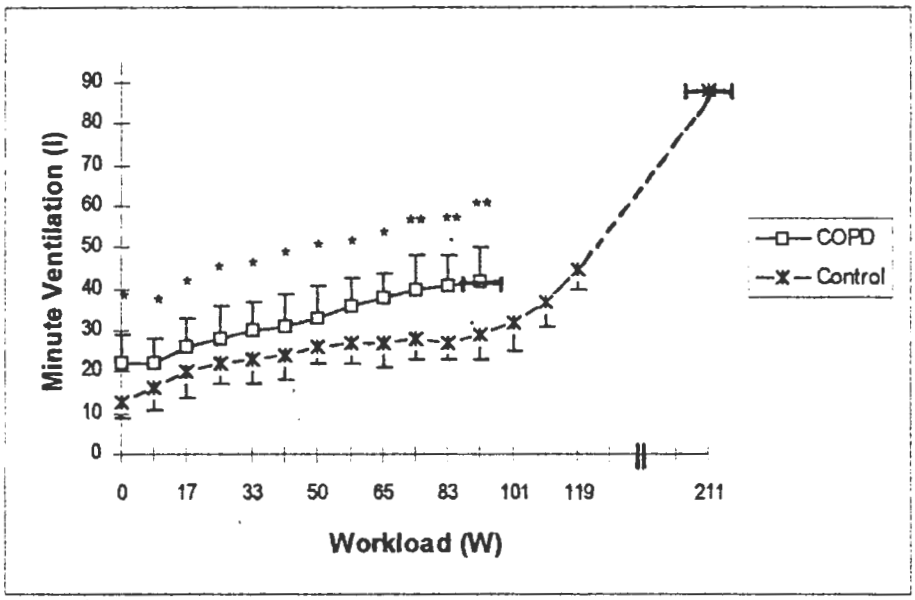

Fig 3.6 Minute ventilation during the incremental cycle test to exhaustion (p=<0.01; * p= <0.05). Data are presented as mean ± SD.**

Heart rate (Fig 3.4), $V0_2$ (Fig 3.5), V_E (Fig 3.6) and arterialised blood lactate concentrations (Fig 3.7) increased over time in response to increased workloads in both groups. However, a comparison of physiological variables at the same absolute workload demonstrated that HR (Fig

3.4), $V0_2$ (Fig 3.5), V_E (Fig 3.6) and arterialised blood lactate concentrations (Fig 3.7) were elevated in the patients with COPD compared to controls during the incremental cycle test to exhaustion. Heart rate was elevated in the patients with COPD compared to controls at the same absolute workload until a workload of 75 Watts; thereafter values were no longer different. $V0_2$ was elevated in the patients with COPD compared to controls until a submaximal workload of 58 Watts; thereafter values were no longer different and in fact $V0_2$ declined in the patients. Arterialised blood lactate concentrations were only different at workloads between 17 to 50 Watts. The most likely reason for lack of significant differences at higher workloads and the decline in $V0_2$ in the patients with COPD after 75 Watts is because seven patients with COPD achieved a maximum workload greater than 42 Watts whilst only four achieved a maximum workload of or greater than 75 Watts. In contrast, all the control subjects exceeded a maximum workload of 75 Watts.

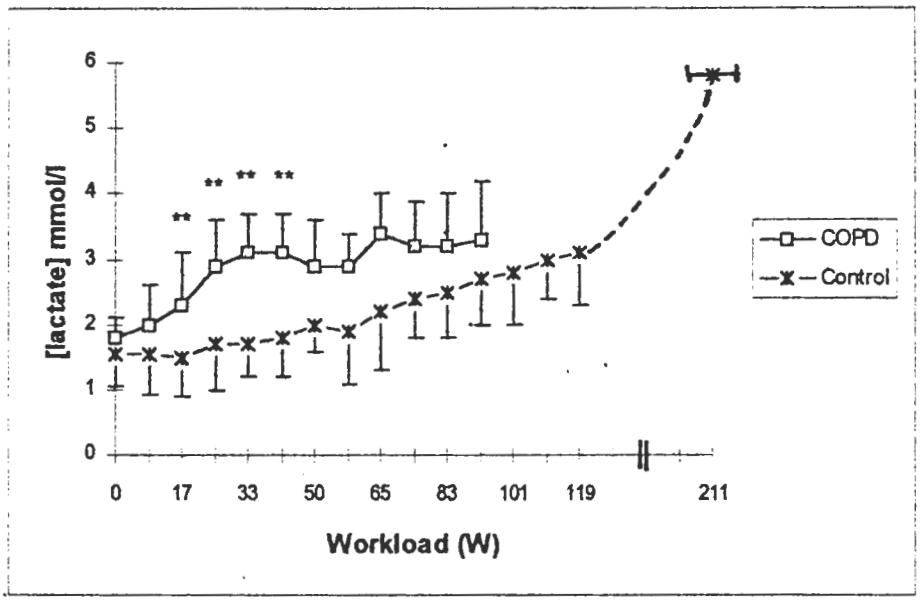

Fig 3.7 Arterialised blood lactate concentrations during the incremental cycle test to exhaustion (p=<0.01). Data are presented as mean ± SD.**

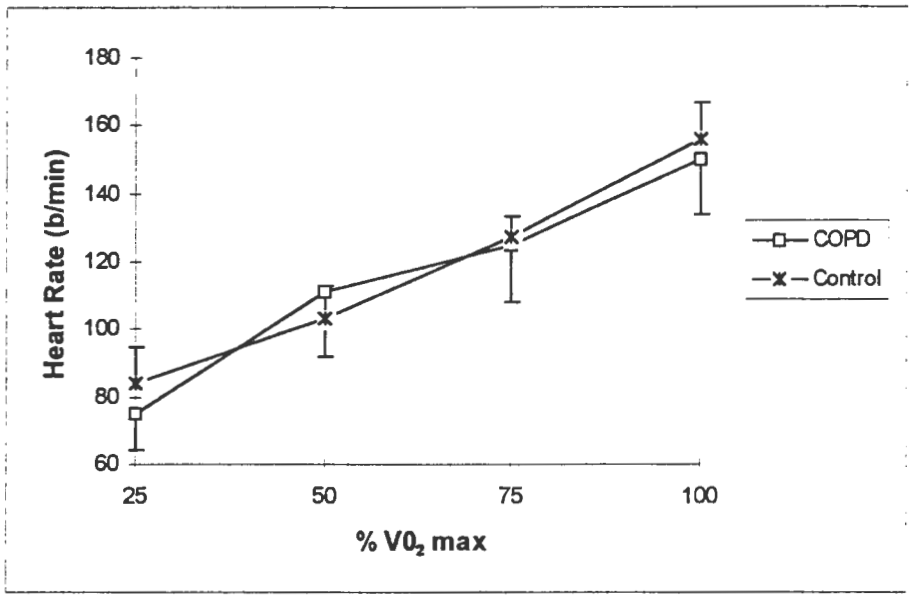

Fig 3.8 Heart rate relative to % $V0_2$ max during the incremental cycle test to exhaustion. Data are presented as mean ± SD.

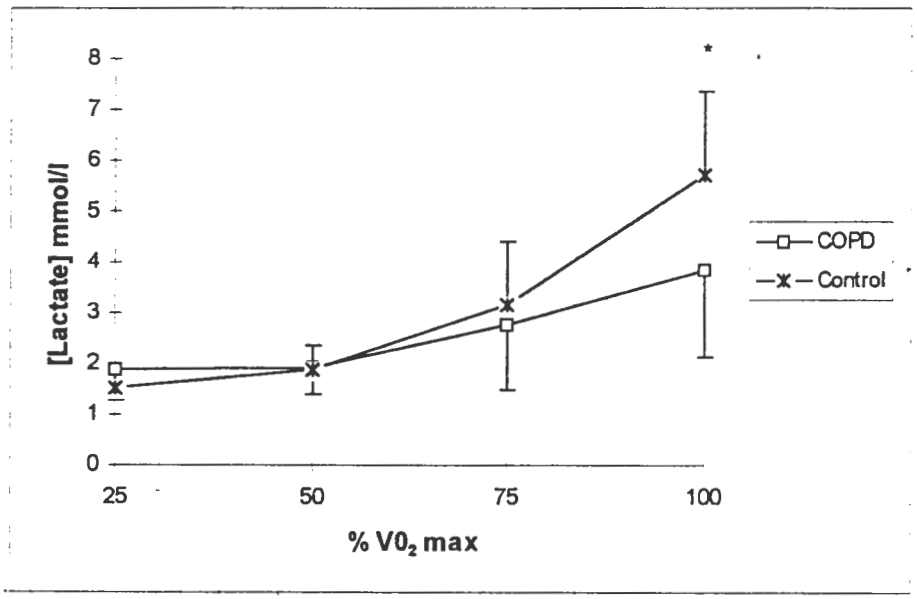

Fig 3.9 Arterialised blood lactate concentrations relative to % $V0_2$ max during the incremental cycle test to exhaustion (* p=<0.05). Data are presented as mean ± SD.

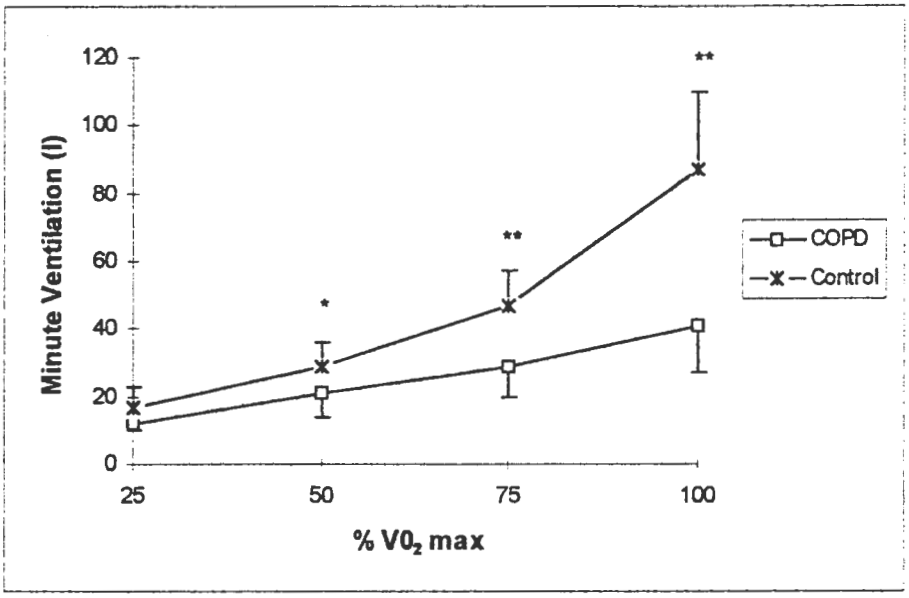

Fig 3.10 Minute ventilation relative to % $V0_2$ max during the incremental cycle test to exhaustion (* p=<0.05; ** p=<0.01). Data are presented as mean ± SD.

In contrast to comparisons made at absolute workloads, comparisons at relative workloads of 25, 50, 75 and 100% of $V0_2$ max demonstrated the following. Firstly, heart rate was not different between the patients with COPD and control subjects during the incremental cycle test to exhaustion (Fig 3.8). Secondly, arterialised blood lactate concentrations were higher at 100% $V0_2$ max (Fig 3.9) and V_E was higher at 50, 75 and 100% of $V0_2$ max (Fig 3.10) in control subjects compared to patients with COPD during the incremental cycle test to exhaustion.

(g) Exercise performance, cardiovascular and respiratory measurements at peak exercise during the incremental cycle test to exhaustion

Cardiorespiratory variables, metabolic responses and perception of effort responses at peak workload are listed in Table 3.5

Table 3.5. Cardiorespiratory measurements, metabolic responses and performance variables at the peak exercise workload during the incremental cycle test to exhaustion in patients with COPD and control subjects

	Exercise time (minutes)	Pk HR (b/min)	Pk SBP (mmHg)	V_E (l/min)	Pk RER (VCO_2/ VO_2)	Pk Post Exercise Plasma [Lactate] (mmol/l)	Pk SaO_2 (%)	Pk VO_2 (mlO_2/kg/min)	Pk RPE (Borg Units)
Patient (mean)	9.9**	150	173*	41.3**	1.1**	5.1**	96.7*	26	6
SD	3.9	16	44	13.5	0.2	2.7	3.0	6	2
Control (mean)	14.4	156	202	87.5	1.4	9.7	98.6	17	5
SD	2.2	11	27	22.1	0.2	2.7	1.2	4	3

Abbreviations: Pk, peak; SBP, systolic blood pressure; HR, heart rate; V_E, minute ventilation; RER, respiratory exchange ratio; Post Exercise Plasma [Lactate]; highest arterialised blood lactate concentration in the five minutes immediately after exercise; SaO_2, saturation of oxygen in arterialised blood; RPE, rating of perceived exertion; *, p<0.05 COPD vs Control; ** p<0.01 COPD vs Control; SD, standard deviation.

VO_2 peak (Fig 3.5), peak workload (Fig 3.4), peak SBP (Table 3.5), peak V_E (Fig 3.6/Table 3.5), peak RER, peak arterialised blood lactate concentrations (Table 3.5) and exercise time to exhaustion (Table 3.5) were lower in the patients with COPD compared to control subjects.

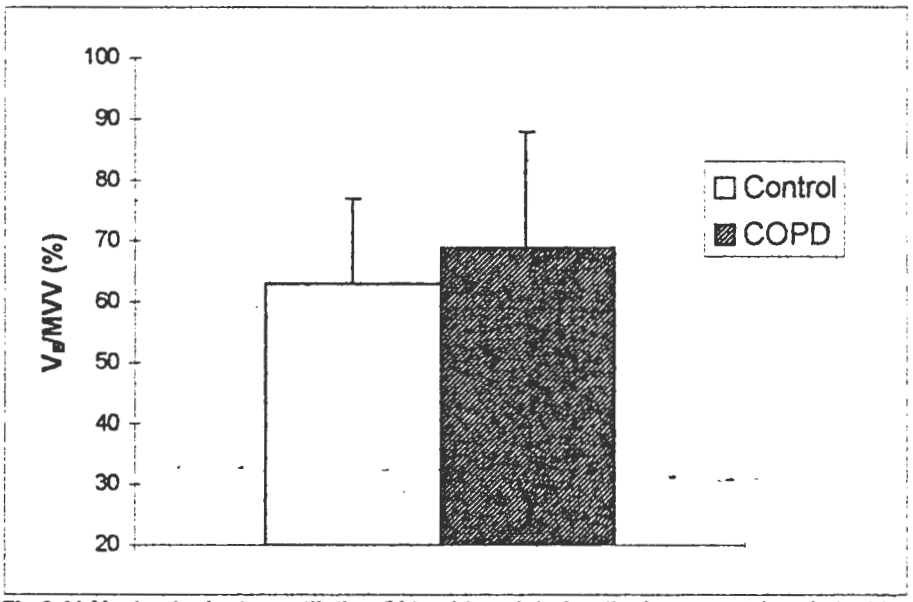

Fig 3.11 Maximal minute ventilation (V_E) achieved during the incremental cycle test to exhaustion as a percentage of maximum voluntary ventilation (MVV) measured at rest. Data are presented as mean ± SD.

Peak V_E recorded during the cycle test calculated as a percentage of MVV measured at rest, was not different between patients with COPD and the control group (Fig 3.11).

Patient arterialised blood gas results at peak workloads are presented in Table 3.6.

Table 3.6. Arterialised blood gas results in patients with COPD and control subjects at peak exercise during the Incremental cycle test to exhaustion.

Patient No:	pH at peak exercise	Lowest $Sa0_2$ (%)	Rest $Sa0_2$ (%)
1	7.40	89	93
2	7.29	89	99
3	7.29	87	95
4	7.38	90	94
5	7.34	87	91
6	-	93	98
7	7.45	98	100
8	7.41	98	-
9	7.32	96	96
10	7.31	92	98
11	7.32	86	93
12	-	-	93
13	7.34	81	91
Mean	**7.35#**	**90 ****	**95.08**
SD	**0.05**	**5**	**3.09**
Controls			
Mean	**7.40**	**95**	**99-100 (norm)**
SD	**0.07**	**3**	**-**

Abbreviations: pH at peak exercise, highest pH recorded during the maximal cycle test to exhaustion; Lowest $Sa0_2$,desaturation level measured during the maximal cycle ergometer test to exhaustion; Rest $Sa0_2$, resting oxygen saturation level prior to start of the cycle test; ** ,p= <0.01 COPD vs control subjects; # , p=0.09 COPD vs control; -, no data obtained for that subject due to difficulty obtaining blood sample or the patient did not participate in a maximum cycle test to exhaustion; SD, standard deviation, norm; norms (West; 1992).

The highest recorded pH tended to be lower (more acidic) in patients with COPD compared to the control subjects. $Sa0_2$ measured by fibreoptic ear oximetry during the maximum cycle test to exhaustion was significantly lower in the patients with COPD compared to the control subjects. Six of the 12 patients with COPD who participated in the incremental cycle test to exhaustion became hypoxaemic to below 90%. Four of the five patients who were hypoxaemic at rest dropped their saturation levels to below 90% during the maximal cycle test. The fifth subject who was

hypoxaemic at rest did not participate in the maximum cycle test. Two subjects who were not hypoxaemic at rest presented with saturation levels below 90% during the cycle test.

To examine the effect of desaturation ($Sa0_2$ below 90%) on exercise tolerance within the group of 12 patients with COPD, the six patients who became hypoxaemic below 90% during the cycle test were compared to the six patients who maintained saturation above 90% during the cycle test. No differences were found between peak $V0_2$ (16 ±4 vs 19 ±4 $ml0_2$/kg/min;NS), peak time achieved (8 ±4 vs 12 ± 3 min; NS) and peak watts achieved (104 ±20 vs 80 ±25, those who maintained saturation vs those who became hypoxaemic; NS) between these two groups. However, RPE was significantly higher in the six patients who became hypoxaemic (4.67 ±1.25 vs. 2 ±1.83 Borg units;p=0.05) compared to the six patients with COPD who maintained saturation during the incremental cycle test to exhaustion.

Six patients terminated exercise due to leg muscle fatigue and six patients terminated due to shortness of breath. The six patients who terminated exercise due to shortness of breath were not the same six patients who became hypoxaemic during exercise. Nine control subjects terminated exercise due to general fatigue, whilst two control subjects experienced excessive leg fatigue. Two of the control subjects' tests were terminated by the physician in attendance due to high systolic blood pressure during exercise.

(h) Skeletal muscle function

(i) Skeletal muscle strength

Peak isokinetic torque of the quadriceps was significantly lower in the patients with COPD compared to the controls (Fig 3.12). Isokinetic peak torque of the hamstrings tended to be lower in the patients with COPD compared with controls (Fig 3.13). However, once corrected for LTV, values were no longer significantly different between groups for either quadriceps (Fig 3.12) or hamstrings peak torque (Fig 3.13).

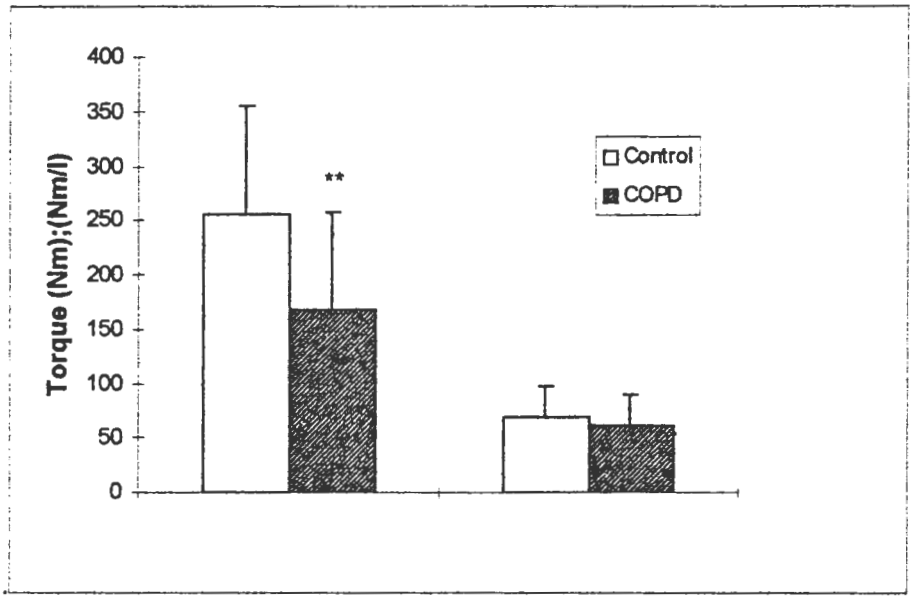

Fig 3.12 Absolute peak torque and peak torque relative to lean thigh volume achieved by the quadriceps muscle during the isokinetic strength test in respective order from left to right (p<0.01). Data are presented as mean ± SD.**

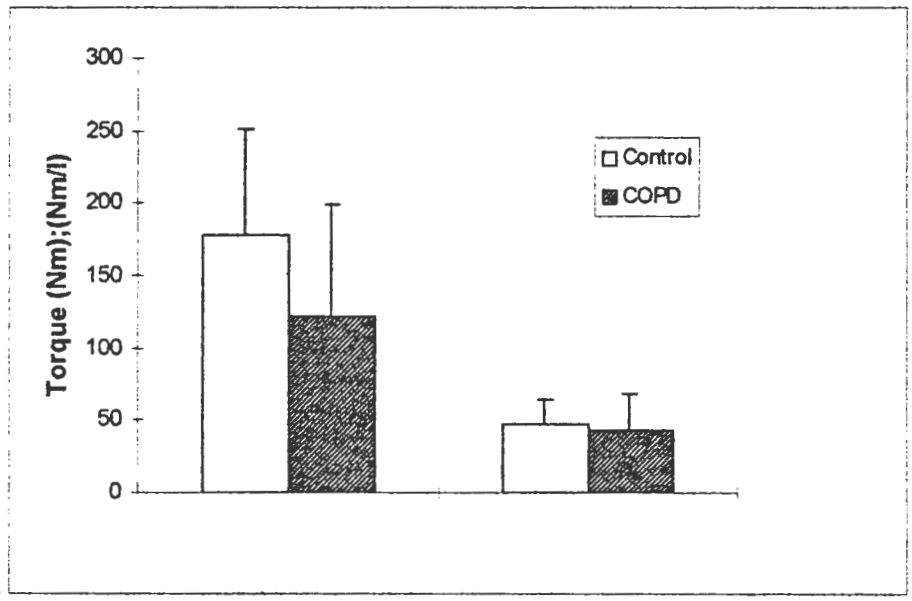

Fig 3.13 Absolute peak torque and peak torque relative to lean thigh volume achieved by the hamstring muscle during the isokinetic strength test in respective order from left to right. Data are presented as mean ± SD.

(ii) Skeletal muscle endurance

Work done during 30 repetitive contractions by the quadriceps was significantly lower in the patients with COPD compared to the control group (Fig 3.14). Even when corrected for LTV, the work done by the quadriceps remained significantly lower in the patients with COPD compared to the control subjects (Fig 3.14).

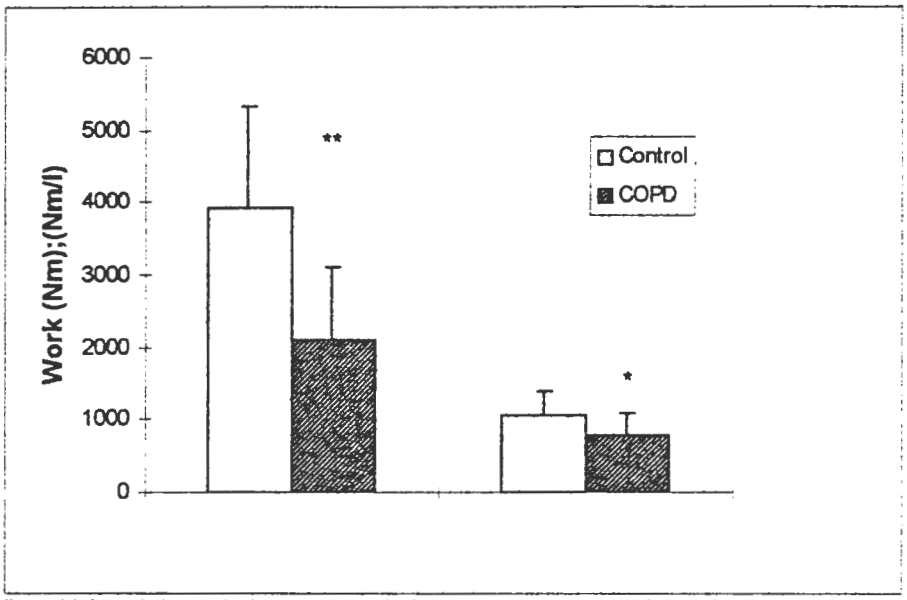

Fig 3.14 Absolute work done and work done relative to lean thigh volume by the quadriceps muscle during the isokinetic endurance test. (, P<0.01 COPD vs Control; * P<0.05 COPD vs Control). Data are presented as mean ± SD and in respective order from left to right.**

Total power generated by the quadriceps during 30 repetitive contractions was lower in the patients with COPD compared with the controls (Fig 3.15). Once corrected for LTV, differences tended towards significance (34 ±15 vs 44 ±13 Nm/l; COPD vs Control; p=0.09).

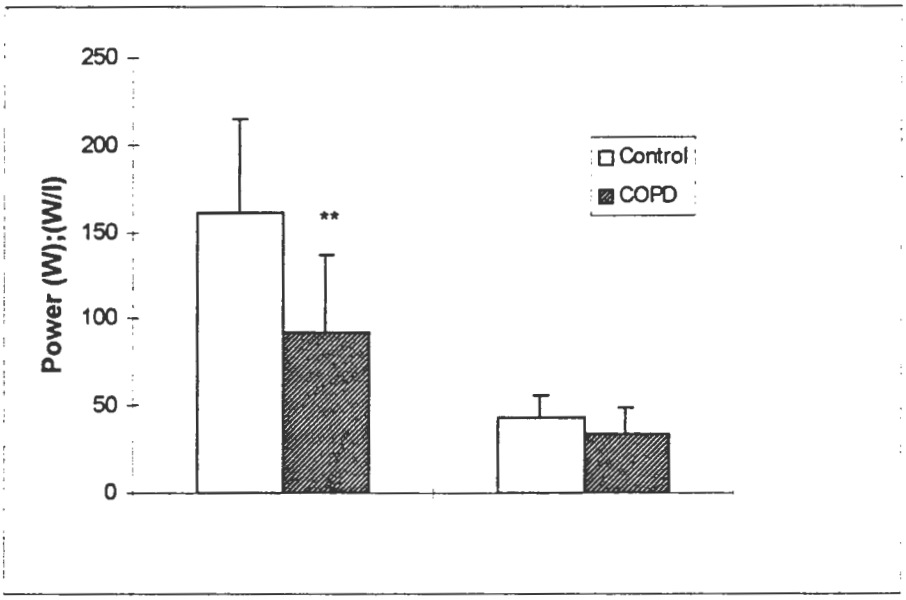

Fig 3.15 Absolute total power and total power relative to lean thigh volume produced by the quadriceps muscle during the isokinetic endurance test. (, P<0.01 COPD vs Control). Data are presented as mean ± SD.**

Peak torque achieved during the isokinetic endurance test was lower in the patients with COPD compared to the controls, prior to (114± 59 vs 187±72 Nm; p<0.01) but not after correction for LTV (42 ±19 vs 51± 21Nm/l; p=NS). Peak torque achieved by the hamstrings during the isokinetic tests of skeletal muscle endurance was not different between groups either before (100±63 vs 141± 60Nm; COPD vs Control; p=NS) or after correction for LTV (37± 22 vs 38± 15Nm/l, COPD vs Control; p=NS).

(iii) Eccentric skeletal muscle function

Eccentric peak torques generated by the quadriceps and the hamstring muscles (Fig 3.16) were lower in the patients with COPD compared to controls. This difference persisted after correction for LTV in the quadriceps (0.9 ±1.2 vs 2.6±0.7Nm/l; COPD vs Control; p<0.05) and in the hamstrings (0.8 ±1 vs. 2.1 ±0.6 Nm/l; COPD vs Control; p=0.05).

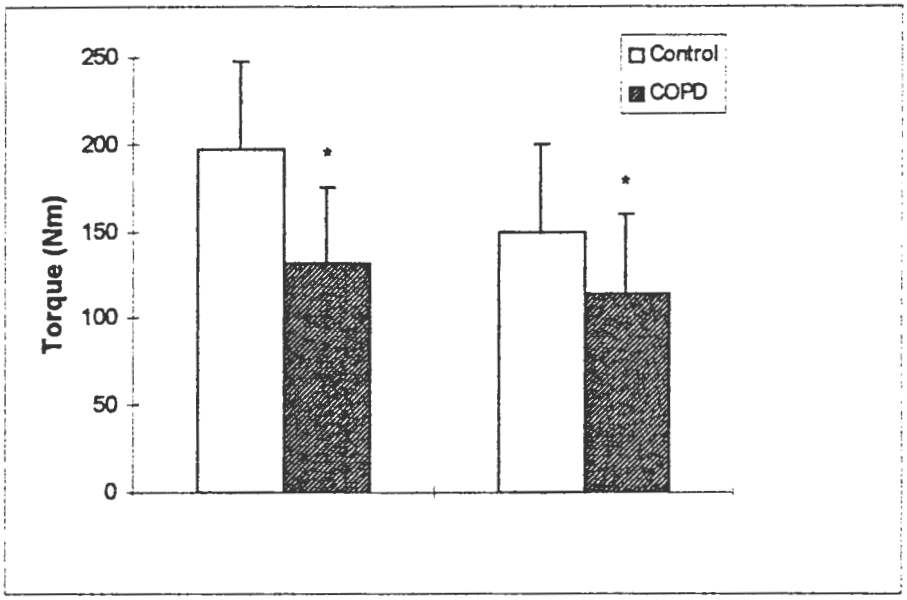

Fig 3.16 Eccentric peak torque generated by the quadriceps and the hamstrings respectively during the eccentric isokinetic muscle test (*, p<0.05 COPD vs Control). Data are presented as mean ± SD.

(j) Results of correlation analysis between measures of exercise tolerance and tests of peripheral skeletal muscle function in patients with COPD.

Correlation analyses was performed between measures of exercise performance (distance covered during the SMWT and exercise time, peak $V0_2$ and peak Watts achieved during the incremental cycle test to exhaustion) and tests of skeletal muscle function in the 12 patients with COPD who completed the incremental cycle test to exhaustion. The results of the correlation analyses are presented in Table 3.7

Table 3.7 Results of a correlation analysis between measures of exercise ability and tests of peripheral skeletal muscle function in thirteen patients with COPD.

	Performance Variables			
Peripheral SM function measurements	**Exercise Duration (mins)**	**Pk $V0_2$ ($ml0_2/kg/min$)**	**Pk WL (W)**	**Dist SMWT (m)**
PKTQ (Nm) (Strength)	r=0.60 n=12 p=<0.05	NS	r=0.65 n=12 p=<0.05	r=0.54 n=12 p=0.05
PKTQ (Nm) (Endurance)	r=0.62 n=12 p=<0.05	NS	r=0.76 n=12 p=<0.01	r=0.56 n=12 p=<0.05
TPQ(Nm)	r=0.64 n=12 p=<0.05	NS	r=0.87 n=12 p=<0.01	r=0.59 n=12 p=<0.05
WDQ (W)	r=0.66 n=12 p=<0.01	NS	r=0.81 n=12 p<0.05	r=0.61 n=12 p=<0.02
ECCQ (Nm)	r=0.83 n=6 p=0.08	NS	NS	r=0.89 n=6 p=0.01

Abbreviations: SM, skeletal muscle; **Pk,** peak; **WL,** workload; **W,** Watts; **Dist,** distance; **SMWT,** six minute walk test ; **m,** metres; **PKTQ (strength),** peak torque achieved by the quadriceps during the isokinetic strength test; **PKTQ (endurance),** peak torque achieved by the quadriceps during the isokinetic endurance test; **Nm,** Newton metres; **TPQ,** total power produced by the quadriceps during the isokinetic endurance test; **WDQ,** work done by the quadriceps during the isokinetic endurance test; **ECCQ,** peak torque produced by the quadriceps during the isokinetic eccentric strength test; **NS,** not significant.

Strong significant correlations were found between work done by the quadriceps, total power produced by the quadriceps during the isokinetic endurance test and peak watts achieved during the incremental cycle test to exhaustion (Table 3.7). Similarly a strong correlation was found between the peak torque generated by the hamstrings during the isokinetic endurance test and peak watts achieved during the incremental cycle test to exhaustion (Table 3.7). Additionally strong correlations were found between the test of eccentric skeletal muscle function and distance covered during the SMWT (Table 3.7). Weaker, but still significant correleations were found between various other exercise performance variables and the tests of skeletal muscle function (Table 3.7). The peak $V0_2$ achieved during the incremental cycle test to exhaustion did not correlate with any of the tests of skeletal muscle function.

4. Discussion

The first important finding of this study was that exercise tolerance was significantly reduced in the patients with COPD during the incremental cycle test to exhaustion and during the SMWT. This finding would support current findings investigating exercise intolerance in patients with COPD (Gallagher et al., 1990; Olopade et al., 1992; Belman 1993; Cooper et al., 1994; Redelmeier et al., 1997). As reported by other authors, V_E, HR, $V0_2$ and arterial blood lactate concentrations were significantly higher at the same submaximal workloads in the patients with COPD during the incremental cycle test to exhaustion (Gallagher et al., 1990; Olopade et al., 1992; Belman 1993; Cooper et al., 1994; Redelmeier et al., 1997). However, when these variables were compared between groups at the same % of maximum $V0_2$, results could be interpreted differently. Arterialised blood lactate concentrations at 100% $V0_2$ max and V_E at 50%, 75% and 100% $V0_2$ max were higher in controls than in the patients, whilst HR was not different between groups. These findings suggest that at the same submaximal workload, patients with COPD are working at a higher percentage of their maximum. Therefore, physiological variables such as V_E, heart rate and arterialised blood lactate concentration are higher. The reported findings of higher physiological variables at submaximal workloads in patients with COPD compared to controls has led to the conception that the exercise intolerance in patients with COPD is due to central factors such as impaired ventilation mechanics (Mahler et al., 1988), impaired gaseous exchange (Degre et al., 1978), increased blood lactate accumulation (Sue et al., 1988) and cardiovascular factors (Fujii et al., 1996). However, when physiological variables are analyzed at the same relative workload, the findings of this study suggest that only V_E may have played a role in the exercise limitation seen in these patients with COPD. V_E was the only variable that remained significantly and consistently lower in patients with COPD when compared with controls at the same relative workload. This would imply a possible limitation in ventilatory mechanics. Findings from this study therefore suggest that the traditional interpretation that increased blood lactate accumulation (Sue et al., 1988) and cardiovascular factors (Fujii et al., 1996). may not be the primary limiting factors during exercise in these patients.

Thus, in order to assess the suggestion that ventilation mechanics may be a limiting factor in the exercise intolerance in the patients investigated in this study, V_E during exercise was measured. If

ventilation mechanics were limiting the patients with COPD during the incremental cycle test, a plateau of V_E towards termination of exercise would be expected. However, no plateau was evident. A large number of the patients achieved their maximum V_E in recovery. The patients were therefore capable of greater ventilation mechanics in recovery than during the exercise test. This may be a reflection of a limitation to ventilatory mechanics during exercise in the patients with COPD.

Additionally, to determine the contribution of ventilatory mechanics to exercise intolerance in patients with COPD, MVV at rest was measured. The average peak V_E achieved for the patients during the incremental cycle test to exhaustion, as a percentage of the average MVV measured at rest was 69%. For the controls it was 62%. If ventilation mechanics were limiting the patients, a V_E at peak exercise close to 100% of the MVV measured at rest would be expected (Gallagher et al., 1990). This further supports the argument that ventilation mechanics are unlikely to be the primary cause of exercise intolerance in patients with COPD. Guyatt (1990) argued that V_E/MVV was not a good measure of the role of ventilatory mechanics during exercise in patients with COPD. He argued that this measure did not consider possible bronchodilation in response to exercise in these patients which would result in an ability to achieve a higher V_E during exercise than MVV at rest. However, pre and post exercise spirometry was conducted on the patients in this study and none of the patients displayed bronchodilation immediately post exercise. It therefore seems unlikely that bronchodilation would have aided V_E during exercise in the patients in this study.

It therefore seems that although ventilatory mechanics may play a role in limiting exercise tolerance in patients with COPD, it seems unlikely that they are the major contributing factor.

Secondly, in order to assess the role of gas exchange in exercise intolerance in patients with COPD, the six patients who became hypoxaemic during the incremental cycle test to exhaustion were compared to the six patients who did not become hypoxaemic. No differences were found for time to exhaustion, peak Watts achieved or peak $V0_2$ achieved during the cycle test between groups. These finding suggest that gas exchange was not a major determining factor for exercise termination in the patients with COPD.

Additionally, the average lowest saturation level for the patients with COPD during exercise was 90%. This is not a level low enough to suggest a pulmonary limitation to exercise. If the literature is reviewed these findings are supported by results indicating no changes reported in $Pa0_2$ concentrations (an indicator of the efficacy of gas exchange) before and after training, yet improved exercise tolerance in patients with COPD (Chester et al., 1977).

RPE was, however, significantly higher in the patients who became hypoxaemic and saturation levels may therefore play a role in perception of fatigue in patients with COPD.

Thirdly, to determine the role of blood lactate accumulation in exercise intolerance in the patients with COPD, arterialised blood lactate concentrations were measured during the incremental cycle test to exhaustion and for the first five minutes of recovery. Other authors have attributed early exercise termination in patients with COPD to excessive blood lactate accumulation at submaximal workloads (Sue et al., 1988). However, although patients responded with higher concentrations of blood lactate at the same absolute workload compared to controls, they terminated exercise at peak blood lactate concentrations significantly lower than the control subjects. In addition, peak blood lactate concentrations during recovery were higher in control subjects compared to patients and blood lactate concentrations at the same relative workload (% $V0_2$ max) were significantly higher in control subjects. If excessive blood lactate accumulation was limiting exercise tolerance in these patients, one would expect higher concentrations of blood lactate at termination of exercise and higher concentrations of blood lactate at the same relative workload in the patients with COPD. This strongly suggests that elevated blood lactate concentrations were not responsible for exercise intolerance and early exercise termination in the patients investigated in this study.

The patients investigated in this study were not in cardiac failure as determined by the medical examination on entry to the study. Therefore it is unlikely that cardiovascular limitations attributed to their exercise intolerance.

Physical inactivity is often hypothesised as the aetiological factor causing exercise intolerance in patients with COPD (Olopade et al., 1992) and other chronic diseases. In an attempt to consider the role of inactivity in exercise intolerance, a physical activity questionnaire was completed. It is an important and interesting finding of this study that although activity levels tended to be different between the patients with COPD and the controls (p=0,07) differences were not statistically significant. When considering the role of inactivity in patients with COPD it is important to consider socio-economic status. Many of the patients came from a socio-economic group in which inactivity would create impossible economic consequences. Patients were dependent on their physical ability for both transport and employment. Many of the patients therefore remained active, despite the physical debilitations of COPD. It would therefore appear that inactivity alone could not account for the exercise intolerance experienced by the patients in this study.

Thus the traditionally central factors of impaired lung mechanics, impaired gas exchange, excessive blood lactate accumulation, physical inactivity and cardiac failure could not account for the exercise intolerance seen in the patients with COPD in this study. In contrast, peripheral factors (skeletal muscle) did appear to contribute to the exercise intolerance in these patients.

Firstly, a number of patients reported leg fatigue during both the incremental cycle test to exhaustion and during the SMWT. Secondly, the correlation analysis demonstrated significant correlations between the various measures of skeletal muscle function and performance variables including: exercise time to exhaustion during the cycle test, peak workload achieved during the cycle test and maximum distance walked during the SMWT. In contrast, peak $V0_2$ (traditionally a measure of heart and lung capacity during exercise) did not correlate with the measures of skeletal muscle function. Finally, the tests of skeletal muscle function demonstrated functional disabilities in the skeletal muscle of the patients with COPD. These functional disabilities were seen during tests of skeletal muscle strength and skeletal muscle endurance. The tests of skeletal muscle function were not limited by central factors as only a small and specific muscle group was active at the time of testing (McArdle et al., 1991). These findings therefore suggest that skeletal muscle is an important contributor to exercise intolerance in patients with COPD.

The existence of skeletal muscle abnormalities in patients with COPD has been suggested by some preliminary research papers (Decramer et al., 1994; Maltais et al., 1997). In addition, skeletal muscle abnormalities have been reported in patients with other chronic diseases (Horber et al., 1986; Horber et al., 1987; Lipkin et al., 1988; Kempeneers et al., 1990; Buller et al., 1991; Minotti et al., 1991; Derman et al., 1992; Diesel et al.,1992; Magnusson et al., 1994)

The patients with COPD in this study presented with significantly lower strength in the quadriceps whilst hamstring strength tended to lower ($p=0.07$) compared to control subjects. Once corrected for LTV, these values were no longer different between groups. This finding suggests that maximal force production per unit of cross sectional area of the skeletal muscle is not different between the patients with COPD and the control subjects. However, due to skeletal muscle wasting seen in patients with COPD, absolute strength of the skeletal muscle is reduced compared to control subjects. This has previously been documented in patients with renal failure (Diesel et al., 1992) and patients with cardiac failure (Lipkin et al., 1988; Kempeneers et al., 1990; Buller et al., 1991; Minotti et al., 1991; Derman et al 1993; Magnusson et al., 1994). It is therefore possible that this finding is common to various chronic diseases.

In contrast to the skeletal muscle strength in the patients with COPD examined in this study, the skeletal muscle endurance was decreased both before and after correction for LTV compared to the control group. This finding suggests that during repetitive contractions of the peripheral skeletal muscle these patients demonstrated a decreased resistance to fatigue. Decreased resistance to fatigue was not due to decreased muscle mass as it persisted once corrected for LTV. Repetitive skeletal muscle contractions of this nature would be performed during activities such as walking and cycling (Jones et al., 1990).. Therefore, it is likely that this decreased resistance to fatigue plays a role in limiting exercise tolerance in patients with COPD. This decreased resistance to fatigue in the skeletal muscle has been reported in patients with renal failure (Diesel et al., 1992) and in patients in cardiac failure (Lipkin et al., 1988; Minotti et al., 1991; Derman et al., 1992) indicating once again that these skeletal muscle abnormalities may be common to various chronic diseases. Additionally, the functional abnormalities seen in the skeletal muscle of the patients with COPD in this study would correspond well with the metabolic abnormalities reported by other

authors in the skeletal muscle of these patients (Jacobbson et al., 1990; Maltais et al., 1997). These findings strengthen the argument that skeletal muscle may contribute to exercise intolerance in patients with COPD.

Lastly, two interesting findings emerge from this study. Firstly, although the hamstrings tended to be absolutely weaker in the patients compared to controls, the difference was not statistically significant. The quadriceps muscles are known to atrophy rapidly whilst the hamstrings are more resistant to atrophy in human subjects (Dubowitz et al., 1985). It is therefore possible that the muscle weakness observed in patients with COPD has not yet reached the stage where the hamstrings have atrophied sufficiently to cause a measurable strength deficit.

Secondly, eccentric strength of the hamstrings and the quadriceps was significant lower in the patients with COPD, both before and after correction for LTV. These results suggest, firstly, a reduced contraction ability during eccentric contractions in the skeletal muscles of the patients. Secondly, that maximal force production per unit of cross sectional area is not within the normal range during eccentric contraction in patients with COPD compared to controls. The importance of eccentric muscle contraction in activities of daily living is established in the normal population (Jones et al., 1990). Therefore, it is likely that eccentric muscle contractions are important in daily living activities of the patients with COPD. However, to our knowledge no other studies have examined eccentric muscle strength in patients with COPD. The sample size used to analyse the eccentric skeletal muscle functional ability in this study was small. This should be considered when interpreting these findings. The results of this preliminary study of the eccentric skeletal muscle in patients with COPD suggest that further research into this area is warranted.

In conclusion, patients with COPD appear to be limited by peripheral rather than central factors during exercise. Strong evidence to support this conclusion was demonstrated during the incremental cycle test to exhaustion, the SMWT and the isokinetic tests of peripheral skeletal muscle strength and endurance. Additional evidence was provided by correlations between various measures of skeletal muscle function and exercise performance variables in the twelve patients with COPD. This conflicts with the more traditional view that exercise tolerance in patients

with COPD is limited by central cardiac, metabolic and respiratory factors (Fujii et al., 1996; Tobin et al., 1988; Belman 1992; Matthay et al., 1992; Patessio et al., 1993).

CHAPTER FOUR

STRUCTURAL ABNORMALITIES OF SKELETAL MUSCLE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE; RELATIONSHIP TO SKELETAL MUSCLE FUNCTION

1. Introduction

In Chapter Three of this dissertation two important concepts were demonstrated. Firstly, functional abnormalities exist in the skeletal muscle of patients with COPD. Secondly, these functional abnormalities play a role in exercise intolerance in patients with COPD. Additionally, in Chapter Three the existence of metabolic abnormalities in the skeletal muscle of patients with COPD was documented (Tada et al., 1992; Wuyam et al., 1992; Thompson et al., 1992; Maltais et al., 1997). These findings therefore suggest that functional and metabolic abnormalities exist in the skeletal muscle of patients with COPD and that these abnormalities may play a role in exercise intolerance. Accordingly, investigation of the structure or histology of such muscle in patients with COPD is warranted.

Very few studies have investigated the histological or structural status of the peripheral skeletal muscle of patients with COPD. Some preliminary evidence is however available. Jacobbson et al., (1990) reported a decline in the percentage of Type I fibres in the quadriceps femoris muscle of eight patients with COPD in respiratory failure and 11 patients not in respiratory failure. Skeletal muscle histological abnormalities have been demonstrated in patients in renal failure and those in cardiac failure (Lipkin et al., 1988; Kempeneers et al., 1990; Buller et al., 1991; Minotti et al., 1991; Derman et al., 1992; Magnusson et al., 1994). However, most studies have focused on metabolic and not structural abnormalities in the peripheral skeletal muscle of patients with COPD.

Therefore, to the best of our knowledge, no studies have provided a detailed histological description of the skeletal muscle of patients with COPD or examined whether the functional abnormalities evident in the skeletal muscle of these patients have a relationship to structural abnormalities in the skeletal muscle.

Therefore the aims of this study were firstly to provide a detailed description of the histological, ultrastructural and morphological status of skeletal muscle in a group of patients with COPD and to compare the morphology, histology and ultrastructure to age matched controls. Secondly, to quantify skeletal muscle structural changes and finally, to determine if a relationship exists between skeletal muscle structural status and (i) skeletal muscle functional ability and (ii) general

exercise intolerance of patients with COPD.

2. Methodology

(a) Skeletal muscle biopsy procedures

Twelve of the 13 patients with COPD described in Chapter Three consented to a skeletal muscle biopsy. Skeletal muscle biopsies of the vastus lateralis muscle were performed according to the methods described in Chapter Two. One biopsy produced inadequate skeletal muscle sample and a second biopsy produced enough sample for light microscopy only. Therefore 11 biopsy samples were successfully obtained for analysis by light microscopy and ten for analysis by electron microscopy from patients with COPD. Eleven control subjects consented to a skeletal muscle biopsy.

(b) Morphometric analysis of light microscopy samples

The investigator completed a morphometric analysis of the light microscopy skeletal muscle biopsy samples according to the methods detailed in Chapter Two. Analysis of skeletal muscle fibre type, size, distribution and predominance was performed in a randomized order. The investigator had no prior knowledge of which samples were from patients with COPD and which samples were from control subjects at the time of analysis.

(c) Histological scoring of skeletal muscle biopsy samples

The specimens were examined initially to ascertain the existence of a pathology and the features of the pathology observed. A skeletal muscle histological scoring system was then used to quantify the pathology observed in the skeletal muscle of the patients with COPD and the control subjects. The skeletal muscle biopsy samples for light microscopic analysis from both patients and controls subjects were numbered, randomized and examined by a histopathologist and the investigator both of whom had no knowledge of whether biopsies were from a control subject or a patient. The scoring system used by Derman et al., (1992) was modified to be made appropriate for the changes that were being observed in the skeletal muscle biopsies being examined. Each specimen was closely examined for the presence of the following characteristics: fibre atrophy,

necrosis, fibre splitting, presence of moth-eaten fibres, fibrosis, nuclear abnormalities including presence of nuclear chains, knots, vesicular and internal nuclei and the presence of ragged red fibres. Each identified abnormality was graded according to the following system; 1 = mild, 2 = moderate, 3 = severe. The skeletal muscle pathology scores were then summed for each specimen.

(d) Electron microscopy analysis of skeletal muscle biopsy samples

Skeletal muscle biopsy samples were then analyzed under electron microscopy by the histopathologist. The histopathologist examined and reported on the biopsy samples in a randomized order.

3. Results

(a) Skeletal muscle fibre morphometry

Skeletal muscle morphometric analysis is displayed in Table 4.1. Morphometric analysis of the skeletal muscle biopsy samples revealed a mean Type II fibre size significantly smaller in patients with COPD compared to the control group (45± 5; range 41 - 55μm; vs 58± 8; range 44 – 66 μm; p =0.01) The mean size of the Type I fibres was not different between the two groups. Two patients had more than 80% Type II fibres which in the vastus lateralis muscle indicates a predominance of Type II fibres (Dubowitz 1985). No control subjects demonstrated a predominance of either fibre type. The average Type II fibre percentage for the patients with COPD tended to be higher than established norms for both genders. Two patients had an average Type I fibre size of greater than 70μm, which indicates possible hypertrophy (Dubowitz 1985). None of the controls had an average Type I fibre size of greater than 67μm.

Table 4.1 Morphometric analysis of Type I and II fibres in the skeletal muscle of patients with COPD compared to the control group and to established norms.

		Fibre size (μm)				
Patient	**Gender**	**Type I**	**Type II**	**Type I (%)**	**Type II (%)**	**Predominance**
1	F	67	43	12	88	Type II
2	M	45	43	43	57	-
3	M	56	44	29	71	-
4	M	73	41	22	78	-
5	M	40	43	28	70	-
6	M	60	44	35	65	-
8	M	76	52	30	70	-
9	M	55	48	34	66	-
10	M	46	42	49	51	-
11	F	56	43	12	88	Type II
13	M	56	55	30	70	-
Mean		**57**	**45****	**30**	**70**	
SD		**11**	**5**	**12**	**12**	
NORMS (Dubowitz 1985)		Males = 61 Females = 53	Males = 62-69 Females = 42-52	Males = 36 Females = 39	Males = 64 Females = 61	
Control						
1	F	60	44	26	74	-
2	M	66	64	38	62	-
3	M	-	-	-	-	-
4	M	58	59	44	56	-
5	M	58	64	31	69	-
6	M	63	66	35	65	-
8	M	59	61	35	65	-
9	F	54	49	46	54	-
10	M	52	54	38	62	-
11	M	59	64	31	69	-
12	M	55	60	58	42	-
Mean		**58**	**59**	**38**	**62**	
SD		**4**	**8**	**9**	**9**	

Abbreviations: Data are presented as mean ±SD. ****, p<0.01;COPD vs control; *****, p<0.05, COPD vs control; **NORMS, normal fibre size for age and gender (Dubowitz 1985).

(b) Light microscopy

The light microscopic descriptions and pathology scores for the skeletal muscle biopsy samples from individual patient and control subjects are presented in Tables 4.2a and 4.2b respectively.

Table 4.2a Light microscopic descriptions from individual skeletal muscle biopsy samples of patients with COPD

Patient	*Description*
1	Variation in fibre size is present with groups of small angulated fibres tending to run in units. Nuclear bags and chains are present. Internal nuclei are increased in number. A necrotic fibre is present as well as several regenerating fibres. The intrafasicular collagen is increased. Type II fibre predominance and most of the atrophied fibres are Type II. Rare small fibres are Type I. Moth-eaten fibres are present on NADH staining.
2	Occasional extremely atrophied fibres are present. Nuclear knots and subsarcolemmal proliferation is present. Some fibre splitting is seen. NADH and SDH staining show moth-eaten fibres particularly amongst the Type II fibres. Lipofuscin is present.
3	There is marked atrophy of fibres with nuclear proliferation and the formation of nuclear bags. Occasional fibres are hypertrophied and there is a large variation in fibre size. An increased number of internal nuclei are present as is subsarcolemmal nuclear proliferation. SDH and NADH staining show moth-eaten fibres predominantly affecting Type I fibres. 35% of Type II fibres are atrophied and 5% of Type I fibres are hypertrophied.
4	A large variation in fibre size is seen, with small fibres tending to occur in groups but also individually. Fibre splitting is present. Intrafasicular collagen appears to be increased. Type II fibre predominance as well as Type II fibre atrophy are seen. Type I fibres seem to be hypertrophied. Internal nuclei are present in increased numbers. Mild fibrosis is present. On NADH staining moth-eaten fibres are seen predominantly in Type I fibres
5	a mild variation in fibre size, some fibre splitting and occasional nuclear knots and chains are present. On NADH staining some fibres are moth-eaten.
6	Numerous flat angulated atrophic fibres are present as are occasional nuclear knots and subsarcolemmal proliferation. Frequent fibre splitting is seen. A few internal nuclei are seen, often in the atrophic fibres. Some Type I fibres have a disturbed pattern on NADH staining.
8	Mild variation in fibre size is noted. On NADH staining some subsarcolemmal accumulations are present. A slight increase in lipid deposits is seen.
9	Frequent atrophic fibres, nuclear knots and fibre splitting are seen. NADH staining shows moth-eaten Type II fibres and Type I fibre subsarcolemmal accumulations.
10	Occasional tiny fibres are present and numerous nuclear knots can be seen. Subsarcolemmal proliferation is present. An increase in internal nuclei is seen. NADH and SDH staining shows subsarcolemmal accumulation.
11	Frequent very atrophic fibres and nuclear knots, some of which are pyknotic are seen. Subsarcolemmal nuclear proliferation, increased number of satellite cells, increased lipofuscin pigment, occasional split fibres, an increase in internal nuclei, occasional necrotic fibres with phagocytes, adipose infiltration, abundant glycogen are seen. Occasional moth-eaten fibres are seen on NADH staining, particularly amongst the Type I fibres. Type II fibre dominance is seen (Ratio I;II 13:97 = 12% Type I fibres).
13	Severe atrophy with tiny fibres is present. Occasional hypertrophied fibres are seen. An increase in nuclei that are pyknotic and nuclear knots are present. Subsarcolemmal proliferation and moderate fibre splitting are seen. A mild increase in internal nuclei is noted. On NADH staining some subsarcolemmal accumulation is present and Type I fibres appear smaller than Type II fibres. Some fibres are moth-eaten on NADH staining.

Table 4.2b	Control subject light microscopic descriptions for skeletal muscle biopsy samples
Control	**Description**
1	Occasional nuclear knots and some moth-eaten Type II fibres are present. Essentially normal skeletal muscle.
2	A moderate number of nuclear knots with tiny fibres are present. Essentially normal skeletal muscle
3	Normal skeletal muscle.
4	Occasional nuclear knots and clumps are seen. Essentially normal skeletal muscle
5	Occasional atrophied fibres, nuclear knots and moth-eaten fibres are seen. Some necrosis is present.
6	Occasional nuclear knots and moth-eaten fibres are present.
8	Severe atrophy with small fibres, nuclear knots, pyknotic nuclei and some nuclei chains are present. Internal nuclei, fibre splitting, occasional ragged red fibres and some large mitochondria are seen.
9	Mild atrophy with occasional nuclear knots is present. Some subsarcolemmal nuclear proliferation and mild subsarcolemmal proliferation are present.
10	Moderate nuclear chains, knots and occasional Type II moth-eaten fibres are present. Essentially normal skeletal muscle
11	Occasional nuclear knots are present. Essentially normal skeletal muscle
12	Moderate atrophy with tiny fibres, mild increase in internal nuclei, moderate number of nuclear knots, severe capillary basement membrane thickening, several severely moth-eaten and degenerative fibres are present.

Light and electron microscopy slides were photographed for graphical depiction of the peripheral skeletal muscle abnormalities. A normal control fibre size variation and structure is presented in Fig 4.1 In contrast, all the patients with COPD displayed a variation in fibre size as presented in Fig 4.3. Nine patients had extreme Type II fibre atrophy involving very small fibres or angulated atrophied fibres (Figs 4.2 and 4.4). One control subject showed severe atrophy, another control subject showed moderate fibre atrophy and two control subjects showed occasional atrophied fibres.

Six patients with COPD showed frequent evidence of fibre splitting (Figs 4.8 and 4.9), whilst only one control subject showed fibre splitting. Seven patients showed an increased number of internal nuclei, some of which were pyknotic (Fig 4.5), whilst two control subjects showed evidence of increased internal nuclei. Severe or moderate nuclear changes including nuclear bags, nuclear knots and pyknotic nuclei were evident in all ten patients with COPD (Figs 4.5, 4.6 and 4.7). Moderate, less frequent nuclear changes were found in all control subjects.

Moth-eaten fibres were found in eight patients (Fig 4.11). Moth-eaten fibres were not selectively

distributed to one fibre type. Three control subjects showed moth-eaten fibres.

Two patients displayed some evidence of fibrosis or regeneration, whilst this was not found in any of the control subjects. One control subject showed ragged red fibres, whilst no patients showed evidence of ragged red fibres. One control subject and one patient with COPD showed necrotic fibres. Two patients showed evidence of increased intrafasicular collagen. Evidence of subsarcolemmal accumulations of mitochondria, glycogen and lipid will be discussed under electron microscopy.

(c) Light microscopy pathology score

The individual skeletal muscle biopsy pathology scores are presented in Table 4.3. The mean pathology score was significantly higher in the patients with COPD compared with the control group. The range of scores in the patients with COPD was five to 13 (arbitrary units) whilst the range for the control group was zero to ten. Two control subjects obtained relatively high skeletal muscle pathology scores, one obtained a score of nine and the other a score of ten. It is interesting to note that subsequent to this skeletal muscle scoring one of these control subjects is undergoing a series of neurological investigations due to the development of a tremor. A similar finding has previously been reported in this laboratory. A control subject scored a skeletal muscle pathology score which was high relative to the other control subjects in a study examining patients with chronic heart failure relative to controls (Derman et al., 1992). Eight months later the control subject was diagnosed with cancer.

Table 4.3 Individual light microscopy skeletal muscle biopsy pathology scores for patients with COPD compared to control subjects

Patient	Atrophy	Necrosis/ regeneration	Fibre Splitting	Internal nuclei	Nuclear Abnormalities	Ragged red	Fibrosis	Moth-eaten	Pathology score (AU)
1	3	1	0	3	3	0	0	3	**13**
2	2	0	1	0	3	0	0	1	**7**
3	3	0	2	3	3	0	0	2	**13**
4	3	0	2	3	3	0	1	2	**14**
5	1	0	2	0	1	0	0	2	**6**
6	3	0	3	0	2	0	0	0	**8**
8	NS								
9	2	0	0	0	3	0	0	1	**6**
10	2	0	0	0	2	0	0	1	**5**
11	3	1	1	2	3	0	0	1	**11**
13	3	0	2	0	3	0	0	1	**9**
Mean									**9****
SD									**3**
Control									
1	0	0	0	0	1	0	0	2	**3**
2	1	0	0	0	2	0	0	0	**3**
3	0	0	0	0	0	0	0	0	**0**
4	0	0	0	0	1	0	0	0	**1**
5	2	1	0	0	1	0	0	1	**5**
6	0	0	0	0	1	0	0	2	**3**
8	3	0	1	2	3	1	0	0	**10**
9	2	0	0	0	1	0	0	0	**3**
10	0	0	0	0	2	0	0	2	**4**
11	0	0	0	0	1	0	0	0	**1**
12	2	0	1	1	2	0	0	3	**9**
Mean									**4**
SD									**6**

Abbreviations: AU, arbitrary units; NS, not scored, the light microscopy slides were accidentally lost by the technicians after reporting, prior to scoring. Data are presented as mean ±SD; **,p<0.01 COPD vs Control.

Table 4.4 Electron microscopic descriptions for individual skeletal muscle biopsy samples in patients with COPD and control subjects.

Patient	Description
1	Glycogen accumulation, focal lipofuscin deposition, degenerate nuclear bags, disrupted myofilaments and z-banding streaming are present. Central nuclei are increased in number and the plasmalemmal basement membrane is redundant.
2	Nuclear knots, fibre atrophy, a moderate number of abnormally shaped and enlarged mitochondria are present. Occasional mitochondria contain paracrystalline rectangular inclusions.
3	Atrophic fibres are present and the sarcolemmal membrane shows a saw-tooth edge. Satellite cells are present as is a small area of fibre disarray. Subsarcolemmal glycogen is present.
4	Inadequate muscle sample for electron microscopy.
5	Muscle contains lipofuscin.
6	Atrophic fibres with patches of thick and thin filaments are shown. Thickened sarcolemmal basement membrane, saw-toothed sarcolemmal membrane, nuclear knots in atrophic fibres, increased satellite cells and some myelin degeneration in mitochondria are shown.
8	Increased lipid accumulation, a few subsarcolemmal accumulations of mitochondria, glycogen and lipid are displayed.
9	Subsarcolemmal accumulations of mitochondria and lipids. Atrophic fibres present, lipofuscin present
10	Enlarged and elongated mitochondria are present, often associated with a lipid vacuole. Occasional subsarcolemmal accumulations can be seen. Areas of fibre streaming are present. The sarcolemmal basal lamina is thickened.
11	Myofibrillar degeneration and disarray are shown with some large mitochondria present.
13	The basement membrane is thickened and reduplicated. Nuclear proliferation, nuclear knots, increased glycogen and some increased fibril dissolution are shown.
Control	
1	The mitochondria have rounded cristae and some are elongated. Some lipid, nuclear and mitochondrial accumulation below the sarcolemma is present. Lipofuscin is present.
2	Occasional "normal" subsarcolemmal accumulations are present.
3	The sarcolemmal membrane has a "saw-tooth" appearance and there is increased glycogen distribution in the area under the sarcolemmal membrane.
4	Mild subsarcolemmal accumulation of mitochondria, glycogen and lipid are present. The sarcolemmal membrane has a "saw-tooth" appearance.
5	Some lipofuscin and subsarcolemmal accumulations are present.
6	Occasional enlarged mitochondria are present.
8	Subsarcolemmal accumulations of large mitochondria and some area of fibre dissolution are present.
9	Occasional subsarcolemmal accumulations of glycogen and mitochondria are present.
10	Subsarcolemmal accumulations of lipid, mitochondria and glycogen are present whilst the mitochondrial are elongated with rounded cristae deposits in them. Lipofuscin is present.
11	Subsarcolemmal accumulations of mitochondria and lipid are present.
12	Moderate amount of fibre dissolution, subsarcolemmal accumulations of mitochondria and glycogen are present. Enlarged, elongated mitochondria and some lipofuscin are present.

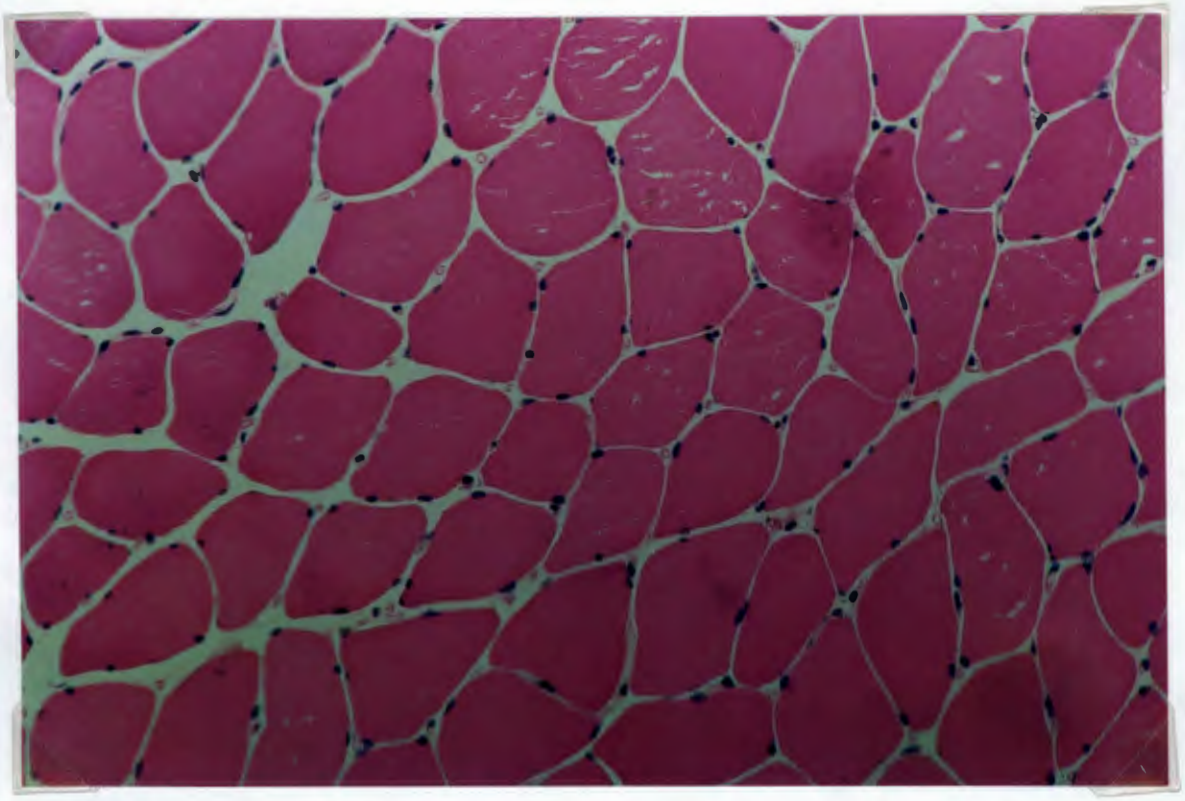

Fig 4.1. A light photomicrograph showing normal fibre size variation in the skeletal muscle of a control subject (H&E x16). Note the structure and regularity of the fibres.

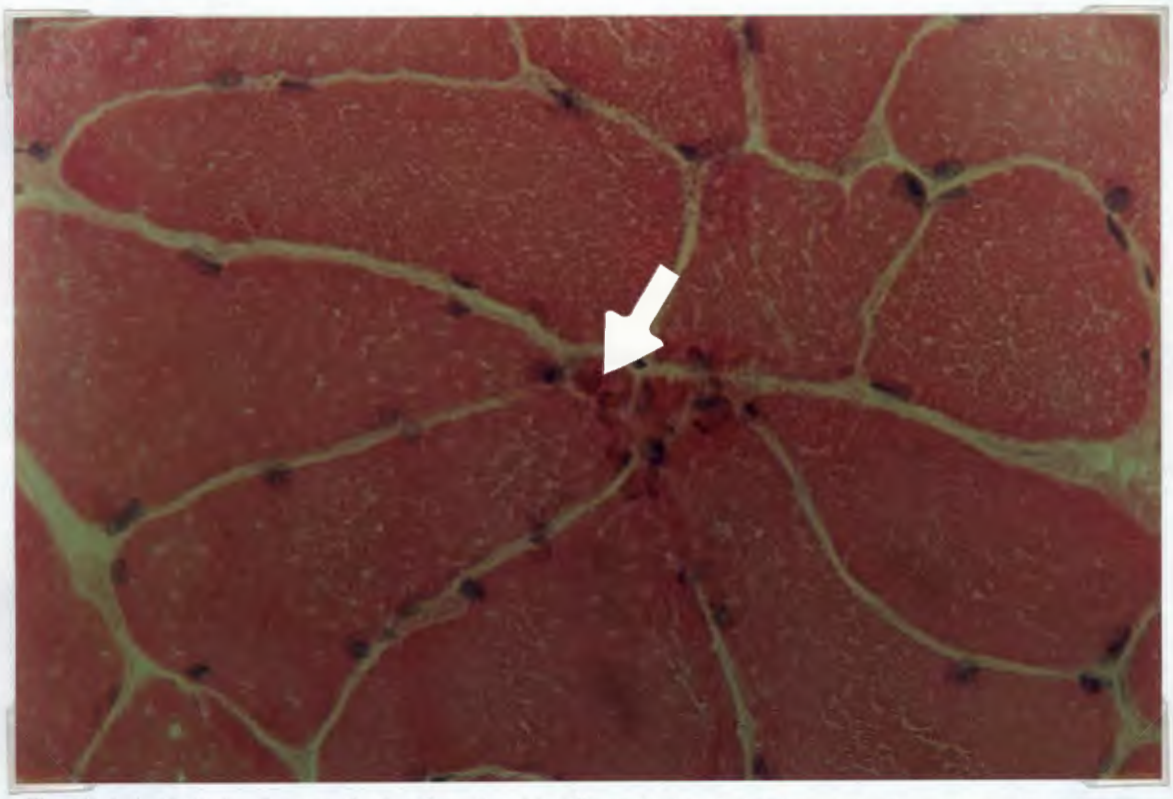

Fig 4.2. A light photomicrograph showing atrophied fibres in the skeletal muscle of a patient with COPD (H&E x40).

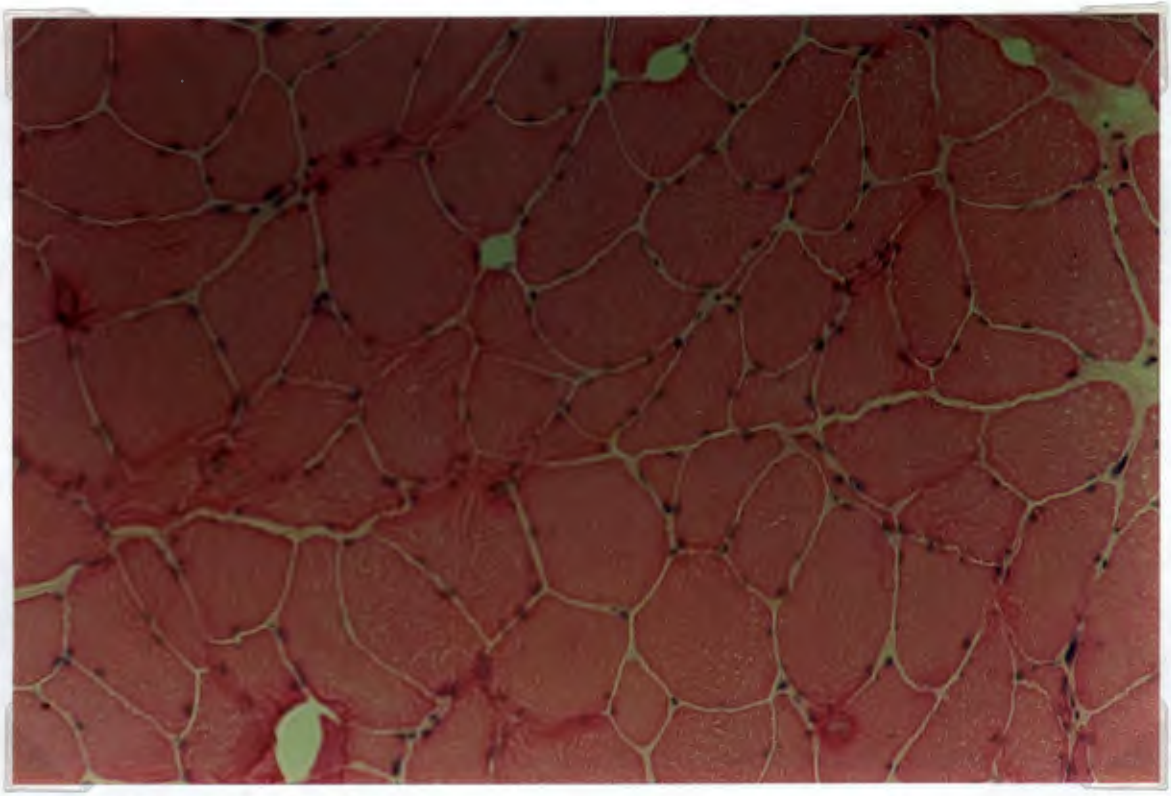

Fig 4.3 A light micrograph showing abnormal fibre size variation in the skeletal muscle of a patient with COPD (H&E x16). Note the differences between fibre sizes and the irregularity of fibre sizes.

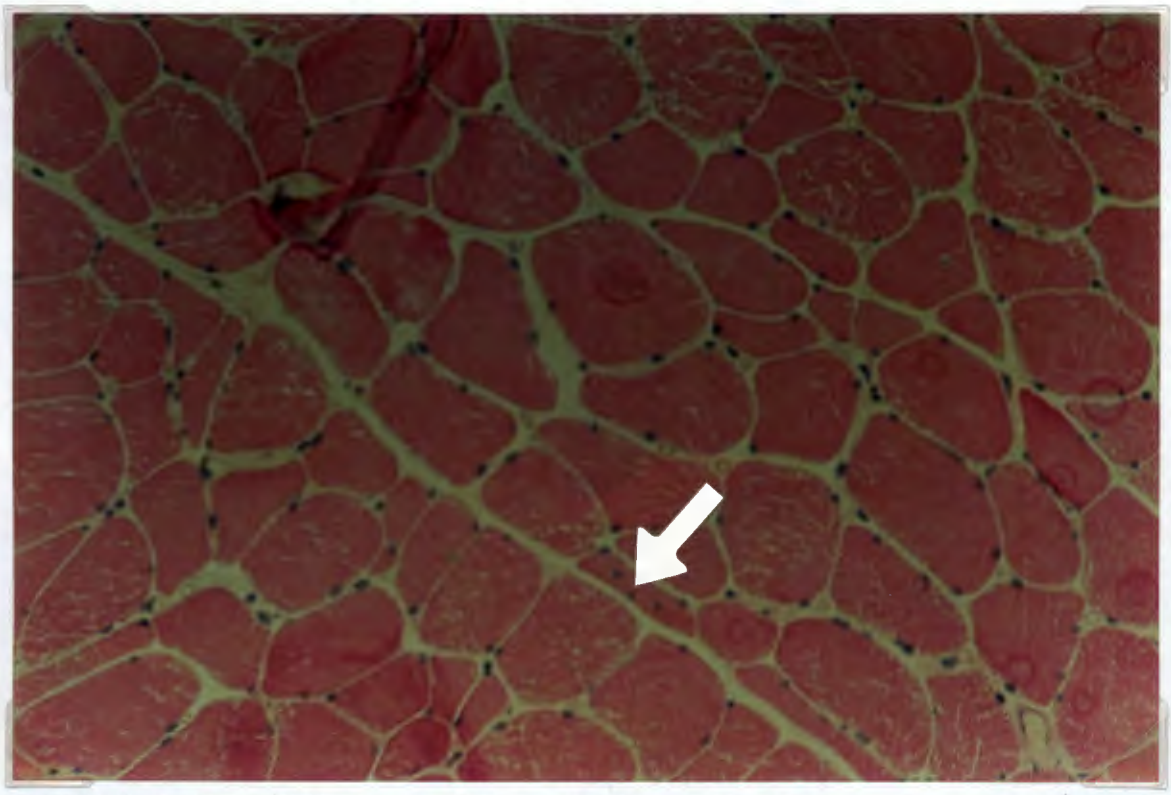

Fig 4.4 A light micrograph showing atrophied, angulated fibres in the skeletal muscle of a patient with COPD (H&E x16).

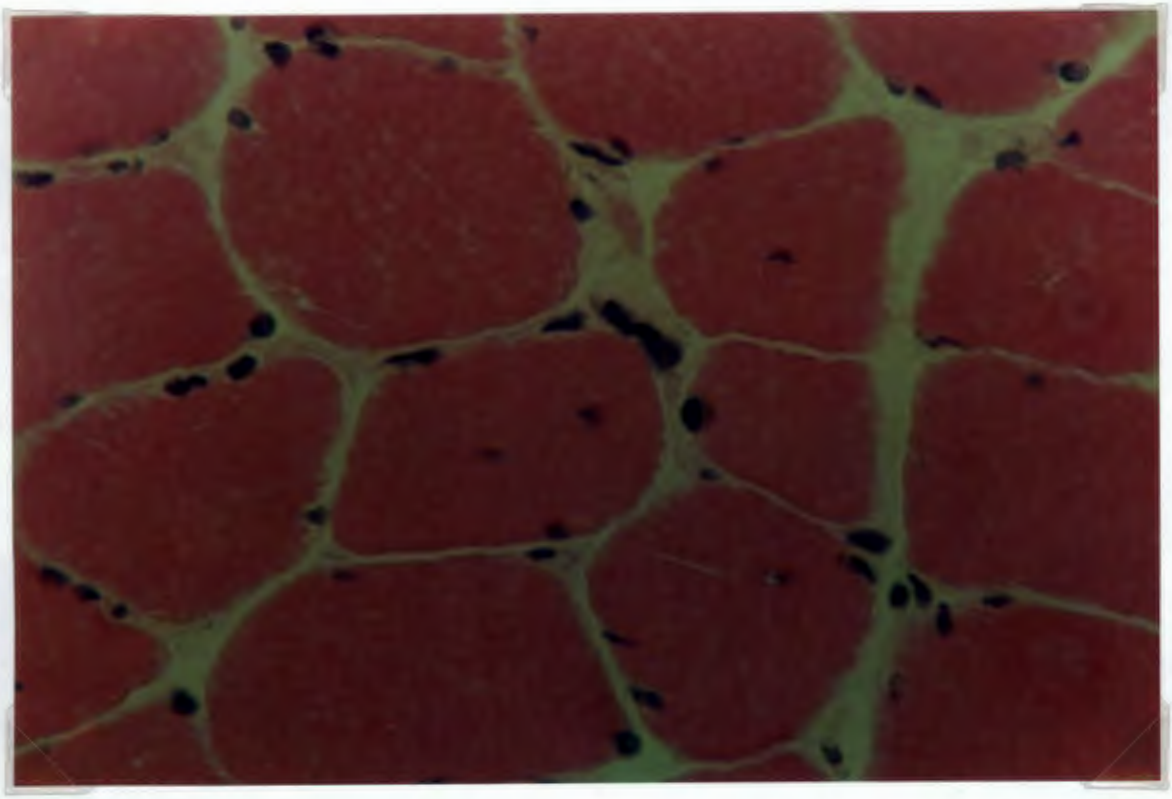

Fig 4.5 A light micrograph showing prominent nuclear chains, nuclear knots and centralized nuclei in the skeletal muscle of a patient with COPD (H&E x40).

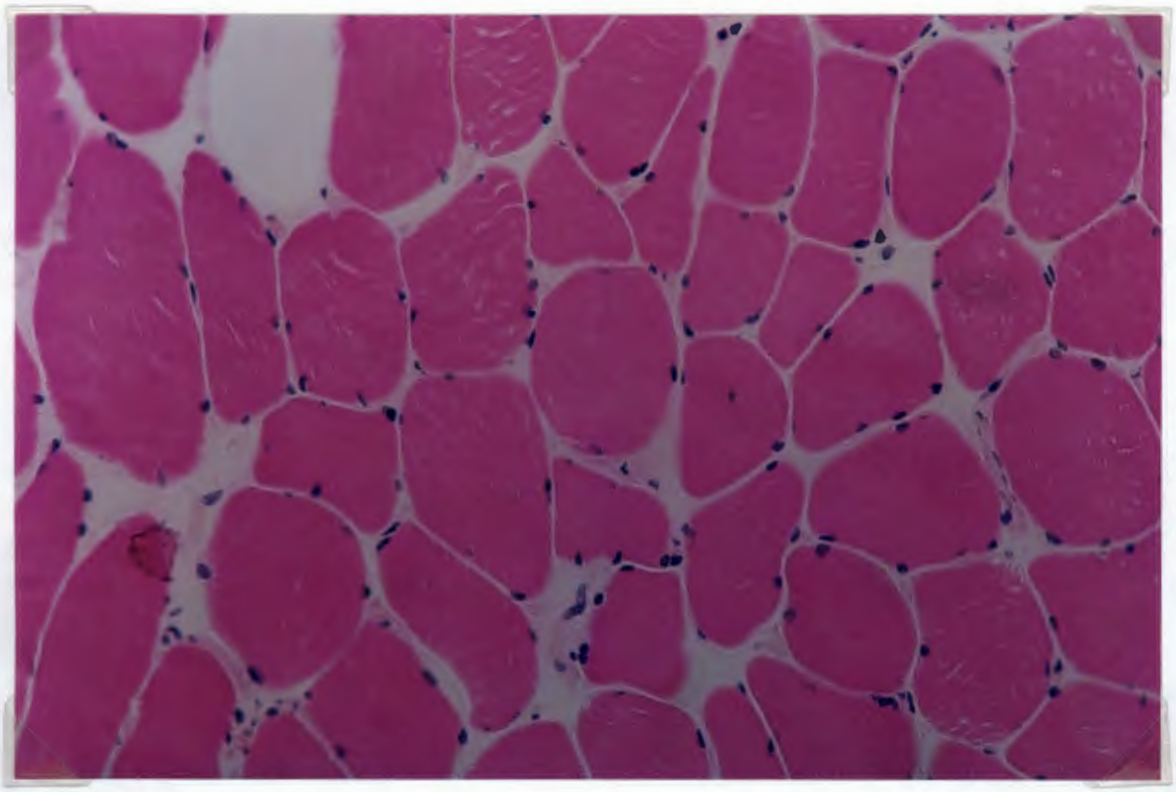

Fig 4.6. A light micrograph showing nuclear bags, chains and knots in the skeletal muscle of a patient with COPD (H&E x16).

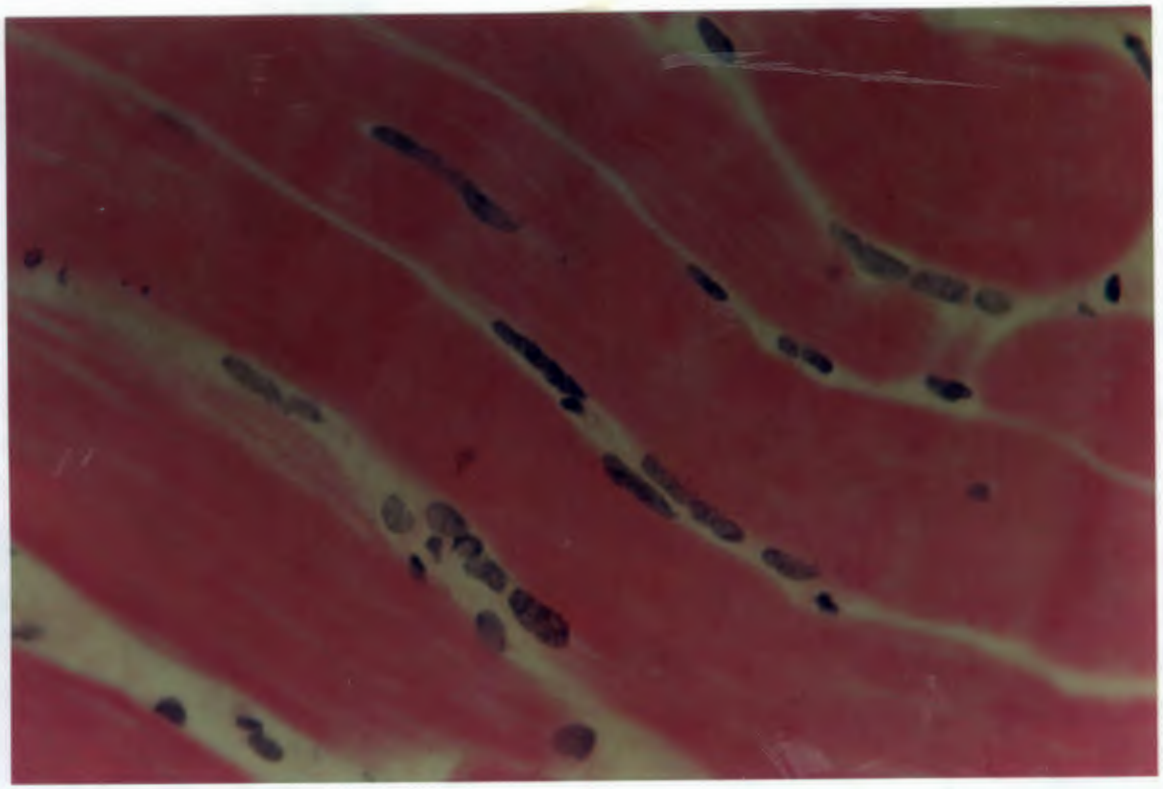

Fig 4.7 A light micrograph showing nuclear chains in longitudinal section in the skeletal muscle of a patient with COPD (H&E x16).

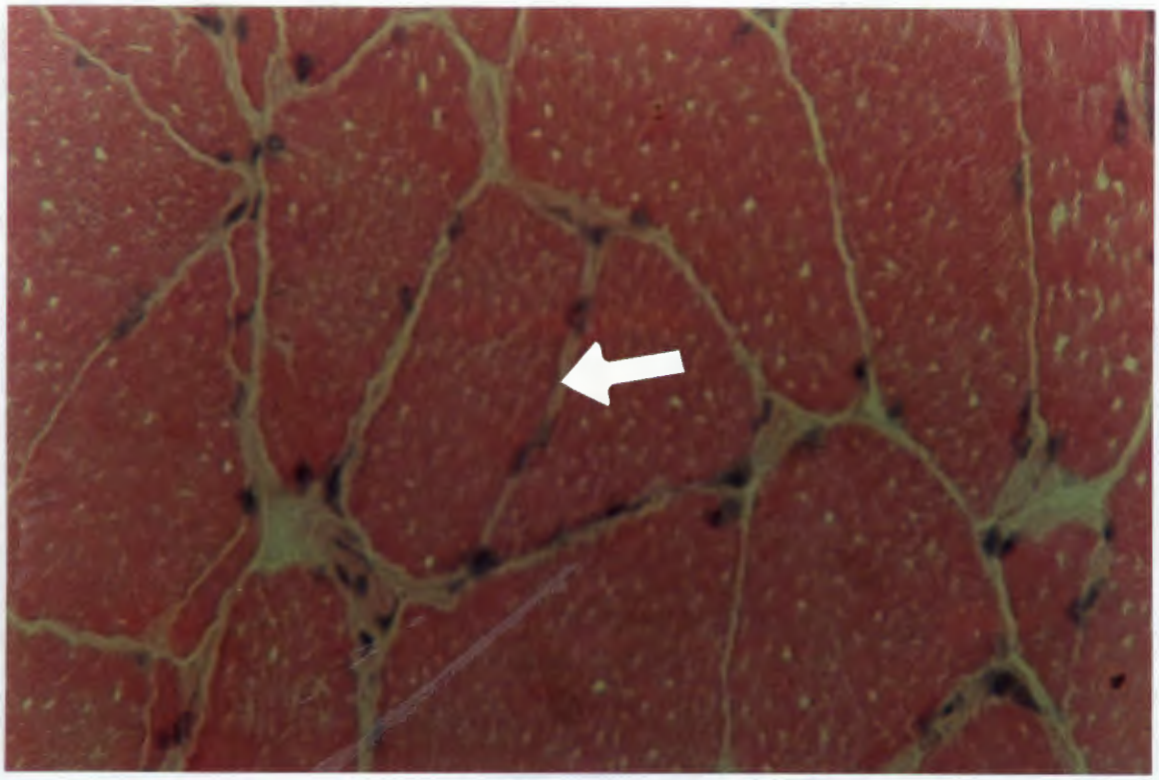

Fig 4.8. A light micrograph showing possible fibre splitting in the skeletal muscle of a patient with COPD (H&E x40).

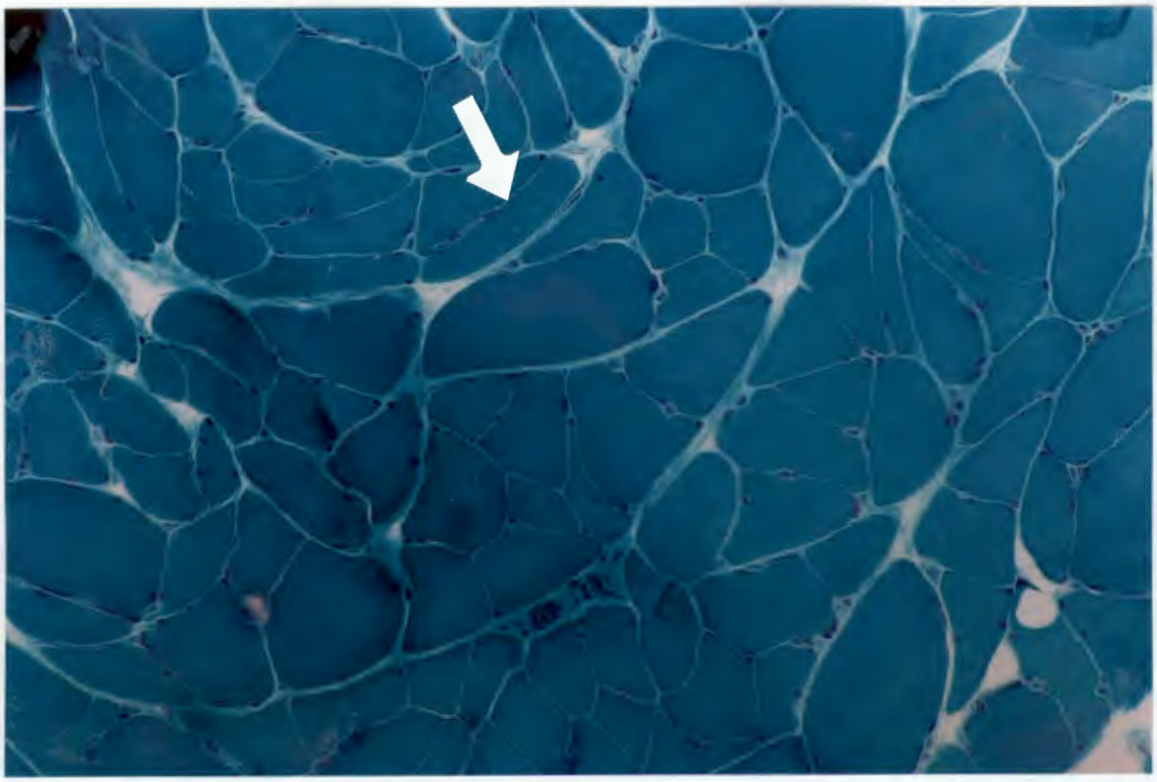

Fig 4.9 A light micrograph showing possible fibre splitting in the skeletal muscle of a patient with COPD (Gomori's Trichrome stain x16).

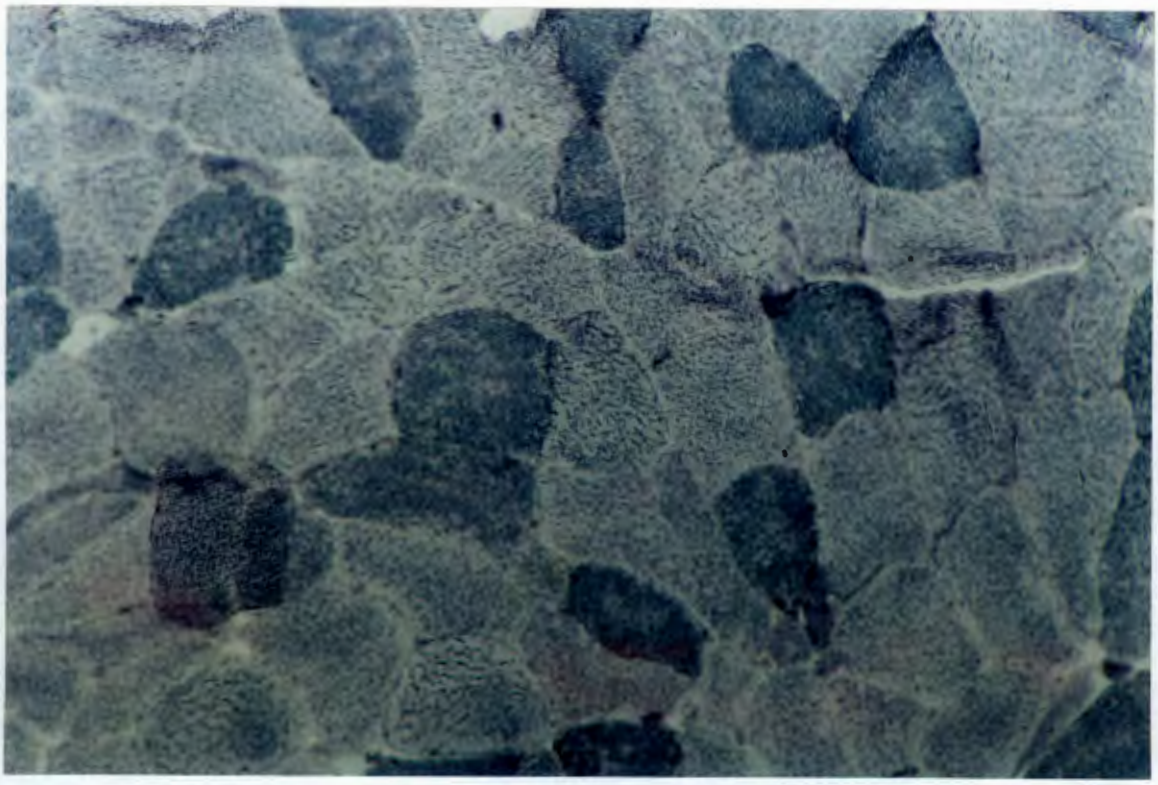

Fig 4.10. An NADH stained light micrograph showing Type II fibre predominance in the skeletal muscle of a patient with COPD (Type II fibres stain a lighter colour on NADH staining) (NADH x16).

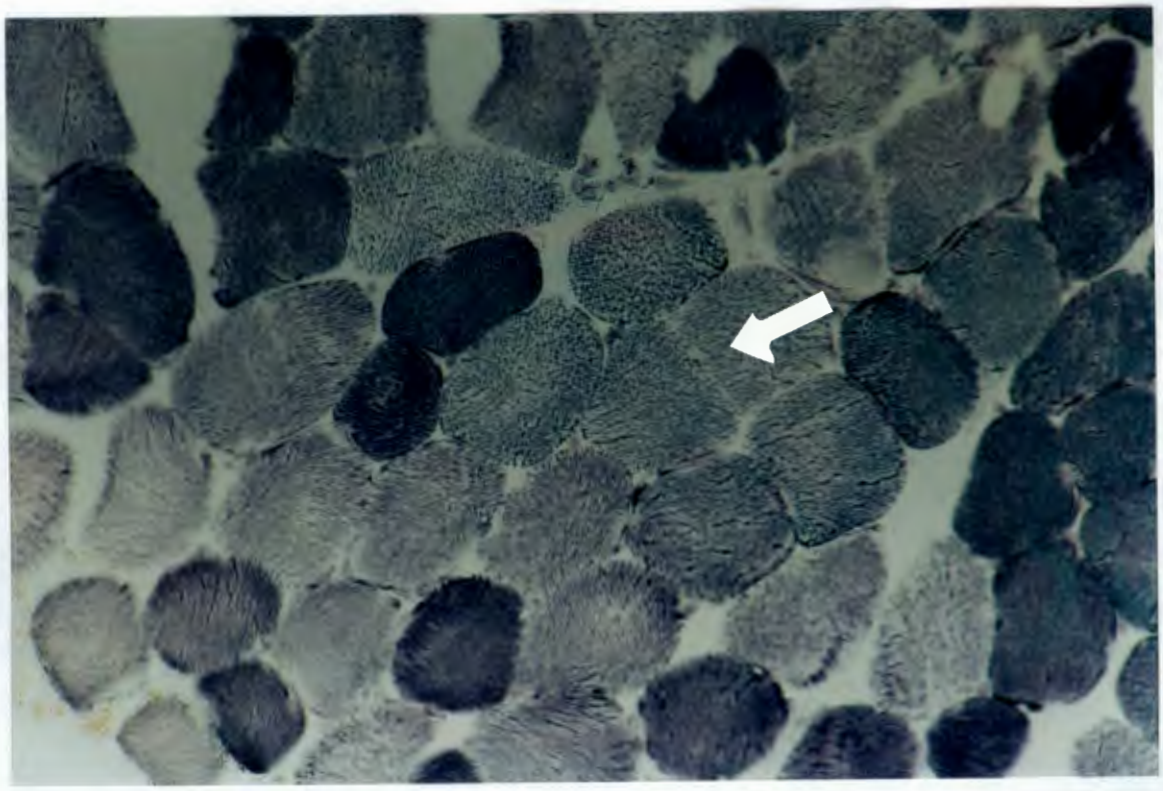

Fig 4.11 A light micrograph showing moth-eaten Type II fibres in the skeletal muscle of a patient with COPD (NADH x16).

(d) Electron microscopy

The electron microscopic descriptions for individual skeletal muscle biopsies are presented in Table 4.4. The ultrastructural changes included fibril dissolution in five patients and two controls (Fig 4.12), subsarcolemmal accumulations and proliferation of either mitochondria, glycogen or lipid were present in almost all biopsies (Figs 4.13 and 4.14). However, the severity was greater in the patients compared to the control group. Saw-tooth sarcolemmal membranes were found in both groups (Fig 4.19), but more frequently in the patients with COPD than in the control group. Satellite cells were found in two of the patients with COPD. Two control subjects showed circular mitochondria cristae (Fig 4.23) whilst one patient with COPD had rectangular paracrystalline mitochondrial inclusions (Fig 4.17). Lipofuscin deposits were found in a number of patients and a number of controls (Fig 4.18).

The changes found on light microscopy such as internal nuclei (Fig 4.16), small atrophic fibres (Fig 4.15) and nuclear bags and chains (Fig 4.20, Fig 4.21, Fig 4.22) were once again more evident in the patients with COPD than in the control group when viewed under electron microscopy.

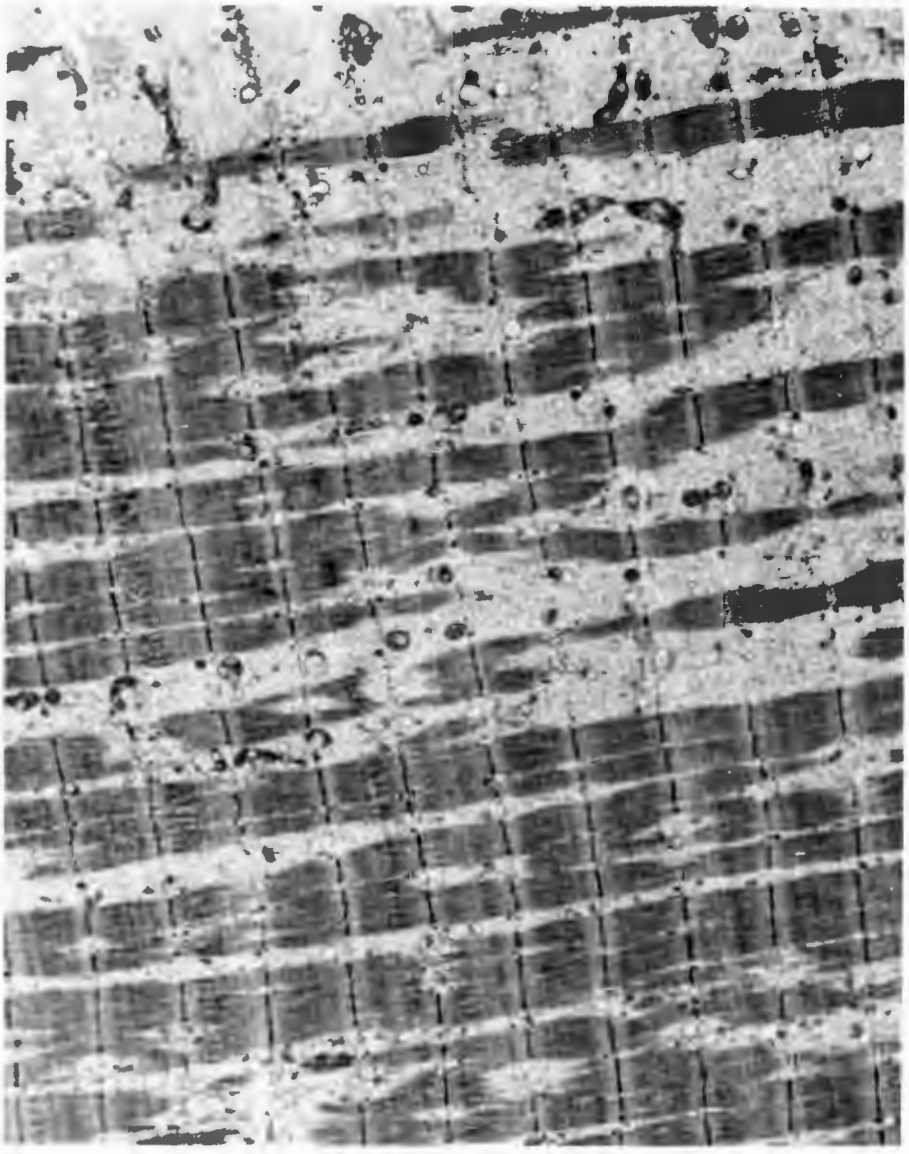

Fig 4.12 An electron micrograph showing fibril dissolution in the skeletal muscle of a patient with COPD (x3000). Note the disruption of the fibre structures by deposits of various substances.

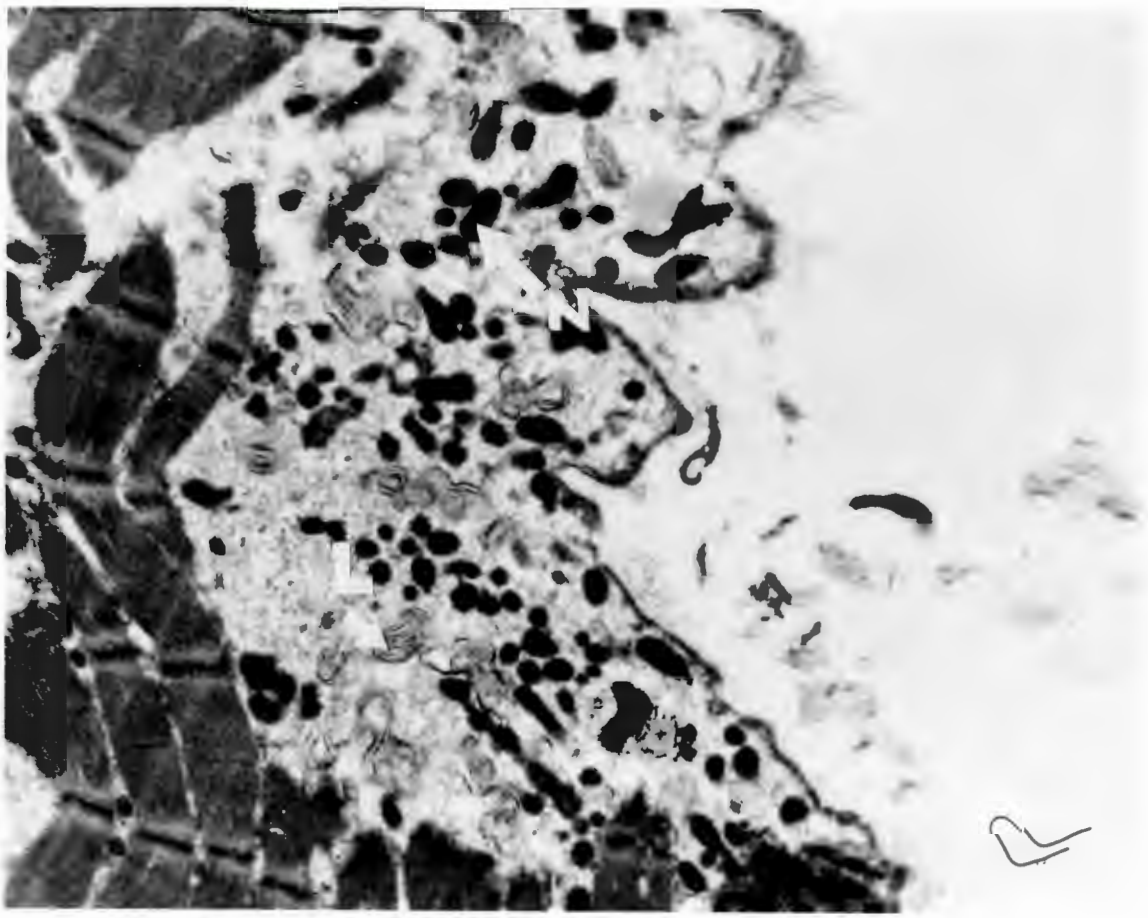

Fig 4.13 An electron micrograph showing subsarcolemmal lipid (L) and nuclear (N) accumulation in the skeletal muscle of a patient with COPD (x4500).

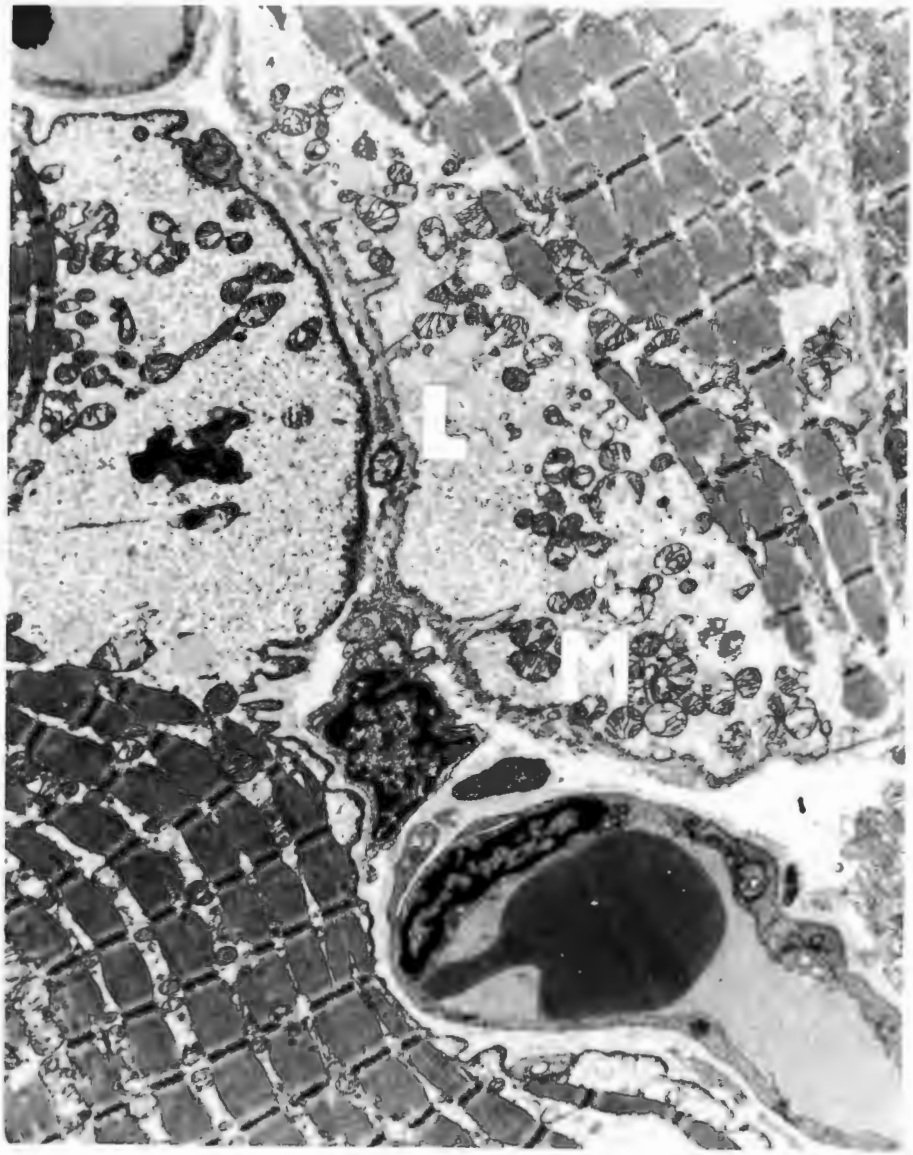

Fig 4.14. An electron micrograph showing subsarcolemmal accumu lation of mitochondria (M) and lipid (L) in the skeletal muscle of patient with COPD (x3000).

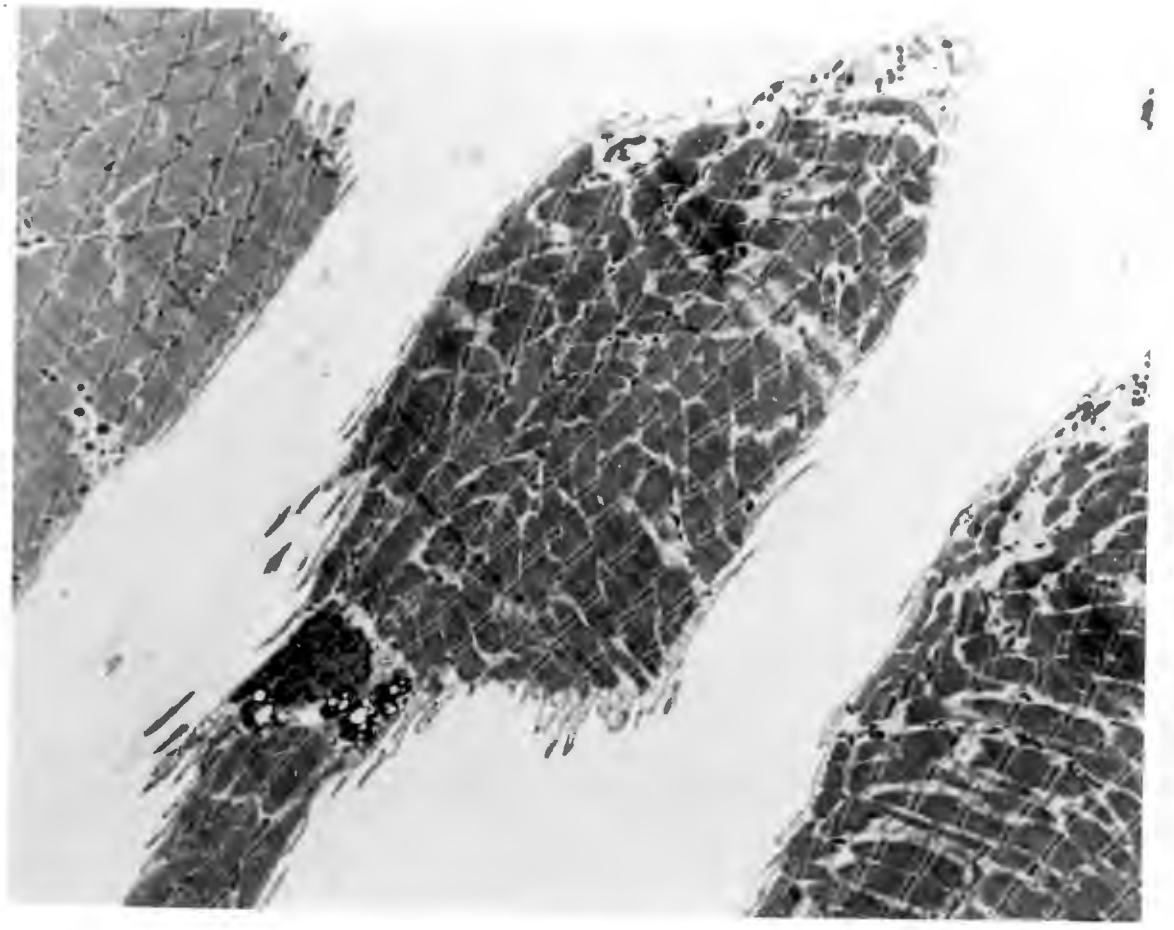

Fig 4.15. An electron micrograph showing small atrophic fibres in the skeletal muscle of a patient with COPD (x2000).

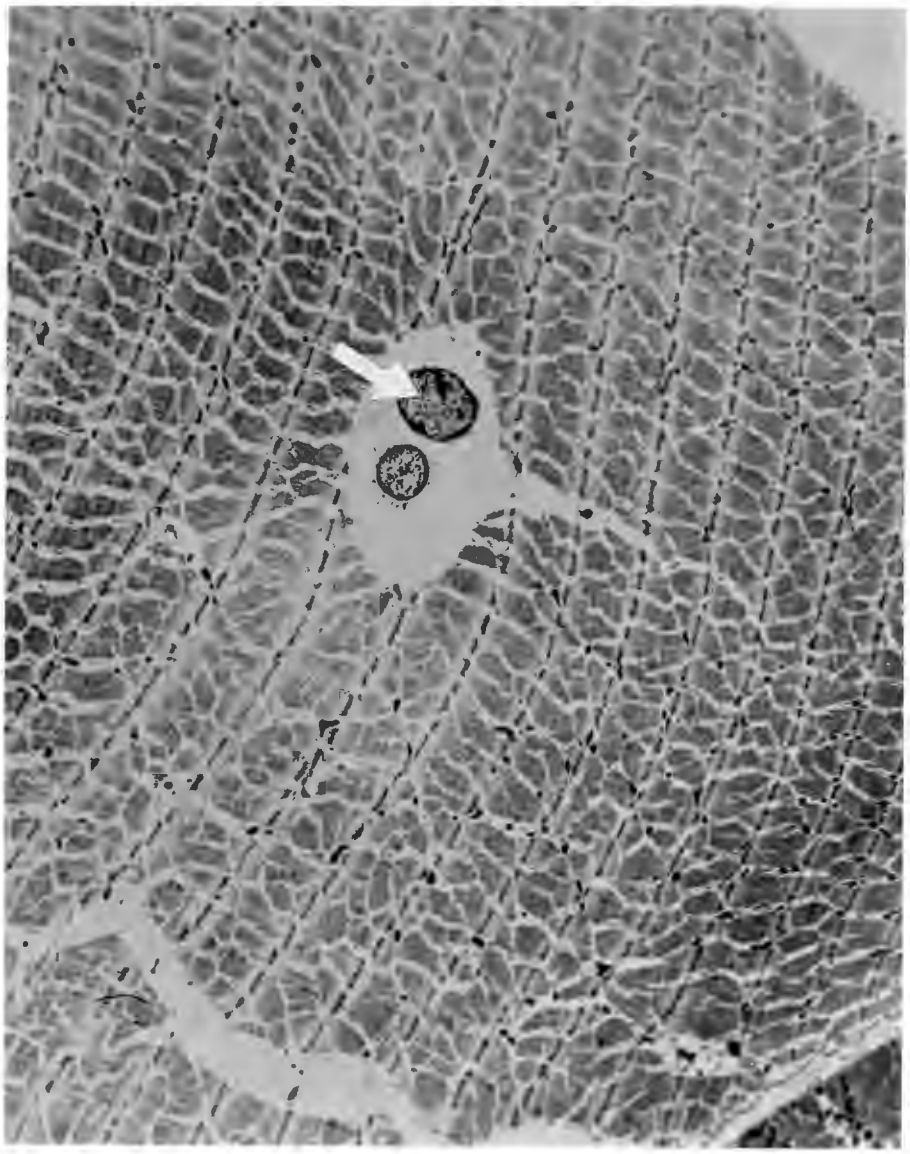

Fig 4.16 An electron micrograph showing centralized nuclei in the skeletal muscle of a patient with COPD (x3000).

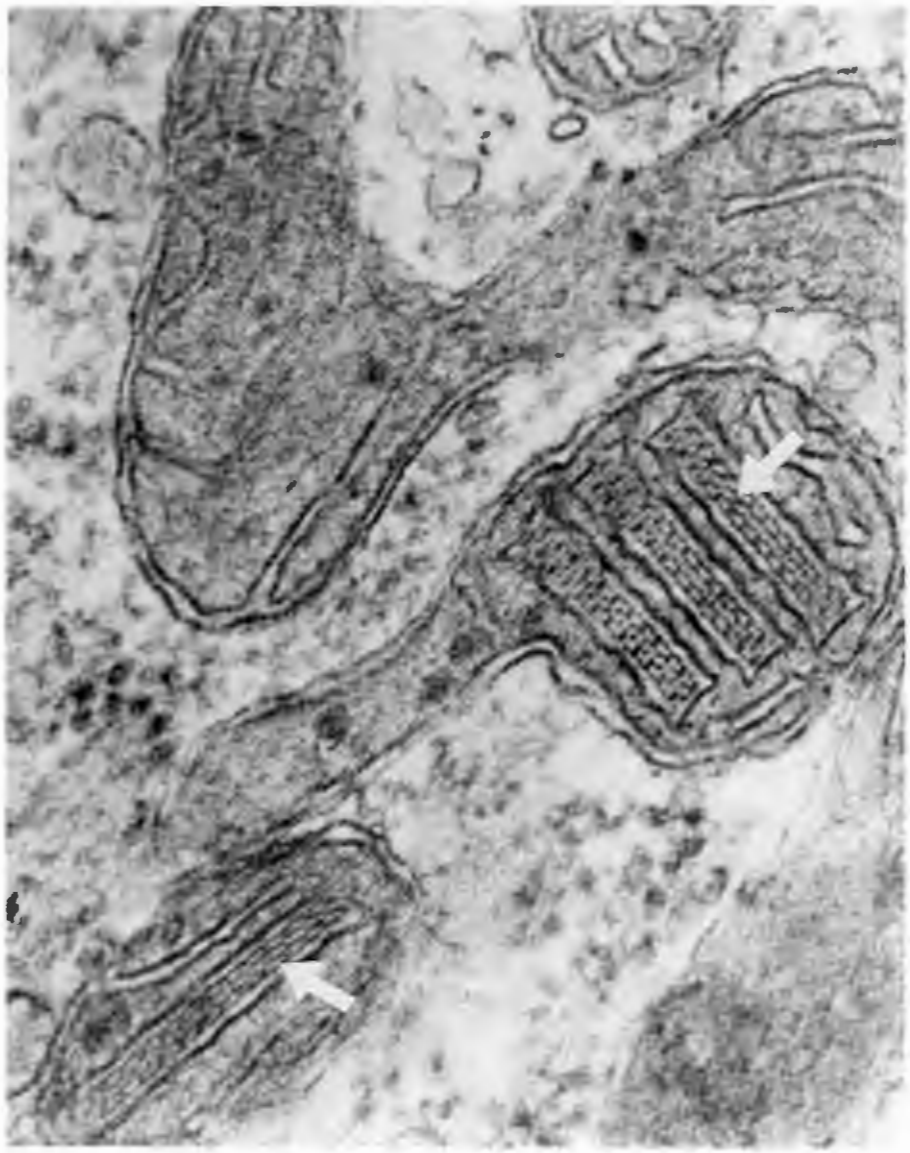

Fig 4.17 An electron micrograph showing rectangular paracrystalline mitochondrial deposits in the mitochondria of a patient with COPD (x4500).

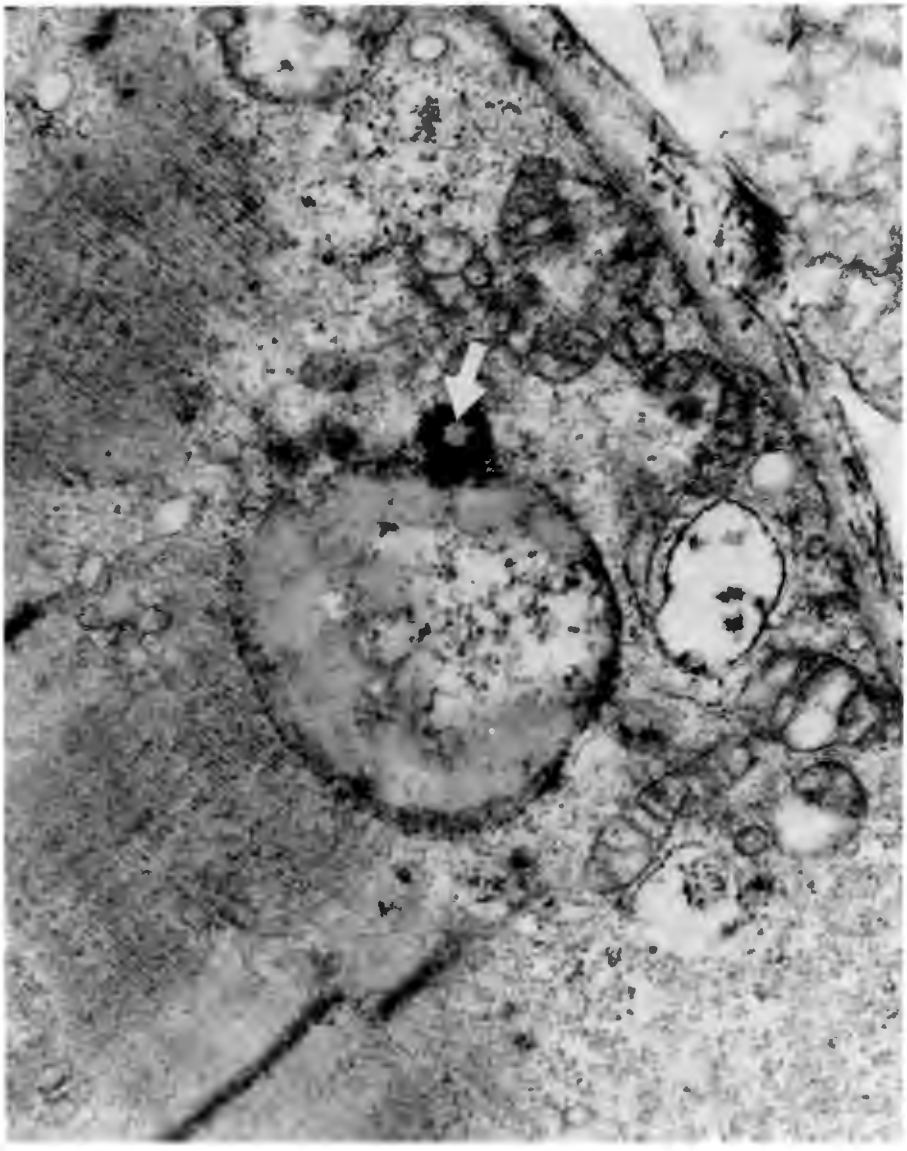

Fig 4.18 An electron micrograph showing lipofuscin in the skeletal muscle of a control subject (x15000).

Fig 4.19 An electron micrograph showing a saw-tooth subsarcolemmal membrane in the skeletal muscle of a patient with COPD (x3000).

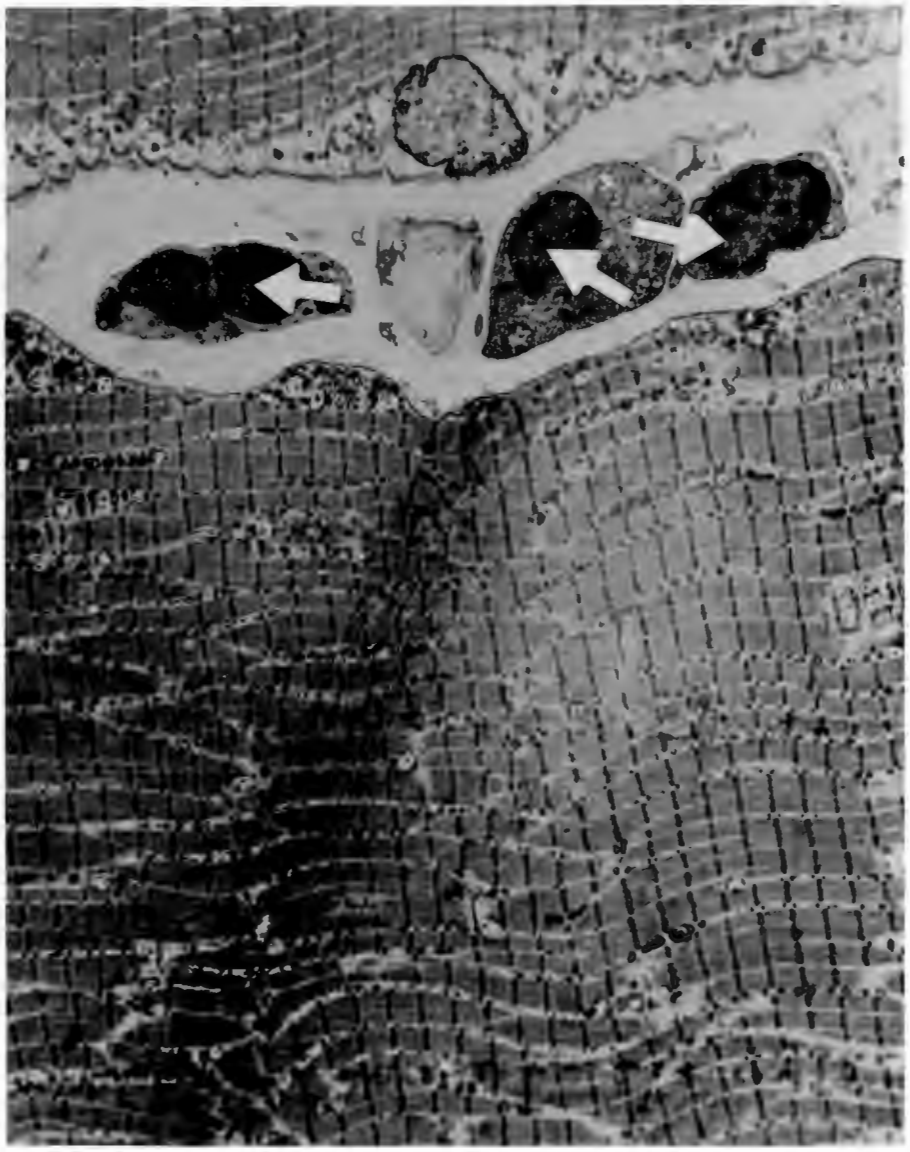

Fig 4.20 An electron micrograph showing nuclear bags in the atrophic fibre in the skeletal muscle of a patient with COPD (x7000). Note that the nuclear bag is almost the full size of the atrophic fibre.

Fig 4.21 An electron micrograph showing nuclear bags and chains in the atrophic fibres in the skeletal muscle of a patient with COPD (x2500).

Fig 4.22 An electron micrograph showing a nuclear bag in an atrophic fibre in the skeletal muscle of a patient with COPD (x15000).

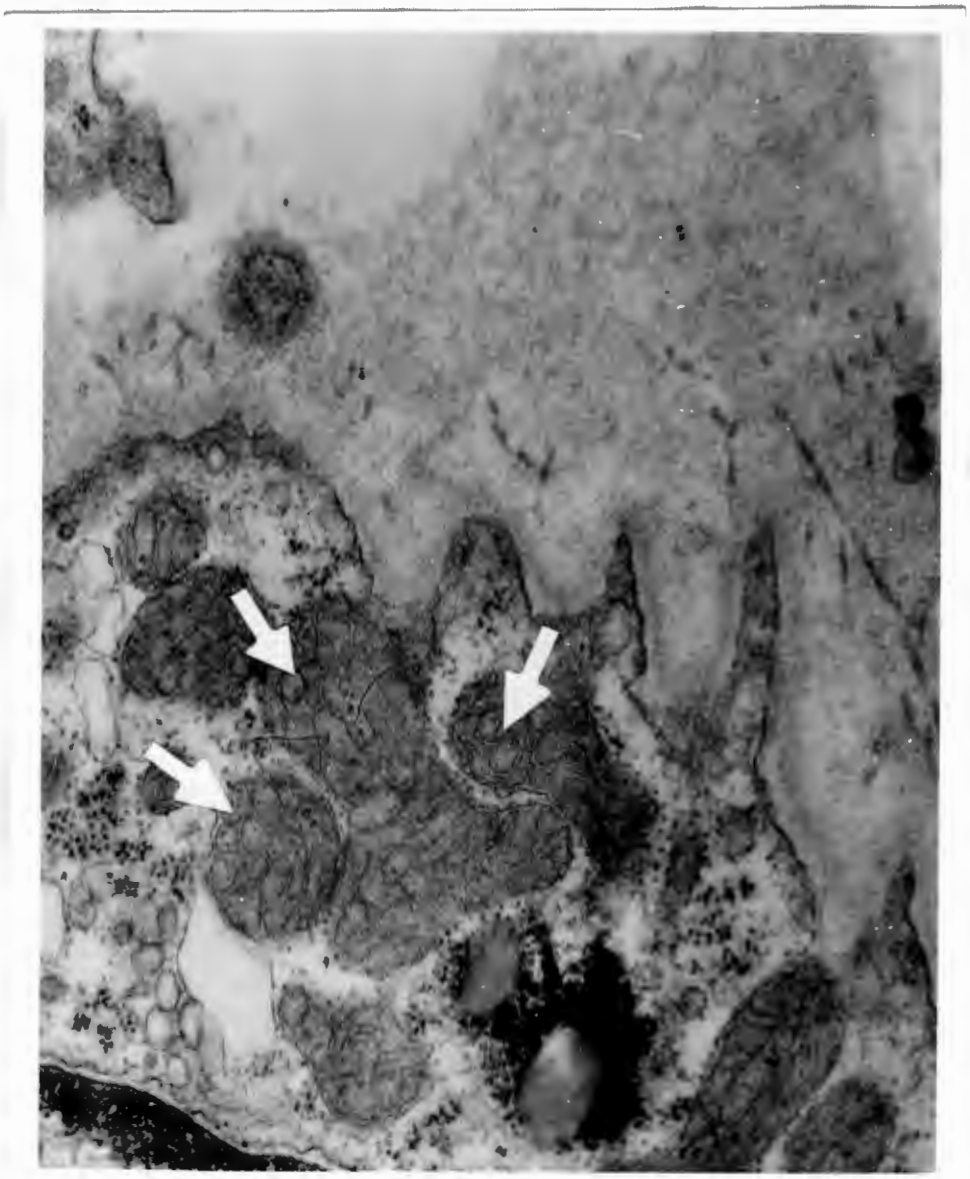

Fig 4.23 An electron micrograph showing circular mitochondrial cristae in the skeletal muscle of a control subject (x15000).

Relationships between skeletal muscle pathology scores, skeletal muscle function and general exercise performance in patients with COPD

To determine if any relationship existed between the observed skeletal muscle structural abnormalities and skeletal muscle function and/or general exercise capacity in the patients with COPD, a correlation analysis was performed between the biopsy pathology scores of the 11 patients with COPD and various measures of skeletal muscle function and general exercise performance.

Table 4.5. Correlation coefficients between skeletal muscle biopsy pathology score and FEV_1, peak exercise variables and measures of skeletal muscle functional ability.

	Correlation with SM biopsy pathology score		
	Patients		Controls
Lung Function Variable	**r Value**	**p Value**	**p Value**
FEV_1 (l)	-0.52	NS	NS
Peak Exercise Variables			
VO_2 peak ($ml0_2$/kg/min)	-0.67	0.04	NS
Peak V_E (l/min)	-0.75	0.02	NS
WLpeak (W)	-0.67	0.04	NS
Maximum exercise time (min)	-0.82	0.01	NS
Measures of SM function			
PKTQ (Nm) (Strength)	-0.69	0.03	NS
PKTQ (Nm/l) (Strength/LTV)	-0.54	0.08	NS
PKTQ (Nm) (Endurance)	-0.79	0.01	NS
PKTQ (Nm) (Endurance/LTV)	-0.63	0.05	NS
TPQ (Nm)	-0.56	0.09	NS
TPQ (Nm/l)	-0.39	NS	NS
WDQ (W)	-0.67	0.04	NS
WDQ (W/l)	-0.57	0.08	
Functional measure of exercise tolerance			
SMWT distance (m)	-0.63	0.05	

Abbreviations: SM, skeletal muscle; FEV_1, forced expiratory volume in 1 second; VO_2 **peak**, peak oxygen consumption achieved during the incremental cycle test to exhaustion detailed in Chapter Three; **Peak** V_E, peak minute ventilation achieved during the incremental cycle test to exhaustion; **WLpeak**, peak work load achieved during the incremental cycle test to exhaustion; **Maximum exercise time**, maximal exercise time achieved during the incremental cycle test to exhaustion; **PKTQ (Strength)**, peak torque produced by the quadriceps muscle during the isokinetic strength tests; **LTV**, lean thigh volume; **l**, litre; **PKTQ (Endurance)**, peak torque produced by the quadriceps muscles during the isokinetic endurance test; **W**, watts; **Nm**, Newton metre; **TPQ**, total power produced by the quadriceps muscle during the isokinetic endurance test; **WD**, work done by the quadriceps muscle during the isokinetic endurance test; **SMWT**, six minute walk test; **m**, metres; **min**, minutes; **r Value**, correlation coefficient.

A significant negative correlation was found between the biopsy pathology scores and the peak exercise variables; V_E and exercise time achieved during the incremental cycle test to exhaustion discussed in Chapter Three. Similarly, a significant negative correlation was found between the biopsy pathology score and peak torque achieved by the quadriceps prior to correction for LTV during the isokinetic endurance test involving 30 repetitive contractions (Fig 4.24).

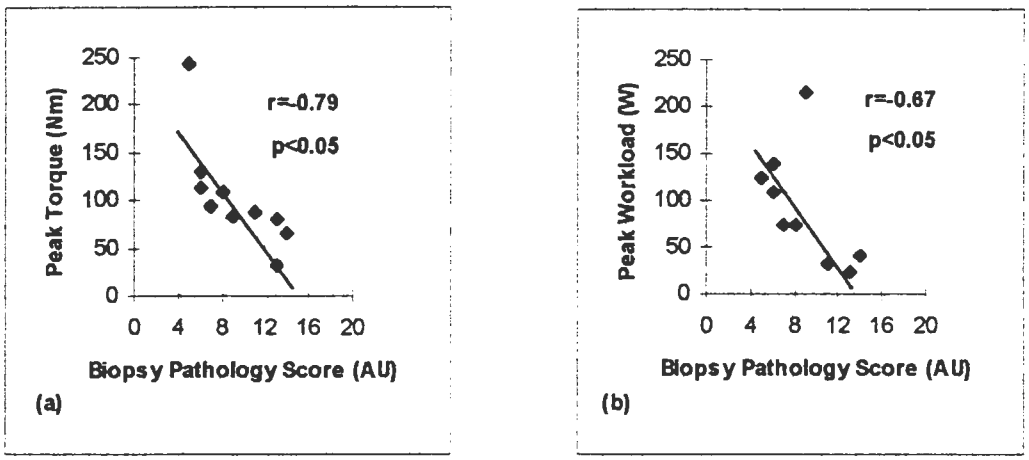

Fig 4.24 Relationship between the biopsy pathology score and (a) peak torque achieved during the isokinetic endurance test (n=10) and (b) peak workload achieved during the incremental cycle test to exhaustion (n=9). Abbreviations: AU, arbitrary units.

Weaker but significant correlations were found between the biopsy pathology scores and the following variables; peak $V0_2$ achieved during the incremental cycle test to exhaustion, peak workload achieved during the incremental cycle test to exhaustion (Fig 4.24), peak torque achieved during the isokinetic strength test of the quadriceps involving two contractions, total power generated by the quadriceps during the isokinetic endurance test involving 30 repeated contractions, work done during the isokinetic endurance test of the quadriceps involving 30 repeated contractions, peak torque achieved by the quadriceps before correction for LTV during the isokinetic endurance test and distance covered during the SMWT.

A correlation analysis was performed between the variables of performance and skeletal muscle function and the biopsy pathology scores of the control subjects. No significant correlations were found (Table 4.5).

Comparison between skeletal muscle structure, function and exercise performance in five patients ingesting prednisone compared to eight patients not ingesting prednisone.

In an attempt to determine the effect of ingesting corticosteroid on skeletal muscle structure, function and exercise performance in patients with COPD a comparison was made between the five patients ingesting prednisone and the eight patients not ingesting prednisone. Results of the comparison are presented in Table 4.6.

Table 4.6. Results of a comparison of skeletal muscle structure, function and general exercise performance between five patients with COPD ingesting prednisone compared to eight patients not ingesting prednisone.

Variable	Patients ingesting prednisone	Patients not ingesting prednisone	Significance level
Measures of SM structure			
Biopsy path score (AU)	11 ±4	7 ±3	p=0.06
Measures of SM function			
PKTQ (Nm) (Strength)	132 ±66	200 ±101	NS
WDQ (W) (Endurance)	1845 ±942	2370 ±1058	NS
Measure of general exercise performance			
VO_2 peak ($ml O_2$/kg/min)	17 ±5	16 ±3	NS
WL peak (W)	73 ±73	89 ±33	NS
SMWT(m)	486 ±209	522 ±117	NS

Abbreviations: SM, skeletal muscle;**Biopsy path score**, biopsy pathology score; **AU**, arbitrary units; **VO_2 peak**, peak oxygen consumption achieved during the incremental cycle test to exhaustion detailed in Chapter Three; **WLpeak**, peak work load achieved during the incremental cycle test to exhaustion; **PKTQ (Strength)**, peak torque produced by the quadriceps muscle during the isokinetic strength tests; **W**, watts; **Nm**, Newton metre; **WDQ**, work done by the quadriceps muscle during the isokinetic endurance test; **SMWT**, six minute walk test; **m**, metres;

No significant difference was found in skeletal muscle function or general exercise performance in the patients ingesting prednisone compared to the patients not ingesting prednisone (Table 4.6). The biopsy pathology score, which is indicative of skeletal muscle structure, tended to be higher (greater pathology) in the patients ingesting prednisone compared to patients not ingesting prednisone (Table 4.6).

4. Discussion

The most important contribution of this study was to document and describe the structural abnormalities seen in the skeletal muscle of patients with COPD. Evidence from analysis of light microscopy, electron microscopy and morphometry of the skeletal muscle biopsy samples of the patients resulted in a detailed description of the abnormalities. The abnormalities seen in the skeletal muscle of the patients with COPD included; severe nuclear abnormalities, Type II fibre predominance, Type II fibre atrophy, fibre splitting, internal nuclei, moth-eaten fibres, subsarcolemmal proliferation and aggregation and, in one patient, mitochondrial paracrystalline deposits. The most frequent pathological features observed in the skeletal muscle of the patients with COPD were abnormalities of the nuclei including nuclear chains, nuclear bags, nuclear knots and pyknotic nuclei. Indeed, nuclear abnormalities were the most outstanding feature within the skeletal muscle of these patients.

Many of the changes documented in the peripheral skeletal muscle of the patients with COPD are non-specific findings and are seen in a variety of disorders. Atrophy of Type II fibres is a non-specific change seen in a variety of muscular disorders and has been described in patients with rheumatoid arthritis, osteomalacia, and thyroid disease as well as in a variety of non-muscular disorders (Edwards et al., 1980). Additionally, moth-eaten fibres are a non-specific change seen in muscular dystrophy, inflammatory disorders of skeletal muscle and spinal muscular atrophy (Edwards et al., 1980). Similarly, paracrystalline deposits are regarded as a relatively non-specific finding, although they are considered an ultrastructural marker of mitochondrial myopathies (Diamauro et al., 1984). As these paracrystalline deposits were found in the skeletal muscle of only one patient in this study, it is possible that this patients had an underlying mitochondrial myopathy. However, the patient did not show any other signs of mitochondrial myopathy such as the presence of ragged red fibres. Furthermore, Lloreta et al., (1996a) described the first case report of paracrystalline deposits in the diaphragm muscle of a patient with COPD. As Lloreta et al., (1996a) did not find these paracrystalline deposits in the latissimus dorsi muscle or the intercostal muscle of the same patient, they suggested selective involvement of the diaphragm muscle and hypothesized that this was due to overuse of the diaphragm in these patients. However, the findings in our study suggests that this abnormality is not selective to the respiratory

skeletal muscles and includes the larger muscles of locomotion.

It is therefore apparent that various non-specific structural abnormalities exist in the skeletal muscles of patients with COPD. It is difficult to determine what might be responsible for this muscle damage. Possible causes include; age, disuse of the peripheral limbs, ischaemia and ingestion of corticosteroid medications (Horber et al., 1986; Decramer et al., 1994).

Age, however was not responsible for the structural abnormalities seen in the peripheral skeletal muscle of the patients investigated in this study. An age and gender matched control group did not show the same degree of muscle damage in their peripheral skeletal muscle. Although some of the structural abnormalities were present in the age matched controls, the extent of the pathology was significantly less. It almost appeared as though the peripheral skeletal muscle of the patients with COPD had aged prematurely.

Similarly, it is unlikely that disuse was responsible for the structural abnormalities reported, for the following reasons. Firstly, as discussed in Chapter Three of this dissertation, the patients with COPD were not significantly less active than the control subjects as measured by the Yale Physical Activity Questionnaire for Older Adults. Secondly, some of the structural changes described in the peripheral skeletal muscle of the patients with COPD in this study have previously been documented in the respiratory muscle of these patients (Campbell et al., 1980). The respiratory muscle in patients with COPD are overused, rather than underused and therefore similar changes in the large locomotor muscles are unlikely to be a result of disuse of the peripheral skeletal muscle. Thirdly, some of the structural abnormalities reported in the locomotor muscles of the patients with COPD are not usually associated with disuse. Fibre splitting and internal nuclei are associated with muscular activity rather than disuse and are signs of regeneration in skeletal muscle (Edwards et al., 1980).

Corticosteroid medication has been reported to cause myopathy of skeletal muscle in patients with chronic disease (Horber et al., 1986). The main feature of steroid myopathy is atrophy of both fibre types (Pleasure et al., 1970). The dose of corticosteroids necessary to induce myopathy is

variable; prednisone 15-100mg daily ranging from one month to five years has been shown to cause a skeletal muscle myopathy (Affifi et al., 1968; Askari et al., 1976). It is unlikely that ingestion of corticosteroid medications was solely responsible for the skeletal muscle pathology seen in the patients in this study for the following reasons: Firstly, the main feature of corticosteroid myopathy, Type I and Type II fibre atrophy, was not observed in the patients examined in this study. In contrast, atrophy seemed to be isolated to the Type II fibres. Horber et al., (1986) reported increased Type II fibre atrophy in renal transplant patients ingesting prednisone. Unfortunately Horber et al., (1986) compared the skeletal muscle biopsies of renal transplant patients ingesting prednisone to skeletal muscle biopsies of normal healthy control subjects. It is therefore difficult to assume that the skeletal muscle structural changes seen in Horber's study (1986) is due solely to prednisone ingestion. Despite the fact that the average time since transplant was 16 months, the effects of the previous renal failure itself cannot be overlooked in relation to the observed skeletal muscle structural changes in the renal transplant patients. Secondly, the patients with COPD ingesting prednisone in this study tended to have greater skeletal muscle pathology than those not ingesting prednisone. However, only five of the 13 patients were ingesting corticosteroid medication, whilst all 13 patients showed skeletal muscle pathology. Therefore prednisone alone could not be responsible for the structural skeletal muscle abnormalities observed in the patients with COPD in this study. Thirdly, the greater structural skeletal muscle abnormalities observed in the patients with COPD ingesting prednisone did not seem to effect the function of the skeletal muscle in these patients. The group of patients with COPD ingesting prednisone did not have significantly greater impairment in skeletal muscle function or exercise tolerance compared to those patients not ingesting prednisone. The results of this dissertation contradict those of Horber et al., (1987) who reports significantly lower isokinetic peak quadriceps force in renal transplant patients ingesting prednisone compared to controls. However, Horber et al., (1987) compared peak quadriceps force in renal transplant patients ingesting prednisone to controls, as opposed to renal transplant patients not ingesting prednisone. Therefore, the observed weakness in skeletal muscle strength in the renal transplant patients may have been due to the effects of the renal disease itself prior to transplantation and not to the prednisone as such. In summary therefore, despite the fact that prednisone ingestion may have contributed, in part, to the structural abnormalities in the skeletal muscle of the patients with COPD

in this dissertation, prednisone ingestion did not seem to effect skeletal muscle functional abnormalities or exercise intolerance in these patients. In addition, although prednisone ingestion may have played a role in the structural skeletal muscle damage seen in patients with COPD in this study, it is unlikely to be the primary cause.

Lastly, the possibility that the patients with COPD in this study have a decreased capillary density, which was unfortunately not measured, cannot be overlooked. Decreased capillary density or normal capillary density coupled with fibre atrophy could retard the delivery of oxygen and nutrients to the muscle cells. This would lead to a relatively ischaemic environment for the muscles cells. However, an ischaemic environment for the peripheral skeletal muscle of the patients with COPD in this study is unlikely to have caused the development of a myopathy in these peripheral skeletal muscles. As previously reported, during ischaemic training, the metabolic profile of skeletal muscle shifts towards facilitating aerobic metabolism with a higher percentage of Type I fibres and a lower percentage of Type II fibres (Esbjornsson et al., 1993). The patients in this study showed an increased percentage of Type II fibres and not an increased percentage of Type I fibres as would be expected in response to an ischaemic environment.

Therefore, the mechanism causing the skeletal muscle pathology documented in the peripheral skeletal muscle of the patients with COPD is not known. Considering the importance of the skeletal muscle pathology and the fact that the pathology may play an important role in the exercise intolerance experienced by these patients, further research investigating the mechanism of this peripheral skeletal muscle pathology in is warranted.

A further important finding of this study was that the skeletal muscle pathology previously reported in the respiratory muscle of patients with COPD (Campbell et al., 1980) is unlikely to be isolated to the respiratory muscles. Previous research has shown pathological structural changes in the respiratory skeletal muscle of these patients (Mizuno et al., 1991; Campbell et al., 1980; Karlson et al., 1992). The changes reported in the respiratory skeletal muscle included: fibre splitting, centralized nuclei, variation in fibre size and atrophy of Type II fibres (Campbell et al., 1980).

These changes have previously been reported to be exclusive to the respiratory skeletal muscle of patients with COPD as a result of selective overwork of the respiratory muscles (Campbell et al., 1980). However, the changes in the respiratory skeletal muscle are identical to the changes reported in the quadriceps femoris muscle of the patients with COPD in this study. This finding therefore suggests that this skeletal muscle pathology occurs not only in the respiratory muscle of these patients but within the larger muscles of locomotion. Further evidence to support the hypothesis that the skeletal muscle abnormalities may not be exclusive to the respiratory muscles of these patients is provided by current literature reports detailing metabolic abnormalities in the oxidative pathways of both the respiratory skeletal muscle (Campbell et al., 1980) and the vastus lateralis muscles (Maltais et al., 1997) of patients with COPD.

An interesting finding from this study is that the pathological changes reported in the skeletal muscle biopsies of patients in this study, including fibre size variation, fibre splitting, internal nuclei and moth-eaten fibres have previously been reported in patients in patients with chronic heart failure and chronic renal failure in this laboratory (Derman et al., 1993; Diesel et al., 1993). However, the skeletal muscle abnormalities reported in patients with COPD can primarily be distinguished from those reported in cardiac failure patients by two observations. Firstly, there was no evidence of thickened basement capillary membranes in the patients with COPD which was the outstanding feature amongst the patients with chronic heart failure and secondly, the severe nuclear abnormalities reported in the patients with COPD were not the most prominent feature in the skeletal muscle of the patients with chronic heart failure.

The second aim of this study was to examine whether the decreased functional ability of the skeletal muscle in patients with COPD as reported in Chapter Three was related to the extent of the structural abnormalities seen in the skeletal muscle in these patients. It would appear that this is indeed the case. A significant relationship exists between the isokinetic strength and endurance measures of skeletal muscle function and the extent of the skeletal-muscle pathology . Patients with the highest pathology scores generated the lowest peak torque, particularly during the test of isokinetic endurance ability involving 30 repeated contractions.

Furthermore, the functional and structural abnormalities seen in the skeletal muscle of the patients with COPD appeared to play a role in their exercise limitation. A relationship between the extent of the skeletal muscle damage and the measures of peak exercise performance during both the incremental cycle test to exhaustion and the SMWT demonstrated the importance of the role that this skeletal muscle damage may play in the exercise intolerance reported in these patients.

The last and unexpected finding in this study was that despite the fact that the skeletal muscle pathology in the patients with COPD clearly correlates to skeletal muscle functional ability and general exercise tolerance, no relationship was found between FEV_1 and the extent of skeletal muscle damage. This raises the question of whether FEV_1 is an accurate predictor of either disease severity or functional capacity in patients with COPD.

Therefore, in summary, structural skeletal muscle abnormalities characterized by severe nuclear abnormalities are present in the peripheral locomotor muscles of patients with COPD. These abnormalities are unlikely to be a result of age, corticosteroid ingestion, disuse of the peripheral skeletal muscle or an ischaemic environment for the skeletal muscle of these patients. The structural abnormalities relate to the functional abnormalities described in Chapter Three and seem to play a role in the exercise intolerance experienced by patients with COPD. Thus, further investigation into the mechanism of this skeletal muscle damage is warranted.

CHAPTER FIVE

PREDICTORS OF EXERCISE CAPACITY IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

1. Introduction

In the last decade, numerous researchers have investigated predictors of exercise capacity in patients with COPD (Bauerle 1985; Gallagher 1990; Rampulla et al., 1992; Maltais et al., 1997). Whilst some researchers have shown that FEV_1 , used historically as a measure of severity of COPD, is indeed a useful predictor of exercise capacity in these patients (Bauerle 1985; Gallagher 1990; Karlsson et al., 1991), others have shown poor or no correlations between FEV_1 and exercise capacity in patients with COPD (Rampulla et al., 1992; Younes 1995; Maltais et al., 1997).

Variables other than FEV_1 have been shown to predict exercise capacity in patients with COPD. These include TLCOsb, which is a measure of pulmonary diffusing capacity, (Karlsson et al., 1991; Anderssen et al., 1992; Wijkstra et al., 1994; Gosselink et al., 1996), V_E (Younes et al., 1995), IC (Younes et al 1995), cardiovascular function (Fujii et al.,1996), blood lactate accumulation (Patessio et al., 1992) and measures of respiratory skeletal muscle strength (Gosselink et al., 1996).

In Chapters Three and Four of this dissertation, evidence that abnormalities in skeletal muscle structure and function contribute to exercise limitation in patients with COPD was provided. It is therefore likely that the skeletal muscle structure and function may play a role in predicting exercise capacity in these patients. Some preliminary research supports this hypothesis. Gosselink et al., (1996) measured peak $V0_2$ during an incremental cycle test and the distance walked during a SMWT in 41 patients with COPD (FEV_1 43±19 %). In addition, lung function, peripheral skeletal muscle strength and respiratory skeletal muscle strength were measured and correlated with distance walked during the SMWT and peak $V0_2$ achieved during the incremental cycle test to exhaustion. Peak quadriceps force accounted for 25% of the variability of the peak $V0_2$ achieved during the incremental cycle test to exhaustion and 40% of the variability of the distance walked during the SMWT. Maximal hand grip strength accounted for 28% of the variability in the peak $V0_2$ achieved and 37% of the variability in the distance walked during the SMWT, whilst FEV_1 did not correlate to exercise tolerance. Therefore, in these 41 patients it would appear that peripheral skeletal muscle function was an important predictor of exercise capacity.

However, research investigating the role of peripheral skeletal muscle function as a predictor of exercise capacity in patients with COPD is sparse. In addition, to the best of our knowledge, no research is available examining the role of peripheral skeletal muscle histology in predicting exercise capacity in patients with COPD. Accordingly, the aim of this study was to examine the role of lung function, peripheral skeletal muscle structure and function, physical activity levels and duration of disease in predicting the exercise capacity of 13 patients with COPD.

2. Methods

A correlation analysis was performed, according to the methods detailed in Chapter Two to determine the relationship between various measures of exercise capacity including 1) peak $V0_2$ achieved during the incremental cycle test to exhaustion, 2) peak workload achieved during the incremental cycle test to exhaustion, 3) distance walked during the SMWT and various possible predictors of this exercise capacity including 1) lung function variables, 2) physical activity level, 3) duration of disease and 4) measures of peripheral skeletal muscle structure and function in 13 patients with COPD. The characteristics of the 13 patients are described in Chapter Three.

3. Results

The results of the correlation analysis are presented in Table 5.1

Table 5.1 Results of the correlation analysis between measures of resting lung function, measures of peripheral skeletal muscle function, miscellaneous variables and parameters of physical performance in patients with COPD.

	Performance Variables		
Lung function variables	Peak $V0_2$ ($ml0_2/kg/min$)	Peak WL (Watts)	SMWT distance walked (metres)
FEV_1 /FVC (%) (Rest)	r=0.72 p<0.01 n=12	NS	r =0.81 p<0.01 n=13
FEV_1 (l) (Rest)	r=0.52 p=0.08 n=12	NS	r=0.59 p<0.05 n=13
TLCOsb (ml/mmHg/min) (Rest)	r =0.81 p<0.01 n=12	r =0.52 p=0.07 n=12	r =0.62 p<0.05 n=13
IC (l) (Rest)	NS	r =0.70 p<0.05 n=10	r =0.58 p=0.07 n=10
$Pa0_2$ (kPa) (Rest)	NS	NS	NS
$PaC0_2$ (kPa) (Rest)	NS	r =0.74 p<0.05 n=9	NS
Lw $Sa0_2$ (%) (During exercise)	NS	NS	NS
Miscellaneous variables			
Physical activity levels	NS	NS	NS
Duration of disease	NS	r =-0.60 p<0.05 n=11	r =-0.65 p<0.05 n=12
Peripheral SM structure			
Biopsy Pathology Score (AU)	r =-0.63 p=0.05 n=10	r =-0.67 p<0.05 n=10	r =-0.63 p=0.05 n=10
Peripheral SM function			
Peak Torque Quads (Strength) (Nm)	NS	r =0.65 p=<0.05 n=12	r =0.54 p=0.05 n=13
Work Done Quads (Endurance (W)	NS	r =0.87 p=<0.01 n=12	r =0.59 p=<0.05 n=13
Total Power Quads (Endurance) (J)	NS	r =0.81 p=0.01 n=12	r =0.61 p=<0.05 n=13
Peak Torque Quads Eccentric (Strength) (Nm)	NS	NS	r =0.89 p=0.01 n=6

Abbreviations: **FEV_1**, forced expiratory volume in 1 second; **FVC**, forced vital capacity; **VO_2 peak**, peak oxygen consumption; **WL**, work load; **IC**, inspiratory capacity measured at rest, **TLCOsb**, transfer factor measured at rest; **$Pa0_2$**, partial pressure of oxygen in the arterial blood at rest; **$PaC0_2$**, partial pressure of carbon dioxide in the arterial blood at rest, **LW$Sa0_2$**, lowest level of oxygen saturation during the maximal cycle test to exhaustion as measured by ear oximetry; **Duration of disease**, length of time in months since diagnosis of COPD; **AU**, arbitrary units; **Biopsy Score**, score given to the extent of pathology from the biopsies of patients with COPD; **SMWT**, six minute walk test; **NS**, not significant

(a) Predictors of peak $V0_2$

FEV_1/FVC accounted for 52% of the variability of peak $V0_2$ achieved, whilst FEV_1 accounted for 27% of the variability of peak $V0_2$ achieved by the patients with COPD during the incremental cycle test to exhaustion. Transfer factor accounted for 66% and the skeletal muscle biopsy pathology score accounted for 40% of the variability of the peak $V0_2$ achieved amongst the patients during the incremental cycle test to exhaustion (Fig 5.2b). No relationship was found between the peak $V0_2$ achieved during the incremental cycle test to exhaustion and; IC, physical activity reported, $Pa0_2$ at rest, $PaC0_2$ at rest, desaturation during exercise, duration of disease and the tests of skeletal muscle function.

(b) Predictors of peak workload achieved

Predictors of peak workload achieved during the incremental cycle test to exhaustion included; TLCOsb, IC, $PaC0_2$ at rest, duration of disease, skeletal muscle biopsy pathology score and measures of isokinetic strength and isokinetic endurance in the knee extensors. No relationship was found between peak workload achieved during the incremental cycle test to exhaustion and FEV_1, FEV_1/FVC, $Pa0_2$ at rest, desaturation during exercise, physical activity reported and eccentric peripheral skeletal muscle function.

(c) Predictors of distance walked during the six minute walk test

The strongest predictors of the distance walked during the SMWT were FEV_1/FVC (Fig 5.1b) which accounted for 66% of the variability and peak torque during the eccentric quadriceps strength test which accounted for 79% of this variability. Weaker predictors of the distance walked during the SMWT included FEV_1 (Fig 5.1a), TLCOsb, IC, duration of disease (Fig 53b), skeletal muscle biopsy pathology score (Fig 5.2a), quadriceps isokinetic strength and quadriceps isokinetic endurance (Fig 5.3a). No relationship was found between the distance walked during the SMWT and $Pa0_2$ at rest, $PaC0_2$ at rest, desaturation during exercise and physical activity reported.

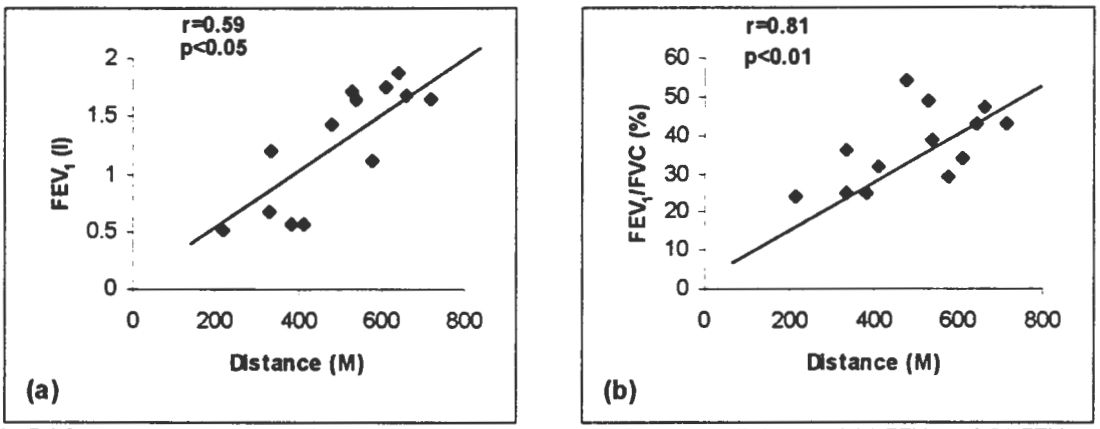

Fig 5.1 Relationship between distance walked during the six minute walk test and (a) FEV_1 and (b) FEV_1/FVC in patients with COPD

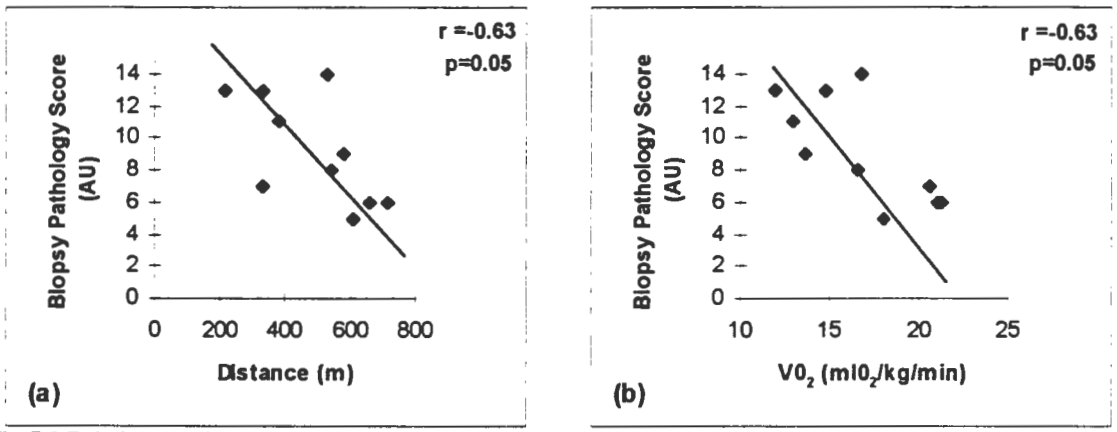

Fig 5.2 Relationship between biopsy pathology score and (a) distance walked during the six minute walk test and (b) peak $V0_2$ achieved during the incremental cycle test to exhaustion in patients with COPD (AU; arbitrary units)

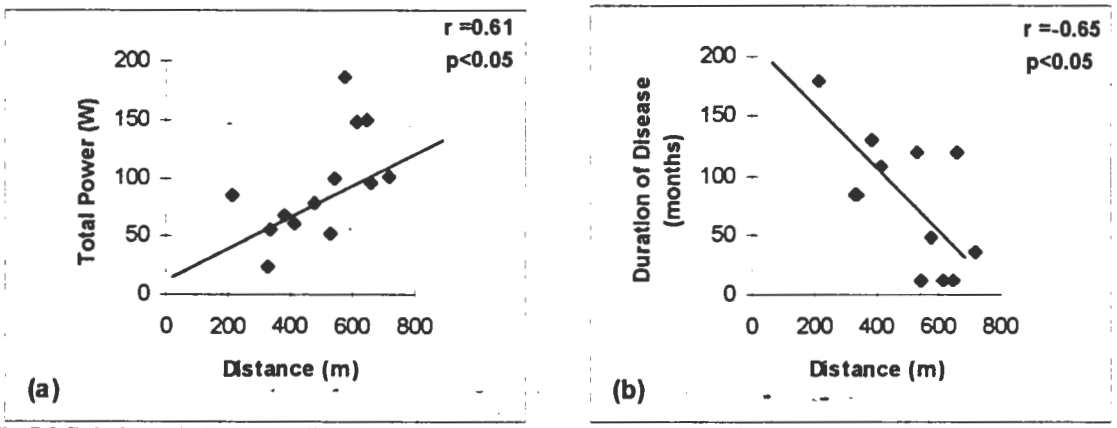

Fig 5.3 Relationship between distance walked during the six minute walk test and (a) total power produced by the quadriceps during the isokinetic test of skeletal muscle endurance and (b) duration of disease in patients with COPD

4. Discussion

The most important finding of this study was that skeletal muscle structure and function contributed to exercise intolerance in patients with COPD. Structural skeletal muscle damage was inversely related to peak $V0_2$, peak workload and distance walked during the SMWT. Additionally, skeletal muscle function positively related to peak workload and peak $V0_2$ achieved during the incremental cycle test to exhaustion in these patients. Gosselink et al., (1996) also showed that both peak quadriceps force and peak handgrip strength were related to exercise limitation in patients with COPD. These findings suggest that skeletal muscle is an important contributor to exercise ability in these patients.

The second important finding was that the lung function measures of TLCOsb, FEV_1/FVC and FEV_1 contributed significantly to exercise intolerance in patients with COPD. The contribution of TLCOsb to exercise limitation in theses patients has been previously reported by other authors (Karlsson et al., 1991; Anderssen et al., 1992; Wijkstra et al., 1994; Gosselink et al., 1996).

Some authors (Rampulla et al., 1992; Maltais et al., 1997; Gosselink et al., 1996) have demonstrated no relationship between FEV_1 and exercise capacity in patients with COPD. In contrast, the findings of this study are similar to those of Gallagher (1990) and Bauerle (1985) who demonstrated a positive relationship between exercise capacity and FEV_1. In deriving the relationship between exercise capacity and FEV_1 in these patients, authors have used different methods of describing the FEV_1 including FEV_1 (l) (Gosselink et al., 1997; Karlsson et al., 1991); FEV_1 as a % of predicted or FEV_1 as a % of FVC (Bauerle et al., 1985). This could account for some of the differences reported in the literature. The results of this study suggest that FEV_1/FVC is a better predictor of exercise tolerance in patients with COPD than FEV_1.

An interesting finding was that duration of COPD contributed to exercise intolerance whilst reported level of physical activity energy expenditure did not appear to contribute to this exercise intolerance. Support for the former finding is shown by an inverse relationship between the duration of COPD and the distance walked during the SMWT and the peak workload achieved during the incremental cycle test to exhaustion. The fact that the subjects' physical activity level

was not related to any of the measures of exercise ability can be interpreted in two ways. Firstly, it is possible that the factors affecting self reported physical activity are different to the factors limiting exercise ability in patients with COPD. Alternatively, the questionnaire used to assess physical activity in this study may not have been a sensitive enough predictor of actual physical activity level in these patients.

A further interesting finding was the significant positive relationship between the knee flexor eccentric function and the distance patients were able to walk during the SMWT. This data needs to be interpreted with caution due to the very small sample size. Further research with a larger sample size is needed to confirm this finding.

In conclusion therefore, it is clear that lung function and skeletal muscle function contribute to exercise intolerance in patients with COPD. However, lung function does not alter after exercise training in these patients (Clark et al., 1996; Younes et al, 1996; Simpson et al., 1992). Accordingly, a factor other than improved lung function must be responsible for the improved functional ability of trained patients with COPD. Chapter Six examines the possibility that improved skeletal muscle structure and function may be responsible for the improved functional ability of trained COPD patients.

CHAPTER SIX

A CASE REPORT OF THE SKELETAL MUSCLE STRUCTURAL AND FUNCTIONAL CHANGES AND EXERCISE CAPACITY IN A PATIENT WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE, BEFORE AND AFTER EXERCISE TRAINING

1. Introduction

Exercise rehabilitation programmes for patients with COPD are a common component of their treatment (Degre et al., 1974; Ferguson et al., 1993; Siafakis et al., 1995). Patients with COPD benefit from regular exercise by demonstrating improvements in functional ability (Morley et al., 1976; Ferguson et al., 1993). However, despite improved functional ability, these patients do not demonstrate improvements in lung function measures of either gas exchange or ventilation mechanics after exercise training (Clark et al., 1996; Younes et al, 1996; Simpson et al., 1992). This finding implies that a factor other than improved lung status must be responsible for the improved functional ability in the patients with COPD after exercise training.

If, as shown in this dissertation, skeletal muscle damage contributes to exercise intolerance in patients with COPD then it could be hypothesized that exercise rehabilitation training reduces this skeletal muscle damage and results in improved exercise tolerance in patients with COPD. Therefore investigation into the responses of these peripheral skeletal muscles to training in patients with COPD is clearly warranted.

The aim of this case study was therefore to examine changes in skeletal muscle structure and function in a patient with COPD before and after exercise rehabilitation.

2. Methodology

One of the patients (AJ) with COPD described in Chapters Three, Four and Five of this dissertation agreed to participate in an exercise rehabilitation training programme for 12 months. Patient AJ had a pack history of 37.5 years and had terminated smoking one year prior to the onset of the study. On entry to the rehabilitation programme and again after 12 months of training AJ underwent; a maximal cycle test to exhaustion, a SMWT, pulmonary function measurements, a skeletal muscle biopsy from the vastus lateralis muscle, isokinetic tests of skeletal muscle strength and endurance and anthropometrical analysis according to the methods detailed in Chapter Two. AJ completed the Yale Physical Activity Questionnaire for Older Adults on entry to the study and after 12 months of exercise rehabilitation training. For the purpose of interim data collection, after three months of training the patient underwent; a skeletal muscle biopsy from the vastus lateralis

muscle, a SMWT and a maximal cycle test to exhaustion. Due to technical failure of the isokinetic apparatus, the isokinetic tests of skeletal muscle functional ability were not recorded after three months of exercise training. The results were compared to an average value for the 13 sedentary control subjects examined in Chapters Three and Four.

Patient AJ participated in exercise rehabilitation at the Respiratory Rehabilitation Programme conducted at the Sports Science Institute of SA, Cape Town, South Africa. The patient attended rehabilitation three times a week , each time for an hour. The patient's training programme is described in Table 6.1.

Table 6.1 Exercise training programme undertaken by patient AJ three times a week for 12 months.

Exercise	Programme at start of rehabilitation	Programme after 12 months of rehabilitation	Description
Walking	150m	2200m	1lap = 74m
Cycling	none	5-10 min.	0-15W (self selected)
Circuit Weight Training Exercises	10 reps 1 set 6 machines	20-30 reps 3 sets 6 machines	Upper and lower limb load constantly upgraded
Jog	none	150m	
Breathing Exercises	Pursed lip breathing performed during exercise and during recovery phases		

The exercise rehabilitation programme was gradually increased by the patient over the 12 month period as his exercise tolerance improved.

3. Results

(a) Resting characteristics of and medication ingested by the patient AJ

Table 6.2 Resting characteristics of, and medication ingested by, the patient with COPD on entry to the study and after three months and 12 months of training.

Training (months)	Mass (kg)	WPAE (kCal)	Medications
0	71	2145	Ipratropium Bromide (when necessary), Budesonide (2 puffs a.m. & 2 puffs p.m.), Theophylline (2 x 5mg daily) & (2 x 5mg daily) Prednisone (2 x 5mg daily)
3	68	Not tested	Ipratropium Bromide (when necessary), Budesonide (2 puffs a.m. & 2 puffs p.m.), Theophylline (2 x 5mg daily) & Prednisone (2 x 5mg daily)
12	68	4467	Ipratropium Bromide (when necessary), Budesonide (2 puffs a.m. & 2 puffs p.m.), & Prednisone (2 x 5mg daily)

Abbreviations: kg, kilogram; WPAE, weekly physical activity expenditure; kCal, kilocalories; mg, milligrams; am, morning; pm, evening.

Patient AJ was a 58 yr old male with a mass of 71kg on entry to the study. He had been diagnosed with COPD 15 years previously and had a smoking pack history of 37.5 years. Patient AJ had been very physically active as a young man and had not smoked for 10 years on entry to the study.

His weight decreased to 68kg after three months of training and remained the same after 12 months of training. After 12 months of exercise training, the patient AJ's weekly physical activity energy expenditure had more than doubled and he was no longer ingesting theophylline (Table 6.1).

(b) Lung function results

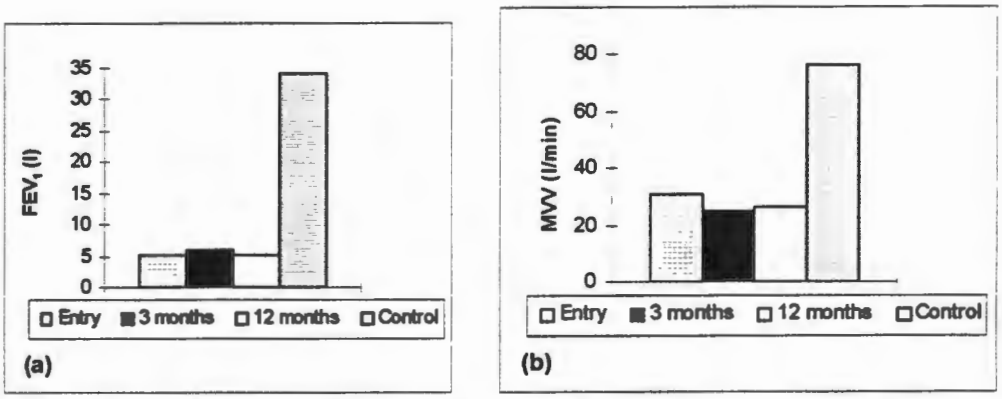

Fig 6.1 (a) FEV_1 and (b) Maximum Voluntary Ventilation (MVV) in patient AJ on entry to the study and after three and 12 months of training; compared to controls.

None of the lung function variables, including FEV_1 or MVV (Fig 6.1),altered substantially after training.

Table 6.3 Pulmonary function test results of the patient with COPD on entry to the study and after 3 and 12 months of training

Training (months)	PEFR (l/min)	TLCOsb (ml/mmHg/min)	PIFR (l/min)	IC (l)	$Sa0_2$ (%)
0	220	14	190	1.4	95
3	267	16	271	1.4	96
12	210	15	110	1.3	97
CONTROL	547	30	354	3.6	99

Abbreviations: PEFR, peak expiratory flow rate; TLCOsb, transfer factor; PEFR; peak expiratory flow rate; PIFR, peak inspiratory flow rate; IC, inspiratory capacity; $Sa0_2$, oxygen saturation in arterial blood as measured by ear oximetry at rest.

(c) Anthropometrical results

Percentage body fat and LTV did not change after three or 12 months of exercise training in the patient AJ.

(d) Peak exercise variables

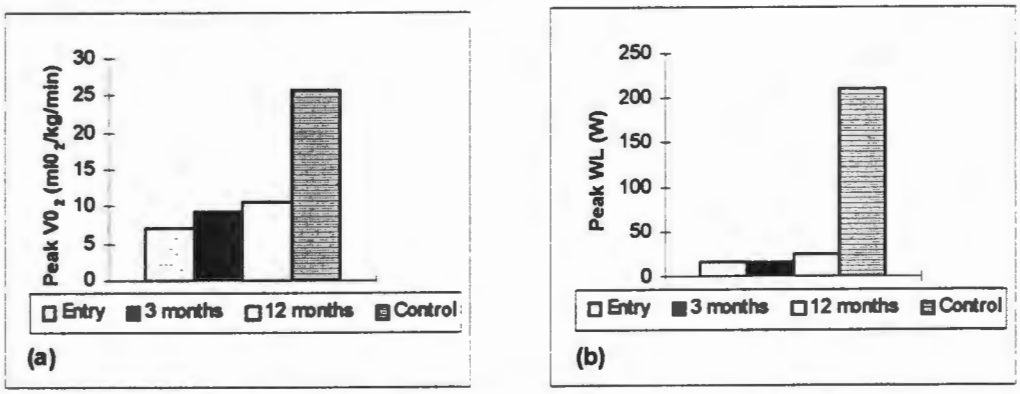

Fig 6.2 (a) Peak $V0_2$ and (b) peak work load achieved by patient AJ during the incremental cycle test to exhaustion on entry to the study and after three and 12 months of training; compared to controls.

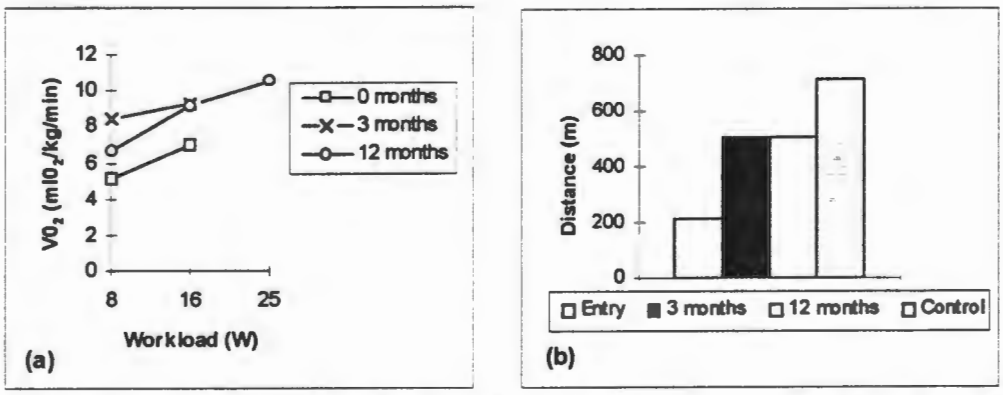

Fig 6.3 (a) $V0_2$ at three cycling work loads and (b) distance covered during the six minute walk test by patient AJ on entry to the study and after three and 12 months of training

Table 6.4 Peak variables achieved during the maximal cycle test to exhaustion in patient AJ on entry to the study and after three and 12 months of training

Training (months)	SBP (mmHg)	V_E max (l)	RER max ($VC0_2/V0_2$)	Plasma [lactate] (mmol/l)	Pk $Sa0_2$ (%)	Lw $Sa0_2$ (%)	Sa02 at Pk Watts
0	190	30	0.84	6.4	98	87	87
3	180	23	0.92	NS	97	88	90
12	170	25	1.06	4.6	98	87	88

Abbreviations: SBP, systolic blood pressure; HR, heart rate; V_E max, peak minute ventilation; RER max, peak respiratory exchange ratio; VCO_2, volume of carbon dioxide expired; Pk $Sa0_2$, highest recording of arterial saturation during the maximal cycle test and recovery; LW $Sa0_2$, lowest recording of arterial oxygen saturation during the maximal cycle test and recovery; $Sa0_2$ at Pk watts, level of oxygen saturation of arterial blood at peak watts as measured by ear oximetry

The maximal cycle test to exhaustion demonstrated an unchanged peak work load after three months of exercise training and an increase of 8 Watts after 12 months of training. Time to fatigue

during exercise did not change in the first three months and increased by one minute after 12 months of training. Heart rate at peak exercise increased from 114b/min to 144b/min during the first three months of training and decreased to 125b/min after 12 months of training. Systolic blood pressure at peak exercise decreased by 10 mmHg at three months and by a further 10 mmHg after 12 months of exercise training.

$V0_2$ (Fig 6.3), V_E, RER and HR were higher after three months and after 12 months of exercise training at a selected submaximal work load in the patient with COPD. Peak $V0_2$ was slightly higher after three months and after 12 months of exercise training (Fig 6.2). Peak RER increased by 0.08 after three months of training and by a further 0.14 after 12 months of training. Neither peak $Sa0_2$, the lowest $Sa0_2$ or the $Sa0_2$ at peak work load changed after exercise training.

(e) Results of the six minute walk test

After three months of training, the distance covered by patient AJ during the SMWT had increased by more than 50% (Fig 6.3), for the same RPE (4; Borg Units) and a similar peak HR (129 vs 124 b/min; 0 vs 3 months). After 12 months of training the distance had increased by only a further 4 metres (Fig 6.3), for a slightly higher peak HR (134 b/min) and a lower RPE (3; Borg Units).

(f) Results of the tests of skeletal muscle function

(i) Muscle strength

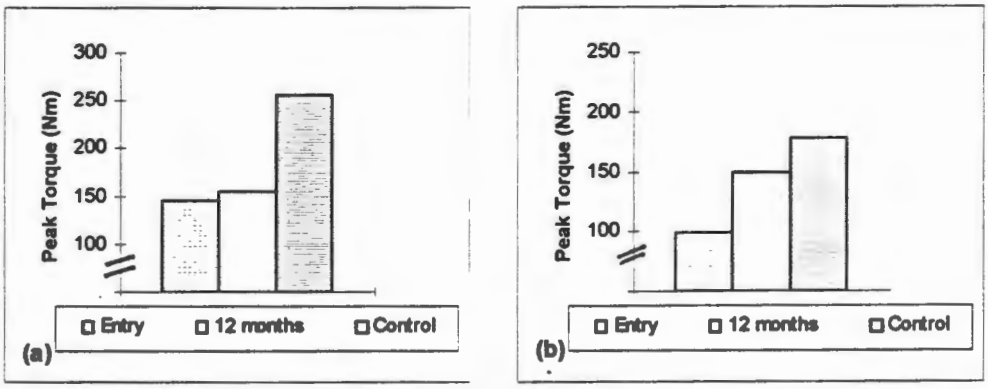

Fig 6.4 Peak torque produced by the (a) quadriceps and (b) hamstrings musculature during the isokinetic strength test by the patient AJ on entry to the study and after 12 months of training, compared to controls

Peak torque generated by the quadriceps and the hamstring muscles during the isokinetic strength test improved after 12 months of exercise training (Fig 6.4).

(ii) Muscle endurance

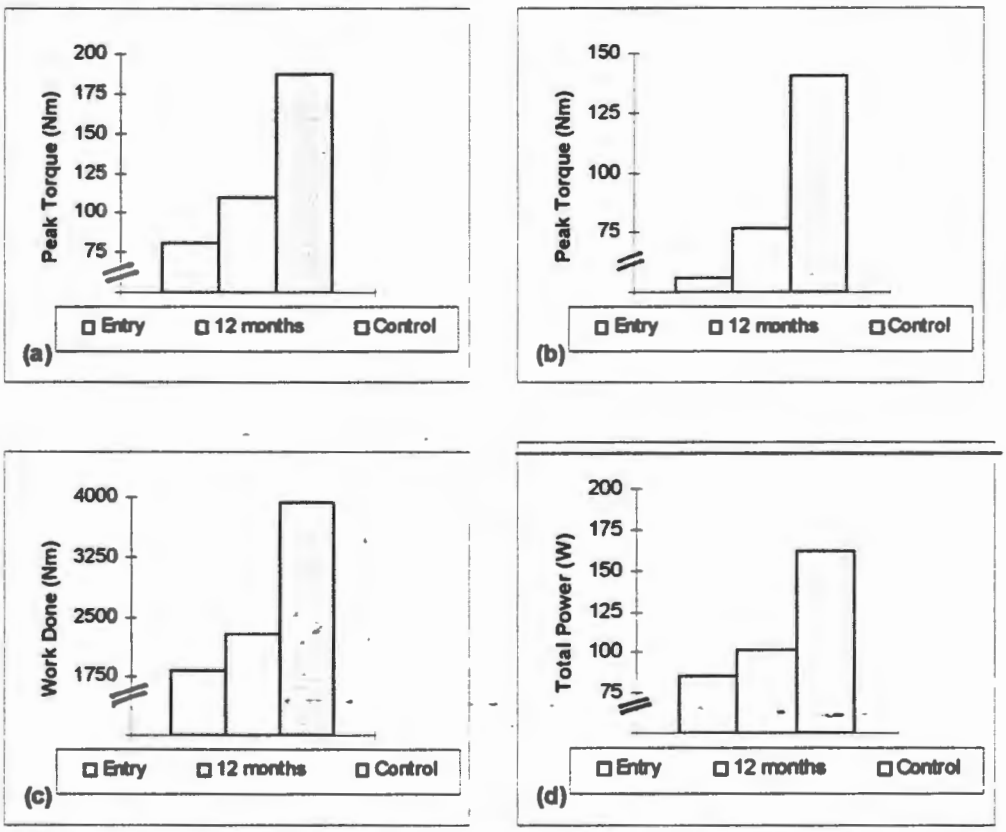

Fig 6.5 Peak Torque produced by the (a) quadriceps and (b) hamstrings musculature, (c) work done and (d) total power generated by the quadriceps during the isokinetic endurance test by the patient AJ on entry to the study and after 12 months of training, compared to controls

All the skeletal muscle function variables of muscle endurance measured during the isokinetic test improved after 12 months of exercise training (Fig 6.5). In particular, marked improvements were noted in work done and total power generated by the quadriceps during the endurance test.

(g) Skeletal muscle structural changes

(i) Light microscopy

Table 6.5 Light microscopy findings of the skeletal muscle biopsy of patient AJ on entry to the study and after three and twelve months of training.

Training (months)	Description	Pathology Score (arbitrary units)
0	Marked atrophy of fibres, with nuclear proliferation and the formation of nuclear bags are seen. Marked fibre splitting is present. Occasional fibres are hypertrophied and there is a large variation in fibre size. There is an increase in both internal nuclei and subsarcolemmal nuclear proliferation. SDH and NADH staining show a moth-eaten appearance affecting predominately Type I fibres	13
3	Numerous atrophic fibres, some only slightly larger than the nucleus are present. A mild increase in internal nuclei, occasional areas of nuclear proliferation, occasional fibre splitting and some nuclear clumps are seen.	4
12	The sections show numerous atrophic fibres, some nuclear bags and clumps and occasional internal nuclei.	5

The biopsy pathology score decreased from 13 AU to four AU after three months of exercise rehabilitation, this decrease was maintained after 12 months of exercise training. The improved biopsy pathology score in the patient AJ was achieved by reductions in fibre atrophy, internal nuclei, fibre splitting, nuclear abnormalities and moth eaten fibres. Fibre structure, size and regularity improved in patient AJ after three months of exercise training (Fig 6.6 and Fig 6.7).

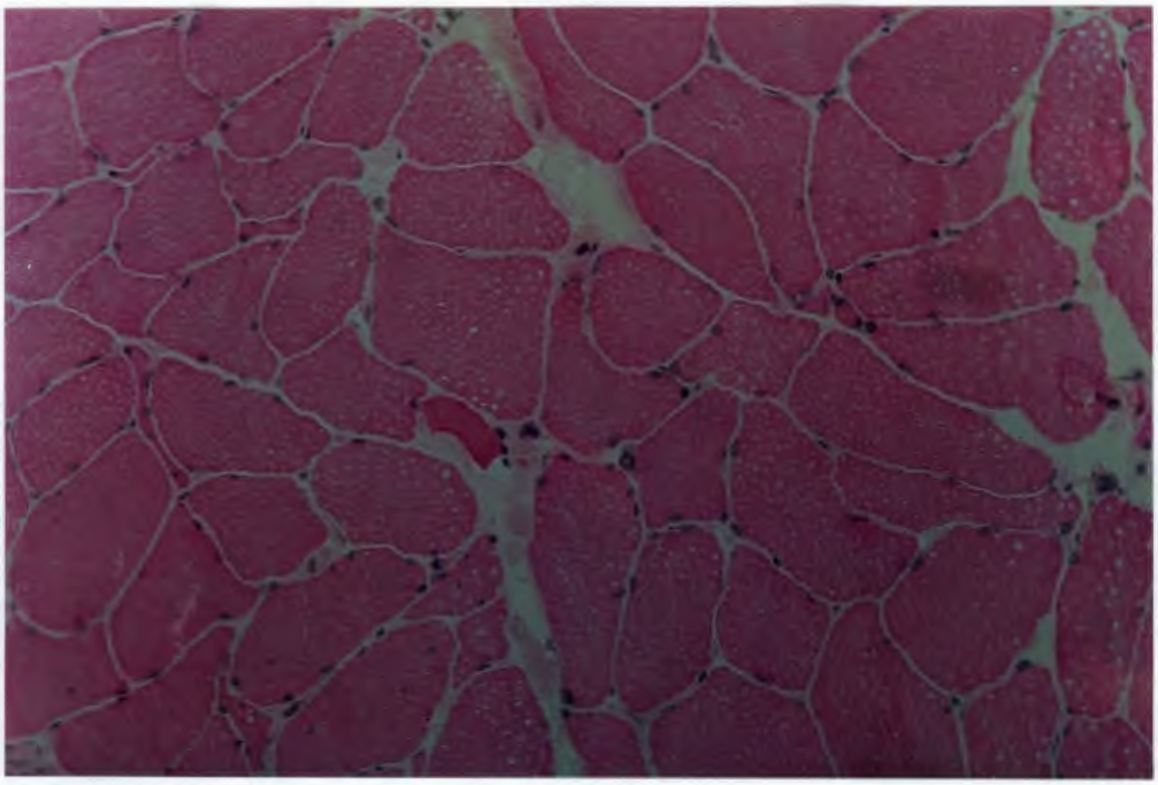

Fig 6.6 A light micrograph of the skeletal muscle biopsy sample of patient AJ prior to exercise training. Note the atrophy and the variation in fibre size and structure. (H&E x16)

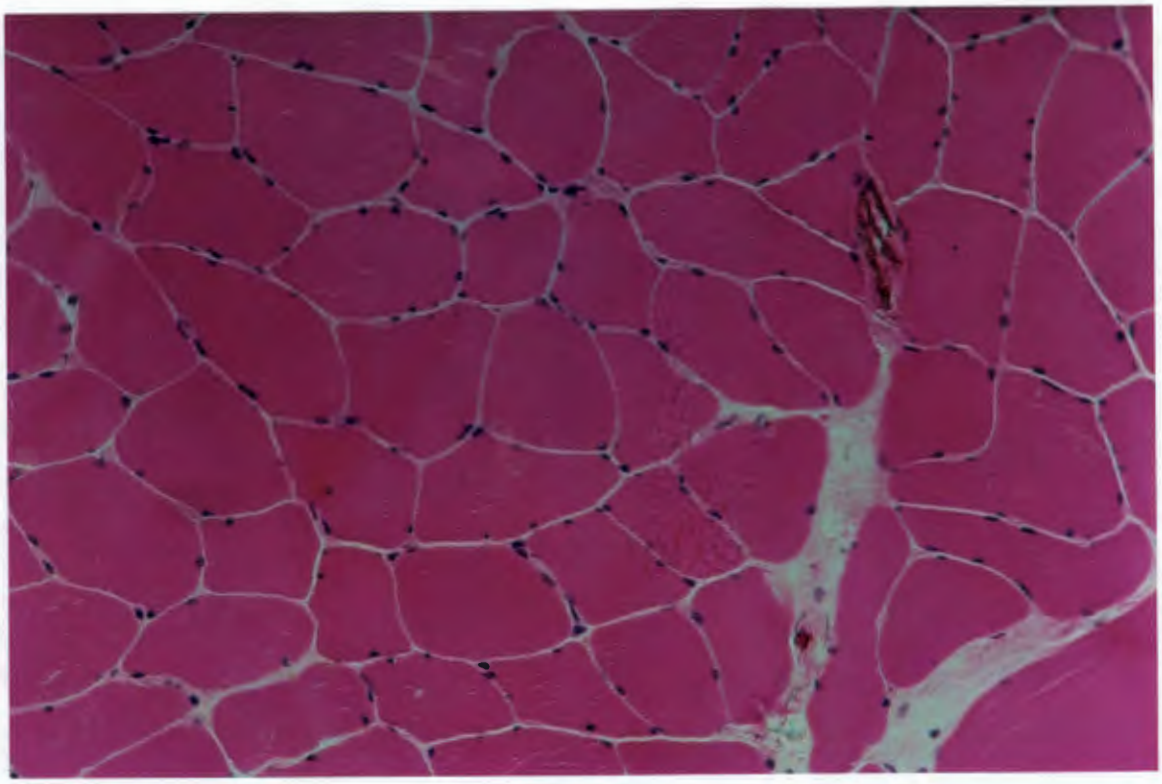

Fig 6.7 A light micrograph of the skeletal muscle biopsy sample biopsy of patient AJ after three months of exercise training (H&E X16). Note the improvement in fibre size variation, atrophy and structure.

(ii) Electron microscopy

Table 6.6 Electron Microscopy descriptions of the skeletal muscle biopsy samples from the patient AJ on entry to the study and after three and 12 months of training.

Training (months)	Description
0	Atrophic fibres are present, the sarcolemmal membrane shows a saw-tooth edge, satellite cells are present and there is a small area of fibre disarray. Subsarcolemmal glycogen is present.
3	Apart from mild streaming of the Z-bands, there is no abnormality on electron microscopy.
12	Mild fibre disarray is present.

The electron microscopy report had improved such that only a mild abnormality of fibre disarray was present after three and 12 months of exercise training.

(iii) Morphometric analysis

Table 6.7 Results of the morphometric analysis of the skeletal muscle biopsy samples from patient AJ on entry to the study and after three and twelve months of exercise training

Training (months)	Type I size (μm)	Type II size (μm)	Type I (%)	Type II (%)	Predominance
0	56 (5% are hypertrophied)	44 (35% are atrophied)	29	71	Normal distribution
3	55	54	29	71	Normal distribution
12	57	55	30	70	Normal distribution
CONTROL	58	58	38	62	

Abbreviations: μm, micrometres

The average size of the type II fibres increased from 44 to 54μm, whilst the type I fibre size remained the after three months of exercise training (Table 6.7). The fibre sizes did not change from three months to 12 months of training. There was no change in the % Type I or % Type II fibres after either three or 12 months of exercise training.

***(d)* Discussion**

The most important finding of this case study was the improved functional capacity demonstrated by patient AJ after exercise training. Patient AJ doubled both the distance he was able to walk during the SMWT and his weekly physical activity energy expenditure after exercise training.

The second important finding of this case study was that this improved functional capacity in patient AJ was not due to changes in either lung function or peak $V0_2$. These two variables did not improve substantially after exercise training. Similarly, the improved functional capacity was not a result of improved gas exchange as demonstrated by the absence of change in either peak or lowest saturation levels during the maximal cycle test after exercise training.

In contrast, both structural and functional changes in skeletal muscle were seen after exercise training in patient AJ. After 12 months of exercise training improvements were shown in the functional strength and endurance of the skeletal muscle of patient AJ. The improved skeletal muscle endurance ability in response to exercise training would correspond with the findings of Maltais et al., (1996) who demonstrated improved skeletal muscle oxidative capacity in patients with COPD after three months of exercise training. Unfortunately, in this case study, functional skeletal muscle ability was not measured after three months of exercise training.

After three months of exercise training the structural status of the peripheral skeletal muscle had improved. This improvement was maintained, but not further improved after 12 months of exercise training. After training, the skeletal muscle abnormalities typically seen in a patient with COPD (as discussed in Chapter Four) such as fibre atrophy and nuclear abnormalities were still present. However, the severity of these abnormalities had decreased. Therefore it would appear that participation in an exercise training programme resulted in some degree of reversal of the abnormalities seen in the skeletal muscle of patient AJ. These finding therefore suggest that the improved functional capacity seen in patient AJ after exercise training was related to the structure and function of his skeletal muscle.

Other interesting and important concepts demonstrated by this case study include: (i) no demonstration of a cardiovascular training effect in the maximum cycle test in patient AJ; (ii) no improvement in peak $V0_2$ after training; (iii) FEV_1 as a predictor of functional ability in a trained COPD patient and (iv) no change in anthropometric data after training. These will be briefly discussed in this numerical order.

Firstly, the $V0_2$, V_E, HR and RER at a set submaximal work load during the cycle test increased after three months of training and after 12 months of training in patient AJ. This increase in physiological variables at a set submaximal work load contrasts to a normal training effect. A lack of the normal cardiovascular training effect in patients with COPD has been reported by other authors (Degre et al., 1974; Chester et al., 1977). Degre et al., (1974) and Chester et al., (1977) attributed this lack of a cardiovascular training effect in patients with COPD to an inability of these patients to train at an intensity high enough to induce a training stimulus. However, patient AJ trained at a HR above 70-80% of his maximum, three times a week for a period of 20-40 minutes. This should be sufficient to induce a cardiovascular training effect (McArdle et al., 1991). A possible explanation for the higher V_E, HR, RER and $V0_2$ at submaximal work loads in this patient with COPD after training is that considering the improved structural status of the skeletal muscle, this patient may have been able to recruit more muscle fibres with a subsequent increase in the physiological response at that particular work load.

Secondly, a training response in a normal healthy control would result in an increase in peak $V0_2$. However, the peak $V0_2$ did not improve substantially in patient AJ after training. A possible explanation for the lack of improvement in peak $V0_2$, peak work load and exercise time to exhaustion after three months of training and minimal improvement after 12 months of training is the tool used for assessment. Peak $V0_2$ may not be an accurate predictor of exercise ability in patients with COPD. A dissociation between a measure of $V0_2$ max and the functional ability of patients with COPD has been reported by other authors (Guyatt et al., 1985; Clark et al., 1996; Milne et al., 1997 unpublished data from this laboratory). Milne et al., (1997) showed that peak $V0_2$ achieved during a maximal test was significantly lower in patients with COPD when a mouthpiece rather than a mask was used. The authors therefore suggested that the mouthpiece may impede

pursed lip breathing and thereby impair exercise capacity. All the maximal $V0_2$ tests conducted in this dissertation used a mouthpiece. Therefore, this patient may have terminated exercise earlier in this laboratory setting than he would have done so in a functional setting such as the SMWT rendering the peak $V0_2$ test an inaccurate predictor of his functional ability.

Thirdly, it is well established that FEV_1 does not improve after exercise training in patients with COPD (Clark et al., 1996; Younes et al, 1996; Simpson et al., 1992). However, FEV_1 is historically used as a measure of functional ability and disease severity in patients with COPD. This case study suggests that in patient AJ, after exercise training, FEV_1 would no longer be a good predictor of the functional ability.

Lastly, it is not surprising that LTV did not change in patient AJ as he was not participating in skeletal muscle **strength** training as such, but rather in skeletal muscle **endurance** training. Improvements in muscle mass were therefore unlikely (Flecker et al., 1997). Patient AJ demonstrated no change in percentage body fat after either three or 12 months of training despite a doubling of weekly energy expenditure. It is quite likely that he increased his food intake to match the increased energy expenditure. This could account for the lack of change in percentage body fat.

In summary therefore it appears that patient AJ derived benefit from exercise rehabilitation and that the improvements seen in his functional capacity after training were most likely due to improvements in the functional and structural status of his skeletal muscle. This case study therefore suggests that just as abnormalities in skeletal muscle may impair functional ability in patients with COPD, improvements in these abnormalities by exercise training increases the functional ability of these patients.

This case study has several limitations. Firstly, a single patient with COPD may not be an accurate predictor of the average response of COPD patients due to the variable nature of the disease. Therefore, group studies are necessary to confirm the findings suggested by this case study. Secondly, a lack of measures of the functional ability of the skeletal muscle after three months of

training made it difficult to determine whether skeletal muscle functional changes played a role in the improved functional capacity of patient AJ after three months of training.

Future research should focus on (i) functional, structural and metabolic changes in the peripheral skeletal muscle of a group of patient with COPD before and after exercise training and (ii) the response of patients with COPD to different types of training programmes, i.e. circuit weight training programmes as opposed to traditional cardiovascular training programmes.

CHAPTER SEVEN

SUMMARY AND CONCLUSIONS

1. Summary

The main aim of this dissertation was to investigate the role of skeletal muscle in the exercise intolerance experienced by patients with COPD. This was investigated in four parts.

Firstly, exercise capacity and skeletal muscle function and the relationship between these two variables were evaluated in a group of patients with COPD. Secondly, the histology, ultrastructure and morphometry of skeletal muscle in these patients was descriptively analyzed and an objective histological scoring system was used to quantify the skeletal muscle pathology observed. The relationship between this skeletal muscle pathology; exercise capability and skeletal muscle function was then analyzed. Thirdly, the ability of skeletal muscle function, lung function, physical activity levels and duration of disease to predict exercise capacity in patients with COPD was evaluated. Lastly, a case study examined changes in skeletal muscle structure and function before and after training in a patient with COPD.

The first study, reported in Chapter Three, demonstrated severely limited exercise tolerance in patients with COPD and provided evidence to suggest that this exercise intolerance was most likely due to peripheral limitations of skeletal muscle as opposed to central limitations of the heart and lungs. Furthermore, a relationship between skeletal muscle function and exercise capacity in these patients supported this hypothesis.

The study reported in Chapter Four showed significant histological and ultrastructural abnormalities in the skeletal muscle of the patients with COPD. The changes were evident on light microscopy and included non-specific changes such as type II atrophy, fibre splitting, moth eaten fibres and a hallmark of nuclear abnormalities including nuclear knots, nuclear chains, nuclear clumps and internalized nuclei . Electron microscopy supported these findings by demonstrating a variety of nonspecific abnormalities including mitochondrial changes, fibril dissolution and accumulation of subsarcolemmal material in both groups, but more prominently in the patients with COPD. The histological abnormalities were quantitatively scored and proved to be significantly higher in patients compared to age matched control subjects. This skeletal muscle pathology score correlated significantly with peak $V0_2$ achieved, peak workload achieved, skeletal muscle function and distance walked during the SMWT. This finding suggests that skeletal muscle structure may

play a role in exercise limitation in patients with COPD.

Chapter five analyzed predictors of exercise capacity in patients with COPD. Both measures of lung function and skeletal muscle function proved to be useful predictors of exercise performance in untrained patients with COPD whilst FEV_1/FVC % proved to be a better predictor of exercise capacity than FEV_1. However, it is well established that exercise capacity in patients with COPD improves after training, yet lung function does not change (Clark et al., 1996; Younes et al, 1996; Simpson et al., 1992).

Therefore, in Chapter Six of this dissertation changes in skeletal muscle structure and function before and after exercise training were analyzed in a case report of a patient with COPD. The results of this case report showed improvements in skeletal muscle structure and function after training. This preliminary finding therefore suggests that improved skeletal muscle structure and function in patients with COPD after training may account, at least in part, for their improved exercise tolerance.

2. Conclusions

This dissertation confirms that severe structural and functional abnormalities exist in the skeletal muscle of patients with COPD and that these may limit exercise capacity in these patients. In addition, the results of this thesis suggest that measures of skeletal muscle structure and function may be useful predictors of exercise capacity in patients with COPD. Some preliminary evidence suggests that the improved exercise capacity in patients with COPD after exercise training may be due to improvements in skeletal muscle structure and function.

Future research should therefore aim to (i) determine the effects of exercise training on skeletal muscle in a group of patients with COPD, (ii) determine how this relates to functional ability and (iii) examine the use of skeletal muscle exercise in patients with COPD who are unable to participate in aerobic endurance training due to the severity of their illness.

CHAPTER EIGHT

REFERENCES

Afifi AK, Bergman RA, Harvey JC. Steroid myopathy: clinical, histological and cytological observations. Johns Hopkins Med 1968;123:158-174

American Thoracic Society. Standardisation of spirometry 1987 update. ATS statement. Am Rev Respir Dis 1987;136:1285-1298

Andersen SJ, Arvidsson U, Fransson L, Nemcek K, Svensson SE. The relationship between the transfer factor obtained at rest, and arterial oxygen tension during exercise, in patients with miscellaneous pulmonary diseases. Journal Int Med 1992;232:415-419

Anderson RJ. Muir's textbook of pathology. 11th edition: 460-466. Edward Arnold, London 1980.

Askari A, Vignos PJ, Moskowitz RW. Steroid Myopathy in connective tissue diseases. Am J Med 1976;61:485-492

Baarends EM, Schols AM, Pannemans DLE, Westerterp KR, Wouters EFM. Total free living energy expenditure in patients with severe chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1997;155:549-555.

Bahler RC, Chester EH, Belman MJ, Baum GL. Multidisciplinary treatment of chronic pulmonary insufficiency, the influence of intrathoracic pressure variations on increases in pulmonary vascular pressure during exercise in patients with chronic obstructive pulmonary disease. Chest 1977;72:703-708

Bauerle O, Younes M. Role of ventilatory response to exercise in determining exercise capacity in COPD. J. Appl Physiol 1995;79:1870-1877

Bellemare F, Grassino A. Force reserve of the diaphragm in patients with chronic obstructive pulmonary disease. J Appl Phsiol 1983;55:8-15

Belman MJ. Factors limiting exercise performance in lung disease. Chest 1992; 5:101-105

Belman MJ. Pulmonary rehabilitation in chronic respiratory insufficiency, exercise in patients with chronic obstructive pulmonary disease. Thorax 1993;48:936-946

Belman JM, Kendregan B. Exercise training fails to increase skeletal muscle enzymes in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 1981;123:256-261

Belman MJ, Mittman C. Ventilatory muscle training improves exercise capacity in chronic obstructive pulmonary disease patients. Am Rev Respir Dis 1980;121:273-280

Bernstein ML, Despars JA, Singh NP, Avalos K, Stansbury DW, Light RW. Reanalysis of the 12-minute walk in patients with chronic obstructive pulmonary disease. Chest 1994;105: 163-167

Brown SE, King RR, Temerlin SM. Exercise performance with added dead space in chronic airflow obstruction. J Appl Physiol 1984;56:1020-1026

Brown SE, Casciari RJ, Light RW. Arterial oxygen saturation during meals in patients with severe chronic obstructive pulmonary disease. South Med J 1983;76:194-198

Brown SE, Milne N, Stansbury DW, Light RW. Effects of digoxin on exercise capacity and right ventricular function during exercise in chronic airflow obstruction. Chest 1984;85:187-191

Buller NP, Jones D, Poole-Wilson PA. Direct measure of skeletal muscle fatigue in patients with chronic heart failure. Br Heart J 1991;65:20-24

Burke L, Deakin V. Clinical sports nutrition. 1st edition: 1-5. McGraw Hill Book Company, Sydney, Australia 1994

Campbell JA, Hughes RL, Sahgal V, Frederiksen J, Shields TW. Alterations in intercostal muscle morphology and biochemistry in patients with obstructive lung disease. Am Rev Respir Dis 1980;122:679-686

Carlson DJ, Ries AL, Kaplan RM. Predictors of maximum exercise tolerance in patients with COPD. Chest 1991;100:307-311

Carone M, Patessio A, Appendini L, Purro A, Czernicka E, Zanaboni S, Donner CF. Comparison of invasive and non-invasive saturation monitoring in prescribing oxygen during exercise in COPD patients. Eur Respir J 1997;10:446-451

Carrieri-Kohlman V, Gormley JM, Douglas MK, Paul SM, Stulbarg SM. Differentiation between dyspnea and its affective components. West J Nurs Research 1996;18:626-642

Carter R, Coast RJ, Idell S. Exercise training in patients with chronic obstructive pulmonary disease. Med Sci Sports Exerc 1991; 24:281-291

Chester EH, Bleman MJ, Bahler RC, Baum GL, Schey G, Buch P. Multidisciplinary treatment of chronic pulmonary insufficiency, the effect of physical training on cardiopulmonary performance in patients with chronic obstructive pulmonary disease. Chest 1977;72:695-702

Clark CJ, Cochrane L, Mackay E, 1996. Low intensity peripheral muscle conditioning improves exercise tolerance and breathlessness in COPD. Eur Respir J 1996;9:2590-2596

Clark TJH, Freedman S, Campbell EJM. The ventilatory capacity of patients with chronic airways obstruction. Clin Sci 1969;36:307-316

Clausen JP, Slausen K, Rasmussen B, Trap-Jensen J. Central and peripheral circulatory changes after training of the arms or legs. Am J of Physiol 1973; 225:675-682

Cockroft A, Beaumont A, Adams L, Guz A. Arterial oxygen saturation during treadmill and bicycle exercise in patients with COPD. Clin Sci 1985;68:327-332

Cooper C. Determining the role of exercise in patients with chronic pulmonary disease. Med Sci Sports Exerc 1995;27:147-157

Dantzker DR, D'Alanzo GE. The effect of exercise on pulmonary gas exchange in patients with severe chronic obstructive pulmonary disease. Am Rev Respir Dis 1986;134:1135-1139

Decramer M, Lacquet LM, Fagard R, Rogiers P. Corticosteroids contribute to muscle weakness in chronic airflow obstruction. Am J Respir Crit Care Med 1994;150:11-16

Decramer M, Gosselink R, Troosters T, Verschuere M, Evers G. Muscle weakness is related to utilisation of health care resources in COPD patients. Eur Respir J 1997;10:417-423

Degre S, Sergysels R, Messin R, Vandermoten P, Salhadin P, Denolin H, De Coster A. Haemodynamic responses to physical training in patients with chronic lung disease. Am Rev Respir Dis 1974;110:395-402

Dempsey JA, Fregosi RF. Adaptability of the pulmonary system to changing metabolic requirements. Am J Cardiol 1985;55:59D- 67D

Derenne JPH, Macklem PT, Roussous CH. The respiratory muscles: mechanics, control and pathophysiology Part I. Am Rev Respir Dis 1978;118:119-133

Derenne JPH, Macklem PT, Roussous CH. The respiratory muscles:mechanics, control, and pathophysiology Part II. Am Rev Respir Dis 1978;118:373-390

Derenne JPH, Macklem PT, Roussous CH. The respiratory muscles:mechanics, control, and pathophysiology Part III. Am Rev Respir Dis 1978;118:581-601

Derman EW, Derman KL, Emms M, St Clair-Smith C, Brink J, Noakes TD. Abnormal skeletal muscle histology and not the ejection fraction predicts exercise tolerance in patients with chronic heart failure. Int Soc for Heart Research, European section meeting. Monduits editions. Int proceedings division 1993;183-188

De Troyer A, Leeper JB, McKenzie DK, Candevia SC. Neural drive to the diaphragm in patients with severe COPD. Am J Respir Crit Care Med 1997;155:1335-1340

De Troyer A, Kelly S, Macklem P, Zin W. Mechanics of intercostal space and actions of external and internal intercostal muscles. J Clin Invest 1985;75:850-857

Diesel WD, Emms M, Knight B, Noakes TD, Swanepoel C, Van Zyl Smit R, Kaschula R, Sinclair-Smith C. Morphological features of the myopathy associated with chronic renal failure. Am J Kid Dis 1993;22:677-689

Diesel WD, Noakes TD, Swanepoel C, Lambert M. Isokinetic muscle strength predicts maximum exercise tolerance in renal patients on chronic hemodialysis. Am J Kid Dis 1990;XVI:109-114

DiMauro S, Bonilla E, Zeviani M, Nakagawa M, DeVivo C. Mitochondrial myopathies. Ann Neurol 1985;17:521-538

Dodd DS, Brancatisano T, Engel LA. Chest wall mechanics during exercise in patients with chronic obstructive airflow obstruction. Am Rev Respir Dis 1984;129:33-38

Donahoe M, Rogers RM, Wilson DO, Pennock BE. Oxygen consumption of the respiratory muscles in normal and in malnourished patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 1989;140:385-391

Drexler H. Skeletal muscle failure in heart failure. Circulation 1992;85:1621-1623

Driver AG, McAlevy MT, Smith JL. Nutritional assessment of patients with chronic obstructive pulmonary disease and acute respiratory failure. Chest 1982;82:568-571

Dubowitz V. Muscle Biopsy - a practical approach. 2nd edition: 3-11. Bailliere Tindall, London 1985

Durnin JVGA, Wormersley J. Body fat assessed from the total body density and its estimation from skinfold thickness: measurements on 481 mean and women aged from 16 to 72 years. Br J Nutrition 1974;32:77-97

Edwards R, Young A, Wiles M. Needle biopsy of skeletal muscle in the diagnosis of myopathy and the clinical study of muscle function and repair. New Engl J of Med 1980;302:260-271

El-Manshawi A, Kilian KJ, Summers E, Jones NL. Breathlessness during exercise with and without resistive loading. J Appl Phsiol 1986;61:896-906

Esbjörnsson M, Jansson E, Sundberg CJ, Sylvén C, Eiken O, Nygren A Kaijser L. Muscle fibre types and enzyme activities after training with local leg ischaemia in man. Acta Physiol Scand 1993;148:233-241

Ferguson GT, Cherniak RM. Management of chronic obstructive pulmonary disease. N Engl J Med 1993;328:1017-1022

Fiaccadori E, Del Canale S, Vitali P, Coffrini E, Ronda N, Guariglia A. Skeletal muscle energetics, acid-base equilibrium and lactate metabolism in patients with severe hypercapnia and hypoxemia. Chest 1987;92:883-887

Fleck SJ, Kraemer WJ. Designing resistance training programmes. 2nd edition. Human Kinetics, Champaign, USA 1997

Fridén J, Seger J, Ekblom B. Sublethal muscle fibre injuries after high-tension anaerobic exercise. Eur J Appl Physiol 1988;57:360-368

Fujii T, Kurihara N, Fujimoto S, Hirata K, Yoshikawa J. Role of pulmonary vascular disorder in determining exercise capacity in patients with severe chronic obstructive pulmonary disease. Clin I Physiol 1996;16:521-533

Gallagher CG. Exercise and chronic obstructive pulmonary disease. Med Clin of North Am 1990;74:619-641

Gertz I, Hedenstierna G, Hellers G, Wahren J. Muscle metabolism in patients with chronic obstructive lung disease and acute respiratory failure. Clin Sci and Molec Med 1977;52:395-403

Gimenez M, Predine E, Marchand M, Servera E, Ponz JL, Polu JM. Implications of lower and upper limb training procedure in patients with chronic airway obstruction. Chest 1992;101:279S-288S

Goodnight-White SJ, Miller CC, Haber ES, Klein PD, Fletcher EC. Lactate kinetics in severe COPD - implications of an abnormal aminopyrine breath test. Chest 1992;101:268S-273S

Gosselink R, Troosters T, Decramer M. Peripheral muscle weakness contributes to exercise limitation in COPD. Am J Respir Crit Care Med 1996;153:976-980

Gutierrez G, Pohil RJ, Narayana P. Skeletal muscle O_2 consumption and energy metabolism during hypoxemia. Am Physiol Soc 1989;2117-2123 (no volume given)

Gutmann L, Blumenthal D, Gutmann L, Schochet SS. Acute type II myofibre atrophy in critical illness. Neurology 1996;46:819-821

Guyatt GH, Berman LB, Townsend M, Pugsley S, Chamger L. A measure of quality of life for clinical trials in chronic lung disease. Thorax 1987;42:773-778

Guyatt GH, Thompson JP, Berman BL, Sullivan MJ, Townsend M, Jones NL, Pugsley SO. How should we measure function in patients with chronic heart and lung disease? J Chron Dis 1985;38:517-524

Guyton AC. Textbook of medical physiology. 5th edition: 576-579. WB Saunders Company, Philadelphia, London, Toronto 1976.

Harber P. Respiratory disability. The uncoupling of oxygen consumption and disability. Clin Chest Med 1992;13:367-376

Horber FF, Hoppeler H, Herren D, Claassen H, Howald H, Gerber C, Frey FJ. Altered skeletal muscle ultrastructure in renal transplant patients on prednisone. Kidney Int 1986;30:411-416.

Horber FF, Hoopeler H, Schneidegger JR, Grunig BE, Howald H, Frey FJ. Impact of physical training on the ultrastructure of midthigh muscle in normal subjects and in patients treated with glucocorticoids. J Clin Invest 1987;79:1181-1190.

Horvath SM, Borgia JF. Cardiopulmonary gas transport and ageing. Am Rev Respir Dis 1984;129: S68-S71

Jacobsson P, Jorfeldt L, Brundin. Skeletal muscle metabolites and fibre types in patients with advanced chronic obstructive pulmonary disease (COPD), with and without chronic respiratory failure. Eur Respir J 1990;3:192-196

Johnson BD, Dempsey JA. Exercise and sports science reviews - Demand vs capacity in the ageing pulmonary system : 171-210. Williams and Wilkins, Baltimore 1991

Jones DA, Round JM. Skeletal muscle in health and disease . 1st edition: 153-155. Manchester University Press, Manchester, 1990
Jones NL. Dyspnea in exercise. Med Sci Sports Exerc 1984;16:14-19

Jones NL. Normal values for pulmonary gas exchange during exercise. Am Rev Respir Dis 1984;129:S44-S46

Karlsson J, Diamant B, Folkers K. Exercise limiting factors in respiratory distress. Respiration 1992;59:18-23

Kempeneers G, Noakes TD, Van Zyl-Smit R, Myburgh KH, Lambert M, Adams B, Wiggins T. Skeletal muscle limits the exercise tolerance of renal transplant recipients: effects of a graded exercise training program. Am J Kidney Dis 1990;XVI:57-65

Kyroussis D, Polkey MI, Keilty SE, Mills GH, Hamnegard C, Moxham J, Green M. Exhaustive treadmill exercise does not reduce twitch diaphragmatic pressure in patients with COPD. Am J Respir Crit Care Med 1995;152:959-964

Kyroussis D, Polkey MI, Keilty SE, Mills GH, Hamnegard C, Moxham J, Green M. Exhaustive exercise slows inspiratory muscle relaxation rate in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1996;153:787-793

Lane R, Adams L, Guz A. The effects of hypoxia and hypercapnia on perceived breathlessness during exercise in humans. J Physiol 1990;428:579-593

Larsson L, Grimby G, Karlsson J. Muscle strength and speed of movement in relation to age and muscle morphology. Am Physiol Soc 1979;451-455

Larsson L. Histochemical characteristics of human skeletal muscle during ageing. Acta Physiol Scand 1983;117:469-471

Leach RM, Davidson AC, Chinn S, Twort CHC, Cameron IR, Bateman NT. Portable liquid oxygen and exercise ability in severe respiratory disability. Thorax 1992;47:781-789

Leblanc P, Bowie DM, Summers E, Jones NL, Kilian KJ. Breathlessness and exercise in patients with cardiorespiratory disease. Am Rev Respir Dis 1986;133:21-25

Lipkin DP, Jones DA, Round JM, Poole-Wilson P. Abnormalities of skeletal muscle in patients with chronic heart failure. Int J Cardiol 1988;18:187-195

Lloreta J, Orozco M, Gea J, Corominas J, Serrano S. Selective diaphragmatic mitochondrial abnormalities in a patient with marked airflow obstruction. Ultrastruct Pathol 1996; 20:67-71 (a)

Lloreta J, Roquer J, Corominas J, Serrano S. Hyperthyroid myopathy with mitochondrial paracrystalline rectangular inclusions. Ultrastruct Pathol 1996;20:61-65 (b)

MacNee W. Oxidants and antioxidants in smoking induced lung disease, part 1. Asthma 1997;June:123-126 (a)

MacNee W. Oxidants and antioxidants in smoking induced lung disease, part 2. Asthma 1997;July:141-143 (b)

Magnusson G, Isberg B, Karlberg K, Sylven C. Skeletal muscle strength and endurance in chronic congestive heart failure secondary to idiopathic dilated cardiomyopathy. Am J Cardiol 1994:73;307-309.

Mahler DA. The measurement of dyspnea during exercise in patients with lung diseases. Chest 1991;101:242S-247S

Mahler DA, Harver A. Prediction of peak oxygen consumption in obstructive airways disease. Med Sci Sports Exerc 1988;20:574-578

Maltais F, Simard A, Simard C, Jobin J, Desgagnes P, LeBlanc P, Janvier R. Oxidative capacity of the skeletal muscle and lactic acid kinetics during exercise in normal subjects and in patients with COPD. Am J Respir Crit Care Med 1996;153:288-293 (a)

Maltais F, LeBlanc P, Simard C, Jobin J, Berube C, Bruneau J, Carrier L, Belleu R. Skeletal muscle adaptation to endurance training in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1996;154:442-447 (b)

Maltais F, LeBlanc P, Jobin J, Berube C, Bruneau J, Carrier L, Breton M, Falardeau G, Belleau R. Intensity of training and physiologic adaptation in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1997;155:555-561

Mancini D, Coyle E, Ferraro N. Skeletal muscle metabolic abnormalities in heart failure are in part due to intrinsic skeletal muscle changes. J Am Coll Cardiol 1989;13:39A

Marin JM, Hussain SNA, Gibbons WJ, Polverino M, Levy RD, Coşio MG. Relationship of resting lung mechanics and exercise pattern of breathing in patients with chronic obstructive lung disease. Chest 1993;104:705-711

Matthews JI, Bush BA, Ewald FW. Exercise responses during incremental and high intensity and low intensity steady state exercise in patients with obstructive lung disease and normal control subjects. Chest 1989;96:11-17

Matthay RA, Arroliga AC, Wiedemann HP, Schulman DS Mahler DA. Right ventricular function at rest and during exercise in chronic obstructive pulmonary disease. Chest 1992;101:255S-262S

Mathur RS, Revill SM, Vara DD, Walton R, Morgan MDL. Comparison of peak oxygen consumption during cycle and treadmill exercise in severe chronic obstructive pulmonary disease. Thorax 1995;50:829-833

McArdle WD, Katch FI, Katch VL. Exercise physiology. Energy nutrition and human performance. Third edition 68-75. Lea & Febiger, Philidelphia, USA 1991

McGavin CR, Gupta SP, Lloyd EL, McHardy GJR. Physical rehabilitation for the chronic bronchitic: results of a controlled trial of exercises in home. Thorax 1997;32:307-311

McGurk SP, Blanksby BA, Anderson MJ. The relationship of hypercapnic ventilatory responses to age, gender and athleticism. Sport Med 1995;19:173-183

Midorikawa J, Hida W, Taguchi O, Okabe S, Kurosawa H, Mizusawa A, Ogawa H, Ebihara S, Kikuchi Y, Shirato K. Lack of ventilatory threshold in patients with chronic obstructive pulmonary disease 1997;64:76-80

Minotti JR, Christoph I, Oka RK, Weiner MW, Wells L, Massie BM. Impaired skeletal muscle function in patients with congestive heart failure: relationship to systemic exercise performance. J Clin Invest 1991;88:2077-2082

Mizuno M. Human respiratory muscles: fibre morphology and capillary supply. Eur Respir J 1997;4:587-601

Möller P, Bergström J, Furst P, Hellström. Effect of ageing on energy-rich phosphagens in human skeletal muscles. Clin Sci 1980;58:553-555

Montes de Oca M, Rassulo J, Celli BR. Respiratory muscle and cardiopulmonary function during exercise in very severe COPD. Am J Respir Crit Care Med 1996;154:1284-1289

Moore GE, Brinker KR, Stray-Gundersen J, Mitchell JH. Determinants of VO_2 peak in patients with end stage renal disease: on and off dialysis. Med Sci Sports Exerc 1992;18-23

Morley M, Cherniak R. Rehabilitation of patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 1976;114:1145-1151

Mungall IPF, Hainsworth R. Assessment of respiratory function in patients with chronic obstructive airways disease. Throrax 1979;34:254-258

Nicholas JJ, Gilbert R, Gabe R, Howland-Auchincloss J. Evaluation of an exercise therapy programme for patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 1970;102:1-9

Noakes T. Lore of running. Third edition: 512-515. Oxford University Press, Cape Town, South Africa 1992

O'Donnell DE, Webb KA, McGuire MA. Older patients with COPD: benefits of exercise training. Geriatrics 1993;48:59-66

O'Donnell DE, Sanii R, Younes M. Improvements in exercise endurance in patients with chronic airflow limitation using positive airways pressure. Am Rev Respir Dis 1988;138:1510-1514

Olopade CO, Beck CK, Viggiano RW, Staats BA. Exercise limitation and pulmonary rehabilitation in chronic obstructive pulmonary disease. Mayo Clin Proc 1992;67:144-157

Owens GR, Rogers RM, Pennock BE. The diffusing capacity as a predictor of arterial oxygen desaturation during exercise in patients with chronic obstructive pulmonary disease. N Eng J Med 1984;310:1218-1221.

Palange P, Galassetti P, Mannix ET, Farber MO, Manfredi F, Serra, P Carlone S. Oxygen effect on O_2 deficit and $V0_2$ kinetics during exercise in obstructive pulmonary disease. J Appl. Physiol 1995;78:2228-2234

Pardy RL, Rivington RN, Despas PJ. The effects of inspiratory muscle training on exercise performance in chronic airflow limitation. Am Rev Respir Dis 1981;123:426-433

Patessio A, Carone M, Ioli F, Donner CF. Ventilatory and metabolic changes as a result of exercise training in COPD patients. Chest 1992;101:274S-278S

Patessio A, Casaburi R, Crone M, Appendini L, Ferdinando Donner C, Wasserman K. Comparison of gas exchange, lactate, and lactic acidosis thresholds in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 1993;148:622-626

Pleasure DE, Walsh GO, Engel WK. Atrophy of skeletal muscles in patients with Cushing's syndrome. Arch Neurol 1970;22:118-125

Polkey MI, Kyroussis D, Keilty S, Hamnegard C, Mills G, Green M, Moxham J. Exhaustive treadmill exercise does not reduce twitch transdiaphragmatic pressure in patients with COPD. Am J Respir Crit Care Med 1995;152:959-964

Raffestin B, Escourrou P, Legrand A, Duroux P, Lockhart A. Circulatory transport of oxygen in patients with chronic airflow obstruction exercising maximally. Am Rev Respir Dis 1982;125:426-431

Rampulla C, Baiocchi S, Dacosto E, Ambrosino N. Dyspnea on exercise: pathophysiologic mechanisms. Chest 1992;101:248S-251S

Redelmeier DA, Bayoumi AM, Goldstein RS, Guyatt GH. Interpreting small differences in functional status: the six minute walk test in chronic lung disease patients. Am J Respir Crit Care Med 1997;155:1278-1282

Rochester DF, Braun NMT, Narinder SA. Respiratory muscle strength in chronic obstructive pulmonary disease. Am Rev Respir Dis 1979;119:151-4

Sanchez J, Brunet A, Medrano G, Debesse B, Derenne J. Metabolic enzymatic activities in the intercostal and serratus muscles and in the latissimus dorsi of middle-aged normal men and patients with moderate obstructive pulmonary disease. Eur Respir J 1988;1:376-383

Schols AM, Soeters PB, Mostert R, Pluymers RJ, Wouters EF. Physiological effects of nutritional support and anabolic steroids in patients with chronic obstructive pulmonary disease. A placebo controlled randomised trial. Am J Respir Crit Care Med 1995;152:1268-1274

Scully RE, Mark EJ, McNeely WF, Ebeling SH, Phillips LD. Case records of the Massachusetts general hospital. N Eng J Med 1997;336:1079-1088

Siafakas NM, Vermiere P, Pride NB, Paoletti P, Gibson J, Howard P, Yernault JC, Decramer M, Higgenbottam T, Postma DS, Rees J. Optimal assessment and management of chronic obstructive pulmonary disease (COPD). Eur Respir J 1995;8:1398-1420

Simard AA, Maltais F, LeBlanc P. Functional outcome of patients with chronic obstructive pulmonary disease and exercise hypercapnia. Eur Respir J 1995;8:1339-1344

Simpson K, Killian K, McCartney N, Stubbing DG, Jones NL. Randomised controlled trial of weightlifting exercise in patients with chronic airflow limitation. Thorax 1991;47:70-75

Sinoway LI, Minotti JR, Dwight D, Pencock JL, Burg JE, Musch TI, Zelis R. Delayed reversal of impaired vasodilation in congestive heart failure after heart transplantation. Am J Cardiol 1988;61:1076-1079

Sogel Schenken N, Burdet L, de Muralt B, Fitting JW. Oxygen saturation during daily activities in chronic obstructive pulmonary disease. Eur Respir J 1996;9:2584-2589

Spiro SG, Hahn HL, Edwards RHT, Pride NB. An analysis of the physiological strain of submaximal exercise in patients with chronic obstructive bronchitis. Thorax 1975;30:415-425

Spiro SG. Exercise testing in clinical medicine. Br J Dis Chest 1977;71:145-172

Stoebner P, Mezin P, Vila A, Grosse R, Kopp N, Paramelle B. Microangiopathy of endoneurial vessels in hypoxemic chronic obstructive pulmonary disease (COPD). Acta Neuropathol 1989;78: 388-395

Sue DY, Wasserman K, Moricca RB, Casaburi R. Metabolic acidosis during exercise in patients with chronic obstructive pulmonary disease. Chest 1988;94:931-938

Tada H, Kato H, Misawa T, Sasaki F, Hayashi, S Takahashi H, Kutsumi Y, Ishizaki T, Nakai T, Miyabo S. 31P-Nuclear Magnetic resonance evidence of abnormal skeletal muscle metabolism in patients with chronic lung disease and congestive heart failure. Eur Respir J 1992;5:163-169

Takahashi M, Hood DA. Chronic stimulation-induced changes in mitochondria and performance in rat skeletal muscle. J Appl Physiol 1993 4:934-941

Taylor DJ, Crowe M, Bore PH, Styles P, Arnold DL, Radda GK. Examination of the energetics of ageing skeletal muscle using nuclear magnetic resonance. Gerontology 1984;30:2-7

Thompson CH, Davies RJO, Kemp GJ, Taylor DJ, Radda GK, Rajagopalan B. Skeletal muscle metabolism during exercise and recovery in patients with respiratory failure. Thorax 1993;48:486-490

Tiep BL, Burns M, Kao D, Madison R, Herrera J. Pursed lip breathing training using ear oximetry. Chest 1986;90: 218-221

Tobin MJ. Respiratory muscles in disease. Clin Chest Med 1988;9:263-284

Wagner PD. Ventilation-Perfusion matching during exercise. Chest 1992;101:192S-198S

Wagner PD, Dantzker DR, Dueck R, Clausen JL, West JB. Ventilation-Perfusion inequality in chronic obstructive pulmonary disease. J Clin Invest 1977;59:203-216

Wagner PD. Determinants of maximal oxygen transport and utilisation. Ann Rev Physiol 1996;58:21-50

Wanke Th, Formanek D, Lahrmann H, Brath H, Wild M, Wagner Ch., Zwick H. Effects of combined inspiratory muscle and cycle ergometer training on exercise performance in patients with COPD. Eur Respir J 1994; 7:2205-2211

Weaver TE, Richmond TS, Narsavage GL. An explanatory model of functional status in chronic obstructive pulmonary disease. Nur Research 1997;46:26-31

West JB. Pulmonary Pathophysiology - the essentials. 4th edition. Williams and Wilkins, Baltimore, USA 1992

West JB. Respiratory Physiology - the essentials. 5th edition. Williams and Wilkins, Baltimore, USA 1995

Wijkstra PJ, Ten Vergert EM, Van der Mark TW, Postma DS, Van Aletna R, Kraam J, Koeter GH. Relation of lung function, maximal inspiratory pressure, dyspnea and quality of life with exercise capacity in patients with chronic obstructive pulmonary disease. Thorax 1994;49:468-472

Wilkinson M, Ranghorn CA, Heath D, Barer G, Howard PA. A pathophysiological study of ten cases of hypoxic cor pulmonale. Q J Med 1988;66:65-85

Woodcock AA, Gross ER, Geddes DM. Oxygen relieves breathlessness in "pink puffers". Lancet 1981; April:907-909

Wuyam B, Payen JF, Levy P, Bensaïdane H, Reutenauer H, Le Bas JF, Benabid AL. Metabolism and aerobic capacity of skeletal muscle in chronic respiratory failure related to chronic obstructive pulmonary disease. Eur Respir J 1992;5:157-162

Yamamoto Y, Hughson RL, Nakamura Y. Autonomic nervous system responses to exercise in relation to ventilatory threshold. Chest 1992;101:206S-210S

Younes M, Whipp BJ, Wasserman K. Exercise pulmonary physiology and pathophysiology. Marcel Dekker, New York 1992: 1-65

CHAPTER NINE

APPENDICES

THE YALE PHYSICAL ACTIVITY SURVEY
FOR OLDER ADULTS

INTERVIEWER: PLEASE MARK TIME: ___ ___ : ___ ___ : ___ ___
HR MIN SEC

INTERVIEWER: (Please hand the subject the list of activities while reading this statement). Here is a list of common types of physical activities. Please tell me which of them you did during a typical week in the last month. Our interest is learning about the types of physical activities that are a part of your regular work and leisure routines.

For each activity you do, please tell me how much time (hours) you spent doing this activity during a typical week. (Hand subject card #1.)

Work	**Time** (Hrs/Wk)	**Intensity Code*** (kcal/min)
Shopping (e.g., grocery, clothes)	_____	3.5
Stair climbing while carrying a load	_____	8.5
Laundry (time loading, unloading, hanging, folding only)	_____	3.0
Light housework: tidying, dusting, sweeping; collecting trash in home; polishing; indoor gardening; ironing	_____	3.0
Heavy housework: vacuuming, mopping; scrubbing floors and walls; moving furniture, boxes, or garbage cans	_____	4.5
Food preparation (10+ mins in duration): chopping stirring; moving about to get food items, pans	_____	2.5
Food service (10+ mins in duration): setting table; carrying food; serving food	_____	2.5
Dish washing (10+ mins in duration): clearing table; washing/drying dishes, putting dishes away	_____	2.5
Light home repair: small appliance repair; light home maintenance/ repair	_____	3.0
Heavy home repair: painting, carpentry, washing/polishing car	_____	5.5
Other: _____	_____	_____+

*Taylor et al., 1978 or McArdle et al., 1981.
+determined by the specified activity

Yardwork	**Time**	**Intensity Code***
Gardening: planting, wedding, digging, hoeing	_____	4.5
Lawn mowing (walking only)	_____	4.5
Clearing walks/driveway: sweeping, shoveling, raking	_____	5.0
Other: _____	_____	_____+

Caretaking		
Older or disabled person (lifting, pushing, wheelchair)	_____	5.5
Childcare (lifting, carrying, pushing stroller)	_____	4.0

Exercise

Brisk walking (10+ mins in duration)	____	6.0
Pool exercises, stretching, yoga	____	3.0
Vigorous calisthenics, aerobics	____	6.0
Cycling, exercycle	____	6.0
Swimming (laps only)	____	6.0
Other: _____	____	____+

Recreational Activities

Leisurely walking (10+ mins in duration)	____	3.5
Needlework: knitting, sewing, needlepoint, etc.	____	1.5
Dancing (mod/fast): line, ballroom, tap, square, etc.	____	5.5
Bowling, bocci	____	3.0
Golf (walking to each hole only)	____	5.0
Racquet sports: tennis, racquet ball	____	7.0
Billiards	____	2.5
Other: _____	____	____+

INTERVIEWER: (please read to subject). I would now like to ask you about certain types of activities that you have done during the past month. I will ask you about how much vigorous activity, leisurely walking, sitting, standing, and some other things that you usually do.

1. About how many times during the month did you participate in vigorous activities that lasted at least 10 minutes and caused large increases in breathing, heart rate, or leg fatigue or caused you to perspire? (Hand subject card #2).

SCORE:
0 = Not at all (go to Q3)
1 = 1-3 times per month
2 = 1-2 times per week
3 = 3-4 times per week
4 = 5+ times per week
7 = Refused
8 = Don't know

FREQUENCY SCORE: = _____

2. About how long do you do this vigorous activity(ies) each time? (Hand subject card #3)

SCORE:
0 = Not applicable
1 = 1-3 times per month
2 = 1-2 times per week
3 = 3-4 times per week
4 = 5+ times per week
7 = Refused
8 = Don't know

DURATION SCORE: = _____
WEIGHT = 5

VIGOROUS ACTIVITY INDEX SCORE:

FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing.)

3. Think about the walks you have taken during the past month. About how many times per month did you walk for at least 10 minutes or more without stopping which was not strenuous enough to cause large increases in breathing, heart rate, or leg fatique or cause you to perspire? (Hand subject card #2.)

SCORE:
0 = Not at all (go to Q5)
1 = 1-3 times per month
2 = 1-2 times per week
3 = 3-4 times per week
4 = 5+ times per week
7 = Refused
8 = Don't know

FREQUENCY SCORE: = _____

4. When you did this walking, for how many minutes did you do it? (Hand subject card #3.)

SCORE:
0 = Not applicable
1 = 10-30 minutes
2 = 31-60 minutes
3 = 60+ minutes
7 = Refused
8 = Don't know

DURATION SCORE: = _____
WEIGHT = 4

LEISURELY WALKING INDEX SCORE:

FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing.)

5. About how many hours a day do you spend moving around on your feet while doing things? Please report only the time that you are actually moving. (Hand subject card #4).

SCORE:
0 = Not at all
1 = less than 1 hr per day
2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hrs per day
7 = Refused
8 = DK

MOVING SCORE = _____
WEIGHT = 3

MOVING INDEX SCORE:

MOVING SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing.)

6. Think about how much time you spend standing or moving around on your feet on an average day during the past month. About how many hours per day do you stand? (Hand subject card #4.)

SCORE:
0 = Not at all
1 = less than 1 hr per day
2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hrs per day
7 = Refused
8 = DK

STANDING SCORE = _____
WEIGHT = 2

STANDING INDEX SCORE:

STANDING SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored a missing.)

7. About how many hours did you spend sitting on an average day during the past month. (Hand subject card #5.)

SCORE:
0 = Not at all
1 = less than 3 hrs
2 = 3 hrs to less than 6 hrs
3 = 6 hrs to less than 8 hrs
4 = 8+ hours
7 = Refused
8 = Don't know
(Responses of 7 or 8 are scored as missing.)

SITTING SCORE = _____
WEIGHT = 1

SITTING INDEX SCORE:

SITTING SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored a missing.)

8. About how many flights of stairs do you climb up each day? (Let 10 steps = 1 flight) _____

9. Please compare the amount of physical activity that you do during other seasons of the year with the amount of activity you just reported for a typical week in the past month. For example, in the summer, do you do moe or less activity than what you reported doing in the past month?

(INTERVIEWER: PLEASE CIRCLE THE APPROPRIATE SCORE FOR EACH SEASON.)

	Lot More	Little More	Same	Little Less	Lot Less	Don't know
Spring	1.30	1.15	1.00		0.70	
Summer	1.30	1.15	1.00		0.70	
Fall	1.30	1.15	1.00		0.70	
Winter	1.30	1.15	1.00		0.70	

SEASONAL ADJUSTMENT SCORE = SUM OVER ALL SEASON / 4

_____

INTERVIEWER PLEASE MARK TIME: _____ _____ : _____ _____ : _____ _____
HR SEC MIN

CARD # 1

Shopping (e.g., grocery, clothes)
Stair climbing while carrying a load
Laundry (time loading, unloading, hanging, folding only)
Light housework: tidying, dusting, sweeping; collecting trash in home; polishing; indoor gardening; ironing
Heavy housework: vacuuming, mopping; scrubbing floors and walls; moving furniture, boxes, or garbage cans
Food preparation (10+ mins in duration): chopping stirring; moving about to get food items, pans
Food service (10+ mins in duration): setting table; carrying food; serving food
Dish washing (10+ mins in duration): clearing table; washing/drying dishes, putting dishes away
Light home repair: small appliance repair; light home maintenance/ repair
Heavy home repair: painting, carpentry, washing/polishing car
Other: _____
Gardening: planting, wedding, digging, hoeing
Lawn mowing (walking only)
Clearing walks/driveway: sweeping, shoveling, raking
Other: _____
Older or disabled person (lifting, pushing, wheelchair)
Childcare (lifting, carrying, pushing stroller)
Brisk walking (10+ mins in duration)
Pool exercises, stretching, yoga
Vigorous calisthenics, aerobics
Cycling, exercycle
Swimming (laps only)
Other: _____
Leisurely walking (10+ mins in duration)
Needlework: knitting, sewing, needlepoint, etc.
Dancing (mod/fast): line, ballroom, tap, square, etc.
Bowling, bocci
Golf (walking to each hole only)
Racquet sports: tennis, racquet ball
Billiards
Other: _____

CARD # 2

0 = Not at all
1 = 1 - 3 x per month
2 = 1 - 2 x per week
3 = 3 - 4 x per week
4 = 4 - 5+ times per week
7 = Refused
8 = Don't know

CARD # 3

0 = Not at all
1 = 1 - 3 x per month
2 = 1 - 2 x per week
3 = 3 - 4 x per week
4 = 5+ times per week
7 = Refused
8 = Don't know

CARD # 4

0 = Not at all
1 = less than 1 hour per day
2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hours per day
7 = Refused
8 = Don't know

METHODOLOGY FOR SKELETAL MUSCLE BIOPSY STAINING TECHNIQUES.

RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL
HISTOLOGY DEPARTMENT

Haematoxylin and Eosin (H&E) stain

1. Place sections in Harris' haematoxylin for 3 minutes.
2. Blue in Scott's tap water substitute or TRIS buffer (pH 10.5) if tap water is acid. Otherwise run in tap water for 2 minutes.
3. Differentiate in 0.2% acid alcohol (HCL-alcohol) until pink – if needed.
4. Re-blue as appropriate (step 2).
5. Place in 1% eosin for 15-20 seconds
6. Wash quickly in distilled water.
7. Dehydrate rapidly.
8. Clear, and mount in synthetic resin.

Harris' haematoxylin

Harris' haematoxylin powder	21.5 g
Absolute alcohol	10 ml
Distilled water	200 ml

Add 4% glacial acetic acid just before use. This increases the precision of the nuclear staining.

Eosin

Eosin	5 g
Distilled Water	100 ml

Dilute to 1% for use.

Alkaline solution (Scott's tap water)

Potassium bicarbonate	2 g
Magnesium sulphate	20 g
Distilled water	1 L

Adenosine triphosphatase (ATPase) stain

Method at pH 9.4

1. Incubate freshly cut sections at 37°C for 30 minutes in the following solution: 5 mg ATP dissolved in a few drops of distilled water. Add 10 ml of 0.1 M glycine/NaCL buffer with 0.75 M $CaCl_2$. Adjust to pH 9.4. Add 0.0309 g/10 ml (20 mM) dithiothreitol solution.
2. Rinse well in distilled water.
3. Immerse in 2% $CaCl_2$ for three rinses, 1 minute each.
4. Rinse well in distilled water.
5. Immerse in dilute (1:10) ammonium sulphide solution for 30 seconds.
6. Rinse well in running tap water.
7. Stain in Harris' haematoxylin for 1 minute and blue in tap water (optional).
8. Dehydrate, clear and mount

Method at pH 4.6 and 4.3

1. Pre-incubate at 4oC in 0.1 M sodium acetate buffer with 10 mM EDTA added, for 10 minutes at pH 4.6 or 4.3
2. Wash in distilled water
3. Precede as for pH 9.4 method

Solutions

0.1 M glycine buffer

0.75 g glycine + 0.585 g NaCl made up to 100 ml with distilled water.

0.1 M glycine/NaCl buffer with 0.75 M $CaCl_2$

50 ml 0.1 M glycine buffer.

10 ml 0.75 M $CaCl_2$

Add approximately 22 ml 0.1mM NaOH until pH 9.4

NADH-tetrazolium reductase (NADH-TR) stain

1. Place fresh sections flat in a petri dish on a damp filter paper.
2. Place 1-2 drops incubating solution onto the section that it is completely covered.
3. Incubate for 30 minutes at 37°C.
4. Wash in distilled water.
5. Fix in 15-20% formalin solution for 10 minutes.
6. Rinse in distilled water.
7. Mount in glycerin jelly.

To remove fat, ascend in acetone (30, 60, 90%) and descend again into distilled water after incubation.

Results

Intramitochondrial deposits of black cobalt formazan indicates site of NAD

Nitroblue tetrazolium stock solution

20 mg/20 ml distilled water

Store at -20°C

NADH stock solution

Nitroblue tetrazolium 1 mg/ml	6.25 ml
0.2 M TRIS buffer pH 7.4	6.25 ml
Cobalt chloride 0.5 M*	1.25 ml
Distilled water	8.75 ml

Store at –20°C (pH 7.4)

* - 0.5 M $CoCl_2$ = 11.9 g/100 ml distilled water.

NADH solution

1 ml of stock solution

1 mg NADH

Oil red O (ORO) stain for lipid

1. Rinse sections in water.
2. Rinse in 60% isopropyl alcohol.
3. Transfer to oil red stain for 10-30 minutes.
4. Differentiate in 60% isopropyl aclohol.
5. Wash in distilled water.
6. Counterstain in Harris' haemotoxylin for 1 minute
7. Rinse in tap water to blue
8. Mount in glycerin jelly.

Results

Nuclei - blue

Fat globules - red

Stock stain

Saturated solution of oil red O in isopropyl alcohol (0.5%).

For use

Dilute 6 ml stock with 4 ml distilled water. Stand for 10 minutes and filter.

Glycerin jelly should only be warm to prevent artefact damage.

· Modified Gomori trichrome stain

1. Fresh cryostat sections air dried for 2-20 minutes.
2. Stain in Harris' haematoxylin for 5 minutes.
3. Rinse in distilled water.
4. Stain in Gomori's trichrome mixture 60 minutes (until green)
5. Differentiate rapidly in 0.2% acetic acid for a few seconds
6. Dehydrate in alcohol series.
7. Clear, and mount in artificial resin

Gomori mixture

0.6 g Chromotype 2R

0.3 g Fast green

0.6g Phosphotungstic acid

1.0 ml Glacial acetic acid

100 ml Distilled water

Trichrome mixture should be made up fresh when staining becomes pale.

Periodic Acid-Schiff technique (PAS) for glycogen

1. Fix sections in acetic ethanol for 5-10 minutes.
2. Wash in distilled water.
3. Place in 0.5% periodic acid for 2-5 minutes to oxidize.
4. Wash in distilled water.
5. Place in Schiff's reagent for 10-15 minutes.
6. Wash in running tap water for 5-10 minutes.
7. Dehydrate in ascending alcohol series.
8. Clear and mount in artificial resin.

Periodic Acid Solution

0.5g periodic acid crystals dissolved in 100 ml distilled water.

Schiff's reagent

Basic fuchsin	1g
Distilled water	200 ml
Sodium metabisulphite	2g
Concentrated HCl	2 ml
Decolourizing charcoal	2g

Boil the water and carefully add basic fuchsin. When dissolved, cool to 50°C and add sodium metabisulphite. Dissolve and allow to cool to room temperature. Add the HCl. Leave in a dark cupboard overnight. Add charcoal and shake for two minutes. Filter and store in a dark bottle in the refrigerator.

PAS control

Incubate control sections in 0.5% α-amylase solution at 37°C for 1 hour. Proceed with PAS stain as routinely carried out.

DAB method for cytochrome oxidase

Incubation medium

3,3' – diaminobenzidine tetrahydrochloride (DAB)	5 mg
50mM phosphate buffer (pH 7.4)	9 ml
(dissolve DAB and phosphate buffer together)	
Catalase solution (20μg/ml)	1 ml
Cytochrom *c* (type II)	10 mg
Sucrose	750 mg

1. Incubate fresh frozen sections for 60 minutes at room temperature (22°C).
2. Wash quickly in distilled water.
3. Dehydrate in ascending alcohol series, clear, and mount in synthetic resin.

Results

Sites of cytochrome oxidase activity – fine brown stain.

Succinate dehydrogenase

1. Incubate at 37°C for 30 minutes in the following solution:

Sodium succinate	100 mg
Nitroblue tetrazolium	10 mg
Phenazine methosulphate	0.25 mg
0.1 M TRIS buffer	10 ml

   The pH is adjusted to 7.0.

2. Extract in acetone, 30, 60, 90, 60 and 30 % in sequence.
3. Rinse in distilled water
4. Mount in glycerine jelly