Exercise Response and the Effect of Supplemental Oxygen during Interval Training on O_2 Uptake Kinetics, Blood Lactate Levels, and Endurance Performance in Patients with Mild, Moderate and Severe Cystic Fibrosis

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Declaration

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Abstract

Background:

Exercise performance is reduced in individuals with moderate to severe cystic fibrosis (CF). A ventilatory mechanical limitation, arterial hypoxemia, cardiovascular abnormalities, alteration in O_2 uptake kinetics and reduced muscle strength may contribute to the reduced exercise performance in individuals with CF patients. The purpose of this research was to compare the exercise response in patients with different CF severities using non- invasive methods, and examine the effect of exercise training with and without O_2 supplementation on exercise performance.

Methods:

Study 1: Adults (n=33) with different severities of CF and healthy controls (n=34) had their body composition, and pulmonary function assessed, and performed a peak exercise test.

Study 2: Adults (n=28) with different severities of CF and healthy controls (n=19) had their muscle strength, pulmonary function, body composition and $\dot{V}O_2$peak assessed, and performed a submaximal exercise tests.

Study 3: Adults with different severities of CF who were randomly assigned to a placebo (n=6) or O_2 supplemental (O_2Suppl) (n=5) group undertook interval training on a cycle ergometer 2 days/week for 8 weeks (single blind). Body composition, pulmonary function, $\dot{V}O_2$peak, muscle strength, time to complete a 6 minute walk and

performance during a constant work load submaximal tests were assessed before and after the training study.

Results:

Peak exercise capacity and muscle strength were reduced and O_2 uptake kinetics was slower in CF patients than healthy controls. The provision of supplemental O_2 during training improved O_2 uptake kinetics and resulted in a decrease in ventilation, respiratory rate and blood lactate levels during exercise.

Conclusion:

Depending on disease severity, the reduction in exercise capacity in CF patients is related to a reduced lean body mass, reduced gas exchange, ventilatory limitation, and an altered cardiovascular response. The provision of supplemental O_2 during exercise training may improve endurance capacity in patients with CF.

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List of Terms and Abbreviations

Term	Description
ASL	Airway surface liquid
AT	Anaerobic threshold
B. cepacia	Burkholderia cepacia
BMI	Body mass index
C (a-v) O_2	Arterial-mixed venous oxygen content
CF	Cystic Fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
cAMP	cyclic adenosine monophosphate
CO	Cardiac output
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
DC	Diffusion capacity
DH	Dynamic pulmonary hyperinflation
D_LCO	Carbon monoxide diffusion capacity
DS	Dead space
EELV	End expiratory lung volume
EFL	Expiratory flow limitation
EILV	End inspiratory lung volume
FEV_1	Forced expiratory volume in one second
FRC	Functional residual capacity
FVC	Forced vital capacity
HIIT	High intensity interval training
HR	Heart rate
HS	Hypertonic saline
IGF-1	Insulin-like growth factor 1
LA	Lactic acid
LV	Left ventricular
MRT	Mean response time
MVV	Maximal voluntary ventilation
NF-kB	Nuclear factor kB

O_2	Oxygen
O_2D	Oxygen deficit
P. aeruginosa	Pseudomonas aeruginosa
$P_{A\text{-}a}O_2$ diff	Alveolar-arterial partial pressure difference
PCr	Phosphocreatine
$P_{ET}CO_2$	End tidal PCO_2
PI	Pancreatic insufficiency
PH	Pulmonary hypertension
PS	Pancreatic sufficiency
$PvCO_2$	Mixed venous PCO_2
RBC	Red blood cell
RR	Respiratory frequency
SpO_2%	Arterial O_2 saturation
SV	Stroke volume
SvO_2	Mixed venous O_2 Saturation
T_E	Expiration time
T_I	Inspiration time
TLC	Total lung capacity
TNF-α	Tumour necrosis factor alphas
$\dot{V}_A$	Alveolar ventilation
$\dot{V}_A/Q$,	Ventilation to perfusion matching
VC	Vital capacity
V_D	Dead space volume
V_D/V_T	Dead space to tidal volume ratio
$\dot{V}_E$	Minute ventilation
$\dot{V}_E/\dot{V}CO_2$	Ventilatory equivalent for CO_2
$\dot{V}_E/\dot{V}O_2$	Ventilatory equivalent for O_2
$\dot{V}O_2$peak	Peak oxygen uptake
V_T	Tidal volume
WR	Work rate

Chapter 1

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease that occurs primarily in Caucasians, with a worldwide incidence rate of 1 in 2,500 to 3,000 live births. It is the most common life-threatening inherited disease in Ireland [1] where the incident rate is the highest in the world, at 1 in 1,353. Males and females are equally affected. The development of sensitive diagnostic techniques and better supportive treatment in multidisciplinary CF care centres, have dramatically increased life expectancy of cystic fibrosis patients in Ireland.

CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on the long arm of chromosome 7 [14]. The CFTR gene codes for a protein that spans the apical membrane of epithelial cells lining the airways, pancreatic ducts, sweat ducts, intestines, biliary tree, and vas deferens, and normally regulates the transport of chlorine and other ions. The chloride ion channel is important in producing sweat, mucus and digestive juices. Normal levels of mucus provide the lungs with a protective mechanism to reduce the risk of infections. In individuals with CF, the absence of this chloride ion channel affects the ability to transport salt and water into and out of cells, which in turn results in the production of dry, thick and sticky secretions that impairs mucociliary clearance (30). A combination of mucus plaques and impaired mucociliary clearance establishes a breeding ground for bacterial infection, particularly in the lungs (13).

Although antibiotics may help to contain infections within the lungs, they usually fail to eradicate the organisms, and destruction of the airways is continual, eventually leading to bronchiolitis, bronchitis, bronchiectasis and eventually fibrosis and irreversible loss of pulmonary function. Chronic lung infection is the principle cause of death in >90% of CF patients [2]. CF also increases susceptibility to malnutrition, pancreatic, liver and gall bladder disease, diabetes, male infertility, and poor growth. Symptoms vary depending on age, prior treatment, types of infections encountered, environmental factors and the extent to which various organs are affected (20).

Treatments for CF include a wide range of therapies based on current infection(s) and disease progression, and include drug based therapies, airway clearance mechanisms, implanted devices, nutritional interventions and exercise. The severity of the illness increases with age, and treatments become progressively more expensive and more difficult to manage. Patients with more severe CF symptoms may require hospitalization for organ transplantation (15) and palliative care.

Exercise intolerance is a characteristic of CF [3]. Studies examining the physiological responses to exercise in CF patients began in the 1970's (44). Research in the late 1980's showed that ventilation and mucus clearance could be improved with exercise (45). Studies in the 1990's started to examine the relation between indices of fitness and survival in CF patients. Nixon et al., [46] found a significant relation between peak oxygen uptake ($\dot{V}O_2$peak) and eight year survival in children with CF. Patients with a $\dot{V}O_2$peak of ≥ 82% of predicted had an 83% chance of survival at 8

years. The 8 year survival rate for patients with a $\dot{V}O_2$peak of 59-81% of predicted was 51% and 28% in those with a $\dot{V}O_2$peak of ≤ 58% of predicted.

The main symptoms of CF that limit exercise performance are dyspnea and fatigue. Impaired lung gas exchange [4], different degrees of lung hyperinflation [5-7], and in more advanced disease, abnormalities of pulmonary circulation [9], may negatively impact on the ability to sustain exercise, especially as the disease becomes more progressive. In addition to alterations in lung function, maladaptive changes in peripheral muscle size and function may also contribute to exercise intolerance in CF [8-10].

Understanding the exercise response in CF is important in order to design and implement appropriate lifestyle interventions. Exercise intolerance in CF is complex and multi-factorial and is dependent on many factors including impaired respiratory system mechanics, arterial hypoxemia, peripheral skeletal muscle dysfunction and cardiovascular function. These contributing factors are interdependent and the predominant contributing factor may vary among patients with different severities of CF. The integrative response during cardiopulmonary exercise testing (CPET) and the assessment of muscle strength are non-invasive sensitive tools than can be used to detect abnormal function and exercise limitation in patients with mild, moderate and severe CF.

Functional capacity as measured by $\dot{V}O_2$peak is a significant predictor of survival in patients with CF [46]. A limitation of maximal testing is that it is dependent on disease status and motivation. Oxygen uptake ($\dot{V}O_2$) kinetics describes the rate of

$\dot{V}O_2$ change at the onset of exercise and is used to determine an individual's ability to adapt to changes in workrate (WR). A slower response of oxygen uptake after an increase in exercise intensity results in a relatively higher oxygen deficit and oxygen debt, which has been linked to premature fatigue and reduced exercise time [131].

Exercise training is an important component of care for patients with CF [11]. Allowing CF patients to train for a longer duration at a high intensity may enhance the training effect. Supplemental oxygen (O_2) has recently been used to overcome the ventilatory limitation during an acute bout of submaximal [12] exercise and during an incremental maximal exercise test [4] in CF patients. The provision of supplemental O_2 during exercise training could delay the ventilatory limitation during exercise without altering lung function or the maximum ventilatory capacity. To date no studies have examined the effect of supplemental O_2 during exercise training on endurance performance training in patients with CF.

Study Aims

The following series of studies will i) compare the exercise response in patients with different CF severities using non- invasive methods, and ii) examine the effect of exercise training with and without O_2 supplementation on exercise performance.

Objectives

1. To determine the role of pulmonary function, arterial O_2 saturation (S_PO_2%), muscle function and O_2 pulse on maximal exercise capacity in adults with mild, moderate and severe CF

2. To compare body composition, peak exercise capacity, muscle strength, and O_2 uptake kinetics in adults with CF and healthy individuals.

3. To compare the effects of an 8 week interval training programme with and without O_2 supplementation on training volume, $\dot{V}O_2$ peak, O_2 uptake kinetics, endurance performance and muscle strength in CF patients.

Hypothesis

1. Ventilatory mechanical limitation and pulmonary gas exchange abnormality will contribute to the reduced peak exercise capacity in CF patients with moderate and severe disease.

2. Cardiovascular abnormalities will contribute to the reduced peak exercise capacity in CF patients with FEV_1 <30% of predicted.

3. Compared to healthy individuals, patients with moderate to severe CF will have a reduction in muscle strength, and prolonged O_2 uptake kinetics, and increased circulating levels of blood lactate during constant load exercise

4. Compared to healthy individuals, patients with mild CF will have similar muscle strength, O_2 uptake kinetics, and blood lactate levels during constant load exercise.

5. Compared to interval training using normal atmospheric oxygen concentration, interval training with supplemental O_2 will result in a greater

training volume, and a greater improvement in $\dot{V}O_2$peak, O_2uptake kinetics, endurance performance, and muscle strength in CF patients

Chapter 2

Literature of Review

Overview of Cystic Fibrosis in Ireland

Cystic fibrosis is an autosomal recessive condition caused by a variable mutation in the CFTR gene [13]. The normal CFTR protein product is an ion channel that is expressed in endothelial cells of the umbilical vein, lung microvasculature, red blood cells, pancreas, lung epithelia, sweat gland, colon, parotid gland, liver, proximal tubules of the kidney, heart myocytes, skeletal muscle and hypothalamus. In CF, the inherited CF gene directs the body's epithelial cells to produce a defective form of the CFTR protein [14] that disrupts epithelial ion transport and can lead to respiratory failure, pancreatic insufficiency, and infertility, as well as a range of other defects.

CF is the most common life-threatening inherited disease in Ireland [1]. The incidence of CF is 1 per 1353 births, which ranks Ireland number one in the world. On a global scale, Ireland also has i) the highest number of CF carriers per head of the population with 1 in 19 people carrying a single copy of the defective CFTR gene, and ii) the largest proportion of families with more than one child suffering from CF.

Over 50 mutations of the CFTR gene are represented in Ireland. The ΔF508, is the common mutation among the Irish population, with 64% and 94% of the population homozygous and heterozygous respectively, for this abnormal allele [1]. The G55ID mutation is the second most frequently-occurring mutation in Ireland. The clinical course of patients with CF is variable and is determined by many interacting

factors. However, advances in the management of CF have led to a significant improvement in survival.

Cystic Fibrosis Transmembrane Conductance Regulator

Structure and Function of CFTR

Epithelial cells that line the airways have ion channels that allow sodium ions to flow into the cell and chloride ions out of the cell into the mucus on the airway surface. Along with an ion pump, the action of the channels results in an excess of chloride ions in the mucus. This creates an ionic gradient that promotes the movement of water through the gaps between the cells, which keeps the mucus moist.

The chloride channel is made from a protein called CF transmembrane regulator (CFTR) protein. It is a 1480 amino acid membrane bound glycoprotein and is a member of the ABC transporter family. It is located predominantly in the apical membrane of epithelia, and is composed of two membrane-spanning domains, two nucleotide-binding domains, and a regulatory domain. The regulatory domain is phosphorylated by protein kinase A (PKA) via a cyclic adenosine monophosphate (cAMP) dependent pathway. The subsequent binding and cleaving of ATP at adjacent sites regulates the opening and closing of the channel, as shown in Figure 2.1.

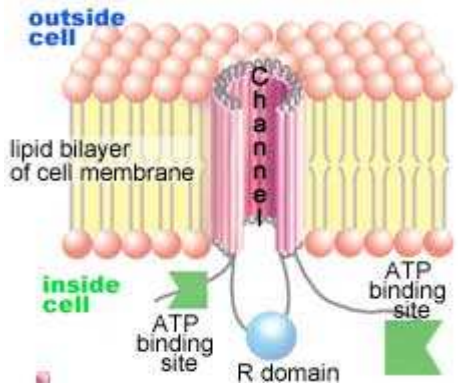

Figure 2.1: Binding and cleaving of ATP at adjacent sites

The CFTR gene is found in region q31.2 on the long arm of human chromosome 7. The normal allelic variant is about 250,000 base pairs long and contains 27 exons. Mutation of the CFTR gene alters the production, structure, or stability of the chloride channel resulting in CF [14].

Mutations in CFTR and Consequences

More than 1000 mutations of the CFTR gene have been identified in people with CF [15]. These mutations can be divided into 5 classes (Figure 2.2).

Class I mutations cause defective protein production. These mutations result in the production of little or no CFTR chloride channels (G542X, 3905 ins T). Class II mutations are associated with defective protein processing, as evident in the ΔF508 mutation. This mutation results in the deletion of a single amino acid at position 508 in the CFTR protein. The resulting abnormal channel is inadequately processed in the endoplasmic reticulum and does not reach the cell membrane to transport chloride ions [14, 17]. The ΔF508 mutation is found in approximately 70% of defective CFTR alleles of patients with CF in the US [14]. Class III mutations are associated with defective regulation. Some mutations cause total loss of ability to be stimulated by

ATP, and in others this ability is reduced (G55ID, G55IS). Class IV mutations are associated with the defective conductance of chlorine ions. Some studies have shown that even in the presence of class IV mutations the CFTR chloride channels are capable of generating normal functional cAMP-regulated chloride channels (R117H, R334W). Finally, class V mutations result in a reduction in the number of CFTR transcripts [18].

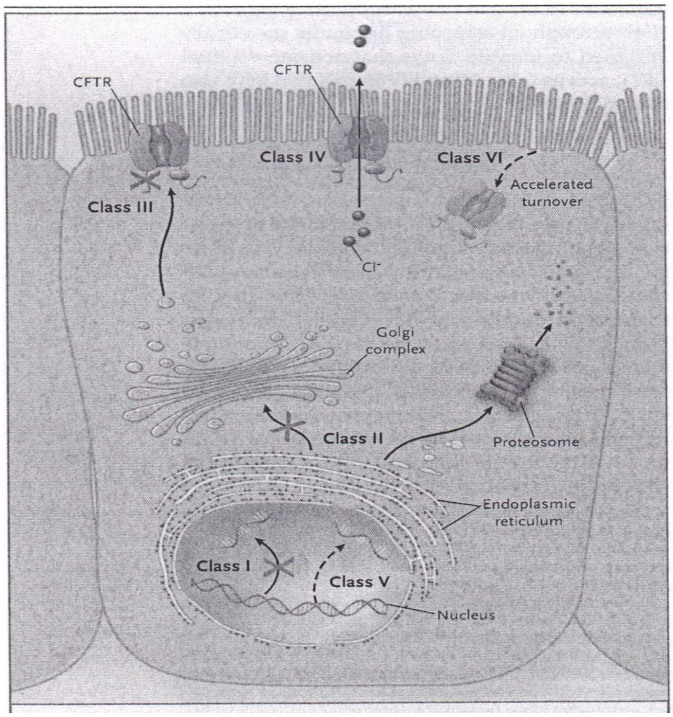

Figure 2.2: Categories of CFTR mutations

Genotype-Phenotype in CF

Cystic fibrosis is characterized by a large variability of clinical expression. Different mutations have varying effects on CFTR function and can result in phenotypes that range from mild to severe [16]. For example, the ΔF508 mutation is associated with a severe phenotype, while other mutations such as R117H, R334W are associated with a milder phenotype [19-20]. In addition, there is substantial variation in disease expression among patients carrying the same mutation.

Clinical Outcome and CFTR Function in the Lungs

Patients homozygous for the ΔF508 mutation have earlier onset of the disease, higher sweat chloride level, and pancreas insufficiency. However, despite the severe nature of this mutation, the severity of pulmonary disease varies among patients. Attempts to link the CFTR mutation to severity of lung disease have been unsuccessful in children and young adults with CF [21-22]. In contrast, adults with CF who have CFTR class I or II mutations on both chromosomes have a reduced forced expiratory volume in one second (FEV_1), reduced forced vital capacity (FVC), and a greater loss of pulmonary function during a 10 year follow up period [23]. In addition, patients in the CFTR class I or II mutations have a higher risk of developing moderate to severe pulmonary disease than patients with at least one CFTR functional class III, IV or V mutation.

Chronic pulmonary infection is the hallmark of CF and respiratory failure is the leading cause of death in 90% of CF patients. Pseudomonas aeruginosa (P. aeruginosa) is the most common pathogen responsible for pulmonary infection in CF. Up to 80% of patients with CF are eventually infected with P. aeruginosa. CF patients who are homozygous for the ΔF508 mutation, have a higher risk for initial P. aeruginosa airway infection than patients with class IV CFTR mutations [24]. In contrast, the prevalence of other common pathogens, such us S. aureus and H. influenzae, are similar across all the CFTR mutations. Burkholderia cepacia (B. cepacia) is a gram-negative bacteria that is also associated with severe airway infection as CF progresses. It is associated with B. cepacia syndrome leading to high fever, bacterial infection and rapid progression to severe pneumonia and death. However, studies have found that B. cepacia is not a

single species but rather a group of closely related species, known as *genomovar*, called B. cepacia complex. At least nine distinctive genomovars of B. cepacia have been identified [25-26]. The majority of CF airways infections with B. cepacia complex are caused by genomovar II, III, and V [27], and the majority of severe infections result from genomovar III [28].

Acute infection is generally associated with inflammation. The airway epithelial cells, which normally expresses the CFTR, directs the inflammatory response. Defects in CFTR are associated with increased production of pro-inflammatory mediators including IL-8, which stimulates the influx of neutrophils into the airway [29]. These neutrophils are the primary effector cells responsible for the pathological manifestations of CF lung disease. Neutrophils release large amounts of proteases (especially elastase) that further stimulate the inflammatory response and the production of O_2 free radicals. Other mediators that play an important role in neutrophil influx in the lung are tumour necrosis factor alphas (TNF-α), IL-1, and IL-6 [30]. Each of these inflammatory mediators are present in high concentrations in CF sputum [31]. Although these mediators may be beneficial in containing bacterial infection by stimulating host defences, their continued overproduction contributes to the pathologic findings in CF. TNF-α enhanced neutrophil oxidation may be responsible for the cachexia associated with CF [32].

For a given bacterial load, the inflammatory response is up to 10 times greater in a CF patient than in an individual with a lower respiratory tract infection, but without the disease. Chronic bacterial infection and excessive inflammation are

responsible for structural damage in the small airways and parenchyma (complex system consisting of alveolar walls, the air-liquid interface and interstitial cells) in CF patients.

From early childhood, parts of the airways in CF patients become thickened and dilated (broncieCtasis). Eventually the lung parenchyma becomes affected by atelectasis (collapse of the alveoli) resulting in a reduction in lung compliance and an increased resistance to blood flow in the pulmonary capillaries of the affected alveoli [28-29]. The progressive structural lung damage eventually leads to end-stage lung disease, which is characterized by a reduction in pulmonary diffusion capacity (DC) resulting in hypoxemia and an increase in the work of breathing. Patients with advanced CF are often O_2 dependent with carbon dioxide (CO_2) retention. They also have an increase in the frequency of exacerbations.

Clinical Outcome and CFTR Function in other Organs

Pancreatic enzymes that are involved in the digestion of food enter the intestine through the pancreatic ducts. In CF, the pancreas produces thick secretions, which block the ducts, resulting in the retention of proenzymes which in turn leads to tissue destruction and fibrosis [33]. CF patients are therefore unable to digest and absorb food properly, leading to weight loss, protein deficiency and deficiency of the fat-soluble vitamins A, D, E, and K [34]. Malnutrition also contributes to the inability to supply energy to meet the requirements associated with lung infections. Since lung infections can lead to reduced appetite and emesis, malnutrition is further enhanced.

Both complications combine to decrease total body mass and lean body mass (LBM) in CF patients [35].

The extent of pancreatic disease also varies among CF patients. Most CF patients suffer from pancreatic insufficiency (PI), but approximately 15% have sufficient pancreatic function (PS) to permit normal digestion [21]. There is some evidence for a genetic basis for the severity of pancreatic disease. More than 50% of the CF-PI patients are homozygous for ΔF508, while none of the CF-PS patients are homozygous for ΔF508, suggesting that ΔF508 is associated with PI [21]. PI is also associated with diabetes. PI is very common in men and women with diabetes [36-37].

Few studies have found a relation between liver disease and CFTR genotype [22, 38]. CF patients who have PI and carry mutations associated with a severe or variable genotype are at increased risk of developing liver disease [38]. The prevalence of abnormal liver function in class IV patients is approximately one third of that found in patients homozygous for ΔF508 [22].

Recently, Lamhonwah et al., [39] found that CFTR is expressed in the sarcoplasmic reticulum of human skeletal muscle. In mice, the lack of CFTR in skeletal muscle has been related to an increase in inflammatory/atrophic gene expression and increased diaphragmatic weakness during pulmonary infection [40].

Treatment

There is currently no cure for CF. Pulmonary disease is the single most important contributing factor to the increased morbidity and mortality associated with CF [33]. While the effect on other organ systems can be contained, arresting the

decline in lung function presents the biggest challenge to clinical management of the disease [33]. Treatment of the lung disease is undertaken with a variety of modalities, including mechanical airway clearance, antimicrobials, bronchodilators, supplemental oxygen, mucolytics, and a variety of other novel treatments including gene therapy.

Treatment for CF patients is primarily directed at controlling lung function, promoting mucus clearance from the lungs, nutritional supplementation and improving functional status. Pancreatic enzymes are taken at meal times to aid digestion. Daily physiotherapy promotes mucus clearance from the lungs and is the cornerstone of managing established pulmonary disease in CF. Whenever possible, lung infections are treated with antimicrobials. Systemic corticosteroids are used to treat airway inflammation [41]. They may also attenuate the decline in lung function [42]. However, chronic use of corticosteroids can reduce muscle mass, negatively impact on muscle function [36], increase body weight due to an increase in fat mass, and cause diabetes. Ideally, treatment with corticosteroids should only be used as a short term intervention during pulmonary exacerbations [41].

Depletion of the airway surface liquid (ASL) is an important factor that also contributes to abnormal mucus clearance in CF. A simple and attractive way to restore ASL is to use an osmotic liquid that draws water into the ASL, therefore minimizing the defect in mucocillary clearance. Hypertonic saline (HS) has been shown to improve FEV_1 [37] and reduce pulmonary exacerbation in older patients with CF. Although much progress has been made in gene therapy, it is presently not a treatment option for patients with CF [43]. Targets for CFTR pharmacotherapy include activation of

chloride secretion and inhibition of sodium absorption. However, results to date have not been promising [43].

Peak $\dot{V}O_2$ and Prognosis in CF

Exercise intolerance is a characteristic of CF. Studies examining the physiological responses to exercise in CF patients began in the 1970's [44]. Research in the late 1980's found that pulmonary ventilation and mucus clearance could be improved with exercise training [45]. Studies in the 1990's started to examine the relation between indices of fitness and survival in CF patients. Nixon et al., [46] found a significant relation between $\dot{V}O_2$peak and eight-year survival in children with CF after adjusting for age, gender, colonization of the respiratory airways by P. cepacia, body mass index (BMI), pulmonary function and end tidal PCO_2 ($P_{ET}CO_2$) at peak exercise. Patients with a $\dot{V}O_2$peak ≥ 82% of predicted had an 83% chance of survival at 8 years. The 8-year survival rate for patients with a $\dot{V}O_2$peak of 59-81% of predicted was 51% and 28% in those with a $\dot{V}O_2$peak of ≤ 58% of predicted.

More recently, Pianosi et al., [47] performed an annual pulmonary function and maximal exercise test in 28 CF children between the age of 8-17 years to determine the relation between changes in lung function and $\dot{V}O_2$peak and survival over 7-8 years. Initial $\dot{V}O_2$peak was not predictive of 7-8 year survival. In contrast, the rate of decline in $\dot{V}O_2$peak and final $\dot{V}O_2$peak were significant predictors of 7-8 year survival. Patients with a peak $\dot{V}O_2$ <32.0 ml·kg $^{-1}$ min^{-1} had an 8 year mortality rate of approximately 60%, whereas none of the children with a $\dot{V}O_2$ peak >45 ml·kg $^{-1}$ min^{-1} died in the follow up period. The decline over time of FEV_1 also predicted mortality. A

decline in $\dot{V}O_2$ peak ≥2.1 ml·kg $^{-1}$ min^{-1} per year, or a decrease in FEV_1 over a 5-year period was associated with an increased mortality rate. Using a shorter follow up period of 5 year, Moorcroft et al., found that $\dot{V}O_2$, FEV_1% predicted and the ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}O_2$) were better predictors of 5-year survival than $\dot{V}O_2$ peak in 92 CF patients [48].

Changes in lung function may also impact on the decrease in $\dot{V}O_2$peak observed in children with CF. Klijn et al., [49] examined changes in nutritional status, lung function (FVC and FEV_1) and $\dot{V}O_2$ peak at baseline and in a 24-month follow-up in 65 CF children between the age of 4 and 18 years. None of the participants had taken antibiotics in the previous 4 months. Nutritional status was assessed by measuring height, weight and fat free mass (FFM). Changes in lung function and, to a lesser extent, nutritional status were positively associated with changes in the aerobic capacity. The change in FEV_1 explained 38% of the variation in $\dot{V}O_2$peak, and the combined change in FEV_1 and FFM accounted for 47% of the variation in $\dot{V}O_2$peak.

Other Prognostic Indicators in CF

FEV_1 is commonly used to assess the mechanical properties of the large and medium-sized airways. In apparently healthy individuals, the FEV_1 accounts for the greatest part of the exhaled volume from a spirometric manoeuvre. In a normal flow-volume loop, the FEV_1 occurs at approximately 75%-85% of the FVC, and is reduced in individuals with obstructive and restrictive lung disorders. In obstructive pulmonary disease, FEV_1 is reduced disproportionately to the FVC. This reduces the FEV_1/FVC ratio below the lower limit of normal indicates airflow limitation. In restrictive

disorders, the FEV_1, FVC, and total lung capacity (TLC) are all reduced, whereas the FEV_1/FVC ratio is normal or even elevated. The annual rate of decline in %pred FEV_1 across the CF population in the US is estimated to be 2% [14, 50]. Moorcroft et al., [51] found a decrease in FEV_1 of 63.0 ml, or 2.4 % predicted per year in patients with mild to moderate disease. Factors that may influence the rate of decline in FEV_1% include nutritional status [52], diabetes, colonization with P. aeroginosa, frequency of pulmonary exacerbations [14, 53] and PI [49].

Carbon dioxide (CO_2) retention during exercise can also be used as a prognostic marker of disease progression in patients with mild to moderate CF [54]. Javadpour et al., [54] found the average decline in FEV_1% per year for the CO_2 retention group was 4.9% compared with 2.3% for the non-CO_2 retention group. Preserving lung function is important considering that the mortality rate for patients with FEV_1<30% predicted [55] is 50% within two years. An FEV_1<30% predicted is also a primary criteria for lung transplantation [56].

Colonisation with P. cepacia is associated with particularly poor prognosis. The mortality rate is approximately 25% within one year of colonisation [55]. Poor nutritional status is also associated with reduced survival. Sharma et al., [57] found that the 7-year prognosis was better in patients with a body weight >85% of ideal than in those with a body weight < 85% of ideal. They suggested that muscle wasting is an excellent predictor of mortality independent of FEV_1% predicted and arterial blood gases ($PaCO_2$ and PaO_2). In contrast, Kerem et al., [55] found that % ideal body weight was not an independent predictor of survival [44]. The two-year mortality rate was

found to be 50% in patients with abnormal resting blood gases (PaO_2 <55 mmHg and $PaCO_2$> 50 mmHg) [54]. Patients with a “high risk” genotype CFTR mutation have a higher mortality rate than patients with a “low risk” genotype CFTR mutation [58].

Some CF patients remain relatively stable for a considerable period of time and then may have rapid lung disease progression. Reduced FEV_1, and a decline in FVC can be observed due to progressive lung scarring, air trapping and increased dead space (DS) ventilation [14].

Physiological Response of the Healthy Respiratory System to Exercise

Exercise intolerance is a primary characteristic of disease progression in CF, and is related to abnormal function of ventilatory, cardiovascular and peripheral muscles. Ventilatory limitation is a dominant contributor to exercise intolerance in more advanced CF disease. The cardiorespiratory system maintains adequate O_2 to the exercising muscle and removes CO_2 during dynamic submaximal exercise in healthy individuals. Despite O_2 desaturation (<10% SvO_2) and CO_2 retention ($PvCO_2$ >80 mm Hg) in mixed venous blood as $\dot{V}O_2$ and $\dot{V}CO_2$ reach maximum levels, the PO_2 and PCO_2 in the blood leaving the lungs remains near resting levels in healthy individuals.

A primary function of the respiratory system during exercise is to ensure normal gas exchange in order to maintain arterial PO_2 and PCO_2 close to resting levels. The time available for alveolar capillary diffusion at peak exercise is shortened due to a large increase in cardiac output. However, pulmonary capillary pressure remains relatively constant due to a decrease in resistance.

Pulmonary ventilation can increase by twenty fold in order to supply O_2 and remove CO_2 from the working muscles during exercise. $PvCO_2$ increases from <50 mmHg at rest to >80 mmHg at maximal exercise. The excess CO_2 must be eliminated in order to maintain arterial PCO_2 and acid base status within the normal range. The alveolar-arterial partial pressure difference ($P_{A-a}O_2$ diff) decreases and alveolar ventilation ($\dot{V}_A$) and DC increases in order to maintain arterial PO_2 at 100 mmHg. In order to maintain arterial PO_2 and PCO_2 within the normal range, ventilatory efficiency is increased by minimizing the increase in respiratory muscle work, pulmonary vascular pressure is maintained within normal limits, and DC is enhanced.

Mechanics of Breathing during Exercise

The work of breathing is the product of pressure and volume changes as shown in Figure 2.3. The volume change refers to the difference in volume of air moved in and out of the lungs. The pressure change is the change in transpulmonary pressure required to overcome both the elastic and resistive work of breathing. The elastic work of breathing is the work performed to overcome the elastic recoil of the chest wall and parenchyma, and to overcome the surface tension of the alveoli [59]. The resistive work of breathing refers to the work undertaken to overcome the tissue and airway resistance. Elastic work increases exponentially as tidal volume increases. When lung compliance decreases, additional force must be generated by the inspiratory muscles in order to expand the lungs.

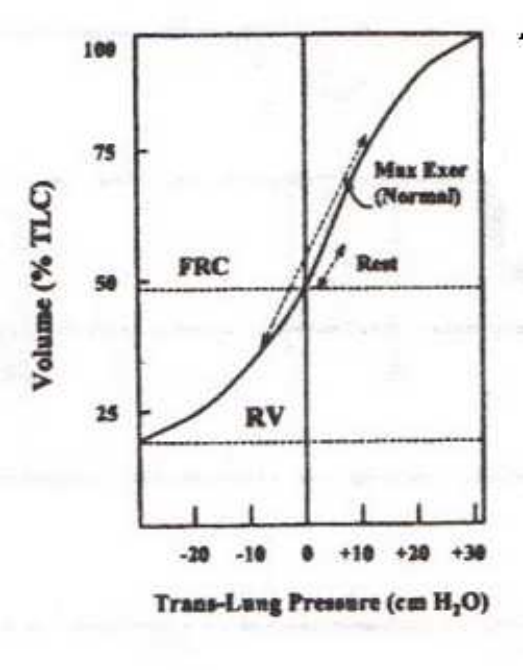

Figure 2.3: Pressure-volume curve in the lungs at rest and during exercise

The respiratory control centre functions to increase ventilation to maintain alveolar O_2 and CO_2 near the resting level while minimizing the mechanical work performed by the respiratory muscles [60]. Minimizing the metabolic cost of breathing will result in an increase in the volume of cardiac output that is available for delivery to other working muscles [61] and delay the onset of dyspnea [62].

During exercise, the increase in $\dot{V}_A$ is proportional to the increase in metabolic rate. An increase in tidal volume (V_T) is primarily responsible for the increase in $\dot{V}_A$ during the transition from mild to moderate exercise. This reduces the DS to tidal volume ratio (V_D/V_T), and minimizes the work of breathing, which is more related to respiratory frequency (RR).

During exercise, the increase in V_T is achieved by encroaching on both the inspiratory and expiratory lung volumes. The balance between the inspiratory and

expiratory flow rate allows the lungs to reduce dynamic end expiratory lung volume (EELV) [63]. Progressive expiratory muscle recruitment ensures that V_T is positioned on the linear portion of the respiratory system's pressure volume loop (20 - 80% of vital capacity) where elastic loading is avoided (Figure 2.3), [63]. This allows V_T to expand to a maximum of 55% of vital capacity (VC), by way of optimizing the length of the diaphragmatic and inspiratory intercostal muscles at the onset of inspiration. As exercise progresses from moderate to higher intensity levels the V_T plateaus and further increases in $\dot{V}_A$ are achieved by increasing RR (Figure 2.4). This reduces the time for inspiration (T_I) and expiration (T_E) [64].

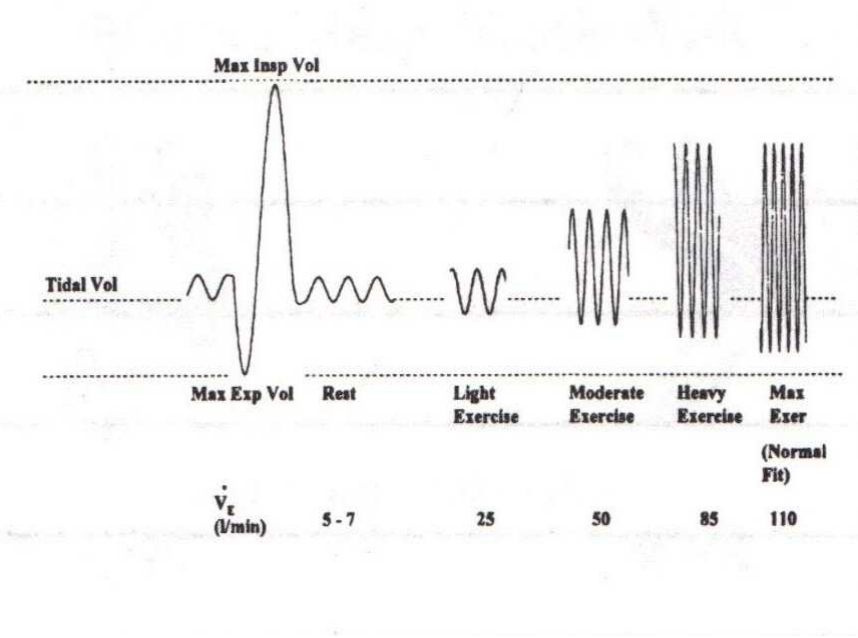

Figure 2.4: Change in breathing pattern during exercise

Control of Alveolar Ventilation: Defence of Alveolar PO_2 and PCO_2

In healthy non-athletic individuals the capacity to increase flow rate and volume at rest is greater than at maximal exercise. This increased resting ventilatory capacity represents a breathing reserve that is not utilized at maximal exercise due to cardiovascular limitations. At maximal exercise, the respiratory muscles can achieve a

ventilation ($\dot{V}_E$) level up to 180 $L \cdot min^{-1}$ while using only 5%-7% of the total body $\dot{V}O_2$ [65]. This efficient breathing response is due to an increase in airway bronchodilatation and a decline in V_D/V_T. Airway bronchodilatation occurs as a result of increased circulating levels of catecholamines. At maximal exercise, V_D/V_T can decrease by approximately 30% of baseline value resulting in almost equal matching between $\dot{V}_E$ and $\dot{V}_A$. Dead space ventilation represents only 13% of total $\dot{V}_E$ at maximal exercise [66].

During low to moderate exercise, the precise regulation of alveolar ventilation relative to metabolic requirement is governed by a feedback signal from exercising muscle to the respiratory pattern generator in the medulla which drives ventilation, and a feed-forward mechanism from higher locomotor centres in the brain [67].

Above 75-80% of maximal exercise capacity, $\dot{V}_A$ increases out of proportion to CO_2 production, resulting in a decrease in arterial PCO_2. The hyperventilatory response to heavy exercise results from stimulation of the carotid chemoreceptors via the metabolic acidosis pathway. The hyperventilatory response helps to minimize the decrease in pH resulting from the accumulation of lactic acid (LA), and increases the alveolar PO_2 and O_2 pressure gradient to protect against the development of arterial hypoxemia. This hyperventilatory response is an "extra" stimulus in addition to the input from the locomotor-linked feed-forward and feedback sources.

Pulmonary Gas Exchange

The efficiency of pulmonary gas exchange from the alveoli to the pulmonary capillary can be assessed by measuring the $P_{A-a}O_2$ diff. The magnitude of the $P_{A-a}O_2$ diff

is determined primarily by the uniformity of $\dot{V}_A$ and pulmonary perfusion (Q). Resting $P_{A-a}O_2$ diff averages 5 to 15 mmHg [68] in normal, healthy non-smoking young adults [69], and may raise another 5 to 10 mmHg by age 70 years. The $P_{A-a}O_2$ diff begins to rise during mild exercise and can increase to 20 - 30 mmHg at maximal exercise in healthy untrained subject, (Figure 2.5) [70]. The increase in $P_{A-a}O_2$ diff is due to a rise in P_AO_2, because $\dot{V}_A$ normally increases out of proportion to $\dot{V}O_2$ and arterial PO_2 is maintained near resting levels.

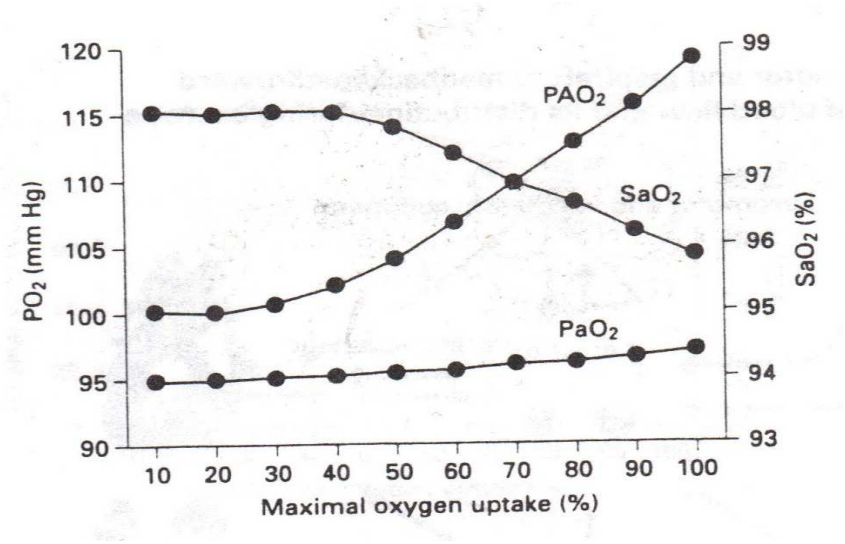

Figure 2.5: PA-aO_2 diff and O_2% saturation during progressive exercise to maximum in healthy young adult

Potential mechanisms responsible for increasing the $P_{A-a}O_2$ diff include poorer mismatching of ventilation to perfusion ($\dot{V}_A/Q$), and/or a reduction in the diffusion of oxygen from the alveoli to the pulmonary capillary. With increasing exercise intensity $\dot{V}_A$ increases out of proportion to Q resulting in $\dot{V}_A/Q$ mismatching. During heavy exercise, $\dot{V}_A/Q$ mismatching is 3 to 4 times higher than at the resting level. An elevated alveolar PO_2 is present across all $\dot{V}_A/Q$ units. The increased O_2 pressure gradient

between the alveoli and pulmonary capillary helps to maintain PaO_2 at normal value despite the large reduction in mixed venous O_2 content [70].

The second contributing factor to the increased $P_{A-a}O_2$ diff during exercise is the right to left shunt of mixed venous blood from the bronchial artery and the coronary vein that enters the arterial system without passing through the lung. A 1-2% extrapulmonary shunt exists in normal lungs. The third potential contributing factor is the failure of mixed venous blood in the pulmonary capillaries to equilibrate with alveolar O_2 pressure. Disequilibrium may occur if the time required for the equilibration is greater than the time that red blood cells (RBC) spend in the gas exchange unit. The average time that a RBC remains in the pulmonary capillary is known as the transit time and is approximately 0.75 seconds at rest. However, only 0.3 seconds is required for the RBC to equilibrate with alveolar PO_2. Although the transit time may decrease to 0.4 seconds during high intensity exercise, it is still efficient to complete oxygenation of haemoglobin [71] in a normal alveolar capillary membrane.

The increase in $P_{A-a}O_2$ diff in healthy men and women may be due to $\dot{V}_A/Q$ mismatching and a small anatomical shunt of mixed venous blood. However, a significant decrease (3-13%) in S_PO_2 has been shown to occur during maximal exercise at sea level in healthy elite athletes [69]. These findings provide evidence that the even in healthy individual the respiratory system may not always be able to maintain normal gas exchange.

The lungs have a number of mechanisms that help to minimize the widening of the $P_{A\text{-}a}O_2$ diff and enhance DC. Firstly, the alveolar capillary diffusion surface can be increased by the recruitment of additional pulmonary capillaries. Secondly, the pulmonary arterioles have a thin flexible wall that helps to maintain a low pulmonary vascular resistance and minimize the increase in pulmonary capillary pressure [72]. Thirdly, pulmonary capillary volume expands up to 3 fold in response to the increase in pulmonary blood flow. This helps to minimize the reduction in transit time.

Ventilatory Response at Rest in CF

CF is a disease characterized by dysfunction of both the small airways (<2 mm) and large airways. Disease progression is associated with parenchyma destruction secondary to bronchietasis [75]. Progressive structural lung damage eventually leads to end stage lung disease accompanied by a severe reduction in pulmonary diffusion. Children with CF have evidence of early small airway damage due to inflammation [73]. This process is irreversible and may also be found in children with CF with a normal pulmonary function.

Increased severity of CF is associated with destruction of the parenchyma, resulting in a reduced lung compliance [76]. An increase in resting respiratory muscle load is found in severe CF patients due to a decrease in lung compliance. Patients with severe CF tend to take small rapid breaths in order to help minimize the increase in elastic force of inspiration and to compensate for the reduction in FEV_1 [76]. However, rapid shallow breathing may increase V_D/V_T resulting in alveolar hypoventilation, hypoxemia and hypercapnia (increased arterial CO_2) [77].

As CF becomes more advanced, the disease may become a heterogeneous disorder characterized by dysfunction of the small and large airways and by parenchyma and vascular destruction, in highly variable combinations. Although the most obvious physiological defect in CF is expiratory flow limitation, Hart et al., [76] found that elastic forces have more effect on ventilatory mechanical constraint than the resistive forces during resting breathing in severe CF patients.

Pulmonary hyperinflation may affect the ventilatory response at rest in children with CF [78]. Pulmonary hyperinflation is present when an expiratory flow limitation reaches a critical level and EELV fails to decline below functional residual capacity (FRC). Pulmonary hyperinflation increases both the work of breathing and the sensation of breathlessness, and impairs respiratory muscle function [79-81]. The reduction in lung compliance and lung hyperinflation decreases resting V_T in advanced CF [78]. However, a recent study found that the presence of static hyperinflation (%RV/TLC) in adolescents with mild to moderate CF did not strongly influence ventilatory constraints during exercise. The static hyperinflation was only a slightly stronger predictor of peak exercise capacity than FEV_1%. [82].

Ventilatory Mechanics and Exercise Intolerance in CF Patients

Pulmonary hyperinflation along with a reduction in resting lung compliance may affect exercise performance in CF patients. During exercise, dynamic pulmonary hyperinflation (DH) is present when EELV is increased above the resting value [6]. Regnis et al., found DH in CF patients with FEV_1 <60% resulted from a combination of flow limitation and an increased ventilatory demand [6-7]. When DH is present, V_T is

positioned close to the TLC on the steep portion of the pressure volume loop resulting in a higher pressure being required to increase V_T for any given volume expansion. During exercise V_T can increase up to 90% of the end inspiratory lung volume (EILV) where further volume expansion is minimal. At a fixed V_T, moderate to severe CF patients rely on increasing RR in order to increase $\dot{V}_E$ [6-7]. DH progressively increases with CF severity due to an increase in airway obstruction. In patients with severe CF, DH may be present during low intensity exercise.

DH may also increase inspiratory muscle loading [83]. Since inspiration begins at higher lung volumes in patients with moderate to severe CF than in healthy individuals, inspiratory muscles must counterbalance the combined inward recoil of the lung and chest wall prior to the initiation of inspiratory flow [6-7]. Higher tidal inspiratory pressures and effort must be generated for any given V_T expansion resulting in an increase in the mechanical work and O_2 cost of breathing.

DH can alter the length-tension relation of the inspiratory muscles, particularly the diaphragm, and compromise their ability to generate pressure [84]. Resting maximal diaphragm strength is reduced by 20% in severe CF patients due to muscle shortening secondary to hyperinflation [84]. Rapid shallow breathing (tachypnea) is also associated with respiratory muscle shortening during exercise resulting in further inspiratory muscle weakness [85]. The combined overloading and reduced maximal strength of the inspiratory muscles leads to premature inspiratory muscle fatigue during exercise in moderate to severe CF patients [81] .

Gas Exchange Abnormality at Rest in CF Patients

Studies that have evaluated gas exchange at rest in patients with CF have been equivocal. An early study found that resting $\dot{V}_A/Q$ ratio is decreased in hypoxemic moderate CF patients, indicating that the alveolar shunt is the primary mechanism responsible for hypoxemia in these patients [86]. It is possible that unventilated alveoli beyond the obstructed small bronchioles (interpulmonary shunt) may be responsible for the impaired gas exchange. In contrast, other investigators found that resting $\dot{V}_A/Q$ ratio is increased in moderate to severe CF patients due to a local hypoperfusion caused by hyperinflation [33, 87].

Gas Exchange Abnormality and Exercise Intolerance in CF Patients

In patients with airway disease, an increased $P_{A\text{-}a}O_2$ diff during exercise typically results from hypoventilation of lung regions relative to their perfusion. Abnormal gas exchange may result in arterial hypoxemia which may limit exercise performance in CF patients with moderate to severe lung disease. CF patients with a severe reduction in resting carbon monoxide diffusion capacity (D_LCO <80% of predicted) are more likely to develop arterial hypoxemia at peak exercise [88]. The severity of arterial hypoxemia during exercise cannot be predicted from resting spirometry [89]. The mechanisms responsible for exercise hypoxemia in CF are not well understood and may be due to ventilation/perfusion mismatching [87-88], intrapulmonary shunting, and alveolar hypoventilation [89].

The ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}O_2$) and CO_2 ($\dot{V}_E/\dot{V}CO_2$) and the V_D/V_T ratio can be used to indicate the ventilatory demand at a given metabolic rate and

therefore provide additional information on ventilatory efficiency. The $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ are dependent on a number of factors that link ventilation to metabolic regulation. An abnormally elevated ventilatory equivalent in the presence of a high $PaCO_2$ may indicate poor distribution of ventilation, areas of the lung with high $\dot{V}_A/Q$ or an increase in DS. The measure of $\dot{V}_E/\dot{V}CO_2$ at the anaerobic threshold (AT) can be clinicaly very usefull. The minimal $\dot{V}_E/\dot{V}CO_2$ is about 25 in healthy young subjects. However, it exceed 30 in older individuals. A $\dot{V}_E/\dot{V}CO_2$ value < 32-34 at or near the AT and <36 at peak exercise in normally found in healthy individuals. Furthermore, the $\dot{V}_E/\dot{V}CO_2$ at the AT is similar to the lowest $\dot{V}_E/\dot{V}CO_2$ ratio when the AT can not be determined during an exercise test [90].

An elevated V_D/V_T ratio is a better predictor of an intrinsic lung abnormality than the ventilatory equivalents. Patients with moderate to severe CF have been shown to have an elevated V_D/V_T at peak exercise due to a low V_T and an increased RR [91]. An elevated V_D/V_T will result in a greater proportion of each breath moving in and out of the airway DS causing arterial hypoxemia.

Role of the Respiratory System in Limiting Exercise Performance in CF

It is not known whether peak exercise capacity is limited by pulmonary mechanisms or gas exchange in moderate to severe CF. McKone et al., [4] compared the effect of adding DS alone, and the combination of DS with supplemental O_2, on peak exercise responses in patients with moderate to severe CF. The increased DS reduced peak exercise capacity with no change in peak $\dot{V}_E$, suggesting a respiratory limitation to exercise. In contrast, peak exercise capacity and peak $\dot{V}_E$ increased when

supplemental O_2 was provided in the presence of added DS, suggesting that hypoxemia rather than pulmonary mechanics are responsible for limiting peak exercise capacity in moderate to severe CF.

In order to stress the ventilatory system, Dodd et al., [92] provided additional DS to the respiratory system during a graded exercise test in patients with mild CF. Peak $\dot{V}_E$ increased through increases in V_T because the participants had an adequate ventilatory reserve to overcome the additional DS. These findings suggest that ventilatory mechanics are not a limiting factor during peak exercise in patients with mild CF. In contrast, Keochkerian et al., [93] found that children with mild to moderate CF developed an expiratory flow limitation during transition from light exercise intensity to peak exercise. These findings suggest that ventilatory mechanics due to DH may be a limiting factor during submaximal exercise and during peak exercise in patients with mild to moderate CF.

Cardiovascular Factors in CF

The effect of CF on cardiac response during exercise is complex and involves many factors. In chronic obstructive pulmonary disease (COPD), severe lung hyperinflation and excessive expiratory muscle recruitment can impair venous return and reduce right ventricular (RV) preload. Several studies have demonstrated an increase in pulmonary vascular resistance during exercise in COPD [94-95] resulting from a reduction in the area of the pulmonary vascular beds, a decrease in compliance, and hypoxemia [96].

The morphological pulmonary vascular changes in CF are similar to that of other hypoxic lung diseases [97]. Ryland et al., [98] studied the heart and lungs of 36 CF children. All patients had some degree of pulmonary artery muscle hypertrophy, arterial sub-intimal fibrosis and thickening of the pulmonary vein wall. The thickness of the muscular wall was significantly related to the degree of RV hypertrophy.

A number of studies indicate that pulmonary hypertension (PH) and RV enlargement occurs in CF patients due to pulmonary vascular remodelling [99-100]. The incidence appears to be higher in patients with severe CF disease. The prevalence of clinical RV disease in CF is unknown, but prognosis is poor once RV failure is evident [101].

Cardiovascular Response during Exercise in CF

Stroke volume (SV) during exercise in affected by CFseverity [102-103]. Radio angiography studies have demonstrated that both RV and left ventricular (LV) ejection fraction may be decreased during exercise in CF. Hortop et al., [104] found a relation between expiratory flow limitation and cardiac performance during exercise in patients with moderate CF disease. It was speculated [104] that the expiratory flow limitation may increase expiratory muscle action resulting in an increase in abdominal and intrathoracic pressure throughout expiration. This may compromise cardiac output (CO) by reducing the venous return and by increasing pulmonary vascular resistance.

Cardiovascular Basis of Exercise Gas Exchange

Measuring respiratory gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) is particularly relevant for evaluating the cardiovascular response during exercise. Oxygen uptake during exercise is a product of CO and the arterial-venous O_2 difference [$c(a\text{-}v)O_2$]. Cardiac output increases during exercise due to an increase in both heart rate (HR) and SV. Widening $c(a\text{-}v)O_2$ depends on the extraction of O_2 from the capillary blood in the exercising muscle, and the distribution of blood flow to the muscle. In the absence of significant anaemia or hypoxemia, maximal $c(a\text{-}v)O_2$ differs very little between healthy and diseased persons [105].

$\dot{V}O$/Heart Rate Relationship (Oxygen Pulse)

The volume of O_2 extracted during each heartbeat is called the oxygen pulse (O_2 pulse) and is equal to the product of SV and $c(a\text{-}v)O_2$. Due to the fact that $c(a\text{-}v)O_2$ at peak exercise does not vary widely between healthy subjects and CF patients with normal RCB and Hb values, the between-subjects variability in O_2 pulse at peak exercise is determined largely by differences in SV [105]. If the O_2 pulse reaches a plateau despite an increase in work rate (WR), it most frequently reflects a low maximal SV [105]. Central factors, particularly those that effect SV including heart disease, deconditioning, ventilatory limitation, and possibly even the hemodynamic consequence of dynamic hyperinflation may decrease O_2 pulse. Factors that effect $c(a\text{-}v)O_2$ including abnormal O_2 utilization, desaturation and anaemia may also reduce the O_2 pulse. Consequently, caution should be taken when interpreting changes in O_2 pulse response as a function of SV when patients are hypoxemic.

HR- $\dot{V}O_2$ Relationship

Among normal subjects, HR increases almost linearly with $\dot{V}O_2$, and reaches a maximal value. In many types of heart disease, the HR increase is relatively steep for a given increase in $\dot{V}O_2$ due to left ventricular dysfunction. Patients with airflow obstruction commonly have a moderately elevated HR at a given $\dot{V}O_2$ due to a reduced SV [106]. The reduction in SV in these patients results from a restriction in cardiac filling due to high intrathoracic pressure during exhalation. Pulmonary vascular disease is also associated with a steep HR response due to pulmonary hypertension (PH). PH may increase the work of the RV and reduce SV [106].

VO_2 in Relation to Work Rate ($\Delta VO_2/\Delta WR$)

Normally $\dot{V}O_2$ increases linearly with increasing external WR during a progressive cycle exercise test. The slope of the relation between $\dot{V}O_2$ and WR is influenced by the change in WR relative to the subject's capacity, but is approximately 8.5-11 $ml \cdot min^{-1} \cdot watt^{-1}$ over the entire range of $\dot{V}O_2$ from unloading cycling through peak exercise. A decrease in the normal relation between $\dot{V}O_2$ and WR implies an abnormal reliance on non-oxidative metabolism. Because the metabolic efficiency of exercising muscles is relatively constant, a reduction in $\Delta\dot{V}O_2/\Delta WR$ usually indicates inadequate O_2 transport, as may occur in individuals with cardiovascular disease or COPD.. However, muscle-related abnormality in O_2 metabolism has been reported in children with mild to moderate CF [10].

Peripheral Muscle Dysfunction and Exercise Intolerance in CF

Muscle dysfunction may contribute to the reduced exercise tolerance observed in CF patients [36, 84, 106-113] and may be due in part to reduced activity levels or immobility due to dyspnea [107]. Muscle dysfunction in CF may include [107-109] muscle weakness [110], decreased number of mitochondria [8, 10, 110-111], and increased reliance on anaerobic glycolysis [8, 111].

Effect of Deconditioning in Normal Skeletal Muscle

Deconditioning is a term used to describe the physiological effects of decreased activity. The physiological effect of immobilisation on peripheral muscle is characterised by atrophy, fibre type re-distribution, altered bioenergetics, and altered capillarization. Atrophy is the process whereby muscle size is reduced, almost exclusively because of reductions in the contractile proteins, actins and myosin. Skeletal muscle mass is normally maintained by a balance between protein synthesis and protein degradation. The signalling pathways that govern muscle atrophy are not fully defined. Nuclear factor kB (NF-kB) is a central regulator involved in the regulation of both the atrophy and hypertrophy signalling pathways [112]. It is down-regulated during muscle atrophy. In general, atrophy is reversible and affects both type I and type II fibres.

Muscle type redistribution represents a change within type II fibres. Type IIa fibres may take on the physiological and biochemical characteristics of type IIb fibres that have less oxidative and more glycolytic potential. There is also evidence that long-

term inactivity and/or chronic disease can alsoresult in a shift between type I to type II fibre type characteristics [113].

In healthy individuals, deconditioning is associated with a reduction in the concentrations of enzyme markers of aerobic oxidative capacity. In contrast, there appears to be no change in the concentration of enzymes involved in anaerobic glycolysis. Deconditioning is also associated with a reduction in the total mitochondrial content and capillary density. Both of these changes may have a negative impact on muscle endurance [114].

Muscle Dysfunction in CF

Moorcroft et al., [115] compared ratings of muscle effort and breathlessness, blood lactate levels and exercise limitation at peak cycle exercise in adults with mild, moderate and severe CF and healthy controls. Ratings of muscle effort were higher than ratings of breathlessness at peak exercise in all patient groups than healthy controls. Although peak work rate was higher in the healthy controls than in the CF patients, there was no difference in peak lactate between the two groups.

Some studies have found evidence of intrinsic abnormality in muscle function in CF independent of deconditioning [8, 10]. Compared to healthy individuals, CF athletes with good nutritional status and a preserved pulmonary function have lower anaerobic power and maximal leg strength, and a reduced mitochondrial oxidative metabolism [8]. Abnormal expression of CFTR in skeletal muscle may be responsible for the reduction in oxidative and anaerobic metabolism due in part to ATPase hydrolysis by CFTR proteins [116]. Other factors that may contribute to peripheral

muscle weakness include malnutrition, systemic inflammation [117], medication (especially corticosteroid) [118], hypoxia, and oxidative stress [119]

Prolonged nutritional depletion is associated with a reduction in muscle mass [120]. The effect of nutritional depletion is greater in type II than type I muscle fibres. Greater atrophy of type II fibres ensures that the tension of the muscle generated during daily activity is preserved. However, maximal strength and endurance may be impaired [121]. Poor nutritional status may limit exercise capacity in CF patients. In patients with severe airway obstruction, Boucher et al., [122] found that physical activity levels were related to nutritional status but not to lung function. Similarly, nutritional status has been identified as a major determinant of anaerobic exercise capacity in CF patients due in part to atrophy of type II fibres [108].

Systemic inflammation may contribute to muscle atrophy in respiratory and non-respiratory chronic diseases. In CF, systemic inflammation is more prolonged than other chronic disease due to endobronchial inflammatory process of the affected lungs. Cell culture models found that pro-inflammatory cytokines, such as TNF-α, induced protein breakdown through activation of NF-kB via an increased production of mitochondrial reactive oxygen species [123-124]. Defrense et al., [117] found a relation between the severity of lung disease in mild to moderate CF and inflammatory cytokines including IL-6 and TNF-α. However, there was no relation between inflammatory cytokines and lean body mass and muscle strength of the diaphragm, biceps and quadriceps.

Steroid-induced myopathy, particularly in type IIb muscle fibres is associated with prolonged use of oral corticosteroids. Recovery following chronic steroid myopathy may take many weeks to months [125]. Chronic steroid use may affect the synthesis of contractile proteins and the turnover of biochemical substrates in skeletal muscles. Corticosteroid may downregulate insulin-like growth factor 1 (IGF-1), and thus may decrease protein synthesis and increase intracellular proteolysis. Daily use of 5.1 $mg \cdot day^{-1}$ of corticosteroid over 12 months has been shown to reduce maximal limb muscle strength in CF patients. The results were independent of the severity of airway obstruction, nutrition and days spent in hospital during the previous 12 months [118].

Chronic or intermittent hypoxia is common in moderate to severe CF patients. In healthy humans, exposure to high altitude hypoxia over weeks and months results in functional, morphological and metabolic changes in the skeletal muscle [126]. Tissue hypoxia limits the production of energy and affects protein synthesis, leading to muscle loss, increased glycolytic activity and reduced oxidative enzyme activity [127]. Hypoxemia can also trigger other pathogenetic mechanisms related to muscle dysfunction, such us an increase in the levels of circulating cytokines and oxidative stress [128].

An imbalance between oxidant and antioxidant capacity of the cells can lead to oxidative damage of protein, lipids, and nucleic acid, a process known as oxidative stress. Oxidative stress can alter muscle contractility, potentially affecting muscle strength, and may contribute to muscle fatigue [119]. Oxidative stress can also

contribute to accelerated protein degradation [129]. Antioxidant supplementation has been shown to improve exercise tolerance in COPD patients [130].

O_2 Uptake Kinetics

O_2 Uptake Kinetics during Constant Load Exercise

The rate of adjustment of oxidative phosphorylation to sudden increases in energy demand has been termed O_2 uptake kinetics. It reflects the integrated response of the ventilatory, cardiovascular, and neuromuscular system to the exercise challenge [131]. The pattern for the increase in pulmonary $\dot{V}O_2$ at the onset of a constant load exercise can be described by the monoexponential function [132]. Three phases are normally considered. Phase I (time delay (TD)) represents the cardiodynamic phase and reflects the increase in pulmonary blood flow without an increase in arteriovenous O_2 difference. It is approximately 15-20 seconds in duration and reflects the time period prior to the blood from exercising muscles entering the pulmonary capillary [133]. Phase II represents the adjustment of the metabolic response to exercise [133]. It reflects the arrival at the lungs of the mixed venous blood and lasts from approximately 15-20 seconds after the onset of exercise to the third minute of exercise [133]. Phase III begins at approximately 3 minutes after the onset of exercise, and reflects the start of the $\dot{V}O_2$ steady state period when the work rate is below the AT. However, if the exercise intensity is above the AT, $\dot{V}O_2$ steady state is delayed or not achieved prior to fatigue ($\dot{V}O_2$ slow component) [134].

Modelling O_2 Uptake Kinetics during Moderate and Heavy Exercise

O_2 uptake kinetics are modelled using non-linear least-square regression techniques. The $\dot{V}O_2$ response data is fitted to a mono-exponential function that includes a single amplitude and time constant (τ) [133]. The mean response time (MRT) indicates the time taken for O_2 to reach 63% of the response amplitude from the onset of exercise with no distinction made for the cardiodynamic phase (phase I) [135]. The normal value for MRT in healthy individuals is approximately 30-40 seconds [136].

The monoexponential fit of the $\dot{V}O_2$ response in phase I and phase II can be described using the following equation:

$$O_2\,(t) = O_2 \text{ baseline} + (A) \times (1\text{-}e\text{-}t/\tau)$$

Where O_2 (t) represents the absolute O_2 at a given time t; O_2 baseline represents the mean O_2 in the baseline period preceding the step transition to the higher work rate; A represents the response $\dot{V}O_2$ steady state amplitude; (1-e-t/τ) is the exponential function describing the rate at which $\dot{V}O_2$ is rising towards the steady state amplitude; t is the time and τ represents the MRT, as shown in Figure 2.6.

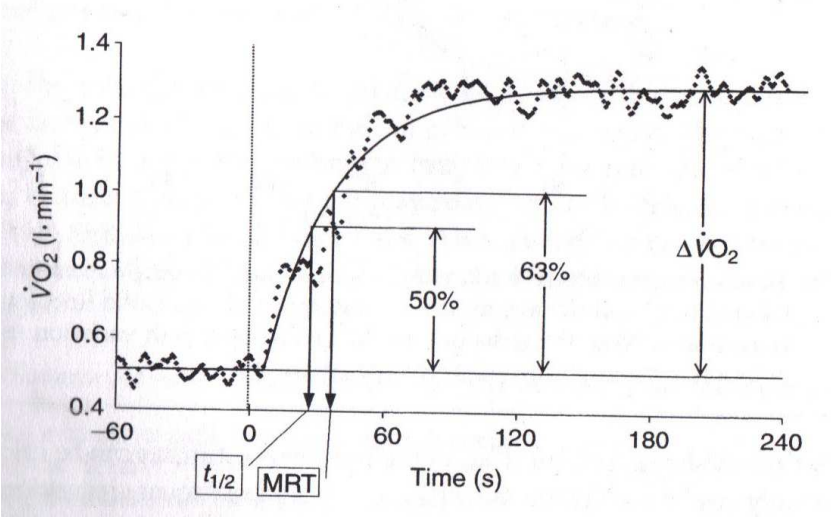

Figure 2.6: Overall VO_2 kinetics expressed as MRT to attain 63% of overall response

Oxygen Deficit

The fact that O_2 uptake kinetics do not increase instantly to a level that meets the $\dot{V}O_2$ demand of submaximal exercise below the AT indicates that an anaerobic energy contribution makes up the difference. The difference between the amount of energy contributed from aerobic ATP production and that required to sustain exercise is called the oxygen deficit (O_2D). The energy provided during the deficit phase of exercise represents a predominance of anaerobic energy transfer. These stores consist principally of energy released through phosphocreatine (PCr) hydrolysis and anaerobic glycolysis [76].

The absolute size of the O_2D is the product of the increase in $\dot{V}O_2$ amplitude (A) and the MRT, from the onset of exercise (O_2D = A x MRT) (Figure 2.7). For a given $\Delta\dot{V}O_2$, the faster the O_2 uptake kinetics (smaller MRT), the smaller the O_2D. Sedentary individuals or patients with chronic cardiovascular and pulmonary disease have a slow O_2 uptake kinetics (large MRT) and high O_2D. A larger MRT is associated with a greater anaerobic contribution to the energy requirement resulting in a greater accumulation

of lactic acid in the exercising muscle. The increase in lactic acid concentration contributes to exercise intolerance [99].

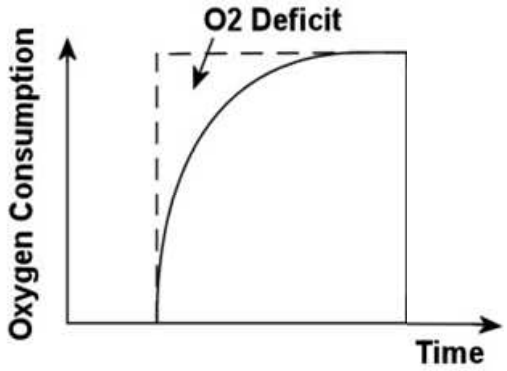

Figure 2.7: O_2 deficit as a function $\dot{V}O_2$ amplitude and MRT

O_2 Uptake Kinetics during Moderate Exercise in Healthy Individuals

The kinetics of the $\dot{V}O_2$ adjustment to a new metabolic requirement is reflected by the capacity of the O_2 delivery to muscle fibres, and the intracellular oxidative metabolism within the muscle fibres [136-137]. In healthy young individuals, O_2 delivery is adequate to support the metabolic requirement in the transition from rest to moderate exercise. The rate of increase in $\dot{V}O_2$ to its asymptote is determined by metabolism within the muscle fibres [131] and is related to enzyme activation and/or concentrations of cellular metabolic controllers.

O_2 Uptake Kinetics during Heavy Exercise in Healthy Individuals

O_2 uptake kinetics during heavy exercise is determined by the magnitude of both the primary component and the $\dot{V}O_2$ slow component [138]. In healthy individuals, the primary component lasts 2 -3 minutes following the onset of exercise and is dependent on the percentage of type 1 oxidative fibres within the exercising muscle. A higher proportion of type 1 fibres may accelerate the primary component

kinetics. The magnitude of the $\dot{V}O_2$ slow component has been described as the difference between $\dot{V}O_2$ at 2 minutes and 6 minutes ($\Delta\dot{V}O_2$ 6-2min) [139-140] or $\dot{V}O_2$ at 3 minutes at the end of the exercise as shown in Figure 2.8 [139]. Putative mechanisms responsible for the $\dot{V}O_2$ slow component during heavy exercise include i) increased cardiac and respiratory muscle work, ii) increased O_2 cost of lactate catabolism [138] and gluconeogenesis [155-156], iii) increased activity/recruitment of IIX fast twitch glycolytic fibres within the skeletal muscle and iv) the pattern of motor unit recruitment [141-142]. The delivery of O_2 to exercising muscle may play a role in limiting O_2 uptake kinetics during heavy exercise [143]. Providing supplemental O_2 during heavy constant load exercise accelerates O_2 uptake kinetics in healthy individuals [144].

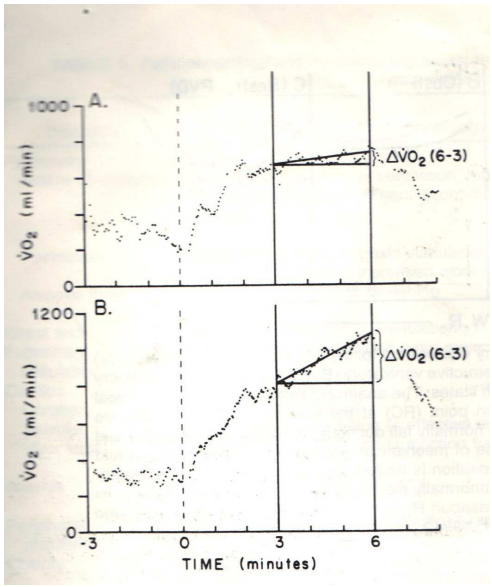

Figure 2.8: $\dot{V}O_2$ slow amplitude during moderate (A) and heavy (B) constant WR exercise for healthy adults

O_2 Uptake Kinetics during Moderate and Heavy Exercise in Pulmonary Disease

O_2 uptake kinetics is slower than normal in chronic diseases such as COPD [145], CF [146], heart failure [147-149], peripheral vascular disease [147], and type II

diabetes [139]. In individuals with COPD, O_2 delivery to exercising muscle is the primary limitation for O_2 uptake kinetics. Providing supplemental O_2 during moderate exercise accelerates O_2 uptake kinetics and reduces the O_2 deficit in hypoxemic COPD [150], but has no effect on non-hypoxemic COPD [151].

Relatively few studies have examined O_2 uptake kinetics in CF. The results were equivocal. Hebestreit et al., [146] found that O_2 uptake kinetics was slower during moderate exercise in mild to moderate CF patients than in healthy controls. The slower O_2 uptake kinetics was related to a reduction in O_2 delivery to the exercising muscle. In contrast, providing supplemental O_2 has been shown to have no effect on O_2 uptake kinetics in mild to moderate CF during moderate exercise [152].

Compared to healthy individuals, O_2 uptake kinetics during heavy exercise may be slower in patients with COPD due to a reduction in systemic and peripheral O_2 delivery to the exercising muscle. Factors responsible for the reduction in oxygen delivery include a higher ventilatory demand, abnormal breathing pattern [153], hypoxemia [150] and an abnormal peripheral vascular response [154].

An impaired pulmonary hemodynamic response may also play a role in the slower $\dot{V}O_2$ kinetics during heavy exercise in COPD patients [155]. A high mean intrathoracic pressure due to increased resistive and elastic loading of the respiratory muscles could dynamically impair the rate of right ventricular filling and left ventricular emptying. The alteration in ventricular function may lead to a reduction in peripheral muscle O_2 delivery and may impair O_2 uptake kinetics [144]. Providing bronchodilator

therapy before heavy exercise reduces lung hyperinflation and accelerates O_2 uptake kinetics in patients with moderate to severe COPD [155].

Exercise Training

As the severity of CF progresses, breathlessness and exercise intolerance are more prevalent. Pulmonary $\dot{V}_E$ and RR consistently account for the greatest amount of variation in rating of perceived exertion during treadmill and cycle ergometer exercise[156]. Indeed, adjustments in respiratory rate during dynamic exercise appear to be one of the primary physiological mediators of respiratory metabolic signals of perceived exertion.

An individual's preferred level of intensity of physical activity depends in part on his or her affective response (pleasure or displeasure) resulting from sensory experience from various parts of the body. When given a choice, individuals will generally adjust their effort intensity during exercise to maximize affect (short duration, high intensity emotion or feeling). The short term rewards of positive affective responses that can be derived from a positive exercise experience might assist people to stay motivated to sustain regular physical activity and avoid drop out. Increased breathlessness at relatively low to moderate levels of physical activity may lead to a reduction in affect during exercise and decrease compliance. As the disease becomes more advanced, patients with CF may spend less time undertaking activities of daily living resulting in peripheral muscle wasting.

Inactivity in healthy individuals has been shown to negatively impact on muscle structure and function [157]. There is accumulating evidence that exercise intolerance

in patients with CF is also associated with abnormalities in skeletal muscle. It is not clear whether the abnormalities are related to deconditioning or to abnormal intrinsic muscle function. Exercise training has the potential to preserve muscle mass, improve exercise capacity and restore patients to the highest level of independent function.

The majority of published studies of exercise training in CF have been short term, involved small numbers of subjects and have not included a control group. In a recent review involving 26 studies, Wilkes et al., [158] examined the effects of prescribed exercise training in addition to medical management in CF. Only 7 of the studies that used a randomized controlled design were included in the analysis. There was evidence of an improvement in exercise capacity and muscle strength but the results were not consistent between studies [11, 156, 159-166]. Shah et al., [108] found that exercise training had no effect on gas exchange, and has only a minor effect on resting pulmonary function in CF.

Moorcroft et al., [106] examined the effects of a one year unsupervised home-based exercise programme in 48 adults with CF. Subjects were randomly assigned to a control group or an exercise group. Heart rate and pulmonary function were also assessed at the beginning and end of the training programme. Blood lactate levels were measured at the end of a submaximal arm and leg ergometry test to assess improvement in fitness. The exercise group received personalized training programmes based on their exercise preferences with the aim of increasing their levels of physical activity. Lower and whole body exercises were focused on aerobic training, and the primary upper body exercise was weight training. Subjects undertook three,

20 minute sessions of upper body and three sessions of lower body exercises per week. In addition to keeping a training diary the subjects also met with an exercise specialist every 4 weeks. FEV_1 in the exercise, and control group declined by 67 ml and 174 ml, respectively. The one year decline in FEV_1 in the exercise group (1.5%) was lower than predicted (2-3 %) [11]. FVC improved by 46 ml in the exercise group and decreased by 167 ml in the control group. Blood levels of lactic acid and heart rates were significantly lower in the exercise group than in the control group during the constant load submaximal leg ergometry test. There was no change in lactate concentrations and heart rates during the arm ergometer test. Orenstein et al., [164] also found n increase in muscle strength and physical capacity in response to a 12-month home-based resistance and aerobic training programme.

Specific Strategies to Overcome Ventilatory Limitation in CF

The challenge in the design of a training programme is to stimulate the cardiovascular system and the musculoskeletal system in order to induce positive adaptive changes [167]. Exercise training programmes should be adapted and designed to the patient's limitation, taking pulmonary and skeletal muscle limitations into account. It is recommended that healthy individuals should exercise 3 to 5 days a week at an intensity between 40 to 75% of peak exercise capacity [168-169]. The majority of patients with mild CF can exercise at these recommended intensities. However, these training intensities may not be tolerated by patients with moderate to severe CF due to the central ventilatory limitation.

Interventions that permit a higher level of ventilation, or reduces the ventilatory requirement at a given level of exercise, may allow CF patients to perform training at a higher intensity [4, 12]. This may lead to enhanced training benefits particularly in moderate to severe patients with FEV_1 <60% predicted. Supplemental O_2 has recently been shown to improve maximal and submaximal exercise endurance in CF patients [4]. The mechanisms responsible for the improvements in exercise performance are not fully understood. It appears that the provision of supplemental O_2 may reduce ventilatory demands and therefore delay the time before reaching ventilatory limitation. A number of mechanisms may help to explain how supplemental O_2 reduces the ventilatory response during acute exercise in patients with pulmonary disease. Firstly, supplemental O_2 may diminish hypoxic drive from peripheral chemoreceptors due to an increase in PaO_2 resulting in a decrease in respiratory rate [151]. Secondly, the reduced metabolic acidosis as a result of improved O_2 delivery to the active muscles may decrease carotid body stimulation [170].

Summary

There is increasing evidence that the exercise intolerance associated with progression of CF severity reflects an integrated abnormality of ventilatory and peripheral muscles. Understanding the complex interface between physiological impairment and disability is important in order to improve our understanding of the role of exercise in the treatment of CF. Exercise training may partly reverse the cardiovascular abnormalities associated with CF. Furthermore, the provision of

supplemental O_2 may potentiate the training effect, and improve exercise tolerance in CF patients.

Chapter 3

Study 1

Breathing Pattern, Pulmonary Gas Exchange, and Cardiovascular Responses during Incremental Exercise in Adults with Cystic Fibrosis

Introduction

Exercise intolerance is well documented in patients with CF [8, 10, 109-110, 115]. Peak exercise capacity in these patients is limited by a number of factors, including impaired respiratory system mechanics [5-7], arterial hypoxemia [4, 88-89], peripheral skeletal muscle dysfunction [106-108, 112], and cardiovascular factors [102-103]. However, the effect of CF on cardiac performance during exercise has received little attention.

It is not known whether peak exercise capacity in moderate to severe CF is limited by pulmonary mechanisms or arterial hypoxemia. Gallagher et al., [4] compared the effect of adding DS alone, or in combination with supplemental O_2, on peak exercise response in patients with moderate to severe CF. The addition of DS volume reduced peak exercise capacity with no change in peak $\dot{V}_E$, suggesting a respiratory limitation to exercise. In contrast, peak exercise capacity and peak $\dot{V}_E$ were increased when supplemental O_2 was provided in the presence of added DS, suggesting that hypoxemia rather than pulmonary mechanics are responsible for limiting peak exercise capacity in moderate to severe CF.

An important determinant of inspiratory muscle length and airway calibre is EELV. An increase in EELV affects both mechanical breathing efficiency and the

distribution of inspired flow. DH is present when an expiratory flow limitation (EFL) reaches a critical level during exercise, and end expiratory lung volume fails to decline below the relaxation FRC [6]. DH increases both the work of breathing and the sensation of breathlessness, impairs respiratory muscle function and decreases exercise capacity [79-81]. An increase in EELV resulting from a combination of EFL and an increased ventilatory demand has been found at peak exercise in CF patients with FEV_1 <60% predicted [5, 171]. DH during exercise may limit V_T and exercise capacity in CF patients with FEV_1 < 60% of predicted [5-6].

Dodd et al., [92] provided additional DS during a graded exercise test in patients with mild CF. Peak $\dot{V}_E$ increased due to the fact that participants had an adequate ventilatory reserve to overcome the additional DS. These findings suggest that ventilatory mechanics are not a limiting factor at peak exercise in patients with mild CF. In contrast, ventilatory mechanics due to DH may be a limiting factor during submaximal exercise and at peak exercise in children with mild to moderate CF[93].

Alterations in peripheral muscle function may also limit exercise performance in CF [106-108]. Moorcroft et al., [115] compared ratings of muscle effort and breathlessness and blood lactate levels at peak cycle exercise in adults with mild, moderate and severe CF and healthy controls. Ratings of muscle effort were higher than ratings of breathlessness at peak exercise in patients with mild to moderate CF but were similar in the severe CF group. Although peak work rate was higher in the healthy controls than the CF patients, there was no difference in peak lactate between the two groups.

The effect of CF on cardiac performance during exercise is complex and multi-factorial and has received little attention. Radio angiography studies have demonstrated that both RV and left ventricular (LV) ejection fraction may be decreased during exercise in CF. Hortop et al., [104] found a relation between expiratory flow limitation and cardiac performance during exercise in patients with moderate CF disease. Endothelial dysfunction may be associated with defective vasodilatation of the pulmonary vessels during exercise [172].

Impaired respiratory system mechanics, arterial hypoxemia and peripheral skeletal muscle dysfunction and cardiovascular factors may all contribute to exercise intolerance in CF. These factors are highly interdependent and may occur in a variety of combinations that differ from patient to patient. The predominant contributing factor may also vary among patients with different severities of CF. Assessment of pulmonary and cardiovascular responses during cardiopulmonary exercise testing (CPET) provides an invasive sensitive tool for the detection of abnormal function and exercise limitation.

The purpose of the present study was to investigate whether maximal exercise capacity in adults with mild, moderate and severe CF is limited by pulmonary mechanisms, arterial hypoxemia or cardiovascular factors. It was hypothesised i) that both a ventilatory mechanical limitation and pulmonary gas exchange abnormality contribute to the reduced peak exercise capacity in CF patients with moderate and severe disease compared to healthy controls, and ii) cardiovascular abnormality also

contributes to the reduced peak exercise capacity in CF patients with FEV_1 <30% of predicted.

Methods

Subjects

Adults (n=33) with different severities of CF and apparently healthy controls (n=34) were recruited. CF patients were recruited from the respiratory department in Beaumont Hospital and were classified as having mild (FEV_1 >60 % predicted), moderate (FEV_1 40-60 % predicted) or severe (FEV_1 <40 % predicted) CF. The diagnosis of CF was based on clinical features, sweat test or genotyping and a pulmonary function test. No other diseases that could limit exercise capacity (cardiovascular, orthopaedic and neuromuscular) were present. The healthy controls were sedentary students recruited from a local university. The nature and risks of the study were explained. A plain language statement was read and informed consent was obtained in accordance with the Beaumont Hospital Ethics (Medical Research) Committee.

Study Overview

Subjects made a single visit to the Cardiovascular Research Unit in DCU. Height, weight, body composition (LBM), and pulmonary function were assessed prior to an incremental symptom-limited peak exercise test.

Preparation

Subjects abstained from alcohol and refrained from strenuous physical activity for 24 hours and fasted for 4 hours prior to the study visit.

Anthropometry

Height and body mass were measured to the nearest 0.1 cm and 0.1 kg respectively using the SECA Stadiometer (Seca model 220 GmbH, Hamburg, Germany). Subjects were barefoot and wore light clothing. BMI was determined as weight (kg) divided by height $(m)^2$.

Body Composition

Double thickness subcutaneous adipose tissue was measured on the right side of the body using skinfold calipers (Harpenden, Cambridge Scientific Industries, MD). The following anatomical sites were used; chest, subscapular, mid-axillary, suprailliac, abdomen, triceps and thigh. A minimum of 2 measurements were taken at each site. If the measurements varied by more than 2 mm, a third measurement was taken. Percent body fat was based on the equation proposed by Jackson and Pollack [173].

Pulmonary Function Test

Standard pulmonary function tests, including spirometry, and measurements of single breath D_LCO (Sensormedics Vmax 229, Sensormedics Corp, CA), were undertaken according to previously described guidelines [174-175] and results were compared to normative values [176]. The spirometry manoeuvre was explained and demonstrated prior to the test.

D_LCO was measured using a Sensormedics Vmax 229 system. Prior to the test, the gas analyzers were calibrated to room air and the breathing tube was flushed with 0.3% CH_4 and 0.3% CO. Following a number of normal tidal breaths, subjects were instructed to make a full exhalation at a moderate flow rate. This was followed by a

complete inhalation and holding of breath for approximately 10 seconds. The subjects were then instructed to exhale completely. The average of two trials was recorded.

Exercise Test

An incremental symptom-limited peak exercise test was performed on an electronically-braked cycle ergometer (Ergoselect 100, Ergoline GmbH) while breathing through a full face mask. The protocol was designed to ensure that subjects reached volitional exhaustion within 8–12 minutes following 3 minutes of rest and 3 minutes unloading cycling. The work rate increment ranged from 5-25 watts·min^{-1} depending on disease severity and fitness level. Respiratory metabolic measures, HR and SpO_2 were continuously recorded throughout the test. Peak oxygen uptake was determined by averaging the two highest consecutive 15 second values [177]. The normal values for peak $\dot{V}O_2$ in men and women were calculated based on the equation of Hansen et al., [178]. The equation for men and women was W*[50.75-0.372(A)], and (W+43)*[22.78-0.179(A)] respectively, where W represents the weight in kilograms, and A represents the age in years.

The $\dot{V}O_2$/WR was calculated based on the equation of Hansen et al., [179]; $\Delta\dot{V}O_2/\Delta WR$ = (peak $\dot{V}O_2$ - unloading $\dot{V}O_2$)/[(T – 0.75)*S, where $\dot{V}O_2$ is measured in ml per minute, T is the time of the incremental exercise, S is the slope of WR increments in watts·min^{-1}, and 0.75 is the time delay before $\dot{V}O_2$ begins to increase in a linear fashion. Predicted O_2 pulse was calculated based on the equation of predicted peak $\dot{V}O_2$/predicted peak HR [179].

The predicted maximal voluntary ventilation (MVV) was calculated as FEV_1*40 [180]. Minute ventilation was expressed relative to $\dot{V}O_2$ and $\dot{V}CO_2$ as ventilatory equivalents ($\dot{V}_E/\dot{V}CO_2$, $\dot{V}_E/\dot{V}O2$). The $\dot{V}E/\dot{V}CO_2$ was calculated at the AT or at the lowest value attained when the AT was not achieved. The V_D volume was calculated based on the equation ($\dot{V}_E$- $\dot{V}_A$)*RR, where $\dot{V}_E$ represents minute ventilation, $\dot{V}_A$ represents alveolar ventilation, and RR represents respiratory rate [177].

Open Circuit Spirometry

Breath by breath expired O_2, carbon oxide (CO_2), ventilatory volume and respiratory exchange ratios (RER) were determined using open circuit spirometry (Innocor, Innovision, Denmark). Airflow was directed by a pneumotach using a differential pressure transducer (Innocor, Innovision, Denmark). The CO_2 and O_2 analysers (Oxigraph Inc, USA) were integrated within the Innocor metabolic system. Gases were sampled at a rate of 120 $ml \cdot min^{-1}$ and analysed by photoacoustic spectroscopy. The response time of the CO_2 analyser was synchronised with the O_2 analyser. The Innocor system was calibrated prior to each test. This involved a gas delay determination that assessed the specific breathing pattern of each subject and an O_2 adjustment to the ambient air. The system was calibrated to the manufacturer's procedures using a 3 L syringe (Series 5530, Hans Rudolph Inc., Germany).

Heart rate and SpO_2% were recorded using a 12 lead ECG (Case 8000, Marquette GE, USA) and pulse oxymetry (Nonin 8500, Nonin Medical, INC, NH, USA) respectively. Blood samples were taken from the earlobe every minute during exercise and used to analyse blood lactate concentration (AccuCheck Softclix Pro Lancet, Accu

Check, Australia). The earlobe was sterilized with a sterile wipe and then pricked with a lancet (AccuCheck Softclix Pro Lancet, Accu Check, Australia) to promote blood flow.

Determination of Anaerobic Threshold

The V-slope method described by Beaver et al. (1986) was used for the determination of anaerobic threshold (AT) [181]. This method involves the analysis of CO_2 elimination ($\dot{V}CO_2$) relative to O_2 uptake ($\dot{V}O_2$). By plotting $\dot{V}CO_2$ against $\dot{V}O_2$, the initial slope of 1.0 is followed by a steeper slope when lactic acid is buffered by bicarbonate, and CO_2 is formed. Two regression lines were plotted; one using the upper data points and the other using the lower data points. The intersection of these two regression lines was used to identify the anaerobic threshold. The AT was expressed as $\%VO_2$ peak predicted value. Figure 3.1 illustrates the V-slope method of determining the AT in a single subject.

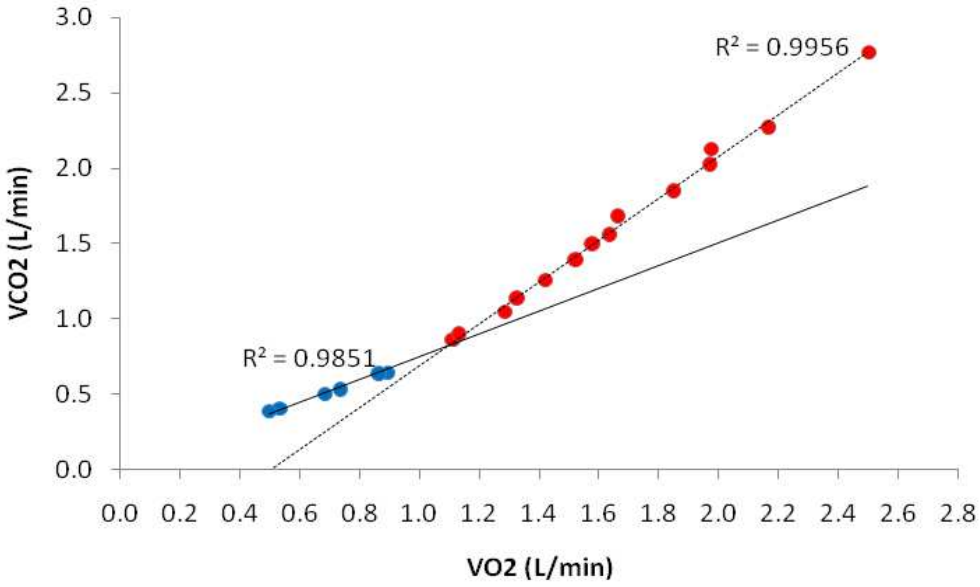

Figure 3.1: Example of the V slope method to determine AT

Determination of the Lactate Threshold

Lactate threshold (LT) was identified as the exercise intensity corresponding to the first significant rise or non-linear increase in blood lactate levels above resting values. A two-phase linear regression model was used to determine the LT. The logarithm (log_{10}) of blood lactate was plotted against the logarithm (log_{10}) of $\dot{V}O_2$ [182]. The lactate threshold was identified as the $\dot{V}O_2$ value corresponding to the intersection of the two least squares regression lines fitted to the data points before and after the onset of a rapid increase in lactate concentration. Figure 3.2 illustrates the method of determining the LT in a single individual.

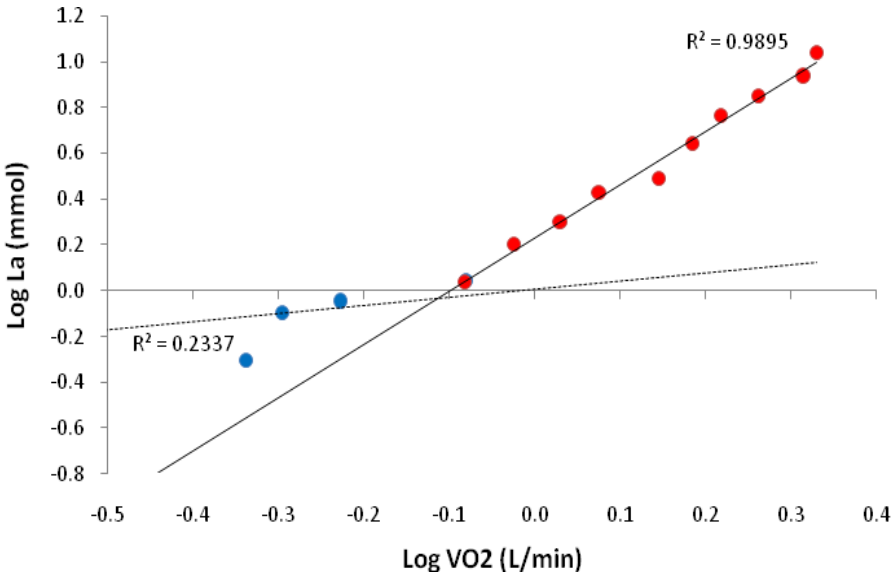

Figure 3.2: Example of log lactate (Log La) method to determine LT

Rating of Perceived Exertion

Overall ratings of perceived exertion were obtained every two minutes using the 15-point Borg category RPE scale. Perceived leg fatigue and dyspnea scores were obtained every two minutes using the Borg CR-10 category RPE scale. Prior to each test a standard set of instructions was read to each subject.

Statistical Analysis

Values are reported as mean ± SD. Independent t-tests or Mann-Whitney U tests were used to compare the physiological responses at peak exercise between the combined CF patients and healthy controls. A one way ANOVA or the Kruskall-Wallis test was used to compare the peak exercise response in patients of varying severity of CF (mild, moderate and severe) and the healthy control group. Post hoc comparisons were made using a Tukey HSD with adjustments for multiple comparisons. P-value of <0.05 was accepted as statistically significant. Data were analysed using SPSS (v17.0, SPSS Inc., IL).

Results

Combined CF vs. Healthy Controls

Anthropometric and Spirometry

Anthropometric and spirometry data are shown in Table 3.1. There was no difference in lean body mass between the two groups. CF patients had a lower FEV_1 %predicted ($p < 0.001$), and FVC % predicted ($p < 0.001$) than healthy controls. A total of 16, 9 and 6 patients had pancreatic insufficiency in the mild, moderate and severe groups, respectively. Of those, 3 patients had diabetes mellitus in the mild group, 2 in the moderate group and 2 in the severe group. Only 1 patient (in the severe group) had osteoporosis. No patient was being treated with oral corticosteroids.

Table 3.1: Subject characteristics

	Experimental Groups	
	Healthy Controls	**Cystic Fibrosis**
Subjects (n)	34 (f=13)	33 (f=9)
Age (yr)	22.2±3.2	24.5±6.2
Weight (kg)	71.6±9.9	65.4±11.6†
Height (cm)	174.6±9.4	172.2±8.0
LBM (kg)	59.0±9.5	55.0±9.0
FEV_1 (L)	4.3±0.7	2.6±1.1 ‡
FEV_1 (% predicted)	106.6±13.8	66.0±25.6 ‡
FVC (L)	5.1±1.0	3.9±1.3 ‡
FVC (% predicted)	108.2±13.2	85.9±22.3 ‡
FEV_1/FVC	84.7±6.7	63.5±13.1 ‡
D_LCO ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	34.5±5.7	30.1±7.6
D_LCO (% predicted)	96.4±9.8	91.1±18.4

Values are mean ± SD; †$p < 0.05$ vs. healthy controls; ‡$p < 0.001$ vs. healthy controls

Peak Exercise Performance

Peak exercise performance values for the CF and control group are shown in Table 3.2. The CF patients had a lower peak $\dot{V}O_2$/LBM ($p<0.001$), and peak lactate ($p<0.001$) than healthy controls. Peak $\dot{V}_E$, V_T, SpO_2%, and O_2 pulse were lower ($p<0.001$) in CF patients than the control group. The AT% based on predicted peak VO_2 was lower ($p<0.001$) in CF patients than the control group.

Table 3.2: Physiological responses at peak exercise in CF and healthy controls

	Experimental Group	
	Healthy Controls	**Cystic Fibrosis**
$\dot{V}O_2$ peak (ml·kg^{-1}LBM min^{-1})	52.2±8.9	35.5±9.5‡
$\dot{V}O_2$ peak %predicted	109.3±16	72.5±18.9‡
Workrate (watts)	251.5±55.3	143.2±62.4‡
Lactate (mmol/L)	10.3±1.7	7.0±2.8‡
AT % Predicted peak VO_2	50.3±8.7	36.1±9.4‡
O_2 Pulse (ml/b)	17.0±4.8	11.8±3.7‡
O_2 Pulse %predicted	108±19	82±18.7‡
$\Delta\dot{V}O_2/\Delta WR$ (ml·min^{-1}·w^{-1})	12.2±2	10.3±2‡
RER	1.2±0.9	1.14±0.1
$\dot{V}_E$ (L·min^{-1})	111.0±29.1	80.1±28.1‡
RR (f·min^{-1})	44.2±8.5	44.8±7.8
$\dot{V}_E$/MVV (%)	64.6±16.6	82.1±18.0‡
V_T, (L)	2.6±0.7	1.8±0.7‡
V_T max/VC	50.0±9.8	45.7±9.3
V_D/V_T	0.15±0.04	0.2±0.06‡
SpO_2 %	97.6±1.0	93.9±4.9‡
$\dot{V}_E/\dot{V}CO_2$ @AT	24±2	30±4‡
P_ETCO_2 (mmHg)	39.6±5.3	39.4±4.8

Values are mean ± SD; ‡p <0.001 vs. healthy controls

Relation between Variables

Peak $\dot{V}_E$/MVV% was inversely related to FEV_1%predicted (r=-0.65; P<0.001) as shown in Figure 3.3. Table 3.3 summarizes the correlations between selected parameters and $\dot{V}O_2$/LBM in CF patients at peak exercise. Peak $\dot{V}O_2$/LBM was related to $V_T$max/VC (r=0.58; p<0.01) (Figure 3.4), and inversely related to $\dot{V}_E/\dot{V}CO_2$ at AT (r=-0.44; p<0.05) (Figure 3.5), and V_D/V_T (r=-0.52; p<0.01) (Figure 3.6). There was no relation between peak $\dot{V}O_2$/LBM and P_ETCO_2. (Figure 3.7). There was positive relation between O_2 pulse %predicted to FEV_1 % predicted (r=0.52; P<0.01) (Figure 3.8).

Table 3.3: Correlation between selected parameters during exercise in CF patients

	$\dot{V}O_2$ (ml kg^{-1} LBM min^{-1})	Lactate (mmol L^{-1})
$\dot{V}_E/\dot{V}CO_2$ @AT	-0.44*	0.81**
V_D/V_T	-0.52**	0.72**
V_T max/VC	0.58**	0.73**
P_ETCO_2 (mmHg)	-0.01	-0.71**

* p <0.05; ** p <0.01

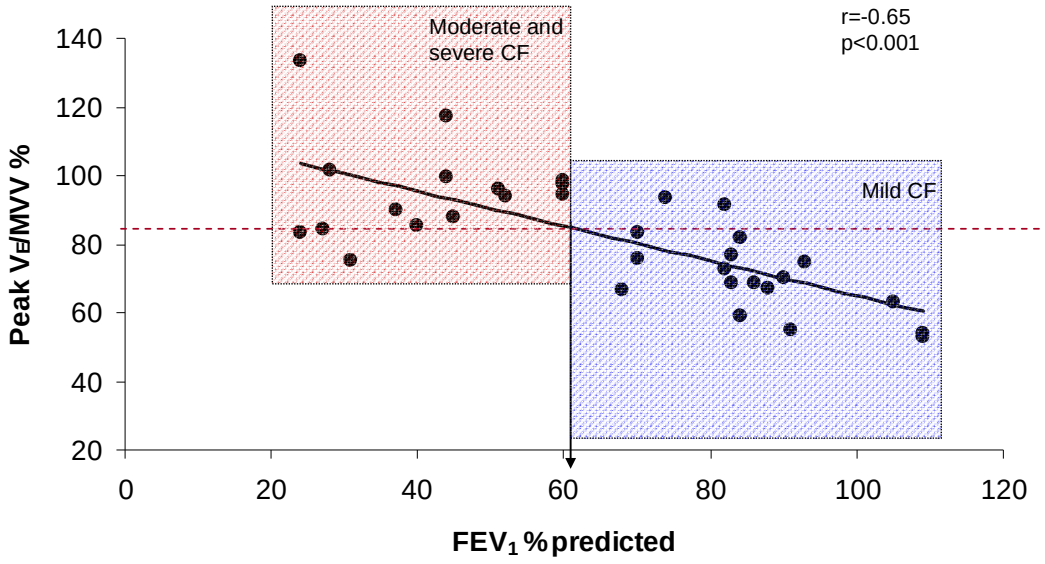

Figure 3.3: Relation between peak $\dot{V}_E$/MVV% and FEV1%predicted in CF patients. The dashed line indicates the $\dot{V}_E$/MVV at 85%

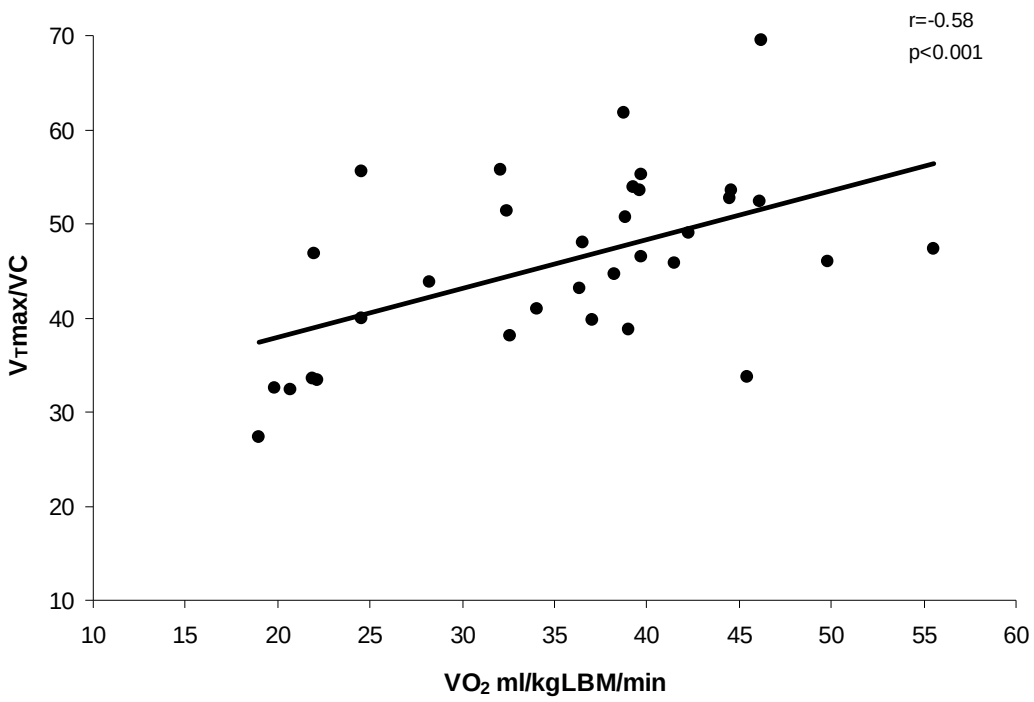

Figure 3.4: Relation between peak $\dot{V}O_2$ ml kg^{-1} LBM min^{-1} and peak V_T max/VC in CF patients

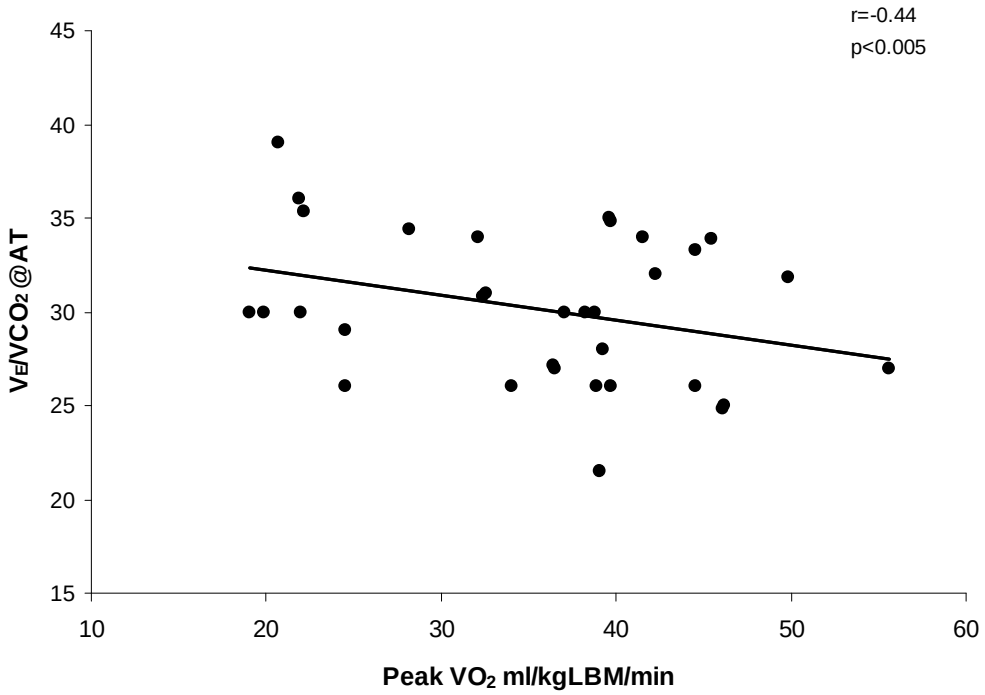

Figure 3.5: Relation between peak $\dot{V}O_2$ ml kg^{-1} LBM min^{-1} and $\dot{V}_E/\dot{V}CO_2$ at the AT in CF patients

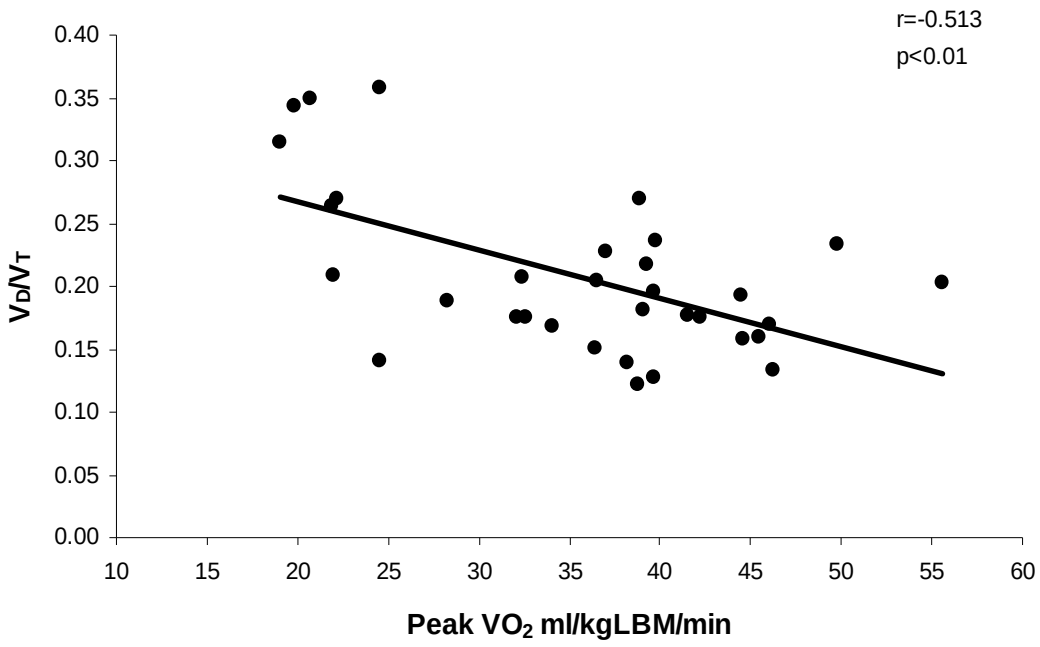

Figure 3.6: Relation between peak $\dot{V}O_2$ ml kg^{-1} LBM min^{-1} and V_D/V_T in CF patients

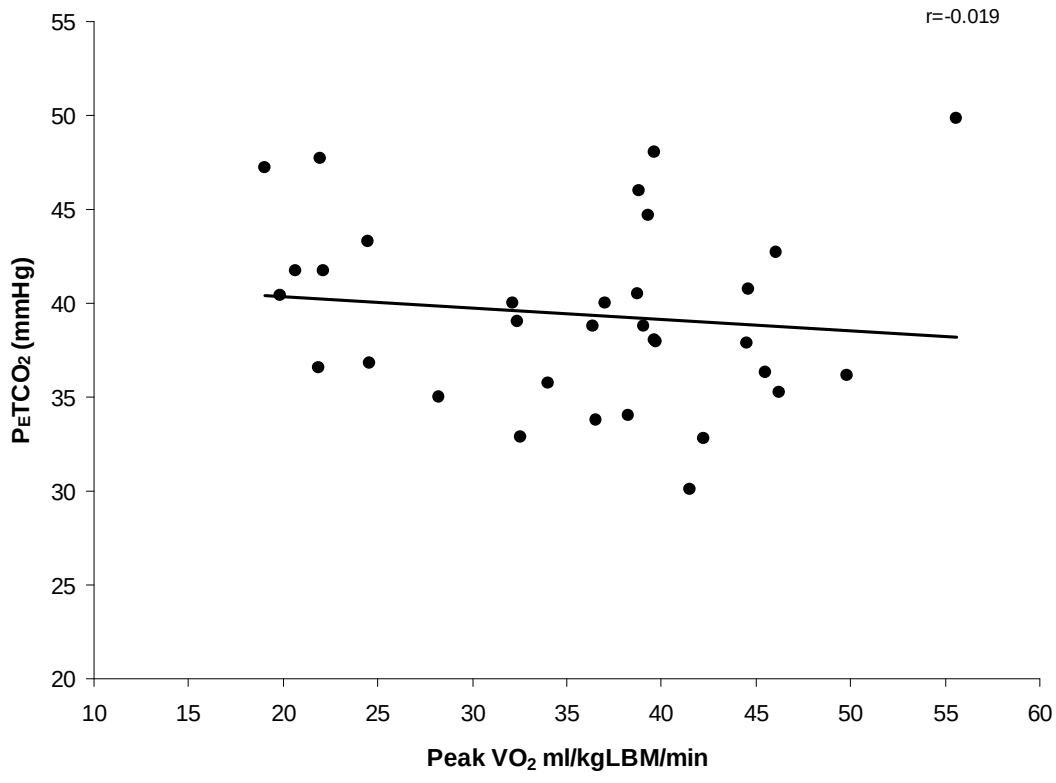

Figure 3.7: Relation between peak $\dot{V}O_2$ ml kg^{-1} LBM min^{-1} and P_ETCO_2 in CF patients

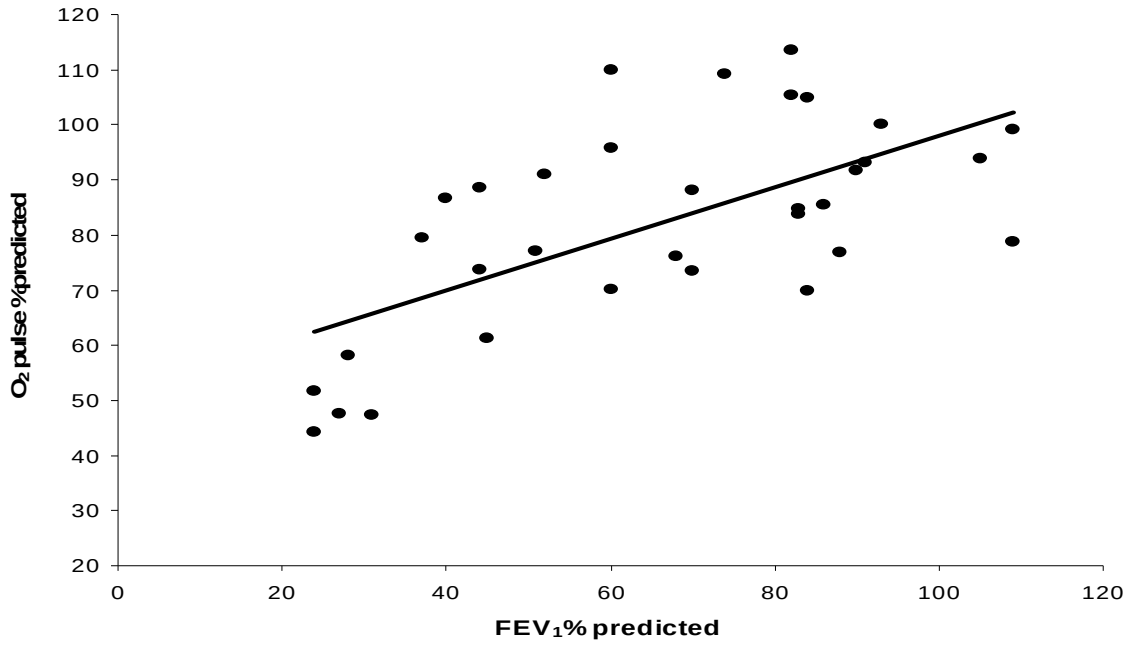

Figure 3.8: Relation between peak O_2 pulse %predicted and FEV_1 %predicted in CF patients

Comparisons between Different CF Severities and the Control Group

Anthropometrics and Spirometry

Anthropometric and lung function data are shown in Table 3.4. Body weight was lower ($p<0.05$) in individuals with severe CF disease than in healthy controls. Spirometry values were higher ($p<0.05$) in the healthy controls than each of the CF groups. Spirometry values were significantly different ($p<0.05$) between each of the CF groups with mild CF > moderate CF > severe CF. D_LCO was reduced ($p<0.05$) in severe CF compared to mild and moderate CF and healthy controls.

Table 3.4: Characteristics of healthy subjects and patients with different CF severities

	Experimental Group			
	Healthy Controls	Cystic Fibrosis		
		Mild	Moderate	Severe
Subjects (n)	33 (f=9)	18 (f=5)	9 (f=3)	6 (f=1)
Age (yr)	22.2±3.2	24.9±6.4	24.8±6.4	24±6.2
Weight (kg)	71.6±9.9	68.9±10.6	64±11.2	57.6±13.0†
Height (cm)	174.6±9.4	174.0±7.8	171.7±6.1	173.4±8.7
LBM (kg)	59.0±9.5	58.2±8.2	52.4±8.3	48.7±9.0
FEV_1(L)	4.3±0.7	3.4±0.8†	2.0±0.5†*	1.0±0.2‡
FEV_1%	106.6±13.8	86.2±12.2†	50.6±7.8†*	28.5±4.9‡
FVC (L)	5.1±1	4.8±1.0†	3.4±0.8†*	2.3±0.5‡
FVC (% predicted)	108.2±13.2	103.0±11.1†	73.5±9.2†*	53.3±6.3‡
FEV_1/FVC	84.7±6.7	71±9†	59.3±8.4†*	47.5±12.9‡
D_LCO ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	34.5±5.7	30.5±4.4	33.3±10	21.6±6.8‡
D_LCO (% predicted)	96.4±9.8	92.5±12.5	99.7±20.3	66±14.5‡

Values are mean ± SD; ‡ p <0.05 different from all the other groups; † p <0.05 vs. healthy;* vs. mild <0.05; f = female

Peak Exercise Performance

Peak exercise performance values are shown in Table 3.5. Severe CF patients had a lower $\dot{V}O_2$ peak/LBM ($p<0.001$) and peak WR ($p<0.001$) than the other groups. There was no difference in peak WR or $\dot{V}O_2$peak/LBM (Figure 3.9) between the mild and moderate CF groups. Peak lactate was significantly different between all groups (Table 3.5).

The AT expressed as %predicted peak $\dot{V}O_2$ was significantly lower in all CF groups than the healthy controls, and was below 40% in all CF groups. O_2pulse was significantly lower in all CF groups than the healthy controls. The pattern of change in

O_2 pulse during the increment test in the severe group was abnormal, reaching a plateau early in the exercise bout despite increasing WR. Although the $\Delta\dot{V}O_2/\Delta WR$ was significantly lower in the severe CF group than the healthy controls, it was still within the normal range. The $\dot{V}_E/\dot{V}CO_2$ @AT was significantly higher in all CF groups than the healthy controls. However there was no difference in P_ETCO_2 between any of the groups during peak exercise. $\dot{V}_E$/MVV% was significantly higher in the moderate and severe CF groups than in the mild CF and healthy controls. Heart rate reserve (HRR) was significantly higher in the severe group than all other groups.

During peak exercise, the severe CF patients had a significantly lower V_T, V_T/VC ratio, higher RR/V_T ratio, and V_D/V_T and a reduced peak SpO_2% than the other CF groups and healthy controls. Compared to the mild CF and healthy control groups, the moderate group had a significantly higher V_D/V_T and SpO_2%. However, the moderate CF patients achieved a normal V_T/VC ratio and a similar peak V_T to the mild CF group (Figure 3.10).

Table 3.5: Physiological responses at peak exercise in different CF severities and healthy controls

	Experimental Group			
	Healthy	Cystic Fibrosis		
	Controls	Mild	Moderate	Severe
$\dot{V}O_2$ peak (ml·kgLBM^{-1}·min^{-1})	52.2±8.9	39.1±6.7 †	37.8±8.7†	21.6±1.9‡
$\dot{V}O_2$ peak (% predicted)	109.3±16	82.2±10.5	74.7±12.8	40.2±8.3
Work rate (watts)	251.5±55.3	172.1±39.3	146.5±63†	51.6±16.3‡
Lactate (mmol·L^{-1})	10.3±1.7	8.8 ±1.7 †	6.5±1.9†*	2.7±0.9‡
AT-Predicted $\dot{V}O_2$ peak (%)	50.3±8.7	37.9±9.9 †	36.3±7.0†	#24.7±3.7‡
$\dot{V}CO_2$ (L/min)	3.6±1	2.7±0.6	2.3±0.9	1±0.2
O_2 Pulse (ml·beat^{-1})	17±4.8	13.6±3.2†	10.8±2.8†	7.3±1.4†*
O_2 Pulse (%predicted)	108±32	90±13†	84±15†	55±13†*
$\Delta\dot{V}O_2/\Delta WR$ (ml·min^{-1}·w^{-1})	12.2±2.2	10.4±1.8	10.9±2.8	9.1±1.5†
RER	1.18±0.93	1.19±0.09	1.14±0.87	0.96±0.11‡
HRR	13.5±9.5	22.8±12.3	24.5±18.8	54.8±13.5 ‡
$\dot{V}_E$ (L/min)	111.0±29.1	95.2±19.2	78±22.6†	38.3±9‡
RR (f/min)	44.2±8.6	41.9±5.4	48.2±7.6	48.2±11.4
SpO_2 %	97.6±1.0	97±1.6	92.3±4.6†*	87±3.9‡
$\dot{V}_E$/MVV (%)	64.6±16.6	70.8±11.8	96.6±9†*	94.6±20.9‡
V_T, (L)	2.6±0.7	2.3±0.5	1.7±0.5†	0.8±0.17‡
$V_T$max/VC	50.0±9.8	48.1±8.7	48.6±7.5	35.3±6.8‡
V_D/V_T	0.15±0.04	0.16±0.02	0.22±0.03†*	0.3±0.52‡
$\dot{V}_E/\dot{V}CO_2$ @AT	23.9±2.1	29±3.7†	31±4†	33±4†
P_ETCO_2 (mmHg)	39.6±5.3	37.3±4.1	40.7±5.1	43.6±3

Values are mean ± SD; ‡ p <0.05 different from all the other groups; †p <0.05 vs. healthy controls; *<0.05 vs. mild CF; # only 3 patients achieved the AT

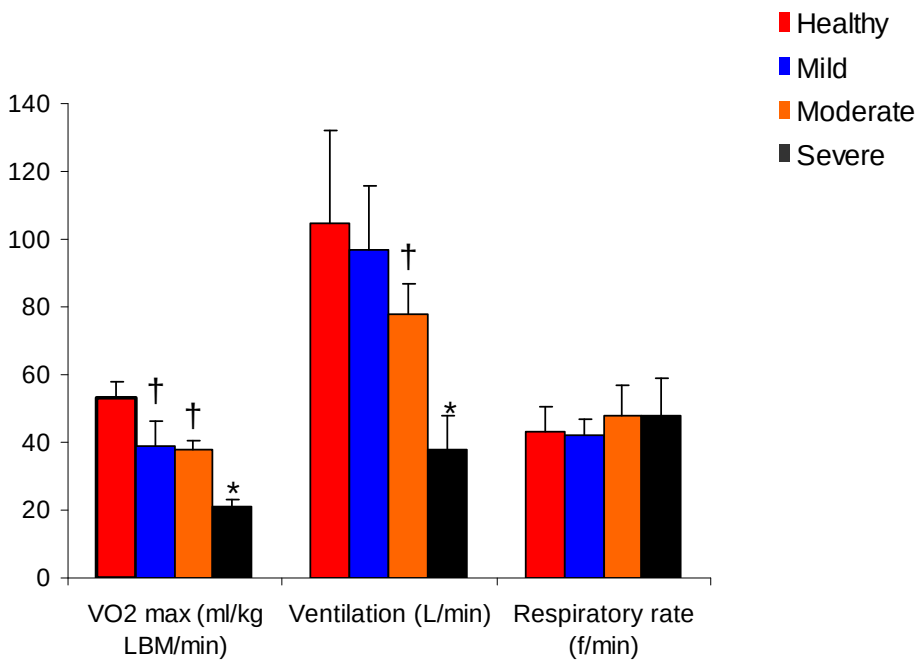

Figure 3.9: $\dot{V}O_2$/LBM, $\dot{V}_E$ and RR at peak exercise in patients with different CF severity and healthy controls
* p <0.05 different from all the other groups; † p <0.05 vs. healthy controls

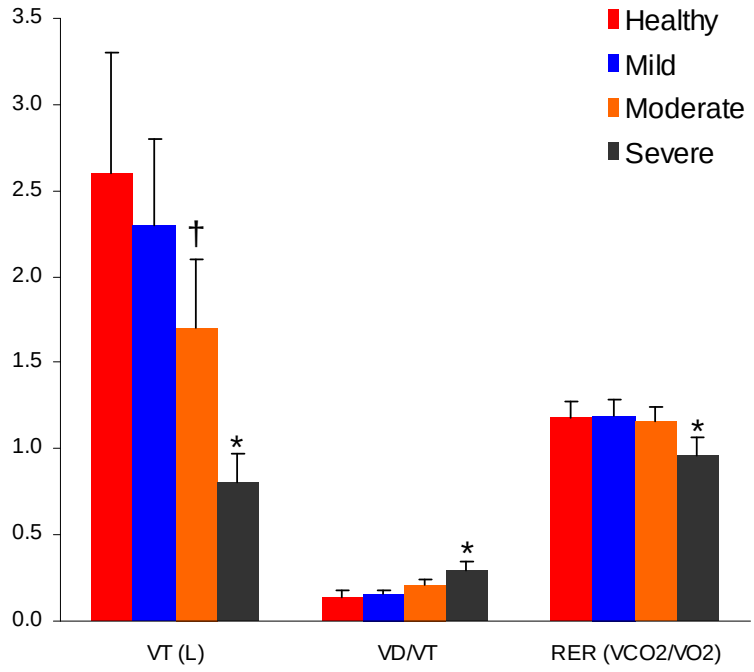

Figure 3.10: V_T, V_D/V_T and RER at peak exercise in patients with different CF severity and healthy controls
* p <0.05 different from all the other groups; †p <0.05 vs healthy controls

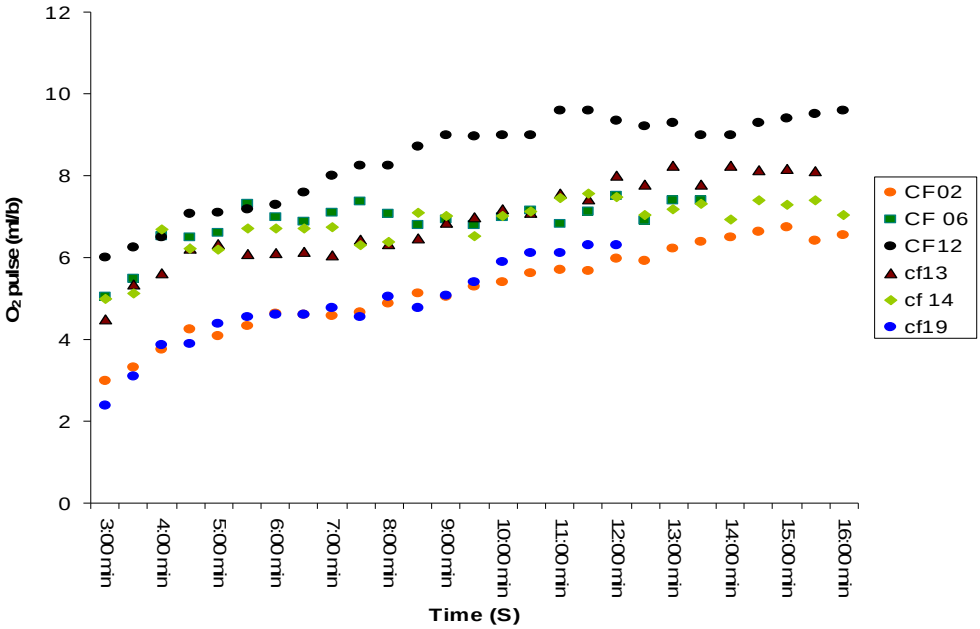

Figure 3.11: Individual changes in O_2 pulse during the incremental exercise test in patients with severe CF*

*All of the patients reached a plateau early during the exercise.

Discussion

The present study examined the effect of pulmonary mechanisms, arterial hypoxemia and cardiovascular factors on maximal exercise capacity in adults with mild, moderate and severe CF compared to healthy controls. The principle findings were; i) ventilatory mechanical limitation, arterial hypoxemia, and cardiovascular factors each contributed to the reduced peak exercise capacity in severe CF patients with FEV_1 <30% predicted, ii) ventilatory limitation was a major contributor to the reduced peak exercise capacity in patients with moderate CF. In addition, deconditioning and a slight reduction in arterial saturation also contribute to an increased ventilatory demand and exercise intolerance in this group and iii) mild CF patients have a reduced peak exercise capacity that seems partly related to deconditioning.

CF is a disease characterized by dysfunction of the small airways [73-74]. As the disease becomes more advanced, a decline in FEV_1 is associated with greater inflammatory processes of the bronchial wall and destruction of the parenchyma. This results in an increase in expiratory flow limitation (EFL) and a reduction in lung compliance during exercise [76]. The capacity to reduce EELV below the FRC is diminished in moderate to severe CF during exercise due to EFL [5]. As a consequence, V_T is positioned close to the TLC on the steep portion of the pressure volume loop. A higher pressure is required to increase V_T at any given volume expansion [59, 183].

Ringis et al., [7] found a lower $\dot{V}O_2$ peak in CF patients with FEV_1 <60% predicted than in healthy controls. There was an increase in EELV during the incremental exercise test resulting in a reduction in V_T and an elevation in the RR.

Normally, the maximum V_T is reached at approximately 55% of VC, allowing optimization of the diaphragmatic and inspiratory intercostal muscle length at the onset of inspiration (61). In the present study, reduced V_T/VC at peak exercise was observed in severe CF patients suggesting a relatively rapid shallow breathing pattern that may increase the overload on the inspiratory muscles. A reduced V_T/VC may reflect the mechanical constraint on V_T expansion during exercise, causing dyspnea and fatigue at a relatively low exercise intensity [5, 7].

An increase in V_D/V_T is one of the major factors responsible for increased ventilatory demands during exercise in patients with lung disease [184]. V_D/V_T is an index of gas exchange efficiency. An increase in V_D/V_T reflects an increased inefficiency of ventilation caused by $\dot{V}_A$/Q mismatching. V_D/V_T is also highly dependent on the breathing pattern. Rapid shallow breathing increases V_D/V_T even without abnormal $\dot{V}_A$/Q [184].

Patients with moderate to severe CF have been shown to have an elevated V_D/V_T during peak exercise due to a low V_T and an increased RR [91]. An elevated V_D/V_T may result in a greater proportion of each breath moving in and out of the airway DS causing arterial hypoxemia. In the present study, patients with severe CF had an abnormal increase in V_D/V_T than the mild and moderate CF groups. The combination of an increase in V_T/VC, and V_D/V_T and arterial hypoxemia at peak exercise may suggest that both a ventilatory mechanical limitation and abnormal gas exchange may contribute to exercise limitation in severe CF patients.

The $\dot{V}_E/\dot{V}CO_2$ at the AT can provide additional information on ventilatory efficiency during exercise. The $\dot{V}_E/\dot{V}CO_2$ was elevated at the AT in all CF severities. However, in patients with severe CF, the elevated $\dot{V}_E/\dot{V}CO_2$ was accompanied by an elevated P_ETCO_2. The abnormally elevated $\dot{V}_E/\dot{V}CO_2$ in the presence of a high P_ETCO_2 decreases the likelihood of a hyperventilation-induced increase in $\dot{V}_E/VCO_2$ and may indicate i) poor ventilatory distribution, ii) areas of the lung with high $\dot{V}_A/Q$, or iii) an increase in DS. These factors may contribute to an increased ventilatory demand at any given exercise capacity and may affect exercise performance in patients with severe CF.

Cardiovascular factors may also affect exercise performance in CF patients. Pulmonary vascular destruction with reduction in the area for gas exchange or a decrease in compliance leads to increased vascular resistance and hypertension in severe CF patients [185]. Endothelial dysfunction in the pulmonary arteries is common in end stage lung disease and may be related to abnormal CFTR function in the pulmonary arteries [186]. An impaired vasodilatory response has been found in pulmonary vessels during exercise, even in patients with mild to moderate CF disease [172].

O_2 pulse is a commonly used non-invasive method to examine the cardiovascular responses during exercise. The peak O_2 pulse was at the lower range of the normal predicted value in the mild and moderate CF groups. There was a reduction in peak O_2 pulse in the severe CF. In addition, the pattern of change in O_2 pulse was abnormal in severe CF, reaching a plateau during early exercise despite an

increasing WR. A low, unchanging O_2 pulse with increasing WR indicates a reduction in maximal SV. There is evidence that the slope of the VO_2-HR relation rather than the maximal O_2 pulse may provide a more reliable estimation of the cardiovascular response during exercise in healthy individuals [187] . Several factors may affect O_2 pulse during exercise. These include heart disease, deconditioning, ventilatory limitation, and possibly even the hemodynamic consequence of dynamic hyperinflation. However, factors that affect the arterial-mixed venous oxygen content including abnormal O_2 utilization, desaturation, and anaemia may also impact O_2 pulse.

An abnormal O_2 pulse pattern in severe CF can be considered an indication of impaired pulmonary hemodynamics. Defective compliance of the pulmonary vascular beds may reduce the O_2 pulse, indicating a reduction in SV during exercise. Several studies have demonstrated resting right ventricular dysfunction in severe CF [188-189]. In severe COPD, right ventricular afterload during exercise is increased because of the increased pulmonary vascular resistance associated with breathing at lung volumes close to total lung capacity. Although left ventricular dysfunction is not typically found in mild to moderate CF, SV during exercise has been shown to be reduced in adults with CF who have moderate to severe lung disease (FEV_1<55% predicted) [102].

The $\Delta\dot{V}O_2/\Delta WR$ may provide additional information on the cardiovascular response during exercise. Clinically, an abnormal slope of the $\Delta\dot{V}O_2/\Delta WR$ relationship is most often due to O_2 transport dysfunction. However, less commonly, O_2 utilization dysfunction may also be associated with an abnormal slope [190]. Indeed, muscle-related abnormality in O_2 metabolism has been found in children with mild to

moderate CF [10]. Although the slope of the $\Delta\dot{V}O_2/\Delta WR$ was reduced in severe CF compared to the healthy controls, but was still in normal value range above 8.3 $ml \cdot min^{-1} \cdot watt^{-1}$ [179]. An abnormal pattern of O_2 pulse combined with a reduced $\Delta\dot{V}O_2/\Delta WR$ may indicate abnormal cardiovascular response related to abnormal pulmonary hemodynamics in patients with severe CF.

Moorcroft et al., [115] found that severe CF patients with an FEV_1 >30% predicted were able to exercise above the LT and achieve a peak lactate value of 5.7 $mmol \cdot L^{-1}$. In the current study, patients in the severe CF group had an FEV_1 <30% predicted and a peak lactate value of 2.7 $mmol \cdot L^{-1}$. Differences in the severity of airway obstruction, ventilatory adaptation to exercise, and cardiovascular responses may help to explain the variation in peak blood lactate between the studies. An FEV_1 <30% predicted may indicate a clinical threshold at which exercise capacity is reduced due to a combination of mechanical ventilatory limitation, abnormal gas exchange, and cardiovascular factors. Indeed, an FEV_1 <30% predicted currently serves as a primary criteria for lung transplantation in CF patients [56].

In the present study, the breathing pattern was similar in patients with mild and moderate CF severities. The moderate CF patients had a $\dot{V}_E/MVV$ value approaching 100, suggesting ventilatory limitation to exercise. However, despite the appearance of a ventilatory limitation, peak exercise capacity was similar to the mild CF group. The ability of the moderate CF group to increase V_T/VC ratio during exercise may help to explain the similar peak exercise capacity in the moderate and mild CF groups. Compared to the control group, patients with moderate CF had a higher

$\dot{V}_E/\dot{V}CO_2$ slope accompanied by a higher V_D/V_T, with no change in peak E_TCO_2, and slight arterial desaturation during peak exercise. Early onset of metabolic acidosis (lower AT), usually reflects deconditioning due to physical inactivity, may also contribute to excessive ventilatory demand [59] causing a reduced breathing reserve during peak exercise.

Alterations in muscle performance may also have contributed to exercise intolerance in CF patients [36, 84, 106-113], and may be due in part to reduced physical activity levels [107]. Muscle dysfunction due to inactivity in CF may include muscle atrophy, muscle weakness [110], decreased number of mitochondria [8, 10, 110-111], and an increased reliance on anaerobic glycolysis [8, 111]. In the present study, the mild CF group had a reduced peak $\dot{V}O_2$, reduced AT, slightly elevated $\dot{V}_E/\dot{V}CO_2$ accompanied by hyperventilation. There was no evidence of pulmonary limitation in the mild CF group during peak exercise. Therefore, it seems that individuals with mild CF have a reduced peak exercise capacity related to deconditioning.

Some studies involving CF have found evidence of an intrinsic abnormality in muscle function independent of deconditioning [8, 10, 109-110]. Compared to healthy individuals, CF athletes with good nutritional status and a preserved pulmonary function have lower anaerobic power and maximal leg strength, and a reduced mitochondrial oxidative metabolism [8]. Abnormal expression of CFTR in skeletal muscle may be responsible for the reduction in oxidative and anaerobic metabolism due in part to ATPase hydrolysis by CFTR proteins [116]. The current study was not

designed to investigate the mechanism responsible for the alterations in muscle performance and further research is required to address this issue.

Limitations of the Study

- MVV was not directly measured, but was estimated by multiplying FEV_1 by 40. It is well established that MVVs estimated using this formula is similar to directly measured values in patients with COPD [180, 191].
- Dynamic hyperinflation was not directly measured during the exercise test. The V_T/VC ratio was used to indicate the ventilatory constraints on V_T expansion during exercise.
- The accuracy and reliability of the non-invasive estimation of SV using O_2 pulse may be limited in patients with severe arterial hypoxemia [187, 192]. Without directly measuring the arteriovenous O_2 content, changes in O_2 pulse as an indicator abnormal SV response may be underestimated.
- The V_D/V_T calculated from expired gas measurements, such as P_ETCO_2, may be underestimated.

Summary

Peak exercise capacity was lower in each of the CF groups compared to healthy controls. The lower exercise capacity in CF patients with an FEV_1 <30% can be partly explained by i) an inability to increase V_T, ii) abnormal gas exchange, and iii) impaired cardiac response (decrease in O_2 pulse). Ventilatory limitation, abnormal gas exchange, and deconditioning were all associated with the lower exercise capacity in

moderate CF. Deconditioning was the primary exercise limitation in patients with mild CF.

Exercising training is an intervention that can be used to break the vicious cycle of skeletal muscle deconditioning, progressive dyspnea, and improve symptoms and activity levels in CF patients. Exercise training can reduce ventilatory demand by decreasing the respiratory rate and improving aerobic capacity. The provision of supplemental oxygen may potentiate the effect of exercise in patients with CF. Supplemental O_2 may reduce DH and improves exercise tolerance in flow-limited patients. The reduction in submaximal $\dot{V}_E$ by reducing the breathing frequency may delay the rate of DH and the onset of critical ventilatory constraints that limit exercise. Supplemental O_2 may also increase SV and exercise tolerance by reducing pulmonary vascular resistance

The results of the present study indicate that increasing V_T in patients with moderate to severe CF, will significantly improve exercise performance. Interventions are required to increases V_T during exercise while at the same time delaying respiratory muscle fatigue.

Chapter 4

Study 2

Skeletal Muscle Function, Blood Lactate and O_2 Uptake Kinetics in Patients with Mild, and Moderate to Severe Cystic Fibrosis

Introduction

Exercise intolerance is well documented in patients with CF. In addition to a ventilatory limitation and/or decreased gas exchange, alterations in muscle function due in part to reduced activity levels or immobility [107, 110, 193] may also contribute to reduced exercise capacity [36, 84, 106-113]. Muscle dysfunction can result in muscle weakness [110], a decreased number of mitochondria [8, 10, 110-111], and increased reliance on anaerobic glycolysis [8, 111].

Muscle weakness is common in CF. The skeletal muscle weakness may be related to peripheral muscle atrophy indicating that muscle disuse is a major cause of muscle weakness [107]. Troosters et al., [109] investigated the relation between physical inactivity, muscle strength and exercise tolerance in adults with mild to moderate CF with an FEV_1 of 64%. Physical inactivity was related to exercise intolerance and skeletal muscle weakness. The impairment in muscle strength and exercise capacity was in excess of that expected due to inactivity. However, in CF patients with an FEV_1 of 94%, peripheral muscle strength was found to be normal [9].

Intrinsic abnormality in muscle function independent of deconditioning has also been found in CF patients [8, 10]. Compared to healthy individuals, CF athletes with good nutritional status and a preserved pulmonary function have lower anaerobic

power and maximal leg strength, and a reduced mitochondrial oxidative metabolism [8]. Abnormal expression of the cystic fibrosis transmembrane conductance regulator (CFTR) in CF skeletal muscle may also play a role in the reduction in mitochondrial oxidative metabolism [116]. Other factors that may contribute to peripheral muscle weakness and alterations in muscle structure include malnutrition, systemic inflammation [117], corticosteroid use [118] and hypoxia [194].

In addition to muscle strength and peak exercise capacity, measuring O_2 uptake kinetics during moderate and/or heavy exercise can provide additional information on O_2 transport and O_2 utilization in skeletal muscle. The increase in pulmonary $\dot{V}O_2$ at the onset of a constant load exercise can be described by the monoexponential function [132]. Three phases are normally considered. Phase I (time delay (TD)) occurs during the first 15-20 seconds of exercise and reflects the increase in pulmonary blood flow without an increase in arteriovenous O_2 difference [133]. Phase II reflects the arrival at the lungs of the mixed venous blood and lasts from approximately 15-20 seconds after the onset of exercise to the third minute of exercise [133]. Phase III begins approximately 3 minutes after the onset of exercise, and reflects the start of the $\dot{V}O_2$ steady state period when the work rate is below the AT.

At exercise intensities above the AT, $\dot{V}O_2$ steady state is delayed or not achieved prior to fatigue [138]. The O_2 uptake kinetics at an intensity above the AT (heavy exercise) are determined by the magnitude of both the primary component and the $\dot{V}O_2$ slow component amplitude [138]. In healthy individuals, the primary component lasts 2-3 minutes following the onset of exercise and is dependent on the

percentage of type 1 oxidative fibres within the exercising muscle. A higher proportion of type 1 fibres may accelerate the primary component kinetics. The magnitude of the VO_2 slow component has been described as the difference between $\dot{V}O_2$ during 3 minutes and 6 minutes of exercise (ΔVO_2 6-3min) [140] as shown in Figure 2.8.

In order to better understand the factors that may affect exercise performance in patients with CF, the aim of the present study was to compare body composition, peak exercise capacity, muscle strength, and O_2 uptake kinetics in healthy adults and those with CF. Individuals with CF have altered pulmonary function, gas exchange dynamics and cardiovascular responses that may alter O_2 uptake kinetics during exercise. In addition, muscle weakness, due primarily to a reduction in LBM is more prevalent as the disease progresses. It is hypothesised that compared to healthy individuals, i) patients with moderate to severe CF will have prolonged O_2 uptake kinetics, increased blood lactate during constant load exercise and a reduction in muscle strength, and ii) patients with mild CF will have similar O_2 uptake kinetics, blood lactate levels during constant load exercise and muscle strength.

Methods

Subjects

Adults (n=28) with different severities of CF and apparently healthy controls (n=19) were recruited. CF patients were recruited from the respiratory department in Beaumont Hospital and were classified as having mild (FEV_1 >60 % predicted), and moderate to severe (FEV_1 <60 % predicted) CF. CF diagnosis was based on clinical features, sweat test or genotyping and a pulmonary function test. No other diseases that could limit exercise capacity (cardiovascular, orthopaedic and neuromuscular) were present. Patients using long-term oral steroid therapy were excluded. The healthy controls were sedentary students recruited from Dublin City University. The nature and risks of the study were explained. A plain language statement was read and informed consent was obtained in accordance with the Beaumont Hospital Ethics (Medical Research) Committee.

Study Overview

Subjects made 4 separate visits to the Cardiovascular Research Unit in DCU. Each session was separated by at least 3 days, and the evaluation was completed within 2-3 weeks. The first visit was used to assess spirometry, single breath D_LCO, LBM and $\dot{V}O_2$peak. During the second and third visit, CF patients and healthy controls performed a constant work load test at 50% or 70% $\dot{V}O_{2.}$peak, separated by approximately 2-3 days. The healthy controls also performed a 10-minute constant load test corresponding to 50% (50 W) and 70% (100 W) of the $\dot{V}O_2$ peak attained by the CF group. The final visit was used to assess muscle strength.

Preparation

Patients abstained from alcohol and refrained from strenuous physical activity for 24 hours and fasted for 4 hours prior to the study visit.

Anthropometry

Height and body mass were measured to the nearest 0.1 cm and 0.1 kg respectively using the SECA Stadiometer (Seca model 220 GmbH, Hamburg, Germany). Subjects were barefoot and wore light clothing. BMI was determined as weight (kg) divided by height $(m)^2$.

Body Composition

Double thickness subcutaneous adipose tissue was measured on the right side of the body using skinfold calipers (Harpenden, Cambridge Scientific Industries, MD). The following anatomical sites were used: chest, subscapular, mid-axillary, suprailliac, abdomen, triceps and thigh. A minimum of 2 measurements were taken at each site. If the measurements varied by more than 2 mm, a third measurement was taken. Percent body fat was based on the equation proposed by Jackson and Pollack [173].

Pulmonary Function Test

Standard pulmonary function tests, including spirometry, and measurements of D_LCO (Sensormedics Vmax 229, Sensormedics Corp, CA), were undertaken according to previously described guidelines [174-175], and the results were compared to normal values [176].

Exercise Test

An incremental symptom-limited peak exercise test was performed on an electronically-braked cycle ergometer (Ergoselect 100, Ergoline GmbH) while breathing through a full face mask. The protocol was designed to ensure that subjects reached volitional exhaustion within 8-12 minutes following 3 minutes of rest and 3 minutes unloading cycling. The work rate increment ranged from 5-25 watts·min^{-1} depending on disease severity and fitness level. Respiratory metabolic measures, HR and SpO_2% were continuously recorded throughout the test. Peak $\dot{V}O_2$ was determined by averaging the two highest consecutive 15 second values. The peak value for $\dot{V}O_2$ and exercise WR were related to normal values [177]. The predicted MVV was calculated as FEV_1*40 [180]. Ventilation was expressed relative to $\dot{V}O_2$ and $\dot{V}CO_2$ as ventilatory equivalents ($\dot{V}_E/\dot{V}CO_2$, $\dot{V}_E/\dot{V}O_2$). Dead space volume (V_D) was calculated based on the equation (V_E-V_A) *RR, where $\dot{V}_E$ represents minute ventilation, $\dot{V}_A$ represents alveolar ventilation, and RR represents respiratory rate [177].

Open Circuit Spirometry

Breath by breath expired O_2, CO_2, ventilatory volume and RER were determined using open circuit spirometry (Innocor, Innovision, Denmark). Airflow was directed by pneumotach using a differential pressure transducer (Innocor, Innovision, Denmark). CO_2 and O_2 analysers (Oxigraph Inc, USA) were integrated within the Innocor metabolic system. Gases were sampled at a rate of 120 ml·min^{-1} and analysed by photoacoustic spectroscopy. The response time of the CO_2 analyser was synchronised with the O_2 analyser. The Innocor system was calibrated prior to each test. This involved a gas

delay determination that assessed the specific breathing pattern of each subject and an O_2 adjustment to the ambient air. The system was calibrated to the manufacturer's procedures using a 3 L syringe (Series 5530, Hans Rudolph Inc., Germany).

HR and SpO_2 were recorded using a 12 lead ECG (Case 8000, Marquette GE, USA) and pulse oxymetry (Nonin 8500, Nonin Medical, INC, NH, USA) respectively. Blood samples were taken from the earlobe every minute during exercise, and at minutes 2, 5, 7, and 10 during the recovery period, and used to analyse blood lactate concentration (AccuCheck Softclix Pro Lancet, Accu Check, Australia). The earlobe was sterilized with a sterile wipe and then pricked with a lancet (AccuCheck Softclix Pro Lancet, Accu Check, Australia) to promote blood flow.

The V-slope method described by Beaver et al. (1986) was used for the determination of AT [181]. A two-phase linear regression model was used to determine the LT. The logarithm (log_{10}) of blood lactate was plotted against the logarithm (log_{10}) of $\dot{V}O_2$ [182].

Rating of Perceived Exertion

Overall ratings of perceived exertion were obtained every 2 minutes using the 15-point Borg category RPE scale. Perceived leg fatigue and dyspnea scores were obtained every 2 minutes using the Borg CR-10 category RPE scale. Prior to each test a standard set of instructions was read to each subject.

Constant Load Exercise Tests

CF patients and healthy controls performed 2 constant work load tests on a cycle ergometer for 10 minutes, separated by approximately 2-3 days. The exercise

tests were performed at an intensity corresponding to 50% (light exercise) and 70% (moderate exercise) of the initial peak $\dot{V}O_2$. Healthy controls performed two additional constant load exercise tests at the same absolute workrate corresponding to 50% $\dot{V}O_2$peak (50W) and 70% $\dot{V}O_2$peak (100W) of the CF patients. For the light exercise, it was assumed that patients exercised at an intensity that was below the AT.

Respiratory metabolic measures, HR and SpO_2% were continuously recorded throughout each test. RPE, dyspnea score and leg fatigue were recorded every 5 minutes and blood samples were taken every minute.

Oxygen Uptake Kinetics

A nonlinear least-square algorithm as described in the following monoexponential equation was used to fit the data from t = 0 sec to t = 600 sec:

$$O_2(t) = O_2 \text{ baseline} + A \bullet (1\text{-}e\text{-}t/\tau)$$

Where O_2 (t) represents the absolute O_2 at a given time t; O_2 baseline represents the mean O_2 in the baseline period (the final 90 seconds of exercise preceding the transition to the higher work rate); A represents the response amplitude and τ represents the MRT. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 seconds of the tenth minute during light exercise, and the final 30 seconds of the sixth minute during moderate exercise. An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. MRT indicates the time taken for O_2 to reach 63% of the response amplitude from the onset of exercise with no distinction made for the various phases of the response [135]. The MRT derived was used to calculate the oxygen deficit (O_2D)

using the equation: $O_2D = MRT*\Delta\dot{V}O_2$, where MRT represents the mean response time, and $\Delta\dot{V}O_2$ represents the amplitude above baseline level. The amplitude of the slow component was described by the difference between $\dot{V}O_2$ during 3 minutes and during 6 minutes of exercise ($\Delta\dot{V}O_2$ 6-3min).

Muscle Strength and Endurance

Subjects performed a bilateral maximal isokinetic and isometric strength test, and a bilateral isokinetic endurance test. Isokinetic strength was determined using concentric contraction of the quadriceps femoris (knee extension) and hamstring (knee flexion) muscle using an isokinetic dynamometer (Biodex System 3, Biodex Medical System, NY, USA). Subjects performed 5 repetitions at $60°\cdot sec^{-1}$ and $180°\cdot sec^{-1}$. The rest period between trials was 90 seconds. The highest peak torque (Nm) generated was recorded.

Maximum voluntary isometric contraction peak torque (Nm) was assessed with the knee flexed at an angle of 60°. Subjects warmed-up with 3 submaximal contractions and then performed 3 consecutive maximal contractions of 5 seconds duration with a 60 second rest period between repetitions. The highest peak torque (Nm) generated was recorded. Muscle endurance was determined using concentric contraction of the quadriceps femoris (knee extension) and hamstring (knee flexion). Subjects performed 20 repetitions at $240°\cdot sec^{-1}$.

Statistical Analysis

Independent t-tests were used to compare the physiological responses at peak exercise between the combined CF patients and healthy controls. A one way ANOVA

was used to compare the peak exercise response in patients of varying disease severity (mild, moderate to severe) and the healthy control group. Post hoc multiply comparison was made using a Tukey HSD. $P<0.05$ was accepted as statistically significant. Values are reported as mean ± SD. Data were analysed using SPSS (v17.0, SPSS Inc., IL).

Results

Anthropometric and Spirometry Data

A total of 28 CF patients and 19 healthy controls completed the study. Approximately one third (n=16) of the CF patients had mild disease severity and 12 had moderate to severe disease severity. Since only 4 of the patients with severe disease severity completed all 4 study visits, the data from the moderate and severe groups were combined for statistical analysis. Anthropometric and spirometry data are summarized in Table 4.1. There was no difference in height, weight, LBM, % fat and DC between the combined CF group and healthy controls. Each of the measured spirometry parameters were lower ($p<0.001$) in the combined CF than healthy controls, and in the moderate to severe CF group and healthy controls. FEV_1% predicted, FVC %predicted and FEV_1/FVC were lower ($p<0.001$) in the mild CF than healthy controls. Diffusion capacity, weight and LBM were significantly different between moderate to severe CF and healthy controls

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Table 4.1: Subject characteristics in different CF severities and healthy controls

	Experimental Group			
	Healthy	**Cystic Fibrosis**		
		Combined	**Mild**	**Moderate & Severe**
Subjects n	19 (f=8)	28 (f=9)	16 (f=4)	12 (f=4)
Age (yr)	22.6±3.2	24.9±5.7†	24.8±6.4	25.9±6.1†
Weight (kg)	71.6±9.9	66.1±11.3	70.4±11.2	60.5±9.3†*
Height (cm)	173.6±8.8	172.2±7.8	174.3±8.5	169.5±6.1
LBM (kg)	58.9±8.8	55.1±8.9	58.5±8.8	50.5±7.7†*
Fat %	17.8±5.3	17.7±7.1	18.5±8.2	16.5±5.2
FEV_1(L)	4.3±0.7	2.6±1.1‡	3.4±0.8†	1.6±0.5‡*
FEV_1%	107.4±13.8	66.4±25.1‡	84.5±14.5‡	42.4±12.4‡*
FVC (L)	5.1±1.0	3.9±1.3‡	4.7±1	2.9±0.8‡*
FVC (% predicted)	107.4±15.6	85.5±19.7‡	98.8±9.2‡	66.5±12.1‡*
FEV_1/FVC	84.7±6.7	64.5±12.7‡	71±8.4‡	55±12.9‡*
D_LCO ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	31.0±3.6	29.8±7.8	31.1±6.4	28.1±9.2‡
D_LCO (% predicted)	96.6±11.5	90.3±18.9	93.6±15.6	86.1±22.3‡

Values are mean ± SD; † p <0.05, ‡ p <0.001, vs. healthy controls, * p <0.05 vs. mild CF

Peak Exercise Performance

Peak exercise performance data are shown in Table 4.2. Pulmonary, metabolic and cardiovascular responses at peak exercise were lower in the combined CF group than in healthy controls ($p<0.001$), and in the moderate to severe group than the healthy controls. Peak exercise capacity, AT% predicted $\dot{V}O_2$ peak and O_2 pulse were significantly lower in patients with mild CF than healthy controls. However, peak ventilatory response was similar between mild and healthy controls.

Peak $\dot{V}O_2$/LBM, AT% predicted $\dot{V}O_2$ peak were similar between mild CF to moderate to severe CF. However, peak O_2 pulse, peak lactate and peak SpO_2% were significantly lower in moderate to severe CF than in mild CF. Peak V_T, V_D/V_T were significantly lower and were accompanied by higher $\dot{V}_E/\dot{V}CO_2$ in moderate to severe CF than in mild CF.

Table 4.2: Physiological response at peak exercise in different CF severities and healthy controls

	Experimental Group			
	Healthy	**Cystic Fibrosis**		
		Combined	**Mild**	**Moderate and Severe**
Subjects n	19 (f=8)	28 (f=9)	16 (f=4)	12 (f=4)
$\dot{V}O_2$ peak (ml·kgLBM^{-1}·min^{-1})	53.0±12.3	36.0±9.1 ‡	39.8±7.8‡	30.9±8.5‡
$\dot{V}O_2$ peak (% predicted)	111.9±19.8	74.4±17.4‡	83.6±12.1‡	62.1±16.1‡
Work rate (watts)	251.5±68.3	147.9±61.4‡	180.2±49.5†	104.8±47.5‡*
Lactate (mmol·L^{-1})	10.3±1.7	7.2 ±2.7‡	8.9±1.7†	4.9±2.0‡ *
AT Predicted $\dot{V}O_2$ peak (%)	49.1±8.0	36.8±9.7‡	38.5±10.6†	34.2±7.7‡
O_2 Pulse (ml·beat^{-1})	17.0±5.6	11.9±3.7†	13.5±3.5†	9.8±2.8‡*
O_2 Pulse (%predicted)	108±32	90±13†	94.5±13.5†	74.7±16.0‡*
$\Delta\dot{V}O_2/\Delta WR$ (ml·min^{-1}·w^{-1})	12.2±2.2	10.4±1.8†	10.1±1.5†	10.5±2.4†
$\dot{V}CO_2$ (L·min^{-1})	3.6±1.2	2.3±0.6†	2.8±0.9†	1.7±0.7‡
RER	1.16±0.1	1.15±0.1	1.19±0.1	1.1±0.1*
HR (b·min^{-1})	187.7±9.3	167.1±18.1‡	173.2±11.7†	159.0±22.3‡
$\dot{V}_E$ (L·min^{-1})	114.9±39.0	82.0±27.0‡	96.0±20.7†	63.4±23.2‡
RR (f·min^{-1})	45.4±10.1	45.7±7.8	42.8±5.5	49.6±8.9
SpO_2%	97.6±1.5	93.9±4.6‡	96.0±4.6†*	91.0±4.7‡ *
$\dot{V}_E$/MVV (%)	66.6±21.7	83.7±19.0‡	72.7±14.2	98.3±14.2‡
$V_{T,}$ (L)	2.6±0.7	1.9±0.5‡	2.2±0.5	1.3±0.5‡*
V_T/VC %	49.6±6.2	46.3±9.4	48.4±8.3	43.5±10.3
V_D/V_T	0.14±0.04	0.2±0.06‡	0.16±0.03	0.25±0.59‡*
$V_E/\dot{V}CO_2$@ AT	23.7±1.9	30.1±4.3‡	28.7±3.8‡	32.2±4.3‡*
P_ETCO_2 (mmHg)	38.9±5.2	39.1±4.8	37.8±5.3	41.0±3.9

Values are mean ± SD; † p <0.05, ‡ p <0.001, vs. healthy controls, * p <0.05 vs. mild CF

Muscle Strength and Endurance

The muscle strength and endurance results are shown in Table 4.3 and Table 4.4 respectively. Quadriceps and hamstring maximal isokinetic strength (60°/s and 180°/s), isometric strength and muscular endurance were significantly higher in the control group than both the combined CF patients and patients with moderate to severe CF. With the exception of left quadriceps peak torque at 60°/s, and right hamstring peak torque at 60°/s and 180°/s, there was no difference in maximal strength or endurance between the patients with mild CF and healthy controls.

Table 4.3: Quadriceps strength and endurance in different CF severities and healthy controls

	Experimental Group			
	Healthy	**Cystic Fibrosis**		
		Combined	**Mild**	**Moderate and Severe**
Subjects n	16 (f=8)	26 (f=5)	14 (f=3)	12 (f=1)
Quadriceps right leg				
Peak torque 60°/s (Nm)	183±55	139±45†	156±45	116±35
Peak torque 180°/s (Nm)	122±39	97±35†	109±40	90±29†
Total work 240°/s (j)	2336±607	1962±716 †	2174±811	1672±447†
Isometric Peak torque 60°/s (Nm)	182±50	155±54†	167±63	136±32 †
Quadriceps left leg				
Peak torque 60°/s (Nm)	180±52	128±40†	140±38†	110±34‡*
Peak torque 180°/s (Nm)	126±35	92±34†	101±44	78±29†
Total work 240°/s (j)	2138±558	1875±726	2078±773	1589±550‡
Isometric Peak torque 60°/s (Nm)	188±63	151±54†	163±61	132±36 †

Values are mean ± SD; † p <0.05, ‡ p <0.001, vs. healthy controls, * p <0.05 vs. mild CF

Table 4.4: Hamstring strength and endurance in different CF severities and healthy controls

	Experimental Group			
	Healthy	**Cystic Fibrosis**		
		Combined	**Mild**	**Moderate and Severe**
Subjects n	16 (f=8)	26 (f=5)	14 (f=3)	12 (f=1)
Hamstring right leg				
Peak torque 60°/s (Nm)	102±26	73±23‡	82±23†	60±16‡
Peak torqu180°/s (Nm)	75±24	58±22†	67±22‡	46±15‡*
Total work 240°/s (j)	1440±427	981±498†	1124±561	773±304‡
Hamstring left leg				
Peak torque 60°/s (Nm)	95±28	67±25†	77±26	54±16‡
Peak torque 180°/s (Nm)	76±23	53±22†	61±22	42±16‡
Total work 240°/s (j)	1310±327	876±451†	1048±508	715±301‡

Values are mean ± SD; † p <0.05, ‡ p <0.001, vs. healthy controls, * p <0.05 vs. mild CF

Exercise O_2 Uptake Kinetics

Light Exercise

CF patients and healthy controls undertook two 10-minute constant load cycle ergometer tests at 50% and 70% $\dot{V}O_2$peak. Healthy controls also performed two additional constant load exercise tests at the same absolute workrate corresponding to 50% $\dot{V}O_2$peak (50W) and 70% $\dot{V}O_2$peak (100W) of the CF patients.

Exercise response O_2 uptake kinetics during the constant load exercise test at 50% $\dot{V}O_2$ peak for CF patients and at 50 W for healthy controls are shown in Table 4.5. MRT was similar between the combined CF and healthy controls. The O_2D per unit work rate was higher ($p < 0.05$) in the moderate to severe CF than mild CF ($p < 0.05$) and healthy controls ($p < 0.05$). SpO_2% at 180 seconds was lower ($p < 0.001$) in the moderate to severe CF group than healthy controls ($p < 0.001$). Exercise response $\dot{V}O_2$ uptake kinetics were similar between mild CF and healthy controls.

Table 4.5: $\dot{V}O_2$ uptake kinetics during light exercise at 50% $\dot{V}O_2$ peak in CF and same absolute workrate in healthy individuals

	Experimental Group			
	Healthy	**Cystic Fibrosis**		
		Combined	**Mild**	**Moderate and Severe**
Subjects n	17 (f=9)	28 (f=5)	16 (f=3)	12 (f=1)
Workrate (W)	50	50±22	61±19	35±16‡*
MRT (s)	36±14	41±11	41±9	42±14
$\dot{V}O_2$ baseline ($ml \cdot min^{-1}$)	421±126	364±78	362±85	368±71
Relative $\dot{V}O_2$ amplitude ($ml \cdot min^{-1} \cdot w^{-1}$)	14.3±1.7	15.1±3.7	13.2±1.6	16.3±4.1†
O_2D (L)	0.4±0.2	0.5±0.21	0.5±0.2	0.4±0.1
Relative O_2D ($L \cdot watt^{-1}$)	0.008±0.03	0.01±0.004	0.008±0.02	0.012±0.05†*
SpO_2 % at minute 3	97.1±0.3	95±2.5	97±2.6	94±2‡*

Values are mean ± SD; † p <0.05, ‡ p <0.001, vs. healthy controls, * p <0.05 vs. mild CF

Moderate Exercise

Exercise response O_2 uptake kinetics for CF patients during the constant load exercise test at 70% $\dot{V}O_2$ peak and for healthy controls at 100 W are shown in Table 4.6. At the same absolute work rate (100 W), MRT was lower ($p<0.001$) between the combined CF and healthy controls. The O_2D per unit work rate was higher ($p<0.001$) in the moderate to severe CF than in mild CF and healthy controls. Lactate levels during the last 15 seconds of the sixth minute were higher in the combined CF (<0.001) than in healthy controls, and in moderate to severe CF than in healthy controls ($p<0.05$).

The $\dot{V}O_2$ slow component response during constant load exercise at 70% $\dot{V}O_2$ peak in CF patients and in healthy controls is shown in Table 4.7. The $\dot{V}O_2$ slow component amplitude per unit work rate was higher ($p< 0.05$) in the combined CF than healthy controls ($p<0.05$), and in moderate to severe CF than in healthy controls ($p< 0.05$). SpO_2% at 360 seconds was lower ($p <0.05$) in the combined CF ($p <0.05$) than in healthy controls, and in moderate to severe CF than in mild CF ($p <0.05$).

Table 4.6: $\dot{V}O_2$ uptake kinetics during moderate exercise at 70% $\dot{V}O_2$ peak in CF and same absolute workrate in healthy individuals

	Experimental Group			
	Healthy	**Cystic Fibrosis**		
		Combined	**Mild**	**Moderate and Severe**
Subjects n	16 (f=8)	26 (f=5)	14 (f=3)	12 (f=1)
Workrate (W)	100	100±42	123±32†	72±32‡*
MRT (s)	39±12	60±10‡	59±8‡	60±11‡
Resting $\dot{V}O_2$ ($ml \cdot min^{-1}$)	402±120	393±93	387±106	399±78
Relative $\dot{V}O_2$ amplitude ($ml \cdot min^{-1} \cdot w^{-1}$)	13.1±1.6	13.2±1.6	12.6±1.15	13.9±2
O_2D (L)	0.8±0.2	1.2±0.5†	1.5±0.5‡	1±0.3 † *
Relative O_2D ($L \cdot watt^{-1}$)	0.008±0.02	0.013±0.003‡	0.012±0.002‡	0.014±0.003‡
Lactate at 6 min ($mmol \cdot L^{-1}$)	3.1±1.5	5.9±2.1‡	6.6±2.3	5.1±1.6†

Values are mean ± SD; † p <0.05, ‡ p <0.001, vs. healthy controls, * p <0.05 vs. mild CF

Table 4.7: Exercise response during the $\dot{V}O_2$ slow component phase at 70% $\dot{V}O_2$ peak in CF and healthy individuals

	Experimental Group			
	Healthy	**CF**		
		Combined	**Mild**	**Moderate and Severe**
Workrate (W)	193±52	100±42	123±32†	72±32‡*
Relative $\Delta\dot{V}O_2$end-3min ($L \cdot min^{-1} \cdot w^{-1}$)	0.871±1.073	1.442±0.761†	1.382±0.648	1.511±0.900†
Lactate at 6 min ($mmol \cdot L^{-l}$)	7.2±1.7	5.9±2.1	6.6±2.3	5.1±1.6†
SpO_2 % at 6 min	97±0.5	93±4†	95±2	90±5†*

Values are mean ± SD; † p <0.05, ‡ p <0.001, vs. healthy controls, * p <0.05 vs. mild CF

Relation between Variables in CF Patients

Quadriceps and hamstring maximal isokinetic and isometric strength were positively correlated with LBM (Figure 4.1) and FEV_1% predicted (Table 4.8). There was a significant relation between quadriceps peak torque 60°/s and $\dot{V}O_2$ peak relative to LBM ($r=0.53$; $p<0.01$). The O_2D per unit WR was inversely correlated ($r=-0.48$; $p<0.05$) to SpO_2% at minute 3 of light constant load (50 W) exercise (Figure 4.2). The MRT during moderate exercise was inversely correlated with the peak O_2 pulse ($r=-0.53$; $p<0.01$).

Table 4.8: Correlation between exercise variable in CF patients

	LBM	**FEV_1% Predicted**
Quadriceps		
Peak torque 60°/s (Nm)	0.78**	0.57**
Isometric Peak torque 60°/s (Nm)	0.71**	0.46*
Total work 240°/s (j)	0.84**	0.54**
Hamstring		
Peak torque 60°/s (Nm)	0.8**	0.55**
Total work 240°/s (j)	0.8**	0.54**

*p <0.05; ** p <0.001

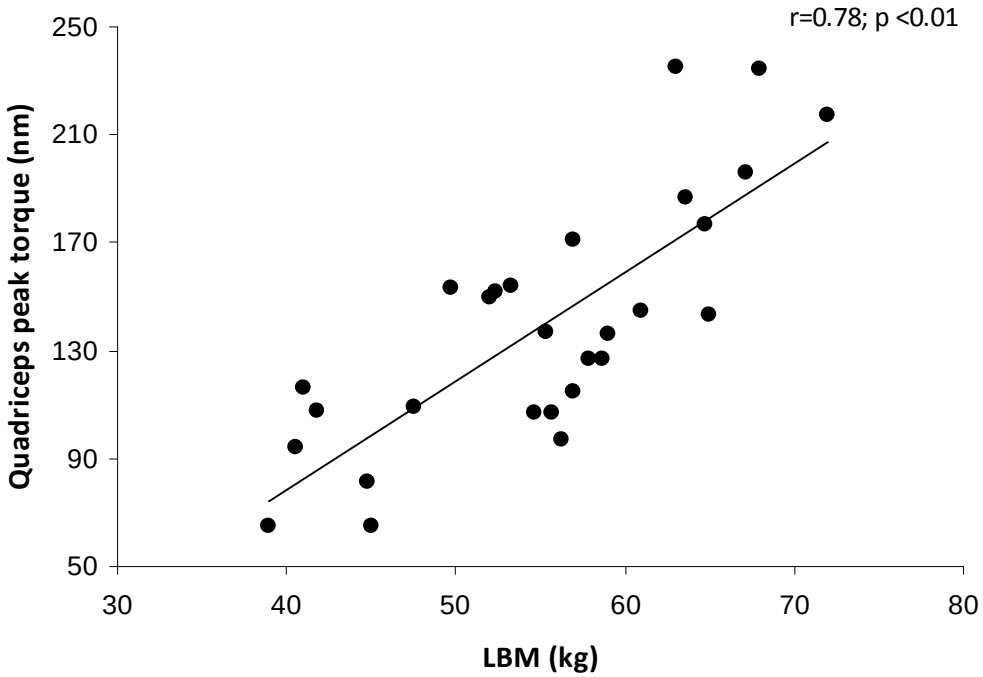

Figure 4.1: Relation between quadriceps peak torque 60°/s and LBM in CF patients

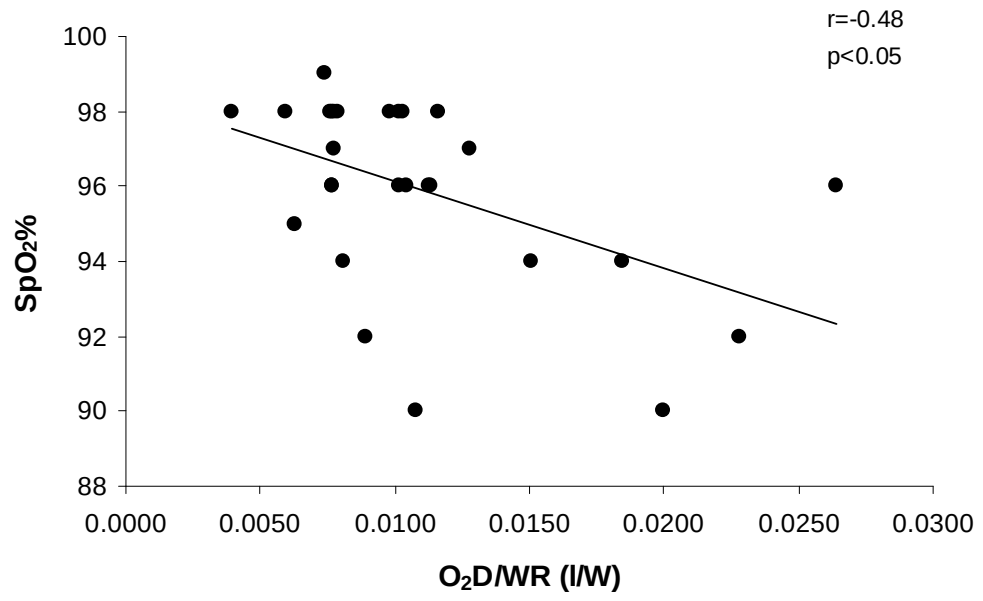

Figure 4.2: Relation between O_2D/WR and SpO_2 % at minute 3 of light exercise in CF patients

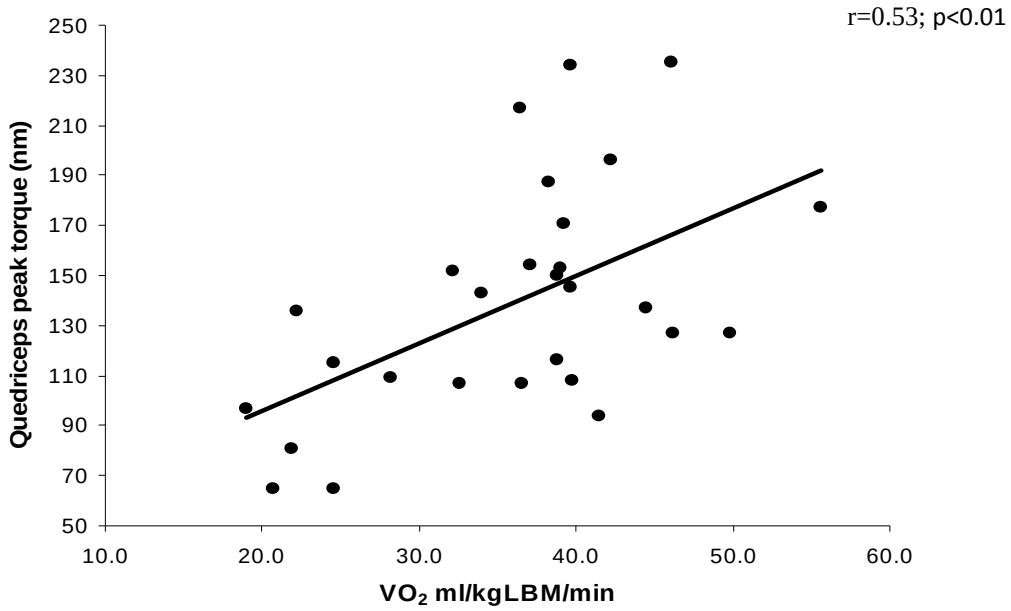

Figure 4.3: Relation between quadriceps peak torque 60°/s and $\dot{V}O_2$peak in mild to moderate CF patients

Discussion

Exercise performance in CF is influenced by a number of factors including body composition, muscle function and O_2 uptake kinetics. The present study compared body composition, muscle strength, and peak exercise capacity in patients with mild, moderate and severe CF and healthy controls. In addition, circulating levels of blood lactate and O_2 uptake kinetics were also assessed during constant load exercise in adults with CF and healthy controls. The data from the moderate and severe groups were combined for statistical analysis and the principles findings were that i) peak exercise capacity was reduced in mild, and moderate to severe CF patients compared to healthy controls ii) muscle strength was lower in patients with moderate to severe CF than healthy controls, iii) there was some evidence of reduced muscle strength in mild CF patients with preserved LBM compared to healthy controls iv) exercise response O_2 uptake kinetics was slower in mild, and moderate to severe CF patients than healthy controls during moderate constant load exercise, and v) circulating levels of blood lactate were higher at the same absolute WR in mild, and moderate to severe CF patient than healthy controls during moderate constant load exercise.

Peak Exercise Capacity

It is well established that exercise tolerance is reduced in CF patients compared to healthy controls [115]. The reasons for the reduction in exercise tolerance are complex. Compared to healthy controls, the patients with moderate to severe CF in the present study were ventilatory limited at peak exercise. They had abnormal gas exchange, reduced O_2 pulse and peripheral muscle weakness. Interestingly, patients

with mild CF and normal LBM, normal O_2 pulse and with no ventilatory limitation had a reduced AT and $\dot{V}O_2$peak. These findings may be related to deconditioning and/or skeletal muscle abnormalities. It is possible that alterations in skeletal muscle metabolism may contribute to the reduced exercise capacity in the mild CF group [10].

The significantly higher $\dot{V}_E/\dot{V}CO_2$ in the mild CF group than the healthy controls may have resulted from an excessively high ventilation. Pianosi, et al., [195] found abnormalities in CO_2 set point during exercise in CF patients. It is possible that the elevated V_E, assuming a normal DS (V_D/V_T = 0.16) may have occurred due to a change in the chemoreceptor set point for $PaCO_2$.[195]. The low $\dot{V}O_2$peak found in the mild CF group may indicate low levels of conditioning that may also help to explain the elevated $\dot{V}_E/\dot{V}CO_2$.

Peripheral Muscle Strength

Muscle atrophy has been shown to have a negative impact on strength in patients with CF [3, 34, 198]. We found that the reduction in lower limb muscle strength and endurance in moderate to severe CF was related to LBM. Despite having a preserved LBM, the mild CF group had a reduction in left quadriceps peak torque at 60°/s, and right hamstring peak torque at 60°/s and 180°/s compared to the healthy controls. These differences may be due to alterations in biochemical or molecular signalling in the skeletal muscle of CF patients. Future investigations should incorporate muscle biopsy sampling in order to examine the changes in the morphological, biochemical and metabolic properties of skeletal muscle in CF.

A number of previous studies have examined muscle function and strength in CF patients [81, 196-197]. In many instances the respiratory muscle in the CF patients had a trained phenotype, probably in response to the increased work of breathing associated with the disease [81, 196-197]. In the present study, quadriceps strength was significantly related to VO_2peak/LBM. Muscle strength is also related to exercise capacity in children with CF, even in the absence of diminished pulmonary or nutritional status [110]. The reduction in muscle strength in the moderate to severe CF group may have contributed to their exercise intolerance in the present study.

Muscle weakness in CF may be related to inactivity [9], malnutrition, systemic inflammation [12], corticosteroids [13], and hypoxia [194]. There is evidence that physical inactivity is an important factor contributing to exercise intolerance and skeletal muscle weakness in adults CF [109]. An increase in systemic inflammation may also contribute to muscle atrophy in respiratory and non-respiratory chronic diseases. Defrense et al., [117] found a relation between the severity of lung disease in mild to moderate CF and inflammatory cytokines, including IL-6 and TNF-α. However, they did not find a relation between inflammatory cytokines and lean body mass and muscle strength of the diaphragm, biceps and quadriceps. After adjusting for FEV_1% predicted and nutrition status, Barry et al., [118] found that the administration of 5.1 mg/day of corticosteroid over 12 a month period resulted in a reduction in muscle strength in CF patients. The use of corticosteroids can be ruled out as a putative mechanism to explain the decrease in muscle strength in the CF group in the present study since none of the study participants were taking oral steroid medication. The current study was not designed to investigate the mechanisms

responsible for muscle weakness in CF, and further research is required to address this issue.

Exercise Response O_2 Uptake Kinetics

Light Exercise

Measurement of O_2 uptake kinetics during light exercise can provide additional information on exercise limitation in CF patients. Adequacy of O_2 delivery during the transient phase following the onset of exercise [137] and/or activity of the intracellular biochemical reaction within the muscle [199] may limit O_2 uptake kinetics. The factors that determine the speed of $\dot{V}O_2$ uptake kinetics in CF at light exercise intensities are not well understood. There was no difference in MRT between patients with CF and healthy controls during light exercise below the AT. However, patients with moderate to severe CF had a larger O_2D per unit WR than mild CF and healthy individuals. This was accompanied by a small reduction in SpO_2% at minute 3 during light exercise. The inverse relation between O_2D per unit WR and SpO_2% at minute 3 may indicate that an impaired O_2 delivery due to abnormal gas exchange may contribute to slower O_2 uptake kinetics in moderate to severe CF. Another potential explanation for impaired O_2 delivery is pulmonary vascular alteration. The resting DC although within the normal range, was significantly lower in the moderate to severe CF patients than the controls. It can be speculated that the DC may deteriorate to a greater extent in moderate to severe CF patients than healthy controls during exercise due to an abnormal recruitment of the pulmonary vascular bed. This may cause a reduction in O_2 delivery to the exercising muscles, which may in turn affect O_2 uptake kinetics in

these patients. The similar MRT between patients with CF and healthy controls is in contrast to the results reported by Hebestreit et al., [146], who found that O_2 uptake kinetics were slower in children with mild CF with a normal SpO2% and a normal $\dot{V}O_2$ peak than healthy children. The difference in age of the CF patients may contribute to the different findings between the two studies.

Moderate Exercise

Measuring O_2 uptake kinetics during moderate exercise may provide additional information on exercise limitation in CF patients due to the additional stress on the cardiopulmonary and peripheral system compared to light exercise. The MRT was slower and lactate levels were higher in the CF group than healthy controls at the same absolute WR. Blood lactate levels were related to the amplitude of the $\dot{V}O_2$ slow component. At the same relative WR (70% $\dot{V}O_2$peak), the $\dot{V}O_2$ slow component amplitude, relative to WR, was higher in patients with CF than healthy controls.

The MRT is an indication of the capacity to deliver O_2 to exercising muscle fibres, and intracellular oxidative metabolism within the muscle fibres [136-137]. The delivery of O_2 to the exercising muscle in moderate to severe CF may be impacted by a reduction in both $\dot{V}_A$ and gas exchange that may result in low arterial O_2 content. In addition, pathological alterations within the pulmonary vasculature may reduce pulmonary blood flow that may negatively impact on CO. In addition to the MRT, the $\dot{V}O_2$ slow component amplitude during moderate exercise was also investigated. Putative mechanisms responsible for the $\dot{V}O_2$ slow component amplitude during moderate exercise include: i) increased cardiac and respiratory muscle work, ii)

increased O_2 cost of lactate catabolism [138], iii) increased activity/recruitment of type IIX skeletal muscle fibres, iv) the pattern of motor unit recruitment [21-22] and v) reduced delivery of O_2 to exercising muscle [143].

In the current study, SpO_2% at the end of exercise was reduced in patients with moderate to severe CF. In contrast to light exercise (50% $\dot{V}O_2$peak), there was no significant relation between SpO_2% and prolonged O_2 uptake kinetics during the bout of constant load moderate exercise. Other factors related to O_2 delivery may slow the O_2 uptake kinetics during moderate exercise in patients with moderate to severe CF. Abnormal pulmonary hemodynamics may alter O_2 delivery to exercising muscle. Pulmonary hypertension has been found in 41% of CF patients with FEV_1<40% [188]. In the present study, there was a negative relation between MRT during moderate exercise and peak O_2 pulse in patients with moderate to severe CF. This relation may suggest that abnormalities of O_2 delivery are a potential mechanism for the reduced O_2 uptake kinetics in this group.

In COPD patients, a higher $\dot{V}_E$, abnormal breathing mechanics [38-39], hypoxemia [150], pulmonary hemodynamics [40] and peripheral vasodilatation [154] may slow the response of systemic and peripheral O_2 delivery to a point where the O_2 uptake kinetics might become limited by O_2 availability. Gaspar et al., [42] found that $\dot{V}O_2$ kinetics during heavy exercise was slower in patients with moderate to severe COPD than healthy individuals due to a reduction in systemic and peripheral O_2 delivery to the exercising muscle. Providing bronchodilator therapy before heavy

exercise reduces lung hyperinflation and accelerates $\dot{V}O_2$ kinetics in patients with moderate to severe COPD [146].

O_2 uptake kinetics in CF may also be affected by a functional deficit within the exercising muscle. The reduced oxidative capacity of skeletal muscle found in CF athletes with good nutritional status and a preserved pulmonary function [111] suggests inefficient O_2 utilization within the skeletal muscle. This metabolic inefficiency may contribute to the slower O_2 uptake kinetics and increased O_2D. The increased O_2D would be expected to elicit a transient reliance on anaerobic energy sources resulting in an increase in lactate production [200].

A reduction in peripheral O_2 delivery to the exercising muscle may also contribute to the higher blood lactate levels found in CF patients during moderate constant load exercise. No published studies have examined peripheral blood flow during exercise in CF. Peripheral blood flow, in not a factor contributing to the early lactate acidosis in COPD [45]. However, the fact that the premature rise in blood lactate during exercise in COPD is related to glycolytic enzyme activity in skeletal muscle [45] indicates that alterations in muscle metabolism may limit exercise performance in this patient cohort. A similar derangement in muscle metabolism may have contributed to the higher circulating levels of blood lactate during moderate constant load exercise, and the lower AT in the CF group than healthy controls.

The present study was not designed to distinguish between muscle deconditioning and alterations in intrinsic muscle metabolism in CF. However, a number of mechanisms have been identified that may help to explain the abnormality

in the skeletal muscle of CF patients. The abnormal O_2 uptake kinetics found in all CF groups may be related to an abnormality in the CFTR protein in skeletal muscle. Recently, Lamhonwah et al., [39] found that CFTR is expressed in the sarcoplasmic reticulum of human skeletal muscle. Lack of CFTR in the skeletal muscle of mice has been related to an increase in inflammatory/atrophic gene expression [40]. A putative defect in the efficiency of mitochondrial oxidative phosphorylation may be related to expression of mutated CFTR. The CFTR protein is involved in ATP hydrolysis [116]. Abnormality in CFTR may reduce ATP hydrolysis causing inefficient oxidative and anaerobic metabolism that may affect O_2 uptake kinetics in CF.

Study Limitations

The mechanisms involved in the reduced O_2 uptake kinetics in CF patients were not investigated due to the requirement of invasive techniques. This will require an invasive study to distinguish between the abnormal O_2 delivery to the exercising muscle and an intrinsic abnormality within the muscle.

The response data when assessing gas and ventilatory variables on a breath-by-breath basis reflects not only the true physiological signal of interest, but also breath-by-breath fluctuations in breathing patterns. Tolerance of exercise stresses may be restricted in moderate to severe CF patients such that the stimulus must be low and thus the response signal is disproportionately small.

Summary

Peak exercise capacity and O_2 uptake kinetics are reduced in patients with mild and moderate to severe CF compared to healthy controls. There was evidence of

muscle weakness in the mild CF group even though they have a preserved LBM and normal pulmonary function. These findings suggest that diminished exercise performance in mild CF is at least partly due to pathophysiological factors in skeletal muscle that cannot fully be explained by muscle deconditioning. In patients with more severe clinical symptoms, the reduction in exercise capacity may be related to a reduced LBM, reduced gas exchange, ventilatory limitation, and an altered cardiovascular response.

Chapter 5

Study 3

Supplemental Oxygen Enhances the Effect of Interval Training on Exercise Tolerance and O_2 Uptake Kinetics in Patients with Cystic Fibrosis

Introduction

Abnormal pulmonary function and decreased gas exchange may contribute to exercise intolerance in patients with moderate and severe CF [115]. Supplemental O_2 has recently been used as a therapeutic modality to overcome the ventilatory limitation during maximal and submaximal exercise in CF patients. Moderate to severe CF patients with arterial hypoxemia during exercise are able to increase their peak $\dot{V}_E$ and $\dot{V}O_2$peak when given supplemental O_2 [4]. Furthermore, providing supplemental O_2 during a bout of exercise at 80% peak WR improves exercise duration by 26% in patients with moderate to severe CF [12]. The improvement in exercise performance following the administration of supplemental oxygen may be due to an alteration in ventilatory response or an improvement in O_2 delivery to the exercising muscle.

In contrast to patients with moderate to severe CF, exercise intolerance in patients with mild CF is due primarily to alterations in muscle function [36, 84, 106-113] secondary to a reduction in physical activity levels [107]. Muscle alterations due to inactivity in mild CF may include atrophy, [110], decreased number of mitochondria [5-8] and increased reliance on anaerobic glycolysis [6-7]. Recently, some studies have

found evidence of an intrinsic abnormality in muscle function which occurs independently of deconditioning in CF [5, 7-9].

High intensity interval training (HIIT) involves repeated bouts of high intensity exercise alternated with rest periods of equal or shorter duration [201]. This type of training allows both sedentary and active individuals to exercise at a higher intensity compared to continuous exercise. Traditionally, HIIT has been used to improve exercise performance in athletes. Recently, studies involving individuals with cardiovascular disease have found that HIIT is more effective that moderate intensity continuous training in improving mitochondrial function, %$\dot{V}O_2$ at the AT, cardiac output, ejection fraction, resting SV, $\dot{V}O_2$peak and time to fatigue (Guiraud et al., 2010). In contrast to cardiovascular disease, it is generally believed that high intensity exercise training is difficult in COPD patients with moderate to severe airway obstruction due to a ventilatory limitation [204]. Providing supplementary O_2 may attenuate the ventilatory limitation and enhance training intensity and duration in these patients [17]. Allowing CF patients to train for a longer duration at a high intensity may enhance the training effect on peripheral muscles and improve endurance exercise performance manifested while breathing room air. To our knowledge no studies have investigated the effects of HIIT on exercise performance in patients with CF.

The present pilot study examined the effects of an 8 week HIIT training programme with and without O_2 supplementation on $\dot{V}O_2$ peak, O_2 uptake kinetics, endurance performance and muscle strength in CF patients. In addition, the study also

compared the effect of interval training with and without O_2 supplementation on the number and duration of high intensity exercise bouts performed at baseline and week 8. All subjects breathed room air during the maximal and submaximal exercise tests performed at baseline and week 8.

It was hypothesized that compared to HIIT while breathing normal room air, HIIT with supplemental O_2 would result in i) a greater improvement in $\dot{V}O_2$peak, O_2uptake kinetics, endurance performance, and muscle strength and ii) a higher number and longer duration of high intensity exercise bouts at week 8.

Methods

Subjects

As a consequence of the practical difficulties in recruiting CF patients with moderate to severe disease, it was decided to also recruit patients with mild disease severity. A total of 11 CF patients (5 female) were recruited to take part in the study. The patients were recruited from the respiratory department in Beaumont hospital. CF diagnosis was based on clinical features, sweat test or genotyping and a pulmonary function test. No other diseases that could limit exercise capacity (cardiovascular, orthopaedic and neuromuscular) were present. The nature and risks of the study were explained. A plain language statement was read and informed consent was obtained in accordance with the Beaumont Hospital Ethics (Medical Research) Committee.

Study Overview

The study used a randomised, single blind design. Patients were randomly assigned to a placebo (n=6) or O_2 supplemental (O_2Suppl) (n=5) group, and were blinded to the treatment. They undertook interval training on a cycle ergometer 2 days per week for 8 weeks. Each session was approximately 1 hour in duration.

Patients made 4 separate visits to the Cardiovascular Research Unit at DCU, before and after the training programme. Each session was separated by at least 3 days. Patients performed the pre and post exercise evaluations while in stable health and not receiving antibiotic medication. The first visit was used to measure body composition, assess spirometry, single breath D_LCO, and $\dot{V}O_2$peak. During the second and third visits, patients performed a constant work load test at 50% or 70% $\dot{V}O_2$peak.

The final visit was used to assess muscle strength and performance in a 6 minute walk test.

Preparation

Patients abstained from alcohol and refrained from strenuous physical activity for 24 hours and fasted for 4 hours prior to each visit.

Anthropometry

Height and body mass were measured to the nearest 0.1 cm and 0.1 kg respectively using the SECA Stadiometer (Seca model 220 GmbH, Hamburg, Germany). Subjects were barefoot and wearing light clothing. BMI was determined as weight (kg) divided by height (m^2).

Body Composition

Double thickness subcutaneous adipose tissue was measured on the right side of the body using skinfold calipers (Harpenden, Cambridge Scientific Industries, MD). The following anatomical sites were used: chest, subscapular, mid-axillary, suprailliac, abdomen, triceps and thigh. A minimum of 2 measurements were taken at each site. If the measurements varied by more than 2 mm, a third measurement was taken. Percent body fat was based on the equation proposed by Jackson and Pollack [173].

Pulmonary Function Test

Standard pulmonary function tests, including spirometry, and measurements of single breath D_LCO (Sensormedics Vmax 229, Sensormedics Corp, CA), were

undertaken according to previously described guidelines [19-20] and results were compared to normative values [176].

Exercise Test

An incremental symptom-limited peak exercise test was performed on an electronically-braked cycle ergometer (Ergoselect 100, Ergoline GmbH) while breathing through a full face mask. The protocol was designed to ensure that subjects reached volitional exhaustion within 8–12 minutes following 3 minutes of rest and 3 minutes unloading cycling. The workrate increment ranged from 5-25 watts·min^{-1} depending on disease severity and fitness level. Respiratory metabolic measures, HR and SpO_2% were continuously recorded throughout the test. Peak oxygen uptake was determined by averaging the two highest consecutive 15 second values. The peak value for $\dot{V}O_2$ and exercise work rate were related to normal value [177]. The predicted MVV was calculated as FEV_1*40 [180]. Minute ventilation was expressed relatively to $\dot{V}O_2$ and $\dot{V}CO_2$ as ventilatory equivalents ($\dot{V}_E/\dot{V}CO_2$, $\dot{V}_E/\dot{V}O_2$). Dead space volume was calculated based on the equation $(V_E\text{-}V_A)$*RR, where $\dot{V}_E$ represents minute ventilation, $\dot{V}_A$ represents alveolar ventilation, and RR represents respiratory rate [177].

Open Circuit Spirometry

Breath by breath expired O_2, CO_2, ventilatory volume and RER were determined using open circuit spirometry (Innocor, Innovision, Denmark). Airflow was directed by pneumotach using a differential pressure transducer (Innocor, Innovision, Denmark). The CO_2 and O_2 analysers (Oxigraph Inc, USA) were integrated within the Innocor metabolic system. Gases were sampled at a rate of 120 ml·min^{-1} and analysed by

photoacoustic spectroscopy. The response time of the CO_2 analyser was synchronised with the O_2 analyser. The Innocor system was calibrated prior to each test. This involved a gas delay determination that assessed the specific breathing pattern of each subject and an O_2 adjustment to the ambient air. The system was calibrated to the manufacturer's procedures using a 3 L syringe (Series 5530, Hans Rudolph Inc., Germany).

HR and SpO_2% were recorded using a 12 lead ECG (Case 8000, Marquette GE, USA) and pulse oxymetry (Nonin 8500, Nonin Medical, INC, NH, USA) respectively. Blood samples were taken from the earlobe every minute during exercise and used to analyse blood lactate concentration (AccuCheck Softclix Pro Lancet, Accu Check, Australia). The earlobe was sterilized with a sterile wipe and then pricked with a lancet (AccuCheck Softclix Pro Lancet, Accu Check, Australia) to promote blood flow.

Rating of Perceived Exertion

Overall ratings of perceived exertion were obtained every 2 minutes using the 15-point Borg category RPE scale. Perceived leg fatigue and dyspnea scores were obtained every 2 minutes using the Borg CR-10 category RPE scale. Prior to each test a standard set of instructions was read to each subject

Constant Load Exercise Tests

Participants performed 2 constant work load tests on a cycle ergometer, for approximately 30 minutes, separated by approximately 2-3 days. The exercise tests were performed at an intensity corresponding to 50% (light exercise) and 70% (moderate exercise) of the initial peak $\dot{V}O_2$. Respiratory metabolic measures, HR and

SpO_2% were continuously recorded and values were averaged during the final 30 seconds of the exercise bout. RPE, dyspnea score and leg fatigue were recorded every 5 minutes and blood samples were taken every minute.

Oxygen Uptake Kinetics

A nonlinear least-square algorithm, as described in the following monoexponential equation, was used to fit the data from t = 0 sec to t = 600 sec:

$$O_2 (t) = O2 \text{ baseline} + A \bullet (1\text{-}e\text{-}t/\tau)$$

where O_2 (t) represents the absolute O_2 at a given time t; O_2 baseline represents the mean O_2 in the baseline period (the final 90 sec of exercise preceding the transition to the higher work rate); A represents the response amplitude and τ represents the mean response time (MRT). The end-exercise $\dot{V}O_2$ was defined as the mean VO_2 measured over the final 30 seconds of the tenth minute during moderate exercise. An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. MRT indicates the time taken for O_2 to reach 63% of the response amplitude from the onset of exercise with no distinction made for the various phases of the response [135]. The MRT derived was used to calculate the O_2 deficit (O_2D) using the equation: $O_2D = MRT*\Delta \dot{V}O_2$, where MRT represents the mean response time, and $\Delta \dot{V}O_2$ represents the $\dot{V}O_2$ amplitude above baseline level.

Muscle Strength and Endurance

Patients performed a bilateral maximal isokinetic and isometric strength test, and a bilateral isokinetic endurance test. Isokinetic strength was determined using concentric contraction of the quadriceps femoris (knee extension) and hamstring (knee

flexion) using an isokinetic dynamometer (Biodex System 3, Biodex Medical System, NY, USA). Subjects performed 5 repetitions at 60°·sec^{-1} and 180°·sec^{-1}. The rest period between trials was 90 seconds. The highest peak torque (Nm) was recorded.

Maximum voluntary isometric contraction peak torque (Nm) was assessed with the knee flexed at an angle of 60°. Patients warmed-up with 3 submaximal contractions and then performed 3 consecutive maximal contractions of 5 seconds with a 60-second rest between repetitions. The highest peak torque (Nm) was recorded. Muscle endurance was determined using concentric contraction of the quadriceps femoris (knee extension) and hamstring (knee flexion). Subjects performed 20 repetitions at 240°·sec^{-1}.

6 Minute Walk Test

The 6-minute walk test was performed indoors, along a flat, enclosed 30 metre corridor with a hard surface, according to ATS guidelines [205]. The patients were instructed to walk as far as possible, turning 180° every 30 metres in the allotted time of 6 minutes. The total distance walked was measured to the nearest metre, and recorded. Sitting HR, lactate level, and SpO_2% were measured before and immediately after the test while in a resting position.

Interval Training Programme

Following a 5-minute warm-up at 5-10 W, the patients undertook approximately 45 minutes of interval training, followed by a 5-minute recovery period. Each interval training session involved a period of high-intensity cycling (HIC) at 70% peak WR followed by 60 seconds of low-intensity cycling (LIC) at 35% peak WR. During

each training session the patients wore a nasal cannula through which medical oxygen (100%) or compressed air were delivered continuously at 3 $L \cdot min^{-1}$.

The first week was used to familiarize patients with the training protocol. HR and SpO_2% were measured at rest and throughout each training programme. Whole blood lactate levels were measured at rest and during the 15 seconds of the final HIC interval phase of each training session. In addition, RPE, dyspnea, and leg fatigue were also measured during the 15 seconds of the final HIC interval phase of each training session.

Statistical Analysis

Baseline anthropometric, pulmonary function, $\dot{V}O_2$peak, peak workrate, total endurance time, and muscle strength data were compared between the two experimental groups using a Mann-Whitney U test. Within group, pulmonary and metabolic responses at peak exercise and at the end of the light and moderate load exercise sessions were compared using Wilcoxan sign rank test. Pulmonary and metabolic responses during the post training constant load moderate (70% $\dot{V}O_2$peak) exercise test were compared to the time the subject stopped exercising during the baseline test (isotime). Significance was accepted at the level of $p<0.05$. Values are presented as mean ± SD. Data were analysed using SPSS (v17.0, SPSS Inc., IL).

Results

Patient Characteristics and Intervention Compliance

Nine of the original 11 participants completed the study. One patient was excluded due to low compliance with the training programme and the other was excluded due to an atrioventricular re-entrant tachycardia. Training sessions had to be rescheduled for 3 patients (2 from the O_2-Suppl group) due to illness. The compliance rate was 100% for the 9 patients who completed the study.

Pulmonary function and LBM for each individual patient are shown in Table 5.1. Age and height were similar in both groups; 28±6 years and 168±5 cm for the O_2Suppl group, and 27±4 year and 170±8 cm for the placebo group, respectively.

Table 5.1: Pulmonary function and LBM for each individual patient before training

	LBM (kg)	**FEV_1% pred**	**FVC % pred**	**D_LCO % pred**	**$\dot{V}_E$/MVV (%)**
O_2Suppl Group					
1	45	31	65	62	94
2	50	86	98	84	72
3	53	44	71	79	117
4	54	84	102	93	59
5	45	45	80	74	88
Mean±SD	**49.6±4.4**	**58.0±25.2**	**83.6±16.2**	**78.4±11.5**	**85.9±22.8**
Placebo Group					
1	39	31	45	47	105
2	50	40	70	95	85
3	41	46	56	91	81
4	72	109	114	100	53
Mean±SD	**50.9±14.9**	**56.5±35.5**	**71.2±30.2**	**83.2±24.4**	**81.4±21.3**

Workrate and HIC Duration

The LIC WR and HIC WR remained constant in the O_2Suppl group (40±22 W and 80±43 W) and the placebo group (42±20 W, 84±58 W) throughout the training period. The total duration of HIC increased ($p<0.05$) by 150% in the O_2Suppl group between week 1 and week 8 (11±2.6 vs.29±2) (Figure 5.1). There was no change in the total duration of HIC between week 1 and week 8 in the placebo group (15±4vs. ±24±9). Blood lactate levels (Figure 5.2) and % peak HR (Figure 5.3) were similar during the final HIC repetition of the first and last training session.

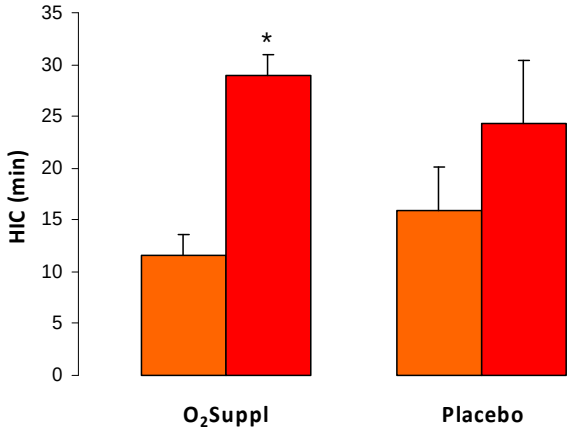

Figure 5.1: Total duration of HIC in the O_2Suppl group and the placebo group between week 1 and week 8 of the training programme
(*p <0.05 vs baseline)

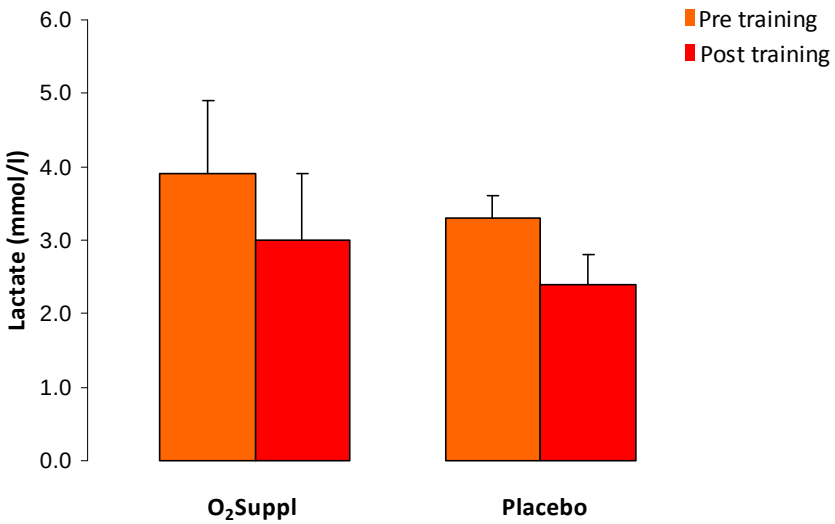

Figure 5.2: Blood lactate level during the final HIC repetition of the last training session in the O_2Suppl group and the placebo group between week 1 and week 8 of the training programme

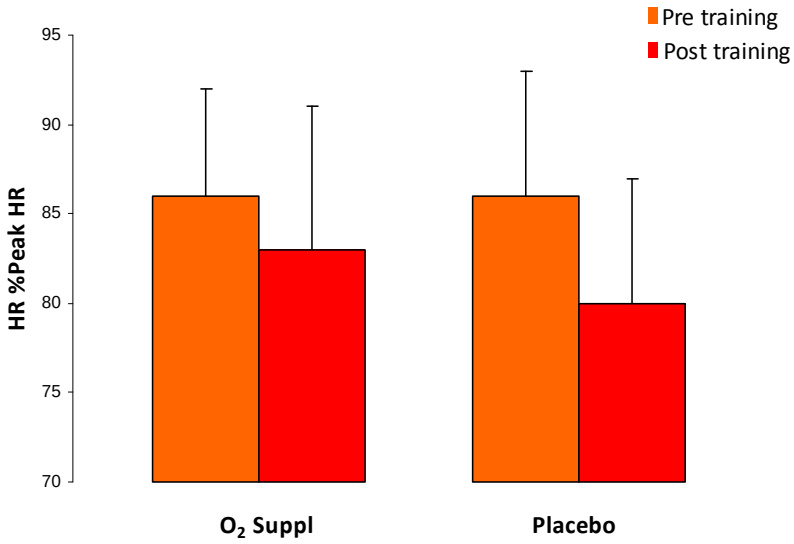

Figure 5.3: Percent of peak heart rate during the final HIC repetition of the last training session in the O_2Suppl group and the placebo group between week 1 and week 8 of the training programme

Exercise Training and Pulmonary Function

There was no difference in weight, LBM or pulmonary function between the two experimental groups at baseline (Table 5.2). Compared to baseline values, FEV_1 % predicted and FVC% predicted were reduced by 8% and 6% respectively ($p < 0.05$) in

the O_2Suppl group, and did not change in the placebo group following the training programme (Table 5.2). Exercise training had no effect on any of the other anthropometric or pulmonary function measurements.

Table 5.2: Weight, height and pulmonary function at rest before and after training

	Experimental Group			
	O_2 Suppl group		**Placebo group**	
	Pre training	**Post training**	**Pre training**	**Post training**
Weight (kg)	58.6±6.8	57.8±7.6	64.2±17.5	64.9±18.0
Lean body mass (kg)	49.6±4.4	48.9±5.1	50.9±14.9	50.7±15.0
FEV_1(L)	2.2±1.1	2.1±1.2 ‡	2.2±1.7	2.2±1.4
FEV_1%	58.0±25.2	53.6±25.8 ‡	56.5±35.5	55.0±27.7
FVC (L)	3.7±1.0	3.5±1.1 ‡	3.2±2.0	3.2±1.8
FVC (% predicted)	83.6±16.2	78.0±16.9 ‡	71.2±30.2	72.0±26.5
FEV_1/FVC	57.2±15.1	55.8±17.0	66.2±13.4	64.0±10.3
D_LCO ml·min^{-1}·mmHg^{-1}	24.9±5.8	25.8±4.8	26.8±10.6	29.0±11.6
D_LCO (% predicted)	78.4±11.5	80.0±11.1	83.2±24.4	90.5±28.2

Values are mean ± SD; ‡ p <0.05 vs. ore training

Metabolic, Pulmonary and Perceptual Responses at Peak Exercise

Metabolic, pulmonary and perceptual responses during peak exercise were similar in both experimental groups at baseline and did not change in response to training (Table 5.3).

Table 5.3: Metabolic, pulmonary and perceptual responses at peak exercise before and after training

	Experimental Group			
	O_2 Suppl group		Placebo group	
	Pre training	Post training	Pre training	Post training
$\dot{V}O_2$ peak ($ml \cdot kg^{-1}LBM \cdot min^{-1}$)	31.9±11.1	33.4±11.2	32.1±5.0	33.8±10.5
Workrate (watts)	116.6±59.4	130.6±70.4	120.7±71.2	137±100.7
Lactate ($mmol \cdot L^{-1}$)	6.2±1.8	6.2±2.4	6.4±3.1	6±2.1
RER	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1
$\dot{V}_E$ ($L \cdot min^{-1}$)	71.3±26.7	71.9±36.3	62.5±29.3	60.6±27.6
$\dot{V}_E$/MVV (%)	85.9±22.8	90.1±21.1	81.4±21.3	77.7±17.4
$V_{T,}$ (L)	1.5±0.8	1.5±0.9	1.6±0.8	1.7±1.3
RR ($f \cdot min^{-1}$)	48.6±9.0	49.3±8.6	44.3±12.1	43.0±13.2
$P_{ET}CO_2$ (mmHg)	35.4±3.1	36.4±4.9	39.8±4.4	41.3±5.4
$\dot{V}_E/\dot{V}CO_2$ @AT	35.7±3.9	35.1±3.8	30±4.8	30±4.3
SpO_2%	93.4±4.3	89.6±7.1	92.5±6.4	91.0±4.7
RPE overall score	15.4±2.5	15.6±3.0	16.0±1.4	16.5±1.0
Dyspnea score	6.6±2.1	6.2±2.3	6.0±1.2	6.2±0.9
Leg fatigue score	7.4±1.9	7.0±2.5	6.0±1.8	6.7±1.7

Values are mean ± SD; ‡ $p < 0.05$ within groups pre and post training

Constant Load Exercise Response

Physiological and perceptual responses during light intensity constant load exercise are presented in Table 5.4. Whole blood lactate, $\dot{V}_{E,}$ and RR were lower ($p<0.05$) at week 8 than baseline in the O_2Suppl group. MRT and O_2D decreased by 22% ($p < 0.05$) and 26% ($p < 0.05$) respectively in the O_2Suppl group following exercise training. Compared to baseline, there was no change in any of the physiological and perceptual responses at week 8 in the placebo group.

Table 5.4: Physiological and perceptual responses during light intensity constant load exercise

	Experimental Group			
	O_2 Suppl group		Placebo group	
	Pre training	Post training	Pre training	Post training
Workrate (W)	37.0±21.0		41.0±23.0	
Time (min)	10.0		10.0	
$\dot{V}_E$ ($L \cdot min^{-1}$)	32.4±2.3	28.1±4.6‡	29.2±2.6	30.2±3.6
$V_{T,}$ (L)	1.0±0.4	1.0±0.4	1.2±0.5	1.1±0.5
RR($f \cdot min^{-1}$)	34.8±11.3	29.6±4.6‡	30.7±12.5	31.2±12.2
$P_{ET}CO_2$ (mmHg)	37.0±2.3	38.8±1.3	38.2±6.6	38.5±6.9
SpO_2%	94.0±1.5	94.4±2.4	94.5±2.6	93.5±2.6
Lactate ($mmol \cdot L^{-1}L$)	2.6±0.8	1.5±0.3‡	1.5±0.2	1.6±2.1
RPE overall score	11.0±1.4	9.8±1.3	10.0±2.9	10.7±2.6
MRT (s)	44.4±9.0	34.0±11.4‡	45.0±16.5	39.2±14.3
O_2D (L)	0.4±0.1	0.3±0.1‡	0.4±0.1	0.33±0.4

Values are mean ± SD; ‡ $p < 0.05$ within groups pre and post training

Effect of Training on Moderate Constant Load Exercise Response

Physiological and perceptual responses during the final 30 seconds of moderate intensity constant load exercise are presented in Table 5.5. Endurance capacity, measured as an increase in exercise time in the constant WR test improved ($p<0.05$) in the O_2Suppl group (Figure 5.4), and did not change in the placebo group (Figure 5.5). The improvement in endurance performance was 65% greater in the O_2Suppl group than in the placebo group. Compared to baseline, $\dot{V}_E$ decreased by 9% at week 8 and there was no change in any of the physiological and perceptual responses during the final 30 seconds of moderate intensity constant load exercise in the placebo group.

Table 5.5: Physiological and perceptual responses during the final 30 seconds of moderate intensity constant load exercise

	Experimental Group			
	O_2 Suppl group		Placebo group	
	Pre training	Post training	Pre training	Post training
Workrate (W)	79±41		88±55	
Time (min)	11.2±2.2	24.5±6.3‡	11.5±5.8	17.8±0.5
HR ($b \cdot min^{-1}$)	155.2±14.2	160.0±11.3	149.0±11.8	149.7±17.7
$\dot{V}_E$ ($L \cdot min^{-1}$)	57.6±16.9	52.6±13.4‡	46.0±8.4	46.5±11.8
$V_{T,}$ (L)	1.4±0.7	1.4±0.62	1.6±0.9	1.4±0.77
RR($f/\cdot min^{-1}$)	41.6±8.0	41.6±7.9	35.5±14.3	40.0±17.7
$P_{ET}CO_2$ (mmHg)	37.0±3.6	37.2±3.8	42.5±10.3	42.0±8.1
SpO_2%	90.2±5.6	89.4±4.4	90.2±8.9	89.5±8.3
Lactate ($mmol \cdot L^{-1}$)	6.3±1.4	5.3±1.3	4.7±0.7	3.8±0.4
RPE overall score	14.4±1.9	14.3±6.2	13.7±1.5	15.2±2.3
Dyspnea score	5.4±1.9	5.2±2.2	4.0±1.1	5.7±2.0
Leg fatigue score	5.2±1.8	4.6±1.5	4.4±1.0	4.7±1.0

Values are mean ± SD; ‡ p <0.05 within groups pre and post training

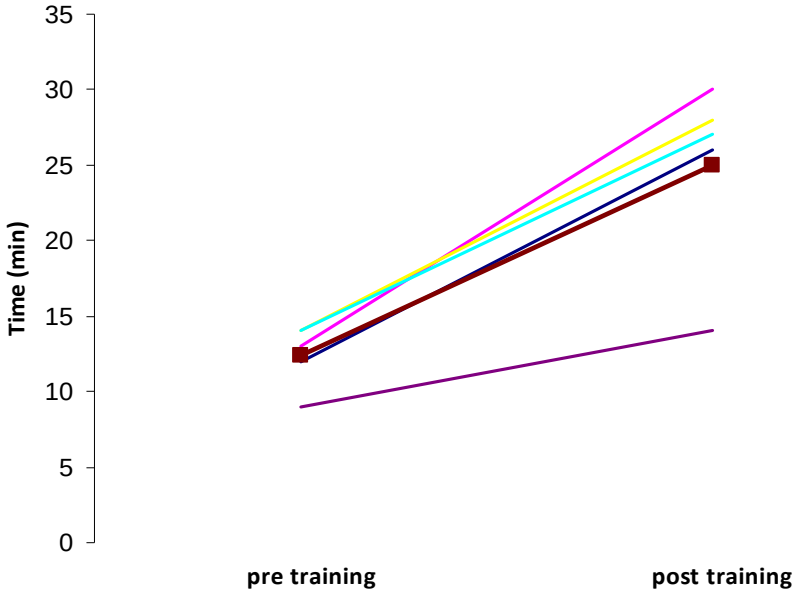

Figure 5.4: Total exercise time for identical moderate work rate before (pre training) and after (post training) the training programme for each individual in the O_2 Suppl group. The bold line represents the mean group value.

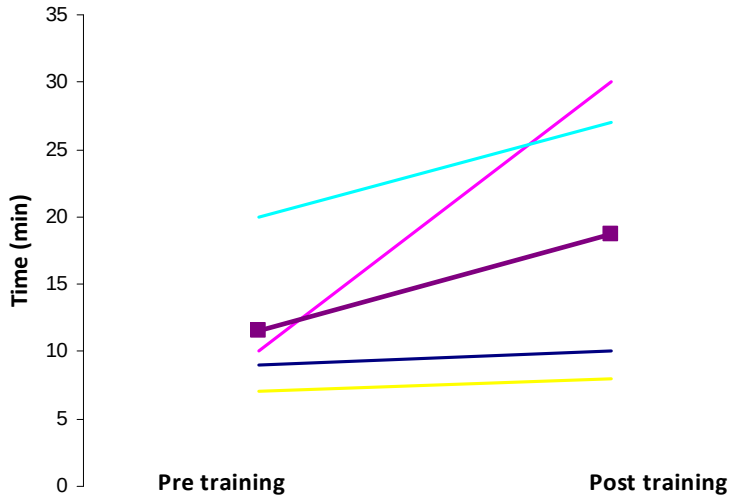

Figure 5.5: Total exercise time for identical moderate work rate before (pre training) and after (post training) the training programme for each individual in the placebo group. The bold line represents the mean group values.

Effect of Training on the Isotime Physiological and Perceptual Responses during Moderate Intensity Exercise

Pulmonary, metabolic and perceptual responses during the post training constant moderate intensity (70% $\dot{V}O_2$peak) exercise test were compared to the time the subject stopped exercising during the baseline test (isotime) (Table 5.6.). In the O_2Suppl group, $\dot{V}_E$ decreased by 15% ($p < 0.05$) (Figure 5.6), RR decreased by 12% ($p < 0.05$) (Figure 5.8), and blood lactate decreased by 28% ($p < 0.05$) (Figure 5.10) at the same time point that the subject stopped exercising during the baseline test. The decrease in $\dot{V}_E$ was accompanied by a 21% decrease in RPE overall score ($p < 0.05$). There was no change in $\dot{V}_E$ (Figure 5.7), RR, (Figure 5.9) and lactate level (Figure 5.11) in the placebo group in response to training.

Table 5.6. Response to moderate intensity constant work rate exercise at identical workrate and exercise duration (isotime)

	Experimental Group			
	O_2 Suppl group		**Placebo group**	
	Pre training	**Post training**	**Pre training**	**Post training**
Subjects (n)	5		4	
Workrate (W)	79±41		88±55	
Time (min)	11.2±2.2		11.5±5.8	
HR ($b \cdot min^{-1}$)	155.2±14.2	152.2±11.7	149.0±11.8	144.0±11.1
O_2 pulse ($ml \cdot beat^{-1}$)	10.0±1.5	9.6±1.4	9.9±1.4	10.0±1.4
$\dot{V}_E$ ($L \cdot min^{-1}$)	57.6±16.9	48.0±11.7‡	46.0±8.4	44.5±10.1
$V_{T,}$ (L)	1.4±0.7	1.4±0.62	1.6±0.9	1.4±0.9
RR($f \cdot min^{-1}$)	41.6±8.0	35.8±6.7‡	35.5±14.3	36.7±16.2
$P_{ET}CO_2$ (mmHg)	37.0±3.6	37.2±6.2	42.5±10.3	40.7±7.1
$\dot{V}_E/\dot{V}CO_2$	33‡2.9	30±2.9‡	30±6	30±4
SpO_2%	90.2±5.6	90.6±3.7	90.2±8.9	89.0±6.7
Lactate ($mmol \cdot L^{-1}$)	6.3±1.4	4.4±0.8‡	4.7±0.7	4.4±0.5
RPE overall score	14.4±1.9	11.8±1.7‡	13.7±1.5	14.7±2.8
Dyspnea score	5.4±1.9	4.0±1.0	4.0±1.1	4.5±1.7
Leg fatigue score	5.2±1.8	4.1 ±1.1	4.4±1.0	6.2±1.3

Values are mean ± SD; ‡ $p < 0.05$ vs pre training

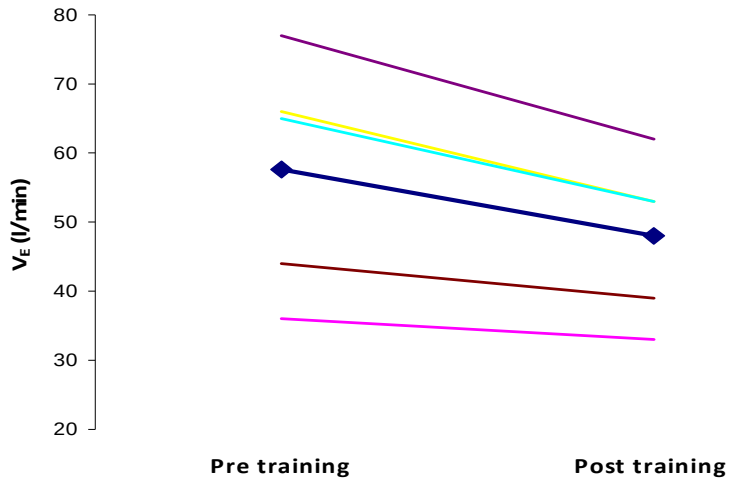

Figure 5.6: Minute ventilation at identical moderate work rate and exercise duration (isotime) before (pre training) and after (post training) the training programme for each individual in the O_2Suppl group.
The bold line represents the mean group value.

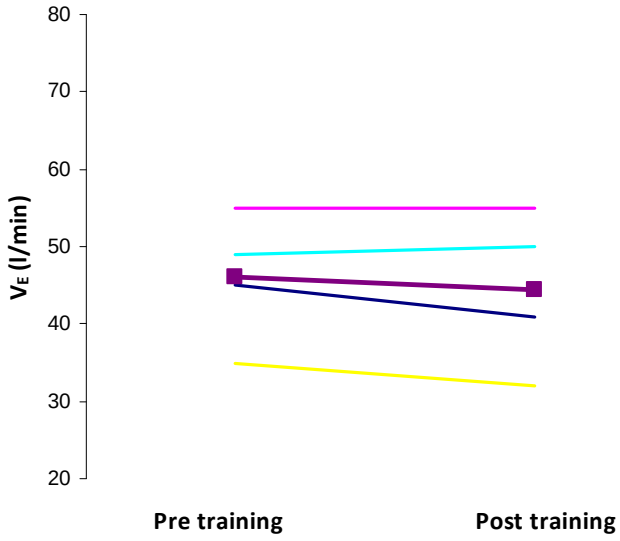

Figure 5.7: Minute ventilation at identical moderate work rate and exercise duration (isotime) before (pre training) and after (post training) the training programme for each individual in the placebo group.
The bold line represents the mean group value.

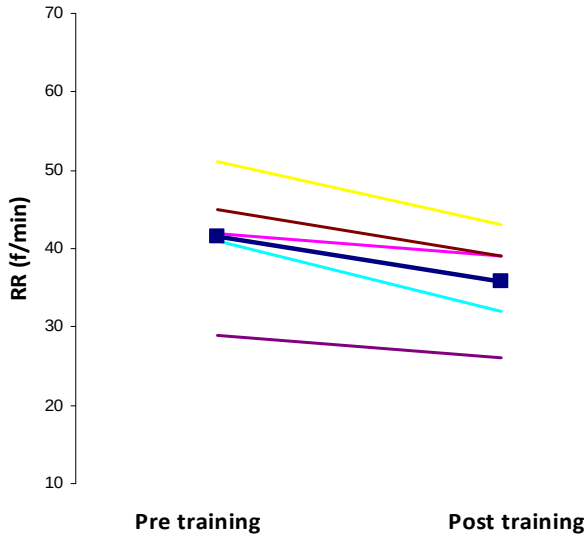

Figure 5.8: Respiratory rate at identical moderate work rate and exercise duration (isotime) before (pre training) and after (post training) the training programme for each individual in O_2Suppl group.
The bold line represents the mean group value.

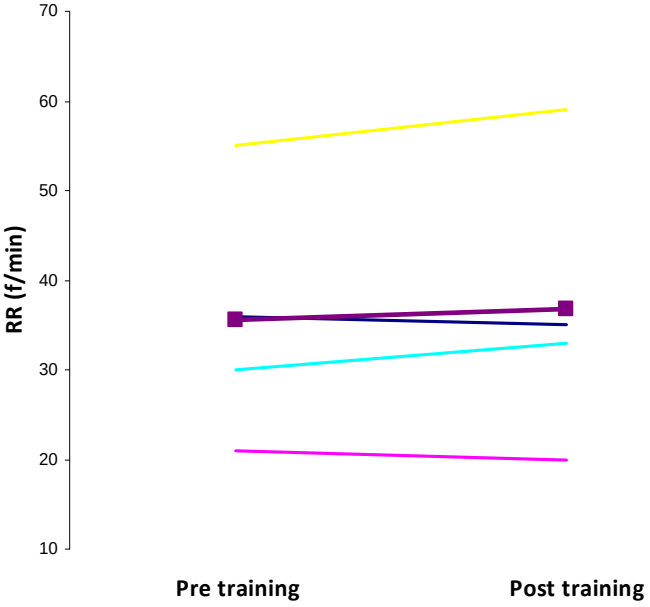

Figure 5.9: Respiratory rate at identical moderate work rate and exercise duration (isotime) before (pre training) and after (post training) the training programme for each individual in the placebo group.
The bold line represents the mean group value.

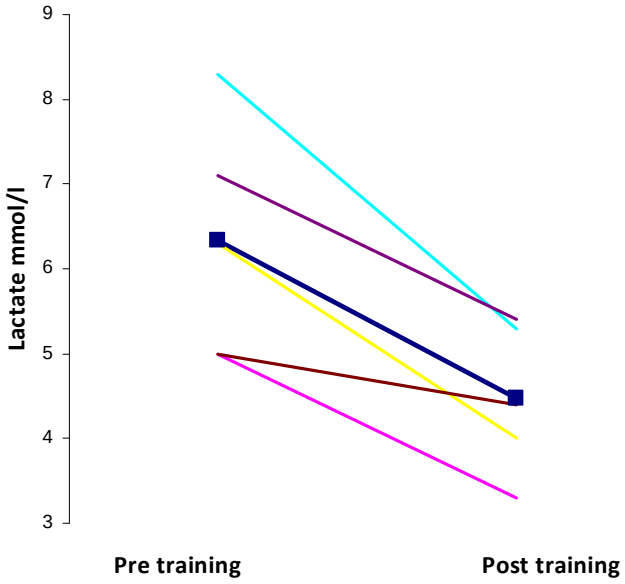

Figure 5.10: Lactate level at identical moderate work rate and exercise duration (isotime) before (pre training) and after (post training) the training programme for each individual in the O_2Suppl group.
The bold line represents the mean group values.

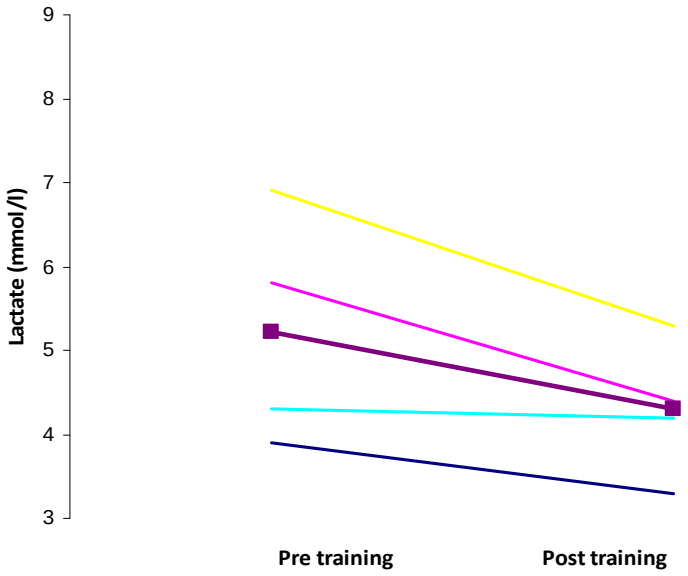

Figure 5.11: Lactate level at identical moderate work rate and exercise duration (isotime) before (pre training) and after (post training) the training programme for each individual in the placebo group.
The bold line represents the mean group values.

Muscle Strength

There was no difference in muscle strength or muscle endurance between the two experimental groups at baseline. There was no change in muscle strength or muscle endurance in either group in response to the interval training programme (Table 5.6).

6 Minute Walk Test

There was no difference in the total distance covered between the two experimental groups at baseline. Compared to baseline, there was an 8% increase ($p<0.08$) in total distance covered in the O_2Suppl group and a 2% decrease in the placebo group (Table 5.6).

Table 5.6: Effect of exercise training on muscle strength

	Experimental Group			
	O_2 Suppl group		Placebo group	
	Pre training	**Post training**	**Pre training**	**Post training**
Subjects (n)	5		4	
Peak torque 60°/s extension (N-m)	107±46	119±36	135±65	128±55
Peak torque 60° isometric (N-m)	113±37	128±25	141±117	147±72
Total endurance Work (J)	1569±619	2005±545	2056±1468	1958±1423
6 minute walking test (m)	580±84	623±58	632±91	619±104

Values are mean ± SD

Discussion

To our knowledge, this pilot study is the first to examine the effects of exercise training with supplemental O_2 on maximal and submaximal exercise performance in CF patients. Patients who were provided with supplemental O_2 were able to maintain the interval training intensity for longer periods compared to patients in the placebo group. The duration of high intensity interval cycling increased by 150% in the O_2Suppl group during the 8-week training programme compared to 53% in the placebo group. Improvements in endurance capacity and ventilatory response to exercise were only evident in the group that received supplemental O_2.

Previous studies have examined the effect of supplemental O_2 on acute exercise performance in patients with CF and chronic obstructive pulmonary disease (COPD). McKone et al., [12] found that exercise duration increased by 26% during a single bout of exercise at 80% peak work rate in patients with moderate to severe CF. They speculated that the improvement in exercise performance may be due to an alteration in ventilatory response or an improvement in O_2 delivery to the exercising muscle. Supplemental O_2 also increases exercise duration during a single bout of exercise at 50% and 75% peak work rate in COPD patients [26-27]. The improvement in acute exercise capacity may to be related to a delay in the initiation of ventilatory limitation as a result of reduced ventilatory demand.

A number of potential mechanisms may help to explain how supplemental O_2 may reduce the ventilatory response during acute exercise in patients with COPD. Firstly, supplemental O_2 may diminish hypoxic drive from peripheral chemoreceptors

due to an increase in PaO_2 resulting in a decrease in respiratory rate [151]. Secondly, the reduced metabolic acidosis as a result of improved arterial O_2 content to the active muscles may decrease carotid body stimulation [170]. Increasing the time available for expiration (T_E) may also reduce RR which may delay the extent of dynamic hyperinflation (DH) [26-29]. Thirdly, pulmonary vasodilatation induced by O_2 may increase CO and muscle O_2 delivery, reduce lactic acid production, and decrease carotid body stimulation [206].

Studies that have examined the effect of exercise training with supplemental O_2 in patients with COPD have been equivocal [17, 30-31]. Rooyackers et al., [207] and Garrod et al., [208] failed to find an additional benefit of supplemental O_2 on WR during training, or peak exercise performance in patients with advanced COPD and exercise hypoxemia. The design of these studies may have contributed to the negative result. In the Rooyackers et al., study, the O_2 training group did not achieve a higher training intensity than the control group. The interval nature of the programme (maximum 3 minute exercise interval followed by a 2 minute rest interval) may have allowed the subjects in the control group to maintain SpO_2% above 90% while exercising at a relatively high work rate. The fact that workrate was not allowed to exceed the level at which SpO_2% fell below 90% may have blunted the potential training benefit.

Improvements in exercise capacity in response to exercise training have been shown to be related to the intensity and duration of training [209]. Subjects in the study by Garrod et al., [208] undertook 45 minutes of exercise training per week for 6

weeks at 80% of the VO_2 peak achieved during a shuttle walk test. No information was provided on the specifics of the training programme, or whether or not the subjects achieved the prescribed training intensity.

Emtner el al., [17], used a double blind randomized control study design to compare the effects of 7 weeks of exercise training with and without supplemental O_2 in patients with severe COPD who did not experience hypoxemia during exercise. Subjects exercised 3 times per week for 45 minutes on a cycle ergometer. Training with supplemental O_2 allowed the patients to train at a higher intensity and improve submaximal exercise performance compared to training under normal atmospheric conditions. The RR was lower and the V_T higher during constant load submaximal exercise under normal atmospheric conditions following the 8-week training programme with supplemental O_2.

In the present study, the circulating levels of blood lactate were the same in both groups during the final HIC repetition at the end of the first training session and the final HIC repetition at the end of the last training session. The fact that blood lactate values remained at a similar level despite a significant increase in HIC interval time over the course of the training programme provides evidence of a physiological training effect in the O_2Suppl group. Compared to pre-training values at the isotime point, V_E, RR, and blood lactate levels were lower in the O_2Suppl group during the post training light constant load exercise test and the moderate constant load exercise test.

In participants who were ventilatory limited during exercise, a reduction in ventilatory demand at a moderate intensity WR may help to explain the improvement

in exercise tolerance following the 8 weeks of exercise training with supplemental O_2. A reduction in the ventilatory demand would allow these patients to exercise for a longer duration before reaching the point of ventilatory limitation. The reduction in ventilatory demand may be due in part to a delay in lactate accumulation. Although arterial blood gases were not measures, it is possible that the decrease in lactate accumulation at the same absolute WR may have resulted in a decrease in arterial hydrogen (H^+) concentration. Lower levels of circulating hydrogen ions should decrease carotid body stimulation and subsequently decrease $\dot{V}_E$ [209].

The reduction in RR may have also contributed to the decrease in ventilatory demand, leading to improved exercise performance in CF patients following exercise training with supplemental O_2. Since 3 of the patients in the O_2Suppl group had mild to moderate hypoxemia during exercise, the reduced RR may have been related to an inhibition of carotid body stimulation resulting from the availability of supplemental O_2 during training. Slowing the RR may lead to reduced dynamic hyperinflation (DH). Since the extent of DH during exercise in flow-limited patients depends on ventilation and the breathing pattern for a given ventilation, a reduction in ventilatory demand by reducing RR should delay the onset of DH. O'Donnell et al., [210] found that a small reduction in ventilation decreased dynamic lung volumes that translated to improvements in symptoms and exercise performance, even in mildly hypoxemic COPD patients [59]. Alison at el., [34] showed that DH occurs in CF patients with FEV_1 <60% predicted. It is possible that the decrease in RR during low and moderate constant load exercise may be associated with a reduction in DH, thus contributing to the overall training benefit in the hypoxemic patients in the O_2Suppl group.

Increasing O_2 delivery to exercising muscles is another putative mechanism that may help to explain the beneficial effect of O_2 therapy during exercise training. Improved muscle O_2 delivery during exercise may attenuate lactic acidosis and, thereby, reduce ventilatory stimulation. As peak O_2 pulse and peak $\dot{V}O_2$ did not change following exercise training in either group, it is unlikely that cardiovascular adaptations could explain the improvement in exercise endurance in the O_2Suppl group following exercise training. Furthermore, the reduction in $\dot{V}_E/\dot{V}CO_2$ and blood lactate levels accompanied by no change in PE_TCO_2 or SPO_2% at isotime exercise, may indicate a greater peripheral (muscle) than central contribution (pulmonary and cardiovascular) to the improvement in exercise performance.

Slow O_2 uptake kinetics have previously been found during acute exercise in patients with CF [146]. The present study is the first to provide information on O_2 uptake kinetics in CF patients following a training programme. MRT was used to determine the O_2 uptake kinetics. MRT is a combination of phase I and subsequent exponential phase II, and is dependent on O_2 delivery to the exercising muscle and muscle O_2 metabolism.

The acceleration in O_2 uptake kinetics found in the O_2Suppl group following the training programme indicates an improvement in skeletal muscle and/or cardiovascular function. Intramuscular changes associated with high intensity training may lead to more efficient oxidative metabolism and faster intramuscular O_2 uptake kinetics following exercise training in healthy individuals [211]. Furthermore, a reduction in lactate level at light and moderate constant load exercise may be

associated with faster O_2 uptake kinetics, indicating an improvement in aerobic metabolism following the training programme. It is possible that the failure to alter O_2 uptake kinetics in the placebo group was due to the fact that they were unable to sustain an exercise intensity required to achieve a sufficient overload to stimulate adaptive changes in the skeletal muscle. It is possible that exercise training with supplemental O_2 may be related to a decrease in ventilatory demand secondary to adaptive changes in skeletal muscle. Although lung volumes were not measured in the present study, it is possible that a decrease in DH may have also contributed to the improvement in endurance performance in response to exercise training with supplemental O_2.

A major limitation of the present study is the small sample size. This was primarily due to the limited patient pool and the time commitment involved in participating in the study. The present findings although interesting and provocative should be considered as preliminary. More extensive research with a larger sample size should be undertaken before recommending the use of supplemental O_2 during exercise training in patients with CF. This preliminary study was designed primarily to assess the effect of supplemental O_2 during exercise training on $\dot{V}O_2$peak, O_2uptake kinetics, endurance performance, and muscle strength in patients with CF. Considering that the study findings indicate a beneficial effect of using supplemental O_2 during exercise training in CF, future studies should examine the putative mechanisms responsible for the enhance training benefits.

Summary

Compared to 8 weeks of HIIT while breathing normal room air, 8 weeks of HIIT with supplemental O_2 resulted in a greater improvement in training volume, O_2uptake kinetics and endurance performance in patients with mild, moderate and severe CF. There was no change in $\dot{V}O_2$peak, muscle strength or muscle endurance in either group in response to the training programme. These finding,s although preliminary and based on a small sample size, indicate that the provision of supplemental O_2 during exercise training may improve endurance capacity in patients with CF.

Chapter 6

Conclusions and Recommendations

The purpose of this research was to compare the exercise response in patients with different CF severities using non- invasive methods, and to examine the effect of exercise training with and without O_2 supplementation on exercise performance. Preliminary evidence is also provided on the beneficial effect of supplemental O_2 during exercise in CF patients.

The results provide important information on both submaximal and maximal exercise response in different CF severities. Evidence is provided to indicate that peak exercise capacity is reduced in individuals with mild, moderate and severe CF compared to healthy controls. Although the sample size was relatively small there was evidence that abnormal mechanical ventilation, reduced pulmonary gas exchange and reduced cardiovascular response may each contribute to exercise limitation in CF patients with FEV_1 <30. The internal and external validity of these findings can be strengthened by using a larger sample size, and employing more invasive techniques to investigate putative mechanisms. For example, measuring DH, which is considered an indication of dynamic ventilatory mechanics may help to explain the reduction in V_T/VC. Therapies such us supplemental O_2 and the use of inhaled bronchodilators before or during exercise partially reverses DH and may represent the first step in improving dyspnea and exercise capacity. Future research is needed to provide a clinical validation of physiological concepts supporting the role of DH on functional status in CF.

Mechanical support ventilation is another intervention that may improve exercise tolerance in CF patients by allowing them to exercise at a higher exercise intensity level. Unloading the respiratory muscle by mechanical support ventilation has been shown to improve blood flow and O_2 transport to the contracting peripheral muscles while reducing quadriceps fatigability in athletes [61, 212]. The interaction between peripheral muscle function and unloading of the respiratory system is an area that requires further research in CF.

In addition to O_2 pulse, the measurement of ccardiovascular and pulmonary hemodynamics would provide a greater insight into the cardiovascular response to exercise in CF. Echocardiography can provide information on CO and pulmonary pressure during exercise. It may help to determine whether a pulmonary vascular response can be considered as a limiting factor in severe CF.

Functional, morphological and biochemical alterations in skeletal muscle due primarily to physical inactivity have been shown to contribute to exercise intolerance in CF patients. In addition, there is also evidence that the impaired exercise capacity in CF may be related that an intrinsic abnormality in muscle function independent of deconditioning. For example, abnormal expression of CFTR in skeletal muscle is associated with a reduction in both aerobic and anaerobic metabolism. The current study used non-invasive methods to assess muscle function, and was not designed to investigate the mechanism responsible for the alterations in muscle performance. Future studies using NMR spectroscopy or muscle biopsy samples should help to distinguish between muscle deconditioning and intrinsic muscle abnormality in CF. In

addition, the morphological, biochemical and molecular changes in response to acute and chronic exercise in individuals with CF should be investigated.

The improvement in endurance capacity and ventilatory response following 8 weeks of exercise training with supplemental O_2 is a novel finding and clinically relevant considering the importance of aerobic fitness in predicting morbidity and mortality. However, the small sample size limits the external validity of the results. The study should be replicated with larger sample size. In addition, the optimal training intensity, duration and frequency when using supplemental oxygen should be determined. If the results of the present study can be replicated in adults, it would be interesting to evaluate the efficacy of supplemental oxygen in children. The development of appropriate strategies to help maintain the training effect in CF should also be investigated.

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Appendix A

CONSENT FORM

Protocol Title:

Effect of Disease Severity on Indices of Fitness in Individuals with Cystic Fibrosis

Please tick the appropriate answer.

I confirm that I have read and understood the Patient Information Leaflet dated 14 October, 2007 attached, and that I have had ample opportunity to ask questions all of which have been satisfactorily answered.

Yes
No

*I understand that my participation in this study is entirely **voluntary** and that I may withdraw at any time, without giving reason, and without this decision affecting my future treatment or medical care.*

Yes
No

I understand that my records may be viewed by individuals with delegated authority from Beaumont Hospital

Yes
No

I understand that my identity will remain confidential at all times.

Yes
No

I am aware of the potential risks of this research study.

Yes

No

I have been given a copy of the Patient Information Leaflet and this Consent form for my records.

Yes
No

FUTURE USE OF ANONYMOUS DATA:

I agree that I will not restrict the use to which the results of this study may be put. I give my approval that unidentifiable data concerning my person may be stored or electronically processed for the purpose of scientific research.

Yes
No

Patient _____ *_____*

Signature *Name in block capitals*

Date:_____

To be completed by the Principal Investigator or his nominee.

I the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a manner that he/she could understand. I have explained the risks involved, the experimental nature of the treatment, as well as the possible benefits and have invited him/her to ask questions on any aspect of the study that concerned them.

_____ _____ _____ _____

Signature: ***Name in Block Capitals:*** ***Qualification:*** ***Date:***

Appendix B

SCREENING VISIT DATA

Name: _____ **Date:** _____

Subject ID: _____

Date of Birth: ____/____/____ **Age:**_____ yrs

Informed consent signed:

Medication list:

Nebulized Medication: _____

Inhaled Medication: _____

Oral Medication: _____

Height: _____ cm RHR:_____ bpm

Weight: _____ kg BP: _____/_____ mmHg

Skinfolds and % Body Fat Measurement

Directions

Use Cambridge Scientific Industries L Skinfold Calipers.
Take measurements on right hand side of the body and rotate sites.

Tester Name: _____

Site	1	2	3	AVG
Tricep				
Subscapular				
Chest				
Midaxillary				
Abdominal				
Suprailiac				
Thigh				
Sum of 7 AVG's (X)				

BD = 1.112 – 0.00043499(sum of 7 skinfolds) + 0.00000055$(x)^2$ – 0.00028826(Age)
X = Sum of 7 site AVG
A = Age in years.
BD=_____.
White males aged 20 – 80. BF% = ((4.95/body density) – 4.50) * 100
White males aged 17 – 19. BF% = ((4.99/body density) – 4.55) * 100

% Body Fat =

% Lean Body Mass =

Spirometry:

	Absolute	% Predicted
FEV1:	_____	_____
FVC:	_____	_____
MVV	_____	_____
D_LCO:	_____	_____

Comments:

Fasting 4 h Yes ☐ No ☐

Resting blood sample: Yes ☐ No ☐

Calibration: Yes ☐ No ☐

O2 Adjust Yes ☐ No ☐

Flowmeter (0.9-1.1) Old _____ New _____

Gas Delay (1400-1700ms) Old _____ New _____

(Gas Delay variance should be no more than 20-40 ms)

Print calibration: Yes ☐ No ☐

Protocol: _____

Stage	**Min**	**Workload**	**RPE-L**	**RPE-C**	**RPE-O**	**Lactate**	**HR**	**BP**
Rest	0-3							
Warm-up	3-6							
Exercise								
1	6-7							
2	7-8							
3	8-9							
4	9-10							
5	10-11							
6	11-12							
7	12-13							
8	13-14							
9	14-15							
10	15-16							
11	16-17							
12	17-18							
13	18-19							

14	19-20							
15	20-21							
R2	22-23							
R5	25-26							
R7	28-29							

Peak Data:

Total time: _____ sec

HR@ AT: _____ bpm

Workload: _____ Watts

%HR@AT _____

VO_2: _____ L/min

VO_2@AT _____ L/min

RER: _____

%VO_2@AT _____

HR: _____ bpm

WL@AT _____ Watts

RPE-O: _____

HR@ LT: _____ bpm

Ve: _____ L/min

%HR@LT _____

Lactate _____ mmol/L

VO_2@LT _____ L/min

AT % VO2 predicted: _____ l/min

%VO_2@LT _____

Comments:

WL@LT _____ Watts

HR@ Vequiv: _____

%HR@Vequiv _____

VO_2@Vequiv _____ L/min

%VO_2@Vequiv_____

WL@Vequiv _____Watts

Appendix C

SUBMAXIMAL TEST DATA COLLECTION

Name: _____ **Date:** _____

Subject ID: _____

Date of Birth: ____/____/____ **Age:**_____ yrs

Height: _____ cm RHR:_____ bpm

Weight: _____ kg BP: _____/_____ mmHg

VO2 max _____ L/min

Visit _____

55% VO_2peak ☐**VO_2(L/min)** _____ **Workload (W)** _____

70% VO_2peak: ☐**VO_2(L/min)** _____ **Workload (W)** _____

Self Selected Intensity: ☐ **VO_2(L/min)** _____ **Workload (W)** _____

Fasting 4 h Yes ☐ No ☐

Resting blood sample: Yes ☐ No ☐

Calibration: Yes ☐ No ☐

O2 Adjust Yes ☐ No ☐

Flowmeter (0.9-1.1) Old _____ New _____

Gas Delay (1400-1700ms) Old _____ New _____

(Gas Delay variance should be no more than 20-40 ms)

Print calibration: Yes ☐ No ☐

Comments:

Stage	Min	Work load	RPE-L	RPE-C	RPE-O	Lactate	HR	BP
Rest	0-3							
Exercise								
1	3-4							
2	4-5							
3	5-6							
4	6-7							
5	7-8							
6	8-9							
7	9-10							
8	10-11							
9	11-12							
10	12-13							
11	13-14							
15	17-18							
16	19-20							
20	22-23							
21	23-24							
25	27-28							

30	32-33							
R2	35-36							
R5	38-39							
R7	41-42							

Comments:

Appendix D

6 Minute Walk Test

Equipment:

1. Countdown timer
2. Lap counter
3. Two small cones to mark turnaround points
4. Chair
5. Worksheets
6. O2 Source
7. Telephone
8. Defibrillator

Patient Prep:

1. Comfortable clothing
2. Appropriate shoes
3. Use of walking aids
4. Light meal in early morning/afternoon before test ok
5. No vigorous exercise 2 hours prior to test

Measurement:

1. Repeat tests should be performed at same time of day.
2. No warm up period
3. Sit resting 10 minutes prior. Check pulse, blood pressure, clothing and shoes
4. Pulse oximetry is optional
5. Stand patient and rate baseline dyspnea and overall fatigue using Borg scale.
6. Set lap counter to 0 and timer to 6 minutes.
7. Instruct patient as follows:
    a. The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk so you will exert yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop and to rest as necessary. You may lean against the wall while resting but resume walking as soon as you are able.
    b. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you, please watch the way I turn with no hesitation.
    c. Demonstrate by walking one lap
    d. Are you ready to do that? I am going to use this counter to keep track of the number of laps you complete. I will click it each time you turn around at this starting line. Remember the object of this is to walk as far as possible or 6 minutes but don't run or jog.
    e. Start on my countdown when you tell me you are ready.
8. Position patient at start line. Stand near the starting line, do not walk with the patient during the test. Start timer when patient begins to walk.

9. Do not talk to anyone during the walk, use even tone of voice and use standard phrases of encouragement. Watch the patient.
    a. Tell patient minutes left and well done or good job. Nothing more.
    b. If patient stops and needs rest, tell them they can lean against the wall and continue when they are able. Do not stop the timer. If they can not or will not continue take the chair to them to sit on and note the distance and time stopped and reason for stopping.
10. When patient returns to the start line, click the counter or mark the paper.
11. when timer is 15 seconds from end tell them in a moment you will tell them to stop, they should stop where they are and you should go to them. When timer ends tell them to stop, go to the patient and take the chair if necessary. Mark the spot with paper or object
12. Record the post walk dyspnea and fatigue levels
13. If using oximeter, measure then remove
14. Record the number of laps from counter
15. Record additional distance as number of meters. Calculate total distance walked to nearest meter and record on worksheet
16. Congratulate patient on their effort and offer a drink of water.

6 Minute Walk Test Report

Date: ____/_____/_____ Time

Lap counter __

Patient name: _____
Patient ID: _____

Gender: M / F
Age: _____
Height _____cm
Weight _____kg
BP _____/_____
Medications taken before the test (dose and time): _____
Supplemental oxygen during the test: NO / YES, flow_____L/min, type_____

	Baseline	**End of Test**	
Time			
Heart Rate			
Dyspnea			Borg scale

Fatigue			Borg scale
Lactate (before and 1 minute after)			
SpO2%			%

Stopped or paused before 6 minutes? NO YES, reason:_____

Other symptoms at end of exercise: angina dizziness hip, leg, calf pain

Number of Laps: _____ (x 60 meters) + final partial lap: _____meters =

Total distance walked in 6 minutes: _____meters

Predicted distance: _____meters Percent predicted: _____%

Comments:

Appendix E

CF Training Programme

Name **Date**

Subject ID

Informed consent form signed

Medication

Hospitalization

Peak Data

Work Rate	Watts	Anaerobic Threshold (watts)
Heart Rate	B/min	Lactate threshold (watts)
VO_2	l/min	
Ventilation	l/min	
SPO_2	%	

Target training Intensity (70% PWR)

Work Rate	Watts
Heart Rate	B/min
VO_2	l/min
Ventilation	l/min
SPO_2	%

Comments

Rest

Sessions	HR	SPO2	BP

Exercise intervals

sessions		Time	WR	HR	SPO2	RPEO	RPEC	RPE L	Lactate
1	warm	5							
	exercise	0-2							
	recovery	2-3							
2									
	exercise	3-5							
	recovery	5-6							
3									
	exercise	6-8							
	recovery	8-9							
4									
	exercise	9-11							
	recovery	11-12							
5									
	exercise	12-14							
	recovery	14-15							
6									
	exercise	15-17							
	recovery	17-18							
7									
	exercise	18-20							
	recovery	20-21							
8									
	exercise	21-23							
	recovery	23-24							

Exercise intervals

sessions		Time	WR	HR	SPO2	RPEO	RPEC	RPEL	Lactate
9									
	exercise	24-26							
	recovery	26-27							
10									
	exercise	27-29							
	recovery	29-30							
11									
	exercise	30-32							
	recovery	32-33							
12									
	exercise	33-35							
	recovery	35-36							
13									
	exercise	36-38							
	recovery	38-39							
14									
	exercise								
	recovery								
15									
	exercise								
	recovery								
16									
	exercise								
	recovery								