Assessment and Interpretation of Aerobic Exercise (Dys)function in Paediatric Patients with Cystic Fibrosis

Submitted by Zoe Louise Saynor
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as a thesis for the degree of Doctor of Philosophy in
Sport and Health Sciences,
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“…all parts of the body which have a function, is used in moderation and exercised in labours in which each is accustomed, become thereby healthy, well-developed, and age more slowly, but if unused and left idle they become liable to disease, defective in growth, and age quickly.”

- Hippocrates, 450 B.C.

“Lack of activity destroys the good condition of every human being while movement and methodological exercise save it and preserve it.”

- Plato, 350 B.C.
ABSTRACT

The purpose of this thesis was to extend our understanding of the assessment and interpretation of aerobic exercise function of paediatric patients with cystic fibrosis (CF). The first investigation sought to establish (1) the validity of traditional criteria to verify maximal oxygen (\(\dot{V}O_{2\text{max}}\)) during a maximal cardiopulmonary exercise test (CPET); and (2) the utility of supramaximal verification (S\(_{\text{max}}\)) to confirm \(\dot{V}O_{2\text{max}}\). Traditional criteria significantly underreported \(\dot{V}O_{2\text{max}}\), whilst S\(_{\text{max}}\) was shown to provide a valid measurement in this patient group. The reproducibility of this CPET protocol, over the short- (48 h) and medium- (4-6 weeks) term, was then established in study two. \(\dot{V}O_{2\text{max}}\) was repeatedly determined with no learning effect over 48 h (typical error (TE): \(\Delta150 \text{ mL}; \Delta9.3\%\)) and 4-6 weeks (TE: \(\Delta160 \text{ mL}; \Delta13.3\%\)). Supplementary maximal and submaximal CPET parameters should be incorporated for a comprehensive evaluation of a patient, however they are characterised by greater variability over time. The influence of mild-to-moderate CF on aerobic exercise function and the matching of muscle O\(_2\) delivery-to-O\(_2\) utilisation during ramp incremental exercise to exhaustion were then examined in study three. Aerobic function was impaired in CF, indicated by very likely reduced fat-free mass normalised \(\dot{V}O_{2\text{max}}\) (mean difference, ±90% CI: -7.9 mL/kg\(^{-1}\cdot\text{min}^{-1}, ±6.1\)), very likely lower \(\dot{V}O_2\) gain (-1.44 mL/min\(^{-1}\cdot\text{W}^{-1}, ±1.12\)) and a likely slower \(\dot{V}O_2\) mean response time (MRT) (11 s, ±13). Arterial oxygen saturation was lower in CF, supporting the notion that centrally mediated O\(_2\) delivery may be impaired during ramp incremental exercise. Although a faster rate of fractional O\(_2\) extraction would be expected in the face of reduced O\(_2\) delivery, this was not observed, suggesting additional impairment in O\(_2\) extraction and utilisation at
the periphery in CF. The fourth study then demonstrated the clinical utility of CPET to assess the response to 12 weeks treatment with Ivacaftor, using a case-based design. Whilst one patient with relatively mild disease demonstrated no meaningful change in \( \dot{V}O_{2\text{max}} \), the second demonstrated a 30% improvement in \( \dot{V}O_{2\text{max}} \), due to increased \( O_2 \) delivery and extraction. Furthermore, changes in aerobic function were detected earlier than spirometric indices of pulmonary function. This study demonstrated that CPET represents an important and comprehensive clinical assessment tool and its use as an outcome measure in the functional assessment of patients is encouraged. Study five investigated the \( \dot{V}O_2 \) kinetics in this patient group. During moderate intensity cycling, the phase II \( \dot{V}O_2 \) time constant (\( \tau \)) (\( p = 0.84 \), effect size (\( ES \)) = 0.11) and overall MRT (\( p = 0.52 \), \( ES=0.33 \)) were not slower in CF. However, both were slowed during very heavy intensity cycling (\( p = 0.02 \), \( ES = 1.28 \) and \( p = 0.01 \), \( ES = 1.40 \), respectively) in CF. Cardiac output and muscle deoxygenation dynamics were unaltered in CF, however, the arterial-venous \( O_2 \) content difference (\( C_{(a-\bar{v})}O_2 \)) was reduced (\( p=0.03 \)) during VH and \( \Delta C_{(a-\bar{v})}O_2 \) correlated with the phase II \( \tau \) (\( r= -0.85; \ p=0.02 \)) and MRT (\( r = -0.79; \ p=0.03 \)) in CF. This study showed that impaired oxidative muscle metabolism in this group is exercise intensity-dependent and mechanistically linked to an intrinsic intramuscular impairment, which limits \( O_2 \) extraction and utilisation. In conclusion, this thesis has provided guidelines for a valid and reproducible CPET protocol for children and adolescents with mild-to-moderate CF, demonstrated the utility of CPET as clinical outcome measure and furthered our understanding of the factors responsible for impaired aerobic exercise function in this patient group.
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<tr>
<td>A</td>
<td>amplitude of the response</td>
</tr>
<tr>
<td>ADL</td>
<td>activity of daily living</td>
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>AT</td>
<td>anaerobic threshold</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>b·min⁻¹</td>
<td>beats per minute</td>
</tr>
<tr>
<td>BSA</td>
<td>body surface area</td>
</tr>
<tr>
<td>c</td>
<td>constant of the [HHb] response that is dependent on ( d )</td>
</tr>
<tr>
<td>c/d</td>
<td>value corresponding to 50% of the total [HHb] amplitude</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>calcium</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>C_{(a-\bar{v})O_2}</td>
<td>arterial-venous oxygen content difference</td>
</tr>
<tr>
<td>CF</td>
<td>cystic fibrosis</td>
</tr>
<tr>
<td>CFRD</td>
<td>cystic fibrosis-related diabetes</td>
</tr>
<tr>
<td>CFTR</td>
<td>cystic fibrosis transmembrane conductance regulator</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>chloride</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CON</td>
<td>controls</td>
</tr>
<tr>
<td>CP</td>
<td>critical power</td>
</tr>
<tr>
<td>CPET</td>
<td>cardiopulmonary exercise test</td>
</tr>
<tr>
<td>CR-10</td>
<td>0-10 category ratio scale</td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>CWR</td>
<td>constant work rate exercise</td>
</tr>
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</table>
$d$ slope of the [HHb] sigmoid
DEXA dual energy X-ray absorptiometry
ECFS European Cystic Fibrosis Society
ECG electrocardiogram
EMG electromyographic
ES effect size
FDA Food and Drug Administration
FFM fat-free mass
$f_0$ baseline [HHb]
FEV$_1$ forced expiratory volume in 1 second
FEV$_1$%predicted forced expiratory volume in 1 second expressed as a percentage of predicted normative values
FVC forced vital capacity
GET gas exchange threshold
[HHb] deoxygenated haemoglobin and myoglobin
HR heart rate
HRCT high-resolution computer tomography
HR$_{peak}$ peak heart rate
La blood lactate
L·min$^{-1}$ litres per minute
LT lactate threshold
MLSS maximal lactate steady-state
mL·kg$^{-1}$·min$^{-1}$ millilitres per kilogram per minute
mL·min$^{-1}$·W$^{-1}$ millilitres per watt per minute
MOD moderate intensity exercise
MRI magnetic resonance imaging
MRT mean response time
$m\dot{V}O_2$ muscle oxygen consumption
\( n \) number of (participants, exercise transitions etc.)

Na\(^+\) sodium

NHS National Health Service

NIRS near infrared spectroscopy

\( O_2 \) oxygen

OUES oxygen uptake efficiency slope

\( \text{OUES}_{100} \) oxygen uptake efficiency slope for the entire duration of the test

\( \text{OUES}_{\text{GET}} \) oxygen uptake efficiency slope below the GET

\( P_0 \) probability

\( \text{PaCO}_2 \) arterial pressure of carbon dioxide

P-CERT pictorial children's effort rating table

PCD primary ciliary dyskinesia

PCr phosphocreatine

PEF peak expiratory flow

\( P_{\text{ETCO}_2} \) end-tidal carbon dioxide partial pressure

\( P_{\text{ETO}_2} \) end-tidal oxygen partial pressure

\( P_i \) inorganic phosphate

PPO peak power output

PRBS pseudo-random binary sequence

\( \dot{Q} \) cardiac output

\( r \) Pearson's correlation coefficient

RER respiratory exchange ratio

RPE rating of perceived exertion

RPD rating of perceived dyspnoea

RPM revolutions per minute

SD standard deviation

SEE standard error of the estimate
$S_{max}$ supramaximal verification

SpO$_2$ arterial oxygen saturation

SRT steep ramp test

SSkF sum of skinfolds

SV stroke volume

$T_1$ test 1

$T_2$ test 2

$T_3$ test 3

TE typical error of the measurement

$TE_{CV\%}$ typical error of the measurement expressed as a percentage of the CV

$TD$ time delay

UK United Kingdom

$V_D/V_T$ ventilatory dead space ratio

$\dot{V}_E$ minute ventilation

$\dot{V}_E/\dot{V}O_2$ ventilatory equivalent for oxygen

$\dot{V}_E/\dot{V}CO_2$ ventilatory equivalent for carbon dioxide

$\dot{V}CO_2$ carbon dioxide output

VH very heavy intensity exercise

$\dot{V}O_2$ oxygen uptake

$\dot{V}O_{2max}$ maximal oxygen uptake

$\dot{V}O_{2peak}$ peak oxygen uptake

VT ventilatory threshold

W watts

$W\cdot min^{-1}$ watts per minute

WR work rate

$\%\Delta$ percentage difference between GET and $\dot{V}O_{2max}$

$\%\Delta[HHb]$ [HHb] normalised to the total amplitude of the response
\( \Delta \) change or difference in a value (delta)

\( \Delta \dot{\text{VO}}_2/\Delta \text{WR} \) functional \( \dot{\text{VO}}_2 \) gain; oxygen cost of exercise

\( \tau \) time constant

[\( x \)] denotes concentration

6MWT 6 minute walk test
 Ion water transport across airway epithelial cells. Chloride (Cl\(^-\)) and sodium (Na\(^+\)) transport across the apical membrane of airway epithelial cells regulate water transport. The “low volume” hypothesis postulates that, in cystic fibrosis patients, Cl\(^-\) transport is impaired and Na\(^+\) absorption is upregulated, which leads to increased water absorption from the airways into the tissue and depletion of the airway surface liquid. Figure taken from Griesenbach & Alton (2015) with permission.

Severe bronchiectasis in a patient with end-stage cystic fibrosis (CF) shown in both chest radiograph (left) and computed tomogram (right) images. For reasons that are not fully understood, the upper lobes are often most severely affected in CF, although this patient has severe bronchiectasis throughout the whole of the right lung. Note the presence of indwelling intravenous catheter (a “port-a-cath”) on the right lateral chest wall. Images taken from Davies et al. (2007) with permission.

Age distribution of deaths in patients with Cystic Fibrosis (CF) in the United Kingdom in 2012. There were 106 recorded deaths in 2012. The median age at death was 28 y (minimum, 5 y; maximum, 72 y; 95% confidence interval, 29 y). At this time the oldest male patient on UK CF Registry was 83 y and the oldest female patient was 81 y. Data taken from the most recent UK Cystic Fibrosis...
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Figure 2.6  Schematic to illustrate the relationship between muscle $O_2$ consumption and pulmonary $O_2$ uptake. $O_2$ is inspired from the atmosphere and delivered by the cardiovascular
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the mitochondria to produce adenosine triphosphate
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to the pulmonary circulation enabling muscle $\dot{V}O_2$ to be
estimated from the pulmonary $O_2$ exchange, although
there will be temporal misalignment owing to the venous
volume and $O_2$ stores between the muscle and lung. VT,
tidal volume; $f$, breathing frequency; SV, stroke volume;
HR, heart rate; $\dot{Q}$, cardiac output; $\dot{Q}CO_2$, muscle $CO_2$
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creatine phosphate; pyr-lac, pyruvate-lactate. Taken
from Jones & Poole (2005) with permission.

Figure 2.7 Schematic to demonstrate the typical response of
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moderate intensity constant work rate exercise. Adenosine triphosphate (ATP) turnover at the cross-
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increases relatively slowly. Note that a steady-state is
attained following ~ 2 min after the increase in exercising
work rate in this example. The red line denotes the
square wave increase in ATP demand necessary to
sustain exercise. Adapted from Jones & Poole (2005)
with permission.

Figure 2.8 Schematic of the rise in muscle (A) and pulmonary $\dot{V}O_2$
(B) at the onset of moderate-intensity exercise. Adapted
from Armstrong & Barker (2009) with permission. The
red line denotes the square wave increase in energetic
(ATP) demand required in order to sustain exercise.
Examples of the O₂ deficit that would be incurred by individuals with different values for the phase II time constant (τ) following the onset of a constant work rate square wave exercise transition. For the same increase in metabolic rate, represented by the $\dot{V}O_2$ achieved in the steady-state (phase III), an individual with fast $\dot{V}O_2$ kinetics would incur a much smaller O₂ deficit than would individuals with slower $\dot{V}O_2$ kinetics. It would be expected that a person with a chronic disease, such as cystic fibrosis, would present with slower $\dot{V}O_2$ kinetics and thereby incur a greater O₂ deficit than their healthy counterparts. Image taken from Jones & Poole (2005) with permission.

Muscle and pulmonary oxygen uptake ($m\dot{V}O_2$ (○ white circles) and $\dot{V}O_2$ (● black circles), respectively) response to knee-extensor exercise performed below (A) and above (B) the gas exchange threshold. The pulmonary $\dot{V}O_2$ data have been time aligned to the $m\dot{V}O_2$ data by moving the data back by the time equal to the time delay of phase I-II of the pulmonary $\dot{V}O_2$ response. Image taken from Krustrup et al. (2009) with permission.

Schematic of the pulmonary oxygen uptake ($\dot{V}O_2$; ● black circles) and muscle phosphocreatine ([PCr]; ○ white circles) responses at the onset of moderate intensity constant work rate exercise in an example participant. Note that the time aligned and modelled pulmonary $\dot{V}O_2$ response closely represents the kinetics of [PCr]. Taken from Rossiter et al. (1999) with permission.
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Figure 2.18  Deoxygenated haemoglobin concentration ([HHb]) plus myoglobin concentration response as a function of relative work rate (WR) for a representative trained (● shaded circles) and untrained (○ open circles) girl during ramp incremental cycling exercise. This data demonstrates a rightward shift of the sigmoidal [HHb] response as a result of training, indicating a better matching of O$_2$ delivery-to-O$_2$ utilisation and the muscle level. Image taken from McNarry et al. (2011) with permission.

Figure 2.19  Sigmoid models of normalised changes in deoxygenated haemoglobin concentration ([HHb]) plus myoglobin concentration (percentage of peak amplitude) as a function of relative (percentage peak) work rate for young adults (YA) versus older adults (OA). Image taken
from Gravelle et al. (2012) with permission.

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Chapter 3 General Methods

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**Equation 2.5** \( \dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + \Delta \dot{V}O_{2,A} \cdot (1 - e^{-\left(t-TD_{0}\right)/\tau}) \)

**Equation 3.1** \( \text{ BMI} = \text{ body mass (kg)} / \text{ stature (m}^2\text{)} \)

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Body fat percentage for all females = 1.33 (triceps + subscapular) – 0.013 (triceps + subscapular)² – 2.5

Equation 3.6  
FFM = body weight – (percentage body fat x body weight) / 100

Equation 3.7  
\[ \dot{Q} = f_c \cdot SVi \cdot BSA \]

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BSA = 0.02465 \times \text{body mass}^{0.5378} \times \text{stature}^{0.3964}

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\[ \dot{V}O_2 / HR_{\text{peak}} \ (\text{mL} \cdot \text{b}^{-1}) = \dot{V}O_2 \ (\text{L} \cdot \text{min}^{-1}) \times 1000 \ \text{mL} / \text{HR} \ (\text{b} \cdot \text{min}^{-1}) \]

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**PhD PUBLICATIONS AND PRESENTATIONS**

**Peer Reviewed Journal Articles**

**Saynor, Z. L.,** Tomlinson, O. W., Barker, A. R., Williams, C. A. (*Submitted for review*). Letter to the Editor: Validity and reliability concerns associated with cardiopulmonary exercise testing young people with cystic fibrosis Response to: Statement on Exercise Testing in Cystic Fibrosis (*Hebestreit et al., 2015 Respiration* Sep 9 [Epub ahead of print]).


**Published Abstracts**


**Conference Presentations**

Verbal presentation at the 11th Australasian Cystic Fibrosis conference, Sydney, Australia.


*Invited Presentations*


Saynor Z. L. (2013). Assessment and interpretation of aerobic function in young cystic fibrosis patients. Invited presentation at Great Ormond Street Children’s Hospital, UK.

Saynor, Z. L. (2011). ‘Exercise is medicine’ – The prognostic value of clinical exercise testing for young cystic fibrosis patients. Invited presentation at the Researching Youth Sport Conference at Brunel University, UK.

Saynor Z. L. (2010). Exercise testing and training for the young cystic fibrosis patient. Invited lecture at Bangor University, UK.

*Awards*


European Cystic Fibrosis Society Young Investigator Travel Grant Award (2012). 35th European Cystic Fibrosis Society Conference, Dublin, Ireland.
ADDITIONAL PUBLICATIONS AND PRESENTATIONS

**Invited Presentations**


**Co-author Conference Presentations**

1.1 Cystic Fibrosis

Cystic fibrosis (CF) is the most common inherited, life-shortening disease amongst the Caucasian population for which there is currently no cure. In 1985 the genetic defect in CF (located on the long arm of chromosome 7) was discovered, with the gene defect identified by full-length sequencing in 1989 (Kerem et al., 1989). Specifically, this defect is expressed as a disruption in the CF transmembrane conductance regulator (CFTR) protein. This protein functions primarily as a cyclic adenosine monophosphate (cAMP)-activated and adenosine triphosphate (ATP)-gated chloride (Cl\(^-\)) channel, found in the membranes of cells that line passageways of the lungs, liver, pancreas, intestines, reproductive tract, and skin. Abnormal CFTR alters the ion transport necessary for proper function of epithelial structure. Consequently, patients are characterised by excessively viscous mucus in almost all organs, including the lungs, gastrointestinal and endocrine systems, which causes a progressive decline in the function of these organs (Cuthbert, 1991).

When this irregularity occurs in the lungs, abnormal thick and dry mucus ensues, resulting in a vicious cycle of bronchial airway obstruction, bacterial infection, and inflammation. The resulting obstructive syndrome causes progressive disability and, as this cycle continues, lung tissue is progressively destroyed, with eventual respiratory failure. Patients therefore experience progressive lung disease, malabsorption and pancreatic insufficiency can cause
malnutrition, steatorrhea and reduced skeletal muscle mass (Davies et al., 2007). Not only does CFTR act as an epithelial chloride channel, it is also located in the vascular endothelium (Tousson et al., 1998), where it is reported to affect the lung endothelial barrier function (Brown et al., 2014) and act as a negative regulator of endothelial inflammatory responses (Scott-Ward & Shute, 2014). As such, endothelial dysfunction has recently been documented in young, relatively well patients with CF (Poore et al., 2013). CFTR is also expressed in skeletal muscle (Lamhonwah et al., 2010), which may have consequences on skeletal muscle function. Although the complexity of CF is becoming increasingly problematic as the life expectancy of patients extends, respiratory failure remains the leading cause of morbidity and mortality in this patient group (Dassios et al., 2015).

CF currently affects over 10,583 people in the United Kingdom (UK), with ~4,000 under the age of 16 y. Although the natural history of CF is a progressive loss of lung function that leads to death, with early diagnosis and improved aggressive therapeutic interventions, survival into the third and fourth decade of life is now common. As such, the most recent UK CF Registry report (2014) predicts a median survival age of 40.1 y for people with CF; a substantial improvement when compared to 8 y of age in 1974, 11 y in 1986 and 29 y in 1992 (Orenstein & Higgins, 2005). This improved outlook is primarily due to earlier diagnosis, the introduction of newborn screening and developments in pharmacological therapies for CF.

The CFTR defect in CF can be classified in 5-6 classes, based on the specific genetic defect, which are outlined in Chapter 2. These classifications range
from complete loss of protein function to marginal dysfunction and play a significant role in the patient’s disease severity (Lubumba et al., 2012). Although traditional treatment regimens focused on alleviating manifestations secondary to CFTR dysfunction in patients with CF, landmark research over recent years has initiated ‘The beginning of effective therapy for cystic fibrosis’ (Davis, 2015). Ivacaftor is a CFTR potentiator that increases the open time of activated CFTR at the cell surface, thereby restoring the Cl⁻-transport activity. Ivacaftor was initially targeted at patients with the Gly551Asp mutation, the third most common genotype in the world (6% of the UK population according to the CFTR2 Global Database). However it has more recently been licensed for patients with other mutations in which the CFTR protein reaches the plasma membrane but does not open appropriately. More recently, a landmark study has demonstrated potential pharmacological therapy, which combined Ivacaftor with an experimental therapy, Lumacaftor (VX-809). This combined therapy (Lumacaftor-Ivacaftor) targets patients homozygous for the most challenging Phe508del CFTR mutation (Wainwright et al. 2015), and in July 2015 was approved as Orkambi® by the Food and Drug Administration (FDA). This marks a new beginning in the treatment and clinical management of individuals living with CF.

1.2 Exercise testing in the clinical management of patients with CF

Whilst the recent increase in the treatment and survival age of patients with CF should be considered a success, this now presents an ongoing challenge for modern medicine. The increased prognosis associated with CF now provides clinical care teams with the challenge of fostering an optimal quality of life (QoL) for this ageing patient population. Developing and enhancing additional
treatment modalities to facilitate this is therefore a clinical priority. Besides pharmacological treatments, exercise forms an integral role in the management of CF and its importance has been recognised since the identification of CF as a clinical syndrome, given that exercise intolerance has always been a hallmark of disease progression (Orenstein & Higgins, 2005). Accordingly, the British Thoracic Society and the Association of Chartered Physiotherapists in Respiratory Care (ATS/ACCP, 2003) recommend exercise should be an integral component of the clinical management of CF. It is important that the utility of exercise testing as a clinical tool is not ignored. The utilisation of exercise should not only be for rehabilitative purposes, but should also include comprehensive exercise assessments of patients’ health and function.

Exercise testing is a valuable investigative tool within both the clinical management and scientific investigation of young people with chronic diseases. Pulmonary function can predict survival in patients with CF (Nixon et al. 1992) and forced expiratory volume in 1 s (FEV₁) and arterial oxygen saturation (SpO₂) are typically used to stratify CF disease severity and referrals for lung transplantation (Augurten et al., 2001; Kerem et al., 1992; Sharples et al., 1993). However, resting measurements cannot accurately predict patients’ exercise performance and how well they will cope with activities of daily living (ADL) that require rapid changes in metabolic rate and co-ordination of the cardiorespiratory and muscular systems. Notably, the European Cystic Fibrosis Society (ECFS) Exercise Working Group has recently promoted cardiopulmonary exercise testing (CPET) as the exercise testing method of choice for patients with CF (Hebestreit et al., 2015). Furthermore, the ECFS Clinical Trials Network Standardisation Committee has recently called for
research assessing the validity, reproducibility and feasibility of outcome measures utilised in the assessment of patients with CF and the most appropriate exercise test for paediatric patients (Bradley et al., 2012).

CPET is considered the gold standard assessment of patients’ aerobic fitness, as it allows for the evaluation of health status and (dys)function, alongside the cause(s) of any change in patients’ clinical and aerobic fitness status (Palange et al., 2007). Furthermore, in patients with CF, clinical utility of CPET derived measurement of peak oxygen uptake ($\dot{V}O_{2\text{peak}}$) has been demonstrated in evaluating patients’ prognosis (Moorcroft et al., 1997; Nixon et al., 1992; Pianosi et al., 2005; Stanghelle et al., 1992), risk of hospitalisation for respiratory exacerbations (Pérez et al., 2014) and QoL (de Jong et al., 1997). Valid and reproducible exercise testing may also provide superior utility to assess how patients respond to clinical therapeutic interventions. For example, intravenous antibiotics (IVABs) or gene mutation targeted therapies, in establishing thresholds for transplantation in more advanced CF, or to assist in the prescription of more individualised exercise training programmes (Stevens & Williams, 2007). Comprehensive analysis of gas exchange, using a treadmill or cycle ergometer, is thus suggested to pose far superior value than current clinical assessments and provide clinicians with more useful information (Ferrazza et al., 2009).

Specifically, CPET is a valuable tool to assess exercise performance and determine the possible underlying cause(s) of exercise limitation in patients with chronic diseases, such as CF (Ferrazza et al., 2009; Palange et al., 2007). The pulmonary, cardiovascular, metabolic, digestive and muscular systems are all
adversely affected by pulmonary disease itself and associated factors, such as hypoactivity (Ferrazza et al., 2009). CPET offers the unique simultaneous evaluation of all of these systems, which are essential for exercise, under conditions of metabolic stress (Wasserman et al., 2004). The principle of CPET for populations with disease is that exercise forces these systems to the limits of their tolerable ranges, enabling observation of abnormal response patterns. The necessary coordination of the respiratory, cardiovascular and muscular systems required for the rapid adjustments when changing from one metabolic rate to another is particularly sensitive to dysfunction at multiple steps along the O₂ transport and utilisation pathway (Wasserman et al., 2004). Therefore, when compared with their healthy counterparts, CPET makes it possible to observe reduced function and any abnormalities in the physiological adaptation to exercise in patient groups (Ferrazza et al., 2009).

1.3 Current provision of exercise testing for patients with CF

Due to the recognised clinical utility of exercise testing within CF, current standards for the management of this disease recommend at least annual exercise testing of patients (Cystic Fibrosis Trust, 2011). Information obtained from such testing should provide clinicians with valuable functional and prognostic insight that common clinical assessment cannot, thereby informing patients’ subsequent clinical management. However, current provision in many centres fails to achieve the recommendation to annually test patients and exercise testing, particularly CPET, remains dramatically underused, despite equipment often being available (Barker et al., 2004; Kaplan et al., 1991; Stevens et al., 2010). A survey of UK CF clinics (Stevens et al., 2010) highlighted that in addition to a mismatch between recommendation and
provision of exercise testing, those tests which are being performed are generally varied, simplistic and crude in nature. These observations are comparable with clinics in Germany (Barker et al., 2004) and the US (Kaplan et al., 1991). Whilst crude exercise tests, such as shuttle walk and step tests, can offer some indication regarding patients’ fitness levels, they are often submaximal, limited in the information they provide and often lack the capability to demonstrate when a submaximal effort has been provided. Current cardiopulmonary evaluations within clinical practice are therefore limited in both their prognostic value and their ability to accurately measures patients’ aerobic fitness levels.

Promisingly, Stevens and colleagues’ survey (Stevens et al., 2010) highlighted that health professionals within UK CF clinics recognise the importance of exercise testing within clinical practice, in line with observations in Germany (Barker et al., 2004; Kaplan et al., 1991). However, before the clinical utilisation of CPET can advance, clarification of the optimal CPET protocol and feasibility of its implementation in the clinical setting is required. Identification of a reproducible and valid exercise test that allows clinical teams to monitor disease progression and/or the response to therapeutic interventions is therefore essential (Stevens & Williams, 2007). To date, no study has formally established an up-to-date ‘gold standard’ valid and reproducible protocol for determining maximal oxygen uptake (\(\dot{V}O_{2\text{max}}\)) in children and adolescents with CF. Until such a study is undertaken, inferences regarding exercise training, pharmacologic interventions and disease-related changes cannot be discerned with certainty. Moreover, knowledge regarding the validity and reproducibility of CPET is needed if strategies are to be devised to enhance implementation of
more detailed cardiopulmonary assessments within the healthcare of young CF patients.

1.4 Assessment of exercise (dys)function in patients with CF

Since the preservation of aerobic fitness and pulmonary function are important for the prognosis and QoL of people with CF, it is important to establish a database regarding the extent of exercise impairment and to identify the factor(s) responsible for impaired aerobic metabolism during exercise. Although previous studies (e.g. Bongers et al., 2012; Bongers et al., 2014; Hjeltnes et al., 1984; Moser et al., 2000) have documented reduced $\dot{V}O_{2\text{max}}$ in paediatric patients with CF, suggesting impaired aerobic exercise function, this requires clarification using a valid and reproducible CPET protocol. Furthermore, although documenting exercise (dys)function in people with CF has received increased attention over recent years (e.g. Bongers et al., 2012; Bongers et al., 2014; de Meer et al., 1999; Moser et al., 2000; Rosenthal et al., 2009; Wells et al., 2011; Werkman et al., 2015), there remains limited evidence regarding which factors are responsible for impaired aerobic exercise function in relatively well children and adolescents with CF.

While impaired aerobic oxidative metabolism has been described in CF patients, it remains unclear whether this is a result of impaired $O_2$ delivery (e.g., Klijn et al., 2003; Moser et al., 2000), the capacity of skeletal muscle to extract and utilise $O_2$ (e.g., de Meer et al., 1995; Kusenbach et al., 1999; Rosenthal et al., 2009; Wells et al., 2011) or a combination of these factors. Since physiological abnormalities in people with CF may affect the transport, extraction and utilisation of $O_2$ to the contracting muscles, assessing the
dynamics of $\dot{V}O_2$ in conjunction with measures at the central and peripheral levels is needed. The past decade has seen the development of non-invasive techniques such as thoracic bioelectrical impedance cardiography, which enables the assessment of cardiac function, and near-infrared spectroscopy (NIRS), which allows the estimated matching of $O_2$ delivery-to-$O_2$ utilisation within the muscle microcirculation to be measured (e.g. DiMenna et al., 2010; Ferreira et al., 2007). Although these non-invasive techniques are suitable for use in paediatric groups, their application within CF-related exercise studies is poor. To date, no study has utilised these techniques in conjunction with the $\dot{V}O_2$ response to investigate the cause(s) of reduced aerobic function during CPET. Such a study will enable the co-ordination and function of the respiratory, cardiovascular and muscular systems to be characterised during exercise. These measurements can offer unique insight into the exercise responses of clinical populations (Mattei et al., 2004; Poole & Jones, 2012), and is key to understanding further the mechanisms by which this disease modulates exercise function of young people with CF.

Whilst exhaustive exercise testing has been a focus of previous research investigating the exercise impairment in people with CF, documenting the kinetics of $\dot{V}O_2$ at the onset and offset of constant work rate (CWR) exercise can also provide a non-invasive window into the metabolic activity of skeletal muscle during exercise (Poole & Jones, 2012). As such, studies investigating this response in children and adolescents with CF would provide a valuable means of developing our understanding of oxidative skeletal muscle metabolism in this group. The collective conclusion from existing literature (for a review see Williams et al., 2014) shows that the dynamics of $\dot{V}O_2$ at exercise onset, and thereby the control of mitochondrial oxidative phosphorylation, is impaired in
CF. However, all previous studies have contained methodological issues (see Chapter 2), such as incorrect handling and analysis of the data, prescription of exercise intensities and the use of semi-supine cycling. Consequently, a robust study measuring the $\dot{V}O_2$ kinetics of children and adolescents with CF is warranted. Documenting the response in conjunction with measures at the central and peripheral levels will also provide important information concerning how well children and adolescents with CF can match O$_2$ delivery-to-O$_2$ utilisation during CWR exercise.

1.5 Objectives of this thesis

In line with the preceding Introduction, through a series of chapters and experimental studies this thesis aims to extend the current evidence base concerning the assessment and interpretation of aerobic exercise function in paediatric patients with mild-to-moderate CF. Specifically, this thesis will:

- Chapter 2 – Provide a comprehensive review of the literature concerning the assessment and interpretation of aerobic exercise (dys)function in patients with CF and the factors purported to play a role in the impairment of aerobic metabolism;
- Chapter 3 - Provide an overview of the general methods utilised in the experimental studies which contribute to this thesis;
- Chapter 4 - Examine the issues surrounding the validity of assessing aerobic exercise function, specifically $\dot{V}O_{2\text{max}}$, in this patient group during exhaustive incremental exercise testing;
- Chapter 5 - Establish the reproducibility of CPET to determine the key parameters of aerobic function in children and adolescents with CF and
provide the typical error (TE) necessary to determine a clinically meaningful change;

- Chapter 6 – Demonstrate, using a case study approach, the clinical application of CPET to assess the effect of a therapeutic intervention (Ivacaftor) on aerobic exercise (dys)function in paediatric CF;

- Chapter 7 – Determine whether children and adolescents with mild-to-moderate CF present with impaired aerobic exercise function using a valid and reproducible ramp incremental exercise test, in addition to exploring $O_2$ delivery and/or $O_2$ extraction limitations;

- Chapter 8 – Provide a comprehensive investigation into whether the pulmonary $\dot{V} O_2$ and muscle deoxygenation (deoxyhaemoglobin + myoglobin ([HHb])) kinetics during moderate (MOD) and very heavy (VH) intensity CWR exercise are impaired in children and adolescents with CF and an exploration into the limiting factor(s).

The experimental hypotheses for the experimental studies are provided in their respective chapters. Finally, Chapter 10 will provide a summary of the primary findings of the experimental studies conducted as part of this thesis, highlighting their novel contribution to the study of exercise (dys)function in young people with CF. Finally, remaining gaps in the evidence base and avenues for future research will be presented.
2.1. Pathophysiology of CF

‘The child will soon die whose forehead tastes salty when kissed’
(Almanac of Children’s Songs and Games. Switzerland, 1857)

CF is an autosomal recessive, predominantly Caucasian, disease caused by a defect on a single gene. In 1985 the genetic defect responsible for CF (located on the long arm of chromosome 7) was discovered and subsequently identified by full-length sequencing in 1989 (Kerem et al., 1989). Specifically, this defect is expressed as a disruption in the CFTR protein. CFTR mutations are currently grouped into 6 classes, depending on the effects elicited on the protein (Wang et al., 2014). These classifications range from complete loss of protein function to marginal dysfunction and play a significant role in determining the magnitude of disease severity (Lubumba et al., 2012). Although the majority of patients with CF are homozygous for the Phe508del (ΔF508) CFTR mutation (Table 2.1), the CF Mutation Database (CFTR1) currently lists 1,991 CFTR mutations.

Table 2.1. The most common single mutations in UK registered (2012) patients with cystic fibrosis. (Data adapted from the UK CF Registry Report, 2013. Approximately 96% of registered patients have been genotyped, with ΔF508 the most common genotype).

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Class</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF508</td>
<td>II</td>
<td>7971</td>
<td>90.7</td>
</tr>
<tr>
<td>G551D</td>
<td>III</td>
<td>471</td>
<td>5.6</td>
</tr>
<tr>
<td>R117H</td>
<td>IV</td>
<td>361</td>
<td>4.3</td>
</tr>
<tr>
<td>G542X</td>
<td>I</td>
<td>307</td>
<td>3.6</td>
</tr>
<tr>
<td>621+1G -&gt;T</td>
<td>I</td>
<td>180</td>
<td>2.1</td>
</tr>
<tr>
<td>N1303K</td>
<td>II</td>
<td>111</td>
<td>1.3</td>
</tr>
<tr>
<td>1717-1G-&gt;A</td>
<td>I</td>
<td>106</td>
<td>1.3</td>
</tr>
<tr>
<td>1898+1G-&gt;A</td>
<td>I</td>
<td>95</td>
<td>1.1</td>
</tr>
<tr>
<td>DI507</td>
<td>II</td>
<td>85</td>
<td>1.0</td>
</tr>
<tr>
<td>R560T</td>
<td>III</td>
<td>83</td>
<td>1.0</td>
</tr>
<tr>
<td>3659delC</td>
<td>II</td>
<td>82</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Classes I-III are generally considered to be more severe than classes IV-VI, which retain varying degrees of CFTR function. The ∆F508 mutation is a class II mutation, which results in defective processing of the CFTR protein. ∆F508 remains to be the most common CFTR mutation in the world, with a prevalence of 91% and 90% in the UK and Ireland, respectively. G542X is a class I mutation, whereby a premature stop codon causes complete lack of protein expression, whilst G551D is an example of a class III mutation, leading to defective chloride channel gating and a more simple target for pharmacological intervention. The G551D mutation is the third most common in the world, with a prevalence of 14% and 6% in the UK and Ireland, respectively. In addition to classic CF, some mutations, generally milder genetic variants which retain residual CFTR function, can lead to isolated milder symptoms. These patients have been classified as atypical CF, non-classical CF, CFTR-related disorders, low-risk genotypes, or mild variant CF (Griesenbach & Alton, 2015).

The CFTR protein functions primarily as a cAMP-activated and ATP-gated chloride channel in the apical membrane of epithelial cells throughout the body. Abnormal CFTR alters the ion homeostasis and, thereby, the ion transport necessary for proper function of epithelial structure (Figure 2.1). Consequently, individuals with CF are characterised by excessively viscous mucus in almost all organ systems, including the respiratory, gastrointestinal, reproductive and endocrine systems, which causes a progressive decline in their function (Cuthbert, 1991). CF therefore affects multiple organs, including the lungs, liver, intestine, pancreas, vas deferens in males, and the skin. People with CF therefore experience progressive lung disease, malabsorption and pancreatic insufficiency, commonly resulting in some degree of malnutrition, steatorrhea and reduced skeletal muscle mass (Davies et al., 2007; Lubamba et al., 2012).
Furthermore, since CF affects the development of the vas deferens and this can also become obstructed by mucus, sperm are not released and infertility characterises males. Mucus can also make fertilisation problematic in females with CF. CFTR also regulates the flow of electrolytes and fluid across cellular membranes and the defective CFTR evident in CF is associated with reduced conductance of Cl⁻ ions across the apical membrane of epithelial cells (Figure 2.1), causing the elevated sodium and Cl⁻ levels which are characteristic of the disease (60 mEq·dL⁻¹ is highly suggestive for diagnosis).

![Figure 2.1](image.png)

**Figure 2.1.** Ion water transport across airway epithelial cells. Choride (Cl⁻) and sodium (Na⁺) transport across the apical membrane of airway epithelial cells regulate water transport. The “low volume” hypothesis postulates that, in cystic fibrosis patients, Cl⁻ transport is impaired and Na⁺ absorption is upregulated, which leads to increased water absorption from the airways into the tissue and depletion of the airway surface liquid. Figure taken from Griesenbach & Alton (2015) with permission.

When this irregularity in ion transport across the epithelial cell membranes occurs in the lungs, abnormally thick and dry mucus ensues, resulting in a vicious cycle of bronchial airway obstruction, bacterial infection, and inflammation. The resulting bronchiectasis causes irreversible anatomic and
histologic pathogenic changes to the airways (Figure 2.2), with grossly dilated airways, thickened bronchial walls and luminal structures commonly evident upon clinical examination. The bronchiectasis in patients with CF is typically more severe than that experienced by patients with non-CF bronchiectasis (Morrisey, 2007). This pulmonary obstructive syndrome causes progressive disability and, as the vicious cycle of infection and inflammation continues, lung tissue is progressively destroyed. This progressive fibrosis and destruction causes a loss of functional lung tissue and, eventually, leads to respiratory compromise and respiratory failure. Despite advances in the clinical treatment of CF, respiratory failure still remains the leading cause of morbidity and mortality in this patient group (Dassios, 2015).

Figure 2.2. Severe bronchiectasis in a patients with end-stage cystic fibrosis (CF) shown in both chest radiograph (left) and computed tomogram (right) images. For reasons that are not fully understood, the upper lobes are often most severely affected in CF, although this patient has severe bronchiectasis throughout the whole of the right lung. Note the presence of indwelling intravenous catheter (a “port-a-cath”) on the right lateral chest wall. Images taken from Davies et al. (2007) with permission.

CF is a complex multisystem disease and, as pulmonary function and life expectancy continue to improve in this patient group, extrapulmonary complications (e.g., hepatic and gastrointestinal issues) will increasingly
contribute to morbidity and mortality. A similar pathologic process to that observed in the lungs occurs in the gastrointestinal tract, pancreas and hepatobiliary system (Lavelle et al., 2015). More specifically, abnormal CFTR function, with reduced bicarbonate and other ion transport levels through the apical surface of epithelial cells, can affect the intestinal tract, including the liver and pancreas (Munck et al., 2015). Thick mucus causes luminal obstruction and pancreatic involvement can result in exocrine and endocrine insufficiency, pancreatic atrophy, and/or fatty replacement.

Exocrine insufficiency reportedly characterises approximately 85% of people with CF, which results in malabsorption of nutrients, including fats and protein. This malabsorption can lead to frequent fatty and foul-smelling stools and abdominal pain. Furthermore, CF has traditionally been associated with malnutrition, secondary to malabsorption, inadequate intake, and increased energy expenditure (Hanna & Weiner, 2015). Nutritional status is also linked to the development of bone disease in CF and poor nutritional status is associated with significantly worse pulmonary function (Steinkamp & Wiedemann, 2002) and malnutrition is associated with a rapid progression of disease and worse prognosis (Sinaasappel et al., 2002; Yen et al., 2013).

A new concern in this aging patient population is the risk of overweight and obesity. One study a decade ago reported that 9% of UK based patients with CF, who are homozygous for the ∆F508 mutation, were overweight and 1% obese (Kastner-Cole et al., 2005). However, a more recent study from the Children’s Hospital in Pittsburgh, United States of America, reported that 15% of their paediatric patients were overweight and 8% were obese, despite the majority being pancreatic insufficient (Hanna & Weiner, 2015). This observation
is comparable with a recent Canadian study in adults with CF, which noted that 18.4% were overweight or obese (Stephenson et al., 2013). As such, whilst nutritional management traditionally includes a high calorie, high fat diet, pancreatic enzyme replacement therapy, vitamin and mineral replacement, and enteral support as required (Schindler et al., 2015), there is increasing clinical interest in muscle maintenance and protein intake (Engelen et al., 2014).

The prevention and management of CF related diabetes (CFRD), which is more common in classes I-III, is also now of increasing clinical concern. The abnormalities of glucose metabolism in CF represent a continuum from normal, through pre-diabetic, to overt diabetes and the pathogenesis is characterised by post-prandial rather than pre-prandial hyperglycaemia (Perano et al., 2014). CFRD impacts both nutritional status and pulmonary function (Bizzarri et al., 2006; Hameed et al., 2015; Moran et al., 2009) and mortality rate is reportedly substantially higher than CF patients without CFRD (Hayes et al., 2015; Moran et al., 2009). As the life expectancy of patients with CF continues to rise, there is new evidence demonstrating vascular complications are seen in both the small and large vessels, with associations with CFRD now being observed (Perano et al., 2015).

CFTR not only acts as an epithelial Cl⁻ channel, but it has also been located in the vascular endothelium (Tousson et al., 1998), where it reportedly affects the lung endothelial barrier function (Brown et al., 2014) and acts as a negative regulator of endothelial inflammatory responses (Scott-Ward & Shute, 2014). In support of this, endothelial dysfunction has recently been documented in young, relatively well patients with CF (Poore et al., 2013). More recently, it has been demonstrated that CFTR is expressed in skeletal muscle cells (Lamhonwah et
al., 2010) and in vitro study of leucocyte mitochondria in patients with CF demonstrates that properties of complex I of the respiratory chain are significantly altered (Dechecci et al., 1988). Furthermore, absence of CFTR from skeletal muscle has been shown to dysregulate calcium (Ca\(^{2+}\)) homeostasis, augment inflammatory or atrophic gene expression signatures and increase diaphragm weakness (Divangahi et al., 2009). CFTR has also been found in ventricular myocytes of several mammalian species, including mice, rats, swine, simians and humans (Duan et al., 1999; Gao et al., 2007; Tilly et al., 1996; Warth et al., 1996). Recent evidence also suggests a role of CFTR in the regulation of cardiomyocyte contraction (Sellers et al., 2010). Furthermore, external factors, such as cigarette smoke, have been shown to alter CFTR function in the lungs as well as extrapulmonary tissues (Cantin et al., 2006; Raju et al., 2013).

2.2. Incidence of CF and predicted survival age

The life expectancy of patients with CF continues to improve due to a combination of aggressive antibiotic treatment, improved emphasis on nutritional status and physiotherapy, and the development of CFTR potentiator treatments. However, CF remains the most common inherited life shortening disease amongst the Caucasian population and currently affects ~ 10,000 people in the UK (with 87% of these included in the most recent UK CF Registry Report (UK Cystic Fibrosis Registry, 2013). Approximately 4,000 of these patients are below the age of 16 y and their treatment is managed within paediatric care. The outlook for people with CF has substantially improved over the last 40 y, with the most recent UK CF Registry report from 2012 (UK Cystic Fibrosis Registry, 2013) reporting a median age at death of 28 y (Figure 2.3)
and predicting a median survival age of 38.8 y and > 57% of patients > 16 y of age either working or studying.

This marks a continued and substantial improvement from the predicted median survival age of 8 y in 1974, 11 y in 1986 and 29 y in 1992 (Orenstein & Higgins, 2005). Despite the improved outlook for the new generation of patients with CF, there were still 106 recorded deaths in the UK in 2012, with a median age of 28 y (Figure 2.3).

More recently, the mortality trends in CF patients across Europe were documented for the first time. It was demonstrated that, across the 27 European countries, the mean age at death and potential years of life lost increased for both genders from 1994 to 2010. However, there is a continued downward trend of CF mortality throughout Europe, with differences by both country and gender.
(Quintana-Gallego et al., 2016). The mean age at death across Europe increased from 17.6 y to 30.3 y in females and from 21.5 y to 29.8 y in males (Figure 2.4), with similar patterns observed in individual countries and the peak age being 20-24 y. The highest age-standardised mortality rates were observed for Ireland and the UK.

A number of factors are responsible for the improved prognosis associated with CF, including developments in specialist centre care, better nutritional support, and improved treatment of pulmonary complications with the aggressive use of antibiotic treatments, improvements in the control of infection and boosts in exocrine pancreatic function, in addition to the development of CFTR-modifying drugs for some genotypes. Furthermore, the implementation of newborn screening for CF in many European countries, North America and Australia has also contributed to the improved outlook for patients with CF. Newborn screening was introduction in the UK in 2007, through the development of the

![Figure 2.4. Mean age of death in Europe between 1994 and 2010. Solid lines represent the regression lines for age-standardised mortality for males (blue) and females (red). Data taken from Quintana-Gallego et al. (2016) with permission.](image-url)
CF UK Newborn Screening Programme Centre, and initial indications suggest that early diagnosis may improve survival, with newborn screened patients having superior weight and pulmonary function later in life than patients diagnosed in response to symptom development (UK Cystic Fibrosis Registry, 2013). Promisingly, of the 63% of children with complete data born in 2012, 79% were identified by newborn screening. Projections of 40 y and > 50 y have been made for the median survival age of CF patients born in 1990 (Elborn et al., 1991) and 2000 (Dodge et al., 2007), respectively. These predictions are not unfounded, particularly when the recent advances in the pharmacological treatment of CF are considered.

2.3 Advancements in the medical treatment of CF

There remains no cure for CF. Traditionally, the clinical management of CF is focused on alleviating the manifestations that are secondary to CFTR dysfunction. IVABs are typically utilised to provide short-term improvements in clinical status and pulmonary function and, in more severe disease, lung transplantation can increase prognosis. Although clinical interventions such as IVABs do provide acute improvements, they are not a long-term management option. Over the past 20 y, a number of therapies (e.g., novel antibiotics, such as inhaled tobramycin and macrolides, novel mucolytics, dornase alpha (a recumbent DNase) and hypertonic saline; Figure 2.4) have progressed from clinical trials to the mainstream clinical treatment of CF (Griesenbach & Alton, 2015). Disappointingly, the development of pulmonary mutation-independent gene therapy for patients with CF has demonstrated no clinical benefit as yet (Griesenbach et al., 2013). However, a particularly exciting area of development
has been the ongoing development of pharmacological treatments to target the defective CFTR responsible for CF.

Over recent years, pioneering research has developed gene mutation targeted therapies which mark the beginning of a new era in the treatment and clinical management of CF. CFTR potentiators are defined as agents that potentiate mutated but apically localised CFTR (Griesenbach & Alton, 2015), which may be of benefit for class III and IV mutations. Initial research has focused on an orally administered CFTR potentiator (Figure 2.4), VX-770, otherwise known as Ivacaftor, and most recently marketed under the name Kalydeco®. Ivacaftor (VX-809) is a CFTR potentiator that increases the open time of activated CFTR at the cell surface, thereby restoring the Cl⁻-transport activity. Ivacaftor was initially targeted at patients with Gly551Asp mutation. However, more recently it has been licensed for patients with other mutations, in which the CFTR protein reaches the plasma membrane but does not open appropriately.

To date, improvements in QoL, incidence of pulmonary exacerbations, respiratory symptoms, pulmonary function, body mass, and biomarkers of CFTR activity (sweat Cl⁻ and nasal potential difference) have been reported following treatment with Ivacaftor in patients heterozygous for the G551D mutation with mild-to-moderate pulmonary disease, without substantial adverse effects (Accurso et al., 2010; McKone et al., 2012; Ramsey et al., 2011). Furthermore, administration of Ivacaftor has revealed improvements in the pulmonary function (5.2 ± 5.6% predicted FEV₁) of severely ill patients (< 40% predicted FEV₁) (Hebestreit et al., 2013) and lung clearance index (95% CI - 2.88 to -1.44) in children aged 6-11 y (Davies et al., 2013). More recently, it has also been demonstrated that Ivacaftor has comparable efficacy in eight other
class III CFTR gating mutations (de Boeck et al., 2014). However, existing evidence suggests that Ivacaftor is ineffective in patients homozygous for F508del (Flume et al., 2012) and that additional treatments are needed to accompany a CFTR potentiator.

**Figure 2.5.** Cystic fibrosis transmembrane conductance regulator (CFTR) pharmacological modulators have different modes of action. A, Read-through compounds which include aminoglycoside antibiotics (e.g., gentamicin, tobramycin) act by suppressing premature termination codons, thus permitting translation to continue to the normal termination of the transcript and thus increasing the total amount of complete CFTR being produced in the cell. B, Correctors (e.g. VX-809 also known as Lumacaftor; VX-661) potentially promote folding of mutant CFTR protein, allowing it to escape ER degradation and reach the cell surface, thus increasing the number of channels present at the plasma membrane. C, Stabilisers include compounds (e.g., HGF) that enhance CFTR retention/anchoring at the cell surface, thus also contributing to increase the number of channels present at the cell surface. D, Potentiators (e.g., VX-770 also known as Ivacaftor) activate CFTR, i.e., increase the probability ($P_0$) of the channel by regulating its gating and possible also the conductance. Taken from Bell et al. (2015).

CFTR correctors (Figure 2.5), agents that correct the processing defect of class II mutations, may therefore present benefit to patients who are homozygous for F508del. The CFTR corrector Lumacaftor (also called VX-809) has been shown to reduce sweat Cl⁻, but not to improve pulmonary function when administered in isolation to patients with the F508del mutation (Clancy et al., 2012). Subsequently, the combination of Ivacaftor and Lumacaftor was assessed in homozygote and compound heterozygote F508del patients, to investigate whether Lumacaftor can transport CFTR to the apical membrane and Ivacaftor
will restore its function (Boyle et al. 2014). The co-administration did improve pulmonary function, however the improvement was less than Ivacaftor treatment in class III mutations and appears less effective in F508del compound heterozygotes (Boyle et al., 2014). More recently, it has been demonstrated that the combination of Lumacaftor-Ivacaftor can provide benefit for patients with CF who are homozygous for the most challenging Phe508del CFTR mutation (Wainwright et al., 2015). Promisingly, in July 2015 this combined therapy was licensed under the name Orkambi® for use in patients with CF.

The identification and licensing of Kalydeco® and Orkambi® represent a milestone in the treatment of CF. These treatments provide the first ‘proof-of-concept’ that the molecular defect in CFTR is targetable and correctable. However, it has recently been highlighted that new outcome measures are needed in the new era of therapeutic CFTR modulation (Bodewes et al., 2015). Bodewes et al. (2015) highlighted that the current clinical end-points of pulmonary function and body mass index (BMI) have limitations in the clinical trials assessing the development of new drugs which target CFTR protein function, largely due to their insensitivity to change and the limited mechanistic insight that they provide. Although the review by Bodewes and colleagues (2015) focused on gastrointestinal endpoints, comprehensive exercise testing may provide other feasible and informative outcome measures. The sensitivity of common clinical assessments, such as spirometry and body mass, to detect change in early disease has been questioned (Welsh et al., 2010). Therefore, a comprehensive assessment of physiological (dys)function of the organ systems under controlled metabolic stress, through detailed exercise testing, warrants further investigation in CF.
2.4 Cardiopulmonary exercise testing as a clinical outcome measure in CF

2.4.1 Current use of CPET within the clinical management of patients with CF

The utilisation of exercise within the clinical management of individuals with CF includes both exercise training and testing. A survey conducted by Stevens and colleagues in 2010 demonstrated that CPET is underutilised as an assessment tool for patients with CF in the UK (Stevens et al., 2010). Of the clinics that responded (51% response rate), only 38.9% of paediatric and 27.8% of adult patients had performed an exercise test in the preceding 12 months, most as part of an annual review process. This is concerning given that the current management guidelines from the UK CF Trust recommend at least annual exercise testing (CF Trust Guidelines) and is not only an issue in the UK, with similar observations across Germany and the US (Barker et al., 2009; Kaplan et al., 1991).

Of the tests performed, the majority were field based tests, including the 6 min walk test (6MW T), 12 min walk test, incremental shuttle walk test, endurance shuttle walk test, and step tests, with only a small number of treadmill and cycle based testing protocols reported (Stevens et al., 2010). This is despite acceptance by clinical and exercise physiologists that maximal CPET provides the most objective assessment of the extent and/or mechanism(s) of exercise (dys)function. Interestingly, CF clinical care teams did recognise the value of exercise testing. When UK clinics were asked to rank the importance of exercise testing on a scale from 1 ‘not important’ to 5 ‘very important’, the mean and median respondent scores were 3.5 and 4.0, respectively. Primary issues precluding the uptake of exercise testing as a standard clinical assessment tool included availability of equipment and issues regarding methodology and
interpretation of the results, due to a lack of expertise and/or training on the topic.

Since pulmonary function may remain stable or improve when high-resolution computed tomography (HRCT) scores worsen (de Jong et al., 2004) and is not a strong predictor of maximal aerobic fitness ($\dot{V}O_{2peak}$) in mild respiratory disease (Pianosi et al., 2005), there is a need for more sensitive and comprehensive assessment methods in paediatric patients with mild-to-moderate CF. Not only does CPET provide a comprehensive physiological evaluation of patients’ (dys)function, but the derived parameters have been shown to provide indices of prognostic stratification in patients with CF. More specifically, a higher $\dot{V}O_{2peak}$ is associated with an improved prognosis (Nixon et al., 1992; Moorcroft et al., 1997; Pianosi et al., 2005), QoL (de Jong et al., 1997) and a reduced risk of hospitalisation for pulmonary exacerbations (Pérez et al., 2014).

In view of this, although CPET has been an established clinical assessment method for decades, the European CF Society (ECFS) Collaborative Exercise Working Group and other international CF associations have this year promoted CPET as the exercise testing method of choice for this patient group (Hebestreit et al., 2015). Moreover, the ECFS Clinical Trials Network Standardisation Committee recently called for research to 1) assess the validity, reproducibility and feasibility of the outcome measures used in the assessment of patients with CF and 2) determine the most appropriate exercise test for paediatric patients (Bradley et al., 2012).

A more standardised approach to CPET as an outcome measure in CF care and research would enable a normative database to develop and thresholds for
‘abnormal’ function and clinical deterioration to be established. Furthermore, establishing a patient’s exercise (dys)function and physiological response profile will enable exercise prescription to be more individualised relative to fitness levels and training goals, since it is currently too generalised and underutilised within UK clinics (Stevens et al., 2010). The implementation of routine CPET in CF will also enable the prognostic value of exercise parameters to be examined alongside more common clinical outcome measures, such as parameters of pulmonary function, body mass, QoL, hospital admissions and drug administration. Furthermore, since there has been a marked improvement in the median survival age of patients with CF over recent years (Dodge et al., 2007), the earlier publications regarding the prognostic value of CPET in patients with CF (Moorcroft et al., 1997; Nixon et al., 1992; Pianosi et al., 2005) are outdated and therefore urgently require updating in the new generation of children and adolescents with CF. Whether submaximal CPET-derived parameters may also provide sensitive predictors or mortality rate in adolescents with CF also warrants further investigation, particularly given recent evidence that the ventilatory equivalent for O₂ ($\dot{V}_E/\dot{V}O_2$) is a strong predictor of mortality rate in adolescents with CF (Hulzebos et al., 2014).

2.4.2 Current use of CPET as an outcome for clinical trials in patients with CF

Pulmonary function and structural investigations and/or measures of nutritional status are traditionally relied upon to measure disease severity and progression in CF, but they cannot predict exercise capacity and are often not sensitive to change in mild-to-moderate disease. As a short-term intervention, IVABs can restore FEV₁ to baseline within 7 days in adult patients (Daniels et al., 2013) and have been shown to improve pulmonary function (FEV₁: + 9.5%) in
paediatric patients following a 14 day course of treatment (Pike et al., 2001). Although IVABs may provide intermediary benefit and patients reportedly feel better, whether pulmonary function is sensitive enough to detect these improvements immediately so that patients can be sent home and/or stop treatment requires further investigation. CPET may therefore provide a more detailed clinical assessment than current standard outcome measures to inform medication and therapy strategies, assist with pre-transplant stratification and evaluate responses to clinical intervention. However, the use of CPET as an outcome in therapeutic trials still remains in its infancy (Bell & Morris, 2010).

To date, a few studies have investigated the impact of a course of IVABs on the exercise function of patients with CF using step and shuttle tests (Pike et al., 2001; Cox et al., 2011). Pike et al. (2001) utilised the 3 min step test and observed reductions in heart rate (HR) and dyspnoea, in addition to increased SpO₂ upon exhaustion. Conversely, although 14 days of home IVABs enhanced pulmonary function in children with CF (FEV₁: + 12 ± 4%), performance on the modified shuttle test did not improve (Cox et al., 2011). Similarly, there is limited evidence concerning the influence of new gene mutation targeted therapies, such as Ivacaftor, on the exercise function of patients with CF. To date, only two of the published clinical trials investigating the efficacy of this new era of treatments have utilised any form of exercise testing, both using the 6MWT. In the case study of a female who was homozygous for the G551D mutation, Harrison et al. (2013) observed an improvement in exercise performance within 2 weeks of treatment, with the distance walked increased from 150 m to 550 m. The majority of this improvement was observed within 2 weeks of initiation of Ivacaftor and continued to increase for the following 50 weeks. This continued improvement is in contrast to the response observed in heterogenous
individuals, who appear to experience a plateau in functional capacity (Davies et al., 2013). However, the subjective nature of the 6MWT provides little insight into the mechanistic explanations of the improvement in aerobic exercise function.

More recently, a case study has demonstrated that Ivacaftor improved maximal aerobic fitness ($\dot{V}O_{2\text{peak}}$) in a 27 y old male with CF (F508del/G551D mutation). Quon et al. (2015) performed CPET pre- and post-treatment and demonstrated a 16% improvement in FEV$_1$, in addition to a 14% improvement in CPET derived $\dot{V}O_{2\text{peak}}$ and significantly reduced exertional dyspnoea. The authors attributed these improvements, at least in part, to improved ventilatory function, including improvements in breathing pattern (increased tidal volume and reduced breathing frequency) and dynamic operating lung volumes (increased inspiratory reserve volume and inspiratory capacity) and decreases in dynamic mechanical ventilatory constraints. However, the authors focused only on pulmonary factors and did not include any other central or peripheral indices, to help us understand the matching of O$_2$ delivery-to-utilisation during exercise.

Despite the promising results shown by administering Ivacaftor in people with CF, no studies have yet assessed the influence of these ground-breaking treatment strategies on the aerobic exercise function of patients using a robust testing protocol. To objectively quantify physical functional changes following different pharmacological treatments, CPET should be included within future research. Furthermore, the utility and sensitivity of CPET versus current clinical outcome measures in clinical trials requires attention. There is also a need for more standardised, comprehensive exercise testing in the evaluation of exercise training programmes in patients with CF.
Supervised exercise training provides several benefits to individuals with CF, including alleviation of dyspnoea and improved exercise tolerance (Cerny et al., 2013), maintained pulmonary function, improved sputum clearance (Dwyer et al., 2011; Hebestreit et al., 2001), training of the respiratory muscles (Houston et al., 2013), delayed reduction in bone mineral density (Tejero García et al., 2011) and improved psychosocial health (Hebestreit et al., 2014). Exercise training may also be important in the management of CFRD, since exercise is known to improve glycaemic control and inflammation in type I diabetes mellitus (Galassetti et al., 2013) and can notably improve glucose tolerance and insulin sensitivity in healthy adolescent boys (Cockcroft et al., 2015). Aerobic fitness and pulmonary function may be modifiable with exercise training interventions in CF (Radtke et al., 2015) and \( \dot{V}O_2_{peak} \) was one of 3 primary outcome measures in the most recent Cochrane review on the topic (Radtke et al., 2015). However, there are concerns regarding the validity of \( \dot{V}O_2_{peak} \) measured using the currently employed CPET protocols.

2.4.3 Current exercise testing protocols for patients with CF

There has been much debate over recent years regarding the most appropriate testing protocol and guidelines to implement for this patient group. A key contributor to the poor uptake of CPET in clinical practice is that the measurement of \( \dot{V}O_2_{peak} \) using CPET remains somewhat of a mystery for many clinicians (Bell & Morris, 2010). Consequently, clinical tests which are being performed are generally simplistic, varied and crude in nature. Maximal and submaximal field tests, such as shuttle walk and step tests are currently the most commonly used in children and adolescents with CF (Stevens et al., 2010). These protocols are attractive for use in the clinical setting, since they are
low cost, simple to administer and do not require specialist ergometers or equipment to measure pulmonary gas exchange and ventilation.

Whilst the modified shuttle test is reportedly valid and reproducible for use in adults with CF (Bradley et al., 1999; Bradley et al., 2000), there is no evidence in paediatric patients with milder disease. However, both the 10 m and 20 m shuttle tests have been validated against treadmill determined $\dot{V}O_{2peak}$ and are reportedly valid and reproducible in paediatric patients with CF (Selvadurai et al., 2003). Shuttle and step tests are, however, limited in the information they provide, since they merely provide a crude indication of performance with gross measurement (e.g., distance walked or number of steps) and the limiting factor(s) (e.g., changes in HR and SpO$_2$) (Lesser et al., 2010; Narang et al., 2003). Recent evidence also shows that distance walked during the 6MWT does not appear to significantly correlate ($r = 0.09$) with $\dot{V}O_{2max}$ in children and adolescents with CF (Lesser et al., 2010). Furthermore, from a practical point of view, many of the healthier new generation of children and adolescents with CF are also now capable of completing even modified versions of these tests, although the proportion is currently not known. Maximal CPET is based on the principle that the respiratory, cardiovascular and muscular systems are forced to the limits of their tolerable ranges, meaning that abnormal response patterns can be observed (Ferrazza et al., 2009). Current crude shuttle and step tests fail to elicit such a response due to their submaximal nature, which is indicated by the fact that 6MWT distance appears to significantly correlate with $\dot{V}O_{2peak}$ in healthy children ($r = 0.59$) but not those with CF ($r = 0.09$) (Lesser et al., 2010).

Over recent years there has been a focus on developing CPET protocols for people with CF that are time efficient and do not require the measurement of
pulmonary gas exchange during exercise, which may increase clinical uptake. The Steep Ramp Test (SRT) has recently been introduced as a simple, short duration incremental exercise test for healthy children and adolescents (Bongers et al., 2013) and those with CF (Bongers et al., 2015; Werkman et al., 2011) and does not require measurements of pulmonary gas exchange (Bongers et al., 2015; Werkman et al., 2011). The primary outcome measure is peak work rate (\(W_{\text{peak}}\)), which is then used to estimate \(\dot{V}O_2\text{peak}\). In healthy children, the SRT is reportedly both a valid and reproducible tool to predict \(\dot{V}O_2\text{peak}\) (Bongers et al., 2013). For the validity assessment, 38 children (17 boys and 21 girls; 1.85 ± 3.20 y) performed the SRT and a regular CPET with respiratory gas analysis, with both tests completed within a two week period. A strong correlation between SRT derived \(W_{\text{peak}}\) and the \(\dot{V}O_2\text{peak}\) achieved during CPET was found \((r = 0.96)\), and yielded the following prediction equation (Equation 2.1):

\[
\dot{V}O_2\text{peak} (\text{mL·min}^{-1}) = (8.262 \ W_{\text{peak}} \ \text{SRT}) + 177.096 \\
(R^2 = 0.917, \ \text{SEE} = 237.4).
\]

Equation 2.1

Reproducibility was assessed in a separate subgroup of participants \((n = 37, 17 \ \text{boys and 20 girls; 13.86 ± 3.22 y})\). An intraclass correlation coefficient (ICC) of 0.99 was reported for \(W_{\text{peak}}\), obtained from two SRTs completed within two weeks, with limits of agreement of +24.5 to -37.5 W.

The validity of the SRT has also been investigated against CPET in 40 children and adolescents (17 boys and 23 girls; 14.7 ± 1.7 y; \(\text{FEV}_1 86 \pm 18\% \ \text{predicted}\)) with CF (Bongers et al., 2015). Although a strong correlation was present between \(W_{\text{peak}}\) attained during the SRT and CPET-derived \(\dot{V}O_2\text{peak}\) \((r = 0.82)\),
peak values for HR (168 ± 14 vs. 182 ± 12 b·min⁻¹), minute ventilation (V̇E) (59.2 ± 19.5 vs. 72.0 ± 20.2 L·min⁻¹) and V̇O₂ (36.9 ± 7.5 vs. 41.5 ± 7.6 mL·kg⁻¹·min⁻¹) were significantly lower when using the SRT compared with CPET and W_peak was higher (252 ± 60 vs. 174 ± 46 W). These findings are in contrast with an earlier study from the same research group that reported similar V̇O₂peak values (38.9 7.4 vs. 38.8 8.5 mL·min⁻¹·kg⁻¹) in adolescents with CF (n = 16, 8 males and 8 females; 14.7 1.7 y; FEV₁ 81 ± 22% predicted) during CPET and the SRT (Werkman et al., 2011). However, the study design in the earlier work by Werkman et al. (2011) differed, with the CPET followed by the SRT. Whilst the SRT may indeed provide an alternative field based test for children and adolescents with CF when gas analysis is not possible, it is limited by the physiological information that it can provide and the short duration of the test. Specifically, the SRT only measures W_peak to estimate V̇O₂peak, and thus does not quantify other parameters of aerobic function (Whipp et al., 1981; Whipp et al., 1982), such as the V̇O₂ mean response time (MRT), gas exchange threshold (GET) and the O₂ cost of exercise (V̇O₂ gain; ΔV̇O₂/ΔWR) and additional markers of ventilatory efficiency. These markers are also effort independent and the potential of submaximal parameters, such as the slope of V̇E/V̇CO₂ (ventilatory drive), to provide superior prognostic indices in adolescents with CF has been raised (Hulzebos et al., 2014).

Efforts have also recently been made to develop CF-specific linear regression models (see Equation 2.2) which can predict V̇O₂peak from the W_peak obtained during cycle based CPET, using the Godfrey protocol (Werkman et al., 2014).

\[
\text{V̇O₂peak (mL·min}^{-1}) = 377.0 - 178.4 \times \text{Sex (0 = female / 1 = male)} + 10.1 \times \text{W_peak}
\]

*Equation 2.2*
Werkman et al. (2014) found a strong prediction model ($R = 0.91$; $R^2 = 0.85$; standard error of the estimate (SEE) = 172.57) to estimate $\dot{V}O_{2\text{peak}}$ (mL·min$^{-1}$) using $W_{\text{peak}}$ and gender in adolescents with CF (FEV$_1$ = 37-147% predicted), when direct $\dot{V}O_{2\text{peak}}$ assessment is not possible. Furthermore, the regression model (Equation 2.2) showed potential to discriminate between patients in different prognosis clusters based on their exercise capacity. To note however, although a 95% prediction interval was reported, the predicted $\dot{V}O_{2\text{peak}}$ was significantly different (107 mL·min$^{-1}$) to directly measured $\dot{V}O_{2\text{peak}}$, however this was a difference that was within the SEE of the model.

The Godfrey protocol (Godfrey, 1971) appears to be the preferred CPET protocol at present for children and adolescents with CF. In line with this, the ECFS have recently recommended the Godfrey cycle ergometer protocol, with monitoring of $\text{SpO}_2$ and pulmonary gas exchange, as the protocol of choice for patients > 10 y of age (Hebestreit et al., 2015). The Australasian and North American consensus statements are anticipated to further support these recommendations. This protocol consists of a 3 min warm-up, followed by ‘step’ increases in work rate each minute until exhaustion. However, some argue that a ramp protocol, whereby exercise intensity is increased linearly throughout exercise rather than step increments each minute, may be more appropriate for children and adolescents with chronic disease, since the linear increase in work rate may be better to depict the progressive changes in $\dot{V}O_2$ during CPET (Bongers et al., 2013).

Another potential issue with the Godfrey protocol (Godfrey et al., 1971) is the work rate increments, which are derived solely based on the individual's height (10 W·min$^{-1}$ < 120 cm; 15 W·min$^{-1}$; 120–150 cm; 20 W·min$^{-1}$ > 150 cm).
Although this method does attempt to individualise work load increments for children and adolescents with CF, it is reported that smaller, healthier children reach exhaustion in approximately 4 min, while larger healthy children exercise for approximately 10 min (Godfrey, 1971). In line with this, there is evidence of short test durations of approximately 4 min within the CF paediatric exercise testing literature (e.g., Kent et al., 2012). Clinically, this protocol may often be modified to counter this issue. Since a test duration of approximately 10 min is suggested to provide optimal cardiopulmonary assessment (Buchfuhrer et al., 1983), recent experimental work sought to determine a CF-specific linear regression model (see Equation 2.3) for children and adolescents to predict $W_{peak}$ based on standard anthropometric and pulmonary function measurements, which could then calculate individualised workload increments to reach volitional exhaustion in ~ 10 min (Hulzebos et al., 2014).

$$W_{peak} (W) = 142.865 + 2.998 \times \text{Age (y)} - 19.206 \times \text{Sex (0 = male, 1 = female)} + 1.328 \times \text{Height (cm)} + 23.362 \times \text{FEV}_1 \text{ (L·min}^{-1})$$

Equation 2.3

$R = 0.89$, $R^2 = 0.79$, SEE = 21.0

When the authors extrapolated the equation to the model validation group there was no significant difference between predicted and measured $W_{peak}$ (164.6 ± 38.6 vs. 166.5 ± 38.1 W, respectively). Furthermore, when the developed protocol (increments per min = predicted $W_{peak}$ / 10) was validated in a subgroup of 14 adolescents with CF, there was no significant difference between measured and estimated $W_{peak}$ (197 ± 50 vs. 186 ± 38 W, respectively). The variance in time to exhaustion was also reduced with use of this protocol compared with the traditional Godfrey method (9.7 – 12.0 vs. 7.8 – 12.0 min, respectively). Hulzebos et al. (2014) therefore concluded that their
model to predict $W_{\text{peak}}$ from standard anthropometric variables is valid and that dividing the predicted $W_{\text{peak}}$ by 10 to determine individualised workload increments for the maximal exercise test will allow participants to achieve true cardiopulmonary exhaustion in the recommended 10 min time frame, which will prevent short test durations that mean additional key parameters of aerobic function cannot be determined. Ultimately, this approach will provide more optimal CF-specific exercise testing of comparable test duration across a wide variety of ages and disease states.

Although there has been increased attention surrounding exercise testing protocols for children and adolescents with CF over the past decade, one primary consideration when choosing the most appropriate exercise testing protocol should be how accurately it depicts aerobic fitness and the key physiological parameters of interest. In addition to the considerations outlined above, there remain some validity concerns regarding the determination of $\dot{V}O_{2\text{max}}$ in children and adolescents with CF using protocols such as the Godfrey. Prior to implementation of CPET within the clinical management of children and adolescents with CF, clarification of the optimal protocol and feasibility within the clinical setting is required (Stevens & Williams, 2007).

2.4.4 Validity considerations for CPET in paediatric patients with CF

$\dot{V}O_{2\text{max}}$ is widely accepted to be the best single measure of an individual’s aerobic fitness; representing the integrated capacity of the pulmonary, cardiovascular and muscular systems to uptake, transport and utilise $O_2$ during intense exercise (Poole et al., 2008). However, there are still issues that confound its interpretation in paediatric populations. Although $\dot{V}O_{2\text{max}}$ is
traditionally identified by a plateau in \( \dot{V}O_2 \) upon exhaustion, despite increasing work rate (Armstrong & Welsman, 1994; Rowland, 1993), it has been established that only a minority of young people display a \( \dot{V}O_2 \) plateau (Armstrong & Welsman, 1994; Armstrong et al., 1996; Barker et al., 2011). As such, the term \( \dot{V}O_{2\text{peak}} \) has been accepted as the standard outcome measure for paediatric populations (Rowland, 1994), defined as the highest \( \dot{V}O_2 \) attained during an exhaustive exercise test. However, the prevalence of a \( \dot{V}O_2 \) plateau upon exhaustion in children and adolescents with CF requires confirmation. Furthermore, despite the reported clinical utility of \( \dot{V}O_{2\text{peak}} \) in CF, its validity using CPET must also be established.

Specifically, given that few children and adolescents satisfy the traditional \( \dot{V}O_2 \) plateau criterion, reliance therefore falls upon secondary criteria, encompassing subjective indicators of effort (sweating, facial flushing, hyperpnoea) and objective secondary criteria (HR, respiratory exchange ratio (RER) and/or post-exercise blood lactate concentration (La\(_{[B]}\)) to verify a maximal effort and confirm \( \dot{V}O_{2\text{max}} \). Such criteria are, however, dependant on arbitrary values and have been deemed unsuitable for healthy children (Barker et al., 2011; Robben et al., 2013), adults (Poole et al., 2008) and ambulatory paediatric patients with spina bifida (de Groot et al., 2009) due to their variability and tendency to underestimate \( \dot{V}O_{2\text{max}} \). It is therefore conceivable that such secondary verification criteria may be equally unsuitable for children and adolescents with CF, however no study has yet investigated their validity in this patient group and their use remains common practice within the CF literature (e.g. Werkman et al., 2011).
Controversy therefore surrounds traditional test procedures and whether they provide a high degree of confidence that ‘true’ $\dot{V}O_{2\text{max}}$ has been obtained (Day et al., 2003; Duncan et al., 1997; Howley et al., 1995; Taylor et al., 1955). A number of recent investigations (Barker et al., 2011; Day et al., 2003; Foster et al., 2007; Hawkins et al., 2007; Midgley et al., 2006; Midgley et al., 2007a; Midgley et al., 2009; Poole et al., 2008; Rossiter et al., 2006; Scharhag-Rosenberger et al., 2011) have reported the utility of a procedure termed the ‘verification phase’ in healthy adults and young people. This method involves a traditional ramp incremental exercise test preceding an individualised supramaximal verification phase ($S_{\text{max}}$), to confirm $\dot{V}O_{2\text{max}}$. Theoretically, if a similar $\dot{V}O_{2\text{peak}}$ is achieved during both tests then the traditional $\dot{V}O_2$ plateau criterion can be satisfied (Hill & Lupton, 1923). The utility, safety and clinical feasibility for children and adolescents with CF are not known. To date, only one study has sought to validate $\dot{V}O_{2\text{peak}}$ in patients with CF using supramaximal exercise (Werkman et al., 2011). However, in this study only adolescents were tested and the SRT was employed as the supramaximal verification phase, rather than an individualised work rate based on the preceding ramp test.

Thus far, one study has established the utility of $S_{\text{max}}$ verification in children and adolescents with a chronic disease. de Groot et al. (2009) investigated the use of a 3 min $S_{\text{max}}$ verification protocol to confirm a maximal effort in twenty children with spina bifida who were ambulatory ($n = 20$, 9 males and 11 females; $10.3 \pm 4.9$ y). In line with data from healthy children (e.g., Barker et al., 2011; Robben et al., 2013), only 65% of the participants achieved a $\dot{V}O_2$ plateau upon exhaustion, 65% met the secondary criteria for HR, whilst 80% achieved an RER greater than 1.00. Only 7/10 children met all three of the secondary verification criteria. When the group was viewed as a whole, no significant
differences were found between the incremental test and $S_{\text{max}}$ derived $\dot{V}O_{2\text{peak}}$ values (34.1 vs. 34.8 mL·kg$^{-1}$·min$^{-1}$, respectively). However, on an individual patient level, 5 children showed a clinically relevant increase in $\dot{V}O_{2\text{peak}}$ with $S_{\text{max}}$ verification testing. Interestingly, of the five children who did not meet $\geq 2$ of the secondary verification criteria, a meaningful increase in $\dot{V}O_{2\text{peak}}$ with $S_{\text{max}}$ was observed. However, conversely, the participant who failed to meet any secondary criteria did not increase $\dot{V}O_{2\text{peak}}$ during the $S_{\text{max}}$ exercise test. Furthermore, of the children who did increase $\dot{V}O_{2\text{peak}}$ during $S_{\text{max}}$, one had met all 3 secondary verification criteria, three had met 2 and one had met 1. This finding that traditional secondary criteria are varied and may significantly underreport $\dot{V}O_{2\text{max}}$ confirms previous findings in healthy children and adolescents (Barker et al., 2011).

No study has formally established an up-to-date ‘gold standard’ valid and reproducible testing protocol for ascertaining ‘true’ $\dot{V}O_{2\text{max}}$ in children and adolescents with mild-to-moderate CF. Until a study is undertaken, inferences regarding exercise training, pharmacologic interventions and disease-related changes cannot be discerned with certainty in this population. The safety and feasibility of implementing such testing is also of clinical interest. A lack of research supported guidelines for $\dot{V}O_{2\text{max}}$ determination may contribute to errors in measurement and interpretation; measures upon which clinical decisions can be made, or an incorrect decision as to whether ‘true’ $\dot{V}O_{2\text{max}}$ has changed in response to therapeutic intervention or disease progression (Midgley et al., 2006). It is important that new conceptual advances within exercise physiology continue to be incorporated within clinical practice. For modern clinical CPET
guidelines to be established, a valid and robust protocol to determine ‘true’ \( \dot{V}O_2 \text{max} \) is essential.

2.4.5 Reproducibility considerations for CPET in paediatric patients with CF

Quantifying reproducibility enables researchers and clinicians to understand the variation associated with outcome measures (Hopkins, 2000) and to determine meaningful changes (Atkinson & Nevill, 1998). Interpreting data in relation to normative data and the TE of the measurement enables researchers and clinicians to determine clinically meaningful changes. To define the minimal physiologic improvement in variables such as \( \dot{V}O_2 \text{max} \), that reflects a disease-related decline or the positive effect of a given therapeutic intervention, the minimally clinically important difference must be established (Ferrazza et al., 2009). Furthermore, reproducibility over time is critical when evaluating the efficacy of CF treatments (e.g., antimicrobials, mucolytics and gene mutation targeted therapies) which may accrue over weeks or months, as well as monitoring exercise training interventions. Consequently, inferences regarding therapeutic interventions or disease-related changes in CPET derived parameters cannot currently be discerned with certainty in these patients until the TE in outcome measures is established. There is some evidence concerning the minimally clinically important difference for more crude or submaximal exercise tests in adults (Barry & Gallagher, 2007; Bradley et al., 2000) and children with CF (e.g. Cunha et al., 2009; Gulmans et al., 1996). However, to date, there are no data concerning the reproducibility of CPET-derived maximal and submaximal outcome measures using a protocol that ensures the determination of ‘true’ \( \dot{V}O_2 \text{max} \) in paediatric patients with mild-to-moderate CF.
Regarding the reproducibility of CPET for people with CF, there is limited data. Two previous studies have reported the utility of incremental CPET, using traditional verification criteria, in adults with CF (Gruet et al., 2010; McKone et al., 1999). McKone et al. (1999) investigated the reproducibility of incremental ramp testing (15 W·min⁻¹) in adults with stable CF (n = 9, 6 males and 3 females; 26.3 ± 8.3 y; FEV₁ 56% predicted). With three tests performed over a 28 day period, there were no significant differences in \( \dot{V}O_2, \dot{V}E, \) respiratory frequency, HR or SpO₂ at rest, exhaustion or 40% and 70% of \( W_{\text{peak}} \) across the three tests. Coefficients of variation for peak \( \dot{V}O_2, \dot{V}E, \) respiratory frequency, HR and SpO₂ of 6.9%, 6.2%, 5.8%, 3.0% and 1.1% were reported, respectively. Similarly, Gruet et al. (2010) reported a coefficient of variation of 8.5% for \( \dot{V}O_{2\text{peak}} \) determined using incremental CPET (12 W·min⁻¹) across a 4 week period in adults with CF (n = 31, 25 males and 6 females; 26.9 ± 6.0 y; FEV₁ 52% predicted).

From a paediatric perspective, only one study has reported the reproducibility of CPET in children with CF (Kent et al., 2012). Using the Godfrey protocol, Kent et al. (2012) sought to determine the coefficients of variation for HR (5.6%), \( \text{SpO}_2 \) (5.2%) and \( W_{\text{peak}} \) (8.9%) in children with CF (n = 16, 9 males and 7 females; 8.7 ± 1.8 y; FEV₁ 88.1 ± 17.4% predicted), using two tests completed within a 1 week period. This study did, however, encompass a number of methodological limitations that confound the interpretation and application of its findings. Firstly, the design was such that an intermittent sprint cycle test was completed within the same testing session as the subsequent incremental CPET. This may have contributed to the short and insufficient ramp test durations recorded (~ 4 min). Furthermore, only limited, parameters of physiological function were presented and no measures of pulmonary gas
exchange were obtained. There is, therefore, a need to investigate the reproducibility of maximal and submaximal CPET parameters in children and adolescents with CF and the utility of $S_{\text{max}}$ verification.

2.5 CPET measurements of interest in the assessment of aerobic exercise (dys)function

2.5.1 Standard parameters of interest from incremental CPET

Although much of the focus over recent years has been on the clinical utility of $\dot{V}O_2^{\text{peak}}$ as the primary outcome measure from CPET in patients with CF, additional maximal and submaximal parameters can offer value. A CPET provides a large number of important parameters, including but not limited to gas exchange and metabolic data ($\dot{V}O_2$, $\dot{V}CO_2$, GET, ventilatory equivalents for $O_2$ ($\dot{V}E/\dot{V}O_2$) and $CO_2$ ($\dot{V}E/\dot{V}CO_2$), $O_2$ pulse, $SpO_2$ via pulse oximetry, end-tidal $O_2$ and $CO_2$), in conjunction with test duration and work rate. These additional parameters are often overlooked in favour of the final maximal parameters obtained at exhaustion, particularly $\dot{V}O_2$, work rate, HR and $SpO_2$. However, submaximal values should assist with diagnostic and prognostic evaluations and need to be incorporated more fully in patient reports (Wasserman et al., 2004). No one single parameter should be used exclusively; rather, it is the integration of the exercise responses as a whole, which adds value when utilising CPET compared with other exercise testing methods.

$\dot{V}O_{2\text{max}}$ represents the gold standard measure of aerobic fitness and provides an indication of how well patients’ lungs, pulmonary and cardiovascular circulation (large and small vessels), and skeletal muscles function in an integrated manner during exercise (see Figure 2.6). However, a more comprehensive
assessment of patients' cardiorespiratory fitness may be gained through quantification of submaximal parameters of aerobic function (i.e., lactate threshold (LT) or its non-invasive equivalent the GET), the kinetics of $\dot{V}O_2$ (MRT) and work efficiency ($\dot{V}O_2$ gain or $\Delta\dot{V}O_2/\Delta\dot{WR}$). These three parameters, combined with $\dot{V}O_{2\text{max}}$, provide the four key parameters of aerobic exercise function (Whipp et al., 1982).

From a practical viewpoint, outcome measures which can assess patients’ function at submaximal intensities, similar to ADLs, are also important.

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**Figure 2.6.** Schematic to illustrate the relationship between muscle $O_2$ consumption and pulmonary $O_2$ uptake. $O_2$ is inspired from the atmosphere and delivered by the cardiovascular system to the working muscles, where it is consumed by the mitochondria to produce adenosine triphosphate (ATP). The deoxygenated blood is then transported back to the pulmonary circulation enabling muscle $\dot{V}O_2$ to be estimated from the pulmonary $O_2$ exchange, although there will be temporal misalignment owing to the venous volume and $O_2$ stores between the muscle and lung. VT, tidal volume; $f$, breathing frequency; SV, stroke volume; HR, heart rate; $\dot{Q}$, cardiac output; $\dot{Q}CO_2$, muscle $CO_2$ production; $\dot{Q}O_2$, muscle $O_2$ utilisation; creat-PO$_4$, creatine phosphate; pyr-lac, pyruvate-lactate. Taken from Jones & Poole (2005) with permission.
Furthermore, submaximal parameters may be especially useful in the clinical environment when patients may be unwilling or unable to provide a maximal effort. Additionally, the GET can improve independently of any changes in $\dot{V}O_{2\text{max}}$ in individuals with CF (Casaburi et al., 1991), and is often used in the prescription of individualised exercise intensities within specific intensity domains (i.e., at a percentage of the GET or using the delta ($\Delta$) concept), as recently demonstrated in paediatric patients with mild-to-moderate CF (Stevens et al., 2011).

Although there is evidence concerning the determination of the GET in adults with CF (Gruber et al., 2014; Sexauer et al., 2003; Shah et al., 1998), there is a need to confirm how identifiable and reproducible these parameters are for use in children and adolescents with mild-to-moderate CF disease. Promisingly, Visschers and colleagues (2015) recently determined the utility and reproducibility of four different non-invasive methods for determining the ventilatory threshold (VT) in 15 children with CF and 15 children with a surgically corrected dextro-transposition of the great arteries. In children with CF, the V-slope, ventilatory equivalent method, and the end-tidal pressure of O$_2$ ($P_{ET}O_2$) method were all comparable and reproducible methods to determine the VT during exhaustive incremental CPET. The V-slope method (Beaver et al. 1986) did show the best inter-rater reproducibility for the $\dot{V}O_2$ at the ventilatory threshold (ICC: 0.92), compared with the ventilatory equivalent (ICC: 0.84) and $P_{ET}O_2$ (ICC: 0.87) methods, however it was recommended that a combination of the three methods may improve the accuracy and reproducibility of determination. The feasibility of this practice may, however, be poor and using all three methods could be particularly time consuming. However, the V-slope method, with verification using the ventilatory equivalent method and $P_{ET}O_2$ is
recommended and has been used within this thesis. The reproducibility of additional submaximal parameters of interest warrants determination.

Ventilatory function is best examined by relating \( \dot{V}_O_2 \) and \( \dot{V}_C_O_2 \) dynamics to \( \dot{V}_E \) (Akkerman et al., 2010), through the slope of the \( \dot{V}_E/\dot{V}_C_O_2 \) response and the \( O_2 \) uptake efficiency slope (OUES). The OUES is useful since it is, theoretically, resistant to early test termination and intra- and inter-rater variability (Akkerman et al., 2010). Although these parameters possess documented utility to identify the presence and severity of ventilatory inefficiency of the heart/lungs and/or response to intervention in heart failure patients, their uptake within the assessment of patients with respiratory disease has been scarce (Bongers et al., 2012). The OUES is suggested to offer a submaximal measure of cardiopulmonary fitness, which could be useful when patients are not able to provide a maximal effort. However, it was recently suggested that this measure is limited by its ability to distinguish between children with mild-to-moderate CF and their healthy peers (Bongers et al., 2012). Bongers et al. (2012) aimed to investigate whether the OUES may provide an alternative to \( \dot{V}_O_2_{peak} \) in children and adolescents with mild-to-moderate CF \((n = 22, 13 \text{ males and } 9 \text{ females; } 15.7 \pm 1.5 \text{ y; FEV}_1 81.5 \pm 15.6\% \text{ predicted})\) when a maximal effort is not possible. Whilst the authors concluded that the OUES is of limited value as a measure of cardiopulmonary exercise capacity in children with mild-to-moderate CF, since the OUES provides an efficiency measure and should not be considered a surrogate for \( \dot{V}_O_2_{max} \), its utility in the assessment of aerobic exercise (dys)function requires further investigation.

Furthermore, questions have been raised regarding whether submaximal parameters may provide better prognostic markers than \( \dot{V}_O_2_{peak} \) in patients with
CF (Hulzebos et al., 2014). Using data from 127 adolescents with CF (n = 127, 77 males and 50 females; 12.7 ± 0.9 y; FEV₁ 77.7 ± 15.6% predicted), Hulzebos et al. (2014) built a multivariate model comprising BMI, FEV₁%predicted, predicted \( \dot{V}O_{2\text{peak}} \) relative to body mass, peak \( \dot{V}E \), peak \( \dot{V}E/\dot{V}O₂ \) and \( \dot{V}E/\dot{V}CO₂ \), and breathing reserve. Subsequently, the Cox proportional hazards method was used to determine the best combination of these parameters to predict mortality and/or the need for lung transplantation in this patient group. The predictors BMI (hazard ratio = 5.54, 95% CI = 1.82-16.83), FEV₁%predicted (hazard ratio = 17.13, 95% CI = 3.76-78.06) and peak \( \dot{V}E/\dot{V}O₂ \) (hazard ratio = 5.92, 95% CI = 1.27-27.63) provided the best prediction model. More specifically, patients with all 3 risk factors had a significantly higher risk of mortality compared with patients presenting with none or 1 and patients with two of the aforementioned factors. However, univariate analyses still revealed predicted \( \dot{V}O_{2\text{peak}} \) relative to body mass to be a significant predictor of mortality in adolescents with CF.

2.5.2 Pulmonary \( \dot{V}O₂ \) kinetics

Although CPET is a clinically useful tool for assessing aerobic exercise (dys)function in children and adolescents with CF, CWR exercise testing protocols may also be insightful. More specifically, although the measurement of maximal aerobic fitness and the response to incremental ramp exercise is of clear clinical and investigational value, children and adolescents with CF rarely exercise at their maximal metabolic rate. Furthermore, exercising at or near \( \dot{V}O₂_{\text{max}} \) does not truly represent the rate at which aerobic energy transfer adapts to the changing metabolic demands faced by the \( O₂ \) transport and utilisation pathways (Figure 2.6) during day-to-day challenges. Accordingly, assessing the speed of the \( \dot{V}O₂ \) response to an altered work rate (termed '\( \dot{V}O₂ \) kinetics') can
provide important information regarding the integrated capacity to transport (see Figure 2.6) and utilise $O_2$ to support the increased rate of ATP turnover in the contracting myocytes and, thereby, provide a non-invasive insight of the contribution of skeletal muscle oxidative metabolism to energy turnover (e.g. Barstow et al., 1996; Brittain et al., 2001; Burnley et al., 2002; Chin et al., 2007; Gerbino et al., 1996; Grassi et al., 1997; Gurd et al., 2006; Jones et al., 2004; Koppo et al., 2004; Pringle et al., 2003). Whilst an indication of the $\dot{V}O_2$ kinetic response at the onset of exercise can be gained by determining the MRT during ramp incremental exercise, a more appropriate assessment of the $\dot{V}O_2$ kinetic response is provided by analysing repeated transitions to a range of submaximal work rates.

At the onset of exercise, an immediate increase in ATP production in the active muscle cell is required in order to meet the increased metabolic demand (Jones & Poole, 2005). However, measurements at the muscle level demonstrate a delayed matching between the ATP supplied by oxidative phosphorylation (Equation 2.4) and the rate of ATP turnover occurring within the myocyte (Figures 2.7 and 2.8a).

$$3 \text{ADP} + 3 \text{P}_i + \text{NADH} + \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow 3 \text{ATP} + \text{NAD}^+ + \text{H}_2\text{O} \quad \text{Equation 2.4.}$$
During a step transition to a higher metabolic rate, muscle $O_2$ consumption ($m\dot{V}O_2$) rises, following an exponential time course until the oxidative rate is coupled to the rate of muscle ATP turnover, assuming that the rate of ATP turnover is constant during exercise. However, since $m\dot{V}O_2$ rises appreciably slower than ATP turnover at the cross-bridges, a steady-state matching between ATP utilisation and ATP supply through oxidative ATP resynthesis might not be achieved until at least 2 min following the onset of exercise (Jones & Poole, 2005; also see Figures 2.7 and 2.8a). This delay has been termed the $O_2$ deficit. The magnitude of the $O_2$ deficit indicates the non-oxidative contributions of ATP resynthesis to the ATP turnover rate. Consequently, a larger $O_2$ deficit is associated with a greater depletion of muscle [PCr] and, particularly at higher work rates, a greater production of lactic acid through anaerobic glycolysis to meet the energy demand during of exercise. A depletion

Figure 2.7. Schematic to demonstrate the typical response of muscle $O_2$ consumption following a 'step' transition to moderate intensity constant work rate exercise. Adenosine triphosphate (ATP) turnover at the cross-bridges increases instantly, however muscle $\dot{V}O_2$ increases relatively slowly. Note that a steady-state is attained following ~ 2 min after the increase in exercising work rate in this example. The red line denotes the square wave increase in ATP demand necessary to sustain exercise. Adapted from Jones & Poole (2005) with permission.
of high-energy muscle phosphates and a reduction in muscle pH have both been implicated in the fatigue process (Fitts, 1994), such that faster \( \dot{V}O_2 \) kinetics should be associated with enhanced exercise tolerance. If people with CF present with prolonged \( \dot{V}O_2 \) kinetics, they would thereby incur a greater \( O_2 \) deficit than their healthy counterparts (Figure 2.9), which may have implications for exercise tolerance.

![Figure 2.8](image)

**Figure 2.8.** Schematic of the rise in muscle (A) and pulmonary \( \dot{V}O_2 \) (B) at the onset of moderate-intensity exercise. Adapted from Armstrong & Barker (2009) with permission. The red line denotes the square wave increase in energetic (ATP) demand required in order to sustain exercise.

Since capturing the \( m\dot{V}O_2 \) kinetic response necessitates invasive and technical procedures, which are inherently unsuitable for use with children and adolescents, it is standard practice to infer \( m\dot{V}O_2 \) by measuring the dynamic changes in breath-by-breath pulmonary \( \dot{V}O_2 \) at the mouth. There are, however, differences in the response profile measured at the muscle versus mouth. Specifically, unlike the exponential rise in \( m\dot{V}O_2 \) measured at the periphery (see Figures 2.7 and 2.8a), the rise in pulmonary \( \dot{V}O_2 \) kinetics during moderate intensity CWR exercise is characterised by three phases (see Figure 2.8b) (Whipp *et al.*, 1982; Whipp & Ward, 1992).
The first of the three phases present in the pulmonary $\dot{V}O_2$ kinetic response during moderate intensity CWR is termed phase I, or the cardio-dynamic phase. This phase indicates an initial rise in $\dot{V}O_2$ due to an immediate rise in cardiac output ($\dot{Q}$) (Barstow et al., 1990; Yoshida et al., 1993), which subsequently increases pulmonary blood flow and perfusion. Although $m\dot{V}O_2$ increases immediately at exercise onset, the rise in $\dot{V}O_2$ measured at the mouth typically takes 15 to 20 s, due to the muscle-to-lung transit delay causing a delayed arrival of deoxygenated blood at the lungs. This point is marked by a fall in $P_{ET}O_2$ and increase in the end-tidal partial pressure of CO$_2$ ($P_{ET}CO_2$), reflecting the arrival of deoxygenated blood at the lungs. The increasing $\dot{Q}$ response then
drives an exponential rise (phase II) in \( \dot{V}O_2 \) towards a new steady-state (phase III) (Whipp & Ward, 1992).

Once the delay caused by the muscle-to-lung transit time has been accounted for, it has been shown that the phase II (or alternatively termed ‘fundamental’) region of the pulmonary \( \dot{V}O_2 \) response during moderate intensity CWR exercise rises with similar kinetics to that of \( m\dot{V}O_2 \) (e.g. Barker et al., 2008; Grassi et al., 1996; Krstrup et al., 2009; Rossiter et al., 2002; Figure 2.10). However, few studies have simultaneously and invasively measured the dynamics of \( m\dot{V}O_2 \) and pulmonary \( \dot{V}O_2 \) at the onset of exercise in humans. Krstrup et al. (2009) were the first to investigate the transit time for blood flow from the contracting muscles to reach the lungs following the onset of knee-extensor exercise performed above and below the GET and, thereby, the contribution from the contracting muscles to pulmonary \( \dot{V}O_2 \). The primary finding of this study was that the phase II \( \tau \) for pulmonary \( \dot{V}O_2 \) reflected the kinetics of \( m\dot{V}O_2 \) at the onset to both exercise above and below the GET (see Figure 2.10). There was also no difference between the amplitude for \( m\dot{V}O_2 \) and pulmonary \( \dot{V}O_2 \) suggesting other tissues are contributing a minimal amount to pulmonary \( \dot{V}O_2 \) during this mode of exercise.

Whilst this type of research is not ethical or feasible in children, attempts have been made to characterise the kinetics of pulmonary \( \dot{V}O_2 \) against the fall in muscle \([PCr]\), taken as a surrogate measure of \( m\dot{V}O_2 \) (Meyer et al., 1988). In healthy adults, Rossiter and colleagues (1999) demonstrated a close kinetic coupling between the fall in \([PCr]\), and rise in phase II of the pulmonary \( \dot{V}O_2 \) response at the onset of prone knee-extensor exercise (see Figure 2.11) using \(^{31}\)Phosphorous magnetic resonance spectroscopy (\(^{31}\)P-MRS). Since the signal
from children during knee extensor exercise was deemed insufficient to compare with the pulmonary $\dot{V}O_2$ response to knee-extensor exercise, Barker et al. (2008) subsequently compared the pulmonary $\dot{V}O_2$ response during upright cycling with the kinetics of muscle [PCr] during prone CWR quadriceps exercise. This study confirmed that the kinetic changes in muscle [PCr] are closely coupled (to within $\pm 4$ s) with the phase II pulmonary $\dot{V}O_2$ response at both the onset and offset of exercise. Thus indirectly suggesting that phase II of the $\dot{V}O_2$ response in children provides an indirect measurement of $m\dot{V}O_2$ kinetics.

Figure 2.10. Muscle and pulmonary oxygen uptake ($m\dot{V}O_2$ (○ white circles) and $\dot{V}O_2$ (● black circles), respectively) response to knee-extensor exercise performed below (A) and above (B) the gas exchange threshold. The pulmonary $\dot{V}O_2$ data have been time aligned to the $m\dot{V}O_2$ data by moving the data back by the time equal to the time delay of phase I-II of the pulmonary $\dot{V}O_2$ response. Image taken from Krustrup et al. (2009) with permission.
A standard exponential equation has been used to characterise this rise in $\dot{V}O_2$ measured at the pulmonary level (Figure 2.8b) at the onset of CWR exercise (Equation 2.5):

$$V\dot{O}_2(t) = \dot{V}O_2 \text{ baseline} + \Delta \dot{V}O_2 A \cdot (1- e^{-(t-TD)/\tau})$$

*Equation 2.5.*

where $\dot{V}O_2 (t)$, $\Delta \dot{V}O_2 A$, $TD$, and $\tau$ represent the value of $\dot{V}O_2$ at a given time ($t$), the $\dot{V}O_2$ prior to the onset of exercise, the amplitude change in $\dot{V}O_2$ from baseline to its asymptote, time delay, and the time constant of the response, respectively.

The mathematically modelled time constant ($\tau$; time taken to achieve 63% of the response amplitude) of the phase II region of the pulmonary $\dot{V}O_2$ response at exercise onset is of particular interest, since it is considered to be an important
determinant of the O$_2$ deficit (see Figure 2.10). The phase II $\dot{V}O_2$ $\tau$ during upright cycling is approximately 20-30 s in healthy children and young adults and reaches a steady state (phase III) with an O$_2$ cost of approximately 10 mL·min$^{-1}$·W$^{-1}$ (e.g., Breese et al., 2012; Koga et al., 1999), however higher $\dot{V}O_2$ gain values have been reported in children (Armon et al., 1990) as well as faster phase II $\tau$ compared with adults (see Barker et al., 2010 for a review).

Although Figure 2.8b depicts the three recognised phases of the pulmonary $\dot{V}O_2$ response at the onset of moderate intensity CWR exercise (Whipp et al., 1982; Whipp & Ward, 1992), the response becomes more complex when more intense exercise is performed (Poole & Jones, 2012; Rossiter, 2011; see Figures 2.11 and 2.12). A steady-state $\dot{V}O_2$ is achieved during moderate intensity exercise, indicating an adequate matching of $\dot{V}O_2$ to the muscle ATP turnover rate. However, during exercise performed above the LT, or its non-invasive equivalent the GET, $\dot{V}O_2$ can increase to higher values than would be predicted based on the work rate, potentially even driving $\dot{V}O_2$ towards $\dot{V}O_2$peak.

Several schemas have been developed to provide a frame of reference for investigating $\dot{V}O_2$ kinetics, within discrete exercise intensity domains that are demarcated by specific physiological parameters (Figures 2.12 and 2.13). In the majority of adult studies (see Figure 2.11), the moderate domain is defined as all work rates that fall below LT, or its non-invasive equivalent the GET or ventilatory threshold. The heavy domain is exercise performed above the LT, whereas severe intensity exercise is demarcated by critical power (CP), which represents the asymptote of the hyperbolic relationship between work rate and time to exhaustion (Poole et al., 1988).
Whilst the above boundaries (Figure 2.12) have been used to demarcate the intensity domains by Jones and colleagues over recent years, a slightly modified schema has also been outlined (see Figure 2.13). The primary difference with the schema presented in Figure 2.13 is evident with the terminology for workrates above CP. Whilst the schematic used by Jones and colleagues refers to these intensities as ‘severe’ and ‘extreme’, Whipp and colleagues term these ‘very heavy’ and ‘severe’. Within this thesis, the terminology presented in Figure 2.13 has been used, since this schema represents the majority of studies in paediatric exercise science.

The delayed and elevated $\dot{V}O_2$ that is seen during exercise above the GET has been termed the $\dot{V}O_2$ ‘slow-component’. The slow-component is of practical and

![Figure 2.12. Schematic of the pulmonary oxygen uptake ($\dot{V}O_2$) response during constant work rate moderate (below the LT), heavy (above the LT) and severe intensity exercise (above CP). Note that the $\dot{V}O_2$ response occurring at exercise onset (phase I) has been omitted. LT, lactate threshold; CP, critical power. Adapted from Jones & Poole (2005) with permission.](image)
functional importance, since it is linked to the progressive loss of muscle homeostasis (Burnley & Jones, 2007; Jones et al., 2010; Rossiter et al., 2002) and muscle contractile efficiency (Barclay et al., 1996; Krustrup, 2004; Woledge et al., 1998). During exercise performed above CP, the $\dot{V}O_2$ slow-component elevates $\dot{V}O_2$ to $\dot{V}O_{2\text{max}}$. The $\dot{V}O_2$ slow-component is therefore related to the muscle fatigue process and a reduction in exercise tolerance.

![Figure 2.13. Schematic of the boundaries of the different domains, demarcated by specific parameters of physiological function (red lines) and the typical pulmonary oxygen uptake ($\dot{V}O_2$) response within these domains (solid and dashed black lines). $\dot{V}O_{2\text{peak}}$, peak oxygen uptake; MLSS, maximum lactate steady-state; CP, critical power; LT, lactate threshold. Image adapted from Armstrong & Barker (2009) with permission.](image)

The functional $\dot{V}O_2$ gain of phase II is also reportedly reduced when the imposed work rate exceeds CP (Wilkerson et al., 2004). For work rates above the GET, the development of the $\dot{V}O_2$ slow-component during exercise increases the $O_2$ cost of exercise to $\sim12-14$ mL·min$^{-1}$.W$^{-1}$ (impaired efficiency) and reflects the fatigue processes occurring within the contracting myocytes (Jones et al., 2011). The higher the work rate is above CP, the lower the magnitude of the $VO_2$ slow-component, such that at work rates close to $VO_{2\text{max}}$
the $\dot{V}O_2$ kinetic response follows a single-exponential function until exhaustion occurs, with the participant reaching their $\dot{V}O_2max$ (Özyener et al., 2001).

Characterising the kinetics of phase II of the $\dot{V}O_2$ response and the magnitude of the $\dot{V}O_2$ slow-component can therefore be particularly insightful when trying to understand the adequacy of oxidative metabolism and mechanistic basis of fatigue and exercise (in)tolerance in both health and disease. However, the impact that exercise intensity has on the $\dot{V}O_2$ kinetic response to exercise requires careful consideration when investigating the effect of diseases, such as CF. For example, the prescription of a single absolute work rate is likely to render patients with CF exercise at a higher percentage of their aerobic fitness than their healthy counterparts, as $\dot{V}O_2peak$ is reduced in this patient group (e.g. Moser et al., 2000). Equally, the prescription of a work rate relative to a percentage of $\dot{V}O_2max$ is flawed, as although the GET appears to be preserved in CF when normalised to $\dot{V}O_2max$ (Thin et al., 2002; Hebestreit et al., 2005) there is a large variability in the position of the GET relative to $\dot{V}O_2peak$ across patients with CF and their healthy peers. Consequently, the use of an absolute work rate or work rate prescribed relative to $\dot{V}O_2peak$ will likely result in participants exercising across the moderate to heavy intensity domains. This practice will alter the pattern of the $\dot{V}O_2$ kinetic response (Figure 2.9) and confound any interpretation of the influence of CF disease upon the dynamics of $\dot{V}O_2$.

An additional issue in paediatric studies is the prescription of exercise intensities above the LT, due to the characteristically low absolute differences in pulmonary $\dot{V}O_2$ measured between the LT and $\dot{V}O_2peak$. This reduces the range of work rates available to prescribe exercise within the heavy or very heavy intensity domains. Furthermore, the CP boundary delineating the heavy and
very heavy domains cannot routinely be assessed in children and adolescents and requires several testing sessions (Barker et al. 2012). Consequently, the ‘delta’ (∆) concept should be utilised to prescribe work rates as a percentage difference between the LT/GET and \( \dot{V}_\text{O}_2\text{peak} \) (Armstrong & Barker, 2009). Typically, ∆ 40% is used to prescribe heavy intensity exercise and ∆ 60-70% is used for very heavy intensity exercise. Prescribing exercise within equivalent intensity domains is essential in order to derive valid physiological inferences regarding the influence that CF disease may have upon skeletal muscle oxidative metabolism.

Dysfunction can occur at any step of the \( O_2 \) transport and utilisation pathway, as typically found in disease (e.g. Spencer et al., 2013), causes a slowing of the \( \dot{V}_\text{O}_2 \) kinetic response at the onset of exercise. For a given metabolic rate, slowed \( \dot{V}_\text{O}_2 \) kinetics mandate a greater \( O_2 \) deficit, increased requirement for substrate level phosphorylation (e.g., muscle [PCr] breakdown, anaerobic glycolysis) and the accumulation of fatigue inducing metabolites (e.g., inorganic phosphate (P_i), hydrogen ions), which can impair patients’ ability to tolerate exercise (Poole & Jones, 2012; Rossiter, 2011). The functional \( \dot{V}_\text{O}_2 \) gain and magnitude of the \( \dot{V}_\text{O}_2 \) slow-component can also provide information regarding aerobic exercise efficiency and the fatigue process(es), respectively. For that reason, measuring the \( \dot{V}_\text{O}_2 \) kinetic response at the onset of exercise and during recovery in patients with CF can provide valuable insight into the mechanism(s) regulating muscle energetics and exercise tolerance.
2.6 Current evidence of aerobic exercise (dys)function in cystic fibrosis

2.6.1 Incremental CPET

In addition to the common clinical symptoms characterising CF (see sections 1.1. and 2.1), early studies show that patients also present with reduced peak exercise performance upon testing (e.g. Almajed & Lands, 2012; Cerny et al., 1982; Cropp et al., 1982; Godfrey & Mearns, 1971; Hjeltnes et al., 1984; Klijn et al., 2003; Moser et al., 2000; Shah et al., 1998). The first studies to document the cardiorespiratory exercise response in people with CF were begun over 40 years ago (Cerny et al., 1982; Cropp et al., 1982; Godfrey & Mearns, 1971). Although more detailed investigations trying to ascertain the factor(s) contributing to exercise dysfunction in this group started over a decade later (e.g. Regnis et al., 1996), much remains to be elucidated regarding the extent and cause(s) of exercise limitation in people with CF. Particularly, there is a need to further characterise the extent of aerobic exercise (dys)function in children and adolescents with mild-to-moderate CF disease. The current knowledge regarding how CF impacts parameters from an incremental maximal CPET, primarily the key parameters of aerobic exercise function (Whipp et al., 1981; Section 2.4.1) is outlined below.

2.6.1.1 Peak $\dot{V}O_2$

Godfrey and Mearns (1971) provided the first detailed overview of the physiological response of patients with CF ($n = 41$, 24 male, 5-21 y) at rest and during exercise. The authors employed the same Godfrey protocol that they had previously applied to the study of healthy children (Godfrey & Davies, 1970), which involved progressive increases in work rate each minute. The ability to
exercise was related to disease severity, with $W_{\text{peak}}$ significantly lower in more severe CF. In the mildest severity group, $W_{\text{peak}}$ was within the normal range. Hjeltnes and colleagues (1984) also provided early suggestions that $\dot{V}O_{2\text{peak}}$ may be lower in CF than healthy male adolescents (15-17 y), however a large variation was observed with values ranging from 40-125% (mean 79%) predicted.

Since this early work, several authors have sought to characterise aerobic fitness of people with CF, with the majority of studies adopting the Godfrey protocol. Specific focus has been on $\dot{V}O_{2\text{peak}}$ or $\dot{V}O_{2\text{max}}$, due to the clinical utility described in sections 1.2 and 2.5.1. In general, patients with CF were traditionally considered to present with a lower $\dot{V}O_{2\text{peak}}$ than their healthy peers upon exercise testing; however there are equivocal data in the literature.

Shah et al. (1998) compared 17 individuals with CF (9 males, 25 ± 10 y, FEV$_1$ 62 ± 21% predicted) and 17 age- and gender-matched controls (7 males, 25 ± 8 y, FEV$_1$ 112 ± 15% predicted) during a CPET whereby work rate increased by 30 W every 30s. Body mass normalised $\dot{V}O_{2\text{peak}}$ was significantly lower in CF than controls (24.6 ± 6.0 vs. 35.5 ± 8.5 mL·kg$^{-1}$·min$^{-1}$, respectively). Savi and colleagues (2015) recently compared 30 adults with mild-to-moderate CF (33.3 ± 9.0 y, FEV$_1$ 71 ± 19% predicted) with 15 healthy controls (29 ± 5 y, FEV$_1$ 109 ± 11% predicted). They reported both a lower absolute (1.91 ± 0.47 vs. 2.66 ± 0.77 L·min$^{-1}$) and relative (28.7 ± 5.0 vs. 35.9 ± 5.0 mL·kg$^{-1}$·min$^{-1}$) $\dot{V}O_{2\text{peak}}$ in CF, differences which remained when age, gender, BMI and FEV$_1$ were corrected for. Perpati et al. (2010) also recorded a reduced $\dot{V}O_{2\text{peak}}$ (29.12 ± 7.02 vs. 35.54 ± 7.31 mL·kg$^{-1}$·min$^{-1}$) in older individuals with CF (9 male, 21 ± 11 y, 77 ±
33% predicted) compared with healthy controls (3 male, 29 ± 4 y, 104 ± 16% predicted), in line with findings in more severe CF disease (Poulio et al., 2001).

From a paediatric perspective, Moser et al. (2000), using an exhaustive incremental cycling protocol (10 W·min⁻¹), compared 22 paediatric patients with CF (8 males, 10.3 ± 0.7 y) and 54 healthy controls (17 males, 9.3 ± 0.1 y). As hypothesised, both absolute (0.96 ± 0.81 L·min⁻¹ vs. 1.47 ± 0.54 L·min⁻¹) and relative (31 vs. 45 mL·kg⁻¹·min⁻¹) VO₂peak were significantly lower in CF than controls, respectively. Keochkerian et al. (2008) also reported significantly reduced body mass normalised VO₂peak in CF versus healthy boys (34.7 ± 8.4 vs. 49.2 ± 4.0 mL·kg⁻¹·min⁻¹, respectively). More recently, Bongers and colleagues (Bongers et al., 2012; Bongers et al., 2014) have also reported a reduced VO₂peak in two groups of adolescents with CF compared with healthy controls using the Godfrey protocol, even following bronchodilation with salbutamol (Bongers et al., 2014).

There are, however, some conflicting reports, suggesting VO₂peak is similar between individuals with CF and healthy controls. For example, Hebestreit et al. (2005) reported similar VO₂peak in a relatively young CF cohort (n = 18, 11 males, 15.8 ± 6.1 y, FEV₁ 71.5 ± 19.5% predicted) compared to their respective controls (n = 15, 8 males, 17.8 7.0 y, FEV₁ 101.0 ± 12.2% predicted). What should be noted is that this patient group ranged from 9 to 33 y of age and the mode of exercise was semi-supine rather than upright cycling. There is, therefore, a need to further investigate the impact of age- and disease severity upon aerobic fitness in CF, particularly since the VO₂peak in the CF cohort was subtly higher than the controls in this study (38.5 ± 7.1 vs. 37.9 ± 5.0 mL·kg⁻¹·min⁻¹). However, a recent study by Wells et al. (2011) also reported a similar
$\dot{V}O_{2\text{peak}}$ in 20 adolescents with CF (10 male) using the Godfrey protocol, compared with healthy controls (44.2 ± 10.9 vs. 44.7 ± 11.6 mL·kg$^{-1}$·min$^{-1}$) with similar habitual physical activity patterns. This raises the question as to whether $\dot{V}O_{2\text{peak}}$ can be maintained at 'normal' levels in young people with CF if physical activity levels are the same as their healthy peers.

Whilst the previously outlined validity considerations regarding the Godfrey protocol should be acknowledged, another factor that should be considered is the normalisation of $\dot{V}O_{2\text{peak}}$ to body composition, specifically FFM. Since patients with CF were traditionally malnourished and nutritional status is a principle concern, normalising for whole body mass may result in undetectable changes in aerobic fitness. In adolescents with CF (14.5 (10-22) y, FEV$_1$ 77.5 (45-123)% predicted), Wideman et al. (2009) observed that absolute (1.33 (0.43-2.37) vs. 2.09 (1.15-3.96) L·min$^{-1}$) and body mass normalised (30.6 (8.5-45.2) vs. 40.6 (29.0-64.5) mL·kg$^{-1}$·min$^{-1}$) $\dot{V}O_{2\text{peak}}$ were significantly reduced in CF compared with their healthy peers (13.8 (10-19) y, FEV$_1$ 91.0 (70-110)% predicted). Several studies conducted by Stevens and colleagues have also demonstrated both reduced body mass normalised and FFM normalised $\dot{V}O_{2\text{peak}}$ in young people with CF compared with their healthy peers (Stevens et al., 2009; Stevens et al., 2011). In a comparison of maximal CPET responses in 16 young patients with CF (6 males, 13.1 ± 3.9 y, FEV$_1$ 89.9 ± 18.9% predicted) compared with 15 healthy controls (6 males, 14 ± 3 y, FEV$_1$ 105.5 ± 9.8% predicted), Fielding et al. (2015) reported no significant differences observed in $\dot{V}O_{2\text{peak}}$ (1.5 ±0.7 vs. 1.8 ± 0.7 L·min$^{-1}$), work rate (119 ± 52 vs. 150 ± 54 W) or HR (175 ± 10 vs. 184 ± 15 b·min$^{-1}$) in CF versus controls, using the Godfrey protocol. Similarly, when normalised for body mass, no differences were evident. Conversely, differences in both $W_{\text{peak}}$ and $\dot{V}O_{2\text{peak}}$ were observed when
normalised for FFM (43.5 ± 7.7 vs. 50.6 ± 7.4 mL·kg⁻¹·min⁻¹), in a CF group who had a significantly lower fat-mass than their healthy peers (8.3 ± 2.6 vs. 11.3 ± 5.0 kg, respectively).

Although Fielding et al. (2015) represents one of the first attempts to normalise \( \dot{V}O_2 \)max to dual-energy X-ray absorptiometry (DEXA) derived FFM, this finding corresponds with previous reports in young patients with CF that have not normalised \( \dot{V}O_2 \)peak to FFM. For example, Poore et al. (2013) recently reported similar absolute (1.44 ± 0.68 vs. 1.81 ± 0.71 L·min⁻¹) and body mass normalised (32.8 ± 6.2 vs. 36.6 ± 8.6 ml·kg⁻¹·min⁻¹) \( \dot{V}O_2 \)peak values in 15 young people with CF (5 males, 12.6 ± 3.4 y, 87.7 ± 22.3% predicted) and 15 healthy matched controls (6 males, 13.6 ± 2.7 y, 104.5 ± 11.8% predicted) using the Godfrey protocol.

The extent of impairment may also depend on disease severity. In a study by de Meer et al. (1999), absolute and FFM normalised \( \dot{V}O_2 \)peak were reduced in patients with moderate CF disease (\( n = 15, 9 \) males; 14.8 ± 1.9 y, FEV₁ 56 ± 12% predicted) compared with healthy controls (\( n = 13, 8 \) males, 15.2 ± 1.9 y, FEV₁ 111 ± 12% predicted; 47 ± 7 vs. 61 ± 7 mL·kg⁻¹·min⁻¹), however this was not the case in patients with milder CF disease (\( n = 13, 8 \) males, 15.3 ± 1.8 y, FEV₁ 100 ± 11% predicted; 57 ± 7 vs. 61 ± 7 mL·kg⁻¹·min⁻¹).

Since previous studies have largely utilised the Godfrey protocol, confirmation of the extent of any reduction in \( \dot{V}O_2 \)peak using a valid and robust protocol is needed. There is also a need to examine specific effects on younger patients, with milder CF, who are habitually active, to explore the impact of CF where sedentariness and deconditioning may well play less of a role, particularly since
\( \dot{V}O_{2\text{peak}} \) typically falls approximately 5–8% per year in this patient group (Pianosi et al., 2005).

2.6.1.2 GET

The GET, VT and AT can all be used to broadly reflect the time at which the LT occurs, however within this thesis, the GET will be taken to reflect the non-invasive equivalent of the LT. The onset of the GET also provides additional information regarding an individual’s aerobic fitness status, with an earlier occurrence indicative of poorer fitness. It has been suggested that an occurrence of this threshold < 50% of predicted \( \dot{V}O_{2\text{max}} \) is associated with deconditioning (Urquart & Vendrausculo, 2015). Promisingly, over recent years authors are presenting both submaximal and maximal parameters of aerobic function; however available GET data are still relatively scarce.

In a study by Groen and colleagues (2010), there was no significant difference in the VT expressed as a percentage of \( \dot{V}O_{2\text{peak}} \) in young people with CF compared to healthy controls (59.7 ± 14.2 vs. 64.2 ± 8.91%). In a recent study by Savi et al. (2015), comparing 30 adults with mild-to-moderate CF (33 ± 3 y, FEV\(_1\) 50-90% predicted) with 15 healthy controls (FEV\(_1\) ≥ 80% predicted), the GET was shown to occur at a similar work rate (80 ± 35 vs. 88 ± 25 W) and corresponding \( \dot{V}O_{2} \) (1.19 ± 0.33 vs. 1.37 ± 0.31 L.min\(^{-1}\)) in CF and their healthy controls, using the V-slope method of determination. This was also seen when data were expressed as a percentage of predicted \( \dot{V}O_{2\text{peak}} \) although this was not measured. Interestingly, Fielding et al. (2015) also reported a similar percentage of \( \dot{V}O_{2\text{peak}} \) for the occurrence of the GET in paediatric patients with CF compared with their healthy counterparts (60 ± 7 vs. 62 ± 7% of \( \dot{V}O_{2\text{peak}} \).
respectively). The data in this study correspond with earlier findings in two groups of adolescents with mild-to-moderate CF compared with healthy controls that there is no difference in the occurrence of the GET relative to $\dot{V}O_{2\text{peak}}$ (67.3 ± 8.8 vs. 67.2 ± 7.7% of $\dot{V}O_{2\text{peak}}$ (Bongers et al., 2012) and 58.0 ± 9.0 vs. 55.0 ± 9.0% of $\dot{V}O_{2\text{peak}}$ (Bongers et al., 2014), respectively).

However, Stevens et al. (2011) observed significant differences in the work rate (69 ± 32 vs. 102 ± 53 W) and body mass normalised $\dot{V}O_2$ (19 ± 4 vs. 26 ± 8 mL·kg$^{-1}$·min$^{-1}$) at the GET in paediatric patients with mild-to-moderate CF. Unfortunately, data expressed as a percentage of $\dot{V}O_{2\text{peak}}$ or $W_{\text{peak}}$ were not provided. The disparity between these studies may well be due to different levels of methodological rigour when identifying the GET. Issues in identification have previously been raised. Thin et al. (2002) presented their absolute GET data in healthy control participants (1.79 ± 0.51 L·min$^{-1}$) and those with mild (1.20 ± 0.56 L·min$^{-1}$), moderate (1.00 ± 0.22 L·min$^{-1}$) and severe CF (0.74 ± 0.25 L·min$^{-1}$) and noted that a VT was only identified in ~50% of their patient group. However, other studies have not had the same problem. For example, Hebestreit and colleagues (2005) observed no difference in a mixed cohort of paediatric and adult patients (67.5 ± 7.6 vs. 65.7 ± 10.5% of $\dot{V}O_{2\text{peak}}$).

As outlined recently by Visschers and colleagues (2014), there is no real consensus regarding the most accurate method to determine the GET in individuals with chronic diseases, such as CF, which may play some part in the limited presentation of this parameter within the literature. However, it has been demonstrated that the GET can be used to obtain an unbiased estimated of the LT which is reproducible (Thin et al., 2002). Furthermore, the V-slope method, ventilatory equivalents and investigating the $P_{ET}O_2$ response can identify the
GET at comparable $\dot{V}O_2$ and work rate values and are equally reproducible (Visschers et al., 2014). There is, however, a need to confirm the influence of mild-to-moderate CF on the GET using rigorous identification procedures.

2.6.1.3 $\dot{V}O_2$ gain

As outlined in section 2.4.1, the slope of the regression of $\dot{V}O_2$ against work rate obtained from an incremental exercise test to exhaustion can provide further insight into aerobic (dys)function, which is independent of effort. This measure provides an index of aerobic exercise efficiency ($O_2$ cost of exercise) (Neder et al., 2001) and has been shown to provide a sensitive parameter of abnormal muscle $O_2$ delivery-to-utilisation during exercise (Gimenes et al., 2011; Hansen et al., 1987; Jones et al., 1998; Meyer et al., 1998; Neder et al., 2001). A reduced ability to increase $\dot{V}O_2$ with work rate indicates an increased reliance on anaerobic glycolysis during exercise. However, as with the GET, the number of studies reporting values for the $\dot{V}O_2$ gain in individuals with CF is limited.

Fielding and colleagues (2015) reported that the $\dot{V}O_2$ gain was significantly lower in paediatric patients with CF compared with healthy controls (8.4 ± 3 mL·min$^{-1}$·W$^{-1}$ vs. 10.1 ± 1.4 mL·min$^{-1}$·W$^{-1}$, respectively), a finding in agreement with earlier observations (Moser et al., 2000). If a lower $\dot{V}O_2$ gain is characteristic of children and adolescents with CF, this would indicate a reduced metabolic capacity to increase $\dot{V}O_2$ in accordance with an increasing work rate. This however needs further confirmation, since there is debate regarding how CF impacts this parameter (e.g. Groen et al., 2010). It would be expected that if young patients with CF have impairment in $O_2$ transport and $O_2$
utilisation during exhaustive, incremental exercise, then the $\dot{V}O_2$ gain would be shallower than their healthy peers.

Using an incremental cycling test, Groen et al. (2010) did not find a significant difference between adolescents with CF and healthy controls ($10.1 \pm 1.3$ vs. $9.2 \pm 1.0$ mL·min$^{-1}$·W$^{-1}$), despite a difference being observed when healthy young people were compared with another clinical group (juvenile dermatomyositis). Although not significant ($p = 0.08$) the authors noted that values tended to be higher in CF than controls. Savi et al. (2015) also reported no difference between CF and healthy adults. Higher values have also previously been presented. de Meer and colleagues (1999) reported higher values for patients with mild ($19.2 \pm 2.3$ mL·min$^{-1}$·W$^{-1}$) and moderate ($18.2 \pm 2.0$ mL·min$^{-1}$·W$^{-1}$) CF, compared with their healthy peers ($16.3 \pm 2.6$ mL·min$^{-1}$·W$^{-1}$), however even the healthy values are higher than would be expected.

It has been proposed that the existing disparity within the literature regarding the influence of CF disease upon the $\dot{V}O_2$ gain may result from the difference in disease severity investigated and methods utilised to determine the $\dot{V}O_2$ gain, specifically whether data was included up to or including data above the GET (Fielding et al., 2015). All of these previous studies have included data from the onset of exercise, which may skew the calculated slope of the response, since it is recommended that the initial 2 min be removed to account for any influence of the $\dot{V}O_2$ MRT. Furthermore, it has been suggested that since the $\dot{V}O_2$ response above the GET is not always linear, it should possibly be excluded from calculations of the $\dot{V}O_2$ gain (Boone & Bourgois, 2012) or the $\dot{V}O_2$ gain could be determined for the regions above and below the GET. To date, Fielding et al. (2015) present the only data concerning the $\dot{V}O_2$ gain calculated
using only the linear data points as recommended by Boone and Bourgois (2012).

2.6.1.4 $\dot{V}O_2$ MRT

The MRT marks the intersection point between the baseline $\dot{V}O_2$ and a backward extrapolation of the linear portion of the $\dot{V}O_2$ response. The kinetic adjustment of $\dot{V}O_2$ at the onset of ramp incremental exercise is fundamentally similar to the MRT measured at the onset of CWR exercise, representing $O_2$ utilisation by the muscle at the onset of exercise. However, this parameter has remained unused within much of the literature investigating aerobic exercise (dys)function in patients with CF. Specifically, data is limited to a single study by Fielding et al. (2015). This study represented one of the first attempts to use the $\dot{V}O_2$ MRT to investigate the adequacy of the $\dot{V}O_2$ kinetic response during incremental exercise in CF. As hypothesised, Fielding et al. (2015) observed a significant slowing of the $\dot{V}O_2$ MRT in CF compared with healthy matched controls (36.1 ± 15.1 vs. 25.0 ± 12.4 s, respectively). Furthermore, the $\dot{V}O_2$ MRT was inversely related to pulmonary function (FEV$_1$/FVC), indicating that better pulmonary function may be associated with faster $\dot{V}O_2$ kinetics in children and adolescents with CF during incremental exercise.

2.6.2 Pulmonary $\dot{V}O_2$ kinetics

As outlined in section 2.5.2, assessing the kinetics of the $\dot{V}O_2$ response to an altered work rate can also provide valuable insight into the integrated capacity of the $O_2$ transport and utilisation pathways to support the increased rate of ATP turnover in the contracting myocytes (see Figure 2.6). Although the $\dot{V}O_2$ kinetic
response is now relatively well documented in healthy children (Armstrong & Barker, 2009), evidence of the influence of CF upon this response is scarce. Although the \( \dot{V}O_2 \) MRT has recently been used to suggest a slowing of the \( \ddot{V}O_2 \) kinetic response during incremental exercise in individuals with CF (Fielding et al., 2015; see section 2.6.1), there is limited data concerning the mathematically modelled \( \ddot{V}O_2 \) response within strict intensity domains in this patient group. Moreover, that which has been conducted comprises a number of methodological issues which must be considered.

In the first study to investigate the \( \ddot{V}O_2 \) kinetic response in CF, Braggion and colleagues (1989) investigated the profile of 10 young people with mild CF (11.1-15.3 y; FEV\(_1\) 77 ± 22% predicted) and 10 healthy age-matched controls (12.2-15.2 y) during 6 min cycling at an intensity equivalent to 1.7 W·kg\(^{-1}\). The kinetics of \( \ddot{V}O_2 \) at the onset and end of the exercise transition were recorded and a monoexponential fit was applied. Although no difference was observed between the groups, a number of methodological issues comprised this study. Specifically, only a single exercise transition was employed to quantify the \( \ddot{V}O_2 \) response and the modelling procedure used did not enable phase II of the response to be isolated which, as previously outlined, is crucial to infer \( \dot{m}\dot{V}O_2 \) dynamics. Additionally, no consideration was given to standardising the exercise intensity domain within and between the groups which, as outlined in section 2.5.2., may render patients with CF exercising at a higher percentage of their aerobic fitness than their healthy counterparts. Barker et al. (2004) also found similar \( \tau \) in patients with CF and healthy controls, when fitting a single monoexponential model to the entire phase I and phase II \( \ddot{V}O_2 \) response.
There are, however, contrasting reports regarding the influence of CF disease upon the kinetics of $\dot{V}O_2$ at the onset of exercise. Massin et al. (2000) investigated the $\dot{V}O_2$ and HR kinetics of paediatric and adult patients with CF ($n = 20$, 10 males; 5-28 y; FEV$_1$ 25-99% predicted). Instead of employing a CWR exercise challenge, participants undertook a pseudo-random binary sequence (PRBS) exercise task. PRBS involves random transitioning between two work rates within the aerobic range and has been validated in healthy children and adolescents and those with cardiac problems (Massin et al., 1998), with the reproducibility for CF patients also reported (Kusenbach et al., 1999). This protocol was employed since the authors deemed it more indicative of ADLs. CF patients were divided into 3 groups, dependent on disease severity assessed using the Shwachman Score (SS) (A, $n = 7$, 5-28 y, SS 85-90; B, $n = 6$, 5-24 y, SS, 70-80; C, $n = 7$, 5-24 y, SS, 30-65). In line with Braggion and colleagues’ (1989) findings in patients with mild CF, the $\dot{V}O_2$ kinetics of patients in group A were not significantly prolonged. However, a reduced $\dot{V}O_2$peak, $\dot{V}O_2$ amplitude and $\dot{V}O_2$ ‘lag time’ were observed in the CF patients in groups B and C and correlated with both FEV$_1$ and the SS. It should, however, be acknowledged that PRBS exercise protocols again prohibit the isolation of the different phases of the $\dot{V}O_2$ kinetic response, do not involve any explicit mathematical modelling of the kinetic parameters and encompass changes in $\dot{V}O_2$ during the recovery period. Furthermore, since a wide age group were pooled together it is difficult to separate the effects of age and disease on the $\dot{V}O_2$ kinetic response. Kusenbach et al. (1999) provided further support to the observations made by Massin and colleagues (2000), reporting slower $\dot{V}O_2$ kinetics in 9 individuals with CF (5 male, 13-31 y, FEV$_1$ 42-88% predicted)
compared with 13 healthy controls (8 male, 9-29 y, FEV\textsubscript{1} 84-143% predicted) during submaximal PBRS cycling exercise.

To provide further clarity regarding the \( \dot{V}O_2 \) kinetic response characterising individuals with CF, Hebestreit \textit{et al.} (2005) sought to investigate the \( \dot{V}O_2 \) kinetics in a group of patients (FEV\textsubscript{1} 37-98% predicted, 9.8-33.8 y) compared with their healthy counterparts (age 9.9-30.8 y). A two stage protocol was employed, consisting of semi-supine cycling at 20 W for 2 min, followed by 3 min cycling at a work rate corresponding to 1.4 W\cdot kg\textsuperscript{-1} or 1.3 W\cdot kg\textsuperscript{-1} for males and females, respectively. This protocol was repeated 2-4 times to improve the signal-to-noise ratio of the \( \dot{V}O_2 \) kinetic response (Whipp \textit{et al.}, 1981). Interestingly, Hebestreit \textit{et al.} (2005) observed a significantly prolonged phase II \( \dot{V}O_2 \tau \) in the CF group compared with the healthy controls (36.8 ± 1.8 vs. 26.4 ± 9.1 s), with no difference in the \( O_2 \) cost of exercise (10.9 ± 1.8 vs. 10.2 ± 1.6 mL\textcdot min\textsuperscript{-1}\textcdot W\textsuperscript{-1}). However, several methodological issues potentially confound the interpretation of this study.

Although Hebestreit and colleagues provided the first attempt to isolate the phase II region of the response and apply an appropriate mathematical model to the data, the prescribed work rate was not classified within a particular exercise intensity domain (see Figures 2.12 and 2.13). As rationalised in section 2.5.2, equating exercise intensity is crucial in the study of \( \dot{V}O_2 \) kinetics, particularly when the substantial inter-individual variability in the occurrence of the LT as a percentage of \( \dot{V}O_{2\text{max}} \) in children and adolescents is considered (Reybrouck \textit{et al.}, 1985). Not prescribing exercise intensity within strict domains therefore increases the possibility that participants both between and within the groups may have been exercising across the moderate and heavy intensity
domains. Secondly, 2-4 repetitions of the exercise protocol were completed on the same day with only 10 min recovery. This raises the possibility that a ‘priming’ effect may have occurred on the \( \dot{V}O_2 \) kinetic response (Burnley et al., 2006), which is likely to be more influential in individuals with slower \( \dot{V}O_2 \) kinetics (Gurd et al., 2005). Furthermore, the exercise mode was semi-supine rather than upright cycling, which may have reduced muscle O\(_2\) delivery during exercise, due to the absence of the gravitational assist to muscle blood flow, and slowed the \( \dot{V}O_2 \) kinetic response (Jones & Burnley, 2005; Jones et al., 2006; Koga et al., 1999; MacDonald et al., 1998). Finally, Hebestreit and colleagues pooled together a wide age group, who will likely span the disease severity spectrum, which has been shown to potentially influence the \( \dot{V}O_2 \) kinetic response (Massin et al., 2000).

In further support of an impact of age and disease severity on the \( \dot{V}O_2 \) kinetic response, Armeniakou et al. (2015) recently reported prolonged \( \dot{V}O_2 \) kinetics during submaximal exercise in adults with CF (\( \dot{V}O_2 \tau \): 42.3 ± 21.5 s) compared with their healthy counterparts (\( \dot{V}O_2 \tau \): 29.3 ± 6.4 s). Furthermore, in line with Massin et al’s (2000) observations, the phase II \( \dot{V}O_2 \tau \) was associated with disease severity, as indicated by the SS, suggesting that this clinical index may provide a useful, independent predictor of the phase II \( \dot{V}O_2 \tau \) in adults with CF ($R^2 = 0.72, P = 0.001$).

In addition to documenting the \( \dot{V}O_2 \) on-kinetics, the recovery kinetics of \( \dot{V}O_2 \) is also important, since they can provide an effort independent insight into skeletal muscle oxidative capacity (Paganini et al., 1997). Pouliou et al. (2001) explored the influence of CF on the \( \dot{V}O_2 \) recovery kinetics of 18 patients (9 male, 23 ± 13 y, FEV\(_1\) 23-128% predicted) versus 11 healthy controls (3 male, 29 ± 4 y).
following an exhaustive treadmill CPET. The recovery of $\dot{V}O_2$, as quantified using a linear slope function to characterise the rapid exponential-like phase during the first minute of recovery following the $\dot{V}O_{2\text{max}}$ test, to be significantly slower in CF. Interestingly, in agreement with previous observations concerning the phase II $\tau$ at the onset of exercise (Armeniakou et al., 2015), Pouliou and colleagues (2001) also showed an association between the recovery of $\dot{V}O_2$ and the SS ($r = 0.81$, $P < 0.001$), which again remained the only predictor after accounting for confounding variables ($\dot{V}O_{2\text{max}}$ and FEV$_1$) in a multivariate analysis. More recently, Perpati et al. (2010) extended these findings from treadmill exercise, demonstrating prolonged $\dot{V}O_2$ recovery kinetics in adults with CF during cycling exercise, again indicated by the first degree slope of $\dot{V}O_2$ during the first minute of recovery following an exhaustive incremental CPET.

Similar results have also been reported in a more homogenous sample of patients with chronic chest diseases (70% CF, FEV$_1$ 82 ± 23% predicted, age 12.7 ± 3.1 y) compared with healthy controls (age 13.2 ± 3.3 y; Stevens et al., 2009). In Stevens et al’s (2009) study, children and adolescents with chronic chest disease were shown to be characterised by a slower $\dot{V}O_2$ recovery following a $\dot{V}O_{2\text{max}}$ test, which correlated with $\dot{V}O_{2\text{max}}$ ($r = -0.39$, $p = 0.04$), when compared with their healthy peers. Furthermore, in CF patients only, the speed of $\dot{V}O_2$ recovery was again associated with the SS ($r = -0.63$, $p = < 0.01$), supporting earlier observations in older patients (Perpati et al., 2010; Pouliou et al., 2001).

There is therefore a need to clarify the impact of CF on the kinetics of $\dot{V}O_2$ in a cohort of children and adolescents with mild-to-moderate disease. Specifically, since almost all chronic diseases that involve one or more of the respiratory,
cardiovascular and/or muscular systems have the potential to slow the $\dot{V}O_2$ kinetic response, confirmation of the impact of mild-to-moderate CF on the dynamics of $\dot{V}O_2$ during exercise within defined intensity domains and using accurate mathematical modelling is warranted. Moreover, the $\dot{V}O_2$ kinetic response during exercise performed above the GET has not formally been documented. Although Hebestreit et al. (2005) reported that their study was investigating the $\dot{V}O_2$ response during moderate intensity cycling, some participants may well have been exercising above the GET and some below. Consequently, the $\dot{V}O_2$ response of people with CF above and below the GET requires investigation. Given earlier suggestions that the kinetics of $\dot{V}O_2$ may be related to $\dot{V}O_{2\text{max}}$ (an independent predictor of prognosis in CF), further characterisation of the response may also be of prognostic utility. There is, therefore, a need for experimentally robust studies to elucidate how CF disease modulates the $\dot{V}O_2$ kinetic profile.

There is some, but limited, evidence to support a contribution from a rate-limiting step in the O$_2$ cascade from the alveoli to the mitochondria. Massin et al. (2000) suggested that O$_2$ delivery, indicated by insufficient SV, plays a role in the altered $\dot{V}O_2$ kinetics that reportedly characterise patients with CF. This was further postulated by Hebestreit et al. (2005), however this was inferred from a correlation between the phase II $\dot{V}O_2$ $\tau$ and SpO$_2$ and this study was not designed to detect abnormalities in muscle metabolism during exercise. Any conditions that impair muscle O$_2$ delivery have the potential to reduce the speed of the $\dot{V}O_2$ kinetic response. However, until physiological information regarding the oxygenation kinetics within the contracting muscles and the balance between O$_2$ delivery and utilisation within the microvasculature of skeletal
muscle are determined in conjunction with pulmonary $\dot{V}O_2$ kinetics, this cannot be confirmed.

2.7 Additional techniques to assess exercise (dys)function in patients with CF

In addition to traditional measurements made via assessments of pulmonary gas exchange and ventilation, several other techniques enable insight into central ($O_2$ delivery) and peripheral function ($O_2$ extraction and utilisation) and have gained popularity over recent years (see Figure 2.14). For example, thoracic electrical bioimpedance cardiography and NIRS now enable valuable insight into cardiac function and central $O_2$ delivery and localised fractional $O_2$ extraction within exercising muscle, which was previously not viable in children and adolescents given their invasiveness. These techniques are outlined below.

![Figure 2.14](image.png)

**Figure 2.14.** Schematic representation of simultaneous measures of pulmonary oxygen uptake ($\dot{V}O_2$), cardiac output ($\dot{Q}$) and muscle oxygenation ($\Delta HbMBO_2$) and deoxygenation ($\Delta HHbMb$) during cycling exercise (A) and the local region of muscle interrogated by near-infrared spectroscopy (NIRS) (B). Image taken from Lai et al. (2009) with permission.
2.7.1 Near-infrared spectroscopy

NIRS provides a non-invasive method to examine the oxygenation status at the skeletal muscle level, at rest and during exercise. This is particularly useful in paediatric research, given that many of the other methods to investigate changes in muscle blood flow, O$_2$ delivery and muscle metabolism are not feasible given their invasiveness and cost (e.g. muscle biopsy, tissue O$_2$ microelectrodes, nicotinamide adenine dinucleotide analysis from exposed tissue surfaces, myoglobin O$_2$ saturation by spectrophotometric analysis). Whilst $^{31}$P-MRS has shown promise in paediatric studies (e.g. Barker & Armstrong, 2010; Willcocks et al., 2010a; Willcocks et al., 2010b; Willcocks et al., 2014), this technique focuses on muscle metabolism, is incredibly costly and limited by the exercise tasks that can be performed and location of the testing environment. NIRS, however, is non-invasive, portable, relatively cheap and enables continuous, real-time measures to be made, thereby providing measurements of dynamic changes in tissue oxygenation in vivo.

NIRS is based on the relative ease with which near-infrared light passes through biological tissues, including bone, skin and muscle. The amount of light recovered after illuminating the tissue depends on the degree of scattering in the tissue and the amount of absorption by the chromophores in the tissue. Only three molecules are known to affect near-infrared light absorption during changes in tissue tension, haemoglobin, myoglobin and cytochrome c oxidase (Boushel & Plantadosi, 2000). When evaluating changes within skeletal muscle tissue, measures of tissue oxygenation are based on the fractional reflection and refraction of near-infrared light by haemoglobin in the microvasculature and myoglobin in the muscle. Each of these measures are dependent on whether
these compounds are bound to O₂. During exercise, this provides insight into the fractional extraction of O₂ in the working muscles.

Whilst early NIRS devices used single-distance continuous-wave light source-detector pairs (McCully & Hamaoka, 2000), more recent models which comprise multi-channel or imaging devices enable measurements of wider areas of interrogation (Queresima et al., 2001; Yamamoto et al., 2001) and have made it possible to image regional differences in skeletal muscle oxygenation and metabolism in different muscle locations (Hamaoka et al., 2011; see Figure 2.15). The development of portable NIRS devices has also meant that measurements can be made more freely during movement (Shiga et al., 1995). For example, a wireless continuous-wave NIRS system, small in size, was developed by Artinis Medical Systems (which will be used within this thesis) to measure muscle oxygenation.

Figure 2.15. Example data depicting the muscle oxygenation response at the m. rectus femoris and m. vastus lateralis during and after isometric knee-extension exercise, as assessed using a multi-channel near-infrared spectroscopy (NIRS) device. Image taken from Hamaoka et al. (2011) with permission.
In intact skeletal muscle, NIRS allows semi-quantitative measurements of haemoglobin plus myoglobin oxygenation (tissue $O_2$ stores) and the haemoglobin volume to be made (Boushel & Plantadosi, 2000). Specifically, NIRS has primarily been used to assess dynamic changes in the status of tissue oxyhaemoglobin ($HbO_2$), deoxy-haemoglobin ($HHb$) and total blood (haemoglobin) volume in brain and muscle tissue. However, the levels of muscle oxygen measured by NIRS are only understood to be a result of the dynamic balance between muscle $O_2$ delivery and consumption (Ding et al., 2001). From the parameters of the response, the investigator can draw conclusions about the balance of $O_2$ delivery and utilisation, but NIRS cannot distinguish between these two factors. The NIRS-derived $[HHb]$ signal therefore provides a measure of estimated skeletal muscle microvascular $O_2$ extraction at the periphery (e.g. DeLorey et al., 2003; Ferreira et al., 2007; Grassi et al., 2003) and, thus, the ratio of local muscle $O_2$ utilisation to microvascular blood flow. NIRS may therefore be useful for understanding muscle oxidative metabolism and alterations caused by changes in aerobic fitness status and/or disease progression in CF.

The profile of the $[HHb]$ signal has been used to describe $O_2$ extraction dynamics during ramp exercise, which in turn permits inferences regarding blood flow within the microcirculation of exercising muscle during this type of exercise (e.g. Boone et al., 2009; DiMenna et al., 2010; Ferreira et al., 2007; McNarry et al., 2011; McNarry et al., 2015). Although pulmonary $\dot{V}O_2$ is known to increase linearly with increasing work rate, following an initial time lag, muscle $\Delta[HHb]$ has been shown to increase in a nonlinear manner (see Figure 2.16). The $\Delta[HHb]$ response to incremental ramp cycling exercise is typically described using a sigmoidal model (e.g. Boone et al, 2009; DiMenna et al.,
2010; Ferreira *et al.*, 2007; McNarry *et al.*, 2011; McNarry *et al.*, 2015), although a bi-linear model is favoured by some (Murias *et al.*, 2013a; Murias *et al.*, 2013b). This response has been well characterised using a sigmoidal (S-shaped) function relative to wrk rate and $\dot{V}O_2$ in healthy individuals ranging from children to the elderly during ramp cycling exercise.

During steady-state exercise, muscle blood flow increases in proportion to muscle $\dot{V}O_2$, with a positive intercept on the $y$-axis (blood flow) that is fibre type dependent (Ferreira *et al.*, 2007). According to the Fick principle, this relationship mandates a hyperbolic increase in fractional $O_2$ extraction with increasing muscle $\dot{V}O_2$ and, by extension, work rate. However, using NIRS-derived [HHb] data as a surrogate for microvascular $O_2$ extraction, the consensus from the literature suggests that a sigmoidal model is better fit to [HHb] data during upright ramp incremental cycling exercise to exhaustion (e.g., Boone *et al.*, 2009; Boone *et al.*, 2010; DiMenna *et al.*, 2010; Ferreira *et al.*, 2007; McNarry *et al.*, 2011; McNarry *et al.*, 2015). Analysis of the $\Delta$[HHb] adjustment during incremental exercise provides information regarding the matching of muscle $O_2$ delivery and extraction during non-steady-state exercise covering a wide range of exercise intensities.
This sigmoidal response has been characterised with three distinct regions; an initial region, slope region and, finally, the plateau region (see Figure 2.16). Assuming the normal linear increase in $\dot{V}O_2$ with incremental work rate, the blunted [HHb] rise within the initial region has been interpreted as evidence that muscle blood flow increases faster that muscle $\dot{V}O_2$ during the initial 2 min of exercise (Ferreira et al., 2007). Conversely, the slope region is characterised by a markedly steeper response, which indicates a greater reliance on fractional $O_2$ extraction as higher work rates are reached. Finally, in most healthy individuals, a plateau occurs, implying an inability for $O_2$ extraction to increase further and implies a linear muscle blood flow to $\dot{V}O_2$ relationship, until the point of exhaustion (Ferreira et al., 2007).

Ferreira et al. (2007) developed computer simulations to assess the sigmoidal [HHb] response and advanced a model whereby the slope and plateau regions provide information that could be useful for assessing localised blood flow within
exercising skeletal muscle and detecting early stages of dysfunction during routine clinical exercise testing. Specifically, Ferreira et al. (2007) suggested that disease-related changes in microvascular function might shift the sigmoid to the left, whereas exercise training and pharmacological treatment that improves vascular function might shift the sigmoid to the right.

To date, there is no evidence concerning the influence of CF disease upon the dynamics of [HHb] during ramp incremental exercise to exhaustion. Considering the hypothesis proposed by Ferreira and colleagues (2007), it may be expected that since CF causes a reduction in aerobic fitness (e.g. Bongers et al., 2012; Bongers et al., 2014; Moser et al., 2000; Wideman et al., 2009), then this may causes a leftward shift of the [HHb] sigmoid. In support of this, Boone et al. (2009) found a rightward shift of the [HHb] response in healthy adults who are trained compared with less well trained counterparts (see Figure 2.17). Boone et al. (2009) demonstrated a rightward shift in the NIRS-derived [HHb] sigmoidal response, measured at the m. vastus lateralis, in healthy trained cyclists \( \left( n = 10 \right) \) compared with healthy physically active students \( \left( n = 11 \right) \). When normalised to the total amplitude of the [HHb] response, the work rate corresponding to 50% of the \( \Delta [\text{HHb}] \) amplitude \( (c/d) \) was the only parameter that differed between healthy trained versus untrained adults, indicating altered \( O_2 \) extraction dynamics as a function of aerobic fitness status. This finding was further supported more recently in a much larger \( \left( n = 64 \right) \) sample of participants (Boone et al., 2016).
The reproducibility of NIRS in children has been documented (Leclair et al., 2010) and over recent years studies have begun to utilise NIRS to investigate muscle oxygenation of healthy children during ramp incremental exercise (McNarry et al., 2011; McNarry et al., 2015). In support of the findings in adults, a rightward shift of the [HHb] sigmoid has been observed in trained versus untrained girls (McNarry et al. 2011; see Figure 2.18). Furthermore, more recently McNarry and colleagues (2015) provided the largest dataset to date ($n = 51; 9.9 \pm 0.6$ y, 31 boys) concerning the typical muscle [HHb] response of healthy children during exercise. This study sought to characterise the $\Delta$[HHb] dynamics in healthy boys and girls during exhaustive incremental exercise.
and whether the reduced $\dot{V}O_{2\text{max}}$ in girls compared to boys was related to altered muscle [HHb] dynamics, as evidenced by an accelerated $\Delta[\text{HHb}]$ response. This study demonstrated that the reduced aerobic fitness observed in girls is partly due to gender-specific changes in muscle fractional O$_2$ extraction dynamics during ramp incremental cycling exercise.

Figure 2.18. Deoxygenated haemoglobin concentration ([HHb]) plus myoglobin concentration response as a function of relative work rate (WR) for a representative trained (● shaded circles) and untrained (○ open circles) girl during ramp incremental cycling exercise. This data demonstrates a rightward shift of the sigmoidal [HHb] response as a result of training, indicating a better matching of O$_2$ delivery-to-O$_2$ utilisation and the muscle level. Image taken from McNarry et al. (2011) with permission.

However, in contrast to the findings regarding training status, recent evidence comparing older (70 ± 3 y) and younger (25 ± 5 y) adults only observed alterations in muscle [HHb] dynamics when the data was expressed relative to absolute work rate. The authors did not observe any significant age-related differences in the $\Delta[\text{HHb}]$ dynamics when expressed as a function of percentage $W_{\text{peak}}$, despite a reduced $\dot{V}O_{2\text{max}}$ in the older participant group (30
vs. 49 mL·kg\(^{-1}·\text{min}^{-1}\) (Gravelle et al., 2012; see Figure 2.19). When the response during CWR exercise was investigated, however, differences in the dynamics of \(\Delta[\text{HHb}]\) between the groups during the on-transient to moderate intensity exercise (80% of the LT) were evident.

![Figure 2.19. Sigmoid models of normalised changes in deoxygenated haemoglobin concentration ([HHb]) plus myoglobin concentration (percentage of peak amplitude) as a function of relative (percentage peak) work rate for young adults (YA) versus older adults (OA). Image taken from Gravelle et al. (2012) with permission.](image)

Although the HHb profile during ramp exercise has been used to describe the effect of body position (DiMenna et al., 2010), trained status (e.g. Boone et al., 2009; McNarry et al., 2011), gender (McNarry et al., 2015) and ageing (e.g. Gravelle et al., 2012; McNarry et al., 2011), there is scare evidence concerning clinical perturbations that might be expected to shift the [HHb] sigmoid leftward. Although NIRS has recently been employed within clinical populations (e.g., Habers et al., 2012; MacDonald et al., 2012), no research has assessed the
influence of clinical perturbations on the sigmoid function of the $\Delta[\text{HHb}]$ response.

From a clinical perspective, NIRS has been used in a variety of populations. NIRS, in combination with $^{31}\text{P-MRS}$ has also been used to determine mitochondrial function and the degree of ischaemia in patients with type II diabetes mellitus and peripheral vascular disease (Malagoni et al., 2010). NIRS has also been used to investigate the effect of bronchodilators (Berton et al., 2010) and $\text{O}_2$ administration (Siquera et al., 2010; Vogiatzis et al., 2009) in patients with chronic obstructive pulmonary disease (Rondelli et al., 2009) and the response to pharmacological treatment (Sperandio et al., 2009) exercise rehabilitation in patients with chronic heart failure (Gerovasili et al., 2009). Whilst NIRS has been used in combination with pulmonary $\dot{\text{VO}}_2$ measurement to explore exercise limitation in patients with metabolic myopathies (Grassi et al., 2007; Grassi et al., 2009), paraplegia (Muraki et al., 2007), type II diabetes (Bauer et al., 2007; Pedersen et al., 2009) and trapezius myalgia (Andersen et al., 2010), it has yet to be applied to people with CF.

Alterations within the $\text{O}_2$ delivery-to-$\text{O}_2$ utilisation relationship would be expected to shift the $\Delta[\text{HHb}]$ sigmoid and, as previously outlined, a leftward shift has been hypothesised for chronically diseased individuals (Ferreira et al., 2007). An inadequate adjustment of muscle blood flow, specifically microvascular blood flow, would require increased $\text{O}_2$ extraction to meet the muscle $\text{O}_2$ requirement for mitochondrial oxidative ATP production. Thus, if $\text{O}_2$ delivery is compromised in children and adolescents with CF during CPET, a more rapid extraction of $\text{O}_2$ at the periphery would be required, thereby shifting the sigmoid to the left compared with a healthy response. Assessing the
dynamics of [HHb] relative to the kinetics of pulmonary $\dot{V}O_2$ during CWR exercise is also warranted. However, to date only a single study has utilised NIRS in the assessment of individuals with CF (Erickson et al., 2015). NIRS was used to measure the recovery rate of muscle O$_2$ consumption of the $m. \text{vastus lateralis}$ following 15 s of electrical stimulation (4 Hz) and subsequent repeated transient arterial occlusions, observing a reduction in maximal muscle $\dot{V}O_2$ in CF versus their healthy counterparts.

2.7.1 Thoracic electrical bioimpedance cardiography

Thoracic electrical bioimpedance cardiography provides a non-invasive, portable, relatively low cost tool, which requires little technical expertise to operate and, thus, is well suited for use in paediatric groups. Thoracic electrical bioimpedance cardiography allows the non-invasive estimation of changes in $Q$, SV and HR at rest and during exercise. $\dot{Q}$ can be measured by passing high frequency, small alternating current through the chest (Warburton et al., 1999). Changes in electrical impedance, caused by pulsatile changes in aortic blood flow, are measured by recording electrodes placed in specific locations on the body. It is assumed that changes in thoracic impedance during systole are related to SV. The PhysioFlow device does not require the measurement of baseline impedance, which is affected by hydration status, blood resistivity, and distance between electrodes. This technique has been shown to provide similar results to direct measures of cardiac function during rest and exercise, including maximal exercise (Charloux et al., 2000; Richard et al., 2001).

Several studies have sought to validate thoracic electrical bioimpedance cardiography against other methods of assessing changes in cardiac indices
during exercise. Moore *et al.* (1992) compared thoracic electrical bioimpedance cardiography (Biomed NCCOM-3) derived $\dot{Q}$ to that measured using the indirect Fick method (CO$_2$ rebreathing technique) in 11 healthy males at rest and during three steady-state intensities (60 W, 120 W and 180 W) of cycling exercise. The $\dot{Q}$ derived using both methods correlated with changes in $\dot{V}O_2$, with a higher correlation for $\dot{Q}$ by the rebreathing technique ($r = 0.94$) than for thoracic impedance cardiography ($r = 0.88$). These results were interpreted to show that thoracic electrical bioimpedance cardiography provides a valid tool for the assessment of $\dot{Q}$ during low and moderate intensity cycling exercise in healthy adults.

Pianosi (1996) sought to investigate the validity of this technique (ICG-M401 impedance cardiograph) versus the CO$_2$ rebreathing technique in paediatric participants. The accuracy of this technique to monitor changes in $\dot{Q}$ was assessed in 30 healthy children during two intensities of CWR cycling exercise (0.5 and 1.5 W·kg$^{-1}$). Given that 80% of the impedance cardiography values were within ±20% of those derived using CO$_2$ rebreathing, it was concluded that this technique provides an accurate estimate of $\dot{Q}$ in healthy children during exercise. Furthermore, using a large sample of healthy young people ($n = 115$; 7-19 y), changes in $\dot{Q}$ and SV (normalised to body surface area (BSA) and expressed as the stroke volume index (SVI)) were measured during an incremental cycling test to exhaustion, in conjunction with changes in $\dot{V}O_2$ measured at the mouth (Pianosi, 2004). When $\dot{Q}$ was regressed against $\dot{V}O_2$, as expected, $\dot{Q}$ increased linearly with $\dot{V}O_2$ in all participants, with the majority demonstrating a gentle, progressive increase in SV in line with work rate. It was concluded that thoracic impedance cardiography has the capacity to accurately
measure changes in $\dot{Q}$ during exercise and provides a useful clinical and research tool in paediatric cardiology and paediatric exercise physiology.

Following validation of thoracic electrical bioimpedance cardiography in healthy young people, Pianosi (1997) sought to assess the accuracy of this technique in 21 young people with CF ($\text{FEV}_1$ 77 ± 21% predicted), compared with CO$_2$ rebreathing with sampling of capillary blood gases, during the same two exercise intensities employed in their earlier work in healthy young people (0.5 and 1.5 W·kg$^{-1}$). In this group, 83% of impedance cardiography values were within ±20% of those measured using CO$_2$ rebreathing. It was concluded that this device also provides rapid, accurate and non-invasive measurements in children with CF.

The reproducibility of using thoracic electrical bioimpedance analysis to determine $\dot{Q}$ during maximal CPET has been evaluated in children (Welsman et al., 2005). Welsman and colleagues investigated the reproducibility of the PhysioFlow device to determine changes in SV and $\dot{Q}$ in 10-11 year olds during maximal ramp incremental cycling exercise. The PhysioFlow device was shown to allow non-invasive, beat-by-beat determination of $\dot{Q}$ and SV, which is feasible for measurements during maximal exercise in children. TEs across three trials, each separated by one week, were 9.3% and 9.3% for $\dot{Q}$ and SV, respectively. The reproducibility statistics demonstrated that, at maximal exercise, this technique falls between Doppler echocardiography (5% variation) and the CO$_2$ rebreathing technique (12% variation).

Despite the demonstrated utility of this method to assess the cardiac function of young people with CF during exercise, its application has been limited. Pianosi
and Pelech (1996) did employ this technique to characterise changes in $\dot{Q}$ and SV in 18 patients spanning the spectrum of mild-to-moderate to severe CF disease (FEV$_1$ 28-80%) compared with 16 healthy matched control participants. Again, $\dot{Q}$ was measured during 3 intensities of cycling, with a similar exercising $\dot{Q}$ reported for both the CF and healthy groups. However, interestingly, SV was reportedly lower in the CF group, particularly those with poorer lung function (FEV$_1$ ≤ 55% predicted). Although further analyses demonstrated body composition and being underweight was also known to play a role, SV was still lower than controls in well-nourished patients with CF with better pulmonary function (FEV$_1$ 56-80% predicted). Since this finding was consistent across the spectrum of pulmonary limitation, even in the absence of malnutrition, another mechanism(s) causing a suppressed exercising SV in CF is speculated. However, this observation requires further investigation during the range of exercise intensities up to $\dot{V}O_2_{max}$. Furthermore, no studies have investigated cardiac indices using thoracic electrical bioimpedance cardiography simultaneously with both $\dot{V}O_2$ and NIRS-derived muscle oxygenation.

### 2.8 Mechanistic basis of aerobic exercise (dys)function in CF

Debate remains regarding the mechanism(s) responsible for impaired aerobic exercise function in patients with CF. Several reviews have been published on the topic in recent years (e.g., Hulzebos et al., 2015; Rand & Prasad, 2012; Williams et al., 2014). In a recent article concerning the utility and interpretation of CPET in CF, Urquart & Vendrusculo (2015) provided a suggested flow-chart to interpret CPET results in individuals with CF (Figure 2.20). However, this schematic outlined ventilatory limitation, deconditioning, submaximal effort and ‘other’ as the possible factor(s) that may impair aerobic fitness status in this
patient group. There has been much debate regarding what these ‘other’ factors are.

A review by Rand and Prasad (2012) outlined a number of factors that likely contribute to reduce aerobic exercise function in patients with CF, including pulmonary function, nutritional status, muscle (dys)function, genotype, habitual physical activity (and the impact gender has upon this), and psychosocial influences. Exercise capacity has also recently been associated with chronic infection and inflammatory status (van de Weert-van Leeuwen et al., 2012).

**Figure 2.20.** Suggested schematic of the interpretation of data from a cardiopulmonary exercise test in patients with cystic fibrosis. Image taken from Urquart & Vendrusculo (2015) with permission.

Typically, the fundamental causes of exercise limitation in pulmonary disease are dyspnoea and leg discomfort (Ferrazza et al., 2009). Although progressive lung disease is the major manifestation of CF, the relationship between
pulmonary function and peak exercise performance is weak (e.g., Klijn et al., 2003; Shah et al., 1988) and, as previously outlined, pulmonary dysfunction and ventilatory abnormalities do not appear to principally modulate the altered aerobic exercise function characterising children and adolescents with mild-to-moderate CF. In patients with mild-to-moderate pulmonary disease, non-pulmonary factors such as low muscle mass, impaired skeletal muscle function, and centrally mediated $O_2$ delivery, seem to predominate in limiting exercise capacity (Moorcroft et al., 2005; Regnis et al., 1996).

2.8.1 Genotype

It is not clear whether genotype per se plays a role in the extent of exercise (dys)function in young patients with CF. There is evidence supporting a significant relationship between the class of the second CFTR mutation and aerobic capacity, anaerobic power and BMI in adolescent patients (~11-17 y) with CF who were heterozygous for the F508del mutation (Selvadurai et al., 2002). Selvadurai et al. (2002) also reported that a second CFTR mutation belonging to either class I or II was associated with reduced aerobic fitness status, anaerobic power and a lower BMI and SS. Conversely, class III, IV and V mutations were associated with higher levels of aerobic fitness, anaerobic power, an increased BMI and less severe disease. Although Selvadurai et al. (2002) observed no significant relationship between pulmonary function and genotype, a more recent study (McBride et al., 2010) observed relationships between reduced pulmonary function and malnutrition and exercise performance, but no relationship with F508del status.
2.8.2 Ventilatory Function

Although impaired aerobic fitness in adults with CF appears to be multifactorial, $\dot{V}O_{2peak}$ has been associated with the magnitude of the ventilatory response during exercise (Pastré et al., 2014). Specifically, in patients with severe airway obstruction ($FEV_1 < 50\%$ predicted), multivariate analysis revealed $FEV_1$ to be a significant predictor of exercise capacity, whereas $\dot{V}_E/\dot{V}CO_2$ ratio at peak exercise was the major determinant of exercise limitation in patients with mild-to-moderate disease ($FEV_1 > 50\%$ of predicted).

High ventilatory dead space ratio ($V_D/V_T$) values during exercise have also been reported in patients with CF (Bongers et al., 2014; Cerney et al., 1982; Coates et al., 1988; Thin et al., 2004; Wilkens et al., 2010). This, in combination with the trend for higher $P_{ETCO_2}$ in patients with CF, indicates abnormal alveolar dead space ventilation (Wilkens et al., 2010), which might explain the higher $\dot{V}_E$ values at rest and during submaximal exercise that have been reported in patients with mild-to-moderate CF disease.

Prevalence of dynamic hyperinflation in adults with mild-to-moderate CF is high and is associated with poorer pulmonary function and exercise tolerance, and increased exertional dyspnoea (Stevens et al., 2013). Due to continuous airflow obstruction, as reflected by a decreased $FEV_1$ and dynamic hyperinflation, adolescents with CF have been found to develop a rapid, shallow breathing pattern at rest (Hart et al., 2002) and during exercise (Keochkerian et al., 2005). This can be accompanied with a decreased ventilatory capacity at $\dot{V}O_{2peak}$ (Keochkerian et al., 2008). This exaggerated ventilatory response with a rapid shallow breathing pattern at rest (Hart et al., 2002) and during exercise (Keochkerian et al., 2008) in adolescents with mild-to-moderate CF could be
due to increased $\dot{V}CO_2$ increasing ventilation during exercise. However, two recent studies investigating young patients with milder CF disease did not observe any differences in the ventilatory response and/or this rapid shallow breathing pattern during exercise (Borel et al., 2014; Bongers et al., 2014).

Although there is some evidence that children and adolescents with static hyperinflation at rest (residual volume to total lung capacity ratio > 30%) seem to be more prone to a ventilatory limitation during exercise (Sovtic et al., 2013; Werkman et al., 2011). Contrasting data has shown that the presence of static hyperinflation in adolescents with CF does not strongly influence ventilatory constraints during exercise and that static hyperinflation is only a slightly stronger predictor of $W_{\text{peak}}$ and $\dot{V}O_{2\text{peak}}$ than airflow obstruction (Werkman et al., 2011).

Furthermore, Bongers et al. (2014) reported no differences in the breathing pattern of young patients with mild CF (FEV$_1$ 78.6 ± 17.3% of predicted) compared with healthy controls (14.3 ± 1.4 y) during a maximal CPET. However, they did observe increased $\dot{V}_E$ at rest and a trend towards lower $\dot{V}_E$ values at maximal exercise. The estimated $V_D/V_T$ ratio and $\dot{V}_E$/work rate ratio were also significantly elevated throughout CPET. Although this increased $\dot{V}_E$/work rate implies an exaggerated ventilatory response, Bongers et al. (2014) importantly documented that this ventilatory response was adequate for $CO_2$ exhalation in paediatric patients with mild-to-moderate CF. This was inferred from a similar $\dot{V}_E/\dot{V}CO_2$-slope, increased RER at submaximal exercise intensities, and the ability to maintain $P_{ET}CO_2$ values within normal ranges throughout the range of exercise intensities that span an exhaustive CPET.
Although Hart et al. (2002) and Keochkerian et al. (2008) reported abnormal breathing pattern in patients with relatively low pulmonary function, the recent work in paediatric patients with milder airway obstruction (Borel et al., 2014; Bongers et al., 2014) suggests that signs of pulmonary insufficiency do not typically characterise children and adolescents with mild CF disease. Although the ventilatory response in young patients with mild-to-moderate CF appears to be adequate, patients still present with significantly reduced \( \dot{V}O_2 \text{peak} \) (e.g., Almajed & Lands, 2012; Bongers et al., 2014; Rand & Prasad, 2012), a reduction which reportedly falls approximately 5–8% per year (Pianosi et al., 2005). As such, understanding the other factors that impair aerobic exercise function in this patient group is important.

2.8.3 Cardiac Function

From a central perspective, early studies indicate impaired cardiac function (Benson et al., 1984; Ionescu et al., 2001; Marcotte et al., 1986; Pianosi & Pelech, 1996). More recently, an inability to augment SV during exercise has also been reported in CF (Rosenthal et al., 2009), which is likely to reduce central \( O_2 \) delivery. Early research suggested that a decreased SV in malnourished individuals with CF was possibly caused by both right and left ventricular dysfunction during stress (Marcotte et al., 1986), without clinical signs or symptoms (Benson et al., 1984). However, this SV was indirectly measured using the Fick equation, which cannot clearly differentiate between right or left ventricular dysfunction.

Further research has sought to characterise ventricular function in this patient group. Of the earlier research, CF patients with normal left ventricular ejection fraction at rest showed compromised ejection fraction during exercise (Benson
et al., 1984; Chipps et al., 1979). Although earlier suggestions indicated that left ventricular dysfunction appears to be rare in CF, even in the most severe pulmonary disease (Fraser et al., 1999; Hirschfield et al., 1979; Panidis et al., 1985; Vizza et al., 1998), there was some evidence of increased right ventricular wall thickness and dimensions and abnormal systolic time intervals (Gewitz et al., 1977; Hirschfield et al., 1979; Rosenthal et al., 1976), with post-mortem data reporting right ventricular hypertrophy in 70% of the children with CF in a study by Marcotte and colleagues (1986). However, since imaging the right hand side of the heart presents a greater challenge, much of the research to date has focused on left ventricular function.

Furthermore, an abnormal right ventricular ejection fraction has been reported using radionuclide angiography (Chipps et al., 1979) and others have shown marked right ventricular dilation and flattening or compression of the ventricular septum in the majority of patients with advanced CF and clinical evidence of right-sided heart failure (Jacobstein et al., 1981). More recently, the presence of significant right ventricular systolic and diastolic dysfunction in the setting of consistent tachycardia and increased $\dot{Q}$ was evidenced in 103 UK based adults with severe CF disease, using two-dimensional and Doppler echocardiography (Florea et al., 2000) and, again, no specific left ventricular abnormalities were detected. Decreased septal and lateral strain rates have also been observed in patients with CF and were found to negatively correlate with the severity of pulmonary impairment (Labombarda et al., 2011).

However, it has been suggested that abnormal right ventricular ejection fraction is only found in patients with severe CF disease (Matthay et al., 1980) and that right and left ventricular systolic function are well preserved in patients with
moderately severe CF (Florea et al., 2000; Panidis et al., 1985). Reports that right-ventricular abnormalities in CF may be caused by pulmonary hypertension offer some support to this (Eckles et al., 2003; Ionescu et al., 2001; Ozcelik et al., 2013). There is also evidence that in adults with severe but stable CF, left and right ventricular function is well maintained in the absence of significant coronary artery disease (Fraser et al., 1999). However, pulmonary hypertension develops in a significant proportion of older patients and is strongly related to $O_2$ status, independent of pulmonary function; and subclinical pulmonary hypertension is associated with increased mortality (Fraser et al., 1999). It has been suggested that right ventricular hypertrophy may be a precursor to ventricular failure in CF; however this appears to be delayed until the terminal stages of the condition.

Few studies to date have documented the cardiac (dys)function of patients with CF during exercise. Pianosi and Pelech (1996) measured $\dot{Q}$ using the indirect Fick method with blood sampling in patients with CF ($FEV_1$ 28-80% predicted) compared with healthy controls, during three intensities of cycling exercise. This study documented that patients with mild-to-moderate CF presented with a significantly lower SV, particularly those with poorer pulmonary function ($FEV_1 \leq 55\%$ predicted). However, the CF group were still able to maintain adequate $\dot{Q}$ during low-to-moderate intensity exercise, through a compensatory increase in HR above that of their healthy peers. However, there was an effect of body weight and malnourishment on SV, although well-nourished patients with poor pulmonary function also had a reduced SV. As such, there is a need to examine the scaling of SV data, normalising for body size using BSA or FFM, and whether exercising differences are still evident. Several other factors have been proposed to contribute to changes in $\dot{Q}$ in CF, including training status,
elevations in pulmonary vascular resistance caused by dynamic hyperinflation during exercise (Wright et al., 1983), reduction in the size of the pulmonary bed because of increased arteriolar wall thickness and reduced arterial density (Ryland & Reid, 1975), and myocardial fibrosis (Oppenheimer & Esterly, 1973). Since there may be some issues with the indirect Fick method in this patient group (Pianosi & Hochman, 1996), alternative assessment methods such as thoracic bioimpedance cardiography warrant further investigation.

Massin et al. (2000) investigated the HR kinetics of 20 severely ill patients with CF (10 male, 5-28 y, FEV₁ < 55% predicted) during PBRS exercise. Extending the observations by Pianosi et al. (1996), Massin and colleagues (2000) reported slower HR kinetics in severely ill adults with CF compared with healthy matched controls, which were attributed to an earlier occurrence of vagal withdrawal during low exercise intensities in CF, with significantly impaired SV. However, in the healthier patients, a higher HR was observed, which concurs with the findings by Pianosi and Pelech (1996).

More recently, reduced exercising SV has also been inferred in patients with CF using respiratory mass spectroscopy (Rosenthal et al., 2009). Whilst it has been proposed that patients with CF can achieve apparently “normal” $\dot{Q}$ in the presence of a lower exercising SV, through elevated HR (Ionescu et al., 2001; Lands et al., 1992), more recent findings suggest that this may only be viable at lower intensities of exercise. Rosenthal et al. (2009) documented a reduced (~ 24%) estimated SV at maximal exercise in young patients with CF, although this was coupled with a similar HR response to healthy individuals.

Due to the presence of pulmonary disease, it is not clear whether these observed changes in ventricular function are simply the result of chronic
pulmonary dysfunction, or are a direct impact of CFTR dysfunction in the heart itself. However, CFTR has been found in ventricular myocytes of various mammalian species, including mice, rats, swine, simians, and humans (Duan et al., 1999; Gao et al., 2007; Tilly et al., 1996; Warth et al., 1996). CFTR may also play a role in the resting membrane potential and regulating the action potential duration, as well as minimising the depolarisation effect of Ca\textsuperscript{2+} entry upon β–adrenergic stimulation (Duan, 2013). An ex vivo study on isolated perfused hearts of CFTR-knockout mice also suggests that CFTR is involved in mediating ischemic preconditioning (Chen et al., 2004). Additionally, CFTR was found to be down-regulated in patients with heart failure (Solbach et al., 2008). In a recent study on isolated neonatal myocytes, Sellers et al. (2010) observed decreased contraction rate and increased Ca\textsuperscript{2+} uptake via the L-type Ca\textsuperscript{2+} channels in the presence of a CFTR inhibitor. Furthermore, increased ventricular pressure and decreased contractile reserve were observed in response to β–adrenergic stimulation in adult CFTR knockout mice (Sellers et al., 2013). Furthermore, changes in myocardial wall thickness and left ventricular chamber size have also been documented (Sellers et al., 2013). However, it was unclear whether these changes can be directly attributed to altered myocyte contractility due to CFTR disruption in the myocytes, or whether these changes in cardiac function are due to systemic loss of CFTR and are secondary to CF complications.

To investigate this question further, Jiang et al. (2015) recently investigated cardiac function of mice with cardiomyocyte-specific or global knockout of CFTR, both at rest and under β–stimulation by MRI in vivo, in addition to in vitro measures of myocyte contractility and Ca\textsuperscript{2+} transients. This study demonstrated that CFTR dysfunction, as evident in CF, leads to increased myocardial
contractility at rest, which may trigger myocardial remodelling in patients with CF, which is independent of pulmonary disease. Despite these observed in vivo changes, there were no differences in myocyte contractility and Ca$^{2+}$ transients. Sellers and colleagues (2010) previously reported increased peak left ventricular pressure and pressure rate at both systole and diastole in CF mice, also suggesting increased myocardial contractility in vivo (Warth et al., 1996). Furthermore, they also observed decreased aortic diameter and increased aortic stiffness by echocardiography, which was further supported by Jiang et al. (2015). The observed increase in myocardial contractility in vivo can be an adaptation to the increased afterload in order to maintain normal $\dot{Q}$. However, the mechanisms leading to aortic constriction and stiffness need further investigation.

Jiang et al. (2015) demonstrated that CFTR disruption leads to increased left-ventricular regional function at baseline, as well as slightly attenuated response to $\beta$–adrenergic stimulation in young CFTR knockout mice, in the absence of pulmonary infection and exacerbation. These observations show that chronically elevated myocardial contractility at baseline may render the hearts of patients with CF more susceptible to untoward remodelling processes that will eventually lead to abnormal left ventricular function that is independent of pulmonary disease, which is supported by the recent observations of early signs of cardiac disease in relatively healthy, young patients with CF (Giacchi et al., 2015).

In a recent study utilising Doppler-echocardiography in adult ($n = CF$) and paediatric ($n = 30$) patients with CF, Giacchi et al. (2015) demonstrated that early signs of potential heart impairment, represented by an increase of
pulmonary blood pressure, are already evidence in young people with CF. Furthermore, in adults with CF, the systolic function of the right ventricle may also be impaired. The authors suggested that these cardiac abnormalities may progressively develop as a consequence of chronic inflammation, caused by the degeneration of pulmonary function.

2.8.4 Skeletal Muscle Function

Compared with healthy controls, reduced peripheral muscle strength has been reported in people with CF (de Meer et al., 1999; Dunnink et al., 2009; Hussey et al., 2002; Sahlberg et al, 2005; Troosters et al., 2009; Vallier et al., 2011), which has been associated with BMI, pulmonary function and inflammatory status (Troosters et al., 2009). However this does not appear to be an independent predictor of respiratory or peripheral muscle strength (Dufresne et al., 2009). Furthermore, more recently, it was demonstrated that reductions in peripheral muscle strength were still present when data was normalised to lean muscle mass (de Meer et al., 1999; Hussey et al., 2002), found to be of contractile origin (Vallier et al., 2011), suggesting that skeletal muscle quality (e.g. the intrinsic force-generating ability) is altered in CF.

From a respiratory muscle perspective, inspiratory muscle strength has been found to be well preserved (Enright et al., 2007) or even higher (Dunnink et al., 2009) in adults with CF compared with their healthy peers, potentially due to an increased work of breathing (Dunnink et al., 2009). There is evidence, however, that reduced FFM is related to a loss in inspiratory muscle strength (Enright et al., 2007) and some studies have reported reduced inspiratory muscle strength in CF (Keochkerian et al., 2005; Lands et al., 1992). Although inspiratory muscle endurance may be reduced in CF and has been associated with
dyspnoea, recent evidence suggests that inspiratory muscle strength is independent of maximal exercise capacity (Leroy et al., 2011).

Moser et al. (2000) hypothesised that the exercise impairment in CF is principally due to a reduced overall muscle mass rather than an abnormality in muscle metabolism. However, reductions in \( \dot{V}O_{2\text{peak}} \) in young CF patients compared with healthy controls could not solely be explained by a specific reduction in muscle size. Specifically, reductions in \( \dot{V}O_{2\text{peak}} \) were still evident when data was normalised to muscle CSA. Consequently, Moser et al. (2000) suggested that impaired \( O_2 \) delivery and/or an intrinsic skeletal muscle abnormality may be responsible for impairing aerobic exercise function in CF. Whilst the potential mechanisms of altered cardiac function in this patient group are covered in section 2.7.3. Several studies have also proposed that the oxidative efficiency of skeletal muscle in individuals with CF may be reduced. The principle factors that could influence the efficiency of oxidative phosphorylation and, thus, the rise in pulmonary \( \dot{V}O_2 \) (and thereby \( m\dot{V}O_2 \)) at the onset of exercise include the transport and delivery of \( O_2 \) to the mitochondria, the provision of necessary substrate into the electron transfer chain and/or feedback control linked to the rise in adenosine diphosphate (ADP) and \( P_i \) released from ATP hydrolysis at the myofibrils. Any of these mechanisms may delay the matching of oxidative ATP resynthesis to the rate of ATP turnover occurring with the myocyte.

An area of particular interest over recent years has been the capacity of CF skeletal muscle to extract and utilise \( O_2 \). Furthermore there is some evidence to support impaired delivery of \( O_2 \) in children and adolescents with CF, it has been hypothesised that patients would therefore present with a compensatory
increase in O$_2$ extraction at the periphery (Ferreira et al., 2007). However, Rosenthal et al. (2009) observed no such response in a study that utilised respiratory mass spectroscopy. Although Rosenthal et al. (2009) could not determine the cause of impaired O$_2$ extraction and/or utilisation in CF skeletal muscle, because no direct peripheral measurements were made, it was suggested that muscle metabolic issues resulting from chronic bronchial sepsis may contribute. However, further confirmation of this response is warranted because inferences at the skeletal muscle level were based upon indirect, interlinked mathematical calculations. Furthermore, although Shah et al. (1997) demonstrated faster HR recovery kinetics (73 ± 6 vs. 86 ± 8 s) and improved supramaximal exercise performance in patients with CF following supplemental O$_2$ compared with room air, data from patients with less severe CF have reported that inhaling hyperoxic gas does not improve either $\dot{V}O_{2peak}$ (Nixon et al., 1990) or $\dot{V}O_2$ kinetics (Kusenbach et al., 1999).

Slower post-exercise muscle [PCr] recovery kinetics, measured using $^{31}$P-MRS, have recently been documented in the m. vastus lateralis of patients with CF (Wells et al., 2011). What was unique to the study by Wells and colleagues (2011) was that they sought to ascertain whether the metabolic abnormalities that reportedly characterise patients with CF are specific to this condition or, rather, are a consequence of chronic respiratory disease in general. Consequently, they investigated twenty patients with CF (FEV$_1$: 92.9 ± 12.7% predicted, age 15.1 ± 1.5 y) and two age-, gender- and habitual activity-matched control groups; healthy controls (FEV$_1$ 94.4 ± 6.6% predicted, age 15.2 ± 1.4 y) and a non-CF respiratory disease control group [primary ciliary dyskinesia (PCD); FEV$_1$ 91.8 ± 9.5% predicted, age 13.8 ± 2.3 y]. In line with earlier findings in the forearm muscle (de Meer et al., 1995), Wells et al. (2011)
reported abnormalities at rest (lower resting [ATP]) and during short bouts of high-intensity exercise (increased end-exercise pH) in patients with CF. Interestingly, this response was not observed in the respiratory disease control group or healthy controls, although both PCD and CF patient groups demonstrated slower [PCr] recovery time constants. This study therefore showed that whilst there are non-specific effects of chronic respiratory disease on skeletal muscle oxidative function in young people, there appear to be some CF-specific abnormalities of skeletal muscle metabolism.

Wells and colleagues (2011) also characterised the efficiency of oxidative exercise function in the skeletal muscle of the forearm during exercise in patients with CF ($n = 8$, 12-17 y) compared with healthy individuals (8-36 y). Intracellular pH and concentrations of phosphate compounds were measured during four steady states of submaximal exercise using $^{31}$P-MRS. This study reported that the efficiency of oxidative ATP synthesis of forearm muscle was 19-25% lower in patients with CF compared with healthy controls. Although de Meer et al. (1995) concluded that an intrinsic role of CFTR to cause abnormal oxidative phosphorylation in CF skeletal muscle was unlikely, this protein has since been expressed within both murine (Fiedler et al., 1992) and human (Lamhonwah et al., 2010) skeletal muscle, as outlined in section 2.1, making a role conceivable (Lamhonwah et al. 2010).

More recently, reduced local muscle oxidative capacity has also been inferred from NIRS-derived [HHb] recovery of $m$. vastus lateralis $O_2$ consumption following 15 s of electrical stimulation and subsequent repeated transient arterial occlusions (Erickson et al., 2015). NIRS was used to measure the recovery rate of $mV\bar{O}_2$ after 15 s of electrical stimulation (4 Hz) and repeated
transient arterial occlusions. Maximal $m\overline{VO}_2$ was reduced in patients with CF (1.82 ± 0.4 L·min$^{-1}$) compared with control subjects (2.13 ± 0.5 L·min$^{-1}$, $P = 0.04$). A significant inverse relationship between age and maximal $m\overline{VO}_2$ was observed in patients with CF ($r = -0.676$, $p = 0.011$) but not in control participants ($r = -0.291$, $p = 0.274$), indicating that patients with CF exhibit a reduction in skeletal muscle oxidative capacity compared with healthy controls. The authors concluded that this reduced skeletal muscle oxidative capacity is accelerated by age and may contribute to exercise (dys)function in patients with CF.

More recently, Werkman et al. (2015) provided the first study that combined NIRS and $^{31}$P-MRS techniques to investigate skeletal muscle oxygenation and metabolism during exercise in 10 paediatric patients with CF with class I-III mutations (5 males, 13.8 ± 1.3 y, FEV$_1$ 92.8 ± 14.6% predicted) versus 10 healthy controls (5 males, 13.7 ± 1.1 y, FEV$_1$ 90.3 ± 13.4% predicted). Using supine cycling exercise, with the upper body set to an approximately 40 degree angle, participants performed a step incremental (0.3 kg·min$^{-1}$ for males and 0.2 kg·min$^{-1}$ for females) cycling test to exhaustion. However, contrary to previous suggestions, Werkman and colleagues did not observe a relationship between exercise capacity and skeletal muscle oxidative metabolism. More specifically, no significant differences in FFM normalised $\overline{VO}_{2\text{peak}}$ were seen between CF and controls. Furthermore, no differences in $^{31}$P-MRS derived [PCr] recovery or [HHb] dynamics were observed, in contrast with previous suggestions (de Meer et al., 1995; Wells et al., 2011).

It has been proposed that deficient sarcoplasmic reticulum CFTR Cl$^-$ channels (Lamhonwah et al., 2010) could perturb electrochemical gradient, leading to
Ca\textsuperscript{2+} homeostasis dysregulation which could alter excitation-contraction coupling. Since this may contribute to increase fatiguability and reduce functional capacity in patients with CF, Gruet et al. (2015) recently sought to determine whether adult patients with mild-to-moderate CF present with altered skeletal muscle contractility and greater muscle fatigue during exercise. Patients performed quadriceps neuromuscular evaluation using single and paired femoral nerve magnetic stimulations and electromyographic (EMG) and mechanical parameters were measured during voluntary and magnetically-evoked contractions at rest, during and after a fatiguing isometric task. Quadriceps muscle CSA was also determined using MRI. In contrast with previous suggestions, Gruet et al. (2015) observed similar skeletal muscle endurance and fatiguability in patients with CF and their healthy counterparts, which is in line with earlier reports (Vallier et al., 2011).

As such, the precise cause(s) of an intramuscular impairment in skeletal muscle oxidative metabolism in people with CF requires further investigation, although several other factors have been proposed. Reduced antioxidant capacity, arising from systemic inflammation and/or oxidative damage, may lower mitochondrial efficiency (Wells et al., 2011). However, it may also be a consequence of the CFTR genetic mutation. As outlined in section 2.1, CFTR is expressed in skeletal muscle cells (Lamhonwah et al., 2010) and there are a number of structural and functional changes in the skeletal muscle of individuals with CF (de Meer et al., 1995; Wells et al., 2011; Lamhonwah et al., 2010). Furthermore, in vitro study of leucocyte mitochondria in patients with CF demonstrates that properties of complex I of the respiratory chain are significantly altered (Dechecci et al., 1988) and the absence of CFTR from skeletal muscle has been shown to dysregulate Ca\textsuperscript{2+} homeostasis, augment
inflammatory or atrophic gene expression signatures and increase diaphragm weakness (Divangahi et al., 2009).

2.9 Summary

Since the preservation of aerobic fitness and pulmonary function are important for the prognosis and QoL of patients with CF, it is important to understand the factors responsible for impairment. This chapter has critiqued current evidence regarding aerobic exercise (dys)function characterising children and adolescents with mild-to-moderate CF. However, it has highlighted a need to clarify the extent of impairment to the key parameters of aerobic exercise function in this patient group. For this to be possible, there is a need to first develop a valid and reproducible CPET protocol to study the physiological responses of young people with CF during exercise that spans the intensity domains, to the point of exhaustion. Chapter 2 has also identified the need for further research investigating the pulmonary $\dot{V}_O_2$ response during CWR exercise of different intensities, demarcated by the GET. According to the Fick principle, the rate of adjustment in $\dot{V}_O_2$ is dictated by $O_2$ delivery and utilisation mechanisms (Figure 2.6), however debate remains regarding the role of respiratory and cardiovascular (dys)function and/or intrinsic muscle abnormalities to impair the response in children and adolescents with CF. The body’s upper limit for $\dot{V}_O_2$ is determined by the maximal $\dot{Q}$, arterial $O_2$ content, fractional distribution of $\dot{Q}$ to the exercising muscles, and the ability of the skeletal muscle to extract $O_2$ (Wasserman et al., 2004). Therefore simultaneous measurements at the central (cardiorespiratory) and peripheral (skeletal muscle) levels are needed to further understand the dynamic matching of $O_2$.
delivery-to-\(\text{O}_2\) utilisation in this patient group during exercise and how this modulates aerobic exercise (dys)function. There is therefore a need for research that utilises additional techniques available, such as NIRS and thoracic electrical bioimpedance cardiography.

2.10 Thesis Objectives

- Study 1 (Chapter 4) will develop a valid CPET protocol for use in paediatric patients with CF. Specifically it will 1) determine whether traditional criteria used to verify \(\dot{\text{VO}}_{2\text{max}}\) in children and adolescents with CF are valid and, 2) investigate the utility of \(S_{\text{max}}\) verification as an alternative method to confirm \(\dot{\text{VO}}_{2\text{max}}\) in children and adolescents with mild-to-moderate CF;

- Study 2 (Chapter 5) will determine the reproducibility of the key parameters of aerobic exercise function derived from CPET in children and adolescents with mild-to-moderate CF over the short-(48 h) and clinically relevant medium-term (4-6 weeks);

- Study 3 (Chapter 6) will investigate the influence of mild-to-moderate CF on aerobic exercise function during exhaustive ramp incremental cycling exercise. Specifically it will investigate 1) any impairment to key parameters of aerobic exercise function and 2) determine whether central and/or peripheral factor(s) modulate any changes in aerobic exercise function;

- Study 4 (Chapter 7) will demonstrate the application of a valid and reproducible CPET protocol as an outcome tool within clinical
interventions. Specifically, it will investigate changes in aerobic exercise function during 12 weeks treatment with Ivacaftor using a case-based design;

- Study 5 (Chapter 8) will investigate the influence of mild-to-moderate CF on aerobic exercise function during CWR exercise. Specifically, it will 1) determine any changes in the kinetics of pulmonary $\dot{V}O_2$ and skeletal muscle [HHb] at the onset of moderate and very heavy intensity cycling exercise and 2) determine the contribution of central and/or peripheral factor(s) to modulate the pulmonary $\dot{V}O_2$ kinetic response in children and adolescents with mild-to-moderate CF during moderate and very heavy cycling exercise.
CHAPTER THREE

General Methods

3.1 Scientific review and ethics approval

All study protocols were assessed by an independent scientific reviewer from the Research and Development Unit at the Royal Devon and Exeter NHS Foundation Trust Hospital (RD&E) (Appendix A). Ethics approval for all work involving patients with CF was obtained from the Local NHS Research Ethics Committees (Appendices B, D, G-I) and experimental procedures for the healthy control participants in Chapter 6 were approved by the University of Exeter Sport and Health Sciences Ethics Committee (Appendix E).

3.2 Funding

All studies were supported by small grants obtained from the Research and Development department at the RD&E (Appendix C).

3.3 Study Participants

Children and adolescents (7-18 y) with CF were recruited from paediatric CF outpatient clinics at the RD&E. The lower age limit was raised to 10 y of age for Chapter 8 to ensure that participants could adhere to the requirements of the investigation. Prior to any invitation to participate, patients’ suitability was assessed by their primary respiratory consultant and the research team using clinical information extracted from routine hospital appointments. Suitable patients were then invited to participate, via their parent(s)/guardian(s). The method of recruitment was voluntary participation through the RD&E. All procedures were explained to patients and their parent(s)/guardian(s) and
supportive written information detailing the exact procedures of each respective study were provided. Potential participants were then contacted 48 hours later via telephone. All participants were informed that voluntary withdrawal was possible at any time. For Chapters 6 and 8, healthy age- and gender-matched controls were recruited from the local area. No group presented with any contraindications to exhaustive exercise.

All participants and their parent(s)/guardian(s) were provided with supportive written documentation in appropriate language (Appendix D), which was substantiated with verbal explanation of the protocol during the familiarisation session. Fully informed written assent (participants < 16 y) and consent was obtained from participants and their parent(s)/guardian(s), respectively (Appendices G-I). Patients between the ages of 16-18 y were deemed capable of providing consent and, therefore, asked to provide consent in addition to their parent(s)/guardian(s).

3.4 Participant inclusion and exclusion criteria

3.4.1 Cystic fibrosis patients

Males and females diagnosed with CF were invited to participate if their pulmonary function and disease profile were considered stable. Specifically, pulmonary function (FEV$_1$) was within 10% of their best in the preceding 2 months and there was no increase in other clinical symptoms or weight loss in the 2 weeks prior to testing. Diagnosis of CF was based on clinical features and was supported by an abnormal sweat test (sweat Cl$^-$ > 60 mmol·L$^{-1}$ > 100 mg sweat) and, where possible, diagnostic genotyping. All CF maintenance medications and therapies were continued throughout each study and were documented. It was also desirable that patients were regularly participating in
physical activity, as recommended as part of the standard clinical care guidelines, although no formal assessment of physical activity status was made due to adherence issues. Participants were also required to be able to understand and cooperate with each study protocol. Patients were excluded if they had any non-pulmonary conditions that may impair exercise ability, such as musculoskeletal disorders and cardiovascular disease or if they were unable to understand or cooperate with the study protocol. Exclusion at familiarisation could also occur due to the onset of an acute infection, issues regarding understanding or cooperating with the study protocol, or the participant and/or their parent(s)/guardian(s) no longer wished to participate.

The clinical profile and disease severity of the young patients with CF were recorded upon enrolment into each experimental study. Information included CF genotype, resting SpO$_2$, BMI, physical activity level (none, school sports, or extracurricular), physical chest examination findings (evidence of clubbing, crackling, wheeze, or hyperinflation), nutritional status and pancreatic sufficiency, chest radiographic findings, pathogens in sputum in the last year (any evidence of Pseudomonas Aeruginosa in accordance with the Leeds criteria (Lee et al., 2003), or other regular pathogens), smoking, medical imaging and DEXA, assessment of liver function and glycaemic control, current treatments and IVABs in the past year, renal function, bone chemistry, dietetic review and any airway clearance methods routinely used. The SS was also used to score general disease profile, scoring four aspects of the disease profile (general activity, physical examination, nutritional status, and chest radiographic findings), with a total of 100 points representing a perfect score of health. The Northern Score provided evidence of radiographic chest findings, with a maximum score of 20 being the most severe.
3.4.2 Healthy control participants

Healthy males and females were recruited as matched controls for each participant with CF. In addition to being age- and gender-matched, the control group were required to have no diagnosis of any respiratory condition or any non-pulmonary condition that may impair exercise ability, no contraindications to performing exhaustive exercise, be regularly participating in physical activity and be able to understand and cooperate with the study protocol. Healthy controls were excluded if they had any pulmonary conditions, any non-pulmonary conditions that may impair exercise ability, such as musculoskeletal disorders (active arthritis, joint or muscle disease) and cardiovascular disease (congenital heart disease or cardiomyopathy), they presented with comorbidities to performing they required exercise, were unable to understand or cooperate with the study protocol due to learning difficulties or otherwise. Controls were also excluded at familiarisation if there was the onset of any infection, any of the above exclusion criteria were met, or the child and/or their parent/guardian was not willing to participate any further.

3.5 Age

Decimal age was calculated, to the nearest 0.1 year, as the difference between the date of birth and the date of the first testing session for each experimental study.

3.6 Pubertal maturity

Pubertal maturity was measured in accordance with the secondary sex characteristics outlined by Tanner (1962), with stage 1 representing pre-pubertal, stages 2, 3 and 4 circum-pubertal, and stage 5 post-pubertal.
Drawings of male and female pubic hair development (Morris & Udry, 1980) made from photographs by Tanner (1975) were used, in conjunction with a written description for each of the 5 stages of male and female pubic hair development underneath each drawing. This measure was self-assessed at home following the last visit for each respective investigation (Appendix J) and was returned to the research team in a sealed envelope. This method has been shown to be valid and reproducible (Chan et al., 2008; Morris & Udry, 1980; Taylor et al., 2001; Norris & Richter, 2005; Schmitz et al., 2004).

3.7 Anthropometry

All anthropometric measurements were made during the familiarisation session for each study. Measurements were taken by the same researcher throughout, with participants wearing shorts and t-shirt/vest and with their footwear removed.

3.7.1 Body mass

Body mass was measured to the nearest 0.1 kg using electronic weighing scales (Seca 220; Vogel & Halke, Hamburg, Germany), with footwear removed.

3.7.2 Stature

Stature was measured to the nearest 1 cm using a stadiometer (Seca 220; Vogel & Halke, Hamburg, Germany). With footwear removed, participants were instructed to stand upright with their heels against the stadiometer and feet together. Gentle pressure was applied to the mastoid process, whilst participants were instructed to stand up straight and look forward.
3.7.3 Body mass index

Body mass index was calculated using the following equation:

\[ BMI = \frac{\text{body mass (kg)}}{\text{stature (m}^2\text{)}} \]

Equation 3.1.

3.7.4 Skinfold measurements

Skinfold measurements were taken on the right hand side of the body using spring loaded callipers (Harpenden; British Indicators, Burgess Hill, UK) with another adult present. Triplicate measurements were taken at the bicep, tricep, subscapular and suprailiac regions. Skinfolds were held between the thumb and forefinger for ~ 2 s prior to the measurement being recorded, to the nearest 0.1 mm. Repeat measurements were separated by at least 1 min, to minimise skinfold compression. The median or triplicate measures at each site was then calculated and used to determine the sum of skinfolds (SSkF). Measurements were taken in accordance with recommended guidelines (Eston et al., 2009).

The measurements obtained at the tricep and subscapular regions were then used to determine FFM using pubertal stage and gender specific equations (Slaughter et al., 1988; see Equations 3.2-3.6). This method has been shown to be suitable for use in paediatric patients with CF (Wells et al., 2008):

Body fat percentage for pre-pubertal males:

\[ \text{Body fat percentage} = 1.21 \times (\text{triceps + subscapular}) - 0.008 \times (\text{triceps + subscapular})^2 - 1.7 \]

Equation 3.2
Body fat percentage for circum-pubertal males:

Body fat percentage = 1.21 (triceps + subscapular) – 0.008 (triceps + subscapular)² – 3.4

Equation 3.3

Body fat percentage for post-pubertal males:

Body fat percentage = 1.21 (triceps + subscapular) – 0.008 (triceps + subscapular)² – 5.5

Equation 3.4

Body fat percentage for all females:

Body fat percentage = 1.33 (triceps + subscapular) – 0.013 (triceps + subscapular)² – 2.5

Equation 3.5

FFM was then calculated as follows;

\[ FFM = \text{body mass} - (\text{percentage body fat} \times \text{body mass}) / 100 \]

Equation 3.6

3.8 Pulmonary function

Forced vital capacity (FVC), FEV₁ and peak expiratory flow (PEF) were assessed using flow-volume loop spirometry (Micromedical Microloop 3535, Numed, Sheffield, UK), with participants seated. All spirometric pulmonary function assessments were performed in line with recommendations by the British Thoracic Society (BTS, 1994) and with verbal encouragement provided during all manoeuvres. The best of three consistent (< 5% variability) exhalations was documented and expressed as a percentage of predicted using
appropriate reference data for young Caucasians (Stanojevic et al., 2009). Values were deemed technically and clinically ‘acceptable’ if the reported values did not exceed the next greatest by > 5%.

Participants were instructed to place their lips tightly around the antibacterial mouthpiece. Following three initial resting breaths, they were directed to breathe in as deeply as possible, and then to ‘blow out’ into the equipment as hard and as fast as possible until no further air could be exhaled. A minimum of three ‘acceptable’ manoeuvres were performed, with a minimum rest period of 30 s between attempts, to minimise respiratory fatigue.

3.9 Infection control and patient safety during exercise

In line with standard clinical practice, segregation was ensured during recruitment discussions at outpatient clinics and throughout any subsequent visits relating to all research studies presented herein. All equipment was cleaned using appropriate bacteriocidal wipes and sterilised following each use and the testing environment was cleaned following each participant and the room ventilated for > 4 hours.

Participants were monitored throughout exercise for any significant development of symptoms, such as severe discomfort or dyspnoea. Fingertip SpO₂ was also monitored on a beat-by-beat basis via pulse oximetry (NONIN, Avant 4000, NONIN Medical Inc.), at rest, throughout exercise and recovery. Supplemental O₂ was available if cases of severe hypoxemia presented, however this was not warranted in any of the presented 6 investigations. Medical personnel were aware when testing was taking place and could be contacted in the event of any adverse symptoms during or following exercise. If patients did experience severe or persistent symptoms during testing, a
respiratory clinician would have provided a medical examination, however this was not needed during any of the testing.

### 3.10 Familiarisation session

All participants were required to attend a preliminary familiarisation visit prior to involvement in the research studies. The aim of this session was to obtain fully informed written consent and assent (depending on participant’s age), to familiarise participants and their parents/guardians with the equipment, experimental procedures and the testing environment. For Chapter 8, this visit was also used to obtain information necessary to set the exercise intensities for the subsequent visits.

During this visit, the principal investigator talked through the information sheet and study protocol again, to ensure that all parties were clear about the study requirements. Following this, consent to participate was obtained. Anthropometric data (see section 3.7) and resting pulmonary function (see section 3.8) were then recorded. Next, participants were familiarised with exercising on a cycle ergometer and maintaining a constant cadence both against a constant and incremental work rate. Appropriate adjustments were made to the ergometer seat, handlebar and pedal cranks for each participant and recorded for subsequent visits (see section 3.11).

### 3.11 Exercise testing

All testing of patients with CF was conducted within temporary exercise laboratories established at the RD&E. Healthy age- and gender matched control participants were tested at the Children’s Health and Exercise Research Centre at the University of Exeter. All repeat testing in the same participants was
performed at the same time of day (± 2 h). Participants were instructed to arrive in a rested and hydrated state, > 2 h postprandial and having refrained from consuming caffeine for > 2 h.

3.12 Maximal cardiopulmonary exercise testing

3.12.1 Equipment

All exercise testing was performed on a cycle ergometer (Lode Excalibur or Lode Corival, Groningen, The Netherlands; Figure 3.1).

![Figure 3.1. The equipment used during all exercise testing which comprises this thesis. Consent was provided to include these photographs within this thesis. (Consent for photography and publication was obtained from all of these participants and their parent(s)/guardian(s)).](image)

3.12.2 Protocol

In all studies comprising this thesis, CPET involved a combined ramp incremental and supramaximal test to exhaustion (see Figure 3.2). For the initial ramp protocol, the ramp rate (10-25 W·min⁻¹) was individually targeted to each participant to produce test durations of 8-12 min, based on information concerning the participant's physical activity and clinical status and their familisation on the cycle ergometer. Following a 3 min warm-up, cycling at 20
W, work rate was increased. A cadence of 70-80 rpm was maintained throughout. Volitional exhaustion was defined as a drop in cadence ≥ 10 rpm for 5 consecutive seconds, despite strong verbal encouragement. Five min active recovery, cycling at 20 W, and then 10 min passive seated recovery then followed. The S_max test was then performed. S_max consisted of 3 min warm-up, cycling at 20 W, followed by a ‘step’ transition to a CWR equivalent to 110% W_peak output achieved during the preceding ramp test. Upon exhaustion from the S_max, 5 min active recovery (20 W) was undertaken (Figure 3.2).

![Figure 3.2. An example pulmonary oxygen uptake (V̇O₂) response during the combined ramp incremental and supramaximal cardiopulmonary exercise testing protocol used to determine V̇O₂max.](image)

### 3.13 CWR exercise testing

#### 3.13.1 Equipment

In Chapter 8, CWR exercise was performed on the same equipment as previously outlined for CPET.
3.13.2 CWR Testing Protocol

For Chapter 8, participants attended the laboratory on four subsequent occasions at a similar time of day to complete ‘step’ change CWR exercise tests within the MOD and VH intensity domains (Figure 3.3). All CWR tests consisted of 6 min of pedalling at 10 W, followed by an instantaneous transition in power output, intended to elicit a \( \dot{V}O_2 \) amplitude corresponding to 90% of the GET and \( \Delta 60\% \) (60% of the difference between the GET and \( \dot{V}O_{2\text{max}} \)) for MOD and VH intensity exercise, respectively, following correction for the \( \dot{V}O_2 \) lag time (Whipp et al., 1981).

This equated to MOD work rates of 58 ± 24 W and 73 ± 35 W for CF and CON, respectively. During VH exercise CF and CON worked at 121 ± 43 W vs. 150 ± 64 W, respectively. A cadence within the range of 70-80 rpm was maintained during all exercise and MOD and VH exercise. Part 1 and part 2 were separated by 30 min seated and 10 min active recovery (total 40 min), in order to remove any priming influence of the preceding exercise. Although at least 45 min is reportedly needed in healthy adults to eliminate a priming effect of prior heavy exercise on the subsequent \( \dot{V}O_2 \) response (Burnley et al., 2006), 40 min was the

![Figure 3.3](image.png)

**Figure 3.3.** Schematic illustration of the exercise testing protocol. MOD, moderate-intensity exercise (90% GET); VH, very heavy-intensity exercise (\( \Delta 60\% \)).
cut-off time due to time restraints on the testing facility and patient time. Each visit was separated by 48-72 hours.

Isolating and characterising an acceptable estimation of $m \dot{V}O_2$ kinetics is complicated in young people, due to the inherently low pulmonary $\dot{V}O_2$ amplitude and larger inter-breath fluctuations, resulting in $\dot{V}O_2$ dynamics with low signal-to-noise properties (Armstrong & Barker, 2009; Potter et al., 1999). Previous work in healthy young people has demonstrated that averaging 4-10 step transitions can yield 95% confidence intervals in the phase II $\dot{V}O_2 \tau$ within approximately 5% (Barker et al., 2008; Fawkner et al., 2002). Therefore, in Chapter 8 four transitions were completed for all conditions to improve the signal-to-noise.

3.14 Measurement of pulmonary gas exchange

A metabolic gas analyser (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany; Figure 3.1) was used to measure $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, $\dot{V}_{E}/\dot{V}O_2$ and $\dot{V}_{E}/\dot{V}CO_2$, respectively. Prior to each test, the metabolic cart was calibrated using gases of known concentration (15.0% $O_2$ and 5.0% $CO_2$) and a 3 L calibration syringe (Hans Rudolph, Kansas City, MO) was used to calibrate the turbine volume transducer. Furthermore, prior to each test, the analyser recorded ambient air temperature and pressure. Breath-by-breath changes in pulmonary gas exchange and ventilation were measured at rest and throughout exercise. It is acknowledged that there are differences between all commercially available gas analysis systems in the method of capture and measurements process. As such, the same system was used throughout all testing. Furthermore, when appropriately calibrated the accuracy of measuring volume and gas fractions
are 2% and 0.1%, respectively and the reproducibility of the Metalyzer 3B system has previously been documented in healthy subjects (Meyer et al., 2001), with a marginally smaller variability than the Metamax 3B system. Intra-class correlation coefficients of 0.98, 0.98 and 0.97 have been documented for $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}E$, respectively, when using the Metalyser 3B.

### 3.15 Measurement of cardiac parameters

Cardiac parameters were non-invasively measured on a beat-by-beat basis using thoracic bioelectric impedance cardiography (PhysioFlow, PF-05, Manatec Biomedical, Paris, France; Figure 3.1). This technique uses a high-frequency (75 kHz) and low-magnitude (1.8 mA) current across the thorax, to enable changes in thoracic impedance during the cardiac cycle to be recorded. $\dot{Q}$ was determined in accordance with Equation 3.7:

$$\dot{Q} = f_c \times SVi \times BSA$$

*Equation 3.7.*

where $f_c$ was HR based on the measurement of the R-R interval as determined by the ECG first derivative $dECG/dt$, BSA was body surface area (BSA) calculated using the Haycock equation (Haycock et al., 1978; see Equation 3.8) and SVi was the stroke volume index (SVI).

$$BSA = 0.024265 \times \text{body mass}^{0.5378} \times \text{stature}^{0.3964}$$

*Equation 3.8.*

To establish the thoracic impedance signal, electrodes (Blue Sensor R; Ambu, Ballerup, Denmark) were positioned on the forehead and base of the neck above the supraclavicular fossa, and two were positioned on the xiphoid process. Another set of two electrodes was used to determine a single electrocardiograph signal at the V1 and V6 positions. This pattern of electrode
placement adheres to recommendations for paediatric practice (Welsman et al., 2005). All skin sites were cleaned prior to electrode contact.

Prior to testing and following 10 min of seated rest, the device was autocalibrated in accordance with the manufacturer’s guidelines. Blood pressure was determined manually using a sphygmomanometer and stethoscope with the participant seated and relaxed on the cycle ergometer. The participant’s mean resting blood pressure, age, stature and body mass were then entered into the software. Autocalibration then established the basic impedance waveforms over 30 cardiac cycles. This technique has previously been validated in children with CF (Pianosi et al., 1997). Impedance cardiography measures of $\dot{Q}$ at two exercise intensities (0.5 and 1.5 W·kg$^{-1}$) were compared to $\dot{Q}$ derived using the indirect Fick method, involving CO$_2$ rebreathing with sampling of capillary blood gases. Thoracic bioelectrical impedance cardiography derived values were reportedly within ± 20% of values measured using the indirect Fick method. Regarding the reproducibility of this device, coefficients of variation of 9.3% have been reported in healthy children for both $\dot{Q}$ and SV at peak exercise during ramp incremental exercise testing (Welsman et al., 2005). It has also been documented that this technique is relatively insensitive to minor changes in electrode placement (Tan et al., 2006).

### 3.16 Measurement of muscle oxygenation

In Chapters 6-8, muscle oxygenation status was non-invasively assessed from the HHb dynamics of the m. vastus lateralis using NIRS (Portamon, Artinis Medical Systems). This system has previously been used in children (e.g. McNarry et al., 2011; McNarry et al., 2014) and consists of an emission probe, with three light sources emitting two wavelengths of light (760 and 850 nm) and
a photon detector. The intensity of incident and transmitted light was recorded continuously at 10 Hz and used to estimate [HHb]. Evidence was recently provided to demonstrate that the kinetics of the NIRS-derived deoxygenation signal in the superficial and deeper muscle regions provides an index of local O₂ extraction kinetics within the intramuscular microcirculation during exercise, rather than intramyocyte myoglobin (Koga et al., 2012). As such, changes in the deoxygenation signal should be taken to indicate changes in local muscle O₂ extraction. The wireless emitter-detector unit was placed over the *m. vastus lateralis*, midway between the greater trochanter and lateral epicondyle of the femur. The area of interrogation was initially cleaned and shaved and, following marking of the placement area, the device was secured with tape (KinesioTex®) and a dark elastic bandage, to minimise extraneous light interference with the near-infrared signal. The Portamon device has been reported to produce acceptable reproducibility estimates (Shadgan et al., 2009).

### 3.17 Measurement of blood lactate concentration

In Chapter 4, a fingertip capillary blood sample (~5 µL) was taken within 30 s of volitional exhaustion from the incremental ramp test and analysed for the concentration of whole blood lactate (La[B]) using a portable device (Lactate Pro, Arkray, Japan). This device has been shown to strongly correlate (*r = 0.99*) with lactate determined using the YSI device (YSI 1500, Yellow Springs Instruments, Australia) (Medbø et al. 2000; Pyne et al., 2000).

### 3.18 Measurement of SpO₂

Transcutaneous SpO₂ was non-invasively measured at the fingertip using pulse oximetry (NONIN, Avant 4000, NONIN Medical Inc., USA).
3.19 Measurement of effort and dyspnoea

Subjective ratings of perceived exertion (RPE) and dyspnoea (RPD) were determined upon exhaustion using the pictorial children’s effort rating table (P-CERT) (Lamb et al., 2000) and the 0-10 category ratio (CR-10) scale (Borg, 1982; Appendix M), respectively. Prior to each study, participants were thoroughly familiarised with all rating scales during their habituation session. The P-CERT (Appendix L) was used throughout, since the scale best depicts incremental exercise.

3.20 Determination of the key parameters of aerobic function

3.20.1 Determination of $\dot{V}O_2^{\text{max}}$

In study 1, the presence of a $\dot{V}O_2$ plateau was determined using methodology that have been previously employed in a paediatric population (Barker et al., 2011; Day et al., 2003). Concisely, a linear regression was plotted over the ‘linear’ portion of the $\dot{V}O_2$ response. The $\dot{V}O_2$ profile at exhaustion was then characterised by extrapolating this linear regression function to exhaustion and isolating the final 60 s of data, to examine the residuals against the extrapolated line (see Figure 3.4). A negative residual indicated a deceleration in $\dot{V}O_2$ against power output and was considered a ‘plateau’ when the magnitude of the residuals was $\geq 5\%$ of the projected $\dot{V}O_2$ (i.e. $\dot{V}O_2$ was $\leq 95\%$ of the projected $\dot{V}O_2$). A positive residual $\geq 5\%$ of the projected $\dot{V}O_2$ represented acceleration and positive or negative residuals $< 5\%$ of the peak power output projected $\dot{V}O_2$ were categorised as linear responses (Barker et al., 2011).
Secondary verification criteria (\(\dot{V}O_2\) at an RER > 1.00 (Gulmans et al., 1997; Klijn et al., 2003), 1.10 (Moser et al., 2000), a HR of 180 b\(\cdot\)min\(^{-1}\) (Gulmans et al., 1997; Klijn et al., 2003) and 95% age-predicted HR\(_{\text{max}}\) (Stevens et al., 2009; Stevens et al., 2011)) were taken from key studies implementing CPET within patients with CF, whilst La\(_{[B]}\) ≥ 6 mmol\(\cdot\)L\(^{-1}\) is often utilised in paediatric physiology. Subsequently, the highest 15 s averaged \(\dot{V}O_2\) obtained during the ramp and S\(_{\text{max}}\) tests was taken to represent \(\dot{V}O_2\)\(_{\text{max}}\).

Scaling of physiological data is particularly important in paediatric groups, in order to create a ‘size free’ parameter that controls for any influence of maturation (Baxter-Jones et al., 2005). Whilst allometry, regression and traditional ratio standard methods are available, in all chapters comprising this
thesis, a log-linear allometric model yielded a scaling component close to unity for FFM, meaning the ratio standard method for normalising $\dot{V}O_{2\text{max}}$ was appropriate. This traditional method involves expressing physiological measurements as a ratio standard, adjusting the function for body size (i.e., expressing $\dot{V}O_{2\text{max}}$ relative to body mass).

### 3.20.2 Determination of the GET

Purpose built software was used to identify the GET (LabVIEW, National Instruments, Newbury, UK; Figure 3.5). The first 60 s of data following the onset of the ramp forcing function and data following the respiratory compensation point (RCP) were removed. A plot of $\dot{V}CO_2$ against $\dot{V}O_2$ was then produced (Figure 3.5a). The GET was identified as the intersection point between the two regression lines, i.e. a disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$, in accordance with the V-slope method (Beaver et al., 1986). The GET was then visually confirmed using the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$ of observation of the $P_{ETCO_2}$ response (Wasserman et al., 2004; see Figure 3.5b). The GET was independently identified by two experienced researchers and expressed both in absolute terms and as a percentage of $\dot{V}O_{2\text{max}}$. As mentioned earlier, both of these methods are appropriate for use in children with CF (Visschers et al., 2015).
3.20.3 Determination of the $\dot{V}O_2$ gain

The functional $\dot{V}O_2$ gain ($\Delta\dot{V}O_2/\Delta WR$) was determined through regression of the linear portion of the $\dot{V}O_2$ response versus power output. The linear portion of the

Figure 3.5. Example dataset from a paediatric patient with cystic fibrosis in the purpose built software (LabVIEW, National Instruments, Newbury, UK) used to identify the gas exchange threshold (GET). Following averaging of the data and removal of the respiratory compensation point, Panel A shows the inflection point identified by the software in a plot of expired carbon dioxide ($\dot{V}CO_2$) against pulmonary oxygen uptake ($\dot{V}O_2$). This intersection point between the two regression lines marks a disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$, in accordance with the $V$-slope method (Beaver et al., 1986). Panel B then demonstrates the subsequent identification and confirmation of this point using the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$ (Wasserman et al., 2004).
test was determined by removing the first 120 s and final 120-180 s of the test, depending on test duration. The decision was verified by visual observation of the data by two trained researchers.

### 3.20.4 Determination of the $\dot{V}O_2$ MRT

The $\dot{V}O_2$ MRT was determined by backward extrapolating the linear function of the $\dot{V}O_2$ response as a function of time to the point where this line intersected baseline $\dot{V}O_2$ (Figure 3.6).

![MRT 55 s](image)

**Figure 3.6.** Example identification of the pulmonary oxygen uptake ($\dot{V}O_2$) mean response time (MRT) in a paediatric patient with cystic fibrosis. In this case, the derived MRT was 55 s. The black solid line represents the backward extrapolation of the regression line fitted through the linear portion of the $\dot{V}O_2$ response.

### 3.21 Determination of the OUES

The OUES for the entire exercise duration (OUES$_{100}$) and up to the GET (OUES$_{GET}$) were derived from the slope of the linear function between $\dot{V}O_2$ (mL·min$^{-1}$) and log $\dot{V}_E$ (L·min$^{-1}$) (Baba et al., 1996).
3.22 Determination of ventilatory drive

The $\dot{V}_E/\dot{V}CO_2$–slope (ventilatory drive) was calculated using linear regression of the $\Delta\dot{V}_E/\Delta\dot{V}CO_2$ response during the entire ramp test (Tabet et al., 2003).

3.23 Determination of the $O_2$ pulse

The $O_2$ pulse ($\dot{V}O_2/HR_{peak}$) was determined using the following standard equation:

$$\dot{V}O_2/HR_{peak} (\text{mL} \cdot \text{beat}^{-1}) = \dot{V}O_2 (\text{L} \cdot \text{min}^{-1}) \times 1000 \text{ mL} / \text{ HR (beats} \cdot \text{min}^{-1})$$

Equation 3.9

3.24 Determination of the arterial-venous $O_2$ content difference

The arterial-venous $O_2$ content difference $[C(a-v)O_2]$ was estimated via rearrangement of the Fick equation (Equation 3.10):

$$C(a-v)O_2 = \dot{V}O_2 / \dot{Q}$$

Equation 3.10

3.25 Analysis of muscle oxygenation response during ramp incremental exercise

For studies 3 and 4, muscle [HHb] data were interpolated to 1 s intervals and averaged to 15 s time bins for the entire test duration. Responses were then normalised to the total amplitude of the response ($\% \Delta[HHb]$), such that 0% represented steady-state values observed during the period of baseline cycling and 100% represented the highest average (i.e., $\Delta[HHb]_{peak}$) (Boone et al., 2009; Gravelle et al., 2012). The response was then expressed as a function of absolute and relative $W_{peak}$ and $\dot{V}O_{2max}$. Although other research groups report a bilinear fit of [HHb] data during ramp incremental exercise (Spencer et al., 2012), preliminary statistical analyses (GraphPad Prism; GraphPad Software,
San Diego, CA) revealed that a sigmoid function provided a superior fit to the [HHb] response when compared with bilinear or hyperbolic curve fitting procedures. The $\Delta$[HHb] response to incremental ramp cycling exercise was therefore described using a sigmoidal model (Equation 3.11) in line with previous studies (Boone et al., 2009; Ferreira et al., 2007; McNarry et al., 2011), as follows:

$$y = f_0 + \frac{A}{1 + e^{-(c+dx)}}$$  

Equation 3.11

where $f_0$ represents baseline [HHb], $A$ represents the amplitude of the response, $d$ represents the slope of the sigmoid, $c$ represents the constant that is dependent on $d$, and $c/d$ represents the value corresponding to 50% of the total amplitude, respectively.

### 3.26 Analysis of $\dot{V}O_2$ kinetic response during CWR exercise

In studies 5 and 6, breath-by-breath changes in $\dot{V}O_2$ for each exercise bout were analysed using methodology previously utilised in paediatric studies (e.g. Barker et al., 2014; Barker et al., 2010; Breese et al., 2012; Fawkner & Armstrong, 2004a). Data for each exercise transition was initially examined for fluctuations that were > 3 standard deviations (SD) from a local moving mean and any errant breaths were excluded. Subsequently, the four repeat transitions for both MOD and VH intensity conditions were linearly interpolated to 1 s data, time aligned to the onset of exercise (i.e., $t = 0$ s) and ensemble averaged to improve the signal-to-noise ratio (Figure 3.7).
The 1 s averaged $\dot{V}O_2$ response for the MOD and VH intensity conditions for each participant were then baseline corrected, by subtracting the mean $\dot{V}O_2$ between -60 and -5 s from the exercise response. To remove the influence of the cardiodynamic phase (phase I) on the analysis of the $\dot{V}O_2$ response, following visual inspection of $\dot{V}O_2$ versus time, the first $21 \pm 3$ s and $17 \pm 4$ s of

**Figure 3.7.** Example profile of the pulmonary oxygen uptake ($\dot{V}O_2$) kinetic response of a paediatric patient with cystic fibrosis. Panels A and B represent the $\dot{V}O_2$ kinetic response to 4 averaged transitions during moderate intensity exercise. Note that averaging to 5-s intervals, as demonstrated in Panel B, improves the signal-to-noise ratio from Panel A and, therefore, the confidence associated with the derived kinetic parameters. The red line denotes the monoexponential fit and the black trace denotes the residuals of the response.
the MOD data and the first $19 \pm 5$ s and $16 \pm 2$ s for the VH response were omitted in CF and healthy participants, respectively. For moderate intensity exercise, the phase II portion of the $\dot{V}O_2$ response (5 s averaged data) was then characterised using Equation 3.12 (GraphPad Prism; GraphPad Software, San Diego, CA):

$$\dot{V}O_2(t) = \Delta \dot{V}O_2 A \cdot (1 - e^{-(t-TD)/\tau})$$

Equation 3.12

where $\dot{V}O_2(t)$, $\Delta \dot{V}O_2 A$, $TD$, and $\tau$ represent the value of $\dot{V}O_2$ at a given time ($t$), the amplitude change in $\dot{V}O_2$ from baseline to its asymptote, time delay, and the time constant of the response, respectively.

For very heavy intensity exercise, it was necessary to account for the $\dot{V}O_2$ slow-component. In line with previous recommendations (Rossiter et al., 2002), the fitting window was constrained to exclude all data following the visually determined onset of the slow-component, the onset of which was determined using a purpose written computer program (LabVIEW, version 6.1; National Instruments, Newbury, UK); see Figure 3.8. A non-linear equation was initially fit up to the first 60 s of exercise and then increased iteratively by 1 s until it encompassed the entire exercise bout (Rossiter et al., 2011). The estimated $\dot{V}O_2 \tau$ for each fitting window was plotted against time to visually identify the point at which the $\dot{V}O_2$ slow-component began, which was defined as the point at which a plateau in the estimated phase II $\tau$ was followed by a consistent and progressive increase (Fawkner & Armstrong, 2004b; Rossiter et al., 2001), as shown in Figure 3.8. The phase II parameter estimates from Equation 3.12 were then resolved by least-squares nonlinear regression (GraphPad Prism; GraphPad Software, San Diego, CA).
The magnitude of the $\dot{V}O_2$ slow-component was determined as the difference between the mean of the final 30 s of VH intensity exercise and the phase II asymptote. The $\dot{V}O_2$ slow-component amplitude was expressed both in absolute terms and relative to end-exercise $\dot{V}O_2$. To provide a description of the overall kinetic response for both intensities of exercise, the MRT was also derived, by constraining the $TD$ in Equation 3.12 to 0 s and fitting from exercise onset to 6 min. The functional gain of the phase II $\dot{V}O_2$ response was also determined for both MOD and VH intensities, by dividing the asymptotic phase II $\dot{V}O_2$ amplitude by the change in work rate above baseline. The end-exercise $\dot{V}O_2$ gain was calculated in a similar manner.

**Figure 3.8.** Example visual identification of the $\dot{V}O_2$ slow-component during very heavy intensity cycling exercise in a paediatric patient with cystic fibrosis. The vertical green line denotes the onset of the slow component, defined as the point at which a plateau in the estimated phase II $\tau$ was followed by a consistent and progressive increase (~120 seconds in this case).
An example profile for the pulmonary $\dot{V}O_2$ response of a child with CF during MOD and VH intensity cycling is provided in Figure 3.9:

**Figure 3.9.** Example profile of the pulmonary oxygen uptake ($\dot{V}O_2$) kinetic response of a female paediatric patient (13.8 y; 1.47 m; 41.5 kg; $\Delta F508/2186delA$) with cystic fibrosis during moderate (●) and very heavy (○) intensity cycling exercise. The vertical dotted lines represent the different phases (I, II and III) of the $\dot{V}O_2$ kinetic response. Notice that during moderate intensity exercise, following phase II (~120 s), the $\dot{V}O_2$ response has attained a steady-state. In contrast, during very heavy exercise, following phase II (~120 s), a steady-state is not achieved due to the emergence of a $\dot{V}O_2$ slow-component, which increases the $O_2$ cost of exercise towards $\dot{V}O_{2\text{max}}$, which in this patient was 1.68 L·min$^{-1}$. Figure taken from Williams et al. (2014).

**3.27 Analysis of muscle oxygenation during CWR exercise**

As with ramp incremental exercise, NIRS data was collected at 10 Hz during CWR exercise. Data were subsequently interpolated to 1 s intervals and expressed as a change, in arbitrary units (a.u.), from baseline. Subsequently, [HHb] profiles were averaged into 5 s time bins, time aligned to the onset of the exercise, and ensemble averaged to yield a single response for both exercise
intensities. The dynamics (fast and slow-component phases) of the [HHb] response for moderate and very heavy intensity exercise were then modelled in a similar manner to the methods outlined above for $\dot{V}O_2$, with slight modification. Since the [HHb] response increases with exponential-like properties following a short delay, data were modelled in line with the methods outlined by DeLorey et al. (2003). The time at which the exponential-like increase in [HHb] occurred was determined as a 1 SD increase above baseline and, following the removal of the data preceding this exponential-like increase, Equation 3.12 was applied to resolve the [HHb] $TD$ and $\tau$. For both MOD and VH, the presence of a slow-component was identified using the methods outlined above for $\dot{V}O_2$. When a slow-component was present, the fitting window was constrained to the time of onset. The overall [HHb] MRT was also determined as the sum of the $TD$ and $\tau$ over the fast component of the response.

3.28 Analysis of the cardiac response during CWR exercise

In studies 5 and 6, beat-by-beat changes in HR, SV and $\dot{Q}$ were linearly interpolated on a second-by-second basis, time aligned to the onset of exercise and ensemble averaged to yield a single averaged file for each participant for both moderate and very heavy intensity exercise. Additionally, the $C_{(a,\overline{v})}O_2$ was estimated via rearrangement of the Fick equation [$C_{(a,\overline{v})}O_2 = \dot{V}O_2 / \dot{Q}$] and averaged as above. In line with existing recommendations (Dewey et al., 2008), SV and $\dot{Q}$ were normalised to FFM to determine the SVI and CI. Initial log-linear allometric modelling deemed that the ratio standard method was an appropriate method of scaling for cardiac parameters, since the derived scaling component was close to unity for FFM. To provide a description of the changes in HR, SV, $\dot{Q}$ and $C_{(a,\overline{v})}O_2$ during both MOD and VH intensity exercise, data were averaged
to 30 s time bins. To provide an index of muscle O$_2$ availability, the increase in $\dot{Q}$ relative to $\dot{V}$O$_2$ ($\dot{Q}/\dot{V}$O$_2$) was also determined at 30 s intervals during MOD and VH intensity exercise.
CHAPTER FOUR

A Protocol to Determine Valid $\dot{V}O_{2\text{max}}$ in Young Cystic Fibrosis Patients

This experimental study has been disseminated as follows:


**4.1 Introduction**

Exercise testing is a valuable investigative tool in young people with chronic diseases, such as CF. Although lung function traditionally measures disease severity, it cannot accurately predict exercise capacity (Nixon *et al.*, 1992; Stevens *et al.*, 2010). Current standards for CF management therefore recommend at least annual exercise testing (Day *et al.*, 2003), however current provision is unsatisfactory (Barker *et al.*, 2004; Stevens *et al.* 2010). CPET, incorporating measurement of pulmonary gas exchange, provides the most
precise measure of exercise capacity (\(\dot{V}O_{2\text{max}}\)) in mild-to-moderate CF. Furthermore, \(\dot{V}O_{2\text{max}}\) holds suggested value in prognostic stratification of patients (Nixon et al., 1992).

\(\dot{V}O_{2\text{max}}\) represents the integrated capacity of the pulmonary, cardiovascular and muscular systems to uptake and utilise O\(_2\) during intense exercise and is traditionally identified by a \(\dot{V}O_2\) plateau upon exhaustion despite an increasing work rate (Rowland et al., 1993). Since only a minority of young people display this response (Astorino et al., 2009; Barker et al., 2004; Rowland et al., 1993), the term \(\dot{V}O_2\text{peak}\) is commonly used, defined as the highest \(\dot{V}O_2\) attained during an exhaustive test (Barker et al., 2011). To verify a ‘maximal effort’, reliance therefore falls upon secondary criteria, encompassing subjective indicators of effort (sweating, facial flushing, hyperpnoea) and objective secondary criteria (HR, RER and/or La\(_\text{B}\)). Unfortunately, most previous studies in CF have not specified their verification criteria. In those which have, there is some disparity, with objective criteria including RER > 1.00 (Gulmans et al., 1997; Klijn et al., 2003) and > 1.10 (Nixon et al., 1992) and HR criteria of 180 b·min\(^{-1}\) (Gulmans et al., 1997; Klijn et al., 2003) and 95% of age-predicted maximum (Stevens et al., 2009; Stevens et al., 2011). Such criteria are dependent on arbitrary values which often underestimate ‘true’ \(\dot{V}O_{2\text{max}}\) and have thus been deemed invalid for healthy children (Barker et al., 2011) and young spina bifida patients (de Groot et al., 2009). It is conceivable that secondary criteria are equally unsuitable for young CF patients. Documenting a valid \(\dot{V}O_{2\text{max}}\) is crucial to the clinical utility of CPET within CF. Accepting submaximal values could distort clinical interpretation and underestimate patients’ prognosis, influencing decisions regarding clinical intervention and/or exercise prescription.
It is important that new conceptual advances within exercise physiology continue to be incorporated within clinical practice. A procedure termed the ‘verification phase’, where CPET is followed by an individualised supramaximal ‘step’ test to exhaustion, can ensure the valid determination of $\dot{V}O_{2\text{max}}$ in healthy children (Barker et al., 2011), sedentary adults (Astorino et al., 2009), active middle-aged and older adults (Dalleck et al., 2012; Rossiter et al., 2006) and adolescent spina bifida patients (de Groot et al., 2009). Supramaximal exercise denotes exercise above the highest peak power achieved during a preceding exhaustive CPET. While $S_{\text{max}}$ has been safely implemented in paediatric spina bifida patients (de Groot et al., 2009) and patients with chronic heart failure (Bowen et al., 2011), the utility, safety and feasibility for young CF patients is unknown. This study aimed to establish the validity of CPET derived $\dot{V}O_{2\text{max}}$ and the utility of $S_{\text{max}}$ to provide a robust measure of $\dot{V}O_{2\text{max}}$ in young CF patients. We hypothesised that: 1) traditional verification criteria would significantly underestimate ‘true’ $\dot{V}O_{2\text{max}}$; and 2) the $\dot{V}O_{2\text{max}}$ obtained during a traditional incremental ramp test would not significantly differ to that from a subsequent $S_{\text{max}}$ verification test, thus providing a valid measure of $\dot{V}O_{2\text{max}}$.

4.2 Methods

4.3.1 Participants, anthropometry and pulmonary function

Fourteen young patients (Table 4.1) with mild-to-moderate CF, regularly partaking in physical activity as is suggested by clinical disease management guidelines, participated in this study. Inclusion criteria comprised a diagnosis of CF based on clinical features, an abnormal sweat test (sweat Cl$^- > 60 \text{ mmol} \cdot \text{L}^{-1}$
and genotyping. Stable pulmonary function within 10% of best in the preceding 6 months and no symptomatic increase or weight loss within 2 weeks was mandatory. Unstable non-pulmonary comorbidities and/or acute infection warranted exclusion. Disease severity was graded using the SS as part of patients’ annual clinical review (Table 4.1). Ethics approval was granted by the South West NHS Research Ethics Committee and informed written consent and assent obtained from parents/guardians and patients, respectively. Body mass was measured to the nearest 0.1 kg and stature to the nearest 0.01 m. Pulmonary function, assessed via spirometry (MicroMedical MicroLoop 3535), determined FVC and FEV₁ (Table 4.1). Pubertal staging was self-assessed (boys ≥ 10 y and girls ≥ 8 y) according to pubic hair classification (Tanner et al., 1962) (Table 4.1) following testing.

4.3.2 Exercise protocol

Exercise was performed on cycle ergometers [Lode Excalibur, Groningen, The Netherlands; Lode (paediatric)]. Following 3 min warm-up (20 W), patients completed an incremental ramp test, whereby resistance increased at a predetermined rate (10-25 W·min⁻¹), ensuring ~ 8-12 min test durations. Patients maintained ~ 70-80 rpm until volitional exhaustion, defined as a drop in cadence > 10 rpm for 5 consecutive seconds despite strong verbal encouragement. $W_{\text{peak}}$ was recorded upon exhaustion. Five minutes warm-down (20 W) and 10 min seated recovery followed. $S_{\text{max}}$ was subsequently undertaken, whereby 3 min cycling (20 W) preceded a ‘step’ transition to a CWR equivalent to 110% of $W_{\text{peak}}$. This work rate was maintained until exhaustion, followed by 5 min recovery (20 W).
TABLES

Table 4.1. Patients’ baseline anthropometric and clinical data.
<table>
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<th>Pubertal maturity (y)</th>
<th>Age (y)</th>
<th>Stature (m)</th>
<th>Body mass (kg)</th>
<th>BMI (kg·m⁻²)</th>
<th>CFTR genotype</th>
<th>C. PSA</th>
<th>SS</th>
<th>Northern scoreᵇ</th>
<th>FVC [% predicted (L)]</th>
<th>FEV₁ [% predicted (L)]</th>
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<td><strong>(4.31)</strong></td>
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Values are means ± SD, with the range also displayed where suitable, unless otherwise stated. BMI, body mass index; CFTR, cystic fibrosis transmembrane conductance regulator; C. PSA, chronic Pseudomonas aeruginosa infection; I, intermittent; F, free; C, chronic; N, never; SS, Shwachman score - scoring 4 separate aspects of the disease profile: general activity; physical examination; nutritional status; and chest radiographic findings, using the most recent clinical review information. A total of 100 points represents a perfect score of health; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s.ᵃ According to Leeds Criteria, “chronic”, >50% of the preceding 12 months were P. aeruginosa culture positive; “intermittent”, ≤50% of the preceding 12 months were P. aeruginosa culture positive; “never”, no growth of P. aeruginosa for the previous 12 months, having previously been P. aeruginosa culture positive; “free”, P. aeruginosa has never been cultured.ᵇ Provides evidence of radiographic chest findings. Maximum score is 20, with 20 being the most severe.
4.3.3 Exercise test measurements

Gas analysers were calibrated using gases of known concentration, and the turbine volume transducer using a 3 L calibration syringe (Hans Rudolph, Kansas City, MO). Breath-by-breath pulmonary gas exchange and ventilation (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany; Metasoft v.3.9.7) were measured using a face mask and, following 1 s interpolation, averaged to 15 s time bins which was subsequently used for all parameters. When appropriately calibrated the accuracy of measuring volume and gas fractions are 2% and 0.1%, respectively. The highest 15 s stationary average represented $\dot{\text{V}}\text{O}_2\text{peak}$.

HR was determined at 5 s intervals (PhysioFlow, PF-05, Manatec Biomedical, Paris, France) and $\text{HR}_\text{peak}$ taken as the highest 15 s mean value. $\text{SpO}_2$ was determined on a beat-by-beat basis via pulse oximetry (Avant 4000, NONIN Medical Inc., USA). A fingertip capillary blood sample (~5 µL) was taken within 30 s of exhaustion following the ramp and analysed for $\text{La}_{[\text{B}]}$ (Lactate Pro, Arkray, Japan). Subjective ratings of RPE and RPD were recorded upon exhaustion using the P-CERT (Lamb et al., 2000) and the CR-10 scale (Borg, 1982), respectively.

4.3.4 Data analysis

The presence of a $\dot{\text{V}}\text{O}_2$ plateau was determined using methodology more comprehensively described elsewhere (Chapter 3; Day et al., 2003; Rossiter et al., 2006). Briefly, analysis requires a linear regression over the 'linear' portion of the $\dot{\text{V}}\text{O}_2$ response. The $\dot{\text{V}}\text{O}_2$ profile at exhaustion was subsequently characterised by extrapolating this linear regression function to exhaustion and isolating the final 60 s of data to examine the residuals against the extrapolated line. A negative residual
indicated a deceleration in $\dot{V}O_2$ against power output and was considered a ‘plateau’ when the magnitude of the residuals was ≥ 5% of the projected $\dot{V}O_2$ (i.e. $\dot{V}O_2$ was ≤ 95% of the projected $\dot{V}O_2$). A positive residual ≥ 5% of the projected $\dot{V}O_2$ represented acceleration and positive or negative residuals < 5% of the $W_{peak}$ projected $\dot{V}O_2$ were categorised as a linear responses (Ruf et al., 2010). Secondary verification criteria ($\dot{V}O_2$ at an RER of 1.00 (Gulmans et al., 1997; Klijn et al., 2003) and 1.10 (Moser et al., 2000), a HR of 180 b∙min$^{-1}$ (Gulmans et al., 1997; Klijn et al., 2003) and 95% age-predicted HR$_{max}$ (Stevens et al., 2009; Stevens et al., 2011)) were selected within the current study based on their use within CF patients, whilst La$_{at[B]}$ ≥ 6 mmol·L$^{-1}$ is often utilised in paediatric studies.

### 4.3.5 Statistics

Data are expressed as means and standard deviations unless otherwise stated and significance set at $p < 0.05$. Paired samples $t$-tests determined mean differences between ramp and $S_{max}$ $\dot{V}O_{2peak}$. Linear regression and Bland and Altman limits of agreement analysis (Bland & Altman, 1999) (mean bias and 95% confidence limits [95% CL]) examined the agreement between ramp and $S_{max}$ $\dot{V}O_{2peak}$. A greater than 9% increase was considered a ‘meaningful’ change between ramp and $S_{max}$ derived $\dot{V}O_{2peak}$ (see section 2.4.5 for further details). This value is considered the typical within-subject short-term variation of $\dot{V}O_{2max}$ in paediatric CF patients, established using unpublished data from within our laboratory. Analyses were performed using SPSS v.18.0 (Chicago, Illinois, USA) and GraphPad Prism (GraphPad Software Inc., San Diego, California, USA).
4.3 Results

Mean ramp test duration was 9 min 27 s ± 3 min 16 s, resulting in a $W_{peak}$ of $174 ± 84$ W. Mean $\dot{V}O_2_{peak}$ was $1.83 ± 0.78$ L·min$^{-1}$ (34.23 ± 6.57 mL·kg$^{-1}$·min$^{-1}$). Ramp $\dot{V}O_2_{peak}$ was not significantly different to the $\dot{V}O_2_{peak}$ predicted by the linear extrapolation of the $\dot{V}O_2$-power output relationship ($1.83 ± 0.84$ L·min$^{-1}$; $p = 0.99$). Mean goodness of fit ($R^2$) for the linear function was $0.84 ± 0.19$. Analysis of patients’ $\dot{V}O_2$-power output profiles revealed a single plateau upon exhaustion, with 13 patients characterised by a linear $\dot{V}O_2$ response (Table 4.2). The mean ‘gain’ ($\Delta \dot{V}O_2/\Delta WR$) of patients’ $\dot{V}O_2$ response to the ramp test was $7.81 ± 1.57$ mL·min$^{-1}$·W$^{-1}$.

All patients satisfied the RER > 1.00 and >1.10 criteria (Table 4.2). However, the $\dot{V}O_2$ at a RER of 1.00 ($0.99 ± 0.47$ L·min$^{-1}$) was lower than that recorded at exhaustion ($1.83 ± 0.78$ L·min$^{-1}$; $p < 0.001$), representing only 54% of $\dot{V}O_2_{peak}$. Similarly, the $\dot{V}O_2$ at RER of 1.10 ($1.36 ± 0.59$ L·min$^{-1}$; $p < 0.001$) were lower than $\dot{V}O_2$ upon exhaustion, representing only 74% of $\dot{V}O_2_{peak}$. Bland and Altman analysis demonstrated the RER of 1.00 and 1.10 criteria to underestimate $\dot{V}O_{2max}$ by a mean bias of $-1.10$ L·min$^{-1}$ (95% CL: -1.68 to -0.01 L·min$^{-1}$, Figure 4.1a) and $-0.47$ L·min$^{-1}$ (95% CL: -1.02 to 0.08 L·min$^{-1}$, Figure 4.1b), respectively. Mean $La_{[B]}$ following the ramp was $9.5 ± 13.1$ mmol·L$^{-1}$ ($n = 13$). Ten patients satisfied the ≥ 6 mmol·L$^{-1}$ criteria (Table 4.2).
### Table 4.2. Ramp test responses in relation to traditional verification criteria.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (y)</th>
<th>Ramp</th>
<th>$\bar{V}_O_2$ peak (L·min$^{-1}$)</th>
<th>$\bar{V}_O_2$ plateau (L·min$^{-1}$)</th>
<th>$\bar{V}_O_2$ at RER &gt;1.00 (L·min$^{-1}$)</th>
<th>$\bar{V}_O_2$ at RER &gt;1.10 (L·min$^{-1}$)</th>
<th>$\bar{V}_O_2$ at 180 b·min$^{-1}$ (L·min$^{-1}$)</th>
<th>$\bar{V}_O_2$ at 95% age-predicted HR (L·min$^{-1}$)</th>
<th>$La_{[B]} \geq 6$ mmol·L$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>13.4</td>
<td>No</td>
<td>2.32</td>
<td>No</td>
<td>0.96</td>
<td>1.58</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>16.7</td>
<td>Yes</td>
<td>3.78</td>
<td>Yes</td>
<td>2.30</td>
<td>3.09</td>
<td>2.76</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>13.4</td>
<td>No</td>
<td>2.05</td>
<td>No</td>
<td>0.79</td>
<td>1.23</td>
<td>1.94</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>7.6</td>
<td>No</td>
<td>0.84</td>
<td>No</td>
<td>0.47</td>
<td>0.73</td>
<td>N/A</td>
<td>N/A</td>
<td>DNC</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>9.9</td>
<td>No</td>
<td>1.39</td>
<td>No</td>
<td>0.73</td>
<td>1.08</td>
<td>1.39</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>11.2</td>
<td>No</td>
<td>1.74</td>
<td>No</td>
<td>1.12</td>
<td>1.42</td>
<td>1.50</td>
<td>1.50</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>13.9</td>
<td>No</td>
<td>2.16</td>
<td>No</td>
<td>1.25</td>
<td>1.53</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>12.2</td>
<td>No</td>
<td>1.18</td>
<td>No</td>
<td>0.83</td>
<td>1.06</td>
<td>1.11</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>11.1</td>
<td>No</td>
<td>1.03</td>
<td>No</td>
<td>0.71</td>
<td>0.94</td>
<td>N/A</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>16.1</td>
<td>No</td>
<td>1.72</td>
<td>No</td>
<td>0.96</td>
<td>1.45</td>
<td>1.45</td>
<td>1.37</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>14.9</td>
<td>No</td>
<td>2.65</td>
<td>No</td>
<td>1.50</td>
<td>1.89</td>
<td>2.40</td>
<td>2.28</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>7.8</td>
<td>No</td>
<td>1.11</td>
<td>No</td>
<td>0.83</td>
<td>0.79</td>
<td>1.02</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>16.6</td>
<td>No</td>
<td>2.16</td>
<td>No</td>
<td>0.75</td>
<td>1.32</td>
<td>2.16</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>18.4</td>
<td>No</td>
<td>1.49</td>
<td>No</td>
<td>0.71</td>
<td>0.96</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
</tr>
</tbody>
</table>

DNC, did not consent to blood sampling for assessment of end-exercise blood lactate concentration ($La_{[B]}$); N/A, not achieved; -, Loss of PhysioFlow data. $\bar{V}_O_2$ peak, peak oxygen uptake; RER, respiratory exchange ratio; HR, heart rate ($n = 12$); $La_{[B]}$, blood lactate concentration; ramp; incremental ramp test.
Figure 4.1. Bland and Altman plots for the secondary criteria typically used during CPET of young CF patients. Plots show the mean bias (floating dotted line) and 95% confidence limits (floating dashed lines) for the oxygen uptake (\(\dot{V}O_2\)) recorded at an RER of \(\geq 1.00\) (A), an RER of \(\geq 1.10\) and (B) a heart rate of 180 beats·min\(^{-1}\) (C) compared with the actual \(\dot{V}O_2\) recorded at exhaustion from the traditional ramp test in absolute terms (top row) and as a percentage of the difference (bottom row).
Due to data loss, HR is presented for 12 patients with a HR_{peak} of 188 ± 12 b·min^{-1}. Nine patients satisfied achieved 180 b·min^{-1} (Table 4.2), whilst 3 attained 95% age-predicted maximum (equating to 196 ± 2 b·min^{-1}; Table 4.2). In those patients satisfying the HR criteria, \dot{V}O_2 at 180 b·min^{-1} (1.75 ± 0.60 L·min^{-1}) and 95% of their age-predicted maximum (1.72 ± 0.50 L·min^{-1}) was lower than that recorded upon exhaustion (1.98 ± 0.84 and 2.04 ± 0.53 L·min^{-1}, respectively), representing 88% and 84% of \dot{V}O_{2peak}, respectively. Bland and Altman analysis revealed 180 b·min^{-1} and 95% age-predicted maximum criteria to underestimate \dot{V}O_{2max} by a mean bias of -0.23 L·min^{-1} (95% CL: -0.85 to 0.39 L·min^{-1}; Figure 4.1c) and -0.32 L·min^{-1} (95% CL: -0.46 to -0.18 L·min^{-1}), respectively.

Mean S_{max} duration was 1 min 23 s ± 0 min 20 s and elicited a similar \dot{V}O_{2peak} to the ramp, despite exercising at a higher power output (191 ± 93 W, Table 4.3). Bland Altman analysis for ramp and S_{max} \dot{V}O_{2peak} revealed a mean bias of -0.00 L·min^{-1} (95% CL: -0.46 to 0.46 L·min^{-1}) or 1.0% (95% CL: -22.5% to 24.5%). No significant differences were observed for HR_{peak}, end-exercise SpO_2, RPE or RPD during ramp and S_{max} testing (Table 4.4).

S_{max} increased \dot{V}O_{2peak} above ramp \dot{V}O_{2peak} in 7 patients (50%) (Table 4.4; Figure 4.2), 3 (21%) of which were deemed clinically important rises (i.e. > 9 % change). Based on this criterion, S_{max} confirmed a valid \dot{V}O_{2max} in 11/14 (79%) patients and identified 3 (21%) cases where a ‘true’ \dot{V}O_{2max} was not obtained, with an average \dot{V}O_2 increase of 20.3 ± 15.7% or 0.33 ± 0.21 L·min^{-1}. No significant relationship existed between S_{max} duration and the difference between the ramp and S_{max} \dot{V}O_{2peak} (r = 0.29; p = 0.32).
Table 4.3. Decision to accept $\dot{V}O_{2\text{peak}}$ using a combined ramp and $S_{\text{max}}$ exercise test.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Ramp $\dot{V}O_{2\text{peak}}$ (L·min$^{-1}$)</th>
<th>$S_{\text{max}}$ $\dot{V}O_{2\text{peak}}$ (L·min$^{-1}$)</th>
<th>$\Delta$ Change (L·min$^{-1}$)</th>
<th>% Change</th>
<th>True $\dot{V}O_{2\text{max}}$ obtained?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (M)</td>
<td>13.4</td>
<td>2.32</td>
<td>2.01</td>
<td>-0.31</td>
<td>-13.4</td>
<td>Yes (ramp)</td>
</tr>
<tr>
<td>2 (M)</td>
<td>16.7</td>
<td>3.78</td>
<td>3.35</td>
<td>-0.43</td>
<td>-8.5</td>
<td>Yes (ramp)</td>
</tr>
<tr>
<td>3 (M)</td>
<td>13.4</td>
<td>2.05</td>
<td>2.31</td>
<td>+0.26</td>
<td>+12.7</td>
<td>No</td>
</tr>
<tr>
<td>4 (F)</td>
<td>7.6</td>
<td>0.84</td>
<td>0.89</td>
<td>+0.05</td>
<td>+6.0</td>
<td>Yes ($S_{\text{max}}$)</td>
</tr>
<tr>
<td>5 (M)</td>
<td>9.9</td>
<td>1.39</td>
<td>1.41</td>
<td>+0.02</td>
<td>+1.4</td>
<td>Yes ($S_{\text{max}}$)</td>
</tr>
<tr>
<td>6 (M)</td>
<td>11.2</td>
<td>1.74</td>
<td>1.57</td>
<td>-0.17</td>
<td>-9.8</td>
<td>Yes (ramp)</td>
</tr>
<tr>
<td>7 (M)</td>
<td>13.9</td>
<td>2.16</td>
<td>1.99</td>
<td>-0.17</td>
<td>-7.9</td>
<td>Yes (ramp)</td>
</tr>
<tr>
<td>8 (F)</td>
<td>12.2</td>
<td>1.18</td>
<td>1.11</td>
<td>-0.07</td>
<td>-5.9</td>
<td>Yes (ramp)</td>
</tr>
<tr>
<td>9 (M)</td>
<td>11.1</td>
<td>1.03</td>
<td>1.10</td>
<td>+0.07</td>
<td>+6.8</td>
<td>Yes ($S_{\text{max}}$)</td>
</tr>
<tr>
<td>10 (M)</td>
<td>16.1</td>
<td>1.72</td>
<td>1.89</td>
<td>+0.17</td>
<td>+9.9</td>
<td>No</td>
</tr>
<tr>
<td>11 (M)</td>
<td>14.9</td>
<td>2.65</td>
<td>2.47</td>
<td>-0.18</td>
<td>-6.8</td>
<td>Yes (ramp)</td>
</tr>
<tr>
<td>12 (M)</td>
<td>7.8</td>
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<td>1.11</td>
<td>0</td>
<td>0</td>
<td>Yes (either)</td>
</tr>
<tr>
<td>13 (F)</td>
<td>16.6</td>
<td>2.16</td>
<td>2.20</td>
<td>+0.04</td>
<td>+1.9</td>
<td>Yes ($S_{\text{max}}$)</td>
</tr>
<tr>
<td>14 (F)</td>
<td>18.4</td>
<td>1.49</td>
<td>2.06</td>
<td>+0.57</td>
<td>+38.3</td>
<td>No</td>
</tr>
</tbody>
</table>

$\dot{V}O_{2\text{max}}$, maximal oxygen uptake; ramp; incremental ramp test; $S_{\text{max}}$, supramaximal exercise test.
Table 4.4. Peak physiological responses during the ramp and \(S_{\text{max}}\) tests.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ramp</th>
<th>(S_{\text{max}})</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\dot{V}O_2) (\text{peak (L\cdot min^{-1})})</td>
<td>1.83 ± 0.78 [0.84-3.77]</td>
<td>1.83 ± 0.69 [0.89-3.46]</td>
<td>0.98</td>
</tr>
<tr>
<td>(\dot{V}O_2) (\text{peak (mL\cdot kg^{-1}\cdot min^{-1})})</td>
<td>34.23 ± 6.57 [25.23-47.54]</td>
<td>34.46 ± 5.65 [23.32-44.42]</td>
<td>0.83</td>
</tr>
<tr>
<td>RER (\text{peak})</td>
<td>1.29 ± 0.10 [1.15-1.49]</td>
<td>1.23 ± 0.12 [0.99-1.34]</td>
<td>0.07</td>
</tr>
<tr>
<td>HR (\text{peak (b\cdot min^{-1}, n=12)})</td>
<td>188 ± 12 [170-208]</td>
<td>183 ± 13 [158-201]</td>
<td>0.08</td>
</tr>
<tr>
<td>(\text{SpO}_2) (%)</td>
<td>96 ± 2 [90-99]</td>
<td>95 ± 3 [90-98]</td>
<td>0.19</td>
</tr>
<tr>
<td>RPE</td>
<td>8 ± 2 [5-10]</td>
<td>8 ± 3 [4-10]</td>
<td>0.37</td>
</tr>
<tr>
<td>RPD</td>
<td>6 ± 2 [2-10]</td>
<td>7 ± 3 [4-10]</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Values are means ± SD, with the range also displayed in parenthesis unless otherwise stated. \(\dot{V}O_2\) \(\text{peak}, \) peak oxygen uptake; RER \(\text{peak}, \) peak respiratory exchange ratio; HR \(\text{peak}, \) peak heart rate; \(\text{SpO}_2, \) end-exercise arterial oxygen saturation; RPE, end-exercise rating of perceived exertion; RPD, end-exercise rating of perceived dyspnoea; ramp; incremental ramp test; \(S_{\text{max}}, \) supramaximal exercise test.
Figure 4.2. The agreement between the peak oxygen uptake (\(\dot{V}O_{2\text{peak}}\)) recorded at exhaustion during ramp and \(S_{\text{max}}\) testing. Plots show the mean bias (floating dotted line) and 95% confidence limits (floating dashed lines) for the \(\dot{V}O_2\) recorded at exhaustion during ramp and \(S_{\text{max}}\) exercise in absolute terms (A) and as a percentage of the difference (B), according to Bland and Altman (1986).

4.4 Discussion

This study sought to establish the validity of traditional \(\dot{V}O_{2\text{max}}\) verification criteria and establish the utility of \(S_{\text{max}}\) in young CF patients. Results revealed four principle findings: 1) a \(\dot{V}O_2\) plateau is rarely observed upon exhaustion; 2) adherence to secondary traditional criteria underreports \(\dot{V}O_{2\text{max}}\); 3) in most cases (78.6%), \(S_{\text{max}}\) did not increase \(\dot{V}O_{2\text{peak}}\) thus confirming a valid \(\dot{V}O_{2\text{max}}\); and 4) \(S_{\text{max}}\) verification identified
those whose initial CPET $\dot{V}O_{2\text{peak}}$ was not a ‘true’ maximum. These findings have significant implications for the assessment and interpretation of CPET in young CF patients in both a clinical and research setting.

In this study only one $\dot{V}O_2$ plateau was documented upon exhaustion during CPET. While this is the first study to document the $\dot{V}O_2$ profile of young CF patients during ramp exercise, Werkman et al. (2011) recorded a plateau in 5 of 16 adolescents with CF during step exercise to exhaustion. However, the latter study employed a fixed $\dot{V}O_2$ plateau criterion ($< 2.1 \text{ mL·kg}^{-1}·\text{min}^{-1}$), which was originally developed on adults during a discontinuous running protocol consisting of steady-state stages (Taylor et al., 1955) and is unlikely to account for the altered O2 cost of exercise reported in young CF patients (Moser et al., 2000). This was accounted for in our study, using an extrapolated linear function from each participant’s $\dot{V}O_2$-power output profile prior to exhaustion. Our findings indicate that young CF patients rarely satisfy the conventional criteria of a $\dot{V}O_2$ plateau during CPET.

Secondary criteria have therefore been adopted to verify $\dot{V}O_{2\text{max}}$ in young people, however they often underreport $\dot{V}O_{2\text{max}}$ or reject a ‘true’ measure within healthy children (Barker et al., 2011; Rowland et al., 1993). Such criteria may be equally unsuitable for clinical paediatric populations. The present investigation confirms this notion. Achieving an RER > 1.00 was the least robust criterion, underestimating on average by 46% (Figure 4.1a). Achieving a RER > 1.10, 180 b·min$^{-1}$ (Figure 4.1c) and 95% of age-predicted HR$_{\text{max}}$ underestimated $\dot{V}O_{2\text{max}}$ by an average of 26%, 12% and 16%, respectively. Although HR criteria appear more robust, their use is limited as five patients (36%) failed to achieve a HR of 180 b·min$^{-1}$, and eleven (79%) failed
to achieve 95% of their age-predicted $HR_{\text{max}}$, one of whom exhibited a $\dot{V}O_2$ plateau. Given the emerging evidence base to reject secondary criteria in healthy children (Barker et al., 2011; Rowland et al., 1993) and adults (Poole et al., 2008) and now young CF patients, their use as a verification tool is limited and should be discontinued.

$S_{\text{max}}$ verification ensures that if the $\dot{V}O_2$peak obtained during a CPET is ‘truly’ maximal, then performing exercise above $W_{\text{peak}}$ from a preceding ramp test should not elicit a further increase in $\dot{V}O_2$, thus satisfying the primary plateau criterion. Limited application of $S_{\text{max}}$ in clinical populations is plausible, as poor exercise tolerance and slow $\dot{V}O_2$ kinetics (Hebestreit et al., 2005) may preclude the attainment of $\dot{V}O_{2\text{max}}$. The present study has demonstrated, however, that $S_{\text{max}}$ can verify $\dot{V}O_{2\text{max}}$ in individuals with CF. Additionally, mean $S_{\text{max}}$ duration was 85 ± 26 s, which is comparable with healthy children (91 ± 26 s) exercising at 105% $W_{\text{peak}}$ (Barker et al., 2011). No adverse incidents were encountered, substantiating previous reports that exercise testing is safe in mild-to-moderate CF (Ruf et al., 2010).

Although mean $S_{\text{max}} \dot{V}O_2$peak was comparable with traditional ramp testing, $S_{\text{max}}$ elicited meaningful rises in 3 of 14 (21%) cases (range 9.9-38.3%; Figure 4.2), findings comparable to young spina bifida (de Groot et al., 2009) [5 of 20 (33%)] and adult chronic heart failure (21%) patients (Bowen et al., 2011). In healthy children, $S_{\text{max}}$ increased $\dot{V}O_2$peak (potentially negligible) in only 1 of 13 cases (8%) (Barker et al., 2011). These findings support the use of $S_{\text{max}}$ as a safe and powerful tool in CF patients to validate $\dot{V}O_{2\text{max}}$ measurement.
Werkman *et al.* (2011) recently examined the feasibility of a SRT to verify $\dot{V}O_{2\text{max}}$ in adolescent CF patients. The authors concluded that $\dot{V}O_{2\text{peak}}$ from traditional CPET reflected ‘true’ $V_{O2\text{max}}$. Although, not discussed by these authors, it is clear that 4 of their 13 patients experienced a potentially meaningful rise in $\dot{V}O_2$ during the SRT (see Figure 2), which is comparable to the $S_{\text{max}}$ increase in $\dot{V}O_{2\text{peak}}$ herein. This supports the present findings that $S_{\text{max}}$ confirmation is an essential addition to traditional CPET to confirm a ‘true’ $V_{O2\text{max}}$ in young patients with CF.

Individual patient data is of interest to the clinician, in that those with the greatest increase in $\dot{V}O_{2\text{max}}$ resulting from $S_{\text{max}}$ were patients about whom there were treatment adherence concerns (e.g. patients 3 and 14). Conversely, those with lower $S_{\text{max}}\dot{V}O_{2\text{peak}}$ versus the ramp were typically physically active and more accomplished in sporting activities (e.g. patients 1 and 6). Although patients 3 and 14 possessed slightly lower $\dot{V}O_{2\text{peak}}$ scores when expressed relative to body mass, their lower fitness are unlikely solely attributable to more severe disease, since superior fitness was recorded for a number of patients with lower scores on all aspects of the disease profile (Table 4.1). Their scores likely represent poor motivation during CPET which may be indicative of motivation in other aspects of their disease management.

Combining a traditional ramp CPET with a $S_{\text{max}}$ test permits the identification of a valid $\dot{V}O_{2\text{max}}$. This protocol can be safely and effectively undertaken within a single laboratory visit and offers clear guidelines and a superior validation of $\dot{V}O_{2\text{max}}$ than current methods. To utilise $\dot{V}O_{2\text{max}}$ in prognostic stratification (Nixon *et al.*, 1992), it is essential that ‘true’ measurements are obtained. Accepting submaximal or rejecting
‘true’ values could distort clinical application and interpretation of CPET. Since healthy adults do not always demonstrate a \( \dot{V}O_2 \) plateau, \( S_{\text{max}} \) may be useful for adult CF patients. However, as more severe disease is associated with aging in CF, the safety and tolerance of \( S_{\text{max}} \) in older patients warrants investigation. Whether a further \( S_{\text{max}} \) test, at a higher percentage of \( W_{\text{peak}} \), could verify \( \dot{V}O_2_{\text{max}} \) in the cases where \( \dot{V}O_2_{\text{peak}} \) increased significantly is also unknown, although utility of a subsequent, more intense verification test to verify \( \dot{V}O_2_{\text{max}} \) has been demonstrated in healthy adults (Scharhag-Rosenberger et al., 2011). From a practical viewpoint, \( S_{\text{max}} \) verification is straightforward to implement as the imposed power output is calculated from ramp test \( W_{\text{peak}} \) on an individual basis and, clinically, may minimise the costs associated with re-tests when the validity of test results is questionable.

4.5 Conclusion

In conclusion, \( S_{\text{max}} \) verification is a safe and well-tolerated tool to determine valid \( \dot{V}O_2_{\text{max}} \) in paediatric patients with CF. Although the present uptake of CPET is poor within the clinical management of young people with mild-to-moderate CF (Barker et al., 2004; Stevens et al., 2010), the ECFS Exercise Working Group have recently recognised such testing as the method of choice when assessing aerobic fitness in this patient population. Consequently, it is recommended that \( S_{\text{max}} \) verification replace traditional criteria for confirming a ‘true’ \( \dot{V}O_2_{\text{max}} \) measurement in young CF patients with mild-to-moderate disease.
4.6 Practical implications

- Aerobic fitness ($\dot{V}O_{2\text{max}}$) measurement can help predict survival in CF.
- Criteria commonly used to confirm $\dot{V}O_{2\text{max}}$ tend to underreport ‘true’ fitness. Conversely, a $S_{\text{max}}$ exercise test can confirm ‘true’ measurements.
- Underreporting $\dot{V}O_{2\text{max}}$ could result in incorrect interpretation of patients’ fitness, prognosis and/or the influence of a therapeutic intervention.
- It is recommended that $S_{\text{max}}$ be adopted when performing CPET on young CF patients in the clinical or research environments.
CHAPTER FIVE

Reproducibility of Maximal Cardiopulmonary Exercise Testing for Young Cystic Fibrosis Patients

This experimental study has been disseminated as follows:


5.1 Introduction

A maximal CPET is considered the ‘gold-standard’ method for evaluating aerobic fitness ($\bar{VO}_{2\text{max}}$) in patients with mild-to-moderate CF. The ECFS Exercise Working Group recently promoted CPET as the exercise testing method of choice for this
patient group. Moreover, the ECFS Clinical Trials Network Standardisation Committee has called for assessment of the validity, reproducibility and feasibility of outcome measures utilised in CF and advocated research into the most appropriate exercise test for paediatric patients (Bradley et al., 2012).

Recently, our research group presented a combined incremental and $S_{\text{max}}$ verification CPET protocol, which is superior at determining valid $\dot{V}O_2\text{max}$ in young CF patients compared to a ramp only protocol (Chapter 4; Saynor et al., 2013a). $\dot{V}O_2\text{max}$ is currently the principle outcome from a CPET, as it has been shown to be an independent predictor of CF patient mortality (Nixon et al., 1992). However, a more comprehensive evaluation of patients’ cardiorespiratory fitness may be gained from CPET, through the quantification of submaximal parameters of aerobic (LT, the kinetics of $\dot{V}O_2$ and work efficiency) and ventilatory ($\dot{V}E/\dot{V}CO_2$ -slope and OUES (Baba et al., 1996)) function. Submaximal outcomes may be especially useful in the clinical environment, as patients may not be able or willing to provide a maximal effort.

Unfortunately, insufficient data exists regarding the reproducibility of CPET in CF patients and that which does exist has utilised testing protocols which cannot verify a ‘true’ maximal effort (e.g., Kent et al., 2012; McKone et al., 1999). Moreover, the only paediatric study to address this issue (Kent et al., 2012) did not measure $\dot{V}O_2\text{max}$. Quantifying reproducibility enables researchers and clinicians to understand the variation associated with outcome measures (Hopkins, 2000) and to determine meaningful changes (Atkinson & Nevill, 1998). Consequently, inferences regarding therapeutic interventions or disease-related changes in CPET derived parameters
cannot currently be discerned with certainty in these patients. Therefore, this study sought to establish the short- (48 h) and medium-term (4-6 weeks) reproducibility of maximal and submaximal indicators of cardiorespiratory fitness using our recently validated CPET protocol.

5.2 Methods

5.2.1 Participants, anthropometry and pulmonary function

Thirteen young patients (Table 5.1) with mild-to-moderate CF were recruited from outpatient CF clinics at the RD&E. Inclusion criteria comprised a CF diagnosis based on clinical features, sweat Cl⁻ > 60 mmol·L⁻¹ / 100 mg and genotyping. Stable pulmonary function within 10% of best in the preceding 6 months and no increase in symptoms or weight loss 2 weeks prior to testing was obligatory. Unstable non-pulmonary comorbidities or acute infections warranted exclusion. Disease severity was graded using the SS (Schwachman et al., 1958) and routine clinical measurements obtained as part of patients’ annual review by their multidisciplinary CF clinical care team (Table 5.1). Ethics approval was granted by the South West NHS Research Ethics Committee and written informed consent and assent obtained from parents/guardians and patients, respectively. Patients arrived at the laboratory in a rested state, at least 2 h post-prandial and having refrained from caffeine for > 2 h. All patients were instructed to continue maintenance medications as usual throughout the duration of their study involvement.

Body mass (Seca 220; Vogel & Halke, Hamburg, Germany) and stature (Seca 220; Vogel & Halke, Hamburg, Germany) were measured to the nearest 0.1 kg and 0.01 m, respectively, at each visit. Skinfolds were measured to the nearest 1 mm on the
right-hand side of the body at the biceps brachii, tricep, subscapula and suprailiac regions (Harpenden; British Indicators, Burgess Hill, UK). FVC and FEV$_1$ were also assessed at each visit to the laboratory, using flow-volume loop spirometry (MicroMedical MicroLoop 3535, Numed, Sheffield, UK). The best of three consistent exhalations (< 5% variability) was recorded, in accordance with the British Thoracic Society (1994) guidelines. All pulmonary function measurements were expressed as a percentage predicted normal, using appropriate reference data (Quanjer et al., 1993).

Table 5.1. Patients’ baseline anthropometric and pulmonary function data upon initiation into the study (n = 13; 4 females).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (mean ± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>12.81 ± 3.26</td>
<td>7.57-18.44</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.53 ± 0.16</td>
<td>1.23-1.74</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>50.89 ± 17.26</td>
<td>24.35-83.50</td>
</tr>
<tr>
<td>BMI (kg·m$^2$)</td>
<td>21.18 ± 3.86</td>
<td>14.19-28.24</td>
</tr>
<tr>
<td>SSkF (mm)</td>
<td>43 ± 13</td>
<td>24-67</td>
</tr>
<tr>
<td>Gender</td>
<td>m = 9, f = 4</td>
<td></td>
</tr>
<tr>
<td>CFTR genotype:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homozygote ∆F508</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>∆F508/P67L</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>∆F508/ 621+IG ➤ T</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>∆F508/ 2184delA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>∆F508/ G551D</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chronic P. Aeruginosa infection$^a$</td>
<td>“chronic,” n = 2;</td>
<td>“free,” n = 5</td>
</tr>
<tr>
<td></td>
<td>“intermittent,” n = 4</td>
<td>“never,” n = 2</td>
</tr>
<tr>
<td>Shwachman score</td>
<td>82 ± 6</td>
<td>67-91</td>
</tr>
<tr>
<td>Northern score$^b$</td>
<td>4 ± 1</td>
<td>2-6</td>
</tr>
<tr>
<td>FVC [% predicted (L)]</td>
<td>103.5 ± 15.0 (3.3 ± 1.2)</td>
<td>79.0-127.0 (1.6-5.1)</td>
</tr>
<tr>
<td>FEV$_1$ [% predicted (L)]</td>
<td>91.7 ± 17.8 (2.7 ± 1.0)</td>
<td>65.0-120.0 (1.4-4.1)</td>
</tr>
</tbody>
</table>

Values are means ± SD, with the range also displayed where suitable, unless otherwise stated. BMI, body mass index; SSkF, sum of skinfolds; CFTR, cystic fibrosis transmembrane conductance regulator; P. Aeruginosa; Pseudomonas Aeruginosa; Shwachman score - scoring 4 separate aspects of the disease profile; general activity; physical examination; nutritional status; and chest radiographic findings, using the most recent clinical review information. A total of 100 points represents a perfect score of health; FVC, forced vital capacity; FEV$_1$, forced expiratory volume in 1 s; $^a$ According to Leeds Criteria, “chronic”, >50% of the preceding 12 months were P. aeruginosa culture positive; “intermittent”, ≤50% of the preceding 12 months were P. aeruginosa culture positive; “never”, no growth of P. aeruginosa for the previous 12 months, having previously been P. aeruginosa culture positive; “free”, P. aeruginosa has never been cultured. $^b$ Provides evidence of radiographic chest findings. Maximum score is 20, with 20 being the most severe.
5.2.2 Exercise testing protocol

Following familiarisation, exercise was performed on a cycle ergometer (Lode Excalibur or Lode Corival, Groningen, The Netherlands). The experimental protocol was identical to our previous study (Chapter 4 - Saynor et al., 2013a), using a combined ramp-incremental and S_{max} verification protocol. Following 3 min warm-up (20 W), patients completed an incremental ramp cycling test, whereby resistance increased at a predetermined rate (10-25 W·min\(^{-1}\)). Ramp rate was dependent on patients’ age, height and fitness level, to elicit ~ 8-12 min test durations. Patients maintained ~ 70-80 rpm until volitional exhaustion, defined as a drop in cadence > 10 rpm for 5 consecutive s despite strong verbal encouragement. Five minutes active (20 W cycling) and 10 min passive seated recovery followed. S_{max} verification of \(\dot{V}O_2\)max was then performed, whereby a 3 min warm-up (20 W) preceded a ‘step’ transition to a CWR equivalent to 110% W_{peak}. This work rate was maintained until voluntary exhaustion. Five minutes active recovery (20 W cycling) completed the CPET.

Following test one (T\(_1\)), all procedures were repeated 48 h (short-term; T\(_2\)) and 4-6 weeks (medium-term; T\(_3\)) later, at a similar time of day. Medium-term clinical stability was monitored T\(_1\)-T\(_3\), with disease considered unstable if a pulmonary exacerbation developed, a change in pulmonary medications was required, chest signs on physical examination altered, or a ≥ 10% decline in pulmonary function was recorded.
5.2.3 Exercise testing measurements

*Gas analysis.* Prior to each test, the metabolic cart (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany) was calibrated using gases of known concentration, and the turbine volume transducer using a 3 L calibration syringe (Hans Rudolph, Kansas City, MO). Breath-by-breath pulmonary gas exchange and ventilation were measured and averaged to 15 s time bins. The highest 15 s stationary average $\dot{V}O_2$ from the ramp and $S_{\text{max}}$ protocols was then taken to represent $\dot{V}O_{2\text{max}}$ (Chapter 4; Saynor *et al.*, 2013).

*Submaximal gas exchange parameters.* The LT was non-invasively identified using the GET (Beaver *et al.*, 1986) and confirmed through visual inspection of the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$ (Wasserman, 1984). The $\dot{V}O_2$ MRT was determined using the time from the onset of the ramp test to the intersection point between the baseline $\dot{V}O_2$ and a backwards extrapolation of the slope of $\dot{V}O_2$ as a function of time. The $\dot{V}O_2$ ‘gain’ ($\Delta\dot{V}O_2/\Delta\text{WR}$) was determined by regression of the ‘linear’ portion of the $\dot{V}O_2$ response against power output. The OUES for the entire exercise duration ($\text{OUES}_{100}$) and up to the GET ($\text{OUES}_{\text{GET}}$) were derived from the slope of the linear function between $\dot{V}O_2$ (mL·min$^{-1}$) and log $\dot{V}_E$ (L·min$^{-1}$) (Baba *et al.*, 1996). The $\dot{V}_E/\dot{V}CO_2$–slope (ventilatory drive) was calculated using linear regression during the entire CPET (Tabet *et al.*, 2003).

*Additional measures:* HR was determined every 5 s (PhysioFlow, PF-05, Manatec Biomedical, Paris, France), with the highest 15 s value taken as $\text{HR}_{\text{peak}}$. Fingertip SpO$\text{\textsubscript{2}}$ was measured on a beat-by-beat basis via pulse oximetry (NONIN, Avant 4000, NONIN Medical Inc., USA). Subjective ratings of RPE and RPD were recorded...
upon exhaustion using the P-CERT and CR-10 scales, respectively, the methodology for which is described elsewhere (Chapter 3; Saynor et al., 2013).

5.2.4 Data analysis and statistics

Data are expressed as means and standard deviations unless otherwise stated. Reproducibility was assessed using a downloadable spreadsheet (Hopkins, 2000). Following initial analyses to ensure distribution normality and heteroscedasticity, paired samples t-tests examined differences between tests with significance set at \( p < 0.05 \). Change in the mean, intraclass correlation coefficients (ICCs), absolute TE and TE expressed as a percentage of the coefficient of variation (TE\(_{CV\%}\)), were calculated (with 90% confidence limits) for short- \((T_1 - T_2)\) and medium-term \((T_1 - T_3)\) pairwise comparisons.

5.3 Results

One patient was lost to follow-up at \( T_3 \), due to reasons unrelated to the study. Table 5.1 summarises patients’ \((n = 13)\) baseline physical characteristics. Clinical stability was defined by symptoms, changes in patients’ treatment, spirometric variables and body mass over the course of the study \((T_1 - T_3)\). All patients remained clinically stable and with no change in symptoms, treatment, body mass \([50.89 \pm 17.26]\) vs. \(50.98 \pm 17.17\) kg; \(p = 0.63\)], BMI \([21.23 \pm 7.79]\) vs. \(21.18 \pm 7.61\) kg·m\(^{-2}\); \(p = 0.97\)] or pulmonary function \([FVC: 3.12 \pm 1.08]\) vs. \(3.03 \pm 1.04\) L; \(p = 0.10\); \(FEV_1: 2.53 \pm 0.88\) vs. \(2.48 \pm 0.87\) L; \(p = 0.10\)]. Stability predated \(T_1\) (i.e. recruitment) and was maintained beyond \(T_3\).
Maximal and submaximal physiological responses from the CPET are presented in Table 5.2. Short- (T₁-T₂) and medium-term (T₁-T₃) reproducibility data from CPET derived measures are presented in Tables 5.3 and 5.4 respectively. The reproducibility for $\dot{V}O_{2\text{max}}$ is presented in Figure 5.1. The GET and OUES$_{\text{GET}}$ were identifiable in all patients at T₁ and 12 (92%) patients at T₂ and T₃. MRT was detected in 11 (85%) patients from T₁-T₃.

When compared with this combined approach (ramp and S$_{\text{max}}$), $\dot{V}O_{2\text{max}}$ obtained using the traditional ramp only method was significantly lower at both T₂ [1.76 (0.56) vs. 1.63 (0.52) L·min⁻¹; $p = 0.01$] and T₃ [1.69 (0.55) vs. 1.62 (0.54) L·min⁻¹; $p = 0.07$], with a trend towards significance at T₁ [1.77 (0.57 vs. 1.68 (0.56) L·min⁻¹; $p = 0.07$], as has been previously demonstrated (Chapter 4; Saynor et al., 2013a). Moreover, $\dot{V}O_{2\text{max}}$ using the combined approach was also associated with smaller error over both the short- [TE: 0.15 (0.12-0.23) vs. 0.23 (0.17-0.38) L·min⁻¹; TE$_{\text{CV}}$: 9.3 (6.9-14.3) vs. 13.5 (9.5-23.3) %] and medium-term [TE: 0.16 (0.12-0.25) vs. 0.19 (0.14-0.32) L·min⁻¹; TE$_{\text{CV}}$: 13.3 (9.9-20.9) vs. 15.5 (10.9-26.9) %] when compared with a ramp test in isolation.
<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximal exercise parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\dot{V}O_{2\text{max}}$ (L·min$^{-1}$)</td>
<td>13</td>
<td>1.77 (0.57)</td>
<td>1.76 (0.56)</td>
<td>1.68 (0.55)</td>
</tr>
<tr>
<td>$HR_{\text{peak}}$ (b·min$^{-1}$)</td>
<td>11</td>
<td>190 (12)</td>
<td>186 (14)</td>
<td>186 (19)</td>
</tr>
<tr>
<td>$SpO_2$ (%)</td>
<td>13</td>
<td>95 (3)</td>
<td>96 (1)</td>
<td>96 (3)</td>
</tr>
<tr>
<td>RPE</td>
<td>13</td>
<td>9 (2)</td>
<td>9 (2)</td>
<td>9 (1)</td>
</tr>
<tr>
<td>RPD</td>
<td>13</td>
<td>7 (3)</td>
<td>6 (3)</td>
<td>8 (3)</td>
</tr>
<tr>
<td>Ramp peak power output (W)</td>
<td>13</td>
<td>157 (55)</td>
<td>148 (62)</td>
<td>145 (65)</td>
</tr>
<tr>
<td><strong>Submaximal parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GET (L·min$^{-1}$)</td>
<td>12</td>
<td>1.00 (0.22)</td>
<td>0.93 (0.21)</td>
<td>1.05 (0.29)</td>
</tr>
<tr>
<td>MRT (s)</td>
<td>11</td>
<td>42 (15)</td>
<td>65 (17)</td>
<td>54 (26)</td>
</tr>
<tr>
<td>$V\dot{O}_2$ gain (mL·min$^{-1}$·W$^{-1}$)</td>
<td>12</td>
<td>8.01 (1.36)</td>
<td>8.11 (1.22)</td>
<td>7.73 (2.64)</td>
</tr>
<tr>
<td>$OUES_{100}$ (mL·min$^{-1}$·logL$^{-1}$)</td>
<td>12</td>
<td>803 (227)</td>
<td>789 (181)</td>
<td>799 (218)</td>
</tr>
<tr>
<td>$OUES_{\text{GET}}$ (mL·min$^{-1}$·logL$^{-1}$)</td>
<td>12</td>
<td>797 (223)</td>
<td>730 (188)</td>
<td>756 (389)</td>
</tr>
<tr>
<td>$\dot{V}_E/\dot{V}CO_2$-slope</td>
<td>12</td>
<td>34.13 (4.51)</td>
<td>33.26 (3.25)</td>
<td>32.14 (5.39)</td>
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<tr>
<td>$\dot{V}_E/\dot{V}O_2$ at the GET</td>
<td>12</td>
<td>28.57 (5.45)</td>
<td>28.63 (3.84)</td>
<td>28.09 (4.58)</td>
</tr>
<tr>
<td>$\dot{V}_E/\dot{V}CO_2$ at the GET</td>
<td>12</td>
<td>28.07 (3.96)</td>
<td>29.15 (5.43)</td>
<td>27.95 (5.51)</td>
</tr>
</tbody>
</table>

Values are means ± SD, with the range also displayed unless otherwise stated. $\dot{V}O_{2\text{max}}$, maximal oxygen uptake; $HR_{\text{peak}}$, peak heart rate; $SpO_2$, end-exercise arterial oxygen saturation; RPE, end-exercise rating of perceived exertion; RPD, end-exercise rating of perceived dyspnoea; ramp, incremental ramp test; GET, non-invasive estimate of the lactate threshold which was verified by the ventilatory threshold; MRT, mean response time; $\dot{V}O_2$ gain, oxygen cost of exercise; $OUES_{100}$, oxygen uptake efficiency slope for the entire duration of the ramp test; $OUES_{\text{GET}}$, $OUES$ to the GET; $\dot{V}_E/\dot{V}CO_2$-slope, ventilatory drive; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen uptake; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide.
Figure 5.1. Line of identity plot for pulmonary oxygen uptake ($\dot{V}O_{2}\text{max}$) over both the short- [48 h (5.1a)] and medium-term [4-6 weeks (5.1b)].
Table 5.3. Short-term (48 h) test-retest reproducibility (T1-T2) of CPET derived measures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Change in the mean</th>
<th>p-value</th>
<th>TE</th>
<th>TECV%</th>
<th>ICC</th>
<th>p-value</th>
</tr>
</thead>
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<td><strong>Lung function</strong></td>
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</tr>
<tr>
<td>FVC (L)</td>
<td>13</td>
<td>0.01</td>
<td>0.79</td>
<td>0.08 (0.05-0.11)</td>
<td>3.1 (2.3-4.7)</td>
<td>1.00</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>FEV1 (L)</td>
<td>13</td>
<td>-0.02</td>
<td>0.48</td>
<td>0.07 (0.06-0.11)</td>
<td>2.7 (2.0-4.1)</td>
<td>0.99</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Maximal exercise parameters</strong></td>
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<td></td>
</tr>
<tr>
<td>VO2max (L·min⁻¹)</td>
<td>13</td>
<td>-0.01</td>
<td>0.91</td>
<td>0.15 (0.12-0.23)</td>
<td>9.3 (6.9-14.3)</td>
<td>0.94</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HRpeak (b·min⁻¹)</td>
<td>11</td>
<td>-4</td>
<td>0.14</td>
<td>6 (4-9)</td>
<td>3.2 (2.3-5.1)</td>
<td>0.83</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SpO2 (%)</td>
<td>13</td>
<td>1</td>
<td>0.42</td>
<td>2 (2-3)</td>
<td>2.2 (1.7-3.4)</td>
<td>0.03</td>
<td>0.91</td>
</tr>
<tr>
<td>RPE</td>
<td>13</td>
<td>0.1</td>
<td>0.72</td>
<td>0.5 (0.4-0.8)</td>
<td>7.8 (5.8-12.0)</td>
<td>0.91</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>RPD</td>
<td>13</td>
<td>-1.3</td>
<td>0.09</td>
<td>1.7 (1.3-2.6)</td>
<td>63.7 (45.1-111.0)</td>
<td>0.60</td>
<td>0.05</td>
</tr>
<tr>
<td>Ramp peak power output (W)</td>
<td>13</td>
<td>-9</td>
<td>0.11</td>
<td>14 (11.21)</td>
<td>21.6 (16.0-34.6)</td>
<td>0.95</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Submaximal parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GET (L·min⁻¹)</td>
<td>12</td>
<td>-0.06</td>
<td>0.17</td>
<td>0.11 (0.08-0.16)</td>
<td>11.2 (8.2-17.8)</td>
<td>0.80</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MRT</td>
<td>11</td>
<td>23</td>
<td>&lt;0.01</td>
<td>14 (10-22)</td>
<td>37.8 (26.8-66.7)</td>
<td>0.32</td>
<td>0.39</td>
</tr>
<tr>
<td>VO2 gain (mL·min⁻¹·W⁻¹)</td>
<td>12</td>
<td>0.10</td>
<td>0.84</td>
<td>1.18 (0.84-2.01)</td>
<td>17.4 (12.0-31.2)</td>
<td>0.18</td>
<td>0.62</td>
</tr>
<tr>
<td>OUES100 (mL·min⁻¹·logL⁻¹)</td>
<td>12</td>
<td>-14.12</td>
<td>0.74</td>
<td>100.85 (75.41-156.39)</td>
<td>12.0 (8.9-19.2)</td>
<td>0.79</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>OUESGET (mL·min⁻¹·logL⁻¹)</td>
<td>12</td>
<td>-67.20</td>
<td>0.23</td>
<td>127.89 (95.63-198.31)</td>
<td>17.9 (13.1-39.1)</td>
<td>0.66</td>
<td>0.03</td>
</tr>
<tr>
<td>VE/VO2-slope</td>
<td>12</td>
<td>-0.88</td>
<td>0.42</td>
<td>2.54 (1.90-3.94)</td>
<td>7.8 (5.8-12.3)</td>
<td>0.63</td>
<td>0.03</td>
</tr>
<tr>
<td>VE/VO2 at the GET</td>
<td>12</td>
<td>0.06</td>
<td>0.96</td>
<td>3.19 (2.38-4.94)</td>
<td>10.2 (7.5-16.2)</td>
<td>0.59</td>
<td>0.05</td>
</tr>
<tr>
<td>VE/VCO2 at the GET</td>
<td>12</td>
<td>1.09</td>
<td>0.32</td>
<td>2.57 (1.92-3.99)</td>
<td>8.8 (6.5-14.0)</td>
<td>0.75</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are reported as means (95% confidence intervals). TE, typical error; TECV%, TE expressed as a percentage of the coefficient of variation; ICC, intraclass correlation coefficient. See table 2 for list of abbreviations for exercise outcomes.
Table 5.4. Medium-term (4-6 weeks) test-retest reproducibility ($T_1$-$T_3$) of CPET derived measures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Change in the mean</th>
<th>p-value</th>
<th>TE</th>
<th>$TE_{CV%}$</th>
<th>ICC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung function</strong></td>
<td>13</td>
<td>-0.08</td>
<td>0.09</td>
<td>0.11 (0.08-0.16)</td>
<td>3.4 (2.6-5.2)</td>
<td>0.99</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>13</td>
<td>-0.07</td>
<td>0.07</td>
<td>0.08 (0.06-0.12)</td>
<td>3.4 (2.5-5.1)</td>
<td>0.99</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>FEV$_1$ (L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Maximal exercise parameters</strong></td>
<td>13</td>
<td>-0.09</td>
<td>0.21</td>
<td>0.16 (0.12-0.25)</td>
<td>13.3 (9.9-20.9)</td>
<td>0.93</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>$\dot{V}O_2$ max (L.min$^{-1}$)</td>
<td>13</td>
<td>-0.28</td>
<td>0.72</td>
<td>1.85 (1.31-3.15)</td>
<td>24.5 (16.8-45.0)</td>
<td>0.24</td>
<td>0.40</td>
</tr>
<tr>
<td>HR$_{peak}$ (b.min$^{-1}$)</td>
<td>11</td>
<td>-5</td>
<td>0.49</td>
<td>14 (10-22)</td>
<td>7.8 (5.7-12.7)</td>
<td>0.30</td>
<td>0.38</td>
</tr>
<tr>
<td>SpO$_2$ (%)</td>
<td>13</td>
<td>1</td>
<td>0.60</td>
<td>3 (2-5)</td>
<td>3.1 (2.2-5.2)</td>
<td>-0.28</td>
<td>0.40</td>
</tr>
<tr>
<td>RPE</td>
<td>13</td>
<td>0.3</td>
<td>0.22</td>
<td>0.6 (0.5-0.9)</td>
<td>7.6 (5.7-11.8)</td>
<td>0.85</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>RPD</td>
<td>13</td>
<td>0.3</td>
<td>0.68</td>
<td>1.9 (1.4-2.8)</td>
<td>38.5 (27.9-63.8)</td>
<td>0.47</td>
<td>0.13</td>
</tr>
<tr>
<td>Ramp peak power output (W)</td>
<td>13</td>
<td>-12</td>
<td>0.05</td>
<td>14 (11-22)</td>
<td>19.8 (14.7-31.6)</td>
<td>0.95</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Submaximal parameters</strong></td>
<td>12</td>
<td>0.05</td>
<td>0.40</td>
<td>0.14 (0.11-0.22)</td>
<td>16.8 (12.3-27.2)</td>
<td>0.74</td>
<td>0.01</td>
</tr>
<tr>
<td>GET (L.min$^{-1}$)</td>
<td>11</td>
<td>12</td>
<td>0.26</td>
<td>24 (18-38)</td>
<td>89.4 (60.3-176.7)</td>
<td>-0.33</td>
<td>0.31</td>
</tr>
<tr>
<td>$\dot{V}O_2$ gain (mL.min$^{-1}$.W$^{-1}$)</td>
<td>12</td>
<td>-0.28</td>
<td>0.72</td>
<td>1.85 (1.31-3.15)</td>
<td>24.5 (16.8-45.0)</td>
<td>0.24</td>
<td>0.40</td>
</tr>
<tr>
<td>OUES$_{100}$ (mL.min$^{-1}$.logL$^{-1}$)</td>
<td>12</td>
<td>-4.29</td>
<td>0.92</td>
<td>107.28 (80.21-166.35)</td>
<td>15.3 (11.3-24.7)</td>
<td>0.80</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>OUES$_{GET}$ (mL.min$^{-1}$.logL$^{-1}$)</td>
<td>12</td>
<td>-40.69</td>
<td>0.61</td>
<td>188.78 (141.15-292.73)</td>
<td>45.4 (32.3-78.6)</td>
<td>0.69</td>
<td>0.01</td>
</tr>
<tr>
<td>$\dot{V}E$/$\dot{V}CO_2$-slope</td>
<td>12</td>
<td>-2.00</td>
<td>0.32</td>
<td>4.66 (3.49-7.23)</td>
<td>17.7 (12.9-28.7)</td>
<td>0.13</td>
<td>0.71</td>
</tr>
<tr>
<td>$\dot{V}E$/$\dot{V}O_2$ at the GET</td>
<td>12</td>
<td>-0.47</td>
<td>0.72</td>
<td>3.13 (2.34-4.86)</td>
<td>10.1 (7.4-16.0)</td>
<td>0.66</td>
<td>0.03</td>
</tr>
<tr>
<td>$\dot{V}E$/$\dot{V}CO_2$ at the GET</td>
<td>12</td>
<td>-0.12</td>
<td>0.90</td>
<td>2.38 (1.78-3.69)</td>
<td>9.4 (6.9-14.9)</td>
<td>0.79</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Values are reported as means (95% confidence intervals). TE, typical error; $TE_{CV\%}$, TE expressed as a percentage of the coefficient of variation; ICC, intra-class correlation coefficient. See table 2 for list of abbreviations for exercise outcomes.
5.4 Discussion

The principle finding of this study was that CPET was reproducible when determining \( \dot{V}O_{2\text{max}} \) [short-term (T1-T2) ICC: 0.94; medium-term (T1-T3) ICC: 0.93], with no significant learning effect and short- and medium-term TEs of 150 mL (\( \Delta \) 9\%) and 160 mL (\( \Delta \) 13.3\%). Of the additional maximal parameters, HR (3.2\%, 7.8\%), \( \text{SpO}_2 \) (2.2\%, 3.1\%) and RPE (7.8\%, 7.6\%) appear to hold acceptable short- and medium-term reproducibility, respectively. Submaximal measures were identifiable in most cases, with the \( \dot{V}/\dot{VCO}_2\)-slope (7.8\%), \( \dot{V}/\dot{VCO}_2 \) at the GET (8.8\%), \( \dot{V}/\dot{VO}_2 \) at the GET (10.2\%), the GET (11.2\%) and OUES\(_{100}\) (12.0\%) demonstrating promising reproducibility over 48 h. However, an increased TE\(_{\text{cv\%}}\) was observed for submaximal parameters at 4-6 weeks, with three (\( \dot{V}O_2 \) gain, OUES\(_{\text{GET}}\), \( \dot{V}O_2 \) MRT) TEs increasing above 20\% (24.5\%, 45.4\%, 89.0\%, respectively). Excluding the latter two variables (OUES\(_{\text{GET}}\) and \( \dot{V}O_2 \) MRT), good short- and medium-term agreement was observed for all measures, highlighting the potential for CPET outcomes to be used to monitor disease progression and/or the effect of therapeutic interventions.

Our data contribute significantly to the literature because the reproducibility of \( \dot{V}O_{2\text{max}} \) has not been established in CF using a valid protocol. Reproducibility over time is crucial when evaluating the efficacy of treatments (e.g. antimicrobials, mucolytics and gene mutation targeted therapies) which may accrue over weeks or months, as well as monitoring exercise training interventions (4-6 weeks). To our knowledge, only one study has examined the reproducibility of \( S_{\text{max}} \) verified (treadmill) \( \dot{V}O_{2\text{peak}} \) in a paediatric clinical population (de Groot et al., 2011), reporting an 8.2\% (100 mL) variation in young spina bifida patients over a 2 week period. Using a solitary traditional ramp test, variations of 6.9\% (McKone et al., 1999) and 8.5\% (Gruet et al.,
have been reported over 4 weeks in CF adults for $\dot{V}O_{2\text{peak}}$. The reproducibility estimate for $\dot{V}O_{2\text{max}}$ in the present study is therefore similar (9.3% and 13.3%) to these earlier studies (Gruet et al., 2010; McKone et al., 1999) and confirms CPET as a reproducible assessment tool. Whilst the compromised validity of performing traditional ramp tests, such as the popular Gofrey protocol, in isolation has previously been demonstrated (Chapter 4; Saynor et al., 2013a) and substantiated herein, the present study in paediatric CF patients also highlights a larger within-subject variation in $\dot{V}O_{2\text{max}}$ over both the short- (13.5 vs. 9.3 %) and medium-term (15.5 vs. 13.3 %) when compared with the combined ramp and $S_{\text{max}}$ approach. Only one study has previously investigated CPET reproducibility in CF children (Kent et al., 2012), but is limited due to methodological concerns. Firstly, only three outcome measures ($W_{\text{peak}}$, $\text{SpO}_2$ and HR) were obtained, offering limited interpretation of aerobic fitness. Moreover, an intermittent sprint cycle test preceded the ramp test, which likely caused fatigue and may explain, in part, their short mean ramp test duration (~4 min).

Outcome measures which can assess patients’ ability to perform at intensities similar to ADLs are also important. Submaximal measures hold specific value when maximal exercise performance is limited by ventilatory capacity and/or effort (Baba et al., 1996; Barker et al., 2011). Furthermore, the GET can improve independent of $\dot{V}O_{2\text{max}}$ (Casaburi et al., 1991; Wasserman, 1984) and facilitates the identification of individualised exercise intensities within specific intensity domains (i.e. at a %GET or %Δ) for young CF patients (e.g. Stevens et al., 2011). The present study employed a cluster of measures and two independent observers to identify the GET in 12 of 13 (92%) patients for all tests, with TE of 11.2% (or 110 mL) and 16.8% (or 140 mL).
over the short- and medium-term, respectively. Using similar methodology, our laboratory has previously reported a similar GET detection rate (100%) in healthy children, with a reproducibility estimate of ~ 8% (Fawkner et al., 2002). The present findings challenge previous reports suggesting difficulties in non-invasively detecting the GET and ventilatory threshold in patients with chronic respiratory disease and airflow limitation (e.g. Sexauer et al., 2003), likely due to the mild disease severity and subsequently normal ventilatory drive of our patients. The $\dot{V}O_2$ gain was associated with reasonable TEs of 17.4% and 24.5% over the short- and medium-term, respectively. The $\dot{V}O_2$ MRT was associated with considerably greater short- (37.8%) and medium-term (89.4%) variation. These submaximal measures, especially the MRT, may therefore be less useful than the GET.

Ventilatory efficiency is best described by relating $\dot{V}O_2$ and $\dot{V}CO_2$ dynamics to $\dot{V}_E$ (Arena et al., 2012). The $\dot{V}_E$-$\dot{V}O_2$ relationship is optimally described through the OUES (Arena et al., 2012), which is theoretically resistant to early test termination and intra- and inter-observed variability (Akkerman et al., 2010). In the current study, OUES$_{100}$ was detectable in all tests and the OUES$_{\text{GET}}$ detectable in all patients at T$_1$ and 92% at T$_2$ and T$_3$. Short- and medium-term TEs of 12.0% and 15.3% were associated with the OUES$_{100}$, compared with 8.3% documented in adult CF patients over a 4 week period (Gruet et al., 2010). Similar variations of 7.8% and 17.7% were documented for the $\dot{V}_E$/$\dot{V}CO_2$-slope in the present study. As the OUES$_{\text{GET}}$ was associated with increased short- (17.9%) and medium-term (45.4%) error and lower detection rate compared to the OUES$_{100}$, the OUES$_{100}$ appears a more robust outcome measure.
The present study provides the reproducibility for maximal and submaximal parameters over the short- and medium-term. Our data denote that \( \dot{V}O_{2\text{max}} \) changes exceeding 9% (150 mL) and 13% (160 mL) may indicate a change attributable to therapeutic intervention or disease progression over the short- and medium-term, respectively. The TE must, however, be considered relative to an established smallest worthwhile change (SWC), to estimate how many participants are needed to observe a ‘meaningful’ effect (Atkinson & Nevill, 1998; Hopkins, 2000; de Vet et al., 2006).

Using Hopkins’ formula (Hopkins, 2000) for the estimation of sample size \([n = 8 (CV^2/d^2)]\), \( CV \) and \( d \) can be substituted for TE and SWC, respectively. While the present study has documented the \( CV \), the value of \( d \) is uncertain for CPET outcomes in CF. Cox and Elkins (2011) recently raised concerns regarding how ‘clinically worthwhile’ exercise training interventions are for patients with CF, given that the SWC for outcome measures had yet to be established. However, the mean annual rate of \( \dot{V}O_{2\text{max}} \) decline could, for example, be used to determine the SWC in \( \dot{V}O_{2\text{max}} \), since it reportedly predicts CF patient survival (Pianosi et al., 2005). Using Pianosi and colleagues’ annual \( \dot{V}O_{2\text{max}} \) decline and the fitness of our similarly aged patients, an increase of \( \sim 6\% \) in \( \dot{V}O_{2\text{max}} \) relative to baseline fitness would be required to prevent a meaningful drop in prognostic stratification. Using 6% as the SWC and a 13.3% TE, 5 patients would be required to detect a change in \( \dot{V}O_{2\text{max}} \) from a 4-6 week intervention that would be considered meaningful and clinically worthwhile.

Determining the extent to which changes in outcome measurements relate to a given reference measure is essential to the clinical utility of CPET. Responsiveness to
intervention has been conceptually described as a signal-to-noise ratio (Atkinson & Nevill, 1998; de Vet et al., 2006; Hopkins, 2000), whereby the TE represents the ‘noise’ and any intervention-induced effect, the ‘signal’. Data concerning $\dot{V}_O_{2\text{max}}$ responsiveness within CF are sparse (Selvadurai et al., 2002). Of the available evidence, studies have reported training-related improvements in $\dot{V}_O_{2\text{max}}$ ranging from ~10-20% (e.g. Hebstreit et al., 2010; Hulzebos et al., 2010; Selvadurai et al., 2002). Using our established long-term TE, the aerobic training improvement could be considered meaningful with a signal-to-noise ratio of ~1.5:1.0 for an ~20% improvement, but questionable, with a signal-to-noise ratio of ~0.8:1.0 for a 10% improvement. Unfortunately, the signal-to-noise ratio for most parameters is unknown. If future intervention studies provided more comprehensive CPET data, this would permit more informed data interpretation, as researchers could select measurements with higher signal-to-noise ratios, whilst also considering their sensitivity.

Standardising CPET procedures will enable a larger empirical database of CF patients to accumulate and, longitudinally, enhance our understanding of the link between physiological dysfunction during exercise and patients’ prognostic stratification. Whilst $\dot{V}O_{2\text{peak}}$ possesses recognised prognostic value (Nixon et al., 1992), the $\dot{V}_E/\dot{V}CO_2$-slope and OUES have demonstrated superior prognostic information in other clinical populations (e.g. Arena et al., 2012; Kasikcioglu et al., 2009) and warrant investigation in CF, particularly given that although patients remained clinically stable throughout the present study, increased medium-term noise was associated with submaximal parameters. This may indicate value in
detecting subtle clinical changes, which current clinical assessments cannot. CPET to assess therapeutic interventions also requires investigation.

5.5 Conclusion

In conclusion, \( \dot{V}O_{2\text{max}} \) was reproducible over 48 h (\( \Delta150 \text{ mL; } \Delta9.3\% \)) and 4-6 weeks (\( \Delta160 \text{ mL; } \Delta13.3\% \)). Supplementary maximal and submaximal parameters should be incorporated to comprehensively assess aerobic exercise function. The present study provides a reproducible CPET protocol for young patients with mild-to-moderate CF and will inform sample size and power calculations when planning interventional studies that use cardiorespiratory fitness as an endpoint.

5.6 Practical implications

- Maximal CPET is a reproducible clinical assessment tool that can be used to assess changes in aerobic exercise function in children and adolescents with mild-to-moderate CF.
- A change > \( \Delta150 \text{ mL or } \Delta9.3\% \) over a 48 h period would indicate a clinically meaningful change in disease progression or in response to a therapeutic intervention.
- A change > \( \Delta 160 \text{ mL or } \Delta 13.3\% \) over a 4-6 week period would indicate a clinically meaningful change in disease progression or in response to a therapeutic intervention.
- The TE values presented in this study will enable the patient sample size for future interventional studies to be determined.
CHAPTER SIX

Impaired Aerobic Function in Young Patients with Cystic Fibrosis
during Ramp Exercise

This experimental study has been disseminated as follows:


**Poster Presentation:** Saynor, Z. L., Barker, A. R., Oades, P. J. & Williams, C. A. (2013). Exercise limitation in paediatric patients with mild-to-moderate cystic fibrosis. The University of Exeter Medical School Medical and Health Research Showcase, Exeter, UK.


6.1 **Introduction**

CF is a complex multiorgan genetic disease, expressed as a disruption in the CFTR protein. In conjunction with its clinical presentation, reduced aerobic fitness (typically determined as $\dot{V}O_{2\text{max}}$), is commonly observed in both adult (Freeman *et al.*, 1993) and paediatric patients (Ionescu *et al.*, 2001). Reduced aerobic fitness is of clinical relevance in patients with CF, given its association with longevity (Nixon *et al.*, 1992; Pianosi *et al.*, 2005), QoL (de Jong *et al.*, 1997) and risk of hospitalisation (Pérez *et al.*, 2000).
Key parameters of aerobic exercise function (i.e., $\dot{V}O_{2\text{max}}$, $\dot{V}O_2$ gain, GET, and $\dot{V}O_2$ MRT) (Whipp et al., 1981) have not, however, been comprehensively documented in CF. Moreover, no previous studies have used a valid protocol (Chapter 4; Saynor et al., 2013) to obtain a ‘true’ measure of $\dot{V}O_{2\text{max}}$ in this population. Identifying the limiting factor(s) impairing aerobic function in CF will facilitate the development of more effective strategies to improve longevity and QoL in this aging patient population.

Because the body’s upper limit for $\dot{V}O_2$ use is determined by the maximal $\dot{Q}$, arterial $\dot{V}O_2$ content, fractional distribution of $\dot{Q}$ to the exercising muscles, and the ability of the skeletal muscle to extract $O_2$ (Wasserman et al., 2004), simultaneous measurements at the central (cardiorespiratory) and peripheral (skeletal muscle) levels are required to understand the dynamic matching of $O_2$ delivery-to-$O_2$ utilisation during exercise. Previous studies in CF have, however, largely neglected this complex interaction and based inferences on investigations of isolated organ systems (e.g. divangahi et al., 2009; Ionescu et al., 2001; Lands et al., 1992;).

As a result, debate remains regarding the relative importance of central and peripheral mechanisms to explain the reduced $\dot{V}O_{2\text{max}}$ in patients with mild-to-moderate CF. Expression of CFTR in the human skeletal muscle (Lamhonwah et al., 2010) suggests an intrinsic myocyte metabolic abnormality, which may be specific to CF (e.g. de Meer et al., 1995; Divangahi et al., 2009) or a consequence of chronic respiratory disease (Rosenthal et al., 2009; Wells et al., 2011). In addition, there is evidence to support a central limitation to exercise through a reduction in SV (Pianosi & Pelech, 1996) and, presumably, muscle $O_2$ delivery (Rosenthal et al.,
2009), in paediatric patients with CF. Although a compensatory increase in muscle
O₂ extraction may be expected to occur in the presence of reduced muscle O₂
delivery, this was not observed in a previous study (Rosenthal et al., 2009).
However, further confirmation of this response is warranted because inferences at
the skeletal muscle level were based upon indirect, interlinked mathematical
calculations.

To further understand how disease pathophysiology alters the O₂ delivery-to-O₂
utilisation relationship during exercise, NIRS can provide valuable, non-invasive
insight into peripheral O₂ extraction. Specifically, the profile of the [HHb] signal has
been used to describe O₂ extraction dynamics during ramp exercise, which in turn
permit inferences regarding blood flow within the microcirculation of exercising
muscle (Boone et al., 2009; Ferreira et al., 2007; McNarry et al. 2011; Murias et al.,
2013a; Murias et al., 2013b). Although the [HHb] profile during ramp exercise has
been used to describe the effect of trained status (Boone et al., 2009; McNarry et al.,
2011) and ageing (Gravelle et al., 2012; McNarry et al., 2011), there are no data
documenting the influence of disease on the [HHb] response to exhaustive
incremental exercise. If the supply of blood to the active muscle during exercise is
impaired in CF, as would be indirectly inferred from previous reports of a reduced SV
(Pianosi & Pelech, 1996), an increased rate of fractional O₂ extraction for a given
\( \dot{\text{V}} \dot{\text{O}}_2 \) would be expected (Ferreira et al., 2007).

The purpose of the present study was twofold; 1) to characterise the four key
parameters of aerobic function in paediatric patients with mild-to-moderate CF and 2)
to characterise the dynamic adjustment of NIRS-derived leg muscle [HHb] during
ramp exercise. It was hypothesised that: 1) aerobic exercise function would be impaired in CF, as evidenced by a reduced $\dot{V}O_{2\text{max}}$, slower $\dot{V}O_2$ MRT, earlier occurrence of the GET and shallower $\dot{V}O_2$ gain and, furthermore, that 2) patients with CF would be characterised by more rapid [HHb] dynamics during ramp exercise (i.e., leftward shift), which will correlate with impaired parameters of aerobic function.

6.2 Methods

6.2.1 Participants, anthropometry and pulmonary function

Ten young patients (9 males (Table 6.1)) with stable mild-to-moderate CF disease (CF) regularly partaking in school and/or extracurricular physical activity were recruited from outpatient clinics at the Royal Devon and Exeter NHS Foundation Trust Hospital. CF inclusion and exclusion criteria are detailed elsewhere (Saynor et al., 2013a). Ten healthy age- and gender- matched control participants (CON) were recruited from the local area (Table 6.1). Neither group presented with any contraindications to exhaustive exercise, and CON were free from any pulmonary conditions. Ethics approval was granted by the South West NHS Research Ethics Committee. Informed written consent and assent were obtained from parents/guardians and patients, respectively. Details concerning the CF patients’ disease severity and clinical profile were obtained by their clinician (Table 6.2). All CF maintenance medications were continued as usual throughout the study.

Body mass (Seca 220; Vogel & Halke, Hamburg, Germany) and stature (Seca 220; Vogel & Halke, Hamburg, Germany) were measured to the nearest 0.01 kg and 0.01 m, respectively. Pubertal maturity was determined using Tanner staging (Tanner, 1962). Skinfolds measured to the nearest 1 mm on the right-hand side of the body at
the triceps and subscapula regions (Harpenden; British Indicators, Burgess Hill, UK) and were used to estimate FFM (Slaughter et al., 1988). FVC and FEV$_1$ were assessed using flow-volume loop spirometry (MicroMedical MicroLoop 3535, Numed, Sheffield, UK). The best of three consistent (< 5% variability) exhalations was documented and expressed as a percentage of predicted using appropriate reference data (Stanojevic et al., 2009).

**Table 6.1.** Baseline anthropometric and pulmonary function data for young CF patients (n = 10, 1 female) and healthy age- and gender-matched controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CF (Mean ± SD)</th>
<th>CON (Mean ± SD)</th>
<th>Change, 90% CL</th>
<th>Inference (in CF)</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>12.7 ± 2.8</td>
<td>12.5 ± 2.8</td>
<td>0.2, ±2.2</td>
<td>Unclear</td>
<td>0.07</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.53 ± 0.15</td>
<td>1.58 ± 0.19</td>
<td>-0.05, ±0.14</td>
<td>Unclear</td>
<td>-0.25</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>53.2 ± 20.0</td>
<td>50.5 ± 17.4</td>
<td>2.7, ±14.6</td>
<td>Unclear</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg·m$^{-2}$)</td>
<td>22.0 ± 4.6</td>
<td>19.5 ± 2.7</td>
<td>2.4, ±3.0</td>
<td>Likely higher</td>
<td>0.60</td>
</tr>
<tr>
<td>BSA (m$^2$)</td>
<td>1.51 ± 0.35</td>
<td>1.48 ± 0.35</td>
<td>0.03, ±0.27</td>
<td>Unclear</td>
<td>0.08</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>42.0 ± 14.6</td>
<td>41.3 ± 14.0</td>
<td>0.6, ±11.1</td>
<td>Unclear</td>
<td>0.04</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.36 ± 1.30</td>
<td>3.69 ± 1.33</td>
<td>-0.33, ±1.03</td>
<td>Unclear</td>
<td>-0.24</td>
</tr>
<tr>
<td>FVC (% predicted range)</td>
<td>102 ± 14 (79-123)</td>
<td>106 ± 10 (92-125)</td>
<td>-4, ±10</td>
<td>Unclear</td>
<td>0.28</td>
</tr>
<tr>
<td>FEV$_1$ (L)</td>
<td>2.69 ± 1.12</td>
<td>3.18 ± 1.18</td>
<td>-0.49, ±0.89</td>
<td>Unclear</td>
<td>-0.41</td>
</tr>
<tr>
<td>FEV$_1$ (% predicted range)</td>
<td>97 ± 22 (66-127)</td>
<td>107 ± 10 (96-129)</td>
<td>-10, ±14</td>
<td>Likely lower</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Values are means ± SD. ES; Effect size; CI, confidence intervals; BMI, body mass index; FFM, fat-free mass (calculated using the equation of Slaughter et al. (1988)); FVC, forced vital capacity; FEV$_1$, forced expiratory volume in 1 s. N.B. Parameters of pulmonary function are expressed as a percentage predicted normal using appropriate reference data (Stanojevic et al., 2009).
Table 6.2. Baseline clinical characteristics for the young CF patients ($n = 10$, 1 female).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (mean ± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CFTR genotype:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homozygote ΔF508 (Class I mutation)</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>ΔF508/ 2184delA (Class II Mutation)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>ΔF508/ G551D (Class III mutation)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><strong>Chronic P. Aeruginosa infection</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“chronic,” $n = 2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“intermittent,” $n = 3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“free,” $n = 3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“never,” $n = 2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shwachman score</td>
<td>81 ± 7</td>
<td>67-91</td>
</tr>
<tr>
<td>Northern score&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4 ± 1</td>
<td>2-6</td>
</tr>
<tr>
<td>Pancreatic insufficient</td>
<td>$n = 10$</td>
<td></td>
</tr>
<tr>
<td>CF-related diabetes</td>
<td>$n = 3$</td>
<td>-</td>
</tr>
<tr>
<td>CF-related liver disease</td>
<td>$n = 3$</td>
<td>-</td>
</tr>
<tr>
<td>IVABs (days in last year)</td>
<td>10 ± 15</td>
<td>0-42</td>
</tr>
</tbody>
</table>

Values are means ± SD, unless otherwise stated.

CFTR, cystic fibrosis transmembrane conductance regulator; *P. Aeruginosa; Pseudomonas Aeruginosa*; Shwachman score - scoring 4 separate aspects of the disease profile; general activity; physical examination; nutritional status; and chest radiographic findings, using the most recent clinical review information. A total of 100 points represents a perfect score of health; IVABs, intravenous antibiotics; *According to Leeds Criteria, “chronic”, >50% of the preceding 12 months were P. aeruginosa culture positive; “intermittent”, ≤50% of the preceding 12 months were P. aeruginosa culture positive; “never”, no growth of P. aeruginosa for the previous 12 months, having previously been P. aeruginosa culture positive; “free”, P. aeruginosa has never been cultured.*

<sup>b</sup> Provides evidence of radiographic chest findings. Maximum score is 20, with 20 being the most severe.

6.2.2 Exercise testing protocol

Participants arrived to the laboratory in a rested state, ≥ 2 h postprandial and having refrained from caffeine for ≥ 2 h. A maximal CPET was performed on a cycle ergometer (Lode Excalibur or Lode Corival, Groningen, The Netherlands) using a single session, combined ramp incremental and $S_{max}$ CPET protocol, which has been validated in healthy children (Barker et al., 2011) and children and adolescents with CF (Chapter 4 - Saynor et al., 2013a). This protocol, the reproducibility of which has recently been documented in young CF patients (Chapter 5; Saynor et al., 2013b), involved an exhaustive ramp incremental (10-25 W·min$^{-1}$) cycling test with a
subsequent $S_{\text{max}}$ (110% $W_{\text{peak}}$) test to exhaustion to verify $\dot{V}O_{2\text{max}}$. After a 3 min warm-up period (20 W cycling), participants completed the incremental ramp test while cycling at a cadence of approximately 70-80 rpm until volitional exhaustion, defined as a drop in cadence ≥ 10 rpm for five consecutive seconds, despite strong verbal encouragement. Five minutes active recovery (20 W cycling) and 10 min passive seated recovery then preceded the $S_{\text{max}}$ verification test, which involved a 3 min warm-up (20 W cycling) before a ‘step’ transition to a CWR equivalent to 110% $W_{\text{peak}}$. Upon voluntary exhaustion, 5 min active recovery (20 W cycling) completed the CPET assessment.

6.2.3 Exercise testing measurements

Before each test, the metabolic cart (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany) was calibrated using gases of known concentration and a 3 L calibration syringe (Hans Rudolph, Kansas City, MO) was used to calibrate the turbine volume transducer. Breath-by-breath changes in pulmonary gas exchange and ventilation were measured and averaged to 15 s time bins, with the highest 15 s stationary average from the ramp or $S_{\text{max}}$ tests representing $\dot{V}O_{2\text{max}}$.

6.2.4 Data analysis

The GET in absolute terms and expressed as a percentage of $\dot{V}O_{2\text{max}}$ was non-invasively identified and confirmed through visual identification of the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$. The $\dot{V}O_2$ MRT was determined using the time from the onset of ramp exercise to the intersection point between baseline $\dot{V}O_2$ and a backward extrapolation of the slope of $\dot{V}O_2$ as a function of time (Wasserman et al., 2004). Regression of the linear portion of the $\dot{V}O_2$ response versus power output was
used to determine the functional $\dot{V}O_2$ gain ($\Delta\dot{V}O_2/\Delta WR$). Equation 6.1 was used to determine the $O_2$ pulse ($\dot{V}O_2/HR_{\text{peak}}$).

$$\dot{V}O_2/HR_{\text{peak}} \text{ (mL}$·$b^{-1}) = \dot{V}O_2 \text{ (L}$·$\text{min}^{-1}) \times 1000 \text{ mL} / \text{HR (b}$·$\text{min}^{-1})$$  \hspace{1cm} \text{Equation 6.1.}

**Near-infrared spectroscopy**

HHb dynamics from the *m. vastus lateralis* were noninvasively measured using NIRS (Portamon, Artinis Medical Systems). This system has previously been used in children (McNarry *et al.*, 2011) and consists of an emission probe, with three light sources emitting two wavelengths of light (760 and 850 nm) and a photon detector. The intensity of incident and transmitted light was recorded continuously at 10 Hz and used to estimate [HHb]. Since the NIRS-derived [HHb] signal does encompass contribution from intramyocyte myoglobin and does not solely reflect the microcirculatory compartment [vascular (Hb) deoxygenation] (Koga *et al.*, 2012), the changes in muscle [HHb] should be considered to represent [Hb+Mb]. The wireless emitter-detector unit was placed over the *m. vastus lateralis*, midway between the greater trochanter and lateral epicondyle of the femur. The area of interrogation was initially cleaned and shaved and, after marking of the placement area, the device was secured with tape (KinesioTex®) and a dark elastic bandage, to minimise extraneous light interference with the near-infrared signal.

**Additional measures**

HR was measured on a beat-by-beat basis using the ECG-derived R-R interval (PhysioFlow, PF-05, Manatec Biomedical, Paris, France). Fingertip SpO$_2$ was recorded via pulse oximetry (NONIN, Avant 4000, NONIN Medical Inc., USA).
Subjective ratings of RPE and RPD were determined upon exhaustion using the P-CERT and CR-10 scales, respectively, the methodology for which is described elsewhere.

**HHb modelling procedures**

Muscle [HHb] data were interpolated to 1 s intervals and averaged data (15 s) for the entire test were subsequently normalised to the total amplitude of the response (%Δ[HHb]), such that 0% represented steady-state values observed during the period of baseline (20 W) cycling and 100% represented the highest average (i.e., Δ[HHb]_peak) (Boone et al., 2009; Gravelle et al., 2012). The response was then expressed as a function of absolute and relative W_peak and \(\dot{V}O_{2max}\). Preliminary statistical analyses (GraphPad Prism, GraphPad Software, San Diego, CA) revealed that the sigmoid provided a superior fit to the [HHb] response when compared with bilinear or hyperbolic curve fitting procedures. The Δ[HHb] response to incremental ramp cycling exercise was therefore described using a sigmoidal model (Equation 6.2) in line with previous studies (Boone et al., 2009; Ferreira et al., 2007; McNarry et al., 2011) as follows:

\[
y = f_0 + A / (1 + e^{-(c+d\chi)}) \quad \text{Equation 6.2.}
\]

where \(f_0\) represents baseline [HHb], \(A\) the amplitude of the response, \(d\) the slope of the sigmoid, \(c\) the constant that is dependent on \(d\) and \(c/d\) the value corresponding to 50% of the total amplitude, respectively.
6.2.5 Statistical analysis

Log-linear allometric models were used to adjust $\dot{V}O_{2\max}$ for body size. The log-linear allometric model yielded a scaling exponent close to unity for FFM ($b = 1.03$), meaning the ratio standard method for normalising $\dot{V}O_{2\max}$ was deemed appropriate. Data are expressed as means and standard deviations unless otherwise stated. Independent samples $t$-tests (SPSS v19.0, Chicago, USA) derived $p$-values for subsequent inferential analyses. Inferential statistics, using 90% confidence intervals (CI) and the effect size (ES), were used to derive magnitude-based inferences regarding the true value of the observed effect statistic (Hopkins et al., 2009). Facilitated by a published Microsoft Excel® spreadsheet (Hopkins, 2007), any influence of CF on parameters of the [HHb] response and maximal and submaximal CPET parameters was calculated, using a 90% CI and the ES. Using a smallest worthwhile ES change of 0.2 (Cohen, 1988) and the 90% CI, the likelihood that the observed effect was beneficial (e.g. higher $\dot{V}O_{2\max}$, faster MRT), trivial or harmful (e.g. lower $\dot{V}O_{2\max}$, slower MRT) was reported. The qualitative terms used to inform these decisions were: < 0.5%, ‘most unlikely’; 0.5-5%, ‘very unlikely’; 5-25%, ‘unlikely’; 25-75%, ‘possibly’; 75-95%, ‘likely’; 95-99.5%, ‘very likely’; > 99.5%, ‘most likely’. An effect was deemed trivial when the majority (> 50%) of the 90% CI resided between beneficial and harmful. Conversely, an effect was deemed unclear when the likelihood of a beneficial and harmful effect was > 5%.

Hopkins’ published spreadsheet (Hopkins, 2007) was also used to determine the 90% CI for Pearson’s correlation coefficients to explore the relationship between key parameters of aerobic exercise function (i.e., $\dot{V}O_{2\max}, \dot{V}O_2$ gain, MRT and the GET) and mechanistically linked parameters of muscle $O_2$ extraction (e.g., $d$ and $c/d$ of the
[HHb] response) and O₂ delivery (e.g., end-exercise SpO₂ and O₂ pulse) in CF. Cohen’s thresholds (Cohen, 1988) for small (0.1), moderate (0.3), large (0.5) and very large (0.7) relationships describe the magnitude of correlations.

6.3 Results

Table 6.1 presents participants’ baseline physical characteristics, with Table 6.2 detailing the clinical profile of the patients with CF. BMI was likely higher, whereas FEV₁ (% predicted) was likely lower in CF than CON. Puberal maturity of both groups was as follows: pre-pubertal (n in CF = 3; n in CON = 1), circum-pubertal (n in CF = 7; n in CON = 8) and post-pubertal (n in CF = 0; n in CON = 1).

Maximal and submaximal CPET parameters are presented in Table 6.3. All participants completed CPET without any adverse events. Ramp PPO was possibly lower in CF and likely lower when expressed relative to body mass. As expected, CF presented with very likely reduced VO₂max, when normalised for both body mass and FFM. Furthermore, the VO₂ gain was very likely lower and the VO₂ MRT was likely slowed in CF. The RPD upon exhaustion was most likely higher in CF, respectively.

Parameter estimates for normalised muscle [HHb] as a function of absolute and percentage W_peak (%W_peak) and VO₂max (%VO₂max) are compared in Table 6.4, and the Δ[HHb]-work rate profile for two representative CF and CON matched pairs are shown in Figure 6.1. Any effect of the reduced aerobic fitness in patients with CF upon the slope (d) of the Δ[HHb]-work rate response was mechanistically unclear when expressed either as a function of absolute and percentage W_peak or absolute
and percentage \( \dot{VO}_{2\text{max}} \). Furthermore, the effect of CF upon the absolute and relative work rate and \( \dot{VO}_2 \) corresponding to 50\% [HHb] amplitude \((c/d)\) was mechanistically unclear.
Table 6.3. Maximal and submaximal physiologic responses of young patients with CF and healthy age- and gender-matched controls to ramp incremental cycle exercise.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CF (Mean ± SD)</th>
<th>CON (Mean ± SD)</th>
<th>Change, 90% CI</th>
<th>Inference</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximal exercise parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute $\dot{V}O_{2\max}$ (L·min$^{-1}$)</td>
<td>1.93 ± 0.84</td>
<td>2.21 ± 0.79</td>
<td>-0.29, ±0.63</td>
<td>Unclear</td>
<td>-0.34</td>
</tr>
<tr>
<td>Relative $\dot{V}O_{2\max}$ (mL·kg$^{-1}$·min$^{-1}$)</td>
<td>36.3 ± 7.6</td>
<td>43.9 ± 5.2</td>
<td>-7.6, ±5.1</td>
<td>Very likely lower</td>
<td>-1.11</td>
</tr>
<tr>
<td>$\dot{V}O_{2\max}$/FFM (mL·kg$^{-1}$·min$^{-1}$)</td>
<td>45.5 ± 9.1</td>
<td>53.5 ± 6.4</td>
<td>-7.9, ±6.1</td>
<td>Very likely lower</td>
<td>-0.96</td>
</tr>
<tr>
<td>Breathing reserve (%)</td>
<td>20.4 ± 19.9</td>
<td>12.4 ± 16.2</td>
<td>8.0, ±14.1</td>
<td>Unclear</td>
<td>0.42</td>
</tr>
<tr>
<td>HR$_{\max}$ (b·min$^{-1}$)</td>
<td>192 ± 11</td>
<td>190 ± 13</td>
<td>2, ±10</td>
<td>Unclear</td>
<td>0.18</td>
</tr>
<tr>
<td>$\dot{V}O_2$/HR$_{\max}$ (mL·b$^{-1}$)</td>
<td>9.78 ± 4.71</td>
<td>11.01 ± 3.39</td>
<td>-1.23, ±3.41</td>
<td>Unclear</td>
<td>-0.28</td>
</tr>
<tr>
<td>SpO$_2$ (%)</td>
<td>95 ± 2</td>
<td>97 ± 1</td>
<td>-3, ±1</td>
<td>Most likely lower</td>
<td>-1.63</td>
</tr>
<tr>
<td>Ramp W$_{\text{peak}}$ (W)</td>
<td>176 ± 94</td>
<td>205 ± 82</td>
<td>-30, ±69</td>
<td>Possibly lower</td>
<td>-0.32</td>
</tr>
<tr>
<td>Relative ramp W$_{\text{peak}}$ (W·kg$^{-1}$)</td>
<td>3 ± 1</td>
<td>4 ± 1</td>
<td>0.7, ±0.5</td>
<td>Likely lower</td>
<td>-0.84</td>
</tr>
<tr>
<td>RPE</td>
<td>9 ± 2</td>
<td>10 ± 1</td>
<td>-1, ±1</td>
<td>Unclear</td>
<td>-0.35</td>
</tr>
<tr>
<td>RPD</td>
<td>9 ± 2</td>
<td>6 ± 2</td>
<td>3, ±2</td>
<td>Most likely higher</td>
<td>1.46</td>
</tr>
<tr>
<td><strong>Submaximal exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\dot{V}O_2$ at the GET (L·min$^{-1}$)</td>
<td>1.13 ± 0.41</td>
<td>1.20 ± 0.30</td>
<td>-0.07, ±0.28</td>
<td>Unclear</td>
<td>-0.20</td>
</tr>
<tr>
<td>GET$<em>{%}$ (% of $\dot{V}O</em>{2\max}$)</td>
<td>61.3 ± 10.2</td>
<td>56.7 ± 8.4</td>
<td>4.6, ±7.3</td>
<td>Unclear</td>
<td>0.47</td>
</tr>
<tr>
<td>MRT (s)</td>
<td>49 ± 21</td>
<td>38 ± 11</td>
<td>11, ±13</td>
<td>Likely slower</td>
<td>0.63</td>
</tr>
<tr>
<td>$\Delta\dot{V}O_2$/ΔWR (mL·min$^{-1}$·W$^{-1}$)</td>
<td>7.62 ± 1.67</td>
<td>9.05 ± 1.17</td>
<td>-1.44, ±1.12</td>
<td>Very likely lower</td>
<td>-0.95</td>
</tr>
</tbody>
</table>

Values are means ± SD. RPE, RPD and SpO$_2$ were measured at the end of exercise. $\Delta\dot{V}O_2$/ΔWR, oxygen cost of exercise (efficiency); GET, non-invasive estimate of the lactate threshold which was verified by the ventilatory threshold; GET$_{\%}$, GET expressed as a percentage of $\dot{V}O_{2\max}$; MVV, maximal voluntary ventilation; $\dot{V}O_{2\max}$, maximal oxygen uptake; MVV, maximal voluntary ventilation; $\dot{V}O_2$/HR$_{\max}$, maximal oxygen pulse; $\dot{V}E$/ $\dot{V}CO_2$-slope, ventilatory drive.
Figure 6.1. Sigmoid models of normalised muscle deoxygenation (% Δ[HHb]) during ramp incremental exercise as a function of percentage peak oxygen uptake for two representative young patients with CF (●, black circles) of the leftward and rightward response patterns and their healthy age- and gender-matched control participants (dashed line; ○, white circles).
Correlational analyses within the CF group revealed small relationships between patients’ $\dot{V}O_{2\text{max}}$ and their [HHb] $c/d$, expressed as a function of $\%W_{\text{peak}}$ ($r = 0.14, \pm 0.58$) and $\%\dot{V}O_{2\text{max}}$ ($r = -0.21, \pm 0.57$), respectively. With the exception of the very large relationship between $\dot{V}O_{2}$ gain and [HHb] $c/d$ $\%W_{\text{peak}}$ ($r = 0.70, \pm 0.36$), relationships between $\dot{V}O_{2}$ gain and [HHb] $c/d$ $\%\dot{V}O_{2\text{max}}$ and the GET and MRT with [HHb] $c/d$ $\%W_{\text{peak}}$ and $\%\dot{V}O_{2\text{max}}$ were all small. A moderate relationship was observed between $\dot{V}O_{2\text{max}}$ and end-exercise SpO$_2$ ($r = 0.33, \pm 0.51$) in CF (Figure 6.2), however this was small ($r = 0.20, \pm 0.54$) in the healthy control group. The relationship between $\dot{V}O_{2\text{max}}$ and O$_2$ pulse was large in CF ($r = 0.58, \pm 0.41$; Figure 6.2) and CON ($r = 0.98, \pm 0.00$). Similarly, the relationships between $\dot{V}O_{2}$ gain and end-exercise SpO$_2$ and O$_2$ pulse were moderate ($r = 0.40, \pm 0.49$) and large ($r = 0.65, \pm 0.37$), respectively in CF, however these were small in CON ($r = -0.15, \pm 0.54$ and $r = 0.1, \pm 0.58$, respectively). Very large relationships were also evident between the GET and end-exercise SpO$_2$ ($r = -0.88, \pm 0.16$) and O$_2$ pulse ($r = 0.98, \pm 0.03$) in CF. The relationship between the GET and O$_2$ pulse was very large in CON ($r = 0.92, \pm 0.12$); however that with SpO$_2$ was small ($r = 0.1, \pm 0.55$). A moderate relationship ($r = 0.39, \pm 0.49$) was also evident between the $\dot{V}O_{2}$ gain and MRT in CF, however this was small in CON ($r = 0.01, \pm 0.55$).
Table 6.4. Parameter estimates for normalised muscle deoxygenation ($\Delta[\text{HHb}]$) as a function of absolute and percentage peak work rate during ramp incremental cycling and the absolute and normalised ratio of [HHb]-to-pulmonary oxygen uptake above and below the gas exchange threshold and at exhaustion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter expressed function of</th>
<th>CF ($n = 9$) (Mean ± SD)</th>
<th>CON ($n = 9$) (Mean ± SD)</th>
<th>Change, 90% CI</th>
<th>Inference (in CF)</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$ (%)</td>
<td>$W_{\text{peak}}$</td>
<td>100.1 ± 18.0</td>
<td>96.1 ± 8.1</td>
<td>4.0, ±11.8</td>
<td>Unclear</td>
<td>0.27</td>
</tr>
<tr>
<td>$d$ (%·W$^{-1}$)</td>
<td>$W_{\text{peak}}$</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.0, ±0.1</td>
<td>Unclear</td>
<td>0.40</td>
</tr>
<tr>
<td>$c/d$ (W)</td>
<td>$W_{\text{peak}}$</td>
<td>98 ± 52</td>
<td>112 ± 54</td>
<td>-14, ±44</td>
<td>Unclear</td>
<td>-0.25</td>
</tr>
<tr>
<td>$A$ (%)</td>
<td>$%W_{\text{peak}}$</td>
<td>100.0 ± 17.8</td>
<td>96.1 ± 8.1</td>
<td>3.9, ±11.7</td>
<td>Unclear</td>
<td>0.26</td>
</tr>
<tr>
<td>$d$ (%·$%W_{\text{peak}}^{-1}$)</td>
<td>$%W_{\text{peak}}$</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.0, ±0.0</td>
<td>Unclear</td>
<td>0.16</td>
</tr>
<tr>
<td>$c/d$ (%$%W_{\text{peak}}$)</td>
<td>$%W_{\text{peak}}$</td>
<td>47.1 ± 17.8</td>
<td>47.7 ± 9.1</td>
<td>-0.7, ±12.0</td>
<td>Unclear</td>
<td>-0.04</td>
</tr>
<tr>
<td>$A$ (%)</td>
<td>$\tilde{V}_{O_2\text{max}}$</td>
<td>88.2 ± 10.2</td>
<td>93.6 ± 6.8</td>
<td>-5.4, ±7.2</td>
<td>Likely lower</td>
<td>-0.59</td>
</tr>
<tr>
<td>$d$ (%·L$^{-1}$)</td>
<td>$\tilde{V}_{O_2\text{max}}$</td>
<td>13.3 ± 16.4</td>
<td>6.9 ± 4.3</td>
<td>6.3, ±10.4</td>
<td>Unclear</td>
<td>0.48</td>
</tr>
<tr>
<td>$c/d$ (L)</td>
<td>$\tilde{V}_{O_2\text{max}}$</td>
<td>1.27 ± 0.51</td>
<td>1.36 ± 0.52</td>
<td>-0.10, ±0.43</td>
<td>Unclear</td>
<td>-0.18</td>
</tr>
<tr>
<td>$A$ (%)</td>
<td>$%\tilde{V}_{O_2\text{max}}$</td>
<td>91.6 ± 9.0</td>
<td>93.5 ± 6.8</td>
<td>-1.9, ±6.6</td>
<td>Unclear</td>
<td>-0.23</td>
</tr>
<tr>
<td>$d$ (%·$%_{\text{max}}^{-1}$)</td>
<td>$%\tilde{V}_{O_2\text{max}}$</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.0, ±0.1</td>
<td>Unclear</td>
<td>0.38</td>
</tr>
<tr>
<td>$c/d$ (%$%_{\text{max}}$)</td>
<td>$%\tilde{V}_{O_2\text{max}}$</td>
<td>66.9 ± 8.5</td>
<td>63.6 ± 5.7</td>
<td>3.3, ±6.0</td>
<td>Unclear</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Values are means ± SD unless otherwise stated. Because of technical issues, [HHb] data are presented for nine matched pairs. $A$, amplitude of the change in the deoxygenated haemoglobin ($\Delta[\text{HHb}]$) response; $c$, constant that is dependent upon $d$ and where $c/d$ $x$-value corresponding to 50% $A$, respectively; $d$, slope of sigmoid.
Figure 6.2. The relationship between maximal oxygen uptake (\(\dot{V}O_{2\text{max}}\)) and changes in end-exercise arterial oxygen saturation (SpO\(_2\)) and the maximal oxygen pulse in young patients with cystic fibrosis.
6.4 Discussion

This is the first study to examine the influence of mild-to-moderate CF on the aerobic function and dynamic adjustments in localised muscle (vastus lateralis) fractional oxygen extraction ($\Delta[Hb]$) in paediatric patients during ramp incremental cycling exercise. As expected, CF patients were characterised by impaired aerobic exercise function, as displayed by a very likely reduced body mass or FFM normalised $\dot{V}O_{2\text{max}}$, a very likely lower $\dot{V}O_2$ gain, and likely slower $\dot{V}O_2$ MRT. Contrary to the experimental hypothesis, however, this reduced aerobic fitness status did not have a clear effect upon the dynamics of the $\Delta[Hb]$ during ramp incremental exercise. Specifically, no clear shift in $c/d$ of the [Hb] response was evident when expressed relative to percentage $W_{\text{peak}}$ or $\dot{V}O_{2\text{max}}$ and relationships with the key parameters of aerobic exercise function were small. Indicators of central $O_2$ delivery were, however, altered by CF. Specifically, end-exercise $SpO_2$ was most likely lower and correlated with $\dot{V}O_{2\text{max}}$ in the CF group only. Thus, these data show that the observed changes in the aerobic exercise function of paediatric patients with CF during incremental ramp cycling are likely related to alterations in muscle $O_2$ delivery, with no compensatory adjustment to the dynamics of muscle $O_2$ extraction within the microcirculation.

This study is unique for a number of reasons. It is the first to use a validated protocol (Chapter 4 - Saynor et al., 2013a) to document ‘true’ $\dot{V}O_{2\text{max}}$ in young patients with CF. Consistent with earlier reports (Keochkerian et al., 2008), $\dot{V}O_{2\text{max}}$ in patients with CF was very likely lower than that of CON in this study when normalised for both body mass and FFM. But importantly, the present results are robust because this is the first to include a $S_{\text{max}} \dot{V}O_{2\text{max}}$ verification phase (Chapter 4; Saynor et al., 2013a)
within the CPET protocol, thereby removing the issue of previous studies, where aerobic fitness status may have been underrepresented because of invalid verification criteria (Chapter 4; Saynor et al., 2013a).

Secondly, this study presents, for the first time, the four key parameters of aerobic function (Whipp et al., 1981), allowing a comprehensive assessment of aerobic fitness in this patient group. Of the additional key parameters, patients with CF presented with a very likely reduced \( \dot{V}O_2 \) gain and a likely slowed \( \dot{V}O_2 \) MRT, with no clear effect on the GET. While slower pulmonary \( \dot{V}O_2 \) kinetics have been documented in patients with CF during constant-load, moderate intensity cycling (Hebestreit et al., 2005), the present study extends these findings to the \( \dot{V}O_2 \) response at the onset of ramp incremental cycling. Although no clear influence upon the GET was evident, the functional gain during the moderate-intensity region of ramp exercise was very likely lower in CF, reflecting either an apparently greater skeletal muscle efficiency or impaired muscle \( O_2 \) consumption. Shallower \( \Delta \dot{V}O_2/\Delta WR \) slopes during exercise have previously been reported in patients with CF (Moser et al., 2000), congenital heart disease (Groen et al., 2010) and juvenile dermatomyositis (Groen et al., 2010). However, steeper \( \Delta \dot{V}O_2/\Delta WR \) responses have also been observed in young patients with CF (Groen et al., 2010). It would be misleading to interpret the reduced \( \Delta \dot{V}O_2/\Delta WR \) in the present findings as enhanced aerobic efficiency, particularly given the impairment in other parameters of aerobic function (i.e., \( \dot{V}O_{2max} \) and MRT). Given the moderate relationship between the \( \dot{V}O_2 \) gain and MRT in CF, the lower \( \Delta \dot{V}O_2/\Delta WR \) slope may be related to the slower pulmonary \( \dot{V}O_2 \) kinetics, such that the rise in \( \dot{V}O_2 \) was not sufficiently rapid to respond to the work rate increments during the CPET.
To our knowledge, this study is the first to report the $\Delta$[HHb] dynamics during ramp exercise in paediatric patients with CF. While pulmonary $\dot{V}O_2$ increased linearly with increasing work rate following an initial time lag, muscle $\Delta$[HHb] (reflecting the ratio of muscle blood flow to muscle $O_2$ utilisation (Table 6.4; Figure 6.1) increased in a nonlinear manner. This response was well characterised using a sigmoid function relative to work rate and $\dot{V}O_2$ in both groups in the current study, which is consistent with previous reports in children and young and old adults during ramp incremental cycling exercise (Boone et al., 2009; Gravelle et al., 2012; McNarry et al., 2011).

Contrary to the study hypothesis, the $\Delta$[HHb] dynamics during ramp exercise were similar between CF and CON in the present study. That is, no clear effect of CF upon either the absolute and relative work rate and $\dot{V}O_2$ corresponding to 50% $\Delta$[HHb] amplitude was observed. This is despite previous reports that aerobic fitness status has an effect upon the dynamic balance between $O_2$ supply and demand and, consequently, the sigmoidal pattern of $\Delta$[HHb] (Boone et al., 2009; McNarry et al., 2011). Boone et al. (2009) previously demonstrated that a higher aerobic fitness is associated with a rightward shift of the [HHb] sigmoidal response (relative to %$W_{peak}$) in healthy adults and that the response correlated with parameters of aerobic fitness (i.e., $\dot{V}O_{2max}$ and the GET). The purported mechanism for this rightward shift in the [HHb] response was attributed to a higher oxidative capacity and/or altered muscle fiber distribution. The rate of fractional oxygen extraction has been shown to be influenced by training status and enhanced $O_2$ delivery in trained versus untrained healthy girls (McNarry et al., 2011). Because $\dot{V}O_{2max}$ was meaningfully reduced in patients with CF in the present study, a more rapid increase in $\Delta$[HHb] during ramp exercise would be expected. However, a previous study (Gravelle et al., 2012)
comparing older (approximately 70 y) and younger (approximately 25 y) healthy adults, which observed alterations when expressed relative to absolute power output, did not observe any age-related differences in ∆[HHb] response dynamics when expressed as a function of %Wpeak, despite a reduced $\dot{V}O_{2\text{max}}$ in the older participants (30 vs. 49 mL·kg$^{-1}$·min$^{-1}$).

Some caution may, however, be applied when considering the present findings to suggest that there are no differences between the ∆[HHb] response of healthy and CF children and adolescents. Inter-patient differences (Figure 6.1) in the ∆[HHb] response suggest that the interpretation that the rate of muscle O$_2$ extraction is unaltered by CF may be too simplistic. When the distribution of the 90% CI for the effect of CF on the ∆HHb c/d was expressed relative to percentage $\dot{V}O_{2\text{max}}$, the majority of the 90% CI distribution favoured a reduced rate of extraction (leftward shift: 9%; trivial: 22%; rightward shift: 69%). The unclear statistical outcome is, therefore, likely to reflect the large inter-patient variability present for this outcome (see Figure 6.1). Indeed, inter-patients differences are not improbable given the complex nature and varied clinical presentation of CF disease, meaning further comment on the responses shown in Figure 6.1 may be of clinical interest. Patient A, who has a left shift on the ∆[HHb] response, is a physically mature boy with few complications and excellent pulmonary function. In contrast, patient B (also male), whose ∆[HHb] response is shifted to the right has poorer pulmonary function, a worse chest x-ray score, nutritional concerns, and complications including CF-related liver disease and impaired glucose tolerance. This is reflected in patient B having received 28 dys of intravenous antibiotics, which signifies treatment intensification, within the preceding year. However, despite patient B's poorer clinical profile, his
\( \dot{V}O_{2\text{max}} \) is markedly higher than that of patient A (49.4 vs. 32.6 mL·kg\(^{-1}\)·min\(^{-1}\)), which may have played a role in causing the rightward shift in the \( \Delta [\text{HHb}] \) response dynamics. However, it should be noted that we only found a small relationship between \( \dot{V}O_{2\text{max}} \) and \([\text{HHb}] \ c/d\) in the present study.

Interestingly, the present findings of unaltered \([\text{HHb}]\) response dynamics for the CF group in the present study are in line with a previous report by Rosenthal et al. (2009), who observed similar \( O_2 \) extraction dynamics during exercise in young patients with CF and their healthy counterparts, despite presenting with impaired aerobic function. When considered in reference to the Fick equation, these data therefore suggest that the impaired aerobic exercise function characterising young patients with CF is caused by a reduction in \( O_2 \) delivery. Altered cardiac function (Benson et al., 1984; Ionescu et al., 2001; Pianosi & Pelech, 1996) and an inability to augment SV during exercise (Rosenthal et al., 2009), which are likely to reduce central \( O_2 \) delivery, have previously been documented in patients with CF, and the most likely lower \( \text{SpO}_2 \) in the present study provides further support. Although it has been propositioned that patients with CF can achieve apparently “normal” \( \dot{Q} \) in the presence of reduced SV during exercise, through elevated HR (Ionescu et al., 2001; Lands et al., 1992), this compensation only seems viable at submaximal exercise intensities, as both CF and CON had similar HR responses at maximal exercise in the present study. In accordance with this findings, a reduced (approximately 24%) estimated SV (derived using respiratory mass spectroscopy) at maximal exercise in young patients with CF coupled with a similar HR response to healthy controls has previously been documented (Rosenthal et al., 2009).
Although it has been hypothesised that patients limited by O₂ delivery during exercise would present with a compensatory increase in O₂ extraction at the local level (Ferreira et al., 2007), both this study and the previous study by Rosenthal et al. (2009), using respiratory mass spectroscopy, observed no augmentation of O₂ extraction in the face of inadequate O₂ delivery during exercise (Rosenthal et al., 2009). Although the previous authors could not determine the cause of this because no direct peripheral measurements were made, it was suggested that muscle metabolic issues resulting from chronic bronchial sepsis may contribute. Importantly, the present study utilising NIRS corroborates the observations of Rosenthal et al. (2009) using a more direct measurement technique.

The relationships between parameters of aerobic exercise function and mechanistic parameters indicative of O₂ delivery and extraction further emphasise the importance of O₂ delivery in explaining the impaired aerobic function in young patients with CF. Although the relationships between key parameters of aerobic exercise function and the rate of peripheral fractional O₂ extraction were small, stronger relationships with parameters of O₂ delivery were evident. Furthermore, while SpO₂ did not correlate with FFM normalised $\dot{V}O_{2\text{max}}$ in healthy controls, a moderate correlation with end-exercise SpO₂ was observed in patients with CF, along with a large relationship between $\dot{V}O_{2\text{max}}$ and the O₂ pulse. The relationships between $\dot{V}O_2$ gain and end-exercise SpO₂ and O₂ pulse were also moderate and large, respectively. Similarly, very large relationships were evident between the GET and end-exercise SpO₂ and O₂ pulse.
There are several limitations of NIRS, which must be acknowledged. First, measurements are restricted to a specific area of interrogation over a, in this case, single heterogenous and superficial muscle, which may not represent whole body skeletal muscle blood flow responses. However, the muscle deoxygenation response measured in the superficial and deeper muscle fibres using NIRS has been shown to reflect muscle oxygenation as measured using phosphorous quenching derived microvascular $O_2$ partial pressure within the same region of muscle (Koga et al., 2012). Although inter-site variation in the [HHb] response cannot be directly rectified, the device was secured to the same anatomical region for all participants to eradicate inter-individual regional differences within the m. vastus lateralis. Although the influence of adipose tissue at the area of interrogation was not directly determined, in line with recommendations, responses were standardised to the total [HHb] amplitude to provide a physiologic normalisation (Boone et al., 2009). Finally, the generalisability of these findings should be viewed in light of the small sample of Northern European patients with CF recruited for this study.

6.5 Conclusion

To conclude, this was the first study to examine the influence of mild-to-moderate CF on key parameters of aerobic function in paediatric patients. As expected, paediatric patients with CF presented with impaired aerobic exercise function compared with that of their healthy counterparts. Specifically, $\dot{V}O_2\text{max}$ and the $\dot{V}O_2$ gain were very likely reduced and the MRT likely slowed. However, in contrast to the study hypothesis, NIRS derived [HHb] dynamics during ramp incremental cycling exercise were similar between CF and CON. The present findings support the notion of
centrally mediated $O_2$ delivery principally limiting the aerobic exercise function of young patients with CF during ramp incremental cycling exercise.

6.6 **Practical implications**

- Aerobic fitness ($\bar{V}O_2$max) measurement can help predict survival in CF.
- Even relatively well, habitually active children and adolescents with mild-to-moderate CF present with impaired aerobic exercise function compared with their healthy peers.
CHAPTER SEVEN

The Effect of Ivacaftor in Adolescents with Cystic Fibrosis (G551D mutation): An Exercise Physiology Perspective

This experimental study has been disseminated as follows:


7.1 Introduction

CF is caused by mutations in the gene encoding the CFTR protein. Traditional therapies focus on alleviating manifestations secondary to CFTR dysfunction. A new oral treatment (Ivacaftor, Vertex Pharmaceuticals, Boston, Massachusetts) has been licensed specifically for those with the G551D-CFTR mutation. Ivacaftor, a CFTR "potentiator", increases the open time of activated CFTR at the cell surface, restoring Cl⁻-transport activity of the G551D-CFTR protein (van Goor *et al.*, 2009).

To date, sustained improvements in QoL, incidence of pulmonary exacerbations, respiratory symptoms, pulmonary function, body mass, and biomarkers of CFTR activity (sweat Cl⁻ and nasal potential difference) have been reported following treatment with Ivacaftor in patients that are heterozygous for the G551D mutation with mild-to-moderately impaired pulmonary function, without substantial adverse effects (*Accurso et al.*, 2010; *Davies et al.*, 2013; *McKone et al.*, 2012; *Ramsey et al.*, 2015).
More recently, administration of Ivacaftor has also revealed clinical improvements in severely ill patients (Hebestreit et al., 2013) and a G551D homozygote (Harrison et al., 2013).

Although common clinical assessments such as spirometry and body mass provide key endpoints for the evaluation of new CF treatments, their sensitivity to detect change in early disease has been questioned (Welsh, 2010). Furthermore, measurements of lung function cannot accurately predict patients' exercise capacity. Aerobic fitness (\(\dot{V}O_{2\text{max}}\)) is of particular clinical importance in patients with CF given its association with longevity (Nixon et al., 1992; Pianosi et al., 2005), QoL (de Jong et al., 1997) and reduced risk of hospitalisation (Pérez et al., 2014). However, exercise testing as an outcome in both physical therapy practice and therapeutic trials remains in its infancy (Bell & Morris, 2010). Understanding how the clinical alterations evident following pharmacological or physical therapy treatment translate to changes in patients' physical function is important.

Only 1 previous study investigating the effects of Ivacaftor has incorporated an exercise testing measure (Harrison et al., 2013), documenting a 292% (+ 410 m) improvement from baseline in the distance achieved during the 6MWT in a female adult (G551D homozygote) following 12 months of treatment. Although tests such as this are common practice within physical therapy for individuals with CF, a number of methodological issues accompany these crude tests, which must be considered when used in this context. First, these tests are often subjective and submaximal in nature and fail to quantify physiologically a maximal effort. Second, the derived parameters are limited to HR and SpO\(_2\), which are often not presented and do not
provide physiological data to support the mechanism(s) responsible for any observed change.

Maximal CPET, incorporating measurement of gas exchange and ventilation, provides the most precise measurement of aerobic fitness. Much of the value of CPET resides in its capacity to describe the integrated function of the pulmonary, cardiovascular and muscular systems during exercise. Moreover, in addition to \( \dot{V}O_2 \text{max} \), additional key parameters of aerobic exercise function can also be obtained, such as the \( O_2 \) cost of exercise (exercise efficiency) (Whipp et al., 1981). In response to the ECFS Clinical Trials Network Standardisation Committee’s call to assess the validity, reproducibility and feasibility of outcome measures to be used in CF, a valid protocol for use with young patients with CF was recently presented (Chapter 4 -Saynor et al., 2013a). Furthermore, the TEs associated with the derived outcome measures have since been presented (Chapter 5 - Saynor et al., 2013b), enabling meaningful change from therapeutic or physical therapy interventions to now be ascertained. However, to our knowledge there are no reports of effect of Ivacaftor on patients’ aerobic fitness assessed using the reference standard CPET.

The purpose of this report was to provide novel data from CPET in 2 teenage patients with CF (\( \Delta F508/G551D \)) treated with Ivacaftor to demonstrate (1) the effects of Ivacaftor on aerobic exercise function and (2) the possible factor(s) modulating this response. By answering these questions, the report will provide novel data on the utility and feasibility of CPET as a clinical outcome measure.
7.2 Methods

7.2.1 Participants, anthropometry and pulmonary function

Case A: A 14 y old female climbing enthusiast had presented with neonatal meconium ileus requiring bowel resection. She suffered a complicated clinical course, with early *Pseudomonal* and then *Stenotrophomononal* respiratory infections, allergic bronchopulmonary aspergillosis, and more recently *Mycobacterium abscessus* infection that could not be eradicated. Despite preserved pulmonary function (FEV\(_1\) 92% predicted), thoracic HRCT detailed extensive bronchiectasis and consolidation in right middle and lingual lobes (Figure 7.1a). BMI was 20.3 kg·m\(^2\) (> 50\(^{th}\) centile). Sweat Cl\(^-\) measured 104 mmol·L\(^{-1}\) pre-treatment. Routine maintenance medications included the following: pancrelipase (10, 000 and 40, 000 in various combinations with meals and snacks), vitamin E (200 units alternate days), vitamin A and D gel (1 daily), ursodeoxycholic acid (450 mg twice daily), polyethylene glycol solution (1 sachet daily), azithromycin (500 mg daily), doxycycline (100 mg once daily), meropenem (nebuliser 250 mg twice daily), dornase alpha (nebuliser 2.5 mg once daily), hypertonic saline (7% nebulised [4 ml] once or twice daily with physical therapy), beclomethasone (200 μg twice daily via spacer), salbutamol (2-4 puffs when required for wheezing), and amphotericin (nebulised 20 mg alternate days, nonliposomal formulation).

Case B: An active 16 y old male presented at the age of 16 months with recurrent respiratory infections and failure to thrive. He has suffered recurrent *Pseudomonal* infections from an early age but has remained well with aggressive treatments (FEV\(_1\) 108% predicted). Thoracic HRCT showed widespread bronchiectatic changes but without significant consolidation (Figure 7.1b). BMI was 19.7 kg·m\(^2\) (50\(^{th}\) centile).
Sweat Cl− measured 107 mmol·L−1 pre-treatment. Routine maintenance medications included the following: pancrelipase (10,000 and 40,000 in various combinations with meals and snacks), vitamin A and D gel caps (3 daily), vitamin E (200 units daily), vitamin K (10 mg daily), Fortisip Compact nutritional supplement (with Creon 2 daily), colomycin (nebulised 2 mega units mixed with gentamicin 80 mg twice daily), dornase alpha (nebulised 2.5 mg once daily), fluocloxacillin (500 mg twice daily).

Figure 7.1. High-resolution computed tomography images for case A and case B pretreatment with orally administered ivacaftor. Case A microbiology: *Mycobacterium abscessus, Stenotrophomas maltophilia*, allergic bronchopulmonary aspergillosis in remission. Case B microbiology: Intermittent *Pseudomonas Aeruginosa* and previous *Achromobacter xylosidans*. 
Body mass (Seca 220; Vogel & Halke, Hamburg, Germany) and stature (Seca 220; Vogel & Halke, Hamburg, Germany) were measured to the nearest 0.01 kg and 0.01 m, respectively. FEV₁ and FVC were assessed using spirometry (MicroMedical MicroLoop 3535). The best of three consistent (< 5% variability) exhalations was documented and expressed as a percentage of predicted reference data (Stanojevic et al., 2009).

7.2.2 Description of Intervention

The main goal of this intervention was to assess the influence of orally administered Ivacaftor treatment (150 mg 12 hourly) on CPET-derived measures of aerobic exercise function in 2 young patients with CF in conjunction with common clinical outcome measures. To monitor the effects of treatment, the 2 teenage patients, both compound heterozygotes (G551D/ΔF508), underwent routine clinical assessments for a period of 20 weeks.

In addition to this, CPET was performed before and after (6 and 12 weeks) initiating orally administered Ivacaftor treatment to assess whether any change in aerobic exercise function was evident and, if so, the physiological factor(s) responsible for this. These time points for reassessment were implemented to enable comparison of intervention-induced changes with the TEs of the CPET measurements established in this patient population over this time period to identify clinically meaningful changes (Chapter 5; Saynor et al., 2013b). The patients continued their normal maintenance medications as required and continued with their typical physical activity and nutritional intake patterns. Additional measurements of central \( O_2 \)
delivery) and peripheral (O₂ extraction / utilisation) factors that can influence \( \dot{V}O_{2\text{max}} \) were also obtained to understand the mechanism(s) responsible for any change.

### 7.2.3 Exercise testing protocol

Participants were instructed to arrive at the exercise laboratory in a rested state, > 2 h postprandial and having refrained from caffeine for > 2 h. Following thorough familiarisation with the equipment and requirements of the visit, a maximal CPET was performed on a cycle ergometer [Lode Excalibur or Lode Corival, Groningen, The Netherlands]. A single-session protocol, encompassing a ramp incremental test (10-25 W·min\(^{-1}\)) and a S\(_{\text{max}}\) (110% \( W_{\text{peak}} \)) verification phase that has been validated in this patient population (Chapter 4; Saynor et al., 2013a) was used. Following a 3 min warm-up (20 W cycling), the incremental ramp test was completed until exhaustion whilst pedalling between 70 to 80 rpm. Exhaustion was defined as a drop in pedal speed of more than 10 rpm for 5 consecutive seconds, despite strong verbal encouragement. Participants then completed 5 min active recovery (20 W cycling) and 10 min passive seated recovery before completing the S\(_{\text{max}}\) verification test. S\(_{\text{max}}\) involved a 3 min warm-up (20 W cycling), an exhaustive ‘step’ transition to a CWR equivalent to 110% \( W_{\text{peak}} \) from the ramp test, followed by 5 min active recovery (20 W cycling).

### 7.2.4 Exercise testing measurements

**Pulmonary gas analysis.** Prior to each exercise test, a metabolic cart (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany) was calibrated using gases of known concentration, and the turbine volume transducer using a 3 L calibration syringe (Hans Rudolph, Kansas City, MO). Breath-by-breath pulmonary gas exchange and
ventilation were measured and averaged to 15 s time bins. The highest 15 s stationary average $\dot{V}O_2$ from the combined ramp and $S_{max}$ exercise tests was taken to represent $\dot{V}O_{2max}$, a safe and appropriate $\dot{V}O_{2max}$ verification criterion in this population (Chapter 4 - Saynor et al., 2013a). The primary outcome measure, given its clinical important in CF, was $\dot{V}O_{2max}$. However, additional submaximal parameters of aerobic fitness were also derived. The LT was noninvasively identified using the GET (Beaver et al., 1986) and confirmed through visual inspection of the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$. The $\dot{V}O_2$ gain ($\Delta \dot{V}O_2/\Delta WR$), a measure of exercise efficiency, was determined by regression of the linear portion of the $\dot{V}O_2$ response against power output.

Additional mechanistic measures: Thoracic bioelectrical impedance cardiography (PhysioFlow, PF-05, Manatec Biomedical, Paris, France), which has been validated in CF (Pianosi, 1997), was used to noninvasively measure beat-by-beat HR, SV and $\dot{Q}$, which was subsequently averaged to 15 s time intervals. $C_{(a-\overline{v})O_2}$, a measure of $O_2$ extraction, was estimated via rearrangement of the Fick equation:

$$(C_{(a-\overline{v})O_2}) = \frac{\dot{V}O_2}{\dot{Q}}$$

$SpO_2$ at the fingertip was measured on a beat-by-beat basis via pulse oximetry (NONIN, Avant 4000, NONIN Medical Inc., USA). Subjective ratings of RPE and RPD were recorded upon exhaustion using methodology described elsewhere (Chapters 4 and 5; Saynor et al., 2013a; Saynor et al., 2013b). All procedures and protocol were approved by the institutional ethics committee and informed parental consent and participant assent were obtained prior to the commencement of the study.
7.3 Results

The 2 patients’ clinical and exercise characteristics at baseline and in response to 12 weeks of treatment with Ivacaftor are presented in Table 7.1. Figure 7.2 presents the percentage change in BMI, FEV₁ and \( \dot{V}O_{2\text{max}} \) during 12 weeks of treatment. BMI and FEV₁ were then monitored during follow-up to week 20. The magnitude of change in these measurements is presented in relation to the established TE of measurement using these procedures over a 4-6 week period. All exercise testing was well tolerated with no adverse events, and all tests satisfied the criteria for the provision of a maximal effort. However, case B reported to his 12 week CPET feeling fatigued.

Case A

This patient experienced 2 successive URTIs (weeks 3 and 10) during treatment. Despite this, her pulmonary function and body mass were maintained when she would typically deteriorate. Following the first 6 weeks of treatment, her weight had increased from 48.5 kg to 50.5 kg, while predicted FEV₁ increased from 92% to 96%. A fall in her sweat Cl⁻ (104 to 21 mmol·L⁻¹) was noted at this time point. Following 6 weeks of treatment her body mass normalised \( \dot{V}O_{2\text{max}} \) had increased by 6.4% from baseline, which was not considered a clinically a meaningful improvement since it resides within the TE of this measurement (Chapter 5; Saynor et al., 2013b). End-exercise SpO₂ upon exhaustion increased from 92% to 96%. Because of a combination of leg fatigue (9 out of 10) and dyspnoea (rating of 7 out of 10) CPET was terminated.

At the 12 week assessment, subjectively she reported feeling better and more “energetic” and was slightly more productive with airway clearance physiotherapy.
Her FEV₁ (%predicted) and body mass showed moderate though convincing increases (+ 4.7% relative and + 1.7 kg, respectively). A small increase in sweat Cl⁻ (21 to 35 mmol·L⁻¹) was evident. Although there was minimal influence upon W_peak, subjective ratings of exertion and dyspnoea or the additional submaximal parameters of aerobic exercise function (GET and V̇O₂ gain) at this time point, her body mass normalised V̇O₂max had increased by 30.3% from pre-treatment baseline. This substantial increase was deemed clinically meaningful since the change over this 6 week period exceeded the TE (13.3%) of measurement established over this duration (Chapter 5 - Saynor et al., 2013b). Furthermore, end-exercise SpO₂ had improved to 98% from 95% pre-treatment. By this point, her W_peak had also increased by 9.0% (12 W) and her rating of dyspnoea had improved from 7 to 5. Little change was detected in the submaximal parameters of aerobic exercise function.
Figure 7.2. Percentage change from baseline in body mass index, forced expiratory volume in 1 second (percentage predicted (Stanojevic et al., 2009)) and body mass normalised maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) in 2 patients with cystic fibrosis patients (CF) with the G551D-CFTR mutation [A (14 y female; ● black circles) and B (16 y male; ○ white circles)] at the start of Ivacaftor (day 0) and following 6, 12 and 20 weeks of treatment. Exercise testing was not performed at 20 weeks and the magnitude of change is presented in relation to the typical error of measurements in young patients with CF over a 4 to 6 week period (Saynor et al., 2013b).
Table 7.1. The clinical and exercise-based response of 2 paediatric cystic fibrosis patients (A, 14 y female; B, 16 y male) with the ΔF508/G551D mutation to 6 and 12 weeks of oral Ivacaftor treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case A Pre-Ivacaftor</th>
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<th>Case A 12 weeks post</th>
<th>Case B Pre-Ivacaftor</th>
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<tr>
<td>FEV₁ (%) predicted</td>
<td>2.53 (92)</td>
<td>2.62 (96)</td>
<td>2.65 (97)</td>
<td>4.19 (108)</td>
<td>4.33 (112)</td>
<td>4.65 (120)</td>
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<td>[L·min⁻¹ (%predicted)]</td>
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<tr>
<td>FVC (%) predicted</td>
<td>3.20 (100)</td>
<td>3.36 (105)</td>
<td>3.32 (107)</td>
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<td>[L·min⁻¹ (%predicted)]</td>
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<td>FEF₂₅₋₇₅ (%) predicted</td>
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<td>2.38 (67)</td>
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<td>4.77 (106)</td>
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<td>107</td>
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<td>172.0</td>
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<tr>
<td>Absolute  VO₂max (L·min⁻¹)</td>
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<td>1.95</td>
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<td>202</td>
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<td>19.0</td>
<td>15.5</td>
<td>18.2</td>
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<tr>
<td>a·VO₂ diff. (mL·min⁻¹·100mL)</td>
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<td>13.6</td>
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<td>96</td>
<td>98</td>
<td>96</td>
<td>96</td>
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<tr>
<td>RPD</td>
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<td>6</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>9</td>
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<tr>
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<td>129</td>
<td>148</td>
<td>220</td>
<td>225</td>
<td>240</td>
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<tr>
<td>VO₂ at the GET (L·min⁻¹)</td>
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<td>0.85</td>
<td>0.87</td>
<td>1.32</td>
<td>1.28</td>
<td>1.09</td>
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<tr>
<td>%GET (% of VO₂max)</td>
<td>60.12</td>
<td>52.99</td>
<td>44.55</td>
<td>51.05</td>
<td>54.78</td>
<td>44.72</td>
</tr>
<tr>
<td>ΔVO₂/ΔWR (mL·min⁻¹·W⁻¹)</td>
<td>7.20</td>
<td>8.00</td>
<td>7.40</td>
<td>9.32</td>
<td>8.46</td>
<td>6.52</td>
</tr>
</tbody>
</table>

Values are means ± SD, with the range also displayed unless otherwise stated. %predicted, percentage predicted (Stanojevic et al., 2009);
Of the factors which can affect case A’s $\dot{V}O_{2\text{max}}$, a change was observed in both central ($O_2$ delivery) and peripheral ($O_2$ extraction) indices. With regard to $O_2$ delivery, a slight reduction in HR was evident at weeks 6 and 12 (205 b·min$^{-1}$ to 202 b·min$^{-1}$ at both tests). However, since SV was increased at both time points [52 mL·b$^{-1}$ to 56 (+ 7%) and 60 (+ 15%) mL·b$^{-1}$], $\dot{Q}$ was improved as a consequence [10.6 L·min$^{-1}$ to 10.8 and 11.6 L·min$^{-1}$ (+ 2% and + 9%, respectively)]. Arterial $O_2$ desaturation upon exhaustion was also reduced during the 12 weeks of treatment, with $SpO_2$ rising from 92% to 96% and 98% at weeks 6 and 12, respectively. Estimated $O_2$ extraction ($C_{(a-e)}O_2$) was also increased at both week 6 [+ 1 mL·min$^{-1}$·100mL (+ 8%) and week 12 [+ 3 mL·min$^{-1}$·100mL (+ 23%)]. This change in physiological function detected through CPET following 12 weeks of treatment preceded the later rise detected in FEV$_1$ (+ 19% from baseline) following 20 weeks (Figure 7.2). Her body mass also increased further to 52.4 kg (+ 3.9 kg from baseline) at this stage.

**Case B**

This patient was clinically well throughout treatment and body mass and pulmonary function remained stable. Following 6 weeks of treatment, his weight remained stable at 58.3 kg while lung function (FEV$_1$) improved from 108% to 112% predicted. A notable fall in sweat Cl$^-$ (107 to 58 mmol·L$^{-1}$) was also evident in this patient at this time point. A modest improvement in his body mass normalised $\dot{V}O_{2\text{max}}$ from baseline was evident (+ 3.4%); however, this was not considered clinical meaningful. End-exercise $SpO_2$ was unchanged at 96% and CPET was terminated because of both leg fatigue (RPE of 9) and dyspnoea (RPD of 7).
Following 12 weeks of treatment, he reported feeling clinically well; however he was tired because of heavy school and football workloads over the preceding weeks. In a patient who has difficulty maintaining weight, he had gained 0.5 kg by week 12. FEV₁ had also increased from 108% predicted at baseline to 120% predicted and sweat chloride concentration has reduced further to 43 mmol·L⁻¹. Although W_peak increased by 9% from baseline (20 W) and SpO₂ at exhaustion had improved from 96% to 98%, his body mass normalised V̇O₂max was marginally reduced (- 5.1% from baseline) as were the submaximal indicators of aerobic fitness. However, this should not be considered a true impairment of aerobic exercise function as it is within the TE of these measurements (Chapter 5 - Saynor et al., 2013b). While his perceived dyspnoea upon exhaustion was higher (7-9), RPE remained stable at 9.

Although modest improvement was observed in his systemic O₂ delivery (Q̇ and SpO₂), this appeared to fluctuate around baseline. Maximal Q̇ was 19.0 L·min⁻¹ at baseline and then 15.5 and 18.2 L·min⁻¹ following 6 and 12 weeks of treatment, respectively. HR and SV remained relatively stable at week 6 (- 3 b·min⁻¹ and + 5 mL·b⁻¹, respectively). However, at 12 weeks his SV was increased to 104 mL·b⁻¹ and maximal HR was substantially lower at 175 b·min⁻¹, meaning Q̇ was not particularly influenced. Furthermore, following increased extraction at 6 weeks (+ 3 mL·min⁻¹·100mL), this was near baseline by week 12 (- 0.2 mL·min⁻¹·100mL). No clinically significant change in SpO₂ was observed. Continued clinical monitoring to week 20 then revealed a steady increase in body mass to gain 3.2 kg from baseline and increase the relative change from baseline in FEV₁% predicted to 6.7%.
7.4 Discussion

The aim of this case report was to describe the effects of orally administered Ivacaftor on the aerobic exercise function and clinical profile of 2 teenage patients with CF (A, 14 y old female; B, 16 y old male) who were heterozygous for the G551D mutation. Furthermore, this report aimed to demonstrate the utility of CPET as a clinical outcome measure. Following 12 weeks of treatment with Ivacaftor, both patients showed substantial improvements in sweat Cl\(^-\). Despite case A experiencing 2 successive URTIs, pulmonary function and body mass were maintained when she would typically deteriorate. Case B was clinically well throughout treatment, with his body weight and lung function stable throughout. Following 12 weeks of treatment, no meaningful change was observed in \(\dot{V}O_{2\text{max}}\) in case B. In case A, however, \(\dot{V}O_{2\text{max}}\) increased by 30.3%, which should be considered clinically meaningful because it is 20% greater than the 4 to 6 week TE associated with this measurement. This improvement resulted from both enhanced muscle \(O_2\) delivery and muscle \(O_2\) extraction.

There could be numerous explanations for the varied response observed between these patients. Firstly, at outset, both patients presented with mildly impaired pulmonary function. However, case A’s pulmonary function was a little lower, evidence of active underlying infection with \(M. Abscessus\) was present and thoracic HRCT identified more severe lung damage with patchy parenchymal inflammatory changes. Although established lung damage cannot directly be rectified, this patient may well have had more to gain from this new, transformational treatment. In an earlier Ivacaftor clinical trial cohort (Ramsey et al., 2011), improvements in sweat Cl\(^-\) and FEV\(_1\) were seen to plateau after 2 weeks. Because case B’s pulmonary function
at baseline was higher than the patients in this initial study by Ramsey et al. (2011), this may explain why a plateau was observed in his response.

Conceivably, an individual ceiling effect for \( \dot{V}O_{2\text{max}} \) improvements may exist, whereby relatively fit patients have less to gain in the absence of exercise training, and that case B’s original fitness status resided around this threshold. As such, case A’s \( \dot{V}O_{2\text{max}} \) normalised to body mass was lower than case B at baseline, of which gender difference may be a factor. An impact of overreaching or chronic fatigue in case B also cannot be excluded. Although a higher \( W_{\text{peak}} \) was documented, this patient reported to the exercise laboratory for his week 12 CPET feeling tired, due to school and football workloads. His lower maximal HR (\( \sim 20 \text{ b} \cdot \text{min}^{-1} \)) compared with his previous CPETs may support this. Interestingly, this reduced response has previously been observed in this patient when he previously performed 2 CPETs over a short-term period. This stresses the importance of CPET standardisation when interpreting “true” physiological changes in results. Although measures of \( O_2 \) delivery, extraction and minimum SpO\( _2 \) in this patient all fluctuated around baseline, without meaningful change, SV was elevated following 12 weeks. However, given that maximal HR was lower, his resulting \( Q \) was not increased.

To our knowledge, the only existing evidence of Ivacaftor’s effect upon aerobic fitness was demonstrated in a 19 y old G551D homozygote with poor pulmonary function using the 6MWT [292\% (+ 410 m) improvement from baseline] (Harrison et al., 2013). However, in contrast to this study, the shuttle walk assessment by Harrison et al. (2013) was undertaken following 12 months of treatment. However, although only presented graphically, the authors’ figure indicates that exercise
testing was also performed at approximately 2 and 10 weeks. Interestingly, the majority of the patient’s improvement in aerobic fitness occurred within the time period spanning these 2 time points (approximately +225 m (week 2) and +310 m (week 10) from baseline, respectively). Only further longitudinal study would confirm the inter-patient variability observed within the present case report and determine whether Ivacaftor could sustain patients’ aerobic fitness following initial improvements. However, data from the Harrison et al. (2013) study are promising, presenting a modest but steady improvement from approximately 10 weeks to 52 weeks of treatment. Of additional interest is the relatively fixed status of the submaximal indices of aerobic function (GET and $\dot{V}O_2$ gain) in contrast to the acute improvement in $V_{O2max}$. Whether these parameters respond over a longer duration warrants further exploration.

The data from this study are clinically useful for a number of reasons. Firstly, they provide novel data regarding the mechanisms by which Ivacaftor may enhance patients’ physiological function during exercise. The magnitude of change in case A was particularly impressive given that 1) she was in a state of URTI during the majority of her treatment, 2) improvements cannot simply be attributable to a learning effect or initial submaximal effort, because both patients were thoroughly familiarised with the protocol that encompasses a verification phase to confirm “true” $V_{O2max}$ (Chapter 5; Saynor et al., 2013a); and 3) no exercise training intervention was undertaken outside the patients’ typical physical activity routine.

Sparse data exists concerning the magnitude of change in $V_{O2max}$ of young patients with CF following pharmacological or exercise interventions. To date, only 1 previous
study has demonstrated a meaningful improvement in \( \dot{V}O_{2\text{max}} \) in young patients with CF and this was following an intense 6 week period of exercise training. Hulzebos et al. (2011) reported a “meaningful” improvement (19%), following a high-intensity cycling exercise training programme. This training intervention resulted in an indication of enhanced \( O_2 \) delivery to the active muscle tissue, evidenced by the \( O_2 \) pulse. It was suggested that \( O_2 \) extraction was also influenced; however, only data for the \( \dot{V}O_2 \) gain was presented, which provides a measure of submaximal \( O_2 \) consumption and exercise efficiency.

An additional important purpose of the present report was to demonstrate the utility of CPET to make inferences regarding therapeutic interventions or disease-related changes. Although case A reported feeling better and more “energetic” at week 12, no clinical improvement was detected using standard spirometric indices until week 20. However, CPET did document substantial improvement in her physiological function. The fact that her \( \dot{V}O_{2\text{max}} \) improvement was out of proportion with early pulmonary function changes, although the latter did pick up during extended follow-up, demonstrated the capacity of this integrated testing to detected subtle changes in patients that are relatively well earlier than common clinical outcomes. Furthermore, although more common clinical exercise tests are often cost-effective and easily conducted, a CPET can provide a wealth of mechanistic information that cannot be derived from standard clinical assessments or crude exercise tests such as shuttle walk or step protocols. In addition, although such tests can be used to estimate \( \dot{V}O_{2\text{max}} \), they are likely to underestimate aerobic fitness and cannot truly verify a maximal effort. Owing to its merits, the ECFS Exercise Working Group recently
promoted CPET as the exercise testing method of choice where possible for this patient group.

Aerobic fitness is an important clinical parameter in CF and should become an important outcome within the physiotherapy assessment of patients. Although the present report focused on the utility of CPET to assess the response to Ivacaftor, more common practices such as IVABs and physical therapy interventions warrant detailed assessment. For example, the present patient reporting feeling more energised is a common response during treatment with IVABs, particularly electively. However, patients must often continue treatment until a change in pulmonary function is observed. CPET may provide a more sensitive outcome measure to detect subtle changes earlier.

It is acknowledged that case study data are limited in their generalisability to the wider patient population. Furthermore, the follow-up time was relatively short and it would have been of interest to have performed CPET at week 20. In addition, no measurements of habitual physical activity were obtained to see whether improved exercise capacity translated into increased levels or intensity of physical activity.

Given the ongoing change in stance from the ECFS regarding the clinical relevance of CPET in CF, it is likely that this will become a routine assessment method over the coming years. If more physical therapists involved in the management and treatment of this condition can adopt this form of testing, this would be of great benefit. It is hoped that this study demonstrates how insightful and relatively straightforward
CPET is and will encourage more physical therapists to adopt it in clinical practice and as an investigative tool.

7.5 Conclusion

These cases demonstrate that not only does Ivacaftor have a substantial beneficial effect on the sweat Cl− of patients with CF and the G551D mutation, but clinically meaningful improvement in aerobic fitness can also be observed in the absence of exercise training. These changes manifest earlier than current clinical outcomes and result from both improved muscle O2 delivery and extraction during exercise. However, a fitness threshold may exist whereby patients who are relatively fit experience less or no improvement. Importantly, this case review highlights that CPET can provide an additional important clinical outcome measure to assess functional change and with this the mechanism(s) responsible for change. CPET can detect substantial changes in aerobic fitness, which may occur independently from adaptations in pulmonary function, as was evidenced in 1 of the present patients. To objectively quantify the influence of pharmacological or physiotherapy interventions on patients’ physiological function, the use of CPET is encouraged. CPET should be included within future, long-term research demonstrating its utility within physiotherapy practice, pharmacological and/or exercise interventions.

7.6 Practical implications

- Aerobic fitness (VO2max) measurement can help predict survival in CF.
- Ivacaftor may be able to significantly improve VO2max in young patients with mild-to-moderate CF and the G551D mutation in the absence of exercise training.
• Ivacfator may improve both cardiac function, which will improve the delivery of O₂, and also the ability of skeletal muscles to extract and use O₂ during exercise.

• CPET should be included within future, long-term research demonstrating its utility within clinical practice, pharmacological or exercise interventions.
CHAPTER EIGHT

Impaired Pulmonary \( O_2 \) Uptake Kinetics in Young Patients with Cystic Fibrosis depend on Exercise Intensity

This experimental study has been disseminated as follows:


**Poster Presentation:** Saynor, Z. L., Barker, A. R., Oades, P. J. & Williams, C. A. (2013). Exercise limitation in paediatric patients with mild-to-moderate cystic fibrosis. The University of Exeter Medical School Medical and Health Research Showcase, Exeter, UK.


8.1 Introduction

\(  \dot{V}O_{2\max} \) is clinically important in patients with CF, given associations with prognosis (Pianosi *et al.*, 2005), risk of hospitalisation (Pérez *et al.*, 2014) and health-related QoL (de Jong *et al.*, 1997). \( \dot{V}O_{2\max} \) by definition does not, however, represent the rate at which aerobic energy transfer adapts to the changing metabolic demands facing \( O_2 \) transport and utilisation during everyday life. In contrast, assessing the dynamic adjustment in pulmonary \( \dot{V}O_2 [\tau \text{ for the primary component (phase II)}] \) at the
onset of exercise provides a non-invasive insight into \( m\dot{V}O_2 \) dynamics (Krustrup et al., 2009) and the breakdown of muscle [PCr] (Barker et al., 2008; Rossiter et al., 2002). Consequently, this parameter can provide insight into the factor(s) mediating muscle metabolic function and the integration of the respiratory, cardiovascular and muscular systems can also be characterised. Although the \( \dot{V}O_2 \) kinetic response and mechanism(s) regulating the rate of rise are relatively well documented in healthy children, there is limited evidence in young people with CF.

Slower \( \dot{V}O_2 \) kinetics have been reported in children and adolescents with CF during incremental (Fielding et al., 2015; Saynor et al., 2014), PRBS (Kusenbach et al., 1999) and CWR exercise (Armeniakou et al., 2015; Hebestreit et al., 2005). However, in contrast a similar response to healthy controls has also been documented in young people with CF (11-15 y) during intense exercise (Braggion et al., 1989). Methodological issues may explain these disparities. Firstly, during incremental and PRBS exercise the phase II portion of the \( \dot{V}O_2 \) response was not isolated, which is critical to reflect the kinetics of \( m\dot{V}O_2 \) (Krustrup et al., 2009). Secondly, the CWR exercise study by Hebestreit and colleagues (2005) did not prescribe work rate within physiologically defined exercise intensity domains, semi-recumbent cycling was used which may negate muscle \( O_2 \) delivery during exercise, and a mixed age group of 10-33 y, which would comprise a range of pulmonary function characteristics, were tested (Hebestreit et al., 2005).

According to the Fick principle, the rate of adjustment in \( \dot{V}O_2 \) is dictated by \( O_2 \) delivery and utilisation mechanisms. Although the Fick equation has been used to provide mechanistic interpretation during incremental exhaustive exercise in CF (e.g.
Chapter 7; Saynor et al., 2014), few studies have applied this to understand how CF modifies the \( \dot{V}O_2 \) kinetic response to CWR exercise (Williams et al., 2014). Slower \( \dot{V}O_2 \) kinetics in young patients with CF have been linked to inadequate \( O_2 \) delivery (Hebestreit et al., 2005), inferred from \( SpO_2 \). Although young patients with CF may present with early signs of cardiovascular abnormalities (Giacchi et al., 2015; Poore et al., 2013; Tousson et al., 1998), impaired skeletal muscle oxidative capacity in CF has also been shown (de Meer et al., 1995; Erickson et al., 2015; Rosenthal et al., 2009; Saynor et al., 2014a (Chapter 6); Wells et al., 2011). However, contrasting data has reported no differences in skeletal muscle oxidative function in children and adolescents with CF compared with their healthy peers (Werkman et al., 2015). The NIRS-derived muscle deoxygenation (\( \Delta[HHb] \)) signal provides insight into the ratio of local muscle \( O_2 \) delivery to muscle \( O_2 \) utilisation. Thus, changes in muscle HHb are considered to represent changes in muscle \( O_2 \) extraction dynamics during exercise (e.g. Ferreira et al., 2007). Although it has been hypothesised that a more rapid increase in muscle HHb dynamics would be evident in the face of reduced central or muscle \( O_2 \) delivery (Ferreira et al., 2007), as evidenced in Chapter 6, children and adolescents with CF do not appear able to compensate by increasing muscle \( O_2 \) extraction during exhaustive ramp incremental exercise (Rosenthal et al., 2009; Saynor et al., 2014). This raises questions regarding the capacity of CF skeletal muscle to increase muscle \( O_2 \) extraction during exercise, but this has yet to be evaluated alongside pulmonary \( \dot{V}O_2 \) kinetics during CWR cycling exercise.

Consequently, this study aimed to characterise the pulmonary \( \dot{V}O_2 \) kinetic response of children and adolescents with mild-to-moderate CF at the onset of MOD and VH intensity exercise. It was hypothesised that: 1) a longer phase II \( \dot{V}O_2 \) \( \tau \) at the onset of
MOD and VH exercise would be evident in CF; 2) slower $\dot{Q}$ (inferred from the CI) and more rapid [HHb] kinetics would be evident in CF during MOD and VH exercise; and 3) slower $\dot{V}O_2$ kinetics would relate to reduced $\dot{Q}$ and altered muscle $\Delta[Hb]$ dynamics in the CF group.

8.2 Methods

8.2.1 Participants, anthropometry and pulmonary function

Seven young people with stable, mild-to-moderate CF (Tables 8.1 and 8.2) and 7 controls (CON) (Table 8.2) participated. Inclusion and exclusion criteria are detailed in Chapter 3. Neither group presented with any contraindications to exercise. Ethics approval was granted by the South West NHS Research Ethics Committee. Informed written consent was obtained from parent(s)/guardian(s) and participants. Participants attended the laboratory five times over a 2 week period, at a similar time of day and separated by 24-48 h. Participants were advised to arrive rested and hydrated, > 2 h postprandial and having refrained from caffeine for > 2 h.
Table 8.1. Baseline clinical characteristics for the young CF patients upon initiation into the study.

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</tr>
<tr>
<td>∆F508/ P67L</td>
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<td>Chronic <em>P. Aeruginosa</em> infection †</td>
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<td>“intermittent,” n = 2</td>
<td>“never,” n = 1</td>
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<td>Shwachman score</td>
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<tr>
<td>Northern score b</td>
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<td>Pancreatic insufficient</td>
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<tr>
<td>CF-related diabetes</td>
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<tr>
<td>CF-related liver disease</td>
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</tr>
<tr>
<td>IVABs (days in last year)</td>
<td>11 ± 9</td>
<td>0-24</td>
</tr>
</tbody>
</table>

Values are means ± SD, with the range also displayed where suitable, unless otherwise stated. CFTR, cystic fibrosis transmembrane conductance regulator; *P. Aeruginosa*; *Pseudomonas Aeruginosa*; Shwachman score - scoring 4 separate aspects of the disease profile; general activity; physical examination; nutritional status; and chest radiographic findings, using the most recent clinical review information. A total of 100 points represents a perfect score of health; IVABs, intravenous antibiotics; † According to Leeds Criteria, “chronic”, > 50% of the preceding 12 months were *P. aeruginosa* culture positive; “intermittent”, ≤50% of the preceding 12 months were *P. aeruginosa* culture positive; “never”, no growth of *P. aeruginosa* for the previous 12 months, having previously been *P. aeruginosa* culture positive; “free”, *P. aeruginosa* has never been cultured. b Provides evidence of radiographic chest findings. Maximum score is 20, with 20 being the most severe.
Table 8.2. Baseline anthropometric, pulmonary function and maximal cardiopulmonary exercise testing data for young patients and healthy age- and gender-matched control participants upon initiation into the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CF Mean ± SD</th>
<th>CON Mean ± SD</th>
<th>p-value</th>
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<tr>
<td>Gender</td>
<td>5 M, 2 F</td>
<td>5 M, 2 F</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (y)</td>
<td>13.5 ± 2.80</td>
<td>13.6 ± 2.40</td>
<td>0.93</td>
<td>-0.04</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.61 ± 0.20</td>
<td>1.62 ± 0.17</td>
<td>0.92</td>
<td>-0.05</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>60.7 ± 22.8</td>
<td>52.4 ± 17.8</td>
<td>0.46</td>
<td>0.38</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>22.6 ± 4.5</td>
<td>19.4 ± 2.9</td>
<td>0.14</td>
<td>0.79</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>49.5 ± 19.9</td>
<td>40.1 ± 12.5</td>
<td>0.30</td>
<td>0.54</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.91 ± 1.29</td>
<td>4.08 ± 1.50</td>
<td>0.82</td>
<td>-0.12</td>
</tr>
<tr>
<td>FVC (% predictedᵃ)</td>
<td>106 ± 10</td>
<td>107 ± 17</td>
<td>0.82</td>
<td>-0.11</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>3.27 ± 1.00</td>
<td>3.59 ± 1.26</td>
<td>0.61</td>
<td>-0.26</td>
</tr>
<tr>
<td>FEV₁ (% predictedᵃ)</td>
<td>102 ± 6</td>
<td>110 ± 12</td>
<td>0.17</td>
<td>-0.72</td>
</tr>
<tr>
<td>CPET parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>𝜃O₂max (L·min⁻¹)</td>
<td>2.08 ± 0.74</td>
<td>2.51 ± 0.91</td>
<td>0.34</td>
<td>-0.49</td>
</tr>
<tr>
<td>𝜃O₂max (mL·kg⁻¹·min⁻¹)</td>
<td>34.30 ± 8.88</td>
<td>47.75 ± 3.56</td>
<td>&lt; 0.01</td>
<td>-1.79</td>
</tr>
<tr>
<td>𝜃O₂max/FFM (mL·kg⁻¹·min⁻¹)</td>
<td>51.87 ± 34.90</td>
<td>65.52 ± 24.65</td>
<td>0.42</td>
<td>-0.42</td>
</tr>
<tr>
<td>𝜃O₂ at the GET (L·min⁻¹)</td>
<td>1.09 ± 0.31</td>
<td>1.38 ± 0.48</td>
<td>0.20</td>
<td>-0.67</td>
</tr>
<tr>
<td>GET% (% of 𝜃O₂max)</td>
<td>53.7 ± 6.4</td>
<td>55.2 ± 3.3</td>
<td>0.57</td>
<td>-0.28</td>
</tr>
<tr>
<td>Ramp Wpeak (W)</td>
<td>162 ± 61</td>
<td>208 ± 86</td>
<td>0.27</td>
<td>-0.58</td>
</tr>
<tr>
<td>Ramp TTE (s)</td>
<td>546 ± 111</td>
<td>729 ± 113</td>
<td>0.01</td>
<td>-1.52</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>95 ± 3</td>
<td>98 ± 1</td>
<td>0.04</td>
<td>-1.23</td>
</tr>
</tbody>
</table>

Values are means ± SD unless otherwise stated. M, males; F, females; BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; CPET, cardiopulmonary exercise testing;  𝜃O₂max, maximal oxygen uptake; FFM, fat-free mass; GET, gas exchange threshold; MRT, mean response time; Wpeak, peak work rate; TTE, time to exhaustion; Sₘₐₓ, supramaximal verification phase; SpO₂%, arterial oxygen saturation. ᵃAccording to Stanojevic et al. (2009).
8.2.2 Exercise testing protocol

Following anthropometric and pulmonary function measurements, the combined ramp incremental and $S_{max}$ CPET that was established in Chapters 4 and 5 was used to determine $\dot{V}O_2\text{max}$ and the GET (Saynor et al., 2013a; Saynor et al., 2013b). All exercise was performed on a cycle ergometer (Lode, Groningen, The Netherlands).

Participants completed MOD and VH CWR exercise tests, comprising 6 min unloaded pedalling (10 W), followed by transitions to elicit $\dot{V}O_2$ amplitudes corresponding to 90% GET and $\Delta60\%$ (60% of the difference between the GET and $\dot{V}O_2\text{max}$) for 6 min. This equated to MOD work rates of $58 \pm 24$ W and $73 \pm 35$ W for CF and CON, respectively. During VH, CF and CON cycled at $121 \pm 43$ W and $150 \pm 64$ W, respectively. Thirty minutes rest separated the MOD and VH transitions.

8.2.3 Exercise testing measurements

Body mass (Seca 220; Vogel & Halke, Hamburg, Germany) and stature (Seca 220; Vogel & Halke, Hamburg, Germany) were measured to the nearest 0.01 kg and 0.01 m. Skinfold measurements (Harpenden; British Indicators, Burgess Hill, UK) were used to estimate percentage body fat (Slaughter et al., 1988). FVC and FEV$_1$ were assessed using spirometry (Micromedical Microloop 3535, Numed, Sheffield, UK), and expressed as a percentage predicted (Stanojevic et al., 2009).

Breath-by-breath changes in gas exchange and ventilation were determined using a metabolic cart (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany), which was calibrated each test using gases of known concentration and a 3 L syringe (Hans...
Rudolph, Kansas City, MO). Fingertip SpO\textsubscript{2} was measured using pulse oximetry (NONIN, Avant 4000, NONIN Medical Inc., USA). A near-infrared spectrometer (Portamon, Artinis Medical Systems) was used to non-invasively measure [HHb] at the \textit{m. vastus lateralis}. Details regarding this system and the site preparation/placement are provided earlier in this thesis (Chapters 2 and 3). Beat-by-beat changes in HR, SV and \(\dot{Q}\) were measured using thoracic bioelectrical impedance cardiography (PhysioFlow, PF-05, Manatec Biomedical, Paris, France), further details of which are provided in Chapters 2 and 3.

8.2.4 Data analysis

\textbf{CPET parameters of aerobic function.} As validated in Chapter 4, the highest 15 s averaged \(\dot{V}O_2\) from the ramp and \(S_{\text{max}}\) tests was taken to represent \(\dot{V}O_{2\text{max}}\) (Saynor \textit{et al.}, 2013) and was normalised to FFM using the ratio standard method. The GET was identified using the V-slope method (Beaver \textit{et al.}, 1986) and confirmed through visual inspection of the ventilatory equivalents for \(\dot{V}O_2\) and \(\dot{V}CO_2\).

\textbf{Pulmonary \(\dot{V}O_2\) kinetics.} Breath-by-breath changes in \(\dot{V}O_2\) were analysed using methodology previously described by our laboratory (Barker \textit{et al.}, 2010; Breese \textit{et al.}, 2012). The four repeat transitions for both MOD and VH were linearly interpolated to 1 s, time aligned to exercise onset (i.e., \(t = 0\) s) and ensemble averaged. The 1 s averaged \(\dot{V}O_2\) response for the MOD and VH conditions for each participant were then baseline corrected, by subtracting the mean \(\dot{V}O_2\) between -60 and -5 s from the exercise response. The duration of phase I was visually assessed to account for the cardiodynamic contribution to the \(\dot{V}O_2\) kinetic response. The first \(21 \pm 3\) s and \(17 \pm 4\) s of the MOD data and the first \(19 \pm 5\) s and \(16 \pm 2\) s for VH were
omitted in CF and CON participants, respectively. The phase II portion of the \( \dot{\text{V}} \text{O}_2 \) response was then characterised using Equation 2.5 (GraphPad Prism; GraphPad Software, San Diego, CA), where \( \dot{\text{V}} \text{O}_2 \ (t), \Delta \dot{\text{V}} \text{O}_2 \ A, \ TD, \) and \( \tau \) represent the value of \( \dot{\text{V}} \text{O}_2 \) at a given time \( (t) \), the amplitude change in \( \dot{\text{V}} \text{O}_2 \) from baseline to its asymptote, time delay, and the time constant of the response, respectively.

The MRT was derived to describe the overall kinetics during both intensities of exercise, by constraining the \( TD \) in Equation 1 to 0 s and fitting from exercise onset to 6 min. The functional gain of phase II was determined by dividing the phase II \( \dot{\text{V}} \text{O}_2 \) amplitude by the change in work rate above baseline. End-exercise \( \dot{\text{V}} \text{O}_2 \) gain was calculated in a similar manner. For VH exercise, the \( \dot{\text{V}} \text{O}_2 \) slow-component onset and amplitude were determined in line with previous work from our laboratory (Barker et al., 2010; Breese et al., 2010). The \( \dot{\text{V}} \text{O}_2 \) slow-component was expressed in both absolute terms and relative to end-exercise \( \dot{\text{V}} \text{O}_2 \).

Muscle oxygenation. NIRS data were collected at 10 Hz, interpolated to 1 s intervals and expressed as a change, in arbitrary units (a.u.), from baseline. Subsequently, [HHb] profiles were 5 s averaged, time aligned to exercise onset and ensemble averaged to yield a single response. The dynamics of [HHb] were modelled in a similar manner to \( \dot{\text{V}} \text{O}_2 \), with slight modification as detailed in Chapter 3 (Barker et al., 2010).

Heart rate, stroke volume, cardiac output and \( C_{(a\overline{v})\text{O}_2} \). Beat-by-beat changes in HR, SV and \( \dot{Q} \) were linearly interpolated to 1 s, time aligned and ensemble averaged to 30 s. The \( C_{(a\overline{v})\text{O}_2} \) was estimated via rearrangement of the Fick equation \[ C_{(a\overline{v})\text{O}_2} = \dot{\text{V}} \text{O}_2 / \dot{Q} \]. SV and \( \dot{Q} \) were normalised to FFM (Dewey et al., 2008) using the ratio
standard method, to determine the CI and SVI. The \( \dot{Q}/\dot{V}O_2 \) ratio was used to provide an index of muscle \( O_2 \) availability relative to metabolic rate.

### 8.2.5 Statistical analysis

Independent samples \( t \)-tests examined mean differences between CF and CON. Additionally, \( ES (d) \) statistics determined the magnitude of the effect, using a pooled SD. The magnitude of the difference between variables of interest were explored using \( ES \) thresholds of trivial \(< 0.2\), small \( > 0.2\), moderate \( > 0.5\), large \( > 0.8\), and very large \( > 1.0\) \( (Cohen, 1992) \). Changes in HR, SV, \( \dot{Q} \) and \( C_{(a-v)}O_2 \) were analysed using mixed model ANOVA. Significant interactions were followed up using independent samples \( t \)-tests. Pearson’s correlation coefficients assessed relationships between \( \dot{V}O_2 \) kinetics and mechanistic parameters of \( O_2 \) delivery and utilisation. Statistical analyses were performed using SPSS (version 19.0, SPSS, Chicago, IL), with the null-hypothesis rejected at alpha level of 0.05.

### 8.3 Results

**Maximal cardiopulmonary exercise testing:** Descriptive characteristics and CPET data are presented in Table 8.2. There were no differences in body size and composition and pulmonary function between CF and CON. \( \dot{V}O_{2\max} \) was reduced in CF compared with CON when normalised using body mass but not FFM.

**Pulmonary \( \dot{V}O_2 \) kinetics:** The \( \dot{V}O_2 \) responses during MOD and VH are presented in Figure 8.1 and the kinetic parameters in Table 8.3. There was no significant difference in baseline \( \dot{V}O_2 \) between the groups for either MOD or VH exercise (Table 8.3). For MOD, CF had no influence on either the phase II \( \tau \), TD or MRT. However,
the phase II $\dot{V}O_2$ gain was lower in CF. During VH, the $\dot{V}O_2$ MRT and phase II $\tau$ were slower in CF. The phase II $TD$, amplitude and gain and end-exercise $\dot{V}O_2$ were not altered in CF. A $\dot{V}O_2$ slow-component manifested in all VH responses, however the amplitude was similar between CF and CON.

**Muscle oxygenation kinetics:** The group mean data for [HHb] and the corresponding kinetic parameters are shown in Figure 8.2 and Table 8.4, respectively. The [HHb] response of one CF patient (male, 10 y, $\Delta$F508 homozygote) did not display exponential characteristics and was, in addition to their healthy control, excluded from [HHb] analyses. There was no difference between CF and CON for any of the [HHb] kinetic parameters during MOD or VH exercise.

**Heart rate, stroke volume index, cardiac index and $C_{(a-v)}O_2$:** Group mean HR, CI, SVI and $C_{(a-v)}O_2$ dynamics are presented in Figure 8.3. No significant time by disease state interaction effect for SVI was evident during either MOD ($p = 0.09$) or VH ($p = 0.27$). During VH there was a significant interaction between time and disease state for HR ($p = < 0.01$), with follow-up comparisons identifying a higher HR in CF at 30 s ($p < 0.05$). There was a significant main effect for disease state ($p = 0.01$) for CI to be lower in CF during MOD but not VH ($p > 0.05$). There was a time by disease state interaction ($p = 0.03$) for $C_{(a-v)}O_2$ during VH (Figure 8.3), with extraction significantly reduced in CF at 30 s ($p = 0.02$) and a trend towards reduced values at 0 s ($p = 0.07$), 60 s ($p = 0.05$) and 120 s ($p = 0.07$). $\dot{Q}/\dot{V}O_2$ was not different between the groups for either intensity ($p > 0.05$).

**Relationships between $\dot{V}O_2$ kinetics and mechanistic parameters:** During MOD, the phase II $\dot{V}O_2$ $\tau$ significantly correlated with $\Delta$[HHb] ($r = 0.84; p = 0.04$) in CF,
whilst the MOD $\dot{V}O_2$ MRT correlated with $\Delta SVI$ in CON ($r = -0.81; p = 0.03$). During VH, the $\Delta C_{(\bar{v})}O_2$ significantly correlated with the phase II $\dot{V}O_2\tau$ ($r = -0.85; p = 0.02$) and MRT ($r = -0.79; p = 0.03$) in CF. Furthermore, $\Delta Q/\Delta V\dot{O}_2$ ($r = 0.78; p = 0.04$) significantly correlated with the phase II $\dot{V}O_2\tau$ in CF during VH exercise.
Table 8.3. Pulmonary oxygen uptake kinetics in CF and CON during MOD and VH exercise.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CF Mean ± SD</th>
<th>CON Mean ± SD</th>
<th>p-value</th>
<th>ES (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Moderate intensity exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline $\dot{V}O_2$ (L·min$^{-1}$)</td>
<td>0.62 ± 0.13</td>
<td>0.68 ± 0.16</td>
<td>0.47</td>
<td>-0.38</td>
</tr>
<tr>
<td>Phase II $\dot{V}O_2$ $\tau$ (s)</td>
<td>25.2 ± 6.7</td>
<td>26.1 ± 8.4</td>
<td>0.84</td>
<td>-0.11</td>
</tr>
<tr>
<td>Phase II $\dot{V}O_2$ $TD$ (s)</td>
<td>21.7 ± 7.3</td>
<td>14.8 ± 7.1</td>
<td>0.10</td>
<td>0.90</td>
</tr>
<tr>
<td>Phase II $\dot{V}O_2$ amplitude (L·min$^{-1}$)</td>
<td>0.42 ± 0.20</td>
<td>0.64 ± 0.34</td>
<td>0.16</td>
<td>-0.76</td>
</tr>
<tr>
<td>Phase II $\dot{V}O_2$ gain (mL·min$^{-1}$·W$^{-1}$)</td>
<td>8.6 ± 1.3</td>
<td>10.4 ± 1.4</td>
<td><strong>0.03</strong>*</td>
<td>-1.21</td>
</tr>
<tr>
<td>$\dot{V}O_2$ mean response time (s)</td>
<td>44.6 ± 8.0</td>
<td>41.6 ± 8.9</td>
<td>0.52</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>Very heavy intensity exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline $\dot{V}O_2$ (L·min$^{-1}$)</td>
<td>0.68 ± 0.15</td>
<td>0.76 ± 0.19</td>
<td>0.37</td>
<td>-0.46</td>
</tr>
<tr>
<td>Phase II $\dot{V}O_2$ $\tau$ (s)</td>
<td>37.5 ± 10.8</td>
<td>25.1 ± 6.5</td>
<td><strong>0.02</strong>*</td>
<td>1.28</td>
</tr>
<tr>
<td>Phase II $\dot{V}O_2$ $TD$ (s)</td>
<td>14.7 ± 6.1</td>
<td>14.6 ± 3.0</td>
<td>0.96</td>
<td>0.03</td>
</tr>
<tr>
<td>Phase II $\dot{V}O_2$ amplitude (L·min$^{-1}$)</td>
<td>0.97 ± 0.34</td>
<td>1.26 ± 0.54</td>
<td>0.25</td>
<td>-0.60</td>
</tr>
<tr>
<td>Phase II $\dot{V}O_2$ gain (mL·min$^{-1}$·W$^{-1}$)</td>
<td>8.9 ± 0.8</td>
<td>9.2 ± 1.2</td>
<td>0.51</td>
<td>-0.34</td>
</tr>
<tr>
<td>$\dot{V}O_2$ slow-component onset (s)</td>
<td>144 ± 29</td>
<td>117 ± 21</td>
<td>0.07</td>
<td>0.99</td>
</tr>
<tr>
<td>$\dot{V}O_2$ slow-component amplitude (L·min$^{-1}$)</td>
<td>0.19 ± 0.15</td>
<td>0.16 ± 0.13</td>
<td>0.74</td>
<td>0.17</td>
</tr>
<tr>
<td>$\dot{V}O_2$ slow-component relative amplitude (%)</td>
<td>9.0 ± 6.3</td>
<td>6.7 ± 3.8</td>
<td>0.43</td>
<td>0.40</td>
</tr>
<tr>
<td>End-exercise $\dot{V}O_2$ (L·min$^{-1}$)</td>
<td>1.83 ± 0.59</td>
<td>2.18 ± 0.83</td>
<td>0.38</td>
<td>-0.46</td>
</tr>
<tr>
<td>End-exercise $\dot{V}O_2$ gain (mL·min$^{-1}$·W$^{-1}$)</td>
<td>9.9 ± 1.3</td>
<td>10.3 ± 0.9</td>
<td>0.56</td>
<td>-0.30</td>
</tr>
<tr>
<td>$\dot{V}O_2$ mean response time (s)</td>
<td>74.6 ± 19.4</td>
<td>51.6 ± 8.3</td>
<td><strong>0.01</strong>*</td>
<td>1.40</td>
</tr>
</tbody>
</table>
Table 8.4. Muscle oxygenation kinetics of young CF patients and healthy control participants at the onset of moderate and very heavy intensity cycling exercise.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CF (Mean ± SD)</th>
<th>CON (Mean) ± SD</th>
<th>p-value</th>
<th>ES (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Moderate intensity exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline [HHb] (a.u.)</td>
<td>-0.01 ± 0.08</td>
<td>0.01 ± 0.03</td>
<td>0.74</td>
<td>-0.17</td>
</tr>
<tr>
<td>Phase II [HHb] τ (s)</td>
<td>12.5 ± 8.8</td>
<td>9.0 ± 5.0</td>
<td>0.42</td>
<td>0.44</td>
</tr>
<tr>
<td>Phase II [HHb] TD (s)</td>
<td>14.8 ± 3.3</td>
<td>11.9 ± 5.6</td>
<td>0.30</td>
<td>0.57</td>
</tr>
<tr>
<td>[HHb] mean response time (s)</td>
<td>27.3 ± 7.6</td>
<td>20.9 ± 2.2</td>
<td>0.08</td>
<td>0.99</td>
</tr>
<tr>
<td>Phase II [HHb] amplitude (a.u.)</td>
<td>1.63 ± 1.73</td>
<td>2.76 ± 1.75</td>
<td>0.29</td>
<td>-0.60</td>
</tr>
<tr>
<td>[HHb] slow-component onset (s) a</td>
<td>104 ± 19</td>
<td>103 ± 30</td>
<td>0.95</td>
<td>0.04</td>
</tr>
<tr>
<td>[HHb] slow-component amplitude (a.u.) b</td>
<td>0.30 ± 0.58</td>
<td>0.51 ± 0.50</td>
<td>0.43</td>
<td>-0.33</td>
</tr>
<tr>
<td>[HHb] slow-component relative amplitude (%) b</td>
<td>11.37 ± 6.90</td>
<td>18.10 ± 5.27</td>
<td>0.25</td>
<td>-0.86</td>
</tr>
<tr>
<td>End-exercise [HHb] (a.u.)</td>
<td>1.99 ± 2.12</td>
<td>3.12 ± 1.91</td>
<td>0.35</td>
<td>-0.52</td>
</tr>
<tr>
<td><strong>Very heavy intensity exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline [HHb] (a.u.)</td>
<td>0.00 ± 0.03</td>
<td>0.02 ± 0.03</td>
<td>0.60</td>
<td>0.29</td>
</tr>
<tr>
<td>Phase II [HHb] τ (s)</td>
<td>13.0 ± 12.4</td>
<td>9.4 ± 3.9</td>
<td>0.51</td>
<td>0.34</td>
</tr>
<tr>
<td>Phase II [HHb] TD (s)</td>
<td>11.3 ± 2.7</td>
<td>9.9 ± 2.7</td>
<td>0.41</td>
<td>0.46</td>
</tr>
<tr>
<td>[HHb] mean response time (s)</td>
<td>24.2 ± 11.9</td>
<td>19.3 ± 1.8</td>
<td>0.34</td>
<td>0.49</td>
</tr>
<tr>
<td>Phase II [HHb] amplitude (a.u.)</td>
<td>4.11 ± 4.70</td>
<td>6.20 ± 3.07</td>
<td>0.39</td>
<td>-0.48</td>
</tr>
<tr>
<td>[HHb] slow-component onset (s) c</td>
<td>151.0 ± 73.4</td>
<td>96.0 ± 29.03</td>
<td>0.18</td>
<td>0.84</td>
</tr>
<tr>
<td>[HHb] slow-component amplitude (a.u.) c</td>
<td>1.15 ± 0.65</td>
<td>1.69 ± 0.48</td>
<td>0.17</td>
<td>-0.85</td>
</tr>
<tr>
<td>[HHb] slow-component relative amplitude (%) c</td>
<td>26.5 ± 13.6</td>
<td>21.6 ± 6.2</td>
<td>0.49</td>
<td>0.40</td>
</tr>
<tr>
<td>End-exercise [HHb] (a.u.)</td>
<td>5.13 ± 5.12</td>
<td>7.81 ± 3.43</td>
<td>0.31</td>
<td>-0.56</td>
</tr>
</tbody>
</table>

a Four participants per group, two healthy controls presented with a monoexponential response and their matched patients were subsequently also removed from the slow-component analyses; b three per group, two healthy controls presented with a monoexponential response and one CF patient had an abnormal negative response following the slow-component onset, therefore their matched patients were subsequently also removed from the slow-component analyses; c one CF patient presented with a monoexponential response and her healthy match was subsequently also removed from the slow-component analyse analyses.
Figure 8.1. Mean pulmonary oxygen uptake ($\dot{V}O_2$) profile for cystic fibrosis (○ white circles) versus healthy (● black circles) children and adolescents during moderate (A, C) and very heavy (B, D) intensity cycling exercise. Figures C and D provide the normalised to end-exercise so that the differences in the phase II region of the $\dot{V}O_2$ response can be observed. The vertical dotted line illustrates the onset of exercise from a 10 W baseline. Data are presented as 5 s averages.
Figure 8.2. Mean muscle deoxygenation ([HHb]) profile for cystic fibrosis (○ white circles) and healthy (● black circles) young people during moderate (A,C) and very heavy (B,D) intensity cycling exercise. Figures C and D provide the normalised to end-exercise so that the differences in the phase II region of the [HHb] response can be observed. The vertical dotted line denotes the onset of exercise from a 10 W baseline. Data are presented as 5 s averages.
Figure 8.3. Group mean heart rate (A), fat-free mass (FFM) normalised stroke volume (B), FFM normalised cardiac output (C) and FFM normalised arterial-venous O₂ content difference [C(a−v)O₂] (D) dynamics of young cystic fibrosis patients (○ white circles) and healthy age- and gender-matched controls (● black circles) during moderate (1) and very heavy (2) intensity cycling exercise. The vertical dotted line denotes the onset of exercise from a 10 W baseline. Data are mean and SD and 30 s averages. * denotes $P < 0.05$, i.e. significant mean difference between CF patients and healthy controls, whilst + denotes a statistical trend ($p = 0.07$).
8.4 Discussion

This is the first study to examine the dynamics of \( \dot{V}O_2 \) in children and adolescents with mild-to-moderate CF at the onset of MOD and VH intensity cycling exercise, relative to adjustments in central \( O_2 \) delivery and localised muscle (\( m. vastus lateralis \)) \( O_2 \) extraction. The novel findings from this study were that 1) pulmonary \( \dot{V}O_2 \) kinetics were slowed in CF during VH but not MOD; 2) there was no differences in muscle [HHb] kinetics between CF and CON during MOD and VH exercise; 3) during VH exercise only, \( C(a\text{-}\overline{V})O_2 \) was reduced in CF within the initial 60 s of exercise onset, and 4) the change in \( C(a\text{-}\overline{V})O_2 \) during VH exercise was significantly correlated with the phase II \( \dot{V}O_2 \) \( \tau \) and MRT in CF. Collectively, these findings support the notion that impaired muscle oxidative metabolism in young CF patients is dependent on the intensity of exercise and principally limited by muscular factors, which limit the extraction and utilisation of \( O_2 \) during VH exercise.

Contrary to our hypothesis, neither the phase II or overall \( \dot{V}O_2 \) kinetics were slowed during MOD intensity cycling in young people with CF. This is consistent with early observations in similarly aged patients (11.1-15.3 y) with mild airway obstruction during 6 min cycling at 1.7 W·kg\(^{-1}\) (Braggion et al., 1989). In contrast, two studies have documented slower \( \dot{V}O_2 \) dynamics during exercise in patients with CF (Hebestreit et al., 2005; Kusenbach et al., 1999), however methodological issues may explain this disparity. Kusenbach et al. (1999) employed PRBS exercise which fails to isolate phase II of the \( \dot{V}O_2 \) response. Although Hebestreit et al. (2005) utilised CWR exercise and isolated phase II, work rate was not prescribed within physiologically defined intensity domains. This meant that patients were likely to be exercising across the MOD-severe intensity domains which, if the intensity was...
above the GET, would be consistent with our present findings of slowed $\dot{V}O_2$ kinetics during exercise performed above the GET. Hebstreit and colleagues (2005) also used semi-supine exercise, which may reduce muscle perfusion and slow $\dot{V}O_2$ dynamics (Koga et al., 1999). Finally, the combination of adult and paediatric patients (10-33 y) could have contributed to slow $\dot{V}O_2$ kinetics, since slower phase II kinetics were recently documented in adults with more advanced CF (22 ± 4 y) during submaximal cycling (Armeniakou et al., 2015).

An interesting finding in this study was that the influence of CF on oxidative muscle metabolism appears to be exercise intensity dependent. This is based on the finding that the phase II $\dot{V}O_2\tau$ and MRT were slowed only during VH exercise and the $ES$ was very large (> 1.0). This is of clinical importance, since slower $\dot{V}O_2$ kinetic response will incur a greater $O_2$ deficit and a greater degree of substrate-level phosphorylation (increased lactic acid and [PCr] breakdown) and the accumulation of fatigue-inducing metabolites (e.g., $P_i$ and $H^+$ ions), which may impair exercise tolerance, especially during VH exercise, in young people with CF. An exercise intensity dependence to the impaired oxidative metabolism in CF corresponds with earlier observations in adolescent patients during a 90 s high-intensity exercise challenge, but not shorter duration or less intense exercise (Wells et al., 2011). This may reflect the greater physiological challenge to mitochondrial aerobic metabolism elicited by higher intensities of exercise.

The longer phase II $\dot{V}O_2\tau$ of patients with CF (10-33 y; FEV$_1$: 37-98% predicted) has previously been linked to inadequate $O_2$ delivery, inferred by a significant relationship with SpO$_2$ (Hebestreit et al., 2005). In the current study, bulk blood flow
(\(\dot{Q}\)), as inferred using the CI, was not profoundly altered during either MOD or VH exercise in CF. Furthermore, \(\dot{V}O_2\) kinetics were not mechanistically linked to the CI and SVI dynamics in this group of patients, despite previous reports that early signs of cardiac dysfunction may present in paediatric patients with CF (Giacchi et al., 2015; Saynor et al., 2014a – Chapter 6). Although CFTR is involved in the regulation of cardiomyocyte contraction (Sellers et al., 2010) and gene mutation targeted therapies have been shown to increase SV in adolescents with CF (Saynor et al., 2014b – Chapter 7), the current study suggests that central \(O_2\) delivery does not principally limit \(\dot{V}O_2\) kinetics in young CF patients. This is further supported by research demonstrating that elevating \(SpO_2\) through the inspiration of hyperoxic gas does not improve the kinetics of \(\dot{V}O_2\) in patients with CF (Kusenbach et al., 1999). However, it must be acknowledged that only central indices of \(O_2\) delivery, which are relatively poor indicators of \(O_2\) delivery at the local muscle level during exercise (Murias et al., 2013), were obtained in these studies.

However, considering the findings in the present study, the impaired \(\dot{V}O_2\) kinetics during VH exercise herein were related to the capacity of skeletal muscle to extract and utilise \(O_2\). For the first time, this study investigated the [HHb] dynamics of young CF patients during CWR exercise, with similar kinetics observed between the groups. If muscle \(O_2\) availability was limiting oxidative metabolism in CF, a compensatory acceleration in the rate of \(O_2\) extraction would be expected (Ferreira et al., 2007). This was not observed in the present study and this finding corresponds with earlier studies during incremental exercise using both NIRS (Saynor et al., 2014a – Chapter 6) and respiratory mass spectroscopy (Rosenthal et al., 2009). Whilst this finding shows that the rate of \(O_2\) extraction taking place was
not different in CF, [HHb] does not reflect the amount of $O_2$ extraction taking place. This can be physiologically interpreted from the $C_{(a-v)}O_2$ parameter.

Interestingly, we observed a significant reduction in $C_{(a-v)}O_2$ in CF during the early stages of VH exercise (see Figure 8.3D), which corresponds with the timing of the phase II portion of the $\dot{V}O_2$ response. Furthermore, $\Delta C_{(a-v)}O_2$ significantly correlated with the phase II $\dot{V}O_2$ and MRT during VH in CF only. These findings suggest that the amount of muscle $O_2$ extraction and utilisation is impaired in this patient group near the onset of exercise and is mechanistically linked to the dynamics of $\dot{V}O_2$. These findings support previous speculations regarding a peripheral limitation slowing $\dot{V}O_2$ kinetics in patients with CF (Hebestreit et al., 2005). This $O_2$ extraction and utilisation impairment may be explained by structural and functional changes in skeletal muscle that are evident in CF (de Meer et al., 1995; Lamhonwah et al., 2010; Wells et al., 2011). Although a recent study has provided conflicting data (Werkman et al., 2015), slower post-exercise [PCr] recovery kinetics, measured using $^{31}$Phosphorous magnetic resonance spectroscopy, suggest impaired muscle oxidative capacity in both the $m. vastus lateralis$ and forearm muscle (de Meer et al., 1995; Wells et al., 2011). More recently, reduced local muscle oxidative capacity was inferred from the recovery of $m. vastus lateralis$ $O_2$ consumption following 15 s of electrical stimulation and subsequent repeated transient arterial occlusions (Erickson et al., 2015). Evidence of CF-specific muscle metabolic abnormalities (lower [ATP] and [ATP]:[PCr] at rest and significantly higher end-exercise pH values) (Wells et al., 2011) also support the present suggestions regarding a muscular abnormality in this patient group.
The cause(s) of an intramuscular impairment in CF are currently unknown, although several factors have been proposed. Reduced antioxidant capacity, arising from systemic inflammation and/or oxidative damage, may lower mitochondrial efficiency (Wells et al., 2011). However, it may also be a consequence of the CFTR genetic mutation. CFTR is expressed in skeletal muscle cells (Lamhonwah et al., 2010) and in vitro study of leucocyte mitochondria in patients with CF demonstrates that properties of complex I of the respiratory chain are significantly altered (Dechecci et al., 1988). Furthermore, absence of CFTR from skeletal muscle has been shown to dysregulate Ca\(^{2+}\) homeostasis, augment inflammatory or atrophic gene expression signatures and increase diaphragm weakness (Divangahi et al., 2009). Conversely, improving CFTR (dys)function using Ivacaftor shows potential to improve aerobic exercise function in adolescents with CF (Saynor et al., 2014b – Chapter 7). However, the impact of impaired vascular function on the ability of people with CF to deliver O\(_2\) locally for extraction also requires further investigation, given recent evidence that vascular endothelial (dys)function is associated with a poorer \(\dot{V}O_{2\text{max}}\) in young people with CF (Poore et al., 2013) association with \(\dot{V}O_{2\text{max}}\) in this patient group.

Whilst the present study provides the first robust investigation of the \(\dot{V}O_2\) kinetic response in young CF patients, there are a number of limitations inherent to the employed measurement techniques. The considerations regarding NIRS exercise measurements in this population have been outlined in greater detail in Chapters 2, 3 and 6 of this thesis and elsewhere (Saynor et al., 2014a). However, these include a restricted, heterogenous and superficial area of interrogation and possible inter-site variation in [HHb]. To minimise these limitations, the NIRS device was secured to the
same anatomical region of all participants to eradicate inter-individual regional differences within the \textit{m. vastus lateralis} and \([\text{HHb}]\) responses were standardised to the total \([\text{HHb}]\) amplitude to provide a physiologic normalisation (Boone \textit{et al.}, 2009). Although the utilised thoracic impedance cardiography device has been validated in CF patients (Pianosi, 1997), this technique provides a non-invasive estimate of SV and more detailed echocardiography indices of ventricular function, in addition to further measurements of vascular endothelial function would be insightful. Further, since CFTR is expressed in human vasculature and vascular endothelial dysfunction has been related to $\dot{\text{V}}\text{O}_{2\text{max}}$ in young CF patients (Poore \textit{et al.}, 2013), contribution to altered $\dot{\text{V}}\text{O}_2$ kinetics warrants further investigation. Finally, since muscle fibre type composition and recruitment were not measured herein, discrepancies in fibre type composition and recruitment strategies between the groups cannot be excluded.

These findings help us to further understand how young people with CF respond to the increased metabolic demand during activities of daily living and fatiguing exercise. Whilst children and adolescents with mild-to-moderate CF appear to respond in a similar manner to their healthy counterparts during MOD exercise, the slowed $\dot{\text{V}}\text{O}_2$ kinetics at the onset of exercise above the GET may well be linked to reduced exercise tolerance, which should be considered by the exercise practitioner when considering exercise prescription strategies for this patient group. Promisingly, identifying the rate limiting determinant(s) of pulmonary $\dot{\text{V}}\text{O}_2$ kinetics in individuals with CF may provide viable targets for intervention.
8.5 Conclusion

To conclude, this study demonstrates that the \( \dot{V}O_2 \) kinetics of paediatric patients with CF are slowed during VH but not MOD intensity cycling exercise. Impaired skeletal muscle oxidative metabolism in this patient group is intensity dependent and appears to be mechanistically linked to an intrinsic intramuscular impairment which limits \( O_2 \) extraction and utilisation. Identifying the rate limiting determinant(s) of pulmonary \( \dot{V}O_2 \) kinetics in individuals with CF may provide viable targets for intervention in the future.

8.6 Practical implications

- Pulmonary \( \dot{V}O_2 \) kinetics may reflect better the demands of daily activities.
- Young people with mild-to-moderate CF appear able to maintain normal oxidative muscle function during moderate intensity exercise performed below the GET.
- Skeletal muscle oxidative metabolism is impaired during higher intensities of exercise and may lead to an earlier onset of fatigue than their healthy peers.
- This impairment appears to be caused by an abnormality within the exercising muscle, preventing it from extracting and using \( O_2 \) as well as healthy young people.
CHAPTER NINE

Summary, Conclusions, Recommendations and Directions for Future Research

9.1 Summary of experimental chapters

The primary objective of this thesis was to further our understanding concerning both the assessment and interpretation of aerobic exercise (dys)function in paediatric patients with CF. Specifically, the studies addressed the following:

1. Investigate the validity of $\dot{V}O_{2\text{max}}$ and the utility of $S_{\text{max}}$ verification in young patients with CF (Chapter 4);
2. Establish the reproducibility of CPET in young patients with CF (Chapter 5);
3. Investigate the influence of CF on the aerobic exercise function of young patients during ramp incremental exercise to exhaustion (Chapter 6);
4. Use a case study design to explore the effect of a CFTR potentiator on the aerobic exercise (dys)function of young patients with CF (G551D mutation) (Chapter 7);
5. Determine whether mild-to-moderate CF slows the $\dot{V}O_2$ kinetics of young patients with CF at the onset of moderate and very heavy intensity exercise and whether this is mechanistically linked to $O_2$ delivery and/or utilisation (Chapter 8);

The experimental chapters in this thesis provide a significant contribution to the literature in this area and offer important information to develop exercise testing guidelines and viable targets for therapeutic intervention. This chapter will briefly
summarise the primary findings of each of the experimental chapters and their contribution. Following this, the overall experimental findings and their impact to this field of research will be synthesised. Finally, experimental considerations and recommendations for future research will conclude this thesis.

9.1.1 Chapter 4

The purpose of this study was to compare the validity of a traditional ramp incremental CPET protocol to determine $\dot{V}O_{2\text{max}}$, with secondary verification criteria, versus the utility of $S_{\text{max}}$ verification in paediatric patients with mild-to-moderate CF. At the time of this investigation, no study had explored this concept in this patient group. The strengths of this study included 1) the use of commonly used secondary verification criteria from the CF exercise literature; 2) the use of a $S_{\text{max}}$ verification phase to provide evidence of how well patients tolerate this intensity of exercise and whether this provides a valid measure of $\dot{V}O_{2\text{max}}$.

In this study, only one patient exhibited a $\dot{V}O_2$ plateau upon exhaustion during an isolated single ramp test. This low prevalence is in line with evidence presented by Werkman and colleagues (2011). These findings highlight that the use of the traditional primary plateau criteria to confirm $\dot{V}O_{2\text{max}}$ is not suitable in paediatric patients with CF, which is in line with recommendations for healthy young people (Barker et al., 2011). What this study also demonstrates is that the use of other traditional secondary verification criteria, such as HR and RER, will significantly underreport $\dot{V}O_{2\text{max}}$ in paediatric patients with CF. Therefore, this study demonstrated that the previously reported low $\dot{V}O_{2\text{peak}}$ values in young people with CF, that were determined using secondary verification criteria, warrant further investigation to determine the true extent of any impairment. What this study also demonstrated,
however, is that a $S_{\text{max}}$ verification phase provides a useful alternative that can confirm whether a ‘true’ $\dot{V}O_{2\text{max}}$ is achieved or not, with a combined ramp and $S_{\text{max}}$ protocol identifying a meaningful (> 9%) increase (9.9 – 38.3%) in $\dot{V}O_{2\text{peak}}$ in 3 of 14 cases in the present study. Clinically, if used as part of the annual review, a misleading value such as this would lead to incorrect interpretation of the data (i.e. a patient’s health and physiological function is declining when it is not) and, possibly, result in unnecessary clinical intervention that is aimed at improving $\dot{V}O_{2\text{max}}$. This finding extends earlier observations in healthy children and adolescents (Barker et al., 2011) and those with spina bifida (de Groot et al., 2009), which advocate that $S_{\text{max}}$ verification testing should be used to verify $\dot{V}O_{2\text{max}}$ during CPET of paediatric groups.

Whilst current clinical exercise testing protocols, such as shuttle and step tests, are limited in their capacity to ensure a maximal effort and the scope of physiological interpretation they convey, this study also raised concerns regarding the validity of current CPET protocols. The present findings highlight that CPET results should be interpreted with caution when traditional verification criteria are used, particularly in the clinical environment where clinical management strategies may be changed in response and it is recommended that $S_{\text{max}}$ verification be included when an incremental test is performed. At present, this protocol has not been incorporated into clinical management guidelines; however the recent ECFS consensus statement marks an improvement with the recommendation for clinical CPET in this patient group (Hebestreit et al., 2015).
In order to implement CPET as a clinical assessment tool, it is necessary to determine the TE in key parameters of aerobic exercise (dys)function in this patient group over relevant time periods, e.g. the short-term or the duration of routine clinical interventions, such as IVABs (~ 4-6 weeks). Having established a valid protocol in Chapter 4, the purpose of Chapter 5 was to determine the reproducibility of this CPET protocol in young patients with CF over both the short- (48 h) and medium-term (4-6 weeks). This is a pertinent question given that reductions in $\dot{V}O_2$peak over time are clinically relevant and previously suggested to be around 5-8% per year. Furthermore, it is vital to assess the true magnitude of improvement caused by any clinical or exercise-related intervention. It is therefore important to identify the typical reproducibility of aerobic fitness status over time given the cyclic nature of CF exacerbations.

The strengths of this study include; 1) the implementation of a valid CPET protocol to determine $\dot{V}O_2$max, in addition to other key parameters of aerobic exercise function; 2) the assessment across clinically relevant time periods; 3) presentation of all of the key parameters of aerobic exercise function (Whipp et al., 1981; Whipp et al., 1982) in addition to other physiologically useful parameters, such as SpO$_2$ and HR.

Data from this study demonstrated that $\dot{V}O_2$max can be repeatedly determined, with no learning effect, in young patients with CF over both the short- (48 h; 9.3% TE$_{CV\%}$) and medium-term (4-6 weeks; 13.3% TE$_{CV\%}$). It is important to note that all patients were thoroughly familiarised with the testing protocol and environment in order to achieve these TEs and this should be a key consideration in research and clinical practice alike. A greater variance was, however, evident over a longer period of time.
(4-6 weeks). Importantly, this study also revealed that a combined ramp and $S_{\text{max}}$ protocol also demonstrated improved reproducibility over both the short- ($\text{TE}_{\text{CV}}\%$: 9.3 (6.9-14.3) vs. 13.5 (9.5-23.3)%) and medium-term ($\text{TE}_{\text{CV}}\%$: 13.3 (9.9-20.9) vs. 15.5 (10.9-26.9)%)) when compared with traditional CPET protocols.

Prior to this study, the reproducibility of CPET had not been established in young patients with CF using a valid protocol (Kent et al., 2012; McKone et al., 1999). Although submaximal parameters were identifiable in most cases, some should be approached with caution (e.g. the $\text{V}\dot{O}_2$ MRT) when being used to monitor disease progression and/or the effect of therapeutic interventions given their increased variability. Taken together, this study established limits of variability to determine clinically meaningful changes over the short- and medium-term for CPET outcomes in young patients with CF, using a superior testing protocol to those currently employed. This application was subsequently demonstrated in experimental Chapters 6 and 7. From a clinical impact perspective, the outcomes of the studies presented in Chapters 5 and 6, have resulted in the RD&E adopting the protocol to use in a clinical setting as part of patients’ annual clinical review. More recently, these protocols have also been embedded within the annual review of paediatric and adult patients with CF (> 400 patients) and non-CF bronchiectasis patients under the clinical management of the Southampton General Hospital and Queen Alexandra Hospital (Portsmouth) and hopefully marks a change in the UK, whereby more CF clinics will adopt this form of exercise testing.

9.1.3 Chapter 6

Following the development of valid and reproducible CPET methodology in Chapters 4 and 5, the objective of Chapter 6 was to provide a comprehensive overview of the
physiological response of young patients with CF during ramp incremental cycling exercise. As outlined in section 2.6.1., maximal aerobic fitness is reportedly reduced in young people with CF in addition to abnormalities in other key parameters of aerobic exercise function. Given the protocol issues outlined in Chapters 4 and 5, there was a need to present an accurate reflection of the response of this patient group during this type of exercise.

The novelty of this study includes: 1) the use of a valid and reproducible CPET protocol for which the TE s are known; 2) the comprehensive simultaneous evaluation of breath-by-breath changes in $\dot{V}O_2$, muscle deoxygenation ([HHb]) and thoracic bioimpedance cardiography derived HR, SV, and $\dot{Q}$ during exercise; 3) appropriate sigmoidal mathematical modelling of the [HHb] data during ramp incremental cycling exercise to provide insight into the matching between $O_2$ delivery and utilisation; 4) investigation of the relationships between these mechanistically important parameters of $O_2$ delivery and extraction and the parameters of aerobic exercise function derived from the CPET.

This study demonstrated that, even when a valid and reproducible CPET protocol is used, FFM normalised $\dot{V}O_{2\text{max}}$ is reduced in relatively well children and adolescents with mild-to-moderate CF compared with their healthy counterparts. Despite previous debate, the $\dot{V}O_2$ gain was reduced and the $\dot{V}O_2$ MRT was slower in CF. These findings suggest that the key parameters of aerobic function are impaired in paediatric patients with CF during ramp exercise. This study also investigated the mechanism(s) responsible for the observed changes in aerobic exercise function in this patient group. From a central perspective, in contrast, some parameters of central $O_2$ delivery were altered in CF; specifically, end-exercise SpO$_2$ was reduced
and there were moderate-strong relationships with parameters of aerobic exercise function.

Finally, this study was the first to examine the influence of mild-to-moderate CF on the dynamic adjustments in localised skeletal muscle (vastus lateralis) fractional O$_2$ extraction in paediatric patients during exercise. Although it has been hypothesised that O$_2$ extraction at peripheral skeletal muscle is accelerated during exercise when the delivery of O$_2$ is inadequate, this study demonstrated that this is not the case is young people with CF during exhaustive incremental cycling exercise. Specially, the dynamics of [HHb] were not altered compared to healthy controls, with only a weak relationship evident with key parameters of aerobic function.

Collectively, these findings support the notion that central O$_2$ delivery is the principle modulator of aerobic function in paediatric patients with CF during exhaustive ramp incremental cycling exercise. The inadequacy of CF skeletal muscle to compensate for inadequate O$_2$ delivery also raises questions regarding whether there is a reduced ability of CF skeletal muscle to extract O$_2$. This proposition does complement previous suggestions that the oxidative capacity of skeletal muscle in CF may be impaired (de Meer et al., 1995; Erickson et al., 2015; Moser et al., 2000; Rosenthal et al., 2009).

9.1.4 Chapter 7

The purpose of Chapter 7 was to evaluate, for the first time, the effects of 12 weeks treatment with an orally administered CFTR potentiator (Ivacaftor) on the aerobic exercise function of two teenage patients with CF (G551D mutation). Using a case study design, the CPET methodology established in Chapters 4 and 5 was used to
examine whether the impairments documented in Chapter 6 can be reversed by this
ground-breaking new pharmacological treatments. It has been proposed that both
altered cardiac function and skeletal muscle oxidative function may contribute to
impair aerobic exercise function in patients with CF. As outlined in section 2.1,
evidence of CFTR expression on cardiac and skeletal muscle tissue is now available
(Duan et al., 1999; Gao et al., 2007; Lamhonwah et al., 2010; Tilly et al., 1996;
Warth et al., 1996). Since Ivacaftor has been shown to restore CFTR function in
patients with the G551D mutation, this intervention should provide some insight into
the role of dysfunctional CFTR to the aerobic exercise function of patients with CF.

Whilst this study adopted a case study approach, due to the prevalence of this CFTR
mutation within the patient network and availability and cost of this drug, the novelty
of this study includes: 1) this study provides novel data demonstrating the utility of
CPET as an outcome measure in clinical trials; 2) analysis at the patient level, which
is clinically important and this personalised medicine approach allows more
measurements to be tracked over time; 3) the utilisation of CPET in conjunction with
the standard clinical outcome measures in clinical trials; 4) the utilisation of a valid
and reproducible CPET protocol for which the TEs are known; 5) the comprehensive
simultaneous evaluation of breath-by-breath \( \dot{V}O_2 \), muscle deoxygenation ([HHb]) and
thoracic bioimpedance cardiography derived HR, SV, and \( \ddot{Q} \) during exercise; 6) the
investigation of the relationships between these mechanistically important
parameters of \( O_2 \) delivery and extraction and the kinetics of pulmonary \( \dot{V}O_2 \).

This study provides novel data demonstrating the utility of CPET as an outcome
measure in clinical trials. Although no clinically meaningful improvement in \( \dot{V}O_{2\max} \)
was evident in a patient with relatively mild disease CF disease (despite a 9%
improvement), a 30% improvement was demonstrated in the second case with more established pulmonary disease, which was substantially greater than the TE of measurement for this period established in Chapter 5 (13.3%). Of further importance, this patient experienced two successive URTIs during the assessment period and her physical activity was much lower than normal due to feeling unwell. Despite this, in addition to the improvements in aerobic fitness, her pulmonary function and body mass were maintained during a time when she would typically deteriorate and she ‘felt more energetic’ upon testing. Notably, this improvement in $\dot{V}O_{2\text{max}}$ was out of proportion with early changes in pulmonary function. This demonstrated that not only is CPET is a sensitive tool to monitor the extent and cause(s) of change following clinic interventions, such as Ivacaftor, it may in fact be more sensitive than current spirometric indices, however this requires confirmation in trials with more participants. Mechanistic data from this study suggest that the observed improvement in aerobic exercise function during CFTR potentiator treatment was due to both enhanced $O_2$ delivery and $O_2$ extraction, which is an exciting finding given that both of these appeared to be impaired during CPET in Chapter 6. These findings raise speculations regarding the role of CFTR, which is expressed in skeletal muscle, cardiac tissue and the endothelium, to impair aerobic exercise function characterising this patient group and highlight the need for further research in this area.

9.1.5 Chapter 8

The purpose of this investigation was to further explore the aerobic exercise (dys)function characterising young patients with CF, by exercising participants within specific physiologically defined CWR intensity domains. More specifically, this study
sought to investigate previous suggestions that pulmonary \( \dot{V}O_2 \) kinetics are slowed in patients with mild-to-moderate CF during moderate intensity exercise (Hebestreit et al., 2005; Kusenbach et al., 1999), indicating impaired oxidative metabolism. Of additional interest was the response to higher intensity exercise and which factor(s) principally limit this response and, by inference, skeletal muscle oxidative metabolism.

The strengths of this study include: 1) the prescription of exercise within defined intensity domains; 2) documenting responses during both moderate and very heavy intensity exercise; 3) the comprehensive simultaneous evaluation of breath-by-breath \( \dot{V}O_2 \), muscle deoxygenation ([HHb]) and HR, SV, and \( \dot{Q} \) during exercise; 4) the ensemble averaging and interpolation of at least 4 transitions within each intensity domain; 5) the correct modelling and isolation of phase II and the slow component (where relevant) within both \( \dot{V}O_2 \) and [HHb] data; 6) the investigation of the relationships between the kinetics of pulmonary \( \dot{V}O_2 \) and measures of \( O_2 \) delivery (\( \dot{Q} \) and SpO\(_2\)) and \( O_2 \) extraction and utilisation ([HHb] kinetics and \( C_{(a-v)}O_2 \) dynamics during exercise).

This study provides new data demonstrating that, contrary to previous reports (Hebestreit et al., 2005), the \( \dot{V}O_2 \) kinetics of children and adolescents with mild-to-moderate CF are not significantly slower than healthy controls at the onset of moderate intensity cycling exercise. Conversely, during exercise performed above the GET both the overall \( \dot{V}O_2 \) MRT and the kinetics of phase II were significantly slowed in young patients with CF compared with their healthy peers. Further examination of mechanistic parameters of central and peripheral function revealed that, while indices of central \( O_2 \) delivery (SV and \( \dot{Q} \)) and the kinetic parameters of the
muscle [HHb] response were not significantly altered in CF, the $C_{(a-v)}O_2$ was reduced. This finding, coupled with the fact that $\Delta C_{(a-v)}O_2$ correlated with the very heavy phase II $\dot{V}O_2$ $\tau$ and MRT in CF only, suggest that the slowing of the $\dot{V}O_2$ response at this intensity is mechanistically linked to impaired muscle $O_2$ extraction and utilisation rather than central $O_2$ delivery.

To further the findings in Chapter 6 during ramp incremental exercise, collectively the data in Chapter 8 indicate that the modulation of oxidative muscle metabolism in young patients with CF is dependent upon the intensity of exercise imposed, with significant impairment evident during exercise above but not below the GET. Furthermore, alterations in skeletal muscle oxidative metabolism during intense exercise appear to be principally regulated by an intrinsic intramyocyte abnormality, aspects of which may be specific to CF and not simply a consequence of chronic respiratory disease (Rosenthal et al., 2009).

9.2 Synthesis of findings and discussion of candidate mechanism(s) responsible for impaired aerobic exercise function in paediatric patients with CF

The following section will synthesise the findings of the experimental chapters and the potential mechanism(s) responsible for the observed findings regarding altered aerobic exercise function in this patient group.

9.2.1 The development of an exercise testing protocol to assess aerobic exercise (dys)function in paediatric patients with mild-to-moderate CF
Although comprehensive exercise testing remains underused in the clinical management of patients with CF in the UK (Stevens et al., 2010), the recent promotion of routine CPET for this patient group by the ECFS Exercise Working Group (Hebestreit et al., 2015) and Australasian and North American groups marks an international change in stance regarding its value for patients with CF. Prior to this thesis, the ECFS Clinical Trials Network Standardisation Committee called for assessment of the validity, reproducibility and feasibility of outcomes measures utilised in CF and advocated research into the most appropriate exercise test for paediatric patients (Bradley et al., 2012). These recent guidelines recommend the Godfrey cycle ergometer protocol, incorporating measurement of pulmonary gas exchange and SpO₂ (and HR and ECG where possible), as the exercise testing method of choice for patients with CF over the age of 10 y. However, Chapter 4 of this thesis provides compelling evidence, corroborating findings in healthy children and adolescents (Barker et al., 2011), that adherence to traditional verification criteria and accepting $\dot{V}O_2\text{peak}$ as a ‘true’ representation of $\dot{V}O_2\text{max}$ in paediatric groups brings with it validity concerns. It is therefore important that the authors of the recently published consensus document regarding the use of CPET in CF (Hebestreit et al., 2015) acknowledge the findings presented within this thesis.

Specifically, adherence to traditional verification criteria was shown to drastically underestimate $\dot{V}O_2\text{max}$. Conversely, it was demonstrated that a simple $S_{\text{max}}$ verification phase can confirm $\dot{V}O_2\text{max}$ in most cases and, more importantly, can identify those patients who may provide a submaximal effort and present with poor motivation to exercise to exhaustion. This was of particular interest to the clinician involved in this work, since the quantification of a patient’s lack of effort at times was related to poor adherence to their treatments and provided data to substantiate
discussions with their parents. Furthermore, not only can the combination of a traditional ramp and $S_{\text{max}}$ test permit measurement of a valid $\dot{V}O_{2\text{max}}$, but Chapters 4-8 have demonstrated that this protocol can be safely and effectively administered in this patient group within a single hospital visit and may be more sensitive that current clinical outcome measures in the assessment of clinical practice and/or interventions (Chapter 7). For the findings of this thesis to be implemented within clinical practice, the next step is to conduct a well powered study which compares the currently available protocols (e.g. Godfrey, SRT, ramp and combined ramp and $S_{\text{max}}$), in addition to other modes of exercise (e.g. the Bruce treadmill protocol).

To extend the findings in Chapter 4, Chapter 5 has also demonstrated that $\dot{V}O_{2\text{max}}$ can be repeatedly determined with no learning effect. The reproducibility of additional parameters of aerobic exercise function and clinical utility were also established. Prior to this thesis, only a single study had reported the reproducibility of CPET in children with CF, using the Godfrey protocol and with several other methodological issues confounding the data (Kent et al., 2012). This thesis now provides clear guidelines concerning the clinically meaningful change required in these outcome measures over the short- and medium term, to enable inferences regarding therapeutic interventions, such as a course of IVABs, nutritional intervention, or inspiratory muscle or whole-body training programmes. These TEs can also be compared with disease-related changes over time, however there is a need to establish the typical changes in aerobic fitness in this patient group using a longitudinal follow-up design. Whilst the recent position stand from the ECFS Exercise Working Group (Hebestreit et al., 2015) does represent an exciting shift in clinical practice, this thesis highlights a number of important testing considerations that should be considered by those looking to implement clinical CPET.
Furthermore, Chapters 6-8 have also provided clear evidence of how useful non-invasive techniques such as thoracic bioelectrical impedance cardiography and NIRS can be used to provide insight into the adequacy of O₂ delivery and utilisation relative to the metabolic demand during exercise in this patient group. Chapter 7 provided evidence, through a case based approach, of how easily a comprehensive CPET can be used to further understand the mechanism(s) by which clinical interventions influence patients' physiological (dys)function and/or QoL. Further research is now needed in larger scale clinical trials. Furthermore, CPET was shown to detect changes earlier than a number of the current routinely used clinical outcome measures. The recent recommendation for clinical CPET by the ECFS now marks an opportunity for international assessments of a number of routine clinical interventions in this patient group. There is a clear need to investigate further how ground breaking treatments such as Kalydeco® and Orkambi® may influence aerobic exercise function in individuals with CF and the mechanism(s) responsible. This is particularly timely given that at the time of writing this, the European Medicines Agency recommend the use of both treatments across the European Union; with Orkambi® licensed in those ≥ 12 y with two copies of ΔF508 and that Kalydeco® should be licensed for use in those with one copy of R117H aged ≥ 18 y, in addition to being extended to children age ≥ 2 y with one of the other 9 gating mutations that are covered by the current license.

There is also a need to utilise these more detailed CPET guidelines in the assessment of exercise training programmes. Although the Cochrane review by Bradley and Moran (2008) suggested that there are some short- and long-term benefits of exercise training for individuals with CF; disappointingly this was only based on the findings from seven trials. There is, therefore considerable scope for
more robust training studies with better, more comprehensive and standardised exercise assessments to evaluate the optimal training strategies for health and aerobic fitness of individuals with CF.

9.2.2 The aerobic exercise (dys)function characterising paediatric patients with cystic fibrosis

Based on early evidence that maximal exercise performance is reduced in individuals with CF (e.g. Almajed & Lands, 2012; Cerny et al., 1982; Cropp et al., 1982; Godfrey & Mearns, 1971; Hjeltines et al., 1984; Klijn et al., 2003; Moser et al., 2000; Shah et al., 1998), it was unsurprising that Chapter 6 observed a reduced \( \dot{V}O_2 \)max in children and adolescents with CF. However, this confirmation was essential given the protocol issues outlined in Chapter 4. This thesis has provided for the first time evidence that even relatively well, habitually active young people with CF present with impaired aerobic exercise function during ramp incremental exercise, with maximal parameters verified supramaximally rather than using the currently recommended Godfrey protocol (Hebestreit et al., 2015). Although Hebestreit et al. (2005) reported similar \( \dot{V}O_2 \)peak values for patients with CF and healthy controls, in a mixed adult and paediatric group, and Wells et al. (2011) made similar observations in adolescents with similar habitual activity patterns, it is possible that there may have been issues with the testing protocols, given the requirement to verify \( \dot{V}O_2 \)peak even in healthy children and adolescents (Barker et al., 2011). There is, however, a need to examine further the influence of CF-related comorbidities (e.g. CFRD, osteoporosis, cardiac abnormalities) and age upon the impaired aerobic exercise function characterising these patients.
Chapter 6 provides for the first time data indicating a slowing of the $\dot{V}O_2$ MRT during incremental exercise, which has since been confirmed (Fielding et al., 2015). This study by Fielding and colleagues also confirms the present finding (Saynor et al., 2014a – Chapter 6) that a shallower functional $VO_2$ gain appears to characterise this patient group. Prior to this thesis, the limited evidence regarding the $\dot{V}O_2$ kinetic response in CF was reported during CWR and PBRS exercise (e.g., Hebestreit et al., 2005; Kusenbach et al., 1999). Of additional interest was how this patient group respond during exercise within physiological defined intensity domains. Based on previous reports of a slowing of the phase II $\dot{V}O_2$ $\tau$ during moderate intensity semi-recumbent cycling (Hebestreit et al., 2005), it was hypothesised that children and adolescents with CF would present with slower $\dot{V}O_2$ kinetics during both moderate and very heavy intensity cycling, suggesting a slower adjustment of oxidative muscle metabolism and increased requirement of substrate level phosphorylation ([PCr] breakdown and anaerobic glycolysis).

Chapter 8 has provided novel insight into an exercise intensity-dependence on the impaired oxidative muscle metabolism characterising children and adolescents with CF during cycling exercise. This is of clinical and practical importance, since slower $\dot{V}O_2$ kinetics will incur a greater $O_2$ deficit and a greater degree of intracellular perturbation. Ultimately, this may well result in an earlier termination of exercise that is performed at these higher intensities. Interestingly, this finding does correspond with an earlier observation by Wells et al. (2011). Using $^{31}$P-MRS derived measurements of the m. vastus lateralis during three different exercise protocols, Wells and colleagues sought to determine the bioenergetic function at rest and following low-, moderate- and high-intensity leg extension exercise. Interestingly, skeletal muscle oxidative capacity was impaired in adolescents with CF following 90
s of high-intensity exercise, but not shorter or less intense exercise (Wells et al., 2011). This was suggested to reflect the greater physiological challenge to mitochondrial aerobic metabolism elicited by higher intensities.

Since exercise performed above the LT may also mandate the recruitment of higher-order muscle fibres. From a practical perspective, there is compelling evidence that endurance athletes tend to have a later occurrence of the LT and CP (Jones & Poole, 2008), which causes a reduction in the magnitude of the VO$_2$ slow-component. There is a need for further research to determine the most-effective and tolerable type of endurance training (i.e., mode, duration, volume, intensity) to improve aerobic fitness in this patient group, however shifting the GET should perhaps also be a primary goal of this interventional work. This may consequent patients training at these higher intensities of exercise in order to improve. Higher intensity training specifically aimed at improving the metabolic efficiency of fatiguing and, particularly, higher-order fibres may also be of benefit to this patient group and has been shown to be more effective than moderate-intensity training in healthy individuals (e.g Bailey et al., 2009; Krstrup et al., 2010) and can improve VO$_{2\text{max}}$ in adolescents with CF (Hulzebos et al., 2011).

Although the kinetics of VO$_2$ in children and adolescents with CF were not impaired during moderate intensity exercise, the current thesis cannot rule out the possibility of physiological differences during exercise below the GET. Whilst this thesis shows that the control of oxidative metabolism is similar between the groups during exercise performed below the gas exchange threshold, there were subtle differences in the physiological response to exercise which became profoundly different at higher exercise intensities. It is possible that subtle differences are evident during
moderate intensity exercise. Given that a close temporal coupling has been reported between the kinetics of phase II \( \dot{\text{VO}}_2 \) and muscle [PCr] in children and adolescents during exercise (Barker et al., 2008), future research should now utilise techniques such as \( ^{31}\text{P}-\text{MRS} \) to further develop the work conducted by Wells and colleagues (2011) and information presented in this thesis, to further characterise the potential exercise-intensity dependence of impaired muscle metabolism apparently characterising young, relatively well patients with CF.

9.2.3 The factor(s) modulating aerobic exercise (dys)function in paediatric patients with cystic fibrosis

This thesis provides important simultaneous measurements at both the central and peripheral levels to enable us to further understand the dynamic matching of \( \text{O}_2 \) delivery-to-\( \text{O}_2 \) utilisation during exercise testing which spans a range of exercise intensities, which previous studies have largely neglected to do (e.g., Divangahi et al., 2009; Ionescu et al., 2001; Lands et al., 1992). The present thesis also provides novel data concerning the \( \dot{\text{VO}}_2 \) kinetic response in young people with CF. It was beyond the scope of this thesis to further investigate the potential role of genotype upon exercise (dys)function, given the large patient numbers that would be required. Furthermore, since recent work in paediatric patients with milder airway obstruction (Bongers et al., 2014; Borel et al., 2014) suggests that signs of pulmonary insufficiency do not typically characterise children and adolescents with CF disease and the ventilatory response is adequate, the present thesis focused on further explaining the role of “other factors”, such as cardiac (dys)function, arterial hypoxaemia and the dynamic balance between \( \text{O}_2 \) delivery and utilisation within the microvasculature of exercising skeletal muscle to impair aerobic exercise function.
In individuals with chronic diseases such as CF, it has been proposed that a ‘tipping point’ may exist, following which the oxidative muscle metabolism (inferred from the phase II kinetics of Ŷ\(\dot{O}_2\)) becomes dependent on the delivery of \(O_2\). Although Hebestreit et al. (2005) observed an inverse correlation between end-exercise Sp\(O_2\) and the phase II Ŷ\(\dot{O}_2\) \(\tau\), in line with our initial observations between Ŷ\(\dot{O}_2\)\(_{max}\) and \(\Delta\text{SpO}_2\) during ramp exercise (Chapter 6), implying that Ŷ\(\dot{O}_2\) kinetics are principally modulated by muscle \(O_2\) availability, the present data (Chapter 8) during moderate intensity CWR cycling present an opposing viewpoint. Perhaps an important methodological consideration concerning the suggestions of Hebestreit and colleagues (2005) is that children, adolescents and adults with CF were pooled together within the same patient sample, within which a wide spectrum of disease severities was likely present. This issue was minimised within the present thesis, by using a more homogenous sample. Supporting this, correlations were also evident between the kinetics of Ŷ\(\dot{O}_2\) and pulmonary function and Ŷ\(\dot{O}_2\)\(_{max}\) in the study conducted by Hebestreit and colleagues (2005), suggesting an additional role of deconditioning and respiratory factors in their sample. Although Chapter 6 reported a very likely reduced Sp\(O_2\) in young patients with CF at Ŷ\(\dot{O}_2\)\(_{max}\) and the phase II Ŷ\(\dot{O}_2\) \(\tau\) of patients with CF has previously been mechanistically linked to inadequate \(O_2\) delivery, inferred by an inverse relationship between Sp\(O_2\) (Hebestreit et al., 2005), elevating Sp\(O_2\) through inspiration of hyperoxic gas does not appear to improve either Ŷ\(\dot{O}_2\)\(_{peak}\) (Nixon et al., 1990) or speed Ŷ\(\dot{O}_2\) kinetics during PRBS exercise (Kusenbach et al., 1999) in patients with CF. These findings imply that arterial hypoxaemia is not likely a primary modulator of aerobic exercise (dys)function in relatively well children and adolescents with CF, thereby implicating cardiovascular and muscular factors.
In Chapter 8, bulk blood flow (SVI and CI) was not significantly reduced during either moderate or very heavy CWR exercise. Furthermore, parameters of pulmonary \( \dot{V}O_2 \) kinetics were not mechanistically linked to parameters of \( O_2 \) delivery in this group of patients, despite previous reports that early signs of cardiac dysfunction may be present in paediatric patients with CF (Giacchi et al., 2015; Chapter 6 – Saynor et al., 2014a). However, the non-invasive technique employed in the present thesis is less sensitive and comprehensive than exercise echocardiographic measurements and these may warrant further investigation in children and adolescents with CF.

Cardiac (dys)function should not be excluded completely as a contributing factor to exercise (dys)function in young patients with CF until this further research is undertaken, given that CFTR is involved in the regulation of cardiomyocyte contraction (Sellers et al., 2010). To provide further support, Chapter 7 demonstrated, albeit through a case study approach, that CFTR targeted pharmacological therapies may have the potential to improve estimated SV in adolescents with CF in the absence of any exercise training. Furthermore, Chapter 6 highlighted the potential role of a centrally mediated \( O_2 \) delivery limitation to limit young people with CF at exhaustion during an exhaustive ramp incremental cycling, with a most likely lower SpO\(_2\) and reduced \( O_2 \) pulse, although a similar maximal HR could be achieved, in line with previous findings (Lands et al., 1992).

For the first time, Chapters 6 and 8 have documented the \( O_2 \) extraction dynamics of paediatric patients with CF during ramp incremental and CWR exercise, with similar \([HHb]\) kinetics observed between the groups during all exercise challenges. However, of particular interest 1) the sigmoidal \([HHb]\) response was not shifted by CF disease during ramp exercise despite central \( O_2 \) delivery being reduced (Chapter 6) and 2) \( C_{(a,\bar{v})}O_2 \), considered to reflect the amount of \( O_2 \) extraction, was significantly
reduced early on during exercise in CF during very heavy CWR cycling, corresponding with the phase II region of the \( \dot{V}O_2 \) response (Chapter 8). Furthermore, \( \Delta C_{(a,\dot{V})O_2} \) significantly correlated with the very heavy phase II \( \dot{V}O_2 \) \( \tau \) and MRT in children and adolescents with CF. Additionally, the phase II \( \dot{V}O_2 \) \( \tau \) also correlated with \( \Delta[HHb] \) during moderate intensity exercise. 

The present thesis therefore extends previous observations using respiratory mass spectroscopy (Rosenthal et al., 2009) that, even in the face of inadequate \( O_2 \) availability, the exercising skeletal muscle of children and adolescents with mild-to-moderate CF does not appear able to respond by increasing the rate of fractional \( O_2 \) extraction, as has been hypothesised (Ferreira et al., 2007).

The impaired oxidative metabolism during CWR exercise above the GET therefore appears to be principally regulated by a more local issue regarding the capacity of skeletal muscle to extract and utilise \( O_2 \). In line with the present observations, a role of peripheral limitation to mediate the \( \dot{V}O_2 \) kinetics of patients with CF has also previously been implicated (Hebestreit et al., 2005). Since both structural and functional changes in skeletal muscle are evident in CF (e.g., de Meer et al., 1995; Lamhonwah et al., 2010; Wells et al., 2011), this role is conceivable. Of additional interest is whether there may be a genetic component to the muscle bioenergetic abnormalities evident in individuals with CF during exercise. Although this information is outside the scope of this thesis, a recent study conducted by Wells and colleagues (2011), which included both healthy and a non-CF respiratory diseased (primary ciliary dyskinesia (PCD)) control groups, demonstrated that whilst both respiratory disease groups presented with significantly slower [PCr] recovery kinetics following high-intensity exercise, CF-specific muscle metabolic abnormalities were also observed. Specifically, CF patients exhibited a lower resting [ATP]:[PCr]
ratio and significantly higher end-exercise pH values compared to both healthy children and adolescents and those with PCD.

Additional support for altered skeletal muscle oxidative metabolism comes from $^{31}$P-MRS based measures of both the *m. vastus lateralis* and exercising forearm muscle, which have reported slower [PCr] recovery kinetics following exercise and, thereby, impaired muscle oxidative metabolism in adolescents with CF (de Meer *et al.*, 1995; Wells *et al.*, 2011). More recently, reduced skeletal muscle oxidative capacity was documented using NIRS (Erickson *et al.*, 2015). Erickson *et al.* (2015) measured a 15% reduction in *m. vastus lateralis* O$_2$ consumption following 15 s of electrical stimulation and subsequent repeated transient arterial occlusions. However, recent opposing data by Werkman and colleagues (2015) again questions the extent of these potential intramyocyte abnormalities.

Impaired muscle metabolism in paediatric patients with CF is not inconceivable and would likely be multifactorial. Reduced antioxidant capacity, arising from systemic inflammation and/or oxidative damage, may lower mitochondrial efficiency (Wells *et al.*, 2011). However, altered function may also be a direct consequence of the CFTR genetic mutation, given than CFTR has been shown to be expressed in skeletal muscle cells (Lamhonwah *et al.*, 2010). Furthermore, *in vitro* study of leucocyte mitochondria in patients with CF demonstrates that properties of complex I of the respiratory chain are significantly altered (Dechecci *et al.*, 1988). Absence of CFTR from skeletal muscle has also been shown to dysregulate Ca$^{2+}$ homeostasis, augment inflammatory or atrophic gene expression signatures and increase diaphragm weakness (Divangahi *et al.*, 2009). In support of this mechanism, Chapter 7 has demonstrated the potential of Ivacaftor to improve aerobic exercise function in adolescents with CF, albeit using a case study design, through both augmented SV
and $O_2$ extraction ($C_{(a-v)}O_2$) occurring within the microvasculature of the *m. vastus lateralis* during exercise.

Furthermore, a recent larger scale study (Edgeworth *et al*., 2015 unpublished abstract) substantiates the potential of Ivacaftor to significantly improve $\dot{V}O_{2\text{max}}$ in adults (18-65 y) with more severe CF (FEV$_1$ 21-110% predicted), in the absence of exercise training. Edgeworth *et al.* (2015, unpublished abstract) enrolled 20 adult patients with CF into a placebo-controlled crossover study of Ivacaftor over a 4 month period, with a further 3 month open-label extension. A stepwise increase in $\dot{V}O_{2\text{max}}$ was observed, with a percentage increase from baseline of 8.0% at the end of the trial and 12.1% following the open label extension. Whilst this is within the TE determined in children with CF within the present thesis, further research is needed to confirm whether this is similar in adults with CF. Importantly, these improvements occurred independent of exercise training, changes in pulmonary function and sweat Cl$, suggesting that these CFTR therapies are having their effect on the cardiovascular and/or muscular systems, as suggested by Chapter 7 of the present thesis.

### 9.3 Recommendations for future research

As previously highlighted, there is now a need for further well-powered research directly comparing the accuracy of the Godfrey protocol, SRT, ramp testing and a combined ramp and $S_{\text{max}}$ protocol to determine $\dot{V}O_{2\text{max}}$, in addition to the other key parameters of aerobic exercise function, in paediatric and adult patients with CF. Establishing this information will then enable us to explicitly provide a toolkit of CPET protocols, which are ranked on their accuracy and the comprehensive data that they can provide the user with. Confirming the findings regarding the utility of $S_{\text{max}}$
verification in adults with CF will also then allow us to further develop our understanding of how the progression of CF disease may further impair aspects of aerobic exercise function.

There is also a need to employ additional investigative techniques to further document the impact of CF disease at both the central and peripheral level during exercise. Since NIRS only indicates the dynamic balance between O$_2$ delivery and utilisation within the microvaculature of the skeletal muscle, there is a need for further research to investigate the influence of CF disease on the skeletal muscle metabolic, pH and blood oxygenation response to exercise of different intensities, demarcated by physiologically defined thresholds (i.e., the GET or its equivalent when using $^{31}$P-MRS; the intracellular threshold). It is suggested that further utilisation of $^{31}$P-MRS imaging techniques will provide the necessary non-invasive window to monitor, in real time, the key variables involved in energy metabolism under both rest and exercise conditions. Specifically, in vivo interrogation of the muscle phosphates and pH during rest and exercise at a high sampling resolution will enable further investigation of any influence of CF disease upon muscle metabolism. Since recent data by Werkman et al. (2015) has further added to the debate regarding whether a skeletal muscle abnormality is present in individuals with mild CF, this work is warranted. Inclusion of a non-CF bronchiectatic respiratory disease control group, a limitation in the present thesis, would also enable any CF-specific abnormalities to be further characterised. Furthermore, other non-invasive imaging techniques, such as blood oxygen level-dependent (BOLD) imaging could be used to detect any alteration in the muscle tissue temporal dynamics (i.e., perfusion and oxygenation) during exercise of different intensities.
Given the conflicting findings between the present thesis and Hebestreit and colleagues (2005) concerning the \( \dot{V}O_2 \) kinetic response of individuals with CF, there is a need to further clarify the control of skeletal muscle metabolism at the onset of different exercise intensities in paediatric patients spanning the disease severity spectrum. There is also a need to explore the role of other CF co-conditions, such as CFRD, to further impact aerobic exercise function. The role of vascular endothelial (dys)function and inflammation on the aerobic exercise (dys)function of this patient group also warrants further attention. Recent evidence has proposed a role of endothelial (dys)function to modulate the aerobic fitness of children and adolescents with CF (Poore et al., 2013). Using the flow-mediated dilation technique, Poore et al. (2013) documented vascular endothelial dysfunction in paediatric patients with CF compared with their healthy counterparts, which was associated with aerobic fitness status (\( \dot{V}O_{2\text{max}} \)). It is currently unclear whether endothelial dysfunction may also be a direct consequence of the CFTR gene polymorphism or rather a consequence of a CF-related inflammation and oxidative stress. However, currently unpublished data (Scott-Ward et al., unpublished abstract) suggests that the genetically defective protein (CFTR) in vascular endothelial cells does contribute to endothelial dysfunction. Given that endothelial function is an important clinical concern, given the risk of vascular complication in end-stage CF, its role in the impaired aerobic exercise function documented in this thesis and the potential of exercise as a low-cost intervention to improve this therefore warrants further investigation. The role of altered cardiac function and contribution of CFTR to this also requires further investigation using more advanced techniques, such as echocardiography, genetic profiling and animal/human tissue models.
One factor that was not considered in the present thesis is the potential role of fibre type differences and altered recruitment patterns during exercise. Unfortunately, there are few studies measuring the muscle activity of patients with CF during exercise (Gruet et al., 2010; Vallier et al., 2011) and, to our knowledge, there is no data concerning the fibre type recruitment strategies of young CF patients compared with their healthy counterparts during moderate and very heavy exercise. Since fibre type composition and recruitment strategies were not measured in this thesis, discrepancies between the groups and a role in the altered \( \dot{V}O_2 \) kinetics at higher work rates cannot be excluded. It is known that cycling at higher work rates mandates the recruitment of muscle fibres with increased metabolic diversity (i.e. type II, IIA and IIX fibres) and, therefore, features of the underlying muscle fibre type distribution influence \( \dot{V}O_2 \) kinetics above the GET (Pringle et al., 2003). Slower \( \dot{V}O_2 \) kinetics characterise humans with a higher proportion of ‘less efficient’ type II fibres during heavy-severe intensity exercise (Pringle et al., 2003) and type II fibres have a greater \( O_2 \) extraction requirement to increase \( \dot{V}O_2 \). Given that \( O_2 \) extraction dynamics were impaired in CF in this thesis (Chapters 6 and 8), fibre type differences is not likely a primary factor regulating the response. However, differential recruitment of muscle fibres either at the onset and/or during exercise may contribute to the intensity dependence of the CF-related differences in muscle energetics evident in Chapter 8. Further research in this area is therefore warranted.

10.4 Recommendations for clinical practice

Obtaining a valid \( \dot{V}O_{2\text{max}} \) measurement is critical and particularly important in paediatric groups. Whilst the importance of calibration of metabolic carts is widely
acknowledged with regard to the accuracy of CPET-derived data, thorough familiarisation and the choice of testing protocol should be vital considerations for those choosing to implement CPET as a clinical assessment tool in patients with CF. From a practical viewpoint, familiarisation with the expectations of the protocol and what it feels like to maintain a steady cadence is essential and should not be overlooked. The choice of protocol and verification criteria are also fundamental considerations to obtain a ‘true’ representation of $\dot{V}O_{2\text{max}}$. However, at a time when the ECFS are recommending the Godfrey protocol, the present thesis has demonstrated that a verification phase should be included to confirm $\dot{V}O_{2\text{max}}$. Furthermore, a key benefit of the ramp incremental protocol is that 1) the test spans the range of exercise intensities and, therefore, 2) derives both important maximal and submaximal parameters of aerobic exercise (dys)function and the mechanism(s) regulating this. This can help to prevent premature test termination and short test durations, which have previously been observed with the CF exercise testing literature (e.g., Kent et al., 2012). There is, therefore, a need for research that directly compares the different CPET protocols, in addition to different modes of exercise, to enable further recommendations regarding the best protocol for this patient group.

These recommendations have significant implications for the assessment and interpretation of CPET in clinical and research settings. To utilise $\dot{V}O_{2\text{max}}$ in prognostic stratification and the assessment of clinical or research interventions in CF, it is essential that ‘true’ measurements are obtained. Accepting submaximal efforts with significantly distort the clinical interpretation of patients’ aerobic fitness status. From a practical viewpoint, $S_{\text{max}}$ is straightforward to implement and, clinically, may minimise the costs associated with re-tests when the validity of a test
is questionable. There is, however, a need to assess the appropriateness of this form of testing in older patients with more advanced respiratory disease, who may also have more serious cardiac (dys)function (e.g. Giacchi et al., 2015).

Interpreting data in relation to normative values and established TEs enables researchers and clinicians to determine meaningful changes. Reproducibility over time is critical when evaluating the efficacy of CF treatments (e.g., antimicrobials, mucolytics and gene mutation targeted therapies) which may accrue over weeks or months, as well as monitoring exercise training interventions. Data in this thesis has provided the reproducibility of CPET derived submaximal and maximal parameters using a valid protocol, the TE of which was also improved when compared with an incremental test using traditional verification criteria. Determining the extent to which changes in outcome measures relate to normative data is fundamental to the utility of CPET. Establishing robust normative data for both young people with CF and their healthy counterparts therefore represents an important next step.

Furthermore, it is recommended that the wealth of parameters provided by CPET is not ignored. Although these indices are often overlooked in favour of maximal end-exercise measurements, submaximal parameters should assist with diagnostic and prognostic evaluations. Furthermore, no single parameter should be used exclusively, rather it is the integration of the exercise responses as a whole which will add value when utilising CPET to assess physiological (dys)function.

10.4 Conclusion

The present thesis provides a series of experimental studies addressing issues relating to the assessment of aerobic exercise (dys)function, typical clinical
presentation and the factor(s) modulating this response in paediatric patients with mild-to-moderate CF. Consequently, this thesis has made a significant and novel contribution to our understanding of how to assess and interpret aerobic exercise (dys)function in this patient group. New and original insights are presented regarding an apparent exercise intensity-dependence of the influence of mild-to-moderate CF disease on skeletal muscle oxidative metabolism in children and adolescents. This thesis is timely, given the recent promotion of CPET within the clinical management of patients with CF by the European, North American and Australasian CF associations and recognition regarding the importance of exercise and physical activity within CF management:

“…Over the past years, evidence for the beneficial effects of exercise on lung health and aerobic exercise capacity is strengthening. Despite the fact that most of the knowledge is based on small studies, the observed effects are encouraging and there is no reason why exercise should not be implemented in all patients’ care.”

- Hebestreit et al. (2015a).

“…Children with cystic fibrosis should be encouraged to take part in as much physical activity as possible, ideally types of exercise that leave you out of breath, like running, swimming, football or tennis.”

- UK Cystic Fibrosis Trust, 2015.

In view of this, it is important that those utilising exercise testing consider and understand the validity and utility of their chosen outcome measures and their associated error, to make decisions regarding clinically meaningful changes in patients’ function with better clarity. Furthermore, those conducting exercise testing
as an outcome measure, particularly in the research environment, should include additional key parameters of aerobic exercise function and consider the use of other non-invasive assessment techniques, such as NIRS and \( \dot{V}O_2 \) kinetics, to provide mechanistic insight into the response to pharmacological and/or physiotherapy intervention practices. This will enable further understanding of the physiological mechanism(s) responsible for changes in patients’ function, as a result of disease progression and/or pharmacological or therapeutic interventions. Systematic exercise testing and training will also enable a larger, more comprehensive epidemiological profile of the exercise (dys)function characterising this new generation of aging patients with CF and national normative reference data, such as that established in the Netherlands (Bongers, 2013), to be established for UK based patients.
CHAPTER TEN

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APPENDIX A

Independent Scientific Review Approval Letters

Studies 1 and 2

Royal Devon and Exeter NHS Foundation Trust

Dr Patrick Oades
RD&E
Wonford

26 October 2010

Dear Dr Oades

Study title: The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population

Your protocol has been returned from Independent Scientific Review.

According to this assessment we are pleased to say that the protocol does meet minimum standards and V2 (please change the version number and dates on the protocol) of the protocol has full scientific approval.

Please use this document and your comment response sheet as part of your submission to ethics

Best wishes

Yours sincerely

Lisa Bowern
R&D Coordinator

Enc
Studies 5 and 6

Royal Devon and Exeter
NHS Foundation Trust

Professor Craig Williams
Children's Health & Exercise Research
Centre, University of Exeter

Miss Zoe Saynor
Children's Health & Exercise Research
Centre, University of Exeter

Date 13.08.12

Dear Craig,

Study Reference: CW/13/08/12

Title: The influence of disease status and 'priming' exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate and high-intensity cycling exercise in paediatric patients with cystic fibrosis.

Your protocol has been returned from Independent Scientific Review. According to this assessment we are pleased to say that the protocol does meet minimum standards. Please use this document as part of your submission to ethics.

If you have any feedback on the Scientific Review process please contact Rohan Chauhan at Rohan.Chauhan@rdeft.nhs.uk.

Best wishes,

Rohan Chauhan
NHS Research Advisor/RDS Consultant
APPENDIX B

Ethics Approval for Studies 1 - 6

Studies 1 and 2

NATIONAL RESEARCH ETHICS SERVICE

South West REC

(formerly Farnborough REC)

South West REC Centre

Level 2, Block B

Weymouth

Livestock Unit

Bristol

BS1 3PU

Telephone: 0117 942 1534

30 December 2010

Professor Craig Williams

Associate Director of Children's Health & Exercise Research Centre and Senior Lecturer in

Exercise Physiology,

School of Sport & Health Sciences,

University of Exeter

Children's Health & Exercise Research Centre,

St Luke's Campus,

Heavitree Road,

Exeter

EX1 2LU

Dear Professor Williams

Study Title: The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population

REC reference number: 10/H0107/78

Thank you for your letter of 20 December 2010, responding to the Committee's request for further information on the above research and submitting revised documentation. The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.
Dear Craig,

Thank you for your application for ethical approval:

Proposal 5 2012/111 (26/10/11)

Title: Oxidative and cardiovascular responses during exercise in patients with cystic fibrosis compared to age and gender matched controls

Applicant: Associate Professor Craig Williams with Dr Alan Barker, Dr Partrick Oades (Consultant Paediatrician) and Zoe Saynor (Research Student)

The proposal (reviewed on the e-Ethics system) was discussed by the Committee. The Committee advised that the application should be amended as follows:

i. The parent/guardian information sheet needs to be reduced from 8 pages to 2-3 and should include less technical terminology and abbreviations and lay titles.
ii. This section of the study does not require detailed information regarding cystic fibrosis, or information specifically related to the NHS and its procedures, such as reference to keeping the information gathered for 15 years, it is usually 5.
iii. A risk assessment needs to be completed for the study of lung function using a micro loop.
iv. Craig Williams should be listed as Dr or Associate Professor rather than Professor v. It needs to be made clear on the information sheet where the study is taking place, is it at CHERC or at the RD&E hospital?
vi. There is a discrepancy between the adult and the child forms, the adult forms suggest that they will be able to complete their child’s form with them, whereas the child’s form suggests they will be able to complete the form separately. Please also ensure the information in these sheets match that in the child sheets. Point 12 should read “what if my child or I”.

vii. In the parent’s letter it states that the study will be a “novel insight for both you and your child”. This appears to try and sell the study and may not be the case.

Decision: The Committee agreed to PROVISIONALLY APPROVE the proposal until July 2012 but required the amendments to be returned to Lauren Hitchman for approval by DW prior to the commencement of the study.
12 October 2012

Professor Craig Williams
Associate Director of Children's Health & Exercise Research Centre and Senior Lecturer in
Exercise Physiology
School of Sport and Health Sciences, University of Exeter
Children's Health & Exercise
Research Centre, St Luke's Campus,
Heavitree Road, Exeter
EX1 2LU

Dear Professor Williams

Study title: The influence of disease status and 'priming' exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate and high-intensity cycling exercise in paediatric patients with cystic fibrosis

REC reference: 12/SW/0270

Thank you for your letter of 2nd October 2012, responding to the Committee’s request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.
Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.rdfforum.nhs.uk](http://www.rdfforum.nhs.uk).

Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
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<tr>
<td>Covering Letter</td>
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<td>02 October 2012</td>
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<tr>
<td>Covering Letter</td>
<td></td>
<td>20 September 2012</td>
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<tr>
<td>Evidence of insurance or indemnity</td>
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<td>01 April 2012</td>
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<tr>
<td>Investigator CV</td>
<td></td>
<td>15 August 2012</td>
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<tr>
<td>Letter from Sponsor</td>
<td></td>
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<tr>
<td>Letter of invitation to participant</td>
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<td>Other: Participant interest form</td>
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<td>Other: CV Dr Alan Barker</td>
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<tr>
<td>Other: CV Dr Patrick Oades</td>
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<tr>
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<td>Other: Certificate of Ethical Approval (Healthy Control Participants)</td>
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<td>Other: Face mask Specifications</td>
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<tr>
<td>Participant Consent Form: Patient (10 - 15 yrs) assent form</td>
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<tr>
<td>Participant Information Sheet: Patient (10 - 15 yrs) Information Sheet</td>
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<tr>
<td>Referees or other scientific critique report</td>
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<td>13 August 2012</td>
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</table>
Health Research Authority

Response to Request for Further Information 24 September 2012
Response to Request for Further Information 20 September 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review — guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

Please quote this number on all correspondence

12/SW/0270

With the Committee’s best wishes for the success of this project

Yours sincerely

Dr Mike Shere
Chair

Email: ubh-tr.southwest5@nhs.net

Enclosures: “After ethical review — guidance for researchers”

Copy to: Mr. Christopher Gardner
Miss Gyan Lang, Research & Development Royal Devon & Exeter
NHS Foundation Trust
APPENDIX C

Funding

Studies 1 and 2

Royal Devon and Exeter NHS Foundation Trust

Dr Patrick Oades
RD&E
Wonford

26 October 2010

Dear Dr Oades

Study title: The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population

Your protocol has been returned from Independent Scientific Review.

According to this assessment we are pleased to say that the protocol does meet minimum standards and V2 (please change the version number and dates on the protocol) of the protocol has full scientific approval.

Please use this document and your comment response sheet as part of your submission to ethics

Best wishes

Yours sincerely

Lisa Bowem
R&D Coordinator

Eric
14 August, 2012

Dear Craig,

**Study Title:** The influence of disease status and ‘priming’ exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate and high-intensity cycling exercise in paediatric patients with cystic fibrosis

Thank you for applying for a small grant. On this occasion I am pleased to say your application has been successful, therefore Research and Development will provisionally meet costs of up to £9,330 for the expenses of this study based on the conditions set out below. This will be allocated in 2 installments to reflect the financial year. Please contact Mike Clark (Research Accounting Officer) on ext 6970 or mike.clark@nhs.net for further details re breakdown of costs.

**It is expected that researchers will submit their full application to Ethics copying in R&D within 2 months from the date of this offer. If no application is received the grant offer will be withdrawn and offered to other applicants.**

Your protocol has been assessed including an Independent Scientific Review. According to this assessment, the protocol does meet minimum standards. Please liaise with the R&D Office in relation to the application for both ethical and Trust Approval if you have not already done so.

In order to secure funding you will need to contact the relevant manager in your department and complete a **FAL form (or if external, invoice R&D through your organisation/dept).**

Once you have gained Trust approval a signed form will be sent to you. On completing the relevant section you must forward to the R&D Management Accountant in finance in order to release the funds.

Researchers must provide a summary of their study results if successful in obtaining a small grant.

In the meantime, congratulations on your successful application.

Best wishes,

![Signature]

Chris Gardner
Directorate Manager, Research and Development

**Studies 4 and 5**
APPENDIX D

Written Supportive Information for Participants and Parents/Guardians (Studies 1 and 2)

A study about fitness of children

Children’s Letter

Dear Child,

We would like to ask you to take part in a study at the hospital. Please read this sheet very carefully before you decide to take part or not.

What is the study about?

- The study will help us find out how fit you are by looking at how much oxygen you use during and after three exercise tests.

What will happen?

- Each exercise test will involve you pedalling on an exercise bike.
- During the exercise tests you will wear a mouthpiece and nose clip.
- Before and after each test you will breathe out into a plastic tube so we can see what your lungs are doing.
- You will also be asked if you would like to do a fingertip prick test. This is where we take a small spot of blood from a tiny prick of your fingertip.
- This would be no more than one on each of the 3 visits.
- You do not have to do the fingertip prick test if you don’t want to and you can still take part in the exercise tests.
Are the exercise tests safe?

- The exercise tests are very safe.
- Your doctor will look at your health records before you start any exercise tests to see if it is safe for you to take part.
- A medical team will be ready during the exercise tests just in case there are any problems.

What else will happen on each visit to the hospital?

First Visit
- On your first visit to the hospital we will measure your height, your height when you are sitting down and your weight.
- We will measure your skin folds at your arm, back and hip. This is done by very gently pinching the skin using a small instrument. This does not hurt but may tickle.
- You will then be shown the equipment that will be used during the following visits
- Finally, you will be given the chance to practice cycling on the exercise bikes and feel what it is like when pedalling is both easy and hard

Second Visit
- The second visit will involve your first exercise test
- Before you have to do any exercise you will be asked to rest
- The exercise test will involve you first pedalling on an exercise bike for 6 minutes when the pedals are quite easy to turn
- You will then be asked to pedal on the exercise bike for as long as you can until you can’t pedal anymore.
- The pedals on the bike will get harder to turn the longer you pedal.
- You will wear a mouthpiece and nose piece during the cycling.
- You will then stay on the bike and pedal easily for 5 minutes, then you will sit down and rest for 10 minutes while still wearing the mouthpiece and nose clip.
- You will then be asked if you would like to do the fingertip prick test.
- You will then get back onto the bike and be asked to cycle for as long as you can again, to check that you cannot go for longer than you did before.
- We will then give you a drink and a biscuit and you can rest before going home.

Third and Fourth Visits
- The next 2 visits will be exactly the same as your 2nd visit
- You will simply be asked to perform the same exercise test and the same measurements will taken

After you have finished taking part in the study
- If you are a girl aged 8 or over or a boy aged 10 or over, we also need to know how developed your body is. To do this we will ask you to go into a private room, look at yourself and answer a question about how far your body has
developed. The question is an easy choice out of 5, and nobody will be present in the room.

What else will you have to do if you do decide to take part?

1. For 2 – 3 days before each visit you must not do any hard exercise.
2. You can eat and drink as normal but do not drink or eat foods which contain caffeine such as tea, coffee, coca-cola, dark/milk chocolate for the 3 hours before coming to see us.
3. Please wear the same clothing (e.g. PE kit, T-shirt, shorts and trainers) each time you visit.

Do you have to take part?

• It is up to you to decide whether or not to take part.
• If you take part you are still free to drop out at any time and without giving a reason.
• If you drop out or do not take part it will not affect the way your doctor and healthcare team treat you.
• If you do not want to have the fingertip prick tests done you can still take part.

What should you do if you want to take part or have any questions?

1. If you would like to take part you must have your parent/carer’s permission by getting them to complete the consent form.
2. You must also complete a form and return it to a member of the research team.
3. If you have any questions please get in touch with a member of the research team who are on the bottom of this page.

Thank you for reading this sheet.
The Research Team.

Miss. Zoe Louise Saynor  
(Children’s Health & Exercise Research Centre, University of Exeter)  
Tel (work): (01392) 264889  
Tel (mobile): (07984) 638788  
E-mail: zls202@exeter.ac.uk

Dr. Patrick J. Oades  
(Consultant Paediatrician, Royal Devon and Exeter Healthcare NHS Trust)  
Tel: (01392) 402665  
E-mail: patrick.oades@rdehc-tr.swest.nhs.uk

Professor Craig. A. Williams  
(Children’s Health & Exercise Research Centre, University of Exeter)  
Tel: (01392) 724809  
E-mail: c.a.williams@ex.ac.uk

Dr. Alan Barker  
(Children’s Health & Exercise Research Centre, University of Exeter)  
Tel: (01392) 722766  
E-mail: a.r.barker@exeter.ac.uk
1. Study Title

The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population.

2. Invitation paragraph

You are being invited to take part in a research study. Before you decide whether or not to be involved, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your clinician/GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

If you would like more information regarding participation in research you can access the public information pack published by INVOLVE (a non-profit organization promoting public involvement in the NHS, public health and social care research). This is freely available on their website www.involve.org.uk/ or you can obtain a paper copy by writing to: Involve, Royal London House, 22-25 Finsbury Square, London, EC2A 1DX.

More specialized information regarding participation in clinical research is published by the UK Clinical Research Collaboration (UKCRC). You can access this information on the UKCRC website www.ukcrc.org or request a printed copy from: UKCRC, 20 Park Crescent, London, W1B 1AL.

Thank you for reading this.

3. What is the purpose of the study?
It is recommended that children and teenagers with cystic fibrosis (CF) take part in sports and games to stay fit and healthy. Exercise not only helps improve fitness and enhances their quality of life through socialising with peers and friends, but it may also help them to cope better with their disease. Exercise testing for children with CF is also held in high regard by clinicians. It may provide clinicians with additional prognostic information relating to your child’s disease. Much remains to be clarified, however, regarding the most feasible tests to implement into clinical practice.

Exercise testing offers potential prognostic value that common clinical assessments you may be familiar with, such as measuring resting lung function with a spirometer, cannot. This study will form the basis of future work examining exercise testing and training for children with CF. It is proposed that this study will initiate implementation of annual exercise testing within the paediatric unit (Bramble ward) of the Royal Devon & Exeter hospital (RD&E). Whilst the main aim of this study is to establish the reliability and validity of a particular exercise test, it will also provide information regarding your child’s exercise tolerance which may offer a novel insight for both you and your child.

During exercise breathing becomes faster and harder because your body needs more oxygen to produce energy to move faster. The study will look at how much oxygen your child breathes in and uses during an exercise test performed on an exercise bike.

The results of the study will help to better inform clinicians regarding what exercise tests are suitable for this patient group and to develop better exercise testing procedures for children with CF.

4. Why have I been chosen?

We are interested in looking at children with cystic fibrosis, aged between 7-18 years old, who regularly take part in physical activity.

5. Do I have to take part?

Taking part is entirely voluntary and it is up to you to decide whether or not you wish to take part. If you do choose to take part you will be given this information sheet to keep, and be asked to sign a consent form giving your permission to participate. If you do decide to take part you are still free to withdraw from the study at any time and without giving a reason. A decision to withdraw at any time or a decision not to take part will not affect the standard clinical care you receive.

6. What will happen to me if I take part?

The study will involve 4 trips to an exercise laboratory within the RD&E, spread across a period of approximately 1 month. The initial visit will be a familiarisation session to discuss any queries you may have, obtain informed consent and to familiarise you with the equipment/testing procedures that subsequent exercise testing will involve. We will also use
this session to obtain some information regarding your body composition and to practice exercising on the bike at different intensities. All times will be at times which are suitable for you. This initial familiarisation session should not exceed 2 hours. The 3 subsequent exercise testing sessions will last ~50 minutes each. On each visit to the centre you will be required to complete an exercise test and the following measurements will be taken:

**Respiratory function:**
The volume of oxygen you are breathing in (VO₂)
The volume of carbon dioxide you are breathing out (VCO₂)
The ratio between the amounts of oxygen you are breathing in and carbon dioxide you are breathing out (RER)
The number of breaths you are taking (fV)

**Lung function**
The volume of air your lungs can hold (VC)
The volume of air you can breathe out (FVC)
The volume of air you can breathe out in one second (FEV₁)
The volume of air you are breathing in and out (PEF)

**Other measurements**
Your heart rate (HR)
How tired you think you feel (RPE)
How breathless you think you feel (RPD)
The amount of lactate in your blood ([La⁻][Hb+Mb]) (optional)
Changes in the concentrations of oxy-[Hb+Mb] and deoxy-[Hb+Mb] haemoglobin/myoglobin within the quadriceps muscles of your right leg

**Visit 1**
On the first visit to the laboratory your height, sitting height, and weight will be taken. You will also have your skin folds measured at your arm, back and hip. This is done by very gently pinching the skin and measuring the width of the skin folds. You will then have your lung function measured, this will involve you taking a big breath and breathing into a plastic tube.

You will be familiarised with the equipment to be used during testing and will be encouraged to practice cycling at different intensities on the stationary bike. You can also take this opportunity to ask any questions to the research staff regarding what would be required at the subsequent exercise testing sessions.

**Visit 2**
The second visit will involve the first of the exercise tests. This first exercise test will involve you pedalling on an exercise bike for as long as you can. This exercise test starts off easy but gradually the pedals get harder to turn making the exercise test progressively more difficult. During this exercise test you will have to breathe through a mouthpiece and wear a nose clip, this may seem strange at first but you will soon get used to it. The amount of oxygen present in your blood will also be monitored throughout the session.
During the exercise you will be shown a list of numbers on a chart numbered 6-20, and will be asked to point to a number on the chart which shows how hard or easy you are finding the exercise test. You will also be asked to rate on a different chart how breathless you feel.

After you have finished the first exercise test you will remain on the bike for a 5 minute cool-down period of unloaded pedalling and then be seated down and rested for 10 minutes whilst still wearing the mouthpiece and nose clip. After the exercise test you will have your lung function measured again. You will then be asked for a spot of blood, which will be drawn from your fingertip by a tiny needle prick. This is optional and even if you wish not to give a spot of blood you will still be allowed to participate in the study. The fingertip prick test is used to examine how hard you worked during the exercise test. Following the 15 minutes rest you will be asked to get back onto the bike and to pedal again for as long as possible. The aim of this is to clarify whether or not you could have continued for any longer during the 1st part of the test. This section will last approximately 2 minutes. During both the rest and the second part of the exercise test you will be wearing the same mouthpiece and nose clip as before in the first exercise test. You lung function will be measured before and after both parts.

Visit 3
Forty-eight hours later you will be asked to complete the second exercise testing which will be identical to visit 2.

Visit 4
You will be required to complete the fourth and final exercise test ~4 weeks after their previous (2nd) exercise test. This visit will again be identical to the previous exercise testing sessions, whilst wearing the mouthpiece and nose clip. Again, your lung function will be measured before and after the third exercise test, and afterwards you will have a choice whether or not to do a final fingertip prick test.

Post-testing
We also need to know your maturation (growth and development). This will require you to self-assess your pubertal stage by giving you 5 options. Self-assessment will take place at your home and you will be asked to return the scale in a sealed envelope you will be provided with. N.B. This will only be asked of boys from age 10 and girls from the age of 8.

Please see the diagram on the following page for a full summary of what will happen during each visit.
Visit 1: Familiarisation session
- SS obtained by Dr. Patrick Oades prior to this
- Run through PIS and protocol once more with patient & parent/guardian
- Obtain age, pubertal maturation and anthropometric measurements
- Cycle ergometry familiarisation
- Familiarisation with assessment equipment and scales
TOTAL TIME: Maximum 2 hours

Exclusion or postponement may occur if a change in health status, such as acute infections or an unstable chronic health deems the patient unsafe to take part.

Visit 2: Exercise session 1
- Baseline lung function, deoxy-[Hb+Mb] and HR
- CPET protocol
- Exercise cessation assessment of RPE, RPD and ([La]B)
- 15 min seated rest
- Supramaximal testing
TOTAL TIME: Approx. 50 minutes
N.B. Lung function and SaO2 will be assessed throughout both exercise and rest

Visit 3: 48 hours post-exercise session 1
- Baseline lung function, deoxy-[Hb+Mb] and HR
- CPET protocol
- Exercise cessation assessment of RPE, RPD and ([La]B)
- 15 min seated rest
- Supramaximal testing
TOTAL TIME: Approx. 50 minutes
N.B. Lung function and SaO2 will be assessed throughout both exercise and rest

Visit 4: 6 weeks post-exercise session 1
- Baseline lung function, deoxy-[Hb+Mb] and HR
- CPET protocol
- Exercise cessation assessment of RPE, RPD and ([La]B)
- 15 min seated rest
- Supramaximal testing
TOTAL TIME: Approx. 50 minutes
N.B. Lung function and SaO2 will be assessed throughout both exercise and rest

Post-testing:
- Pubertal maturity self-assessed at home at returned in a sealed envelope to ZS
Figure 1. The assessment procedures for each participant.

7. What else will I have to do?

If you wish to take part in the study we would like you to attend all 4 visits to the laboratory. We ask that you be in a rested state on arrival, and have performed no strenuous exercise in the 2-3 days prior to visits.

On each visit to the laboratory we also ask that you have had sufficient food and drink before you arrive, and wear the same or similar clothes for each exercise test. We also ask that you don’t eat or drink any caffeine 3 hours prior to each visit. However, it is important that you do eat a light meal or snack before each visit (i.e. sandwich, cereal etc.).

8. What are the possible risks of taking part?

The exercise testing is very safe. An assessment of your health will be made before you start the exercise tests to see if it is safe for you to take part. During the exercise tests you will be carefully monitored and observed to ensure your safety and well-being.

9. What are the potential benefits of taking part?

The research is intended to enhance our understanding of how the lung and respiratory function of children and teenagers with CF respond during and after exercise testing. The information we obtain from the study may help to develop better exercise testing programmes with UK CF clinics.

Hopefully, you will take away a positive and enjoyable experience from your involvement in the exercise tests. You will be tested on equipment that is used to monitor the fitness of sports people, and you will get to see how fit you currently are and what intensity of exercise you can tolerate. You will be given the results of your exercise tests and talked through them by a member of the research team. This will hopefully prove an interesting and constructive exercise, particularly if you have a strong interest or involvement in sport and may wish to increase the amount of physical activity you participate in.

10. What happens if I don’t want to continue in the study?

You are free to withdraw from the study at any point without giving a reason. Dropping out of the study will not affect your clinical care in any way or your relationship with the paediatric staff.

If you do decide to not continue with the study then you will not be required to complete any additional exercise tests, or attend any additional visits to the centre that are associated with the research. Results from the exercise tests that you have previously completed will still be available to you.

11. What if something goes wrong?
There are no special compensation arrangements if taking part in this study harms you. Your rights are the same as undergoing research i.e. if you are harmed due to someone’s negligence, then you may have grounds for legal action, but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University complaints procedures will be available to you.

12. Will my taking part in this study be kept confidential?

We have a responsibility to inform you of how we will collect, store and use you information that we gather during the study. The primary concern is that any information that we collect about you will be confidential. All of the this information collected such as your name, date of birth, contact details, details of your health and test results will be transferred to a paper study file, which will be kept in a secure room in the centre in a locked filing cabinet.

Your exercise results will be kept anonymous by assigning them a unique study code and participant number. Only your date of birth will be used to identify the results. The results from the exercise tests are collected on paper and stored in a locked filing cabinet. The exercise results are then transferred from paper and stored on a computer. The only personal information stored on computer will be your date of birth and participant number. Your exercise results will be password protected, as will the computer used to store the information. All the paper and computer files will be stored for 15 years, after this period paper files will be destroyed and computer files erased. Only the researchers involved in the study will have access to your medical records and exercise results.

13. What will happen to the results of the study?

Once the study is completed, which is targeted to be September 2011, you will be sent a summary of our research findings. The research is undertaken with the intention of being submitted as a PhD thesis. It is also the intention to submit the results of the research to relevant journals for publication, and to inform our colleagues of the findings at scientific meetings. In publishing and talking about the study you will not be identifiable.

You will be given the opportunity to comment on the research protocol and testing procedure. This information will be collated and may inform future studies. You will also receive the results and conclusions from the research and are free to request information regarding your individual data.

14. Who is organising and funding the research?

The study is a collaboration between the Paediatric Unit of the Royal Devon and Exeter (RD&E) NHS Foundation Trust Hospital and the Children’s Health and Exercise Research Centre at the University of Exeter. The study is sponsored by the RD&E NHS Foundation Trust. None of the researchers or doctors involved personally receives money for including you in the study.
As a result, a monetary contribution will be provided to go some way towards covering travel expenses.

15. Who has reviewed the study?

The scientific content of the study has been reviewed by the Peninsula Research and Development Support Unit. All research within the National Health Service (NHS) is looked at by an independent group of people, called a Research Ethics Committee (REC). RECs safeguard the rights, safety, dignity and well-being of people participating in research in the NHS. They review applications for research and give an opinion about the proposed participant involvement and whether the research is ethical. The present study has been reviewed and given favourable opinion by the South West 5 (or Frenchay) REC.

16. What should I do if I would like to take part?

If you would like to take part in the study you must give your permission by completing the consent form. You should this form to a member of the research team.

17. What if I have any questions?

If you have any questions please do not hesitate to get in touch with a member of the research team.

18. Contact for further information

If you need further information please contact:

**The Research Team**

Miss. Zoe Louise Saynor (Children’s Health & Exercise Research Centre, University of Exeter)
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Dr. Alan Barker (Children’s Health & Exercise Research Centre, University of Exeter)
Tel (work): (01392) 722766
E-mail: a.r.barker@exeter.ac.uk
PARENT/GUARDIAN INFORMATION SHEET
FOR
THE RELIABILITY AND VALIDITY OF MAXIMAL CARDIOPULMONARY
EXERCISE TESTING AS A PROGNOSTIC TOOL WITHIN THE YOUNG CYSTIC
FIBROSIS POPULATION
Version number: 2
Date: 20/12/10

1. Study Title

The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population.

2. Invitation paragraph

Your child is being invited to take part in a research study. Before you decide whether or not to be involved, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your clinician/GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

If you would like more information regarding participation in research you can access the public information pack published by INVOLVE (a non-profit organization promoting public involvement in the NHS, public health and social care research). This is freely available on their website www.involve.org.uk/ or you can obtain a paper copy by writing to: Involve, Royal London House, 22-25 Finsbury Square, London, EC2A 1DX.

More specialized information regarding participation in clinical research is published by the UK Clinical Research Collaboration (UKCRC). You can access this information on the UKCRC website www.ukcrc.org or request a printed copy from: UKCRC, 20 Park Crescent, London, W1B 1AL.

Thank you for reading this.

3. What is the purpose of the study?
It is recommended that children and teenagers with cystic fibrosis (CF) take part in sports and games to stay fit and healthy. Exercise not only helps improve fitness and enhances their quality of life through socialising with peers and friends, but it may also help them to cope better with their disease. Exercise testing for children with CF is also held in high regard by clinicians. It may provide clinicians with additional prognostic information relating to your child’s disease. Much remains to be clarified, however, regarding the most feasible tests to implement into clinical practice.

Exercise testing offers potential prognostic value that common clinical assessments you may be familiar with, such as measuring resting lung function with a spirometer, cannot. This study will form the basis of future work examining exercise testing and training for children with CF. It is proposed that this study will initiate implementation of annual exercise testing within the paediatric unit (Bramble ward) of the Royal Devon & Exeter hospital (RD&E). Whilst the main aim of this study is to establish the reliability and validity of a particular exercise test, it will also provide information regarding your child’s exercise toleration which may offer a novel insight for both you and your child.

During exercise breathing becomes faster and harder because your body needs more oxygen to produce energy to move faster. The study will look at how much oxygen your child breathes in and uses during an exercise test performed on an exercise bike.

The results of the study will help to better inform clinicians regarding what exercise tests are suitable for this patient group and to develop better exercise testing procedures for children with CF.

4. Why has my child been chosen?

We are interested in looking at children with cystic fibrosis, aged between 7-18 years old, who regularly take part in physical activity.

5. Does my child have to take part?

Taking part is entirely voluntary and it is up to you to decide whether or not your child takes part. If you do allow your child to take part you will be given this information sheet to keep, and be asked to sign a consent form giving your child permission to participate. If you do decide to allow your child to take part you are still free to withdraw your child from the study at any time and without giving a reason. A decision to withdraw your child at any time or a decision not to take part will not affect the standard clinical care your child receives.

6. What will happen to my child if I allow them to take part?

The study will involve 4 trips to an exercise laboratory within the RD&E, spread across a period of approximately 1 month. The initial visit will be a familiarisation session to discuss any queries you or your child may have, obtain informed consent/assent and to familiarise both you and your child with the equipment/testing procedures that subsequent exercise testing will involve. We will also use this session to obtain some information regarding your
child’s body composition and to practice exercising on the bike at different intensities. All
times will be at times which are suitable for you. This initial familiarisation session should
not exceed 2 hours. The 3 subsequent exercise testing sessions will last ~50 minutes each. On
each visit to the centre your child will be required to complete an exercise test and the
following measurements will be taken:

**Respiratory function:**
The volume of oxygen your child is breathing in (VO$_2$)
The volume of carbon dioxide your child is breathing out (VCO$_2$)
The ratio between the amounts of oxygen your child is breathing in and carbon dioxide he/she is
breathing out (RER).
The number of breaths your child is taking (fv).

**Lung function**
The volume of air your child’s lungs can hold (VC).
The volume of air your child can breathe out (FVC).
The volume of air your child can breathe out in one second (FEV$_1$).
The volume of air your child is breathing in and out (PEF).

**Other measurements**
Your child’s heart rate (HR).
How tired your child thinks he/she feels (RPE).
How breathless your child thinks he/she feels (RPD)
The amount of lactate in your child’s blood ([La$^-$_B] (optional)
Changes in the concentrations of oxy-[Hb+Mb] and deoxy-[Hb+Mb] haemoglobin/myoglobin within the quadriceps muscles of your child’s right leg

**Visit 1**
On the first visit to the laboratory your child’s height, sitting height, and weight will be taken.
They will also have their skin folds measured at their arm, back and hip. This is done by very
gently pinching the skin and measuring the width of the skin folds. They will then have their
lung function measured, this will involve your child taking a big breath and breathing into a
plastic tube.

Your child will be familiarised with the equipment to be used during testing and will be
couraged to practice cycling at different intensities on the stationary bike. You can also
take this opportunity to ask any questions to the research staff regarding what would be
required at the subsequent exercise testing sessions.

**Visit 2**
The second visit will involve the first of the exercise tests. This first exercise test will involve
your child pedalling on an exercise bike for as long as they can. This exercise test starts off
easy but gradually the pedals get harder to turn making the exercise test progressively more
difficult. During this exercise test they will have to breathe through a mouthpiece and wear a
nose clip, this may seem strange at first but they soon get used to it. The amount of oxygen
present in your child’s blood will also be monitored throughout the session.

During the exercise your child will be shown a list of numbers on a chart numbered 6-20, and
will be asked to point to a number on the chart which shows how hard or easy they are
finding the exercise test. They will also be asked to rate on a different chart how breathless they feel.

After they have finished the first exercise test they will remain on the bike for a 5 minute cool-down period of unloaded pedalling and then be seated down and rested for 10 minutes whilst still wearing the mouthpiece and nose clip. After the exercise test your child will have their lung function measured again. They will then be asked for a spot of blood, which will be drawn from their fingertip by a tiny needle prick. This is optional and even if your child wishes not to give a spot of blood they will still be allowed to participate in the study. The fingertip prick test is used to examine how hard they worked during the exercise test. Following the 15 minutes rest your child will be asked to get back onto the bike and to pedal again for as long as possible. The aim of this is to clarify whether or not they could have continued for any longer during the 1st part of the test. This section will last approximately 2 minutes. During both the rest and the second part of the exercise test your child will be wearing the same mouthpiece and nose clip as before in the first exercise test. Your child’s lung function will be measured before and after both parts.

**Visit 3**
Forty-eight hours later your child will be asked to complete the second exercise testing which will be identical to visit 2.

**Visit 4**
Your child will be required to complete the fourth and final exercise test ~4 weeks after their previous (2nd) exercise test. This visit will again be identical to the previous exercise testing sessions, whilst wearing the mouthpiece and nose clip. Again, their lung function will be measured before and after the third exercise test, and afterwards they will have a choice whether or not to do a final fingertip prick test.

**Post-testing**
We also need to know your child’s maturation (growth and development). This will require them to self-assess their pubertal stage by giving them 5 options. Self-assessment will take place at home and you will be asked to return the scale in a sealed envelope you will be provided with. **N.B. This will only be asked of boys from age 10 and girls from the age of 8.**

Please see the diagram on the following page for a full summary of what will happen during each visit.
Visit 1: Familiarisation session
- SS obtained by Dr. Patrick Oades prior to this
- Run through PIS and protocol once more with patient & parent/guardian
- Obtain age, pubertal maturation and anthropometric measurements
- Cycle ergometry familiarisation
- Familiarisation with assessment equipment and scales
TOTAL TIME: Maximum 2 hours

Visit 2: Exercise session 1
- Baseline lung function, deoxy-[Hb+Mb] and HR
- CPET protocol
- Exercise cessation assessment of RPE, RPD and ([La]B)
- 15 min seated rest
- Supramaximal testing
TOTAL TIME: Approx. 50 minutes
N.B. Lung function and SaO₂ will be assessed throughout both exercise and rest

Visit 3: 48 hours post-exercise session 1
- Baseline lung function, deoxy-[Hb+Mb] and HR
- CPET protocol
- Exercise cessation assessment of RPE, RPD and ([La]B)
- 15 min seated rest
- Supramaximal testing
TOTAL TIME: Approx. 50 minutes
N.B. Lung function and SaO₂ will be assessed throughout both exercise and rest

Visit 4: 6 weeks post-exercise session 1
- Baseline lung function, deoxy-[Hb+Mb] and HR
- CPET protocol
- Exercise cessation assessment of RPE, RPD and ([La]B)
- 15 min seated rest
- Supramaximal testing
TOTAL TIME: Approx. 50 minutes
N.B. Lung function and SaO₂ will be assessed throughout both exercise and rest

Post-testing:
- Pubertal maturity self-assessed at home at returned in a sealed envelope to ZS

Exclusion or postponement may occur if a change in health status, such as acute infections or an unstable chronic health deems the patient unsafe to take part.
Figure 1. The assessment procedures for each participant.

7. What else will my child have to do?

If your child wishes to take part in the study and he/she has your permission we would like your child to attend all 4 visits to the laboratory. We ask that they be in a rested state on arrival, and have performed no strenuous exercise in the 2-3 days prior to visits.

On each visit to the laboratory we also ask that they have had sufficient food and drink before he/she arrives, and wear the same or similar clothes for each exercise test. We also ask that they don’t eat or drink any caffeine 3 hours prior to each visit. However, it is important that they do eat a light meal or snack before each visit (i.e. sandwich, cereal etc.).

8. What are the possible risks of my child taking part?

The exercise testing is very safe. An assessment of your child’s health will be made before they start the exercise tests to see if it is safe for them to take part. During the exercise tests your child will be carefully monitored and observed to ensure their safety and well-being.

9. What are the potential benefits of my child taking part?

The research is intended to enhance our understanding of how the lung and respiratory function of children and teenagers with CF respond during and after exercise testing. The information we obtain from the study may help to develop better exercise testing programmes with UK CF clinics.

Hopefully, your child will take away a positive and enjoyable experience from their involvement in the exercise tests. They will be tested on equipment that is used to monitor the fitness of sports people, and they will get to see how fit they currently are and what intensity of exercise they can tolerate. They will be given the results of their exercise tests and talked through them by a member of the research team. This will hopefully prove an interesting and constructive exercise, particularly if they have a strong interest or involvement in sport and may wish to increase the amount of physical activity they participate in.

10. What happens if my child doesn’t want to continue in the study?

Your child is free to withdraw from the study at any point without giving a reason. Dropping out of the study will not affect your child’s clinical care in any way or your relationship with the paediatric staff.

If your child does decide to not continue with the study then they will not be required to complete any additional exercise tests, or attend any additional visits to the centre that are associated with the research. Results from the exercise tests that they have previously completed will still be available to them.

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Your child’s exercise results will be kept anonymous by assigning them a unique study code and participant number. Only your child’s date of birth will be used to identify the results. The results from the exercise tests are collected on paper and stored in a locked filing cabinet. The exercise results are then transferred from paper and stored on a computer. The only personal information stored on computer will be your child’s date of birth and participant number. Their exercise results will be password protected, as will the computer used to store the information. All the paper and computer files will be stored for 15 years, after this period paper files will be destroyed and computer files erased. Only the researchers involved in the study will have access to your child’s medical records and exercise results.

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16. What should I do if my child would like to take part?

If your child would like to take part in the study you must give your permission by completing the consent form and your child must complete the assent form. You should then return the two forms to a member of the research team.

17. What if my child or me have a question?

If you or your child has any questions please do not hesitate to get in touch with a member of the research team.

18. Contact for further information

If you need further information please contact:

**The Research Team**

Miss. Zoe Louise Saynor (Children’s Health & Exercise Research Centre, University of Exeter)
Tel (work): (01392) 264889
Tel (mobile): (07984) 638788
E-mail: zls202@exeter.ac.uk

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E-mail: patrick.oades@rdehc-tr.swest.nhs.uk

Professor. Craig A. Williams (Children’s Health & Exercise Research Centre, University of Exeter)
Tel (work): 01392 724809
E-mail: c.a.Williams@ex.ac.uk

Dr. Alan Barker (Children’s Health & Exercise Research Centre, University of Exeter)
Tel (work): (01392) 722766
E-mail: a.r.barker@exeter.ac.uk
The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population

Participant interest form

<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Telephone number</th>
<th>Age/DOB</th>
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</tbody>
</table>

Medical conditions:

Activity levels:

Availability:

Other notes:

Zoe Saynor  
M.Phil./Ph.D. Researcher  
Tel: +44 (0) 1392 264889 / 07748207099  
Email: zls202@exeter.ac.uk
Dear

The Children's Health and Exercise Research Centre, in conjunction with Bramble Ward at the Royal Devon & Exeter Hospital, is currently undertaking some exciting research. We are currently looking for children/adolescents aged between 7-18 yrs with cystic fibrosis who would be interested in taking part.

On behalf of our paediatric team our exercise physiologist Zoe Saynor will contact you via telephone to see if you would like to be involved with our research.

An information sheet has been included for you to read and help you decide whether you would like to take part.

Your participation in this research project would be much appreciated. However, you are by no means obliged to take part in this study if you do not wish to. Furthermore, your medical care and individual rights are not affected by a decision to not participate, or subsequently withdraw from the study.

Many thanks for reading this,

Kind Regards,

Dr Patrick Oades
Consultant Paediatrician
Royal Devon and Exeter Healthcare NHS Trust

Miss Zoe Saynor
M.Phil/Ph.D. Researcher
Children's Health and Exercise Research Centre
University of Exeter
A study about fitness of children

Children’s Letter

Dear Child,

We would like to ask you to take part in a study being conducted by the Children’s Health & Exercise research Centre, Exeter. Please read this sheet very carefully before you decide to take part or not.

What is the study about?

- The study will help us find out how fit you are by looking at how much oxygen you use during and after an exercise test.

What will happen?

- The exercise test will involve you pedalling on an exercise bike.
- During the exercise test you will wear a rubber face mask over your nose and mouth.
- Before and after each test you will breathe out into a plastic tube so we can see what your lungs are doing.
- You will also be asked if you would like to do a fingertip prick test. This is where we take a small spot of blood from a tiny prick of your fingertip.
- This would be no more than one.
- You do not have to do the fingertip prick test if you don’t want to and you can still take part in the exercise test.
Are the exercise tests safe?

- The exercise tests are very safe.
- We will check a number of parts of your health records before you start any exercise tests to see if it is safe for you to take part.
- First aiders will be ready during the exercise tests just in case there are any problems.

What else will happen on each visit to the hospital?

Lab Visit – Part 1 (Familiarisation)
- On your visit to the laboratory we will measure your height, your height when you are sitting down and your weight.
- We will measure your skin folds at your arm, back and hip. This is done by very gently pinching the skin using a small instrument. This does not hurt but may tickle.
- You will then be shown the equipment that will be used during the following visits
- Finally, you will be given the chance to practice cycling on the exercise bikes and feel what it is like when pedalling is both easy and hard

Lab Visit - Part 2 (Exercise Test)
- The next part of your visit will involve your exercise test
- Before you have to do any exercise you will be asked to rest
- The exercise test will involve you first pedalling on an exercise bike for 3 minutes when the pedals are quite easy to turn
- You will then be asked to pedal on the exercise bike for as long as you can until you can’t pedal anymore.
- The pedals on the bike will get harder to turn the longer you pedal.
- You will wear a rubber face mask over your nose and mouth during the cycling.
- You will then stay on the bike and pedal easily for 5 minutes, and then you will sit down and rest for 10 minutes while still wearing the face mask.
- You will then be asked if you would like to do the fingertip prick test.
- You will then get back onto the bike and cycle for 3 minutes when the pedals are quite easy to turn
- You will then be asked to cycle for as long as you can again, to check that you cannot go for longer than you did before – this bit of the test will be shorter than the first bit
- We will then give you a drink and a biscuit and you can rest before going home.

After you have finished taking part in the study
- **If you are a girl aged 8 or over or a boy aged 10 or over,** we also need to know how developed your body is. To do this we will ask you to go into a private room, look at yourself and answer a question about how far your body has developed. The question is an easy choice out of 5, and nobody will be present in the room.
What else will you have to do if you do decide to take part?

4. For 2 – 3 days before each visit you must not do any hard exercise.
5. You can eat and drink as normal but do not drink or eat foods which contain caffeine such as tea, coffee, coca-cola, dark/milk chocolate for the 3 hours before coming to see us.
6. Please wear sports clothing (e.g. PE kit, T-shirt, shorts and trainers) when you visit

Do you have to take part?
- It is up to you to decide whether or not to take part.
- If you take part you are still free to drop out at any time and without giving a reason.
- If you do not want to have the fingertip prick tests done you can still take part.

What should you do if you want to take part or have any questions?

4. If you would like to take part you must have your parent/carer’s permission by getting them to complete the consent form.
5. You must also complete a form and return it to a member of the research team.
6. If you have any questions please get in touch with a member of the research team who are on the bottom of this page.

Thank you for reading this sheet.
The Research Team.

Miss Zoe Louise Saynor
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Dr. Patrick J. Oades
(Consultant Paediatrician, Royal Devon and Exeter NHS Foundation Trust Hospital)
Tel: (01392) 402665
E-mail: patrick.oades@rdehc-tr.swest.nhs.uk
PARTICIPANT (16-18 YEARS) INFORMATION SHEET
FOR
OXIDATIVE AND CARDIOVASCULAR RESPONSES DURING EXERCISE IN PATIENTS WITH CYSTIC FIBROSIS COMPARED TO AGE AND GENDER MATCHED CONTROLS
Version number: 2
Date: 02/01/12

1. Study Title: Oxidative and cardiovascular responses during exercise in patients with cystic fibrosis compared to age and gender matched controls.

2. Invitation paragraph
You are being invited to take part in a research study as they may be a match to compare with one of our young cystic fibrosis (CF) patients. Before you decide whether or not to be involved, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your clinician/GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

3. What is the purpose of the study?
This research is intended to enhance our understanding of how children and teenagers with CF respond during and after exercise testing. We know that children with CF find it more tiring to exercise and comparison with somebody who is healthy (i.e. you) will allow us to try and see if there are any differences in the way their body’s respond to exercise.

4. Why have I been chosen?
You are a similar age, height, weight and gender to one of our patients who has been tested.

5. Do I have to take part?
Taking part is entirely voluntary and it is up to you to decide whether or not to be involved. If you do decide to take part you will be given this information sheet to keep and asked to sign a consent form giving your permission and get your parent/guardian to do the same. Following this you are still free to withdraw at any time, without giving a reason.

6. What will happen to me if I allow them to take part?
The study will involve 1 trip to an exercise laboratory within the Children’s Health & Exercise Research Centre, or within your respective school at a time suitable for you and your parent/guardian. The entire testing session will last ~1-1.5 hours. The session will be split into (a) an initial familiarisation session / obtaining some baseline information and (c) the exercise test:
(a) Upon arrival to the laboratory your height, sitting height, and weight will be taken. You will also have your skin folds measured at your arm, back and hip. This is done by very gently pinching the skin and measuring the width of the skin folds. We will then measure your lung function, which simply involves taking a big breath and breathing into a plastic tube. You will be familiarised with the equipment to be used during testing and encouraged to practice cycling at different intensities on the stationary bike. You can also take this opportunity to ask any questions to the research staff.

(b) You will then be required to complete the exercise test and a number of measurements will be taken. This 1st part of the exercise test will involve you pedalling on an exercise bike for as long as you can. This exercise test starts off easy but gradually the pedals get harder to turn making the test progressively more difficult. After you have finished this initial exercise test you will remain on the bike for 5 minutes to allow you to catch your breath. You will then be seated down and rested for 10 minutes, during which we will measure your lung function again. This is optional and you will still be allowed to participate in the study if you decide against this measurement. Following this rest you will be asked to get back onto the bike and to pedal again for as long as possible. The aim of this is to clarify whether or not you could have continued for any longer during the 1st part of the test – this exercise will only last a couple of minutes. Finally, when you have recovered we will assess your lung function for a final time.

Measurements taken:
During exercise you will breathe through a facemask, this may seem strange at first but you soon get used to it. The amount of oxygen present in your blood will also be monitored throughout the session with a sensor taped on their index finger. You will also wear 6 sticky pads, on their forehead, neck and torso to assess your heart’s response to exercise; you will not feel anything during this. You will also wear a small plastic unit on your right thigh, which will be bandaged in place and allows us to examine how much oxygen that you breathe in is being used by the working muscles. During exercise you will be shown a list of numbers on a chart numbered 0-10 and asked to point to the number or picture which shows how hard or easy you think you are exercising. On a different chart you will rate how out of breath you feel. Following the 1st bit of exercise you will be asked for a spot of blood, which will be drawn from your fingertip by a tiny needle prick. Finally, we need to know your maturation (growth and development), requiring you to self-assess your pubertal stage by giving them 5 options. Self-assessment will take place at home and you will be asked to return the scale in a sealed envelope you will be provided with. N.B. This will only be asked of boys from age 10 and girls from the age of 8.

7. What else will I have to do?
If you wish to take part, we ask that you be in a rested state on arrival to the laboratory, having performed no strenuous exercise in the 2-3 days prior. We also ask that you have had sufficient food (i.e. a light snack such as a sandwich) and drink and wear suitable clothing for performing the exercise test. Finally, we ask that you avoid caffeine 3 hours before testing.

8. What are the possible risks of me taking part?
The exercise testing is very safe. An assessment of your health will be made before any involvement to confirm it is safe for you to take part. You will be carefully monitored and observed to ensure your safety and well-being during all testing.

9. What are the potential benefits of me taking part?
Whilst the main aim of this study is to help develop better understanding regarding exercise for young CF patients, it is also hoped that you will take away a positive and enjoyable experience from your involvement. You will be tested on equipment used to monitor the fitness of sports people, and you will get to see how fit you currently are and what intensity of exercise you can tolerate. You will be given the results of your exercise tests and talked through them by a member of the research team. This will hopefully prove an interesting and constructive exercise, particularly if you have a strong interest or involvement in sport and may wish to increase the amount of physical activity you participate in.

10. What if something goes wrong?
There are no special compensation arrangements if taking part in this study harms you. Your rights are the same as undergoing research i.e. if you are harmed due to someone’s negligence, then you may have grounds for legal action, but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University complaints procedures will be available to you.

11. Will my taking part in this study be kept confidential?
We have a responsibility to inform you of how we will collect, store and use your information obtained during the study. All information (such as your name, date of birth, contact details, and test results) will be transferred to a paper study file, which will be kept in a secure room in the centre in a locked filing cabinet and you will be assigned a unique study code and participant number to keep you anonymous. Only your date of birth will be used to identify you. Data will be transferred from paper to be stored on a password protected computer and you will not be identifiable. All files will be stored for 5 years, after which the paper files will be destroyed and computer files erased. Only the researchers involved in the study will have access to your exercise results.

12. What will happen to the results of the study?
Once the study is completed, your parent/guardian will be sent a summary of our research findings. Although this research is undertaken with the intention of being submitted as a PhD thesis, it is also intended that results will be submitted to relevant scientific journals for publication, and to inform our colleagues of the findings at scientific meetings. In publishing and talking about the study, you will not be identifiable. You and your parent/guardian are free to request information regarding your individual data.

13. Who is organising and funding the research?
The study is a part of a larger collaboration between the Paediatric Unit of the Royal Devon and Exeter (RD&E) NHS Foundation Trust Hospital and the Children’s Health and Exercise Research Centre at the University of Exeter. The study is sponsored by the RD&E NHS Foundation Trust. None of the researchers or doctors involved personally receives money for including you in the study.

14. Who has reviewed the study?
The present study has been reviewed and given favourable opinion by the departmental ethics committee within Sport & Health Sciences, University of Exeter.

15. What should I do if I would like to take part?
If you would like to take part in the study you must give your permission by completing the consent form and your parent/guardian must also complete their consent form. You should then return the two forms to a member of the research team.

16. What if I have a question?
If you or your parent/guardian have any questions please do not hesitate to get in touch with the principle investigator (Miss Zoe Saynor) as outlined at the top of page 1.

Thank you for considering taking part in this study. Please read this information carefully, before you sign the consent form. Please take this leaflet home for reference.
1. Study Title: Oxidative and cardiovascular responses during exercise in patients with cystic fibrosis compared to age and gender matched controls.

2. Invitation paragraph
Your child is being invited to take part in a research study as they may be a match to compare with one of our young cystic fibrosis (CF) patients. Before you decide whether or not to be involved, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your clinician/GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

3. What is the purpose of the study?
This research is intended to enhance our understanding of how children and teenagers with CF respond during and after exercise testing. We know that children with CF find it more tiring to exercise and comparison with somebody who is healthy will allow us to try and see if there are any differences in the way their body’s respond to exercise.

4. Why has my child been chosen?
Your child is a similar age, height, weight and gender to one of our patients who has been tested.

5. Does my child have to take part?
Taking part is entirely voluntary and it is up to you to decide whether or not your child is involved. If you do allow your child to take part you will be given this information sheet to keep and asked to sign a consent form giving your permission. Following this you are still free to withdraw your child at any time, without giving a reason.

6. What will happen to my child if I allow them to take part?
The study will involve 1 trip to an exercise laboratory within the Children’s Health & Exercise Research Centre, or within their respective school at a time suitable for you. The entire testing session will last ~1-1.5 hours. The session will be split into (a) an initial familiarisation session / obtaining some baseline information and (c) the exercise test:
(a) Upon arrival to the laboratory your child’s height, sitting height, and weight will be taken. They will also have their skin folds measured at their arm, back and hip. This is done by very gently pinching the skin and measuring the width of the skin folds. They will then have their lung function measured, this will involve your child taking a big breath and breathing into a plastic tube. Your child will be familiarised with the equipment to be used during testing and will be encouraged to practice cycling at different intensities on the stationary bike. You can also take this opportunity to ask any questions to the research staff.

(b) Your child will then be required to complete the exercise test and a number of measurements will be taken. This 1st part of the exercise test will involve your child pedalling on an exercise bike for as long as they can. This exercise test starts off easy but gradually the pedals get harder to turn making the test progressively more difficult. After they have finished this initial exercise test they will remain on the bike for 5 minutes to allow them to catch their breath. They will then be seated down and rested for 10 minutes, during which we will measure their lung function again. This is optional and your child will still be allowed to participate in the study if they decide against this measurement. Following this rest your child will be asked to get back onto the bike and to pedal again for as long as possible. The aim of this is to clarify whether or not they could have continued for any longer during the 1st part of the test – this exercise will only last a couple of minutes. Finally, when your child has recovered we will assess their lung function for a final time.

_Measurements taken:_

During exercise your child will breathe through a facemask, this may seem strange at first but they soon get used to it. The amount of oxygen present in your child’s blood will also be monitored throughout the session with a sensor taped on their index finger. Your child will also wear 6 sticky pads, on their forehead, neck and torso to assess their heart’s response to exercise; your child will not feel anything during this. They will also wear a small plastic unit on their right thigh, which will be bandaged in place and allows us to examine how much oxygen that your child breathes in is being used by the working muscles. During exercise your child will be shown a list of numbers on a chart numbered 0-10 and asked to point to the number or picture which shows how hard or easy they think they are exercising. On a different chart they will rate how out of breath they feel. Following the 1st bit of exercise your child will be asked for a spot of blood, which will be drawn from their fingertip by a tiny needle prick. Finally, we need to know your child’s maturation (growth and development), requiring them to self-assess their pubertal stage by giving them 5 options. Self-assessment will take place at home and you will be asked to return the scale in a sealed envelope you will be provided with. N.B. This will only be asked of boys from age 10 and girls from the age of 8.

7. What else will my child have to do? 
If your child wishes to take part, we ask that they be in a rested state on arrival to the laboratory, having performed no strenuous exercise in the 2-3 days prior. We also ask that they have had sufficient food (i.e. a light snack such as a sandwich) and drink and wear suitable clothing for performing the exercise test. Finally, we ask that they avoid caffeine 3 hours before testing.
8. What are the possible risks of my child taking part?
The exercise testing is very safe. An assessment of your child’s health will be made before any involvement to confirm it is safe for them to take part. Your child will be carefully monitored and observed to ensure their safety and well-being during all testing.

9. What are the potential benefits of my child taking part?
Whilst the main aim of this study is to help develop better understanding regarding exercise for young CF patients, it is also hoped that your child will take away a positive and enjoyable experience from their involvement. They will be tested on equipment used to monitor the fitness of sports people, and they will get to see how fit they currently are and what intensity of exercise they can tolerate. They will be given the results of their exercise tests and talked through them by a member of the research team. This will hopefully prove an interesting and constructive exercise, particularly if they have a strong interest or involvement in sport and may wish to increase the amount of physical activity they participate in.

10. What if something goes wrong?
There are no special compensation arrangements if taking part in this study harms your child. Your rights are the same as undergoing research i.e. if your child is harmed due to someone’s negligence, then you may have grounds for legal action, but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University complaints procedures will be available to you.

11. Will my child’s taking part in this study be kept confidential?
We have a responsibility to inform you of how we will collect, store and use your child’s information obtained during the study. All information (such as their name, date of birth, contact details, and test results) will be transferred to a paper study file, which will be kept in a secure room in the centre in a locked filing cabinet and assigning a unique study code and participant number to keep it anonymous. Only your child’s date of birth will be used to identify them. Data will be transferred from paper to be stored on a password protected computer and your child will not be identifiable. All files will be stored for 5 years, after which the paper files will be destroyed and computer files erased. Only the researchers involved in the study will have access to your child’s exercise results.

12. What will happen to the results of the study?
Once the study is completed, your will be sent a summary of our research findings. Although this research is undertaken with the intention of being submitted as a PhD thesis, it is also intended that results will be submitted to relevant scientific journals for publication, and to inform our colleagues of the findings at scientific meetings. In publishing and talking about the study, your child will not be identifiable. You and your child and are free to request information regarding your child’s individual data.

13. Who is organising and funding the research?
The study is a part of a larger collaboration between the Paediatric Unit of the Royal Devon and Exeter (RD&E) NHS Foundation Trust Hospital and the Children’s Health and Exercise Research Centre at the University of Exeter. The study is sponsored by the RD&E NHS Foundation Trust. None of the researchers or doctors involved personally receives money for including your child in the study.

14. Who has reviewed the study?
The present study has been reviewed and given favourable opinion by the departmental ethics committee within Sport & Health Sciences, University of Exeter.

15. What should I do if my child would like to take part?
If your child would like to take part in the study you must give your permission by completing the consent form and your child must complete the assent form. You should then return the two forms to a member of the research team.

16. What if my child or me have a question?
If you or your child has any questions please do not hesitate to get in touch with the principle investigator (Miss Zoe Saynor) as outlined at the top of page 1.

Thank you for considering taking part in this study. Please read this information carefully, before you sign the consent form. Please take this leaflet home for reference.
Participant Recruitment Flyer
Version Number: 1
Date: 05/10/11

Dear

The Children’s Health and Exercise Research Centre, in conjunction with Bramble Ward at the Royal Devon & Exeter Hospital, is currently undertaking some exciting research. We are currently looking for children/adolescents aged between 7-18yrs, who may be a healthy match for our group of cystic fibrosis patients, who would be interested in taking part.

On behalf of our paediatric team our exercise physiologist Zoe Saynor will contact you via telephone to see if you would like to be involved with our research.

An information sheet has been included for you to read and help you decide whether you would like to take part.

Your participation in this research project would be much appreciated. However, you are by no means obliged to take part in this study if you do not wish to. Furthermore, your individual rights are not affected by a decision to not participate, or subsequently withdraw from the study.

Many thanks for reading this,

Kind Regards,

Miss Zoe Saynor
MPhil, Ph.D. Researcher
Children’s Health and Exercise Research Centre
University of Exeter

Dr Patrick Oades
Consultant Paediatrician
Royal Devon and Exeter Healthcare NHS Trust
Patient’s Letter (10-15 years)

Dear Patient,

We would like to ask you to take part in a study at the hospital. Please read this sheet very carefully before you decide to take part or not.

What is the study about?

- The study will help us find out how fit you are by looking at how much oxygen you use during and after a number of exercise tests and how well you cope when you change from resting to begin some exercise.

What will happen?

- You will visit the hospital 5 times to exercise on a bike
- During the exercise tests you will wear a rubber face mask that covers your nose and chin, a small plastic box will be bandaged onto your right leg, and you will have 2 sticky pads on your head, neck, chest and back which will be attached to plastic wires.
- You will not feel anything from these things other than that they are there and the mask may make your face a bit sweaty
- Before and after each test you will breathe out into a plastic tube (spirometer) so we can see what your lungs are doing – just like you do at the CF clinics
- The first visit will be an exercise test to see how fit you are
• Visits 2-5 will be 2 x 30 minutes of cycling on the bike, with a number of easy rests in between.
• The people who will be testing you will be different to your usual direct care team at the hospital, however they are trained in exercise testing like this and regularly exercise test young people.

Are the exercise tests safe?

• The exercise tests are very safe.
• Your doctor will look at your health records before you exercise to check that it is safe for you to take part.
• A medical team will be ready during the exercise tests just in case there are any problems.

What else will happen on each visit to the hospital?

Visit 1
• On your first visit to the hospital we will measure how tall you are and how much you weigh.
• We will measure your skin folds at your arm, back and hip. This is done by very gently pinching the skin using a small instrument. This does not hurt but may tickle.
• You will then be shown the equipment we will use
• Finally, you will practice cycling on the exercise bike and feel what it is like when pedalling is both easy and hard
• **You will then complete your first exercise test**
• Before you have to do any exercise you will be asked to rest
• The exercise test will involve you first pedalling on an exercise bike for 3 minutes when the pedals are quite easy to turn
• You will then be asked to pedal on the exercise bike for as long as you can until you can’t pedal anymore.
• The pedals on the bike will get harder to turn the longer you pedal
• You will wear the face mask and other equipment during the cycling.
• You will then stay on the bike and pedal easily for 5 minutes, then you will sit down and rest for 10 minutes while still wearing the black box and sticky pads – but we will remove the face mask.
• We will then pop the face mask back on
• You will then get back onto the bike and be asked to cycle for as long as you can again, to check that you cannot go for longer than you did before (this will usually only last 1-2 minutes)
• We will then give you a drink and you can rest before going home.

Visits 2-5
• **The next 4 visits will all be exactly the same as each other**
• You will perform exercise on the bike and the same measurements will taken
• The test will be different to visit 1
• You will be cycling on the bike for just under 30 minutes twice, with a 30 minute rest in between
• The exercise will be made up of bits which are easy, bits which are a little harder and one bit where you will cycle for 6 minutes and it will be quite hard

**After you have finished taking part in the study**
• We also need to know how developed your body is. To do this we will ask you to go look at yourself at home and answer a question about how far your body has developed. The question is an easy choice out of 5. We would also like you to wear an elastic belt with a little plastic box for a week after you have finished your testing, to see how much exercise/play you usually do.

**What else will you have to do if you do decide to take part?**

7. For 2 – 3 days before each visit you must not do any really hard exercise (if possible).
8. You can eat and drink as normal but do not drink or eat foods which contain caffeine such as tea, coffee, Coca-Cola for 3 hours before coming to see us.
9. You will also be asked if you would like to have your photograph taken during testing, for us to use when talking about the findings from this study in the future. This is completely up to you and you do not have to say yes.

**Do you have to take part?**

• It is up to you if you take part.
• If you take part you can drop out at any time and don’t have to give us a reason.

**What should you do if you want to take part or have any questions?**

7. If you would like to take part you must have your parent/carer’s permission by getting them to complete the consent form.
8. You must also complete a form which tells us you would like to take part and return it to a member of the research team.
9. If you have any questions please get in touch with a member of the research team who are on the bottom of this page.

**Thank you for reading this letter.**
The Research Team.
Miss. Zoe Louise Saynor
(Children’s Health & Exercise Research Centre, University of Exeter)
Tel (work): (01392) 264889
E-mail: zls202@exeter.ac.uk

Dr. Patrick J. Oades
(Consultant Paediatrician, Royal Devon and Exeter Healthcare NHS Trust)
Tel: (01392) 402665
E-mail: patrick.oades@nhs.net

Professor Craig. A. Williams
(Children’s Health & Exercise Research Centre, University of Exeter)
Tel: (01392) 724809
E-mail: c.a.williams@ex.ac.uk

Dr. Alan Barker
(Children’s Health & Exercise Research Centre, University of Exeter)
Tel: (01392) 722766
E-mail: a.r.barker@exeter.ac.uk
1. Study Title

The influence of disease status and ‘priming’ exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate and high-intensity cycling exercise in paediatric patients with cystic fibrosis.

2. Invitation paragraph

You are being invited to take part in a research study. Before you decide whether or not to be involved, it is important for you and your parent/guardian to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your clinician/GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this information sheet.

3. What is the purpose of the study?

It is recommended that children and teenagers with cystic fibrosis (CF) take part in sports and games to stay fit and healthy. Exercise not only helps improve fitness and enhances their quality of life through socialising with peers and friends, but it may also help them to cope better with aspects of their disease. It has been reported that CF patients are characterised by reduced aerobic fitness when compared with their healthy counterparts. However, the extent and cause(s) for why patients with CF may find it more tiring during exercise than a healthy person of the same age remains an area of debate.
During exercise, breathing becomes faster and harder because the body needs more oxygen to produce energy to move faster. When we go from rest or performing light exercise to harder exercise necessitating our body to work harder, then the energy (and therefore oxygen) our muscles need to keep working must change quickly. During this change from rest to exercise all of the bodily systems (e.g. the heart, lungs and muscles) which are important during exercise must work together in co-ordination to increase the amount of oxygen/energy which is needed by the muscles working during exercise. If someone can ‘switch on’ this system to produce the necessary energy more quickly when they start to exercise then we know that they will be able to exercise for longer before they become tired.

This study will look at how well you respond during these changes from rest to exercise of different difficulties and how fast you can ‘switch on’ this ‘aerobic energy system’. Understanding this is of importance, as it will help us to know where to focus intervention strategies (e.g. exercise training) to improve the ability of CF patients to tolerate exercise and, subsequently, improve their quality of life.

Whilst the main aim of this study is to gather the above information, it will also provide information regarding your exercise tolerance/fitness which may offer a novel insight for both you and your parent/guardian. Additionally, we know that participating in regular exercise is beneficial to patients with CF and participating in this research project will also serve as 5 sessions of supervised moderate- and high-intensity cycling exercise training for you.

4. Why have I been chosen?

We are interested in young cystic fibrosis patients, aged between 10-18 years old, who have stable disease and regularly take part in physical activity.

5. Do I have to take part?

Taking part is entirely voluntary and it is up to you to decide whether or not you take part. If you do decide to be involved and your parent/guardian give permission, you will be given this information sheet to keep, and be asked to sign a consent form giving your permission to participate (your parent/guardian will do the same). If you do decide to take part you are still free to withdraw from the study at any time and without giving a reason. A decision to withdraw from the study or a decision not to take part will not affect the standard clinical care you receive.

6. What will happen to me if I do decide to take part?

The study will involve 5 trips to an exercise laboratory within the Royal Devon and Exeter NHS Foundation Trust Hospital (RD&E), separated by at least 48 h and ideally completed over a maximum 2 week period (to minimise the chance of any significant in your clinical status or fitness levels). All testing will take place at times which are suitable for you and your parent/guardian and this can be at weekends or after school if preferred. On all 5 visits to the centre, you will be required to complete an exercise test and the following measurements will be taken:
**Respiratory function:**
The volume of oxygen you are breathing in ($\dot{V}O_2$)
The volume of carbon dioxide you are breathing out ($\dot{V}CO_2$)
The ratio between the amounts of oxygen you are breathing in and carbon dioxide you are breathing out (RER).
How much air you are taking in each minute ($\dot{V}E$).

**Lung function**
The volume of air you can breathe out (FVC).
The volume of air you can breathe out in one second (FEV$_1$).
The volume of air you are breathing in and out (PEF).

**Other measurements**
How fast your heart is beating (HR)
How much blood your heart pumps out each time it contracts (SV)
How tired you think you are feeling (RPE).
How breathless you think you feel (RPD)
Changes in the concentration of oxygenated and deoxygenated haemoglobin/myoglobin within the quadriceps muscles of your right leg
The amount of oxygen present in your blood ($SaO_2$)

**Visit 1**
The initial visit will consist of two parts:

1: **Familiarisation session / baseline assessments**
You and your parent/guardian will be given the opportunity to discuss any queries you may have, give your informed consent/assent and to familiarise you and your parent/guardian with the equipment and testing procedures that will be used during exercise testing visits. We will then obtain some information regarding your body composition. Your height, sitting height, and weight will be measured. You will also have your skin folds measured at the front and back of your arm, just below your shoulder blade and at the front of your hip. This is done by very gently pinching the skin and measuring the width of the skin folds. You will then have your resting lung function measured, this will involve you taking a big breath and breathing into a plastic tube and is no different to the lung function tests you routinely perform in clinics. You will then be familiarised with the equipment to be used during testing and encouraged to practice cycling at different intensities on the stationary exercise bike. You can then also take this opportunity to ask any questions to the research staff regarding the requirements of the next exercise testing sessions.

2: **Incremental Exercise Test**
Following the above, you will be required to perform your first exercise test. This initial exercise test will be an ‘incremental ramp cycle test to exhaustion’. This test requires you to pedal on an exercise bike for as long as you can. This exercise test starts off easy but gradually the pedals get harder to turn making the exercise test progressively more difficult, this will feel like you are cycling up a hill which is getting steeper.
During this exercise test you will be required to wear a rubber face mask which covers your nose and chin, which will be connected to a device measuring the oxygen you are breathing in and carbon dioxide you are breathing out. This may seem strange at first but you will soon get used to it and this is a standard piece of kit used when performing this form of exercise test on young people. The amount of oxygen present in your blood will also be monitored throughout the session using a small rubber device placed on the end of your index fingertip. In addition to this, you will wear 6 electrode pads (similar to an ECG if you have ever had or seen one of these), placed on your head, neck, chest and back. These are simply gel pads which will stick to your skin and, when attached to wires, will tell us how your heart is responding during exercise. Finally, you will have a small black plastic device taped and bandaged securely on to your right thigh. This device will simply shine light into the working muscle and allows us to estimate how much of the oxygen you breathe in is being delivered to the muscles that need it when you are working during exercise. Throughout the exercise test (every minute) you will be shown a list of numbers (0-10) corresponding to pictures of a child running up stairs and getting more and more tired. You will be asked to point on the chart to tell us how hard or easy you are finding the exercise. You will also be asked to rate on a different chart how out of breath you feel, again using words and numbers (0-10). This exercise test usually lasts ~8-12 minutes.

After you finish this exhaustive exercise test, you will stay on the bike for a 5 minute ‘cool-down’ period of easy pedalling to recover and then will sit down and rest for 10 minutes. The electrodes and black box will still be attached, however the face mask will be removed when sitting down recovering. During this rest you will have your lung function measured again.

Following this 15 minute rest, you will be asked to get back onto the bike and to pedal again for as long as possible. Unlike the first test, this test will not get gradually harder and harder. This will be set at a similar difficulty to where you stopped during the first test and will typically only last approximately 1-2 minutes. When you become exhausted you will again be asked to stay on the bike and pedal lightly for 5 minutes to allow your body to recover gradually, as you will have been working hard during the test. The aim of this test is check whether or not you could have continued for any longer during the 1st test. During the second part of the exercise test you will wear the same equipment as in the first part test. Your lung function will be measured one last time when you get off the bike.

This first visit will last 1-1.5 h.

Visit 2, 3, 4 and 5:

At least forty-eight hours later you will be asked to complete your second exercise testing session which will be identical to visits 3, 4 and 5 to follow. This visit will again last approximately 1.5 hours, however we will not need to collect the skin fold measurements again or practice using the exercise bike.

These sessions will require you to exercise for 2 x ~30 minutes. However, only 12 minutes of each will be exercise that makes you feel like you are working reasonably hard, the rest will be light exercise which will feel nice and easy to turn the pedals.

The exercise session
You will begin with a 4-minute ‘warm-up’ of easy pedalling. You will then cycle for 6 minutes at an intensity we call ‘moderate’ (this will feel like you are having to work to turn
the pedals, however it should not be too hard or cause you to become exhausted). This will be followed by 6 minutes easy cycling like the warm-up. The ‘intensity’ will then increase to one that is ‘moderate’ again for a further 6 minutes, which will finally finish with 6 minutes of easy cycling. You will then get off the bike and sit down and rest for 30 minutes.

Following this 30 minute rest period, you will get back onto the bike for the second and last part of the exercise test. Again, this will begin with 4 minutes of easy pedalling and then the ‘intensity’ will be increased for the next 6 minutes. However, this time it will be ‘very-heavy’, which means that you will have to work a bit harder to turn the pedals and will feel a bit more out of breath. Following this you will again cycle for 6 minutes which will be nice and easy and the pedals will feel much lighter to turn. The ‘intensity’ will then be increased to the ‘moderate’ one again. Following this you will stay on the bike and recover for a few minutes.

The session will then be complete.

During this exercise session you will have to wear the rubber face mask again. The amount of oxygen present in your blood will also be monitored throughout the session using the same small rubber device placed on the end of your index fingertip during their 1st visit. You will also wear the 6 electrode pads on your head, neck, chest and back and the small black plastic box on your right thigh. At various time points during exercise and at the end of the 4 harder periods of exercise you will be shown the chart which shows how hard or easy you are finding the exercise and asked to rate on the breathlessness chart whether you are feeling out of breath.

Visits 3, 4 and 5 will be identical to visit 2 and will be separated by at least 48 h (ideally within 2-3 days) at a time suitable to you and your parent/guardian.

Post-testing
We also need to know your maturation (growth and development). This will require you to self-assess your pubertal stage by selecting from 5 options on a chart which shows different pictures of pubic hair through the stages of maturation. Self-assessment will take place at your home and your parent/guardian will be asked to return the scale in a sealed envelope they will be provided with. We are also interested in assessing how physically active you are during a typical week, if you and your parent/guardian agree to do so. This will be assessed by wearing a small hip-mounted plastic box on an elastic belt for 7 days, whilst completing a brief diary of the physical activities you perform from the time you wake up to the time you go to bed. The monitor would be removed if you swim or take a bath/shower and when in bed.

7. What else will I have to do?

If you do decide to take part in the study and your parent/guardian has given their permission, we would like you to attend all 5 visits at the exercise laboratory. We ask that you be rested when you arrive, having performed no exhaustive exercise in the 2-3 days before you visit (other than involvement in this study). Any specific questions regarding any exercise training or sport you may be doing prior to testing sessions can be discussed with the research team prior to any involvement in this research study.
On each visit to the laboratory we also ask that you have had enough to eat and drink before you arrive, and that you wear the same or similar clothes for each exercise test (that is sports kit, usually shorts, t-shirt and trainers). We also ask that you don’t eat or drink any caffeine 3 hours prior to each visit. However, it is important that you do eat a light meal or snack before each visit (i.e. sandwich, cereal etc.).

We may ask you if we can take photographs during your exercise tests. The purpose of these photographs is to be possibly used within University of Exeter publicity materials, in Miss Saynor’s PhD thesis and in conference presentations such as posters and PowerPoint presentation. This is entirely voluntary and will in no way affect any subsequent involvement in the study if you and/or your parent/guardian should decline. Permission to take any photographs will be obtained in writing prior to involvement in the study.

8. What are the possible risks of me taking part in this study?

The exercise testing is very safe. An assessment of your health will be made before you start the exercise tests to see if it is safe for you to take part. During the exercise tests, you will be carefully monitored and observed to ensure your safety and well-being.

9. What are the potential benefits of me taking part in this study?

This research is intended to enhance our understanding of how children with CF tolerate exercise and, more specifically, the rapid changes their bodies must deal with when they change between rest and exercise of difference intensities. The information we obtain from the study will help to improve our understanding regarding whether CF disease makes it more challenging for patients to exercise without becoming tired earlier on that someone without CF. It can also help to develop better exercise testing programmes within CF clinics.

Hopefully you will enjoy your experience if you are involved in the exercise tests. You will be tested on equipment that is used to monitor the fitness of sports people, and will get to see how fit you currently are and what intensity of exercise you can tolerate. Visits 2-5 will also act as supervised exercise sessions, which we know to be beneficial for young people with CF. You will be given the results of their exercise tests and talked through them by a member of the research team. This will hopefully prove an interesting and constructive exercise, particularly if you have a strong interest or involvement in sport and may wish to increase how physically active you are. You can also use the 30 minute break to discuss any questions you may have regarding exercise with an exercise physiologist, if you wish to do so.

10. What happens if I do not want to continue in the study?

You are free to withdraw from the study at any point without giving a reason. Dropping out of the study will not affect your clinical care in any way or your relationship with the paediatric staff.

If you do decide not to continue with the study then you will not be required to complete any additional exercise tests, or attend any additional visits to the centre that are associated with
the research. Results from any exercise tests that you have previously completed will still be available to you.

11. What if something goes wrong?

Should taking part in this study harm you in any way, compensation arrangements are under the directive of the University of Exeter. Your rights are the same as any person undergoing research i.e. if you are harmed due to someone’s negligence, then you may have grounds for legal action. If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University complaints procedures will be available to you. In any such case, complaints should be directed to Professor Adrian Taylor, Director of Research for Sport and Health Sciences, University of Exeter [+44 (0) 1392 724747; Adrian.Taylor@exeter.ac.uk].

12. Will my involvement in this study be kept confidential?

We have a responsibility to inform you of how we will collect, store and use your information that we gather during the study. The primary concern is that any information that we collect about you will be confidential. All of this information collected such as your name, date of birth, contact details, details of your health and test results will be transferred to a paper study file, which will be kept in a secure room in a locked filing cabinet. Members of the research team will be outside your direct standard care team and will have access to your data. However, your exercise results will be kept anonymous by assigning them a unique study code and participant number. Only your date of birth will be used to identify the results. The results from the exercise tests are collected on paper and stored in a locked filing cabinet. The exercise results are then transferred from paper and stored on a computer. The only personal information stored on computer will be your date of birth and participant number. Your exercise results will be password protected, as will the computer used to store the information. All the paper and computer files will be stored for 15 years, after this period paper files will be destroyed and computer files erased. Only the researchers involved in the study will have access to your medical records and exercise results.

13. What will happen to the results of the study?

Once the study is completed, which is targeted to be the end of December 2012, the results will be analysed and interpreted and your parent/guardian will subsequently be sent a summary of our research findings. The research is undertaken with the intention of being submitted as a PhD thesis. It is also the intention to submit the results of the research to relevant journals for publication, and to inform our colleagues of the findings at scientific meetings. In publishing and talking about the study you will not be identifiable.

You and your parent/guardian will be given the opportunity to comment on the research protocol and testing procedure. This information will be collated and may inform future studies. You and your parent/guardian will also receive the results and conclusions from the research and are free to request information regarding your individual data.
14. Who is organising and funding the research?

The study is a collaboration between the Paediatric Unit of the Royal Devon and Exeter (RD&E) NHS Foundation Trust Hospital and the Children’s Health and Exercise Research Centre at the University of Exeter. The study is sponsored by the RD&E NHS Foundation Trust and has been supported financially by a small grant award from the RD&E. A monetary contribution will be provided to your parent/guardian to go some way towards covering your travel expenses (45p/miles for first 100 miles travelled and 25p/mile thereafter) and/or parking costs, bus or rail fares when attending testing sessions at the RD&E.

15. Who has reviewed the study?

The scientific content of the study has been reviewed by the Peninsula Research and Development Support Unit. All research within the National Health Service (NHS) is looked at by an independent group of people, called a Research Ethics Committee (REC). RECs safeguard the rights, safety, dignity and well-being of people participating in research in the NHS. They review applications for research and give an opinion about the proposed participant involvement and whether the research is ethical. The present study has been reviewed and given favourable opinion by the South West 5 (or Frenchay) REC.

16. What should I do if I would like to take part?

If you would like to take part in the study you must give your permission by completing the consent form and your parent/guardian must do the same. You should then return the two forms to a member of the research team.

17. What if I have a question?

If you or your parent/guardian have any questions please do not hesitate to get in touch with a member of the research team using the details provided on page 9 and at the top of page 1.

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If you need further information please contact the research team using the details presented on page 9 and at the top of page 1.

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The Research Team

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_N.B. Contact outside of office hours should be made to Miss Zoe Louise Saynor._
1. Study Title

The influence of disease status and ‘priming’ exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate and high-intensity cycling exercise in paediatric patients with cystic fibrosis.

2. Invitation paragraph

Your child is being invited to take part in a research study. Before you and your child decide whether or not to be involved, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your clinician/GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this information sheet.

3. What is the purpose of the study?

It is recommended that children and teenagers with cystic fibrosis (CF) take part in sports and games to stay fit and healthy. Exercise not only helps improve fitness and enhances their quality of life through socialising with peers and friends, but it may also help them to cope better with aspects of their disease. It has been reported that CF patients are characterised by reduced aerobic fitness when compared with their healthy counterparts. However, the extent and cause(s) for why patients with CF may find it more tiring during exercise than a healthy person of the same age remains an area of debate.
During exercise, breathing becomes faster and harder because the body needs more oxygen to produce energy to move faster. When we go from rest or performing light exercise to harder exercise necessitating our body to work harder, then the energy (and therefore oxygen) our muscles need to keep working must change quickly. During this change from rest to exercise all of the bodily systems (e.g. the heart, lungs and muscles) which are important during exercise must work together in co-ordination to increase the amount of oxygen/energy which is needed by the muscles working during exercise. If someone can ‘switch on’ this system to produce the necessary energy more quickly when they start to exercise then we know that they will be able to exercise for longer before they become tired.

This study will look at how well your child responds during these changes from rest to exercise of different difficulties and how fast they can ‘switch on’ their ‘aerobic energy system’. Understanding this is of importance, as it will help us to know where to focus intervention strategies (e.g exercise training) to improve the ability of CF patients to tolerate exercise and, subsequently, should improve their quality of life.

Whilst the main aim of this study is to gather the above information, it will also provide information regarding your child’s exercise tolerance/fitness which may offer a novel insight for both you and your child. Additionally, we know that participating in regular exercise is beneficial to patients with CF and participating in this research project will also serve as 5 sessions of supervised moderate- and high-intensity cycling exercise training for your child.

4. Why has my child been chosen?

We are interested in young cystic fibrosis patients, aged between 10-18 years old, who have stable disease and regularly take part in physical activity.

5. Does my child have to take part?

Taking part is entirely voluntary and it is up to you to decide whether or not your child takes part. If you do allow your child to take part you will be given this information sheet to keep, and be asked to sign a consent form giving permission for your child to participate (they will do the same). If you do decide to allow your child to take part you are still free to withdraw them from the study at any time and without giving a reason. A decision to withdraw your child at any time or a decision not to take part in the first place will not affect the standard clinical care they receive.

6. What will happen to my child if I allow them to take part?

The study will involve 5 trips to an exercise laboratory within the Royal Devon and Exeter NHS Foundation Trust Hospital (RD&E), separated by at least 48 h and ideally completed over a maximum 2 week period (to minimise the chance of any significant change in your child’s clinical status or fitness levels). All testing will take place at times which are suitable for you and your child and this can be at weekends or after school if preferred. On all 5 visits to the centre, your child will be required to complete an exercise test and the following measurements will be taken:
**Respiratory function:**
- The volume of oxygen your child is breathing in ($\dot{V}O_2$)
- The volume of carbon dioxide your child is breathing out ($\dot{V}CO_2$)
- The ratio between the amounts of oxygen your child is breathing in and carbon dioxide he/she is breathing out (RER).
- How much air your child is taking in each minute ($\dot{V}_E$).

**Lung function**
- The volume of air your child can breathe out (FVC).
- The volume of air your child can breathe out in one second (FEV$_1$).
- The volume of air your child is breathing in and out (PEF).

**Other measurements**
- How fast your child’s heart is beating (HR)
- How much blood your child’s heart pumps out each time it contracts (SV)
- How tired your child thinks he/she feels (RPE).
- How breathless your child thinks he/she feels (RPD)
- Changes in the concentration of oxygenated and deoxygenated haemoglobin/myoglobin within the quadriceps muscles of your child’s right leg
- The amount of oxygen present in your child’s blood (SaO$_2$)

**Visit 1**
- The initial visit will consist of two parts:

  1: **Familiarisation session / baseline assessments**
  - You and your children will be given the opportunity to discuss any queries you may have, give your informed consent/assent and to familiarise you/your child with the equipment and testing procedures that will be used during exercise testing visits. We will then obtain some information regarding your child’s body composition. Your child’s height, sitting height, and weight will be measured. They will also have their skin folds measured at the front and back of their arm, just below their shoulder blade and at the front of their hip. This is done by very gently pinching the skin and measuring the width of the skin folds. They will then have their resting lung function measured, this will involve your child taking a big breath and breathing into a plastic tube and is no different to the lung function tests they routinely perform in clinics. Your child will then be familiarised with the equipment to be used during testing and encouraged to practice cycling at different intensities on the stationary exercise bike. You and your child can then also take this opportunity to ask any questions to the research staff regarding the requirements of the next exercise testing sessions.

  2: **Incremental Exercise Test**
  - Following the above, your child will be required to perform their first exercise test. This initial exercise test will be an **incremental ramp cycle test to exhaustion**. This test requires your child to pedal on an exercise bike for as long as they can. This exercise test starts off easy but gradually the pedals get harder to turn making the exercise test progressively more difficult, this will feel like your child is cycling up a hill which is getting steeper.
During this exercise test they will be required to wear a rubber face mask which covers their nose and chin, which will be connected to a device measuring the oxygen they are breathing in and carbon dioxide they are breathing out. This may seem strange at first but they will soon get used to it and this is a standard piece of kit used when performing this form of exercise test on young people. The amount of oxygen present in your child’s blood will also be monitored throughout the session using a small rubber device placed on the end of their index fingertip. In addition to this, they will wear 6 electrode pads (similar to an ECG if you have ever had or seen one of these), placed on their head, neck, chest and back. These are simply gel pads which will stick to the skin and, when attached to wires, will tell us how your child’s heart is responding during exercise. Finally, your child will have a small black plastic device taped and bandaged securely onto their right thigh. This device will simply shine light into the working muscle and allows us to estimate how much of the oxygen your child breathes in is being delivered to the muscles that need it when they are working during exercise. Throughout the exercise test (every minute) your child will be shown a list of numbers (0-10) corresponding to pictures of a child running up stairs and getting more and more tired. They will be asked to point on the chart to tell us how hard or easy they are finding the exercise at that time. They will also be asked to rate on a different chart how out of breath they feel, again using words and numbers (0-10). This exercise test usually lasts ~8-12 minutes.

After your child has finished this exhaustive exercise test, they will remain on the bike for a 5 minute ‘cool-down’ period of easy pedalling to recover and then will sit down and rest for 10 minutes. The electrodes and black box will still be attached, however the face mask will be removed when sitting down recovering. During this rest your child will have their lung function measured again.

Following this 15 minute rest, your child will be asked to get back onto the bike and to pedal again for as long as possible. Unlike the first test, this test will not get gradually harder and harder. This will be set at a similar difficulty to where your child terminated the first test and will typically only last ~ 1-2 minutes. When your child becomes exhausted they will again be asked to stay seated on the bike and pedal lightly for 5 minutes to allow their body to recovery gradually, as they will have been working hard during the test. The aim of this test is to clarify whether or not they could have continued for any longer during the 1st test. During the second part of the exercise test your child will be wearing the same equipment as in the first exercise test. Your child’s lung function will be measured one last time when they get off the bike.

This initial visit will last 1-1.5 h.

Visit 2, 3, 4 and 5

At least forty-eight hours later your child will be asked to complete the second exercise testing session which will be identical to visits 3, 4 and 5 to follow. This visit will again last approximately 1.5 hours, however we will not need to collect the skin fold measurements again or practice using the exercise bike.

These sessions will require your child to perform 2 x ~30 minute periods of exercise. However, only 12 minutes of each will be exercise that makes your child feel like they are working reasonably hard, the rest will be light exercise during which it will feel nice and easy to turn the pedals.
**The exercise session**

Your child will begin with a 4-minute ‘warm-up’ of easy pedalling. They will then cycle for 6 minutes at an intensity we call ‘moderate’ (this will feel like they are having to work to turn the pedals, however it should not be too hard or cause them to become exhausted). This will be followed by 6 minutes easy cycling like the warm-up. The ‘intensity’ will then increase to one that is ‘moderate’ again for a further 6 minutes, which will finally finish with 6 minutes of easy cycling. Your child will then get off the bike and sit down and rest for 30 minutes.

Following this 30 minute rest period, your child will get back onto the bike for the second and last part of the exercise test. Again, this will begin with 4 minutes of easy pedalling and then the ‘intensity’ will be increased for the next 6 minutes. However, this time it will be ‘very-heavy’, which means that your child should have to work a bit harder to turn the pedals and will feel a bit more out of breath. Following this your child will again cycle for 6 minutes which will be nice and easy and the pedals will feel much lighter to turn. The ‘intensity’ will then be increased to the ‘moderate’ one again, which is easier and the same as the 2 moderate periods your child competed at the start of the session. Following this your child will stay on the bike and recover for a few minutes.

*The session will then be complete.*

During this exercise session your child will have to wear the rubber face mask again. The amount of oxygen present in your child’s blood will also be monitored throughout the session using the same small rubber device placed on the end of their index fingertip during their 1st visit. They will also wear the 6 electrode pads on their head, neck, chest and back and the small black plastic box on their right thigh. At various time points during exercise and at the end of the 4 harder periods of exercise your child will be shown the chart which shows how hard or easy they are finding the exercise and asked to rate on the breathlessness chart whether they are feeling out of breath.

Visits 3, 4 and 5 will be identical to visit 2 and will be separated by at least 48 h (ideally within 2-3 days) at a time suitable to you.

**Post-testing**

We also need to know your child’s maturation (growth and development). This will require them to self-assess their pubertal stage by selecting from 5 options on a chart which shows different pictures of pubic hair through the stages of maturation. Self-assessment will take place at home and you will be asked to return the scale in a sealed envelope you will be provided with. We are also interested in assessing how physically active your child is during a typical week, if you and they agree to do so. This will be assessed by wearing a small hip-mounted plastic box on an elastic belt for 7 days, whilst completing a brief diary of the physical activities they perform from the time they wake up to the time they go to bed. The monitor would be removed if they swim or take a bath/shower and when in bed.
7. What else will my child have to do?

If your child wishes to take part in the study and he/she has your permission we would like them to attend all 5 visits at the exercise laboratory. We ask that they be in a rested state on arrival, and have performed no exhaustive exercise in the 2-3 days prior to visits (other than involvement in this study). Any specific questions regarding any exercise training or sport your child may be doing prior to testing sessions can be discussed with the research team prior to any involvement in this research study.

On each visit to the laboratory we also ask that your child has had sufficient food and drink before he/she arrives, and that they wear the same or similar clothes for each exercise test (that is sports kit, usually shorts, t-shirt and trainers). We also ask that they don’t eat or drink any caffeine 3 hours prior to each visit. However, it is important that they do eat a light meal or snack before each visit (i.e. sandwich, cereal etc.).

We may ask your child if we can take photographs during their exercise tests. The purpose of these photographs is to possibly be used within University of Exeter publicity materials, in Miss Saynor’s PhD thesis and in conference presentations such as posters and PowerPoint presentation. This is entirely voluntary and will in no way affect any subsequent involvement in the study if you and/or your child should decline. Permission to take any photographs will be obtained in writing prior to involvement in the study.

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Your child is free to withdraw from the study at any point without giving a reason. Dropping out of the study will not affect their clinical care in any way or your relationship with the paediatric staff.

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16. What should I do if my child would like to take part?

If your child would like to take part in the study you must give your permission by completing the consent form and your child must also complete their assent (<16 y) / consent (16-18 y) form (depending on their age). You should then return the two forms to a member of the research team.

17. What if my child or I have a question?

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The Research Team

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Dr. Alan Barker (Children’s Health & Exercise Research Centre, University of Exeter)
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N.B. Contact outside of office hours should be made to Miss Zoe Louise Saynor.
Patient Recruitment Flyer
Version Number: 1
Date: 11/08/2012

Dear

The Children’s Health and Exercise Research Centre, in conjunction with Bramble Ward at the Royal Devon & Exeter Hospital, is currently undertaking some exciting research. We are currently looking for children/adolescents aged between 10-18yrs with cystic fibrosis who would be interested in taking part.

An information sheet has been included for you to read and help you decide whether you would like to take part. If this research is a project which you feel you may be interested in, could you please complete the provided ‘participant’ interest form. On behalf of our paediatric team our exercise physiologist Zoe Saynor will subsequently contact you via telephone to see if you would like to be involved with our research.

Your participation in this research project would be much appreciated. However, you are by no means obliged to take part in this study if you do not wish to. Furthermore, your medical care and individual rights are not affected by a decision to not participate, or subsequently withdraw from the study.

Many thanks for reading this,

Kind Regards,

Dr Patrick Oades
Consultant Paediatrician
Royal Devon and Exeter Healthcare NHS Trust

Miss Zoe Saynor
M.Phil/Ph.D. Researcher
Children’s Health and Exercise Research Centre
University of Exeter
APPENDIX G

Consent and Assent Forms for Studies 1 and 2

Study Number:
Patient Identification Number for this trial:

ASSENT FORM FOR CHILDREN

(To be completed by the child and their parent/guardian)

The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population

Child (or if unable, parent on their behalf) /young person to circle all they agree with please:

Have you read (or had read to you) about this project [Version 2 dated 20/12/10] Yes/No

Has somebody else explained this project to you? Yes/No

Do you understand what this project is about? Yes/No

Have you asked all the questions you want? Yes/No

Have you had your questions answered in a way you understand? Yes/No

Do you understand it's OK to stop taking part at any time? Yes/No

Are you happy to take part? Yes/No

If any answers are ‘no’ or you don't want to take part, don't sign your name!

If you do want to take part, please write your name and today’s date
Your name ____________________________________________

Date ____________________________________________

Your parent or guardian must write their name here too if they are happy for you to do the project

Print Name ____________________________________________

Sign ____________________________________________

Date ____________________________________________

The doctor who explained this project to you needs to sign too:

Print Name ____________________________________________

Sign ____________________________________________

Date ____________________________________________

Thank you for your help.
**PATIENT (16-18yrs) CONSENT FORM**

**Title of Project:** The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population

**Name of Researchers:** Dr. Patrick J. Oades, Dr. Craig A. Williams, Dr Alan Barker and Miss. Zoe Louise Saynor

**Please initial box**

1. I have read and understand the information sheet [Version 2 dated 20/12/10] for the above study and have had the opportunity to ask questions.

2. I understand that my taking part is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.

3. I understand that sections of my medical records may be looked at by the researchers in the study where it is relevant to my participation, and that this information will be kept secure.

4. I understand that during the period of my participation that hard exercise is to be avoided but that I should eat and drink normally.

5. I am aware that my exercise results will be kept anonymous by giving them a unique study code and participant number, only my date of birth will be used to identify the results. I give consent for my medical records and exercise results to be used as indicated in the parent/guardian information sheet [Version 2 dated 20/12/10]. I understand that my exercise results will be stored for 15 years after which they will be destroyed.

6. I give my consent for to take part in the above study.

________________________   __________________________  
Name of participant    Date     Signature

________________________   __________________________  
Name of parent/guardian    Date     Signature

________________________   __________________________  
Researcher    Date     Signature
PARENT/GUARDIAN CONSENT FORM

Title of Project: The reliability and validity of maximal cardiopulmonary exercise testing as a prognostic tool within the young cystic fibrosis population

Name of Researchers: Dr. Patrick J. Oades, Dr. Craig A. Williams, Dr Alan Barker and Miss. Zoe Louise Saynor

Please initial box

1 I have read and understand the information sheet [Version 2 dated 20/12/10] for the above study and have had the opportunity to ask questions.

2 I understand that my child’s taking part is voluntary and they are free to withdraw at any time, without giving any reason and without his/her medical care or legal rights being affected.

3 I understand that sections of any of my child’s medical records may be looked at by the researchers in the study where it is relevant to his/her participation, and that this information will be kept secure.

4 I understand that during the period of his/her participation that hard exercise is to be avoided but that he/she should eat and drink normally.

5 I am aware that my child’s exercise results will be kept anonymous by giving them a unique study code and participant number, only my child’s date of birth will be used to identify the results. I give consent for his/her medical records and exercise results to be used as indicated in the parent/guardian information sheet [Version 2 dated 20/12/10]. I understand that my child’s exercise results will be stored for 15 years after which they will be destroyed.

6 I give my consent for my child to take part in the above study.

______________________________  ________________  ____________________
Name of participant             Date                  Signature

______________________________  ________________  ____________________
Name of parent/guardian          Date                  Signature

______________________________  ________________  ____________________
Researcher                      Date                  Signature
APPENDIX H

Consent and Assent Forms for Study 3

ASSENT FORM FOR CHILDREN
(To be completed by the child and their parent/guardian)

Oxidative and cardiovascular responses during exercise in patients with cystic fibrosis compared to age and gender matched controls

Child (or if unable, parent on their behalf) /young person to circle all they agree with please:

Have you read (or had read to you) about this project [Version 2 dated 02/01/12] Yes/No

Has somebody else explained this project to you? Yes/No

Do you understand what this project is about? Yes/No

Have you asked all the questions you want? Yes/No

Have you had your questions answered in a way you understand? Yes/No

Do you understand it’s OK to stop taking part at any time? Yes/No

Are you happy to take part? Yes/No

If any answers are ‘no’ or you don’t want to take part, don’t sign your name!

If you do want to take part, please write your name and today’s date

Study Number:
Participant Identification Number for this trial:
Your name

Date

Your parent or guardian must write their name here too if they are happy for you to do the project

Print Name

Sign

Date

The researcher who explained this project to you needs to sign too:

Print Name

Sign

Date

Thank you for your help.
PARTICIPANT (16-18 y) CONSENT FORM

Title of Project: Oxidative and cardiovascular responses during exercise in patients with cystic fibrosis compared to age and gender matched controls
Name of Researchers: Miss Zoe Louise Saynor, Dr. Alan R. Barker, Dr. Patrick J. Oades and Associate Professor Craig A. Williams

Please initial box

1. I have read and understand the information sheet [Version 1 dated 02/01/12] for the above study and have had the opportunity to ask questions.

2. I understand that my child’s taking part is voluntary and they are free to withdraw at any time, without giving any reason and without his/her medical care or legal rights being affected.

3. I understand that sections of any of my child’s medical records may be looked at by the researchers in the study where it is relevant to his/her participation, and that this information will be kept secure.

4. I understand that during the period of his/her participation that hard exercise is to be avoided but that he/she should eat and drink normally.

5. I am aware that my child’s exercise results will be kept anonymous by giving them a unique study code and participant number, only my child’s date of birth will be used to identify the results. I give consent for his/her medical records and exercise results to be used as indicated in the parent/guardian information sheet [Version 1 dated 02/01/12]. I understand that my child’s exercise results will be stored for 15 years after which they will be destroyed.

6. I give my consent for my child to take part in the above study.

_________________________  ___________  ______________________
Name of participant      Date          Signature

_________________________  ___________  ______________________
Name of parent/guardian  Date          Signature

_________________________  ___________  ______________________
Researcher               Date          Signature
PARENT/GUARDIAN CONSENT FORM

Title of Project: Oxidative and cardiovascular responses during exercise in patients with cystic fibrosis compared to age and gender matched controls

Name of Researchers: Miss Zoe Louise Saynor, Dr. Alan R. Barker, Dr. Patrick J. Oades and Associate Professor Craig A. Williams

1. I have read and understand the information sheet [Version 2 dated 02/01/12] for the above study and have had the opportunity to ask questions.

2. I understand that my child’s taking part is voluntary and they are free to withdraw at any time, without giving any reason and without his/her medical care or legal rights being affected.

3. I understand that sections of any of my child’s medical records may be looked at by the researchers in the study where it is relevant to his/her participation, and that this information will be kept secure.

4. I understand that during the period of his/her participation that hard exercise is to be avoided but that he/she should eat and drink normally.

5. I am aware that my child’s exercise results will be kept anonymous by giving them a unique study code and participant number, only my child’s date of birth will be used to identify the results. I give consent for his/her medical records and exercise results to be used as indicated in the parent/guardian information sheet [Version 2 dated 02/01/12]. I understand that my child’s exercise results will be stored for 15 years after which they will be destroyed.

6. I give my consent for my child to take part in the above study.

____________________  _____________  __________________
Name of participant       Date       Signature

____________________  __________________
Name of parent/guardian  Date       Signature

____________________  __________________
Researcher              Date       Signature
APPENDIX I

Consent and Assent Forms for Studies 5 and 6

Study Number: 12/SW/0270
Patient Identification Number for this trial:

ASSENT FORM FOR PATIENTS (10-15 y)
(To be completed by the patient and their parent/guardian)

The influence of disease status and ‘priming’ exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate and high-intensity cycling exercise in paediatric patients with cystic fibrosis

Child (or if unable, parent on their behalf) /young person to circle all they agree with please:

Have you read (or had read to you) about this project [Version 3 dated 02/10/12] Yes/No

Has somebody else explained this project to you? Yes/No

Do you understand what this project is about? Yes/No

Have you asked all the questions you want? Yes/No

Have you had your questions answered in a way you understand? Yes/No

Do you understand that you will be working with people different to your usual care team at the hospital? Yes/No

Do you understand it’s OK to stop taking part at any time? Yes/No
Are you happy to take part? Yes/No

If any answers are ‘no’ or you don’t want to take part, don’t sign your name!

If you do want to take part, please write your name and today’s date

Your name _____________________________

Date _____________________________

Your parent or guardian must write their name here too if they are happy for you to do the project

Print Name _____________________________

Sign _____________________________

Date _____________________________

The person from the research team who explained this project to you needs to sign too:

Print Name _____________________________

Sign _____________________________

Date _____________________________

Thank you for your help.
PATIENT (16-18 y) CONSENT FORM

Title of Project: The influence of disease status and ‘priming’ exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate and high-intensity cycling exercise in paediatric patients with cystic fibrosis

Name of Researchers: Miss Zoe L. Saynor, Dr. Patrick J. Oades, Dr. Alan R. Barker and Prof. Craig A. Williams

Please initial box

1 I have read and understand the information sheet [Version 3 dated 02/10/12] for the above study and have had the opportunity to ask questions.

2 I understand that taking part is voluntary and I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.

3 I understand that sections of my medical records may be looked at by the researchers (who are outside my usual direct clinical care team at the hospital) in the study where it is relevant to my participation, and that this information will be kept secure.

4 I understand that during the period of my participation that hard exercise is to be avoided but that I should eat and drink normally.

5 I am aware that my exercise results will be kept anonymous by giving them a unique study code and participant number, only my date of birth will be used to identify the results. I give consent for my medical records and exercise results to be used as indicated in the patient (16-18 y) information sheet [Version 3 dated 02/10/12]. I understand that my exercise results will be stored for 15 years after which they will be destroyed.

6 I give my consent to take part in the above study.

_________________________  ___________________  ___________________
Name of participant        Date                      Signature

_________________________  ___________________  ___________________
Name of parent/guardian    Date                      Signature

_________________________  ___________________  ___________________
Researcher                 Date                      Signature
PARENT/GUARDIAN CONSENT FORM

Title of Project: The influence disease status and ‘priming’ exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during moderate and high-intensity cycling exercise in paediatric patients with cystic fibrosis

Name of Researchers: Miss Zoe L. Saynor, Dr. Patrick J. Oades, Dr. Alan R. Barker and Prof. Craig A. Williams

Please initial box

1 I have read and understand the information sheet [Version 3 dated 02/10/12] for the above study and have had the opportunity to ask questions.

2 I understand that my child’s taking part is voluntary and they are free to withdraw at any time, without giving any reason and without his/her medical care or legal rights being affected.

3 I understand that sections of any of my child’s medical records may be looked at by the researchers (who are outside their usual direct clinical care team at the hospital) in the study where it is relevant to his/her participation, and that this information will be kept secure.

4 I understand that during the period of his/her participation that hard exercise is to be avoided but that he/she should eat and drink normally.

5 I am aware that my child’s exercise results will be kept anonymous by giving them a unique study code and participant number, only my child’s date of birth will be used to identify the results. I give consent for his/her medical records and exercise results to be used as indicated in the parent/guardian information sheet [Version 3 dated 02/10/12]. I understand that my child’s exercise results will be stored for 15 years after which they will be destroyed.

6 I give my consent for my child to take part in the above study.

____________________  _______________  __________________
Name of participant     Date                  Signature

____________________  _______________  __________________
Name of parent/guardian Date                  Signature

____________________  _______________  __________________
Researcher              Date                  Signature
APPENDIX J

Pubertal Maturation Assessment Documentation

Assessment of Pubertal Maturation letter
Date: __/__/201_

Dear parent/guardian,

Thank you once again for taking the time to attend the laboratory and allowing __________ to complete the exercise testing study. Your attendance is much appreciated. I will be in touch with individual feedback from testing in due course, in addition to a summary from all research published as a result of your attendance (if you wish to receive this).

As mentioned within the ‘parent/guardian information sheet’ we require an assessment of your child’s pubertal maturation. The assessment of physiological and physical change during growth is essential for valid interpretation of human performance and comparison between the different groups involved in this study.

Please find attached ‘maturation assessment procedures – parent/guardian’. Could you please read these and, subsequently, run through them with/pass them on to your child prior to their completion.

I would be very grateful if you could then post this to the address above. Many thanks.

Your compliance is very much appreciated.

Many thanks,

Zoe Saynor
Paediatric Physiology Research Scholar
Tel: +44 (0) 1392 724759
Email: z.l.saynor@exeter.ac.uk
The assessment of physiological and physical change during growth is essential for the valid interpretation of human performance.

**Pubertal maturity will be self-assessed at home following completion of the exercise testing protocol.**

Drawings of male and female pubic hair developed by Morris and Udry (1980) made from photographs by Tanner (1975) will be used. A written description of each of the 5 stages of male and female pubic hair development will be given on the chart as how to select the correct pubertal stage.

The most practical approach to assess maturation was developed by Tanner (1962). Tanner developed a 5-point scale to assess ‘biological maturity’ through observation of secondary sexual characteristics. The scales depicted five or more stages of breast and pubic hair (girls), pubic hair and genitalia development (boys). A limitation is that trained health professionals such as paediatricians and school nurses are typically employed to assess the scales. Subsequently Morris and Udry (1980) developed a self-assessment scale based on Tanner stages and found that children were able to accurately assess their own stage of maturation with correlation coefficients in the range of 60-70% (Matsudo & Matsudo, 1993).

The secondary sex characteristics described by Tanner (1962) will be used to assess pubertal maturity. Pubertal stage 1 will be identified as pre-pubertal, stages 2, 3 and 4 circum-pubertal, and stage 5 post-pubertal.

(Please find these below and circle the drawing which you feel best represents you)
The drawings on this page show different amounts of female pubic hair. A girl passes through each of the five stages shown by these drawings. Please look at each drawing and read the sentences under the drawings. Then choose the drawing closest to your stage of hair development and mark it 1. Then choose the drawing that is next closest and mark it 2.

1. Drawing A
2. Drawing B
3. Drawing C
4. Drawing D
5. Drawing E

There is no pubic hair.

There is a little long, lightly colored hair. This hair may be straight or a little curly.

The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a larger area.

The hair is now as dark, curly, and coarse as that of an adult female. However, the area that the hair covers is not as large as that of an adult female. The hair has not spread out to the thighs.

The hair now is like that of an adult female. It also covers the same area as that of the adult female. The hair usually forms a triangular (△) pattern as it spreads out to the thighs.
MATURATION ASSESSMENT PROCEDURES (MALES) – PARENT/GUARDIAN

The assessment of physiological and physical change during growth is essential for the valid interpretation of human performance.

Pubertal maturity will be self-assessed at home following completion of the exercise testing protocol.

Drawings of male and female pubic hair developed by Morris and Udry (1980) made from photographs by Tanner (1975) will be used. A written description of each of the 5 stages of male and female pubic hair development will be given on the chart as how to select the correct pubertal stage.

The most practical approach to assess maturation was developed by Tanner (1962). Tanner developed a 5-point scale to assess 'biological maturity' through observation of secondary sexual characteristics. The scales depicted five or more stages of breast and pubic hair (girls), pubic hair and genitalia development (boys). A limitation is that trained health professionals such as paediatricians and school nurses are typically employed to assess the scales. Subsequently Morris and Udry (1980) developed a self-assessment scale based on Tanner stages and found that children were able to accurately assess their own stage of maturation with correlation coefficients in the range of 60-70% (Matsudo & Matsudo, 1993).

The secondary sex characteristics described by Tanner (1962) will be used to assess pubertal maturity. Pubertal stage 1 will be identified as pre-pubertal, stages 2, 3 and 4 circum-pubertal, and stage 5 post-pubertal.

(Please find these below and circle the drawing which you feel best represents you)
The drawings on this page show different amounts of male pubic hair. A boy passes through each of the five stages shown by these drawings. Please look at each drawing and read the sentences under the drawing. Then choose the drawing closest to your stage of hair development. Mark a 1 on the line above that drawing. Then choose the drawing that is next closest to your stage of hair development and mark it a 2. In choosing the right picture, look only at the pubic hair, and not at the size of the testes, scrotum, and penis.

1. Drawing A
   - There is no pubic hair at all.

2. Drawing B
   - There is a little soft, long, lightly colored hair. Most of the hair is at the base of the penis. This hair may be straight or a little curly.

3. Drawing C
   - The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a somewhat larger area.

4. Drawing D
   - The hair is now as dark, curly, and coarse as that of an adult male. However, the area that the hair covers is not as large as that of an adult male. The hair has not spread out to the thighs.

5. Drawing E
   - The hair has spread out to the thighs. The hair is now like that of an adult male. It covers the same area as that of an adult male.
Please find enclosed an ActiGraph physical activity monitor (little red unit attached on a black waist belt).

The monitor is already programmed to record your movement and physical activity patterns. Simply attach the black belt around your waist, clipping in place and ensuring this is reasonably tight so as to not move around too much. The red monitor should have the little sticker at the top (make sure it is the right way up) and should be locating just above the hip of your dominant leg – i.e. the leg you would prefer to kick a ball with. Please ensure that monitors are removed when swimming, bathing, showering and before bedtime. Then pop them back on when you wake up the next day.
Activity Log Book

(EXAMPLE) DAY 1:

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>06:00 – 07:00</td>
<td></td>
</tr>
<tr>
<td>07:00 – 08:00</td>
<td></td>
</tr>
<tr>
<td>08:00 – 09:00</td>
<td></td>
</tr>
<tr>
<td>09:00 – 10:00</td>
<td></td>
</tr>
<tr>
<td>10:00 – 11:00</td>
<td></td>
</tr>
<tr>
<td>11:00 – 12:00</td>
<td></td>
</tr>
<tr>
<td>12:00 – 13:00</td>
<td></td>
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<td>13:00 – 14:00</td>
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<td>14:00 – 15:00</td>
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<td>15:00 – 16:00</td>
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<td>16:00 – 17:00</td>
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<td>17:00 – 18:00</td>
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<td>18:00 – 19:00</td>
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<tr>
<td>19:00 – 20:00</td>
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<td>20:00 – 21:00</td>
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<td></td>
</tr>
<tr>
<td>22:00 – 23:00</td>
<td></td>
</tr>
<tr>
<td>23:00 – 00:00</td>
<td></td>
</tr>
</tbody>
</table>
Dear Parent,

Your child is currently wearing a physical activity monitor. Please ensure that monitors are removed prior to swimming, bathing, showering and before bedtime. Could you please make a note of the time your child puts the monitor on each morning and the time it is taken off at night. It would also be helpful if any times the monitor is removed during the day could be recorded. Thank you.

<table>
<thead>
<tr>
<th>Day</th>
<th>Time put on in morning</th>
<th>Time taken off at night</th>
<th>Times removed during the day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
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<tr>
<td>Day 4</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please return the monitor in the envelope provided following the 7-day assessment.

PLEASE ENSURE THE MONITOR IS RETURNED ON: ASAP FOLLOWING 7-DAY ASSESSMENT

Thank you,
Zoe Saynor
07984 658768
zls202@exeter.ac.uk
APPENDIX L

Children’s Effort Rating Scale (P-CERT)
APPENDIX M

Rating of Perceived Dyspnoea Measurement Scale

Borg’s 0-10 Category-ratio (CR-10) rating of perceived dyspnoea scale
(Modification of Borg’s 1962 RPE Scale)

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Nothing at all</td>
</tr>
<tr>
<td>0.5</td>
<td>Very, very slight (just noticeable)</td>
</tr>
<tr>
<td>1</td>
<td>Very slight</td>
</tr>
<tr>
<td>2</td>
<td>Slight (light)</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>Somewhat severe</td>
</tr>
<tr>
<td>5</td>
<td>Severe (heavy)</td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Very severe</td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Very, very severe (almost maximum)</td>
</tr>
</tbody>
</table>

The 0-10 category-ratio (CR-10) scale modified for use to measure dyspnoea. The scale incorporates nonlinear spacing of verbal descriptors of severity that correspond to specific numbers and ratio properties of intensities. A number greater than 10 can be selected by the patient if his or her dyspnoea exceeds ‘very, very severe (almost maximum)’
APPENDIX N

Patient Medical Profile Form

Patient Information (collected by MDT staff member)

Date:  
Name:  
Gender:  
Age [yrs + m (0.0 dec.):  
Height (cm):  
Body mass (kg):  
BMI:  
CF genotype:  
Resting SaO₂ (%):  
Shwachman Score:  
Physical activity level: none / school sports / extracurricular sporting activities

Resting Lung Function (actual measure and % predicted):

<table>
<thead>
<tr>
<th>VC</th>
<th>FVC current (%)</th>
<th>FVC 12 month best (%)</th>
<th>FEV₁ current (%)</th>
<th>FEV₁ 12 month best (%)</th>
<th>FEF₂₅₋₇₅ current (%)</th>
<th>FEF₂₅₋₇₅ 12 month best (%)</th>
<th>PEF current (%)</th>
<th>PEF 12 month best (%)</th>
</tr>
</thead>
</table>

[STUDY REF]
Physical examination (Tick as appropriate):

Clubbing? □ Signs of hyperinflation? □
Crackles? □ Wheeze? □

Nutritional status (Tick as appropriate):

Pancreatic insufficient? □ Pancreatic sufficient? □

Chest Radiographic findings (Tick as appropriate):

Northern score:

Pathogens in sputum in last year (Pseudomonas Aeruginosa):

Chronic PsA □ (>50% of the preceding 12 months were PsA culture positive)
Intermittent PsA □ (≤50% of the preceding 12 months were PsA culture positive)
Free □ (None growth of PsA for the previous 12 months, having previously been PA culture positive)
Never □ (PsA has never been cultured)

Other regular pathogens (Circle as appropriate):

SA HI MRSA Other grm -ve NTM

[STUDY REF]
### Smoking *(Circle as appropriate):*

<table>
<thead>
<tr>
<th>none</th>
<th>active</th>
<th>passive</th>
</tr>
</thead>
</table>

### Other imaging / DEXA? *(Insert relevant notes/information):*

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
</table>

### Assessment of liver function / glycaemic control:

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFRLD?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFRDM?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFRIGT?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal glycaemic control?</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

### Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IVAB days in last year (days):</td>
<td></td>
</tr>
<tr>
<td>Hypertonic saline</td>
<td></td>
</tr>
<tr>
<td>Pulmozyme</td>
<td></td>
</tr>
<tr>
<td>Nebulised antibiotics</td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td></td>
</tr>
<tr>
<td>Other oral antibiotic</td>
<td></td>
</tr>
<tr>
<td>IHCS</td>
<td></td>
</tr>
<tr>
<td>LABA</td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td></td>
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</tbody>
</table>

(STUDY REF)
<table>
<thead>
<tr>
<th>Renal function:</th>
<th>Na:</th>
<th>Creat.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBC:</td>
<td>Hb.:</td>
<td>Neut.:</td>
</tr>
<tr>
<td>Bone chemistry &amp; Mg.:</td>
<td>Mg.:</td>
<td>Ca.:</td>
</tr>
<tr>
<td>PO4:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABPA:</td>
<td>Yes</td>
<td>/</td>
</tr>
<tr>
<td>(If yes):</td>
<td>Confirm in REMISSION when studied (tick):</td>
<td></td>
</tr>
<tr>
<td>Dietetic review:</td>
<td></td>
<td></td>
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<tr>
<td>Supplements:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral?</td>
<td>Yes</td>
<td>/</td>
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<tr>
<td>Gastronomy?</td>
<td>Yes</td>
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</table>
Airway clearance method:

<table>
<thead>
<tr>
<th>Method</th>
<th>☐</th>
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<tbody>
<tr>
<td>PN&amp;PD</td>
<td>☐</td>
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<tr>
<td>ACBT</td>
<td>☐</td>
</tr>
<tr>
<td>Flutter/PEP/Acapella</td>
<td>☐</td>
</tr>
</tbody>
</table>

Date completed: 

Staff member: 

[STUDY REF]