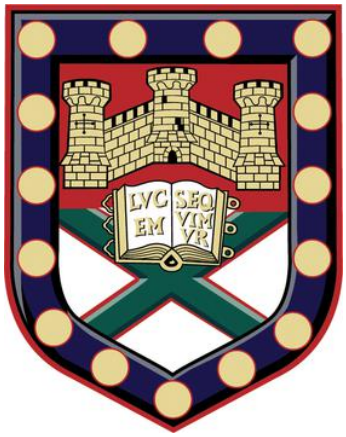
Sex Differences in Parameters of Cardiopulmonary Fitness and their Relationship to Clinical Outcomes in Young People with Cystic Fibrosis



Submitted by Chloe Louise Bland

to the University of Exeter

as a thesis for the degree of Master of Science by Research in

Sport and Health Sciences,

July 2019

UNIVERSITY OF EXETER

ABSTRACT

Background: Females with cystic fibrosis have higher mortality rates than their male counterparts. Pulmonary function defined as forced expiratory volume in 1 s (FEV_1) is a predictor of mortality. However, when pulmonary function is accounted for, females still have a higher mortality rate than males. Independent of lung function, peak oxygen uptake ($\dot{V}O_{2peak}$) is a significant predictor of mortality, with higher values of $\dot{V}O_{2peak}$ relating to a lower risk of mortality. In healthy children, $\dot{V}O_{2peak}$ is significantly different between sexes, however it has yet to be determined whether sex differences in $\dot{V}O_{2peak}$ occur in CF. **Aim:** To identify sex differences in exercise capacity in young people with CF, when appropriately scaled for different body size variables and adjusted for key clinical parameters such as lung function and nutritional and maturity status. **Methods:** 52 young people (29 males and 23 females) aged 8 – 25 y with CF underwent a ramp cycle test to exhaustion. 47 completed an additional supramaximal cycle test to exhaustion at 110 % of ramp test peak power to verify that a true $\dot{V}O_{2max}$ was obtained. $\dot{V}O_{2peak}$ was scaled allometrically and using the ratio standard method. ANCOVAs were utilised to remove the influence of clinical parameters. **Results:** $\dot{V}O_{2peak}$ was significantly lower in females than males for absolute $\dot{V}O_{2peak}$ ($p < 0.001$, 1.41 ± 0.38 $L \cdot min^{-1}$ and 2.17 ± 0.82 $L \cdot min^{-1}$, respectively), $\dot{V}O_{2peak}/BM^{*\beta}$ ($p < 0.001$, 53.28 ± 10.93 $mL \cdot kg^{*0.82} \cdot min^{-1}$ and 73.04 ± 19.57 $mL \cdot kg^{*0.82} \cdot min^{-1}$, respectively), $\dot{V}O_{2peak}/BSA^{*\beta}$ ($p < 0.001$, 814.58 ± 159.05 $mL \cdot m^{2(*1.29)} \cdot min^{-1}$ and 1108.06 ± 296.24 $mL \cdot m^{2(*1.29)} \cdot min^{-1}$, respectively), and $\dot{V}O_{2peak}/Stature^{*\beta}$ ($p < 0.001$, 464.76 ± 86.69 $mL \cdot m^{*2.42} \cdot min^{-1}$ and 627.77 ± 183.28 $mL \cdot m^{*2.42} \cdot min^{-1}$, respectively). These remained significant with the inclusion of co-variates, peak height velocity (PHV), age, body mass index (BMI) and FEV_1.

Conclusions: Irrespective of the scaling method and body size variable used or if adjusted for key clinical parameters (age, PHV, BMI and FEV_1), young females with CF have a reduced $\dot{V}O_{2peak}$ compared to males. Future studies should consider sex differences in exercise capacity as a potential contributor to differences in mortality.

Keywords: respiratory disease, exercise capacity, children, adolescents, cardiopulmonary exercise testing.

ACKNOWLEDGEMENTS

Firstly, I would like to thank both of my supervisors, Professor Craig Williams and Dr Alan Barker. You have given me tremendous support over the past two years in order to help me develop and grow both as a researcher and a person. Without your guidance (and especially your patience!) I would not have been able to achieve what I have during my time in CHERC.

Thank you to my examiners Dr Gary Brickley and Dr Bert Bond for taking the time to examine this thesis.

I would also like to thank Dr Owen Thomlinson. For his everlasting patience with my many statistics questions. To everyone at CHERC, thank you for your friendships, coffee breaks and gym sessions. You made my time in Exeter an experience I will never forget, and I am forever grateful.

Finally, I am extremely indebted to my family (the dogs included!) and James, for all the love and support throughout these past few years to help me get to this point. And to my wonderful parents, you have continually inspired me to be the best I can be, I wouldn't be where I am today if it weren't for you. Without you all, this thesis would not have been possible. I hope it, and I, make you proud.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

Figure 2.7. Schematic based on the Fick Equation to highlight what is known and not known about contributors of exercise capacity/ aerobic fitness. Hb = Haemoglobin; O_2 = oxygen; PO_2 = partial pressure of oxygen; Q = cardiac output; a-vO_2 difference = arterial-venous oxygen difference; $\dot{V}O_2$; maximal oxygen uptake.

Chapter 3

Figure 3.1. Patient inclusion flow diagram.

Figure 3.2. Example of plotting $\dot{V}O_2$ against time linear regression in MSF21 (LabVIEW, National Instruments, Newbury, UK) to obtain $\dot{V}O_{2peak}$. Example data from a patient with cystic fibrosis.

Figure 3.3. Scaling of body size parameters. Significant correlations denoted by *. Males r and *p* value denoted in top right corner of each graph; females denoted in the bottom right corner. 1; absolute $\dot{V}O_{2peak}$ plotted against BM, BSA and stature. 1; absolute $\dot{V}O_{2peak}$ plotted against a: mass, b: body surface area and c: stature. 2; ratio standard scaled $\dot{V}O_{2peak}$ plotted against a: BM, b: BSA and c: stature. 3; allometrically scaled $\dot{V}O_{2peak}$ plotted against a: BM, b: BSA and c: stature. $\dot{V}O_{2peak}$; peak oxygen uptake. BM; body mass. BSA; body surface area. β; beta coefficient.

Figure 3.4. Example data of an individual with cystic fibrosis to determine the gas exchange threshold in MSF21 (LabVIEW, National Instruments, Newbury, UK). Respiratory compensation point, the point at which there is a steeper rate of minute ventilation [$\dot{V}_E$] vs. rate of carbon dioxide production [$\dot{V}CO_2$] (Beaver *et al.,* 1986), was removed before further analysis. **A:** Shows the inflection point

identified by the software on the graph plotting $\dot{V}CO_2$ against oxygen uptake ($\dot{V}O_2$). The intersection of the yellow lines marks the disproportionate increase in $\dot{V}CO_2$ compared to $\dot{V}O_2$, and therefore the gas exchange threshold. **B:** Shows the visual determination of the ventilatory threshold using ventilatory equivalents of $\dot{V}O_2$ and $\dot{V}CO_2$ (identified on the graph by the intercept of the horizontal and vertical lines).

Chapter 4

Figure. 4.1. $\dot{V}O_{2peak}$ distribution in males and females. Lines represent mean and SD. Significant difference highlighted with an *.

Figure 4.2. Correlation between minute ventilation and age. Solid linear line represents the visual point at which the positive correlation becomes negative.

Figure 4.3. Mean oxygen uptake (a) and $\dot{V}O_{2peak}$ allometrically scaled to body mass (b) body surface area (c) and stature (d) for males and females in each of the categories of 1: $FEV_{1\%predicted}$ tertiles and 2: age tertiles. Vertical lines represent SD. Significant main effect of: sex = *; tertiles = #.

Figure 4.4. Adjusted means and ranges for absolute and scaled VO_{2peak} parameters in males and females. Significant differences highlighted with an *.

LIST OF ABBREVIATIONS AND SYMBOLS

6MWT	6-minute walk test
$b \cdot min^{-1}$	Beats per minute
BMD	Bone mineral density
BMI	Body mass index
$Breaths \cdot min^{-1}$	Breaths per minute
cAMP	Cyclic adenosine monophosphate
CF	Cystic fibrosis
CFRDM	Cystic fibrosis related diabetes mellitus
CFTR	Cystic fibrosis transmembrane conductance regulator
CHO	Carbohydrate
Cl^-	Chloride ion
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise test
CSA	Cross sectional area
$E1 \cdot g^{-1}$	Elastase-1 per gramme
FEV_1	Forced expiratory volume in 1s
$FEV_{1\%predicted}$	Forced expiratory volume in 1s as a percent of predicted norms
FFM	Fat free mass
$freq \cdot y^{-1}$	Frequency per year
FVC	Forced vital capacity
$FVC_{\%predicted}$	Forced vital capacity as a percent of predicted norms
GET	Gas exchange threshold
HR	Heart rate
HR_{peak}	Peak heart rate
K^+	Potassium ion

$kg \cdot m^{-2}$	kilogramme per metre squared
$km \cdot h^{-1}$	kilometre per hour
L	Litre
$L \cdot min^{-1}$	Litre per minute
$mL \cdot cm^{-1} \cdot min^{-1}$	Millilitre per centimetre per minute
$mL \cdot kg^{-1} \cdot min^{-1}$	Millilitre per kilogramme per minute
$mL \cdot min^{-1}$	Millilitre per minute
$mL \cdot W^{-1} \cdot min^{-1}$	Millilitre per watt per minute
MM	Muscle mass
$mmol \cdot L^{-1}$	Millimole per litre
Na^{+}	Sodium ion
O_2	Oxygen
OUES	Oxygen uptake efficiency slope
PA	Physical activity
$\dot{Q}$	Cardiac output
RCP	Respiratory compensation point
RER	Respiratory exchange ratio
$rev \cdot min^{-1}$	Revolution per minute
RPD	Rating of perceived dyspnoea
RPE	Rating of perceived exertion
RSM	Ratio standard method
RV	Right ventricle
RV/TLC	Reserve volume to total lung capacity ratio
S_{max}	Supramaximal
SRT	Steep ramp test
SV	Stroke volume
TTE	Time to exhaustion
µg	Microgramme

$\dot{V}CO_{2peak}$	Peak rate of carbon dioxide output
$\dot{V}_E$	Minute ventilation
$\dot{V}_E/\dot{V}CO_2$	Ventilatory equivalent for carbon dioxide
$\dot{V}_E/\dot{V}O_2$	Ventilatory equivalent for oxygen
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_{2max}$	Maximal rate of oxygen uptake
$\dot{V}O_{2peak}$	Peak rate of oxygen uptake
W	watt
$W \cdot 10s^{-1}$	watt per 10 seconds
WR	Work rate
WR_{peak}	Peak work rate
$W \cdot min^{-1}$	watt per minute
$W \cdot s^{-1}$	watt per second

1. INTRODUCTION

Cystic Fibrosis (CF) is a complex, multi-organ, life shortening disease caused by a mutation in the CF transmembrane conductor regulator (CFTR) protein. The disease primarily effects the lungs and digestive system, however the mutated CFTR protein is expressed in the membranes of cells lining the skin and reproductive tracts as well. One in 25 people in the UK are carriers of the recessive gene (~ two million people) leading to 1 in 2,500 babies born with CF in the Caucasian population.

Females with CF have been found to have higher rates of mortality than males with CF. Rosenfeld *et al.,* (1997) used CF registry data in the US from 1988-1992 and reported females below 20 y to have a 60 % greater risk of death than males below 20 y. Interestingly, the sex difference became less significant with increasing age (Rosenfeld *et al.,* 1997), contradicting previous theories that hormonal differences may be the cause of greater mortality rates in females (Gurwitz *et al.,* 1979). Some studies dispute the sex gap entirely, arguing that improved diagnosis and therapies, especially lung airway clearance treatments, have narrowed the sex gap in mortality (Viviani *et al.,* 2011; Verma *et al.,* 2005). However, according to the Cystic Fibrosis Trust registry (2017), median predicted survival age for men and women is still significantly different (47.9 y and 44.2 y respectively). This suggests, despite improved therapies, females are still at a higher risk of early mortality than their male counterparts.

Due to the nature of CF, parameters of lung function have been considered the best predictors of mortality, specifically forced expiratory volume in 1 s (FEV_1) and $FEV_{1\%predicted}$. Those with < 30 % $FEV_{1\%predicted}$ have a 50 % chance of mortality in two years compared to a less than 5 % chance in those with > 60 % $FEV_{1\%predicted}$ (Kerem *et al.,* 1992). Furthermore, Kerem *et al.,* (1992) reported risk

of death with an $FEV_{1\%predicted}$ of 20-30 % to be 18 % higher in females than males with CF, concluding that females with CF have a less favourable mortality for a given FEV_1. This conclusion was supported by Corey *et al.,* (1997), who have shown steeper declines of $FEV_{1\%predicted}$ in females with CF than males with CF. Despite this, $FEV_{1\%predicted}$ only partially accounts for reduced mortality, with females experiencing a ~60 % greater mortality than males when pulmonary function is removed as an explanatory factor (Rosenfeld *et al.,* 1997).

The National Health Service (NHS) in the UK provides vital support, treatment and management for all those with CF, utilising lung function tests to assess the individual. Each year a patient will undergo an annual review of overall health to track progression, which enables continual improvement and individualisation of treatment. All data from annual review is added to the UK CF Trust registry, creating a national database. Treatments include medicines, physiotherapy, nutrition, surgery and exercise. Investigations into exercise in CF started in the 1970s and exercise testing in cardiology was introduced in 1980s. Since then, exercise testing in clinical populations has significantly increased, however it is still described as a specialised test in many CF NHS clinics. In the last few decades exercise initiatives, such as 'Exercise is Medicine' launched in 2007 (Lobelo *et al.,* 2014), have been developed to encourage health care professionals to utilise physical activity (PA) as a treatment and 'prescribe' exercise with continual, routine testing.

Independent of lung function, exercise capacity (expressed through $\dot{V}O_{2peak}$ [peak oxygen uptake][1]), has been shown to be a significant predictor of mortality in CF

[1] $\dot{V}O_{2max}$ is the oxygen intake during an exercise intensity at which actual oxygen intake reaches a maximum, eliciting a plateau. $\dot{V}O_{2peak}$ is the highest $\dot{V}O_2$ attained in a specific test and regardless of the subject's effort (Whipp, 2019) and may not elicit a plateau.

(Radtke *et al.,* 2017; Pianosi *et al.,* 2005; Nixon *et al.,* 1992). A higher value of $\dot{V}O_{2peak}$ relates to lower mortality rates. Only ~30 % of the reduced exercise capacity elicited in CF is explained by pulmonary function (Pastre *et al.,* 2014) indicating there are other factors involved. Few studies have explored sex differences in regards to exercise capacity in CF. Kilbride *et al.,* (2012) reported no significant differences in $\dot{V}O_{2peak}$ when scaled for body mass between males and females with CF (46.7 $mL \cdot kg^{-1} \cdot min^{-1}$ and 41.9 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively). Conversely, Gruber *et al.,* (2011) identified a significant difference between males and females $\dot{V}O_{2peak}$, in absolute and relative terms (32.1 $mL \cdot kg^{-1} \cdot min^{-1}$ and 29.5 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively), however there is a large difference in sample size between the Kilbride and Gruber studies (16 and 344 respectively) which is likely to have caused the differences in significance. Ratio standard scaling is reportedly unsuitable in healthy children, and previous literature in one CF centre reports a broad range of body sizes and compositions (Hanna and Weiner, 2015), raising the question whether scaling for body mass is a sufficient scaling approach in CF. Scaling is especially prominent when assessing differences in the paediatric population due to the influences of puberty on growth and body composition. In healthy children, it has been reported that $\dot{V}O_{2peak}$ normalised for fat free mass (FFM) or muscle mass (MM) elicits a ~5 % and ~1 % lower $\dot{V}O_{2peak}$ in females respectively (Welsman *et al.,* 1997). It may be that a similar phenomenon is seen in the CF paediatric population. In many of these studies, the authors have only accounted for the influence of body mass to assess sex differences in the $\dot{V}O_{2peak}$ response; they failed to account for other clinical factors that could influence results such as genotype, nutritional (Vieni *et al.,* 2013; Fogarty *et al.,* 2012) and maturity status.

Therefore, the purpose of the present thesis is to identify sex differences in exercise capacity in young people with CF, when appropriately scaled for different body size variables and adjusted for key clinical parameters such as lung function, age and nutritional status.

2.1 Cystic fibrosis pathophysiology

CF is an autosomal recessive life shortening disease, primarily targeting the lungs and digestive system. It is the most common genetic disease in the Caucasian population with a prevalence of 1 in 2,500 babies born equating to ~10,400 people in the UK, 53.2 % of which are male (Cystic Fibrosis Trust, 2017c). One in 25 people are carriers of the defective recessive gene (over two million people in the UK), if two carriers have a child there is a 25 % chance they will be born with CF (Fig. 2.1). A median projected life expectancy for a new-born with CF is ~47 years of age in the UK (Cystic Fibrosis Trust, 2017a). In 2013, the median age of death was only 29 years showing the magnitude of development in care and treatment for people living with CF over the last 5 years alone (Cystic Fibrosis Trust, 2016). It is understood that females with CF have a lower life expectancy, living ~3.7 years less than males (Cystic Fibrosis Trust 2017c).

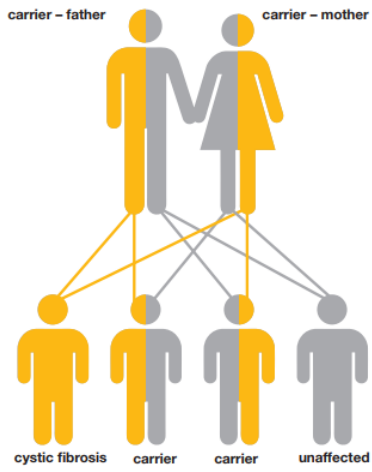


Figure 2.1. The probability of two carrier parents producing a child with CF. (Cystic Fibrosis Trust, 2015b).

The CFTR gene, located on chromosome 7 (Kerem *et al*., 1989), codes for the cyclic adenosine monophosphate (cAMP) - dependent CFTR protein found in epithelial cells that produce mucus, sweat, saliva and digestive enzymes that line most exocrine organs such as the lungs/airways, pancreas and liver (Thomson and Harris, 2008). Healthy CFTR proteins behave as channels for negatively charged chloride ions (Cl^-) moving in and out of cells. Creating a high concentration of solute on one side of the membrane causes water to move from low to high concentrations via osmosis, equalising the gradient. CF is a result of a defective CFTR gene that causes mutation or dysfunction of the CFTR protein (Tucker *et al*., 2017). In a person with CF, the movement of Cl^- is dysregulated causing increased sodium (Na^+) and water retention inside the cell, resulting in thickening of the viscous mucus layer.

There are varying severities of CF due to over 1,700 different mutations of the CFTR gene, which can be classified (class I-VI; Table 2.1 and Fig. 2.2) by how they impact the production, processing, conduction, transportation or volume of the CFTR protein (Cystic Fibrosis Foundation, 2017a). Class I mutations result in no synthesis of a functional CFTR protein and class VI causes accelerated turnover or decreased stability of the CFTR protein at the cell membrane. Of people with CF in the UK, 90 % have at least one allele with the Phe508del class II mutation (Cystic Fibrosis Trust, 2015a), which causes degradation of the protein shortly after synthesis. Consequently, the protein does not reach the surface of the cell membrane (Rowe, Miller and Sorscher, 2005) and cannot carry out its function.

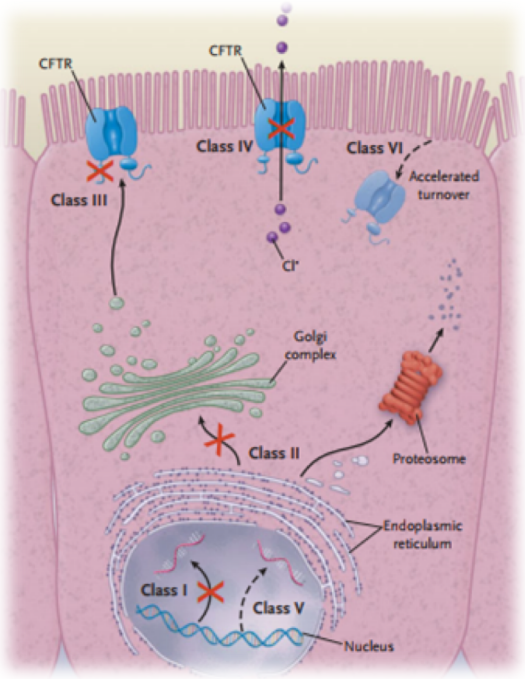


Figure 2.2. Classes of defects in the CFTR gene. No synthesis (class I); defective protein maturation and premature degradation (class II); defective or disordered channel regulation (class III); defective or disordered chloride or channel conductance (class IV); reduced synthesis of CFTR protein (V); decreased stability or increased turnover of CFTR from the cell surface (VI). (Reproduced from Rowe, Miller and Sorscher, 2005).

Table 2.1. Classes of CFTR mutation and defect, with examples of specific mutations. (Boyle and De Boeck, 2013).

Class	Defect	Examples
I	No functional CFTR protein	Gly542X Trp1282X Arg553X 621+1G→T
II	Defective CFTR maturation	PHE508del Asn1303Lys Ile507del Arg560Thr
III	Defective channel regulation	Gly551Asp Gly178Arg Gly551Ser Ser549Asn
IV	Defective channel conductance	Arg117His Arg347Pro Arg117Cys Arg334Trp
V	Reduced CFTR synthesis	3849+10kbC→T 2789+5G→A 3120+1G→A 5T
VI	Increased turnover of CFTR from the cell surface	4326delTC Gln1412X 4279insA

CFTR; Cystic fibrosis transmembrane conductance regulator.

These mutations ultimately lead to many complications and symptoms in CF, of which, the lungs and airways are largely affected. The cilia on CF cells are smaller and flatter so therefore cannot beat away the thickened mucus layer, causing ineffective clearance of bacteria from the lungs (Thomson and Harris, 2008; Fig. 2.3). This leaves people with CF prone to infection, with 25 % of the paediatric population in 2017 developing influenza (Cystic Fibrosis Trust, 2018f), which can

often lead to inflammation caused by trapped bacteria resulting in damage to the airway lining. Gradually, continual reinfection will lead to a progressive decline in pulmonary function, the extent to which this occurs is dependent upon the individual. Females tend to experience a greater decline (Cystic Fibrosis Trust, 2018f).

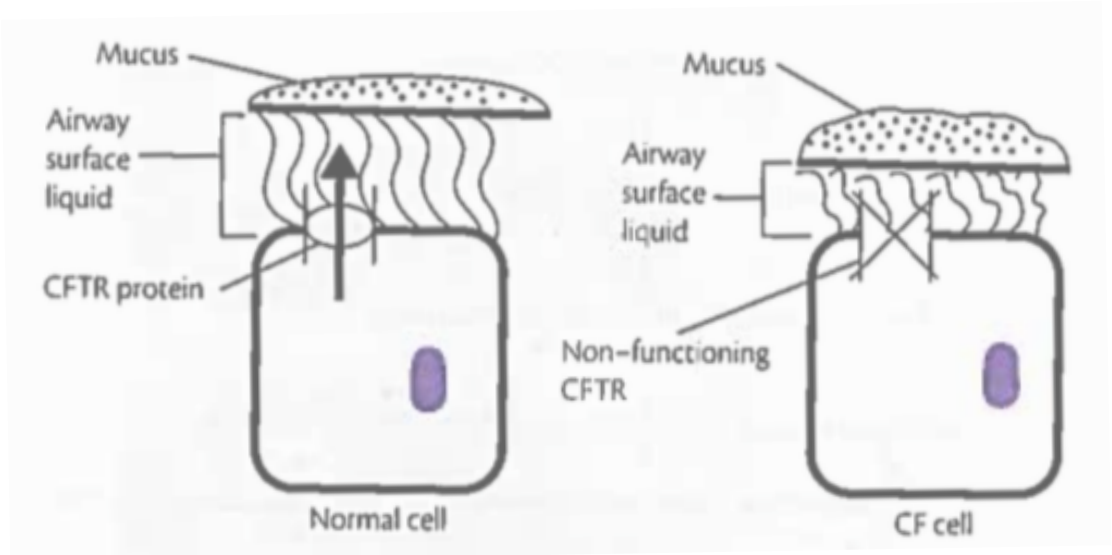


Figure 2.3. CFTR protein in the airways of a healthy and CF cell. (Thomson and Harris, 2008).

A further complication of CF is an increased reserve volume to total lung capacity ratio (hyperinflation). This occurs due to a reduced ability to expire, causing people with CF to work harder than average for a reduced amount of O_2 than those without CF. Due to viral infections, wheezing can be experienced, and some people with CF may develop asthma as a result of narrowing and damage to airways. Other complications include; haemoptysis caused by ruptures in small blood vessels often associated with infection; Pneumothorax and nasal polyps (outgrowths of the mucus membranes as a response to irritation or inflammation).

The leading cause of death in CF is deterioration and failure of the pulmonary system (Flume *et al.,* 2009), therefore management of lung health is the primary

target of treatment. The most common way to assess lung function in children and adults with CF is by spirometry, which measures FEV_1 and forced vital capacity (FVC) (Fig. 2.4). A value of 85 % predicted FEV_1 is the threshold of near normal lung health and is often classified as mild CF (Cystic Fibrosis Trust, 2015).

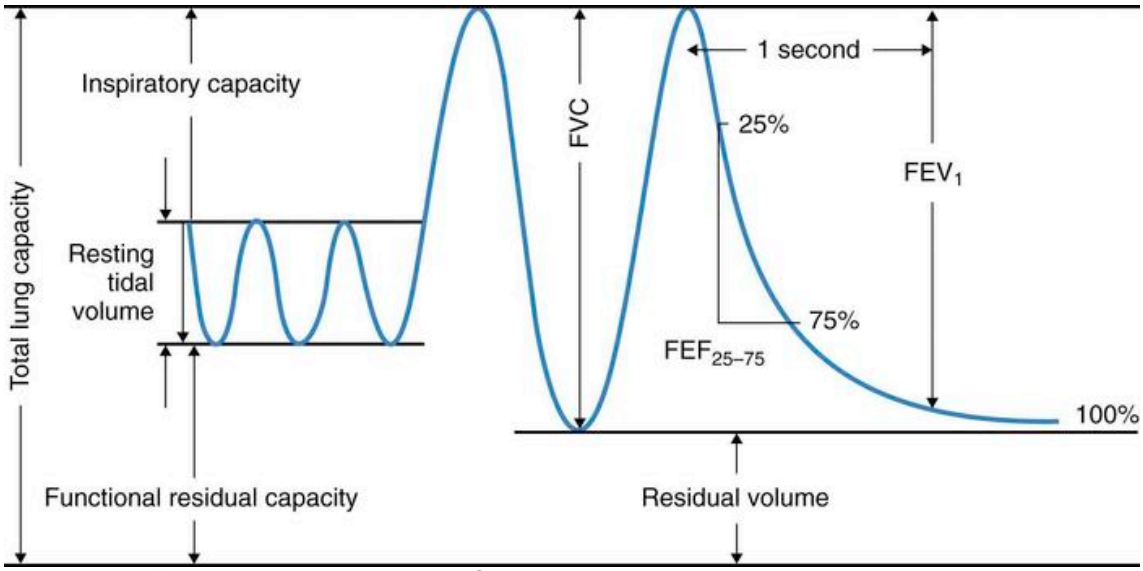


Figure 2.4. Lung Volumes. FVC = Forced vital capacity; FEF_{25-75} = Forced expiratory flow from 25-75 % of vital capacity; FEV_1 = Forced expiratory volume in 1 second. (Metro Health, 2018).

Dysregulation of ions and lack of osmotic drive also affects cells in the pancreas and digestive system. The small channels in the pancreas can become blocked, due to viscous secretions reducing the volume of pancreatic enzymes that reach the small intestine and causing pancreatic insufficiency. However, humans have a large reserve of pancreatic enzymes such that we only require 10 % for normal functioning (Thomson and Harris, 2008), therefore ~10-15 % of people with CF can be pancreatic sufficient (Borowitz *et al.,* 2004). Importantly, these individuals do not have normal pancreatic function and may become pancreatic insufficient with time or severity of the disease. In these pancreatic sufficient individuals,

some of the cells in the pancreas die, leading to inflammation and sometimes pancreatitis. Those with pancreatic insufficiency cannot get pancreatitis as all of the cells die and become fibrotic tissue.

Meconium ileus is a form of bowel obstruction common in the CF new-born population, 90 % of new-borns with meconium ileus are diagnosed with CF (Southampton Children's Hospital, 2016). This is due to decreased intestinal fluid secretion, which makes the meconium (bowel contents) thicker and stickier. Thus, meconium is harder to move through the bowel and often gets stuck at the ileocecal junction (between the small and large intestine) making it difficult for individuals with CF to defecate. Distal intestinal obstruction syndrome, gastro-oesophageal reflux and rectal prolapse are other examples of altered digestive function in CF, further descriptions can be found in Thompson and Harris (2008).

About 40 % of the CF population have liver irregularities, however only 5-10 % experience problems and symptoms (Cystic Fibrosis Trust, 2017b). Intrahepatic bile ducts (transport bile salts from the liver and gallbladder to the duodenum) can become irritated and blocked by thick mucoid secretions (Lamireau *et al.,* 2006) sometimes called gallstones. Eventually liver cirrhosis, a late and severe stage of scarring, can occur causing irreversible damage to the liver (Cystic Fibrosis Trust, 2017b). Scarring causes increasing back pressure of blood flow and enlargement of the spleen and blood vessels, termed portal hypertension.

CF related diabetes mellitus (CFRDM) is present in approximately 2 % of children and 19 % of adolescents in the CF population. There is contrasting evidence relating to differences in sex, with some literature suggesting a ~5 % higher incidence in females compared to than males, when averaged over an age range of 13 to > 35 years (Marshall *et al.,* 2005). In contrast, Moran *et al.,* (2009) reported no sex differences in CFRDM prevalence overall, but found a higher

incidence in females aged 30-39 y. Such contradictory findings may therefore be attributed to using differing age categories. CFRDM occurs due to the scarring of the pancreas. Thus, CFRDM is normally a gradual process starting with the inability to produce sufficient insulin to deal with the demands of large quantities of glucose. In turn, the blood glucose levels remain high for longer periods of time meaning more insulin is needed but not enough is being produced, resulting in glucose intolerance. Furthermore, those with CFRDM may not be able to utilise insulin in the correct way, like those with type 2 diabetes (Cystic Fibrosis Foundation, 2017b). However, as some native insulin is produced, CFRDM is often mild in comparison to other non-CF related diabetes.

Many adults with CF struggle to synthesise a healthy amount of new bone for their age, and therefore develop low bone mineral density (BMD), consequently increasing bone fragility and leaving bones prone to fracture. Healthy people reach peak bone mass shortly after adolescence (> 20 y), of which, in the two-year period only 25% of total skeletal mass is obtained (Smitt and Mehls, 2008), therefore the years prior to peak bone mass (i.e. adolescence) may be crucial in development. Conway *et al.,* (2000) reported as many as 79 % of males and 59 % of females with CF to have osteoporosis or osteopenia in at least one site. It is unclear whether the dysfunctional CFTR gene is directly responsible for low BMD, however, many studies have shown an association between low BMD and disease severity (Cystic Fibrosis Trust, 2007; Buntain *et al.,* 2004; Haworth *et al,* 2002; Conway *et al.,* 2000). Furthermore, people with CF have poor nutrition and digestion/absorption of what they do ingest. Abnormal absorption of fat-soluble vitamins and vitamin D deficiency due to reduced exposure to the sun can lead to osteomalacia or rickets (Cystic Fibrosis Trust, 2007). Lack of key nutrients such

as vitamin D, K and calcium also contribute to the poorer bone health experienced in CF.

Excessive Na^+, Cl^- and potassium (K^+) loss is often experienced in CF, largely through sweating but also vomiting (Thomson and Harris, 2008) and diarrhoea (Gutierrez *et al.,* 2016). In non-CF individuals, Cl^- and Na^+ are reabsorbed through CFTR channel proteins, however in CF these channels are dysfunctional, therefore reabsorption cannot occur (Gutierrez *et al.,* 2016). Furthermore, under normal conditions an increase in extracellular osmolality is a trigger for thirst, however this trigger is reduced in people with CF increasing risk of dehydration. The combination of these two factors makes CF patients more susceptible to dehydration than healthy individuals. The kidneys can sometimes try to compensate by releasing other electrolytes, leaving people with CF feeling lethargic, especially infants and children (Thomson and Harris, 2008). CF can also cause many other complications such as gall bladder disease, urinary incontinence, infertility, arthritis and more. Thus, CF is much more commonly regarded as a multi–organ disease.

2.2 Treatment

CF centres provide care from a multidisciplinary team of specialists to deliver treatment of CF. Medicines can be used to treat different symptoms of CF disease. Medicine, for treatment of the lungs, can be taken three ways; orally, through intravenous methods and/or by inhaling through a nebuliser (Cystic Fibrosis Trust, 2018a), although intravenous methods are typically only used if oral treatment is ineffective. Bronchodilator drugs and steroids, such as corticosteroids or glucocorticoids, are inhaled into the lungs to relax the muscles in the airways, which relieves tightness and makes breathing easier. This can also aid the effectiveness of other medication as air can move more freely through

the pulmonary system. Often working for ~4-6 hours, bronchodilators take 20 minutes to take effect and may have some side effects such as nausea and dizziness (Cystic Fibrosis Foundation, 2018). Mucolytic enzymes can complement bronchodilators as they break down mucus, widening the lumen of the airways and easing clearance from the lungs. Antibiotics are another key medication that treat against bacterial infections in the lungs by inhibiting growth or killing bacteria. Guidance from the Cystic Fibrosis Trust suggests all new-borns diagnosed with CF be placed on anti-staphylococcal antibiotic prophylaxis with flucloxacillin for 3 y. Some patients, such as those deteriorating on normal therapies, can be placed on trials of antibiotics with the aim of slowing pulmonary function decline (Cystic Fibrosis Trust, 2009). Bisphosphonates and the steroid Flixonase can be used to treat other CF related complications such as low BMD and rhinitis (swelling in the nasal airways) respectively (Cystic Fibrosis Trust, 2018a).

Energy needs of people with CF can reach 150-200 % of normal requirements, especially when fighting infections (Cystic Fibrosis Trust, 2018b). Therefore, treatment is in place to aid digestion and absorption of food. Enzyme capsules can be used to assist breakdown of food in the digestive system, however much of this treatment is highly individualised due to age, stature, weight, lung function and PA differences. Energy drinks and other high energy foods can be used to counter unsuccessful digestion, but those with CFRDM have to carefully balance their energy intake with insulin treatments, making it more challenging to meet energy requirements. In most part, hospitals and clinics provide specialist dieticians to aid effective energy balance for those with and without CFRDM.

Physiotherapy is largely used in CF to effectively loosen and clear mucus build up in the lungs, however it can aid with other problems that may add to the strain

of the disease such as back and continence issues (Cystic Fibrosis Trust, 2018c). Autogenic drainage is a technique that uses a series of sighs and breaths to move the mucus from the smaller airways into larger ones, requiring the patient to feel and control the mucus in order to cough and clear it at the optimum point. There are many other techniques to remove mucus (including use of devices to aid removal) and physiotherapy to remove it can be uncomfortable. Therefore, physiotherapists often choose a technique based on what works best with the individual with sessions lasting anywhere between 10 – 60 min. Physiotherapists aim to create a programme of techniques that allow patients to independently carry out treatment, allowing them more freedom as they grow into adulthood.

Organ transplants can be considered when all previous treatments no longer have any impact, and are undertaken when organs, such as lungs or liver, are severely impaired, causing the patient to become unwell, and in turn requiring additional care. Kerem *et al.,* (1992) stated that females, due to their greater risk of mortality for a given FEV_1, may need to be considered for a lung transplant earlier than their male counterparts. Before a transplant operation can ensue, the patient with CF must first be assessed and wait for the organ to become available, which may take some time. On average a person with CF will wait 18 months for an organ and only 2 in 3 people on the organ list will receive a transplant (Cystic Fibrosis Trust, 2018d). A lung transplant can significantly improve quality of life for a person with CF, however they will unfortunately not have the life expectancy of a healthy individual and will require continual treatment for the rest of the body. Of patients who have undergone a lung transplant, 50 % will live at least 5 y after the operation (Cystic Fibrosis Trust, 2018d).

Arguably the least invasive and most ‘normal’ form of treatment is exercise. Any planned and structured activity requiring physical exertion that is carried out to

improve health and fitness is termed exercise; whereas PA is defined as bodily movement, produced by the skeletal muscles, requiring energy expenditure above rest (Caspersen *et al.,* 1985). Swimming, running, football and other sports are all examples of exercise suitable for CF treatment. Using a trampoline is good choice of exercise in children as it is typically seen as enjoyable, however the risk of injury to benefits is often disputed (Barak *et al.,* 2005). Selvadurai *et al.,* (2002) reported a 2 % and 6 % greater increase in $FEV_{1\ \%predicted}$ with aerobic and anaerobic training programmes, respectively, compared with standard chest physiotherapy only. Not only does exercise enhance mucus clearance (McIlwaine, 2007), but it is further useful to increase MM and physical fitness (Cystic Fibrosis Trust, 2018e; Radtke, *et al.,* 2017).

2.3 Exercise testing in cystic fibrosis

Standard spirometry tests only allow assessment of the lung function, but not the stress that exercise or PA puts on the pulmonary, cardiovascular and muscular systems (Ferrazza *et al.,* 2009). Therefore, tests have been developed to assess the complex interaction of the body's systems during exercise. In some cases, field tests are used to assess exercise capacity in CF. Although they require less expensive equipment, field tests tend to use more space and do not provide gas exchange data, which can be useful as it provides quantitative values of key exercise parameters. The six-minute walk test (6MWT) is self-paced and submaximal in all but those with severe CF (Hebestreit *et al.,* 2015). Whereas, incremental shuttle tests such as the 3 min step test, 20 m shuttle test and 10 m shuttle test, are all externally paced with multiple levels designed to assess exercise capacity. Field tests cannot directly measure $\dot{V}O_{2peak}$, however they can be useful in a clinical environment to assess children for lung transplants or following transplants where exercise capacity is limited (Hebestreit *et al.,* 2015).

The steep ramp test (SRT) is performed on a cycle ergometer and estimates $\dot{V}O_{2peak}$ from peak work rate using a validated algorithm (Bongers *et al.,* 2013). Initially, participants undertake a warm-up at 25 W before the resistance increases by 10, 15 or 20 W·10 s^{-1} depending on the stature of the participant (<125 cm, 125-150 cm and >150 cm respectively). Cadence is kept between 60-80 rev·min^{-1}, and maximal exercise is accepted when cadence falls below 60 rev.min^{-1} and participants show subjective signs of intense effort (Bongers *et al.,* 2015). Bongers and colleagues found that the SRT over reported work rate (WR) in both absolute and relative terms when compared to a cardiopulmonary exercise test (CPET) but under reported peak heart rate (HR_{peak}). However, peak WR (WR_{peak}) attained during SRT correlates strongly (r = 0.82) with $\dot{V}O_{2peak}$ attained during a CPET, which could show that the SRT can be used to predict $\dot{V}O_{2peak}$ in CF and may be less demanding as suggested by the ~8 % lower HR_{peak} reported by Bongers *et al.,* (2015). However, rating of perceived exertion (RPE) was not reported in the above study by Bongers, therefore, perceptually the SRT may be more or equally demanding on the participant.

Laboratory tests such as the Bruce treadmill protocol have been developed to directly assess measures of fitness. Originally the Bruce protocol was used to assess aerobic impairment in cardiovascular disease but has since been expanded for use in CF and other pulmonary diseases such as Chronic Obstructive Pulmonary Disease (COPD; Hebestreit *et al.,* 2015). The protocol is a continuous, incremental treadmill test consisting of 3 min stages at a specified gradient and speed (1 = 10 % grade, 2.7 km·h^{-1}; 2 = 12 %, 4 km·h^{-1}; 3 = 14 %, 5.4 km·h^{-1}; 4 = 16 %, 6.7 km·h^{-1}; 5 = 18 %, 8.0 km·h^{-1}, 6 = 20 %, 8.8 km·h^{-1}; 7 = 22 %, 9.6 km·h^{-1}). Some CF patients who have an expected lower exercise capacity can use the modified Bruce Protocol, which has two additional stages

prior to stage 1. These stages are at the same speed as stage 1 but at a lower percent grade (stage 0 = 0 %; stage ½ = 5 %). Outcome variables measured included $\dot{V}O_{2peak}$ and exercise time; WR_{peak} can be calculated by using final percent grade, speed, time at final stage, body mass and gravity. Despite being a valid and reliable exercise test in both healthy children/ adults and in CF populations (Hebestreit *et al.,* 2015), determining WR_{peak} and $\dot{V}O_{2peak}$ on a treadmill is less precise than with cycle ergometers (e.g. Godfrey Protocol) as these parameters are often calculated from test parameters such as exercise time. Test-retest reliability of $\dot{V}O_{2peak}$ is r = 0.94 in healthy children and r = ~0.86 in healthy adults (Hebestreit *et al.,* 2015). Furthermore, the authors failed to report test-retest reliability for CF populations.

The Godfrey protocol has been used in CF with varying degrees of severity, cardiopulmonary impairment and age. The test is a stature dependent, continuous, incremental cycle test to volitional fatigue. Starting at 10 W (stature < 120 cm), 15 W (stature 120-150 cm) and 20 W (stature >150 cm) the electronically braked cycle ergometer holds the power output constant regardless of cadence; however participants are encouraged to maintain a constant cadence throughout. WR increases every minute and outcome measures of $\dot{V}O_2$, SPO_2 and HR are recorded during the last 15 s of each increment. Those with mild or moderate CF can follow this original protocol. However, in severe CF a modified protocol can be recommended using smaller increments i.e. 10 W for FEV_1 < 30 % (Hebestreit *et al.,* 2015). The Godfrey protocol at increments of 15 $W \cdot min^{-1}$ has been shown to be very reproducible over 28 days in adults with CF, but not children with CF, with test retest variations of 6 % WR_{peak} and 6.9 % in $\dot{V}O_{2max}$ (McKone *et al.,* 1999). This protocol has been used to determine $\dot{V}O_{2peak}$, which

has a prognostic value of equal importance to lung function parameters, specifically FEV_1 (Nixon *et al.,* 1992).

CPETs such as the Godfrey and Bruce protocols are the gold standard to assess exercise capacity, as they can assess gas exchange measures, the primary outcome of which is $\dot{V}O_{2max}$. However not all CF patients are able to complete a maximal exercise test, therefore some authors have suggested the use of submaximal measures as an alternative assessment of exercise capacity (Williams *et al.,* 2014). The gas exchange threshold (GET) has been proposed as a non-invasive estimation of the lactate threshold that demarcates moderate and heavy intensity exercise domains. However, the GET can be difficult to detect in chronic respiratory conditions; COPD non-detection is ~40 % (Sue *et al.,* 1988), whereas CF (when 80 % of participants were mild-moderate disease cases) had a reported 15 % non-detection (Thin *et al.,* 2002). Gruet *et al.,* (2010) concluded that the oxygen uptake efficiency slope (OUES), which describes the relationship between $\dot{V}O_2$ and minute ventilation $(\dot{V}_E)$, is a more useful submaximal parameter than the GET. The OUES was developed in 1996 by Baba and colleagues and, despite being shown to be reliable in adults with CF (Gruet *et al.,* 2010), Williams *et al.,* (2018) have concluded the OUES is not a valid alternative to maximal measures of aerobic fitness in children and adolescents with CF. However, the above study by Gruet *et al.,* (2010) had a more biased population favouring males compared to Williams *et al.,* (2018), which may have influenced the significance of results as sex is reportedly a determinant of OUES (Buys *et al.,* 2015). In addition, Marinov *et al.,* (2007) suggested that healthy boys have significantly higher values of OUES than girls.

Irrespective of whether healthy, CF or other disease status, exercise testing does not always elicit a plateau (the primary criteria for $\dot{V}O_{2max}$; Barker *et al.,* 2011) in

the majority of children, leading to the term $\dot{V}O_{2peak}$ being more commonly used when referring to exercise capacity in children. With $\dot{V}O_{2max}$, secondary criteria are often used to determine whether true max has been attained (e.g. heart rate (HR) ±10 $b \cdot min^{-1}$ of predicted max, and respiratory exchange ratio (RER) >1.0). However, Saynor *et al.,* (2013a), reported that secondary criteria often underestimated the true maximum in children with CF. The authors utilised a supramaximal (S_{max}) protocol as an addition to a protocol with only an incremental test to exhaustion, such as the widely used Godfrey protocol (Godfrey *et al.,* 1971).

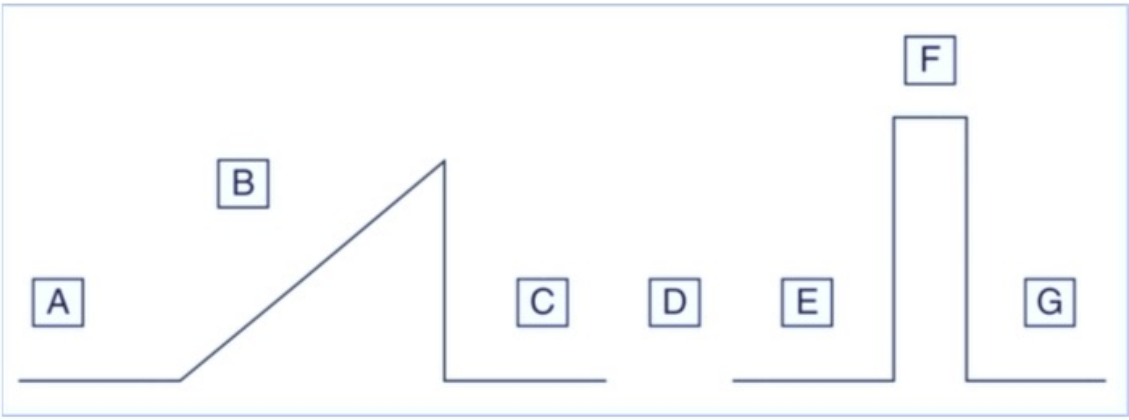


Figure 2.5. Ramp and S_{max} protocol. A: 3 min warm up, 20 W. B: Incremental ramp test, at individualised increments. C: ~5 min unloaded pedalling recovery. D: 10 min recovery off the bike. E: 3 min warm up at 20 W. F: S_{max} bout, 110 % peak power attained during B. G: ~3 min unloaded pedalling recovery (reproduced from Williams *et al*, 2014).

Studies have concluded that incremental tests to exhaustion that make use of secondary criteria can underestimate $\dot{V}O_{2max}$ in healthy populations (Sansum *et al.,* 2019; Poole *et al.,* 2008). Therefore, S_{max} variation of CPET, which can be appended onto the end of most protocols as it includes both an incremental test

to exhaustion prior to a S_{max} bout (Fig. 2.5), can determine a valid and reliable $\dot{V}O_{2max}$ in CF (Saynor *et al.,* 2013a; Saynor *et al.,* 2013b). Saynor and colleagues (2013a) confirmed that a bout at 110 % of peak power attained in an incremental test to exhaustion could verify the 'true' and confirm attainment of $\dot{V}O_{2max}$. Further, a more recent study by Saynor and colleagues (2018) has confirmed the S_{max} to be safe and well tolerated in both children and adults with varying severities of CF. The above study by Saynor *et al.,* (2018) had a relatively small sample population (45 participants), therefore a larger observational study may be required to confirm this conclusion. The ramp stage of the protocol increases in increments individualised to each of the participants (10-25 $W \cdot min^{-1}$) based on previous prediction equations for peak power (Hulzebos *et al.,* 2012) to ensure test duration lasts between 8-12 min. Due to the S_{max} being in the upper region of the severe exercise intensity domain, it generally lasts between 1-4 mins. The S_{max} bout has been validated to determine a 'true' $\dot{V}O_{2max}$ in healthy adults (Dalleck *et al.,* 2012). Sansum *et al.,* (2019) reported that the S_{max} verified $\dot{V}O_{2max}$ in 128 healthy children, and verification is not affected by sex, body mass or cardiorespiratory fitness. Furthermore, in a recent retrospective analysis including children and adults with mild to severe CF, the S_{max} produced a valid measure of $\dot{V}O_{2max}$ in 86 % of children and 96 % of adults (Causer *et al.,* 2018). Traditional controversial criteria for determining $\dot{V}O_{2max}$ such as HR, RER and [BLa] can now be replaced by the S_{max} in healthy children (Sansum *et al.,* 2019) and children/ adults with mild to severe CF (Saynor *et al.,* 2013a).

2.4 Cystic fibrosis and exercise capacity

Exercise capacity or $\dot{V}O_{2peak}$ has been shown to be a key indicator of mortality in the CF population independent of lung function (Hulzebos *et al.,* 2014; Pianosi *et*

al., 2005a; Moorcroft *et al.,* 1997; Nixon *et al.,* 1992). Moorcroft *et al.,* (1997) reported $\dot{V}O_{2peak}$ significantly predicted mortality in 67 adult CF participants, however it was not more useful than FEV_1. Conversely, Nixon and colleagues (1992), identified $\dot{V}O_{2peak}$ as a significant predictor of mortality and FEV_1 did not improve the relationship in the model. A meta-analysis, including 551 patients with CF, concluded a lower $\dot{V}O_{2peak}$ was significantly associated with a 5-fold relative risk increase in mortality (Vendrusculo *et al.,* 2019).

Table 2.2. Generic training guidelines for CF (Reproduced from Williams *et al.,* 2010).

	Patients with mild to moderate CF lung disease	Patients with severe CF lung disease
Recommended activities	Cycling, walking, hiking, aerobics, running, rowing, tennis, swimming, strength training, climbing, roller-skating, (trampolining)	Ergometric cycling, walking, strengthening exercises, gymnastics, and day-to-day activities
Method	Intermittent and steady-state	Intermittent
Frequency	3–5 times per week	5 times per week
Duration	30–45 minutes	20–30 minutes
Intensity	70%–85% HRmax; 60%–80% peak $\dot{V}O_2$; LT; GET	60%–80% HRmax; 50%–70% peak $\dot{V}O_2$; LT; GET
Oxygen supplementation	Indicated, if SaO_2 drops below 90% during exercise	Indicated, if SaO_2 drops below 90% during exercise (cave: resting hypoxia)
Activities to avoid	Bungee-jumping, high diving, and scuba diving	Bungee-jumping, high diving, scuba diving, and hiking in high altitude
Potential risks associated with exercise, and training	Dehydration Hypoxemia Bronchoconstriction Pneumothorax Hypoglycaemia* Hemoptysis Oesophageal bleedings Cardiac arrhythmias Rupture of liver and spleen Spontaneous fractures**	

HRmax: maximum heart rate; peak $\dot{V}O_2$: peak oxygen consumption; LT: lactate threshold; GET: gas exchange threshold; SaO_2: oxygen saturation.
* Depending on the existence of an impaired glucose tolerance.
** Depending on the existence of untreated CF-related bone disease.

There are also reports that $\dot{V}O_{2max}$ is associated with quality of life (de Jong *et al.,* 1997) and reduced hospitalisation in children with CF (Perez *et al.,* 2014). Promoting a higher exercise capacity can improve quality of life for people with CF by helping to clear mucus and increase tolerance of everyday physical tasks (Cystic Fibrosis Trust, 2018e; de Jong *et al.,* 1997). Despite this knowledge there are still no clear and precise guidelines for PA and exercise in CF due to a lack of well controlled trials and large differences amongst protocols (Bradley and Moran, 2008). Williams *et al.,* (2010) provided a useful table of general recommendations (Table 2.2), which can be used to help create a more individualised programme by clinicians. A meta-analysis, including data from 17 centres and 726 patients worldwide, reported contradictory evidence regarding how specific exercise can be beneficial to CF patients. Nevertheless, the overriding message is that there is no evidence to actively discourage exercise in children and adults with CF (Radtke *et al.,* 2017).

Children with mild CF have been shown to have a lower $\dot{V}O_{2peak}$ (- 3.2 $mL \cdot kg^{-1} \cdot min^{-1}$; -7.6 $mL \cdot kg^{-1} \cdot min^{-1}$) than their healthy peers (Vandekerckhove *et al.,* 2017; Saynor *et al.,* 2014 respectively), which is linked to disease severity. Cross sectional data have shown that lower $\dot{V}O_{2peak}$ is significantly associated (p = 0.0001) to a lower FEV_1 in children and adolescents, unfortunately, the authors failed to report the correlation statistic (Pianosi *et al.,* 2005a). Pianosi and colleagues (2005b) collected data in CF children (aged 7-16 years) over a 5-year period. They found that in children, $\dot{V}O_{2peak}$ increased with age but adolescents (and those with FEV_1 < 80 % $_{predicted}$) observed a fall in $\dot{V}O_{2peak}$; those with FEV_1 > 80 % $_{predicted}$ tended to maintain $\dot{V}O_{2peak}$. However, the authors did not consider other clinical factors that could influence results such as genotype, nutritional and maturational status, therefore we cannot infer causality from these results. In

contrast, in a paediatric (8-18 y) longitudinal study over 2 years reported $\dot{V}O_{2peak}$ to increase over the observation period, with change in FEV_1 and FFM explaining 47 % of the variance of the change in absolute $\dot{V}O_{2peak}$ (Klijin *et al.,* 2003). However, the authors in this study failed to account for maturity status, therefore the increase in $\dot{V}O_{2peak}$ may have been the result of multiple other factors such as training and puberty. Currently, there is limited longitudinal evidence reporting the response of exercise related variables in children with CF. Two of the most important factors relating to mortality in CF, lung function and a body size/ nutrition, have been accounted for in the study by Klijin *et al.,* (2003), yet there is still 53 % of variance in CF $\dot{V}O_{2peak}$ that is unaccounted for.

There are many conceivable factors that limit exercise capacity; however, it is unknown which processes in the oxygen transport and utilisation pathway are the biggest contributors to exercise intolerance for children and adults with CF (Fig. 2.6). Due to the primary nature of CF causing obstruction in the airways, there is impairment in the lungs during exercise. People with CF experience a reduced reserve volume to total lung capacity ratio (RV/TLC), which can cause hyperinflation and therefore increased dead space (Hulzebos *et al.,* 2014). This increases demand on the inspiratory muscles to maintain $\dot{V}_E$, increasing the oxygen cost, and therefore causing people with CF to have a higher ventilatory contribution to $\dot{V}O_{2peak}$, thus reducing the respiratory muscle endurance (Leroy *et al.,* 2011). Additionally, dynamic hyperinflation has been shown to reduce exercise capacity in other diseased populations (Zhao *et al.,* 2016). Furthermore, adults and adolescents with CF have an observed higher respiratory muscle strength than predicted because of the greater work of breathing (Dunnink *et al.,* 2009). Moorcroft *et al.,* (2005) found that adults with mild or moderate CF had similar scores for ventilatory parameters as the healthy controls, reporting muscle

soreness as the reason for termination. In severe CF, Moorcroft *et al.,* (2005) reported the opposite, suggesting that it is only in severe CF that lung function is the main reason for exercise intolerance. In addition, ventilatory parameters (such as FEV_1), only explain approximately one third of reduced exercise capacity in CF suggesting other factors contribute to reduced exercise capacity (Pastre *et al.,* 2014).

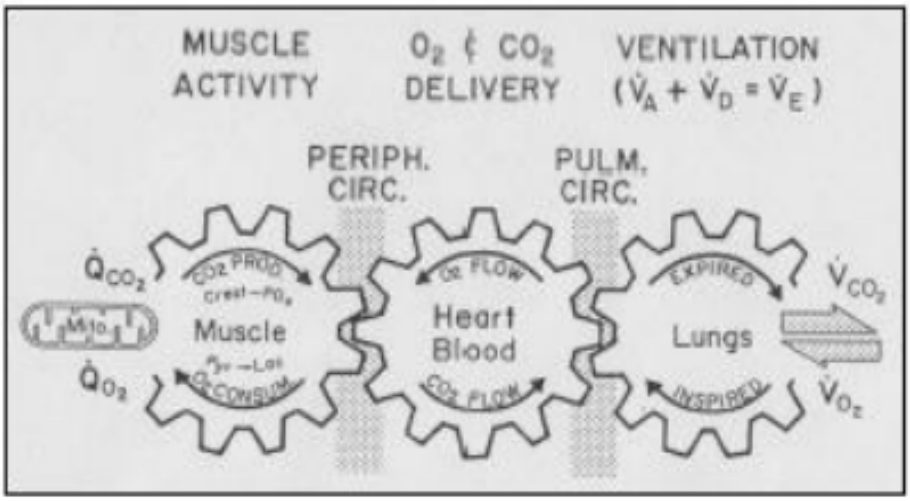


Figure 2.6. The oxygen transport and utilisation pathway showing the integration of muscular, cardiovascular and pulmonary systems (reproduced from Wasserman et al., 1999).

As ventilatory parameters cannot explain fully the reduced exercise capacity in CF, researchers have investigated the role of cardiovascular function. Figure 2.6 shows the integration of the heart, lungs and muscles in the oxygen transportation and utilisation pathway that may contribute to reduced exercise capacity. It has been suggested that cardiopulmonary factors may provide insight into the limitations of exercise for CF patients. During maximal exercise in children with CF there is a reduced stroke volume (SV) which is not sufficiently compensated for by HR, resulting in a reduced cardiac output ($\dot{Q}$; Rosenthal *et al.,* 2009),

whereas during submaximal exercise HR can compensate sufficiently. Although Rosenthal and colleagues assessed $\dot{Q}$ indirectly through the Fick equation (Fig. 2.7), other studies have shown that adults with CF elicit right ventricular (RV) abnormalities (Ionescu *et al.,* 2001) measured through echocardiography. Ionescu *et al.,* (2001) concluded that RV dysfunction is positively correlated with disease severity, which can in turn influence exercise tolerance. In a study by Giacchi *et al.,* (2015) it was reported children with CF to be vulnerable to heart impairment and hypothesised that this was because of the consistent chronic inflammation due to declining lung function. Furthermore, children with CF cannot increase RV ejection fraction sufficiently during exercise (Benson *et al.,* 1984), which increases the risk of pulmonary hypertension and exercise intolerance (Lewis *et al.,* 2013). However, it is unknown whether children with CF elicit pulmonary hypertension during exercise and if this dysfunction influences exercise intolerance.

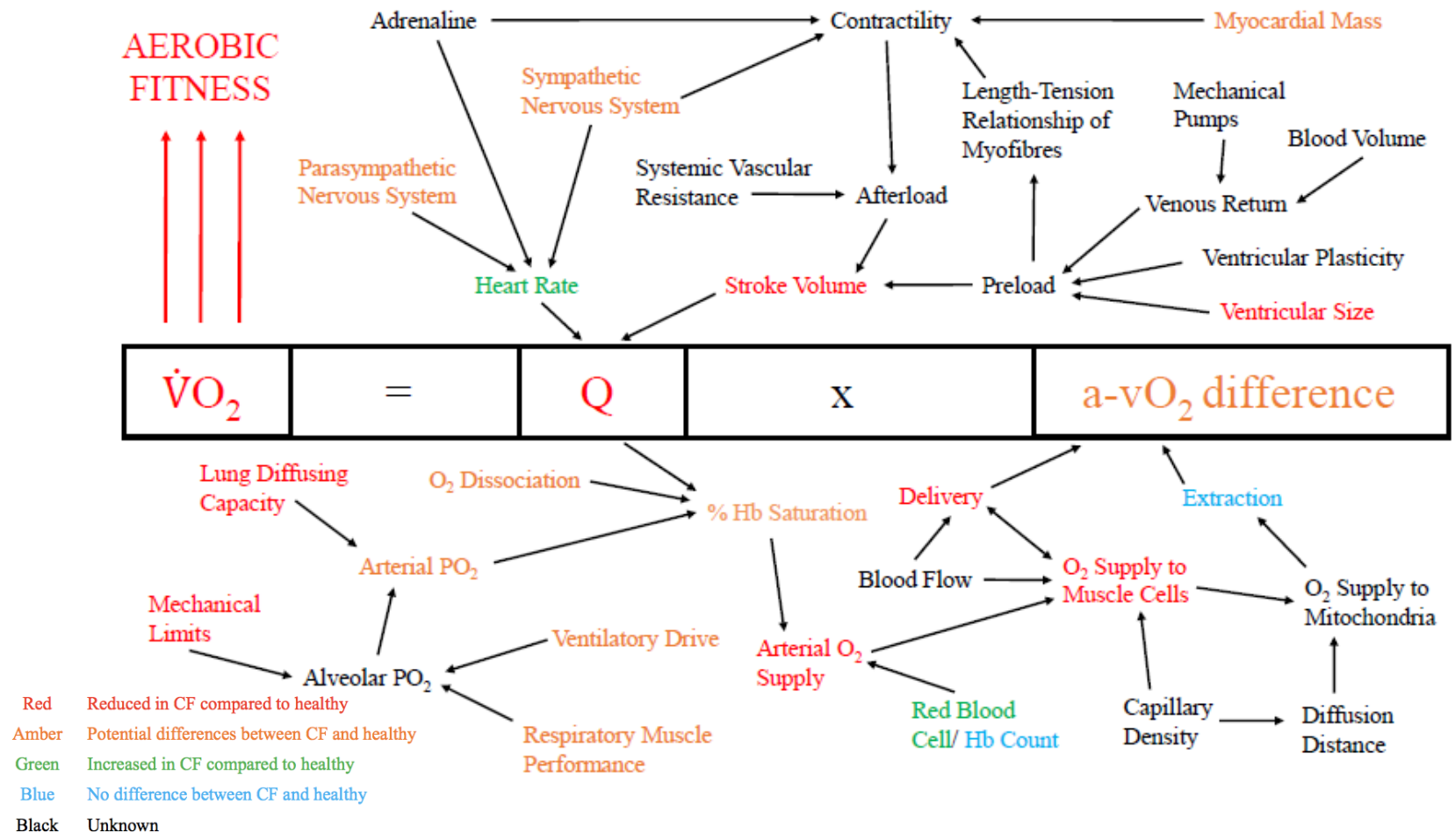


Figure 2.7. Schematic based on the Fick Equation to highlight what is known and not known about contributors of exercise capacity/ aerobic fitness. Hb; Haemoglobin. O_2; oxygen. PO_2; partial pressure of oxygen. Q; cardiac output. a-vO_2 difference; arterial-venous oxygen difference. $\dot{V}O_2$; maximal oxygen uptake.

The CFTR protein has been shown to present in skeletal muscle cells as well as in the pulmonary and digestive systems (Lamhonwah *et al.,* 2010; Divangahi *et al.,* 2009). It is known that muscle strength is positively related to anaerobic (Boas *et al.,* 1996) and aerobic (Klijn *et al.,* 2003; de Meer *et al.,* 1999) capacity. Muscle strength is reduced in young CF males (Hussey *et al.,* 2002) and when muscle size is accounted for there is still ~5 $mL \cdot cm^2 \cdot min^{-1}$ difference in $\dot{V}O_{2max}$ between CF and controls (Moser *et al.,* 2000). However, this study used cross sectional area (CSA) as a measure for muscle size and scaled using the ratio standard method (RSM). CSA is an indirect measure of muscle volume and the RSM has been shown to over scale data, causing heavier children to be penalised and lighter children to have their fitness elevated (Tanner, 1949). Allometric scaling uses an equation ($Y=a.X^b$) to describe the specific relationship between the physiological variable (i.e. $\dot{V}O_2$) and a unit of body size (i.e. fat free mass) to more appropriately remove the influence of body size (Welsman and Armstrong, 2000). Where Y = physiological variable; a = proportionality constant; X = body size measure; and b = power function.

Some studies have found evidence for impaired skeletal muscle function regardless of MM (Erickson *et al.,* 2015; Rosenthal *et al.,* 2009; de Meer *et al.,* 1995). Erickson *et al.,* (2015) reported an age dependent inverse relationship with oxidative capacity and age-related disease progression. Conversely, Werkman *et al.,* (2016) found that there were no metabolic constraints in muscle oxidative capacity. However, both of the aforementioned studies had small cohorts of participants (13 and 10, respectively), measured different outcomes and the study by Erickson *et al.,* (2015) used older participants than Werkman *et al.,* (2016), making it difficult to compare the two. Furthermore, periods of greater inflammation such as infection of Pseudomonas aeruginosa, has been shown to

cause reduced muscle strength and exercise capacity in adolescent CF (Van de Weert-van Leeuwen *et al.,* 2012; Divangahi *et al.,* 2009). When whole body exercise is undertaken, children with mild to moderate CF appear to elicit no differences in the rate of oxygen extraction at the skeletal muscle level compared to healthy matched controls (Saynor *et al.,* 2014). This leads to the conclusion that oxygen delivery and cardiovascular factors are the primary limiting factors in the skeletal muscles (Saynor *et al.,* 2014; Rosenthal *et al*., 2009). In contrast, a later study by Saynor and colleagues (2016) proposed that the changes in aerobic metabolism are intensity dependent, impairments occurring during heavier intensities, linking to an impairment in O_2 extraction and utilization in pediatric CF populations. Most of the literature bases its findings on mild to moderate CF, little or no evidence exists into skeletal muscle metabolism during exercise for those with severe CF (Werkman *et al.,* 2016; Van de Weert-van Leeuwen *et al.,* 2013). Few studies recruit severe CF patients largely due to pragmatic reasons, such as ability to perform the test. This makes it difficult to effectively research all aspects of CF, however it may be that findings would be similar but exacerbated in severe CF.

2.5 Sex differences and exercise capacity

Previous literature has shown that mortality of CF is largely dependent on nutrition or body mass index (BMI; body mass in kg over stature^2 in m) (Vieni *et al.,* 2013; Fogarty *et al.,* 2012; Liou *et al.,* 2001; Huang *et al.,* 1987), lung function (Liou *et al.,* 2001; Corey *et al.,* 1997; Moorcroft *et al.,* 1997; Kerem *et al.,* 1992; Nixon *et al.,* 1992), sex (Liou *et al.,* 2001; Corey *et al.,* 1997; FitzSimmons, 1993) and exercise capacity (Radtke *et al.,* 2017; Pianosi *et al.,* 2005). As both sex and exercise capacity are predictors of mortality, the relationship between the two factors required exploration. It is known that exercise capacity, measured using

$\dot{V}O_{2max}$, differs between sexes in healthy children (McNarry *et al*., 2015), however there is limited evidence in CF populations.

In a population of 248 healthy children aged 8-11 y, Dencker *et al.,* (2007) reported $\dot{V}O_{2peak}$ to be higher in boys than girls, dependent on whether $\dot{V}O_2$ was expressed using scaling (8%) or in absolute terms (18%). Other studies have shown absolute values for $\dot{V}O_{2peak}$ are ~20 % lower in females at a given age. When normalizing for BM, Armstrong *et al.,* (1997) reported significantly higher $\dot{V}O_{2peak}$ in boys than girls (adjusted $\dot{V}O_{2peak}$ means of 1.76 $L \cdot min^{-1}$ and 1.52 $L \cdot min^{-1}$, respectively). However, Davies *et al.,* (1972) proposed that normalizing for body mass may be inappropriate as $\dot{V}O_{2max}$ is related to the volume of muscle utilized during exercise in children. Thus, the percentage difference $\dot{V}O_{2peak}$ is minimised to ~1 % when normalised MM between males and females (Welsman *et al.,* 1997). In children with CF, when normalised for BM, males had higher $\dot{V}O_{2peak}$ (+3.7 $mL \cdot kg^{-1} \cdot min^{-1}$ on average) than females (Pianosi *et al.,* 2005a). It is unknown whether males with CF have a higher $\dot{V}O_{2peak}$ than females with CF when normalised for FFM or MM as previous literature fails to report results for each sex.

Many CF clinics do not take measurements of fat mass and FFM, possibly due to time constraints and the number of additional assessments required and their validity and reliability. However, fat mass and FFM have different properties such as conductivity, permittivity, and density (all greater in FFM). CF patients have an increase in fat mass and muscle wasting in comparison to healthy controls. In healthy individuals, females have higher proportions of fat mass than males suggesting females with CF could be even more likely to have higher proportions of fat mass than healthy males, which may contribute to the increased mortality

of females with CF. Therefore, it could be invalid to measure and compare participants based on whole body mass when compositions could differ between two children of the same total body weight. Furthermore, Singer and colleagues (2014), reported fat mass to be an inflammatory marker in children. Inflammation contributes to cardiovascular disease and insulin resistance (Dedoussis *et al.,* 2010; Berg *et al.,* 2005) suggesting healthy females may be at a higher risk of developing other diseases than males, due to their increased proportions of fat mass. However, Singer *et al.,* (2014) used a cross sectional design to establish causality; a longitudinal approach may be more appropriate to determine the causality between fat mass and inflammatory markers. Furthermore, this study did not include people with CF, who are already susceptible to diseases such as diabetes (Zirbes and Milla, 2009). It would therefore be prominent to identify whether females with CF are at a higher risk of developing diseases, which may contribute to their higher mortality.

Many studies have suggested $FEV_{1\%predicted}$ (Corey *et al.,* 1997; Kerem *et al.,* 1992) to be the best predictor of mortality in children, adolescents and adults with CF (Kerem *et al.,* 1992; Nixon *et al.,* 1992). For a given value of FEV_1, females have a higher mortality, whilst risk of death at an FEV_1 between 20-30 % is 18 % higher in females (Kerem *et al.,* 1992). This is supported by Corey *et al.,* (1997) who reported that females have a steeper slope of decline in $FEV_{1\%predicted}$ than males; a difference of ~20 % FEV_1 predicted at the age of 30. However, this sex difference was only evident after 5 years of age meaning other factors could be influential. Habitual PA has been shown to help slow the rate of decline of lung function and can increase FEV_1 in females, indicating there may be a sex specific response to PA and/or exercise. However, reports in the CF literature show levels of PA to decline during childhood and adolescence, with females becoming less

physically active than males during the pubertal years (Selvadurai *et al.,* 2004). This reduction in PA in females with CF during adolescence may have a deconditioning effect on the body, as PA has been suggested to play a role in maintaining exercise capacity in CF (Savi *et al.,* 2015), resulting in a lower exercise capacity than their male counterparts. This could partly explain why increasing habitual PA in females has a greater influence on lung function than in males.

Although previous studies have reported that pulmonary function may partially account for the increased risk of mortality in females with CF, when removed as a factor, females still have a higher mortality rate (~60 %; Rosenfeld *et al.,* 1997). Independent of lung function, a reduced exercise capacity has been shown to be a predictor of mortality in children, adolescents and young people with CF (Hulzebos *et al.,* 2014; Nixon *et al.,* 1992). It is well established that females with CF are at a greater risk of mortality than their male counterparts at any age (Corey *et al.,* 1997), however sex differences are yet to be explored in relation to exercise capacity despite being an additional predictor of mortality (Hulzebos *et al.,* 2014; Pianosi *et al.,* 2005; Moorcroft *et al.,* 1997; Nixon *et al.,* 1992). In support, Werkman *et al.,* (2014) identified that work peak and sex were significant predictors of $\dot{V}O_{2peak}$, therefore by extrapolation highlighting that there are sex differences in exercise capacity in CF.

Hulzebos *et al.,* (2014) reported the peak ventilatory equivalent ratio of oxygen ($\dot{V}_E/\dot{V}O_2$), an indication of ventilation required for a given $\dot{V}O_2$, to be a significant predictor of mortality, with higher values for those with poor survival. This study described a normal ventilatory drive in adolescent CF, but a higher carbon dioxide (CO_2) production during exercise as result of greater carbohydrate (CHO) oxidation compared to healthy controls. However, they did not identify whether

this value was significantly different between sexes. It is possible that females with CF have significantly different $\dot{V}E/\dot{V}O_2$ to males, due to differences in CHO oxidation, which contributes to higher mortality. Although, in healthy children it has been reported that there are no significant sex differences in $\dot{V}_E/\dot{V}O_2$ (Armstrong *et al.,* 1997).

Whilst there is some literature that reports sex differences in relation to exercise capacity in CF children, few focus on this parameter as a key objective. Given that females with CF have higher mortality rates and the evidence in healthy children regarding a lower exercise capacity in females, it would be prominent to assess this area of research in CF. This line of enquiry may help to produce effective exercise management for females with CF to improve mortality to equal that of males with CF.

2.6 Research questions

Q1: What effect does disease status, as defined by FEV_1, have on sex differences in cardiopulmonary exercise test outcomes?

Q2: What effect does age have on sex differences in cardiopulmonary exercise test outcomes?

Q3: What effect does ratio standard and allometric scaling for different body size variables (body mass, stature and body surface area) have on sex differences in $\dot{V}O_{2peak}$?

Q4: Are sex differences in $\dot{V}O_{2peak}$ (absolute and scaled) apparent after adjusting for lung function and nutritional and maturity status?

3. General Methods

3.1 Ethical approval

This thesis is a retrospective analysis and has been awarded Health Research Authority (HRA) approval (Appendix A). Ethics approval for the access to the retrospective data was obtained from the University of Exeter, Sport and Health Sciences Ethics Committee (Appendix B). All exercise and measures were taken as part of standard care of CF patients at the Royal Devon and Exeter NHS Foundation Trust Hospital (RD&E). Due to the retrospective nature of this thesis, no patients were approached to be a part of the present study but HRA and ethics approval have been sought to ensure it is in line with patient confidentiality and data protection laws. All adult patients (> 18 y) provided prior ethical consent, whilst paediatric patients (≤ 17 y) provided assent and parents or carers provided informed consent.

3.2 Protocol

Following HRA and ethics approval, files were extracted from the hospital server onto an encrypted memory stick. Following transport to the University site (University of Exeter, St. Luke's campus) analysis and interpretation was carried out.

3.3 Study participants

Fifty-two young people (29 males and 23 females, 16.62 ± 4.62 y and 17.52 ± 5.27 y; stature, 166 ± 15 cm and 157 ± 9 cm; body mass, 61.31 ± 18.05 kg and 53.99 ± 12.50 kg, respectively) with CF that have undergone a CPET at the RD&E as part of their annual review were included (Fig. 3.1). Patients were identified by staff at the hospital and anonymised prior to use for the present study. Patients were considered to have CF if tested positive for a sweat test (sweat Cl^- > 60

mmol·L^{-1} > 100 mg sweat) and in 47 of 52 participants, genotyping was used. A total of 46.2 % of the study population were homozygous for F508del mutation compared to 50.2 % of the UK population recorded in the CF Trust registry (Cystic Fibrosis Trust, 2017c). Furthermore, only 19.2 % of the study population presented CFRDM compared to the 32 % and < 25 % reported in adults and children respectively (Cystic Fibrosis Trust, 2017c). CFRDM was diagnosed by an oral glucose tolerance test.

Measuring fecal elastase-1 can identify exocrine status in CF, normal function is described as 200 to > 500 µg E1·g^{-1} stool, whereas mild to moderate insufficiency is 100 – 200 µg E1·g^{-1} stool (National Health Service, 2018). Approximately 79 % of the study population were identified as pancreatic insufficient. Pseudomonas Aeruginosa is considered chronic if the patient has had 3 or more infections in 12 months (National Institute for Health and Care Excellence, 2018). In this study population there were on average 1 ± 2 infections per year.

3.3.1 Inclusion and exclusion criteria. Only those who were considered clinically stable by staff at the hospital were asked to perform a CPET. Patients were free from; musculoskeletal injury, pulmonary exacerbations and intravenous (IV) antibiotics, or at the end of the IV course. Furthermore, patients were willing, able and had the mental capacity to undergo testing. Patients were only included if between 8 – 25 years old.

Some patients were unable to perform the test due to other complications such as bowel cancer, hemoptysis and in some cases obesity where they could not safely perform on a bike and therefore were not included. Participants who were infected with non-tuberculosis mycobacteria could not use the gas analyser due to hygiene and risk of cross infection. Therefore, these patients underwent the

protocol without gas analysis, $\dot{V}O_2$ was then estimated from peak work rate using the equation from Werkman *et al.,* (2014). These patients were not included in the present thesis as measures of gas exchange could not be reported.

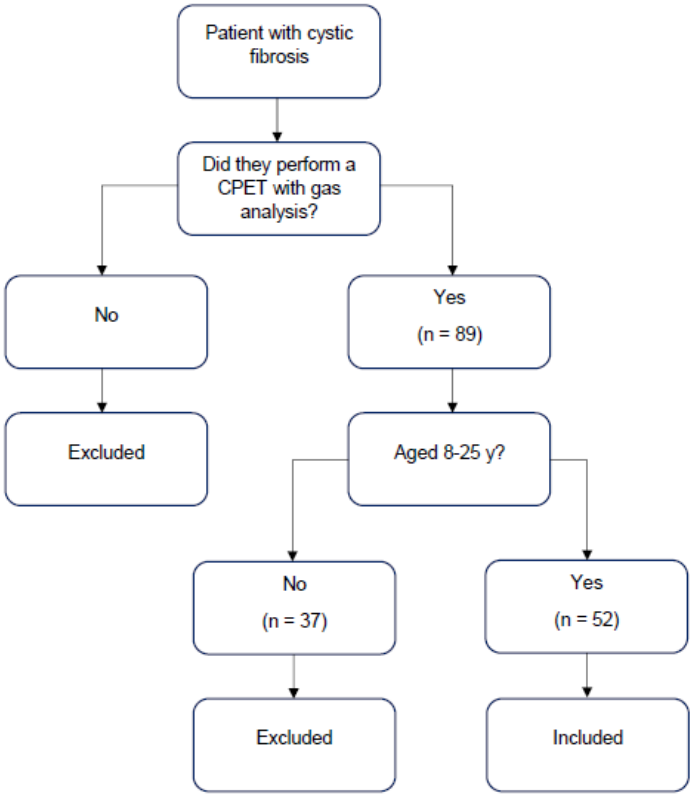


Figure 3.1. Patient inclusion flow diagram.

3.4 Age

Age was calculated as a decimal to the nearest 0.01 year. This indicates the age at completion of the CPET, the difference between date of birth and date of the exercise test. To identify the effect of age in binary terms on sex differences in CPET outcomes and run a two-way ANOVA, age was split into tertiles. Groups were created with 17 (9 male), 18 (13 male) and 17 (7 male) participants, average age of 11.52 y (8.82 - 13.84 y), 16.77 y (13.85 – 18.54 y) and 22.79 y (18.55 – 25.10) respectively.

3.5 Maturity

Pubertal stages based on secondary sex characteristics (based on Tanner criteria), the most common way of reporting sexual maturity, were not reported in the retrospective data, therefore years from peak height velocity (PHV) was used as a marker of (somatic) maturity. PHV can be used as a marker of somatic maturity by identifying the highest growth velocity in stature during adolescence. Often, predictive equations for PHV require sitting height, however as the hospital did not record this, the following equations were used based on stature and age for the children and adolescents (Moore *et al.,* 2015):

Boys Peak Height Velocity (y) = -7.999994 + [0.0036124 x age (y) x stature (cm)]

Girls Peak Height Velocity (y) = -7.709133 + [0.0042232 x age (y) x stature (cm)]

These equations were reported to be valid in healthy boys and girls (R^2 = 0.906, SEE = 0.514 and R^2 = 0.898, SEE = 0.528 respectively; Moore *et al.,* 2015). However, it has yet to be determined if these equations are valid for use in CF populations. Maturity was then identified as pre (< -1 y), post (> +1 y) and circa (-0.99 to +0.99) PHV due to concerns that the error is too great as a continuous score (Mirwald *et al.,* 2002). There is increasing evidence that skeletal age of the hand-wrist is preferred to account for maturity (Malina*,* 2011), however due to the retrospective nature of the present analysis this parameter was not available. Participants aged 18 y and above were considered an adult, and therefore were labelled a post PHV for analysis.

3.6 Anthropometry

All measures were taken within the RD&E by clinic nurses or physiotherapists prior to the exercise test. Typically, sportswear was worn, and footwear was removed in all cases.

3.6.1 Body mass. Body mass was measured using electronic scales (Seca 220; Vogel & Halke, Hamburg, Germany) to the nearest 0.01 kg.

3.6.2 Stature. Stature was measured to the nearest 1 cm. Participants stood upright on a stadiometer (Seca 220; Vogel & Halke, Hamburg, Germany) with their heels against the back and feet together. Gentle pressure was applied to the mastoid process and body of mandible, whilst patients were instructed to stand straight and look forward.

3.6.3 Body mass index. BMI was calculated from the stature and weight measures using the following equation:

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

BMI was reported both as a continuous score and into risk categories. Participants aged 18 y and above were categorised as: < 18.49 $kg \cdot m^{-2}$ = underweight; 18.50 – 24.90 $kg \cdot m^{-2}$ = healthy; 25.00 – 29.90 $kg \cdot m^{-2}$ = overweight, > 30 $kg \cdot m^{-2}$ = obese. The Cystic Fibrosis Foundation (2019) recommends men and women should maintain a BMI of at least 22 and 23 $kg \cdot m^{-2}$ respectively. Those aged below 18 y were classified using cut offs for thinness (Cole *et al.,* 2007) and fatness based on centile curves (Cole *et al.,* 2000). Categories were used to normalise the influence of a mixed adult and paediatric population.

3.6.4 Body surface area. BSA was calculated using the following equation from Haycock *et al.,* (1978):

$$BSA\ (m^2) = body\ mass^{0.5378}\ (kg) \times stature^{0.3964}\ (cm) \times 0.024265$$

This equation is known to reduce the underestimation of child and infant surface area as seen in formulas from Du Bois and Du Bois (1916). The equation was developed on multiple ethnic groups (including Caucasian, the likely majority in this study cohort) and on both healthy and clinical populations.

3.7 Pulmonary function

FVC and FEV_1 were collected via a spirometer (Micromedical Microloop 3535, Numed, Sheffield, UK) and performed to NHS guidelines. Patients were seated with a soft nose clip to prevent air escaping through the nose. Patients may have been asked to have a couple of practice attempts prior to the test. During the test patients were asked to inhale fully and close lips tightly around the mouthpiece before exhaling as quickly and forcefully as possible. Spirometry tests were repeated on average three times per patient, with a minimum of 30 s between tests, to ensure a reliable result.

$FVC_{\%predicted}$ and $FEV_{1\%predicted}$ were calculated by using normative values stated by Quanjer *et al.,* (2012) based on sex, ethnicity and body size. In the case that only absolute values were obtained, % predicted was calculated using a free to download desktop calculator from the Global Lung Initiative website as suggested by Quanjer *et al.,* (2012). Further to this, $FEV_{1\%predicted}$ was categorised into mild (> 70.0 %), moderate (40.0 – 69.9 %) and severe (< 39.9 %) lung function (Cystic Fibrosis Foundation, 2009) for further descriptive data relevant to the clinical setting. For the purpose of running a two-way ANOVA to identify the effect of disease status on sex differences in CPET outcomes, $FEV_{1\%predicted}$ was split into tertiles as FEV_1 categories contained a high proportion (79 %) of participants in a single category.

3.8 Cardiopulmonary exercise testing

3.8.1 Safety and cross infection. As these tests were conducted as part of routine clinical care, all procedures were completed in line with standard clinical practice. Patients did not interact or come to contact with one another at either clinic or physiotherapy departments. Post CPET, all equipment was cleaned with antibacterial wipes and the environment sterilised. At least 1 hour was left between each patient to control for cross infection.

Patients were monitored by a physiotherapist throughout testing for signs of significant symptoms (i.e. dyspnoea identified using the Borg scale). Nurses and medical staff were aware testing was taking place and supplemental O_2 was available if necessary.

3.8.2 Protocol. CPETs were performed on cycle ergometers (Lode Excalibur or Lode Corival, Groningen, The Netherlands) at the RD&E. All CPETs involved an incremental ramp to exhaustion and 47 of 52 participants completed a supramaximal verification stage as suggested by Saynor *et al.,* (2013a) to determine a valid $\dot{V}O_{2max}$ (Fig. 2.5). Patients performed a 3 min warm up at ~20 W prior to the incremental ramp stage. For the ramp, the rate of increase (10-25 $W \cdot min^{-1}$) was predetermined and individualized for each participant based on their PA and clinical status to ensure the duration of the ramp lasted between 8-12 min. Patients were encouraged to maintain a cadence of ~60-80 $rev \cdot min^{-1}$. When cadence dropped > 10 $rev \cdot min^{-1}$ for 5 consecutive seconds despite encouragement, volitional exhaustion was assumed and the ramp stage concluded. A period of active recovery (~5 min) at 20 W followed. At least 10 min passive recovery preceded the S_{max} stage. The S_{max} phase consisted of another 3 min warm up at ~20 W prior to a step transition to 110 % of WR_{peak} attained

during the incremental ramp test. When volitional exhaustion occurred, typically after ~3 min, an optional 5 min active recovery (~20 W) was completed before the termination of the CPET (Fig. 2.5).

3.9 Gas exchange parameters

Metabolic gas analysers (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany) were used to measure parameters of gas exchange ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$) and calibrated to known concentrations of gases. A 3 L calibration syringe (Hans Rudolph, Kansas City, MO, USA) was used to calibrate the turbine volume transducer. At rest and exercise, breath by breath pulmonary gas exchange and ventilation was measured through a face mask, 1 s averages were recorded and subsequently averaged into 10 s for the use of analysis for all parameters. The accuracy of the gas analyser was 2 % (O_2) and 0.1 % (CO_2) when calibrated according to manufacturer's instructions.

3.10 Heart rate

HR was measured continually throughout the CPET via a Bluetooth heart rate monitor (Polar T31 Heart Rate Strap, Polar Electro, Finland) strapped across the chest; around the base of the sternum and between ribs 5/6.

3.11 Determination of parameters

3.11.1 Determination of $\dot{V}O_{2peak}$. Breath by breath data were converted into templates and opened in MSF21 [purpose built software (LabVIEW, National Instruments, Newbury, UK). $\dot{V}O_2$ was plotted against time as 10 s averages, the linear portion of the graph was isolated to plot a regression line (Fig. 3.2) to identify $\dot{V}O_{2peak}$. If the $\dot{V}O_2$ attained during the S_{max} was < 9 % greater than $\dot{V}O_{2peak}$

during the ramp stage, $\dot{V}O_2$ was verified as maximal, as suggested by Saynor and colleagues (2013a).

% change in $\dot{V}O_2$ from ramp to S_{max}

$$= [(S_{max}\ \dot{V}O_{2peak} - \text{Ramp}\ \dot{V}O_{2peak}) \div \text{Ramp}\ \dot{V}O_{2peak}] \times 100$$

As ~10 % of participants did not complete the S_{max} verification $\dot{V}O_{2peak}$ was identified as the largest 10 s average $\dot{V}O_2$ value from the ramp test[2].

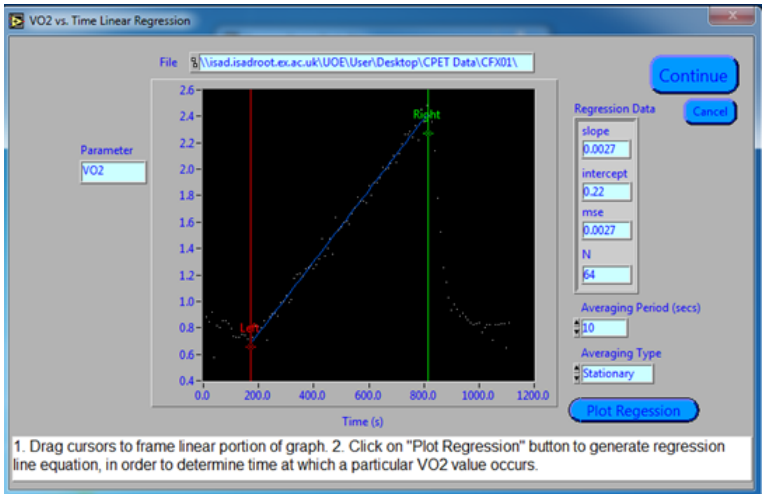


Figure 3.2. Example of plotting $\dot{V}O_2$ against time linear regression in MSF21 (LabVIEW, National Instruments, Newbury, UK) to obtain $\dot{V}O_{2peak}$. Example data from a patient with cystic fibrosis.

Time to exhaustion (TTE) was identified as time from the start of the ramp to exhaustion, the point at which the participant could no longer hold the cadence of ~60-80 rev·min^{-1}.

Scaling of data in the paediatric population is important to identify a relative size free value for parameters such as $\dot{V}O_{2peak}$. This helps to control for the influence

[2] As $\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$ are used interchangeably in the literature, for the purpose of this thesis all highest $\dot{V}O_2$ data will be referred to as $\dot{V}O_{2peak}$.

of growth in children and adolescents (Baxter-Jones *et al.,* 2005). Allometric scaling is considered to be the gold standard method whilst the RSM is still widely used. Prior to scaling, absolute $\dot{V}O_{2peak}$ for each sex was plotted against BM, BSA and stature, producing a scatter plot to determine if scaling was required (i.e. a significant correlation would demonstrate scaling was required). In all cases the plots informed that scaling was required to produce size free values for $\dot{V}O_{2peak}$ (Fig. 3.3). Body size variables (BM, BSA and stature) were then used to scale $\dot{V}O_{2peak}$ for each sex using the RSM (Y/X), however scatter plots and correlations produced showed the RSM did not appropriately remove the influence of body size for stature in both males and females (Fig. 3.3). Log-linear regression analyses were then used to scale $\dot{V}O_{2peak}$ via the allometric method, whereby sex and BM, sex and BSA, sex and stature, were entered as predictor variables in three separate regression models.

The analyses produced a scaling exponent (*b*) which was used in the power function ratio to produce size free values of $\dot{V}O_{2peak}$ (Fig. 3.3). Correlational analyses were run to confirm that measures were size free. The scaling exponents identified were:

$$\dot{V}O_{2peak}/BM^{*b}$$

b = 0.82, 95% CI [0.470-1.178]

$$\dot{V}O_{2peak}/BSA^{*b}$$

b = 1.29, 95% CI [0.726-1.843]

$$\dot{V}O_{2peak}/Stature^{*b}$$

b = 2.42, 95% CI [1.090-3.751]

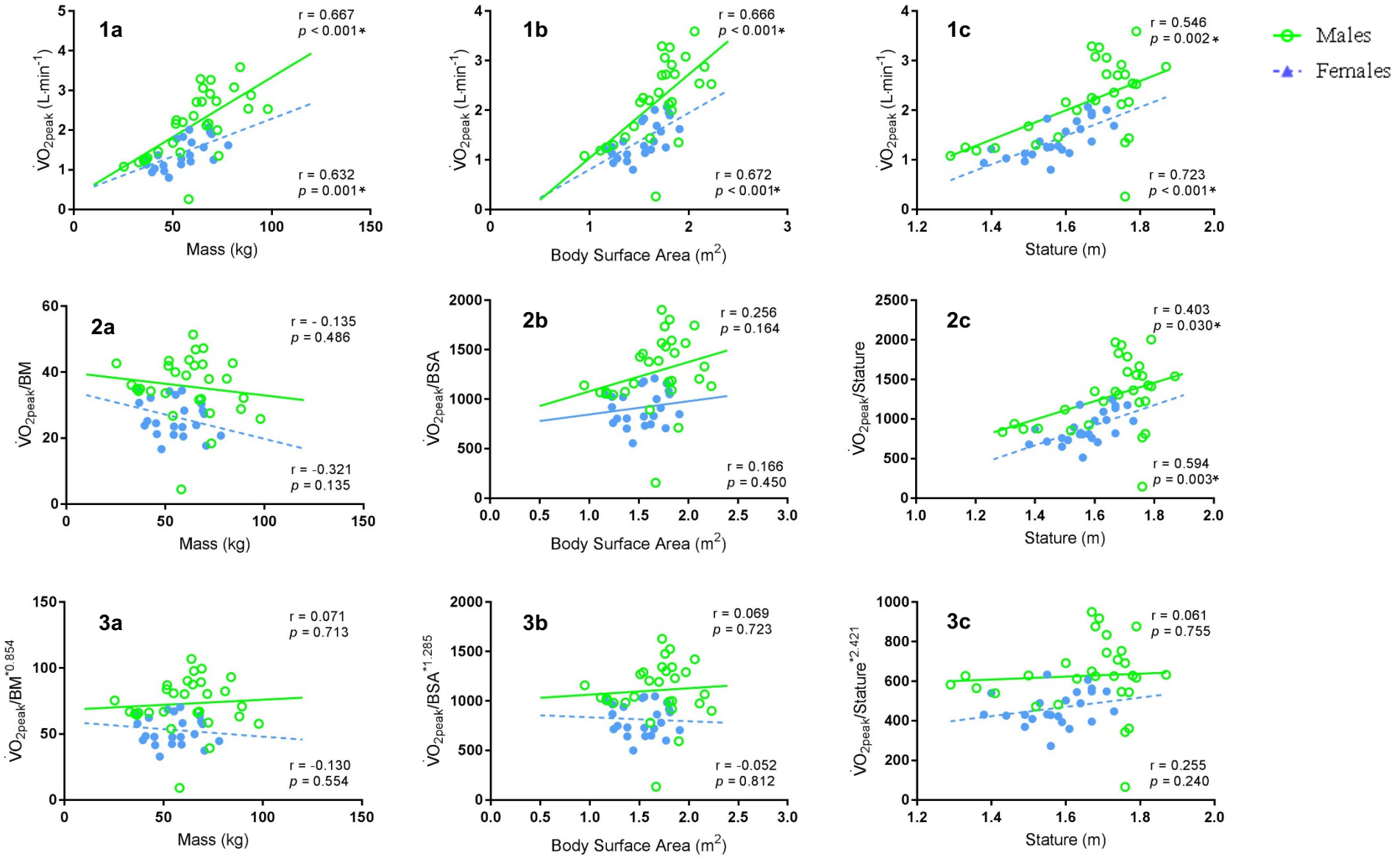

1a
r = 0.667
p < 0.001*
r = 0.632
p = 0.001*
V̇O2peak (L·min-1)
Mass (kg)
1b
r = 0.666
p < 0.001*
r = 0.672
p < 0.001*
V̇O2peak (L·min-1)
Body Surface Area (m2)
1c
r = 0.546
p = 0.002*
r = 0.723
p < 0.001*
V̇O2peak (L·min-1)
Stature (m)
Males
Females
2a
r = - 0.135
p = 0.486
r = -0.321
p = 0.135
V̇O2peak/BM
Mass (kg)
2b
r = 0.256
p = 0.164
r = 0.166
p = 0.450
V̇O2peak/BSA
Body Surface Area (m2)
2c
r = 0.403
p = 0.030*
r = 0.594
p = 0.003*
V̇O2peak/Stature
Stature (m)
3a
r = 0.071
p = 0.713
r = -0.130
p = 0.554
V̇O2peak/BM*0.854
Mass (kg)
3b
r = 0.069
p = 0.723
r = -0.052
p = 0.812
V̇O2peak/BSA*1.285
Body Surface Area (m2)
3c
r = 0.061
p = 0.755
r = 0.255
p = 0.240
V̇O2peak/Stature*2.421
Stature (m)

Figure 3.3. Scaling of body size parameters. Significant correlations denoted by *. Males r and *p* value denoted in top right corner of each graph; females denoted in the bottom right corner. 1; absolute $\dot{V}O_{2peak}$ plotted against BM, BSA and stature. 1; absolute $\dot{V}O_{2peak}$ plotted against a: mass, b: body surface area and c: stature. 2; ratio standard scaled $\dot{V}O_{2peak}$ plotted against a: BM, b: BSA and c: stature. 3; allometrically scaled $\dot{V}O_{2peak}$ plotted against a: BM, b: BSA and c: stature. $\dot{V}O_{2peak}$; peak oxygen uptake. BM; body mass. BSA; body surface area. β; beta coefficient.

3.11.2 Determination of other $\dot{V}O_2$ parameters. Functional $\dot{V}O_2$ gain was determined through regression of the linear profile of $\dot{V}O_2$ response versus power output during the ramp test.

$$\dot{V}O_2 \text{ gain} = \Delta\dot{V}O_2 \div \Delta WR$$

Oxygen pulse, a marker of maximal stroke volume and therefore cardiac fitness, was identified using the following equation.

$$\text{Oxygen pulse} = \max (\dot{V}O_2 \div HR)$$

3.11.3 Determination of the gas exchange threshold and respiratory compensation point. The RCP was visually identified using MSF21 as the first inflection point when plotting $\dot{V}_E$ versus $\dot{V}CO_2$, when no inflection point could be visually identified, the RCP was not obtained. Once identified, the RCP was reported as a % of $\dot{V}O_{2peak}$. All data following the respiratory compensation point (RCP) were removed and a graph of $\dot{V}CO_2$ against $\dot{V}O_2$ was produced (Fig. 3.4 A). The GET was identified using the V-slope method (Beaver *et al.,* 1986); the intersection of two regression lines from a plot of $\dot{V}CO_2$ against $\dot{V}O_2$. In the case

that the GET could not be determined in this way, it was visually determined using the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$ (Fig. 3.4 B). The GET was identified individually by two separate researchers in 1/3 of data sets (researchers were within 10 % of each other, therefore the remaining 2/3 of data was split between researchers for analysis). GET data were presented as an absolute value ($\dot{V}O_2$ at the GET), GET relative to body mass and as a percentage of $\dot{V}O_{2peak}$.

3.11.4 Determination of ventilatory drive. Using 10 s averages during the ramp protocol, the highest ventilatory equivalents at peak exercise were recorded to obtain $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$.To identify $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ slopes, 10 s data from the entire ramp test was input into a computer programme (GraphPad Prism Version 7; GraphPad Software, 171 San Diego, CA) which generates the regression slope automatically by plotting $\dot{V}_E$ vs $\dot{V}O_2$ and $\dot{V}_E$ vs $\dot{V}CO_2$.

3.11.5 Determination of $\dot{V}O_2$ reference values. $\dot{V}O_{2peak}$ values obtained by the participants were compared to equations for calculating reference $\dot{V}O_{2peak}$ values in healthy children (Bongers *et al.,* 2014b). Comparisons between reference values and the participants in the present analysis were made through identifying percentage differences (see appendix C for individual reference values). Participants aged 18 y and above were considered an adult and therefore were not included in the analysis of reference values.

$$\text{Boys } \dot{V}O_{2peak} \text{ (L·min}^{-1}\text{)} = (0.0033 \text{ x } (\text{age}^2)) + (0.1316 \text{ x age}) + 0.084$$

$$\text{Girls } \dot{V}O_{2peak} \text{ (L·min}^{-1}\text{)} = (-0.0022 \text{ x } (\text{age}^2)) + (0.2184 \text{ x age}) - 0.4727$$

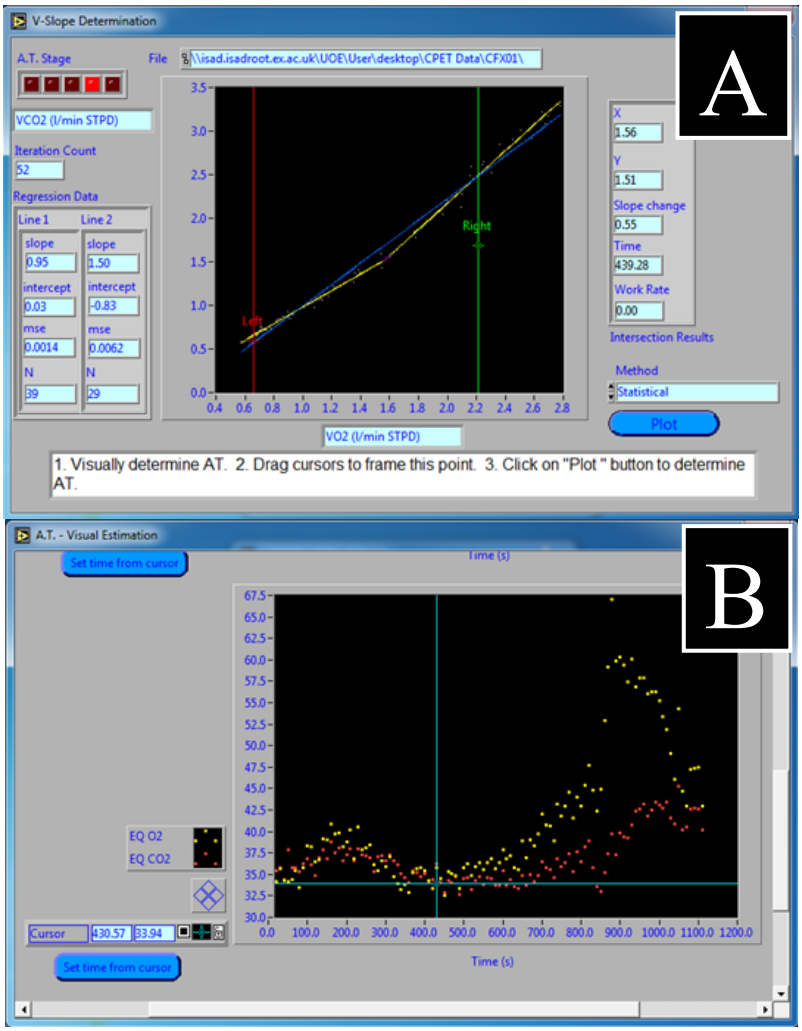


Figure 3.4. Example data of an individual with cystic fibrosis to determine the gas exchange threshold in MSF21 (LabVIEW, National Instruments, Newbury, UK). Respiratory compensation point, the point at which there is a steeper rate of minute ventilation [$\dot{V}_E$] vs. rate of carbon dioxide production [$\dot{V}CO_2$] (Beaver *et al.,* 1986), was removed before further analysis. **A:** Shows the inflection point identified by the software on the graph plotting $\dot{V}CO_2$ against oxygen uptake ($\dot{V}O_2$). The intersection of the yellow lines marks the disproportionate increase in $\dot{V}CO_2$ compared to $\dot{V}O_2$, and therefore the gas exchange threshold. **B:** Shows the visual determination of the ventilatory threshold using ventilatory equivalents of $\dot{V}O_2$ and $\dot{V}CO_2$ (identified on the graph by the intercept of the horizontal and vertical lines).

3.12 Statistical analyses

Data are presented as mean and standard deviation unless otherwise stated with the alpha level set to $p = 0.05$ significance level. Descriptive statistics with mean sex differences in continuous outcome variables determined by independent t-tests. For sex differences in clinical and CPET outcomes, estimates of effect size were calculated using Cohen's D (0.2 = small effect; 0.5 = medium effect; 0.8 = large effect; Cohen 1998). Sex differences in categorical data were identified by the Mann-Whitney test after converting into ordinal data as the assumptions of Chi Square analysis were not met. Relationships were determined between clinical and exercise test parameters using Pearson's correlation coefficient for the whole group and each sex separately. Correlations were considered to be; negligible r = (±) 0.00 to 0.30; weak r = (±) 0.30 to 0.50; moderate r = (±) 0.50 to 0.70; strong r = (±) 0.70 to 0.90; or very strong r = (±) 0.90 to 1.00 as defined by Mukaka (2012). Bi lateral correlational analyses of $\dot{V}_{Epeak}$ and age was identified by splitting the whole group into two equal groups (26 participants in each group) of age (< 17 y and ≥ 17 y).

Separate two-way ANOVA's were run on 52 participants to examine the effect of; sex (male/ female) and lung function (FEV_1 tertiles) [research question 1]; and sex (male/ female) and age (tertiles) [research question 2] on different CPET parameters (dependent variable). For the purpose of analysis, age and lung function ($FEV_{1\%predicted}$) were categorised by tertiles to relate more closely to clinical classification of CF disease severity (i.e. mild, moderate and severe). Tertiles were split through: $FEV_{1\%predicted}$, < 77.48 = 1, 77.49 – 91.49 = 2, > 91.50 = 3 (Fig. 4.6); Age (y), < 13.85 = 1, 13.86 – 18.55 = 2, > 18.56 = 3. Data were not skewed (identified from histograms and P-P plots) and Levene's statistic was not

significant when based on the median, therefore the assumptions of normality and homogeneity of variances were met for the following analyses.

Body size variables (BM, BSA and stature) were used to scale $\dot{V}O_{2peak}$ for each sex using the RSM (Y/X). Log-linear regression analyses were used to scale $\dot{V}O_{2peak}$ via the allometric method; sex and BM, sex and BSA, sex and stature, were entered as predictor variables in three separate analyses to produce a β coefficient. For the purpose of research question 3, independent t-tests were run to identify sex differences scaled $\dot{V}O_2$ values.

To identify sex differences in absolute and allometrically scaled $\dot{V}O_2$ when controlling for age (y), PHV (pre, circa and post pubertal), BMI (underweight, healthy weight, overweight, obese) and FEV_1 (% predicted) (research question 4), ANCOVAs were utilised. Exploratory analyses were undertaken with the removal of PHV as a co variate. Assumptions of the ANCOVA; (1) independence of the co variate and treatment effect, (2) homogeneity of regression slopes, (3) normal distribution and (4) homogeneity of variance were met for all conditions. Partial eta squared effect sizes were defined as small = 0.1, medium = 0.3 or large = 0.5 (Cohen, 1988).

All analyses were performed using SPSS v 25 (Chicago, Illinois, USA) and graphs created in GraphPad Prism 7 (GraphPad Software Inc., San Diego, California, USA).

4.1 Descriptive statistics

4.1.1 Clinical Parameters. Of the 52 CF patients included, 46.2 % and 38.5 % were homozygous and heterozygous for F508del respectively. The median age of the whole group was 17.02 ± 4.89 y, 29 (55.8 %) of which were male. Stature and body mass were 162 ± 13 cm and 58.07 ± 16.12 kg, respectively. Stature was significantly higher in males than females. Males had a significantly higher FVC (L) and FEV_1 (L) than females. Non-significant differences between males and females were noted in: Age (y), time between CPET and annual review (y), BM (kg), BSA (m^2), BMI ($kg \cdot m^{-2}$ and categories), $FVC_{\%predicted}$ and $FEV_{1\%predicted}$ (Table 4.2). The paediatric population consisted of 12 girls and 19 boys, of which 42 % were post pubertal. Whole group categorical data are exhibited in Table 4.1. Full descriptive data of clinical outcomes, with significant sex differences identified from independent t-tests, are presented in Table 4.2. FVC or $FVC_{\%predicted}$ was not recorded in two females and one male.

Table 4.1. Group frequencies of categorical clinical parameters.

Variable		Males	Females	Total (% of population)	*p* value
Genotype	Homozygous	15	9	46.2	0.367
	Heterozygous	10	10	38.5	
	Neither	3	0	5.8	
Pancreatic	Sufficient	3	2	9.6	0.869
	Insufficient	23	18	78.8	
FEV_1 (% predicted)	Severe	2	0	3.8	0.208
	Moderate	2	7	17.3	
	Mild	25	15	78.8	
Diabetes	None	23	12	67.3	**0.032***
	Impaired Glucose Tolerance	1	2	5.8	
	CFRDM	3	7	19.2	
BMI	Underweight	1	1	3.8	0.431
	Normal	19	17	69.2	
	Overweight	8	5	25	
	Obese	1	0	1.9	
Maturity	Pre-Pubertal	6	2	15.4	0.776
	Pubertal	4	6	19.2	
	Post Pubertal	19	15	65.4	
Chronic PA	None	21	16	71.2	0.810
	1-5	5	3	15.3	
	>5	1	2	5.7	

Significant sexes differences obtained using the Mann-Whitney test; significant p values highlighted in bold and *. Data presented as a frequency of sub-groups and a percentage of the whole study population. FEV_1; Forced expiratory volume in 1 s. CFRDM; Cystic fibrosis related diabetes mellitus. BMI; Body mass index. PHV; Peak height velocity. Chronic PA; Chronic pseudomonas aeruginosa infection.

Table 4.2. Sex differences in anthropometric and pulmonary function parameters.

Variable	Whole Group	Males	Females	*p* Value	Effect Size
Age (y)	17.02 ± 4.89	16.62 ± 4.62	17.52 ± 5.27	0.514	0.182
Time Between CPET and AR (y)	0.25 ± 0.32	0.23 ± 0.29	0.29 ± 0.35	0.517	0.187
Stature (cm)	162 ± 13	166 ± 15	157 ± 9	**0.027***	0.728
BM (kg)	58.07 ± 16.12	61.31 ± 18.05	53.99 ± 12.50	0.104	0.446
BSA (m^2)	1.61 ± 0.29	1.67 ± 0.32	1.53 ± 0.22	0.086	0.510
BMI ($kg \cdot m^{-2}$)	21.70 ± 3.76	21.81 ± 3.92	21.56 ± 3.62	0.811	0.066
FVC (L)	3.46 ± 1.26	3.89 ± 1.35	2.89 ± 0.88	**0.005***	0.878
FVC ($L \cdot m^{-1}$)	2.10 ± 0.64	2.31 ± 0.13	1.83 ± 0.47	**0.007***	1.392
FVC (% Predicted)	90.48 ± 17.04	91.28 ± 18.38	89.41 ± 15.46	0.709	0.110
FEV_1 (L)	2.78 ± 1.08	3.14 ± 1.17	2.33 ± 0.77	**0.006***	0.818
FEV_1 ($L \cdot m^{-1}$)	1.69 ± 0.56	1.87 ± 0.60	1.46 ± 0.42	**0.009***	0.792
FEV_1 (% Predicted)	83.66 ± 19.76	86.01 ± 21.01	80.70 ± 18.09	0.341	0.271

Data are presented as mean ± SD. Significant differences between sexes are highlighted in bold and *. p value determined from independent samples T-Tests, alpha level = 0.05. Effect size = Cohen's d. CPET; Cardiopulmonary exercise test. AR; Annual review. BM; Body mass. BSA; Body surface area. BMI; Body mass index; 0 = underweight, 1 = healthy weight, 2 = overweight, 3 = obese. FVC; Forced vital capacity. FEV1; Forced expiratory volume in 1 s.

4.1.2 Cardiopulmonary exercise test parameters. GET was identified in 47 of 52 participants, unidentified in 2 males and 3 females. RCP could not be identified in 9 males and 10 females. HR was not picked up or recorded in 8 males and 11 females. RER could be identified in all but 1 male participant. Mean TTE took 505 ± 142 s (sex differences reported in Table 4.3).

Of the 47 participants that completed the S_{max}, 34 % of $\dot{V}O_{2peak}$ were not verified by the S_{max} bout (62 % of males and 72 % females obtained true $\dot{V}O_{2max}$). No significant differences in S_{max} TTE were observed between males and females (p = 0.189).

Absolute $\dot{V}O_{2peak}$ was significantly (p < 0.001) lower in females than males (1.41 ± 0.38 $L \cdot min^{-1}$ and 2.17 ± 0.82 $L \cdot min^{-1}$ respectively) (Fig. 4.1). Compared to reference $\dot{V}O_{2peak}$ values in healthy children, mean difference in this population was + 17.8 % and + 34.6 % for males and females $\dot{V}O_{2peak}$, respectively (p = 0.010).

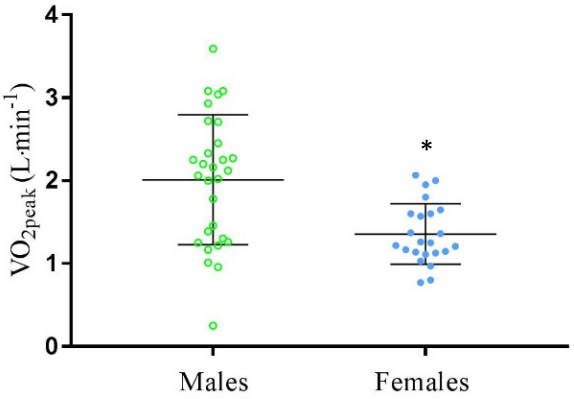


Figure. 4.1. $\dot{V}O_{2peak}$ distribution in males and females. Lines represent mean and standard deviation. Significant difference highlighted with an *.

Males had a significantly higher mean than females in the following parameters: peak power; peak power ramp relative to body mass; TTE ramp; absolute $\dot{V}O_{2peak}$; $\dot{V}CO_{2peak}$; GET (absolute $\dot{V}O_2$ at GET); $\dot{V}O_2$ at GET relative to BM; $\dot{V}_{Epeak}$; tidal volume and oxygen pulse (Table 4.3). At peak $\dot{V}O_2$, females elicited significantly lower values than males for $\dot{V}CO_2$ (1.72 ± 0.58, 2.68 ± 1.14 L·min^{-1}, p = 0.001, respectively) and $\dot{V}_E$ (61.34 ± 22.54, 100.35 ± 41.83 L·min^{-1}, p < 0.001, respectively).

Ramp data revealed non-significant differences between males and females in: $\dot{V}O_2$ gain; GET % $\dot{V}O_{2peak}$; RCP % $\dot{V}O_{2peak}$; $\dot{V}_E/\dot{V}O_2$; $\dot{V}_E/\dot{V}CO_2$; $\dot{V}_E/\dot{V}O_2$ Slope; $\dot{V}_E/\dot{V}CO_2$ Slope; breathing frequency; RER_{peak}; and HR_{peak} (Table 4.3).

Table 4.3. Cardiopulmonary exercise test parameters with sex differences.

Variable	Whole Group	Males	Females	*p* Value	Effect Size
Sub-maximal					
GET ($L \cdot min^{-1}$)	1.07 ± 0.44	1.25 ± 0.50	0.83 ± 0.16	**0.001***	-1.131
GET ($mL \cdot kg^{-1} \cdot min^{-1}$)	18 ± 5	21 ± 5	16 ± 3	**< 0.001***	-1.213
GET (% VO_{2peak})	60.03 ± 9.32	59.28 ± 9.85	61.04 ± 8.68	0.528	0.190
RCP (% VO_{2peak})	94.02 ± 4.14	94.45 ± 3.66	93.36 ± 4.86	0.466	-0.253
$\dot{V}_E/\dot{V}O_2$ Slope	48.36 ± 11.93	46.59 ± 11.24	50.51 ± 12.64	0.247	0.328
$\dot{V}_E/\dot{V}CO_2$ Slope	36.59 ± 5.21	35.52 ± 5.32	37.89 ± 4.89	0.107	0.464
Maximal					
Peak Power (W)	164 ± 74	194 ± 78	126 ± 47	**0.001***	1.056
Peak Power ($W \cdot kg^{-1}$)	2.79 ± 0.84	3.16 ± 0.78	2.34 ± 0.69	**< 0.001***	1.114
TTE (s)	505 ± 142	563 ± 139	432 ± 111	**0.001***	1.041
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	1.83 ± 0.75	2.17 ± 0.82	1.41 ± 0.38	**< 0.001***	-1.189
$\dot{V}O_2$ Gain ($mL \cdot min^{-1} \cdot W^{-1}$)	6.99 ± 1.76	7.23 ± 1.78	6.75 ± 1.73	0.328	-0.100
$\dot{V}CO_{2peak}$ ($L \cdot min^{-1}$)	2.32 ± 1.03	2.75 ± 1.10	1.77 ± 0.60	**< 0.001***	-1.106
$\dot{V}_E$ ($L \cdot min^{-1}$)	89.52 ± 37.22	104.71 ± 40.49	70.37 ± 20.94	**0.001***	-1.065
$\dot{V}_E/\dot{V}O_2$ ($L \cdot min^{-1}$)	68.32 ± 17.83	69.39 ± 19.72	67.02 ± 15.56	0.642	-0.133

$\dot{V}_E/\dot{V}CO_2$ (L·min^{-1})	47.28 ± 5.72	46.40 ± 6.07	48.35 ± 5.20	0.230	0.345
BF (breaths·min^{-1})	60.78 ± 8.90	62.64 ± 9.80	58.43 ± 7.14	0.090	-0.491
Tidal Volume (L)	1.79 ± 0.69	2.03 ± 0.73	1.48 ± 0.50	**0.003***	-0.879
RER_{peak}	1.60 ± 0.33	1.66 ± 0.34	1.54 ± 0.31	0.189	-0.369
HR_{peak} (b·min^{-1})	177 ± 17	177 ± 19	176 ± 14	0.942	-0.060
Oxygen Pulse (mL·min^{-1})	11 ± 4	12 ± 5	8 ± 2	**0.009***	-1.050

Data are expressed as mean ± SD. Significant differences between sexes are highlighted in red and *. Alpha level = 0.05, p value obtained by independent samples T-tests. Effect size = Cohen's d. $\dot{V}O_{2peak}$; Peak oxygen uptake. $\dot{V}O_2$ gain; Oxygen cost of exercise. $\dot{V}CO_{2peak}$; Peak carbon dioxide output. GET; Gas exchange threshold. RCP; Respiratory compensation point. $\dot{V}_E$; Minute ventilation. $\dot{V}_E/\dot{V}O_2$; Ventilatory equivalent for oxygen uptake. $\dot{V}_E/\dot{V}CO_2$; Ventilatory equivalent for carbon dioxide. $\dot{V}_E/\dot{V}O_2$ slope and $\dot{V}_E/\dot{V}CO_2$ slope; Ventilatory drive. TTE; Time to exhaustion. BF; Breathing frequency; RER_{peak}; Peak respiratory exchange ratio. HR_{peak}; Peak heart rate.

4.1.3 Correlations between CPET and clinical parameters. For the whole group, a strong and positive correlation was observed between FEV_1 (L) and GET ($L \cdot min^{-1}$; r = 0.71, p < 0.001), and a moderate correlation was identified between FEV_1 (L) and peak power relative to body mass (r = 0.63, p < 0.001).

Simple linear correlation between age and $\dot{V}_{Epeak}$ revealed a weak but positive correlation r = 0.32, *p* = 0.023, which remained significant when analyses were run separately for males (r = 0.435, *p* = 0.018) and females (r = 0.430, *p* = 0.040). Visual analysis of data points revealed $\dot{V}_{Epeak}$ declines after ~ 18 y of age (Fig. 4.2). Bi lateral correlational analyses showed a significant positive correlation between $\dot{V}_{Epeak}$ and participants aged 8-16 y (r = 0.71, *p* < 0.001); and a significant negative correlation between $\dot{V}_{Epeak}$ and participants aged 17-25 y (r = -0.515, *p* = 0.007).

Pearson's correlation analyses produced coefficients between clinical and $\dot{V}O_{2peak}$ (absolute and relative) parameters for each sex (Table 4.4). $\dot{V}O_{2peak}$ was significantly correlated to BMI in males only. BM, BSA and stature all significantly correlated to $\dot{V}O_{2peak}$ in both males and females. Age did not significantly correlate with any $\dot{V}O_{2peak}$ parameters.

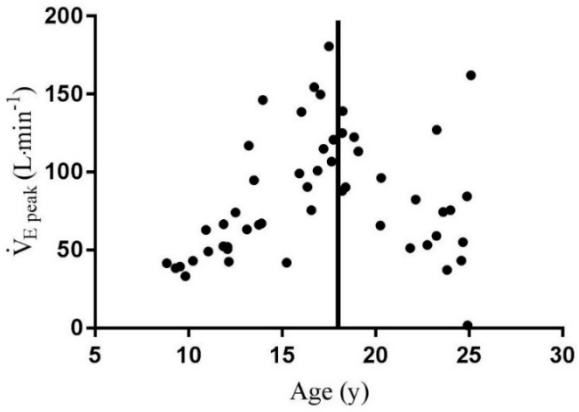


Figure 4.2. Correlation between minute ventilation and age. Solid linear line represents the visual point at which the positive correlation becomes negative.

Table 4.4. Correlations between clinical and $\dot{V}O_{2peak}$ parameters in males and females.

$\dot{V}O_{2peak}$ parameter	FEV_1 % predicted		FVC % predicted	
	Males	Females	Males	Females
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	r = 0.504, ***p* = 0.005***	r = 0.576, ***p* = 0.004***	r = 0.422, ***p* = 0.025***	r = 0.530, ***p* = 0.013***
$\dot{V}O_{2peak}$/ BM ($L \cdot min^{-1} \cdot kg^{-1}$)	r = 0.604, ***p* = 0.001***	r = 0.514, ***p* = 0.012***	r = 0.402, ***p* = 0.034***	r = 0.367, *p* = 0.102
$\dot{V}O_{2peak}$/ BSA ($L \cdot min^{-1} \cdot m^2$)	r = 0.622, ***p* < 0.001***	r = 0.623, ***p* = 0.002***	r = 0.466, ***p* = 0.012***	r = 0.507, ***p* = 0.019***
$\dot{V}O_{2peak}$/ Stature ($L \cdot min^{-1} \cdot m^{-1}$)	r = 0.562, ***p* = 0.002***	r = 0.597, ***p* = 0.003***	r = 0.461, ***p* = 0.013***	r = 0.508, ***p* = 0.019***
$\dot{V}O_{2peak}$/ $BM^{*\beta}$	r = 0.622, ***p* < 0.001***	r = 0.576, ***p* = 0.004***	r = 0.439, ***p* = 0.020***	r = 0.448, ***p* = 0.042***
$\dot{V}O_{2peak}$/ $BSA^{*\beta}$	r = 0.634, ***p* < 0.001***	r = 0.587, ***p* = 0.003***	r = 0.545, ***p* = 0.015***	r = 0.444, ***p* = 0.044***
$\dot{V}O_{2peak}$/ $Stature^{*\beta}$	r = 0.631, ***p* < 0.001***	r = 0.586, ***p* = 0.003***	r = 0.500, ***p* = 0.007***	r = 0.416, *p* = 0.061

Correlation coefficient identified from Pearson's correlation analysis. Alpha level = 0.05. Significant *p* value highlighted in bold and *. $\dot{V}O_{2peak}$; peak oxygen uptake. FEV_1; forced expiratory volume in 1 s. FVC; forced vital capacity. BM; body mass. BSA; body surface area.

4.2. Research question 1 and 2

(The effect of disease status [1] and age [2] on CPET outcomes)

Two separate two-way ANOVAs were run to address research aims 1 and 2; sex and lung function in one model, sex and age in another. An interaction effect of age tertiles and sex was noted for RCP (% $\dot{V}O_{2peak}$). No interaction effects of sex and FEV_1 tertiles or sex and age tertiles were noted in any other of the ANOVAs. Main effects and effect sizes are reported below (Table 4.5). Mean and standard deviations of $\dot{V}O_{2peak}$ (absolute and allometrically scaled) for each category of FEV_1 tertiles and age tertiles are reported in figure 4.3.

FEV_1 tertiles significantly explains variance in: $\dot{V}O_{2peak}$ ($L \cdot min^1$), $\dot{V}O_{2peak}/BM^{*\beta}$, $\dot{V}O_{2peak}/BSA^{*\beta}$, $\dot{V}O_{2peak}/Stature^{*\beta}$, TTE (s), $\dot{V}CO_{2peak}$ ($L \cdot min^1$), GET (% $\dot{V}O_{2peak}$) and tidal volume (L). Significant effects of age tertiles are noted in; $\dot{V}O_{2peak}$ ($L \cdot min^1$), $\dot{V}O_{2peak}/Stature^{*\beta}$, peak power (W), TTE (s), $\dot{V}CO_{2peak}$ ($L \cdot min^1$), GET (L), $\dot{V}_{Epeak}$ ($L \cdot min^{-1}$), $\dot{V}_E/\dot{V}O_2$ ($L \cdot min^{-1}$), tidal volume (L) and RER_{peak} (Table 4.5).

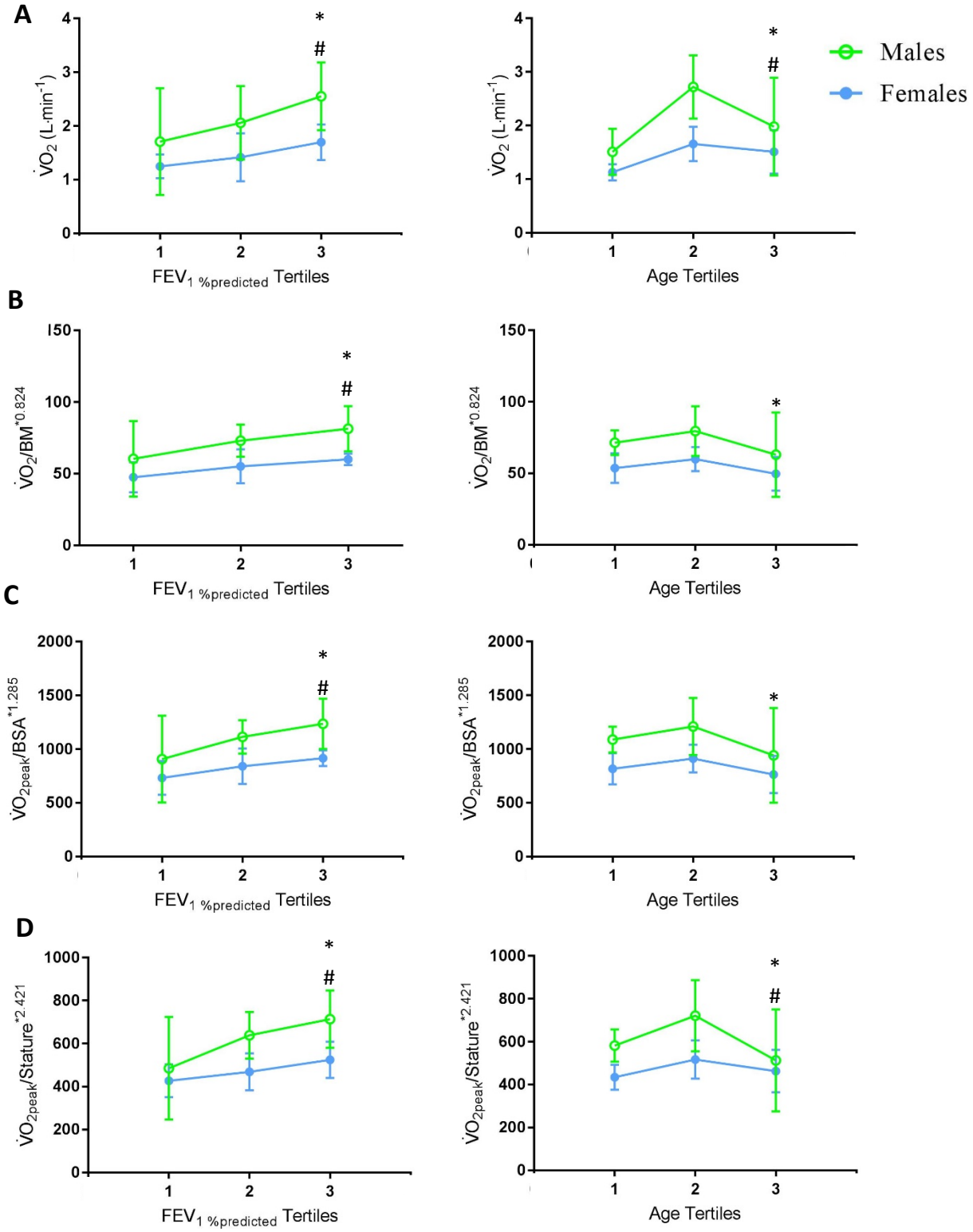


Figure 4.3. Mean oxygen uptake (a) and $\dot{V}O_{2peak}$ allometrically scaled to body mass (b) body surface area (c) and stature (d) for males and females in each of the categories of 1: $FEV_{1\%predicted}$ tertiles and 2: age tertiles. Vertical lines represent SD. Significant main effect of: sex = *; tertiles = #.

Table 4.5. Main effects and effect sizes of two-way ANOVA in young people with CF.

Dependent Variable	Independent variables: Sex and FEV_1 Tertiles			Independent variables: Sex and Age Tertiles		
	Main Effect of Sex	Main Effect of FEV_1 Tertiles	Adjusted R^2	Main Effect of Sex	Main Effect of Age Tertiles	Adjusted R^2
$\dot{V}O_{2peak}$ ($L \cdot min^{1}$)	***p* = 0.001***	***p* = 0.020***	0.33	***p* < 0.001***	***p* < 0.001***	0.51
$\dot{V}O_{2peak}/BM^{*\beta}$	***p* < 0.001***	***p* = 0.013***	0.35	***p* < 0.001***	*p* = 0.079	0.29
$\dot{V}O_{2peak}/BSA^{*\beta}$	***p* < 0.001***	***p* = 0.010***	0.36	***p* = 0.001***	*p* = 0.064	0.30
$\dot{V}O_{2peak}/Stature^{*\beta}$	***p* < 0.001***	***p* = 0.005***	0.37	***p* = 0.001***	***p* = 0.024***	0.34
$\dot{V}O_2$ gain ($mL \cdot min^{-1} \cdot W^{-1}$)	*p* = 0.509	*p* = 0.160	0.07	*p* = 0.626	*p* = 0.182	0.01
Peak power (W)	***p* = 0.003***	*p* = 0.090	0.23	***p* < 0.001***	***p* < 0.001***	0.51
Peak power ($W \cdot kg^{1}$)	***p* = 0.003***	*p* = 0.087	0.27	***p* = 0.001***	*p* = 0.451	0.19
TTE (s)	***p* = 0.002***	***p* = 0.048***	0.28	***p* = 0.002***	***p* = 0.022***	0.28
$\dot{V}CO_{2peak}$ ($L \cdot min^{1}$)	***p* = 0.002***	***p* = 0.050***	0.27	***p* < 0.001***	***p* < 0.001***	0.52
RCP (% VO_{2peak})	*p* = 0.473	*p* = 0.261	-0.13	***p* = 0.046***	***p* = 0.011***	0.32
GET ($L \cdot min^{1}$)	***p* = 0.002***	*p* = 0.619	0.17	***p* < 0.001***	***p* < 0.001***	0.50
GET ($mL \cdot kg^{1} \cdot min^{1}$)	***p* < 0.001***	*p* = 0.946	0.20	***p* = 0.001***	*p* = 0.914	0.20
GET (% $\dot{V}O_{2peak}$)	*p* = 0.816	***p* = 0.035***	0.08	*p* = 0.733	*p* = 0.715	0.05
$\dot{V}_{Epeak}$ ($L \cdot min^{1}$)	***p* = 0.002***	*p* = 0.060	0.27	***p* < 0.001***	***p* < 0.001***	0.53
$\dot{V}_E/\dot{V}O_2$ ($L \cdot min^{-1}$)	*p* = 0.886	*p* = 0.332	-0.02	*p* = 0.859	***p* = 0.034***	0.12

$\dot{V}_E/\dot{V}CO_2$ (L·min^{-1})	p = 0.539	p = 0.068	0.06	p = 0.401	p = 0.267	0.01
$\dot{V}_E/\dot{V}O_2$ Slope	p = 0.310	p = 0.982	-0.08	p = 0.274	p = 0.958	0.01
$\dot{V}_E/\dot{V}CO_2$ Slope	p = 0.221	p = 0.284	0.01	p = 0.120	p = 0.113	0.07
Tidal volume (L)	**p = 0.013***	**p = 0.014***	0.26	**p = 0.001***	**p < 0.001***	0.54
BF (breaths·min^{-1})	**p = 0.047***	p = 0.336	0.03	p = 0.075	p = 0.312	0.02
HR_{peak} (b·min^{-1})	p = 0.669	p = 0.610	0.05	p = 0.962	p = 0.223	-0.06
RER_{peak}	p = 0.405	p = 0.070	0.09	p = 0.420	**p = 0.005***	0.23
Oxygen Pulse (mL·min^{-1})	**p = 0.033***	p = 0.195	0.16	**p = 0.009***	p = 0.064	0.34

Significant main effects are expressed by p value highlighted in bold and *. Alpha level set to 0.05.; $\dot{V}O_{2peak}$; peak oxygen uptake. $\dot{V}O_{2peak}/BM^{*\beta}$ maximal oxygen uptake scaled to body mass and raised to the exponent 0.82. $\dot{V}O_{2peak}/BSA^{*\beta}$; maximal oxygen uptake scaled to body surface area and raised to the exponent 1.29. $\dot{V}O_{2peak}/Stature^{*\beta}$; maximal oxygen uptake scaled to stature and raised to the exponent 2.42. TTE; time to exhaustion. $\dot{V}CO_{2peak}$; peak carbon dioxide output. RCP; respiratory compensation point. GET; gas exchange threshold. $\dot{V}_{Epeak}$; peak minute ventilation. BF; breathing frequency. HR_{peak}; peak heart rate. RER_{peak}; peak respiratory exchange ratio.

4.3. Research question 3

(Effect of scaling on sex differences in $\dot{V}O_{2peak}$)

$\dot{V}O_{2peak}$ allometrically scaled (Fig. 3.3) for BM, BSA and stature was significantly lower in females than males (Table 4.6).

Table 4.6. $\dot{V}O_{2peak}$ sex differences, with allometric scaling.

Variable	Males	Females	*p* Value
$\dot{V}O_{2peak}/BM^{*\beta}$	73.04 ± 19.57	53.28 ± 10.93	**< 0.001***
$\dot{V}O_{2peak}/BSA^{*\beta}$	1108.06 ± 296.24	814.58 ± 159.05	**< 0.001***
$\dot{V}O_{2peak}/Stature^{*\beta}$	627.77 ± 183.28	464.76 ± 86.69	**< 0.001***

Data are presented as mean ± SD. Significant differences between sexes are reported from independent samples t-test and expressed by *p* value highlighted in bold and *. Alpha level set to 0.05.; $\dot{V}O_{2peak}/BM^{*\beta}$; peak oxygen uptake scaled to body mass and raised to the power 0.82. $\dot{V}O_{2peak}/BSA^{*\beta}$; peak oxygen uptake scaled to body surface area and raised to the power 1.29. $\dot{V}O_{2peak}/Stature^{*\beta}$; peak oxygen uptake scaled to stature and raised to the power 2.42.

4.4 Research question 4

(Effect of scaling and co variates on sex differences in $\dot{V}O_{2peak}$)

ANCOVAs were run on 52 participants to examine the effect of sex on absolute and scaled $\dot{V}O_{2peak}$ when controlled for covariates; age (y), PHV (pre, pubertal and post), BMI (underweight, normal, overweight and obese) and $FEV_{1\%predicted}$ as a continuous variable (Table 4.7).

When run without covariates for absolute $\dot{V}O_{2peak}$, sex explained approximately 25 % of variance. Inclusion of all co-variates explained 67 % of variance, with sex

accounting for ~21 %. Sex, PHV, BMI and $FEV_{1\%predicted}$ had positive relationships with absolute $\dot{V}O_{2peak}$, age had a negative relationship. A significant effect of age (F (1, 46) = 12.151; p = 0.001) was noted when PHV was removed from the absolute $\dot{V}O_{2peak}$ model. Age was non-significant with the removal of PHV in all other models.

Age and BMI had a negative relationship with $\dot{V}O_{2peak}/BM^{*\beta}$ and $\dot{V}O_{2peak}/BSA^{*\beta}$. PHV, sex and $FEV_{1\%predicted}$ had positive relationships with $\dot{V}O_{2peak}/BM^{*\beta}$ and $\dot{V}O_{2peak}/BSA^{*\beta}$. Age had a negative relationship with $\dot{V}O_{2peak}/Stature^{*\beta}$, PHV, BMI, sex and $FEV_{1\%predicted}$ had positive relationships with $\dot{V}O_{2peak}/Stature^{*\beta}$.

Sex and FEV_1 significantly explained; 67 % variance in absolute $\dot{V}O_{2peak}$, 56 % variance in $\dot{V}O_{2peak}/BM^{*\beta}$, 55 % variance in $\dot{V}O_{2peak}/BSA^{*\beta}$ and 52 % of variance in $\dot{V}O_{2peak}/Stature^{*\beta}$ (Table 4.7). Age, PHV and BMI did not significantly explain variance in any of the $\dot{V}O_{2peak}$ parameters.

Adjusted means were higher in males than females in all absolute and scaled $\dot{V}O_{2peak}$ parameters (Fig. 4.4). Mean for males and females: $\dot{V}O_{2peak}$, 2.14 $L \cdot min^{-1}$ and 1.44 $L \cdot min^{-1}$; $\dot{V}O_{2peak}/BM^{*\beta}$, 7.28 $ml \cdot kg^{*0.82} \cdot min^{-1}$ and 54.23 $ml \cdot kg^{*0.82} \cdot min^{-1}$; $\dot{V}O_{2peak}/BSA^{*\beta}$, 1094.812 $ml \cdot m^{2(*1.29)} \cdot min^{-1}$ and 831.292 $ml \cdot m^{2(*1.29)} \cdot min^{-1}$; $\dot{V}O_{2peak}/Stature^{*\beta}$, 616.44 $ml \cdot m^{*2.42} \cdot min^{-1}$ and 479.04 $ml \cdot m^{*2.42} \cdot min^{-1}$, respectively.

Table 4.7. ANCOVA models for absolute and relative $\dot{V}O_{2peak}$ for young people with CF.

	Co variates	F	*p*	η_p^2
Absolute $\dot{V}O_{2peak}$ (adjusted R^2 = 0.67)				
	Age	(1, 46) = 1.62	0.210	
	PHV	(1, 46) = 21.01	**< 0.001***	
	BMI	(1, 46) = 1.69	0.200	
	FEV_1	(1, 46) = 24.41	**< 0.004***	
	Sex	(1, 46) = 32.22	**< 0.001***	0.41
$\dot{V}O_{2peak}/BM^{*\beta}$ (adjusted R^2 = 0.56)				
	Age	(1, 46) = 3.128	0.084	
	PHV	(1, 46) = 4.168	**0.047***	
	BMI	(1, 46) = 3.591	0.064	
	FEV_1	(1, 46) = 22.825	**< 0.001***	
	Sex	(1, 46) = 15.374	**< 0.001***	0.36
$\dot{V}O_{2peak}/BSA^{*\beta}$ (adjusted R^2 = 0.55)				
	Age	(1, 46) = 2.979	0.091	
	PHV	(1, 46) = 3.554	0.066	

	BMI	(1, 46) = 1.839	0.182	
	FEV_1	(1, 46) = 22.322	**< 0.001***	
	Sex	(1, 46) = 23.375	**< 0.001***	0.34
$\dot{V}O_{2peak}$/Stature$^{*\beta}$ (adjusted R^2 = 0.52)				
	Age	(1, 46) = 1.971	0.167	
	PHV	(1, 46) = 4.272	0.044*	
	BMI	(1, 46) = 1.831	0.183	
	FEV_1	(1, 46) = 20.218	**< 0.001***	
	Sex	(1, 46) = 17.327	**< 0.001***	0.27

F values reported as (degrees of freedom) = F value. Alpha level set to 0.05, significant results are highlighted in bold and by *. η_p^2; partial eta squared. $\dot{V}O_{2peak}$; peak oxygen uptake. $\dot{V}O_{2peak}$/BM$^{*\beta}$; maximal oxygen uptake scaled to body mass and raised to the exponent 0.82. $\dot{V}O_{2peak}$/BSA$^{*\beta}$; maximal oxygen uptake scaled to body surface area and raised to the exponent 1.29. $\dot{V}O_{2peak}$/Stature$^{*\beta}$; maximal oxygen uptake scaled to stature and raised to the exponent 2.42. PHV; peak height velocity. BMI; body mass index. FEV_1; forced expiratory volume in 1 s.

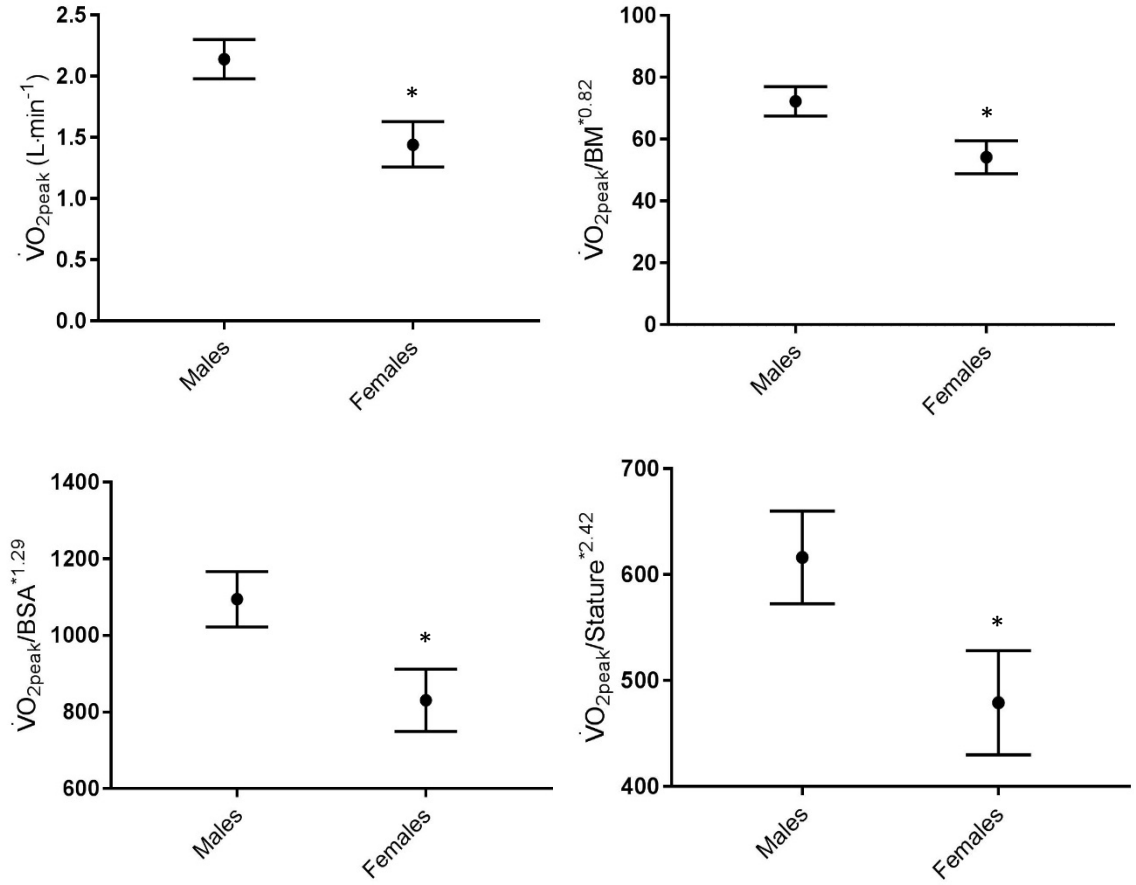


Figure 4.4. Adjusted means and ranges for absolute and scaled VO_{2peak} parameters in males and females. Significant differences highlighted with an *.

The key aim of this thesis was to identify sex differences in CPET parameters in young people with CF. To address this aim, four key findings were identified: (1) There are no sex differences in $\dot{V}O_{2peak}$ and other CPET parameters in relation to disease status defined by $FEV_{1\%predicted}$ (tertiles); (2) Age and sex interacted to significantly explain differences in RCP (% $\dot{V}O_{2peak}$). No significant interaction effects were noted for any other CPET outcomes; (3) A novelty of this study showed that allometry appropriately removed the influence of body size in all parameters (BM, BSA and stature). When allometrically scaled for BM, BSA and stature, females had a lower $\dot{V}O_{2peak}$ than their male counterparts; (4) After adjusting for various co-variates (lung function, nutritional and maturational status) sex differences still remained in absolute $\dot{V}O_{2peak}$, $\dot{V}O_{2peak}/BM^{*\beta}$, $\dot{V}O_{2peak}/BSA^{*\beta}$ and $\dot{V}O_{2peak}/Stature^{*\beta}$, but there was still ~50 % variance left unexplained.

5.1 Sex differences in exercise parameters

The findings in this thesis are consistent with previous literature in healthy children reporting females to have a ~0.3 $L \cdot min^{-1}$ lower exercise capacity than their male counterparts (Armstrong *et al.,* 1997). However, the cause of such a difference is still under debate. When scaled using allometry and controlled for co-variates (FEV_1, BMI, PHV and age), $\dot{V}O_{2peak}$ was significantly affected by sex (females elicited lower values) indicating there may be mechanistic differences between males and females with CF. Klijin *et al.,* (2003) proposed FFM to better adjust for body size in children with CF, explaining up to 9 % further variance than other body size variables, (such as BM, BSA and stature which were used in the

present study) due to the effect that CF has on muscle development (Boas *et al.,* 1996). Despite this, allometrically scaled BM, BSA and stature appropriately removed the influence of body size in the present analysis (Fig. 3.3). Klijin *et al.,* (2003) did not consider the influence of maturation or age, reporting 47% of variance in exercise capacity was due to FEV_1 and FFM. Wirth and colleagues (1978) reported $\dot{V}O_{2peak}$ to be higher in healthy boys than girls and increase with maturity, however the increase and sex difference was likely due to the influence of body size as sex differences in $\dot{V}O_{2peak}$ were non-significant when related to body mass. In the present analysis PHV was used to account for maturity status as the thesis population included paediatric participants. Although, PHV may not be a valid measure as calculations were established in healthy children and may not transfer in CF populations due to the complications with nutrition and stunted growth that some people with CF experience (Hankard *et al.,* 2002). Unfortunately, commonly reported markers of sexual maturity such as Tanner stages, were not available to complement the estimate of PHV. No differences between males and females were noted in Smax verification, secondary criteria (HR_{peak} and RER_{peak}) and breathing frequency, suggesting females and males were working equally intensively. Therefore, it is very unlikely that $\dot{V}O_{2peak}$ is lower in females due to a lack of effort or motivation in the present analysis. Similar conclusions have been previously reported on the cycle ergometer and treadmill in healthy children (Welsman *et al.,* 1997).

Armstrong (2006) provided reference values for peak $\dot{V}O_2$ in boys and girls, 48-50 $mL \cdot kg^{-1} \cdot min^{-1}$ and 35-45 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively, which was higher on average by ~35 % than the mean in this population (33 $ml \cdot kg^{-1} \cdot min^{-1}$ and 25 $ml \cdot kg^{-1} \cdot min^{-1}$ for boys and girls respectively). Additionally, Adegboye *et al.,* (2011) recommended that for metabolic health in non-diseased 15 year old boys and

girls, a minimum peak $\dot{V}O_2$ of 46 mL·kg^{-1}·min^{-1} and 33 mL·kg^{-1}·min^{-1} should be obtained, respectively. When Bongers' reference equations for healthy children were utilised (Bongers *et al.,* 2014b; Appendix C), males and females with CF in the present study had 17.8 % and 34.6 % lower $\dot{V}O_{2peak}$ (respectively) than they would if healthy. Therefore, the population in the present analyses were less fit than their healthy counterparts based on predictive equations. However, Bongers' reference equations were not developed in the CF population, and were identified from a Dutch population which may not transfer to a British population. Compared to CF populations, the participants in this analysis had on average a 0.21 L·min^{-1} and 6.48 ml·kg^{-1}·min^{-1} lower $\dot{V}O_{2peak}$ than participants in a study by Saynor *et al.,* (2014). Although, Saynor and colleagues (2014) identified $\dot{V}O_{2peak}$ in only 10 children with CF and a mean age ~4.3 y younger than the present analysis, the age difference might explain the small discrepancy in $\dot{V}O_{2peak}$ despite equivalent protocols being used and participants from the same region of the UK. However, the present analysis appears to show an increase in $\dot{V}O_{2peak}$ with age, therefore the lower exercise capacity despite a higher average age could suggest a difference in disease severity between the two populations. Furthermore, participation in the study by Saynor *et al.,* (2014) was voluntary, offering a potential selection bias towards milder disease severity and aerobically fitter CF cohort. It is difficult to identify exactly where this population fits in terms of relative fitness due to the age range of the present population spanning from childhood, adolescence into early adulthood. However, when comparing FEV_1 tertiles (Fig. 4.3) the lowest tertile includes a range of 29.59 to 77.33 % predicted FEV_1. This suggests that in terms of disease severity, this population was relatively fit and healthy as > 70 % is considered a mild disease status. Significant differences seen between males and females in terms of $\dot{V}O_{2peak}$ may only be

identifiable within the patients with more mild to moderate disease severity as they are most similar to a healthy population than those with severe CF. There is limited research investigating the severe CF population, but it is possible that the sex differences in $\dot{V}O_{2peak}$ may not be as noticeable in severe CF participants, as in mild to moderate CF.

In this population, females with CF have a significantly greater percentage difference between their absolute $\dot{V}O_{2peak}$ and absolute $\dot{V}O_{2peak}$ reference values from Bongers *et al.,* (2014b) than males (-34.6 % and -17.8 % difference respectively). This exacerbates the already significantly lower exercise capacity in healthy females experienced. Therefore, the implications of a lower exercise capacity in healthy females is widened further by CF in females. Due to the relatively healthy CF population, as defined by $FEV_{1\%predicted}$, this additional separation between males and females in terms of exercise capacity may be increased in a severe CF population. However, it is important to note that the Bongers reference values are based on age and sex alone, neglecting other possible factors such as body size variables, which may account for the greater percentage difference $\dot{V}O_{2peak}$ experienced in females.

In a cross-sectional study involving 83 participants (47 males) it was reported that those with CF and a glucose intolerance had a lower exercise capacity than those without a glucose intolerance (Foster *et al.,* 2018). In the present analysis twice as many females had CFRDM than males, suggesting a possible explanation for the lower exercise capacities seen in females. However, it may be that those people with CFRDM, or a glucose intolerance, have a reduction in PA due to the demands of managing CFRDM, and therefore a reduction in exercise capacity rather than a direct causal relationship.

Although $\dot{V}O_{2peak}$ was significantly lower in females, $\dot{V}O_2$ gain (males 7.2 ± 1.8 $mL \cdot W^{-1} \cdot min^{-1}$ and females 6.8 ± 1.7 $mL \cdot W^{-1} \cdot min^{-1}$) was not significantly different between sexes. It is documented that CF patients have impaired oxygen uptake kinetics compared to their healthy counterparts, with average functional gain of CF patients equalling 8 ± 3 $mL \cdot W^{-1} \cdot min^{-1}$ (Fielding *et al.,* 2015), similar to the whole group average $\dot{V}O_2$ gain in the present analysis (7 ± 2 $mL \cdot W^{-1} \cdot min^{-1}$). Therefore, despite no differences between sexes, this population may have experienced impaired oxygen uptake kinetics compared to the healthy population. However, the CF population in the study by Fielding *et al.,* (2015) did have a lower gain than the healthy controls, which alongside a delayed $\dot{V}O_2$ response, was attributed to impaired oxygen transport and utilisation in the muscles.

Peak power, absolute and relative to body mass, were significantly lower (~68 W and ~1 $W \cdot kg^{-1}$ respectively) in females than males. This finding shows that, although only a small difference when presented relative to body mass, females are less effective at producing power, however FFM or MM would be a more appropriate measure to identify whether this is a purely muscle compositional constraint. It is well documented that adult males and females have different body compositions with males eliciting higher proportions of muscle mass than females (Schorr *et al.,* 2018; Bredella, 2017; Abe *et al.,* 2003), however, this is heavily dependent on maturation when researching children (Loomba-Albrecht and Styne, 2009). It may be that females have less FFM/ MM than males and therefore cannot produce the same power per unit of body size. There have been reports of structural differences in muscles types between sexes in mice (Eason *et al.,* 1985) and healthy human populations (Staron *et al.,* 2000) with the expression of type II fibres of the vastus lateralis muscle being more prominent

in males than females (Haizlip *et al.,* 2015; Staron *et al.,* 2000). Fast twitch muscle fibres in the vastus lateralis (quadriceps), may be particularly important towards the latter stages of incremental maximal testing on cycle ergometers, where the participant is working anaerobically with greater load. Further studies have shown some differences in contractile properties between healthy men and women (Gandevia 2001). However, many of these studies have been isolated to single muscles, with different muscles used between studies, making it difficult to generalize findings to the entire body. Furthermore, Blimkie and Sale (1998) described no sex differences in muscular force in healthy cohorts once the influence of muscle cross sectional area is considered. The CF literature does not report whether there are sex differences in skeletal muscle properties, likely due to research aiming to identify differences between CF and healthy controls. People with CF have been reported to have a reduced oxidative work performance (de Meer *et al.,* 1995), similar contractility, fatigability (Gruet *et al.,* 2016) and lower maximal torque in the vastus lateralis and medialis than healthy controls, this was reported to be due to less developed musculature rather than a direct result of the CFTR defect (Stein *et al.,* 2016). It could be that males and females with CF have differences in muscular properties due to differences in sex rather than the impact of CF.

Submaximal parameters such as GET absolute and relative ($ml \cdot kg^{-1} \cdot min^{-1}$) were significantly lower (~0.4 $L \cdot min^{-1}$ and ~5 $mL \cdot kg^{-1} \cdot min^{-1}$ respectively) in females than males. However, these parameters are closely linked to absolute value of $\dot{V}O_{2peak}$ so may be biased, as females have a lower $\dot{V}O_{2peak}$. Conversely, GET as a percent of $\dot{V}O_{2peak}$ was not significantly different between sexes, this parameter removes the influence of absolute $\dot{V}O_{2peak}$ attainment.

Females have a significantly lower peak $\dot{V}_E$ than males accompanied by no difference in breathing frequency, suggesting females are unable to inhale in as much air as males or do not inhale as deeply to compensate. This could explain part of the lower peak $\dot{V}O_2$ in females, however this again may simply be due to the influence of body and lung size. In healthy children, stature is reported to be the main predictor of vital capacity (Bhatti *et al.,* 2014). Females in the present analysis were significantly ~10 cm (p = 0.027) smaller in stature and experienced ~0.5 L lower tidal volume, supporting the idea that lung size could be the reason for a lower $\dot{V}_{Epeak}$ in females.

Previous literature has reported no significant differences in $\dot{V}_E/\dot{V}CO_2$ slope between CF and healthy controls (age, sex and anthropometrically matched; Bongers *et al.,* 2014a) however $\dot{V}_E/\dot{V}CO_2$ slope values in the present analysis were ~5-7 units higher than the CF group in the study by Bongers *et al.,* (2014a). This suggests the group in the present analysis may have greater dead space fraction and/ or lower partial pressure of CO_2 as seen in healthy subjects with greater $\dot{V}_E/\dot{V}CO_2$ slope values (Sun *et al.,* 2002). There were no significant differences between males and females in terms of $\dot{V}_E/\dot{V}O_2$ (absolute and slope) and $\dot{V}_E/\dot{V}CO_2$ (absolute and slope) despite ventilatory equivalents of O_2 and CO_2 significantly predicting mortality in children with CF (Hulzebos *et al,* 2014) and females having a higher mortality (Dodge *et al.,* 2007; Liou *et al.,* 2001; Corey *et al.,* 1997; Rosenfeld *et al.,* 1997) which factors such as nutrition or pulmonary function cannot explain (FitzSimmonds, 1993). Furthermore, literature in healthy adults has reported significant sex differences in $\dot{V}_E/\dot{V}CO_2$ during an incremental cycle test to exhaustion (Neder *et al.,* 2001). The results in this thesis are consistent with previous findings in healthy children (Armstrong *et al.,* 1997). In addition, Tucker *et al.,* (2019) reported no sex differences in $\dot{V}_E/\dot{V}O_2$ (peak) and

$\dot{V}_E/\dot{V}CO_2$ (peak and slope) in people with CF at baseline, but females exhibited a greater decline in ventilatory equivalents (p = 0.001, p = 0.002 and p = 0.016 respectively) than males over ~3 years, despite no change in $\dot{V}O_{2peak}$. The authors attributed this to reduced CO_2 production and/ or reduced sensitivity to arterial CO_2 in females with CF. Furthermore, Tucker and colleagues (2019) suggested that deteriorations in ventilatory dynamics may precede the decline in exercise capacity and therefore be a more sensitive prognostic tool than $\dot{V}O_{2peak}$ for monitoring mortality between sexes. However, in the above study by Tucker et al., (2019), no significant differences were reported in $\dot{V}O_{2peak}$ absolute or relative to body mass which are inconsistent with the present findings. This may be due to; stricter exclusion criteria, a smaller sample size (20 compared to 52), raw data averaged into 30 s (rather than 10 s averages) and no S_{max} verification in the Tucker study compared to the present analysis.

Females demonstrated significantly lower values for oxygen pulse ($\dot{V}O_2$/ HR) in the present analysis (~4 $mL \cdot min^{-1}$), which is likely due to the higher $\dot{V}O_{2peak}$ in males combined with no sex differences in HR. This implies less oxygen is taken up with each cardiac contraction in females, although females may have significantly smaller ventricles than males causing this phenomenon. Comparisons between the sexes were not significantly different in terms of BM, BSA or BMI indicating males and females may have similar heart sizes by extrapolation. However, previous studies in participants without heart disease reported stature to significantly affect heart size (Pfaffenberger *et al.,* 2013). In the present study, females were significantly smaller than males supporting the idea that they had smaller hearts. In patients with coronary heart disease, oxygen pulse has been reported to add no additional information than $\dot{V}O_{2peak}$ in relation to fitness or prognosis (Laukkanen *et al.,* 2006), suggesting perhaps this

parameter is negligible. However, previous literature in CF has identified ventricular dysfunction both in relation to (Giacchi *et al.,* 2015; Ionescu *et al.,* 2001) and independent of (Sellers *et al.,* 2013) disease status. Furthermore, in a variety of different patient populations, sex differences are apparent in parameters of ventricular dysfunction (Ferreira *et al.,* 2015; Coutinho *et al.,* 2013; Hameedullah *et al.,* 2012), therefore females with CF may have greater ventricular dysfunction compared to males.

$\dot{V}O_{2max}$ was verified if $\dot{V}O_{2peak}$ determined from the S_{max} was < 9 % outside of the ramp $\dot{V}O_{2peak}$ as reported by Saynor *et al.,* (2013a). In the present population only 66 % of the 47 participants that underwent the S_{max} were verified to have attained true $\dot{V}O_{2max}$, compared to ~90 % in a healthy population (Saynor *et al.,* 2013a). However, the CPET data was taken as the first CPET recorded at the NHS Trust Hospital, some participants may have never before performed an exercise test or been on an exercise bike and others may have moved from a previous hospital where they were subject to CPETs. Offering the possibility that there may have been a learning effect to the protocol between the ramp and S_{max} bout, enabling greater effort in the S_{max} bout. Average peak HR, a surrogate measure of effort, increased from 177 bpm to 179 bpm from ramp to S_{max} bout suggesting this learning effect may have been minimal. Additionally, Saynor *et al.,* (2013b) reported no learning effect of maximal incremental cycle tests. $\dot{V}O_{2max}$ was reproducible over 8 hours and 4-6 weeks in children with cystic fibrosis.

Sub maximal measures such as GET (% $\dot{V}O_{2peak}$), $\dot{V}_E/\dot{V}CO_2$ slope and $\dot{V}_E/\dot{V}O_2$ slope were not different between males and females suggesting no difference at a submaximal level, however differences at maximal level ($\dot{V}O_{2peak}$) are noted. This is similar to findings in healthy populations that suggest multiple similarities between males and females at submaximal intensities (Deschenes *et al.,* 2006)

and higher stroke volume in males being attributable to greater heart sizes (Wilmore *et al.,* 2001; Wiebe *et al.,* 1998). Some studies have noted greater HR of females during submaximal exercise (Seebauer *et al.,* 2003; Wilmore *et al.,* 2001), which is postulated to compensate for a lower stroke volume. Furthermore, Deschenes and colleagues (2001) reported healthy males to have higher (p = 0.04) utilisation of CHO sources than healthy females at prolonged submaximal intensities, due to higher RER values in males. However, RER was not significantly different between males and females at any point (S_{max}, ramp or peak $\dot{V}O_2$) in the present analyses, suggesting similar efficiency of utilising fats and CHO between the sexes at maximal exercise. These results support findings at maximal exercise in healthy trained athletes (Goedecke *et al.,* 2000), however both the study by Goedecke *et al.,* (2000) and the present study did not account for menstrual cycle in females, which may alter substrate utilisation due to hormonal fluctuations (Devries et al., 2006; Zderic et al., 1985). Although, the above studies used very different sample groups than the present thesis.

Absence of interaction effects was noted for sex in relation to $FEV_{1\%predicted}$ (tertiles) and all but one parameter (RCP % $\dot{V}O_{2peak}$) for sex in relation to age (tertiles). This suggests that the influence of sex on cardiopulmonary exercise variables is not reliant on age or disease status as defined by lung function. From both of these models, sex can statistically account for variance in all $\dot{V}O_{2peak}$ parameters. These results complement those of the t-test results, however there are exceptions and differences in p values between the two different two-way ANOVA models. Although the interaction effect is non-significant, it is still accounted for in the model, potentially making the main effects noted subtly different than if run without the interaction. Whilst it can be suggested that sex influences variance in a number of CPET outcomes ($\dot{V}O_{2peak}$ absolute and

relative, peak power [W and $W \cdot kg^{-1}$], TTE, $\dot{V}CO_{2peak}$, RCP % $\dot{V}O_{2peak}$, GET [$L \cdot min^{-1}$ and $ml \cdot kg^{-1} \cdot min^{-1}$], $\dot{V}_{Epeak}$, tidal volume, BF and oxygen pulse) not all of these outcomes have statistically significant group means between males and females (see Table 4.3. for significant t-test results).

5.2 Influence of lung function on exercise parameters

Irrespective of whether male or female, $FEV_{1\%predicted}$ tertiles significantly explained variance in many CPET parameters including $\dot{V}O_{2peak}$ absolute and scaled using allometry. The relationship between lung function and exercise capacity has been previously noted by Pastre *et al.,* (2014), who stated that an impaired $\dot{V}O_{2peak}$ was more pronounced in participants with a low FEV_1. Tucker *et al.,* (2017) reported preservation or improved lung function following an acute bout of maximal exercise and this improvement was related to peak work rate and $\dot{V}_{Epeak}$. Although the present analyses did not include follow up tests of lung function, no significant relationship was noted between lung function and $\dot{V}_{Epeak}$ or peak power, which is in contrast to the above study by Tucker and colleagues.

A strong positive correlation between FEV_1 and GET was observed ($r = 0.71$), associating the greater lung function the greater submaximal performance. It is difficult to infer a direction of causality of the correlation due to the cyclical effect of one parameter on the other. A person may not be conditioned, resulting in a lower GET (Ghosh, 2004), and therefore lung function declines. We know maximal exercise training can improve respiratory function (Tucker *et al.,* 2017). This improvement in respiratory function is attributed to improvements in voluntary and involuntary muscle strength and efficiency of muscles utilising oxygen (Hulke *et al.,* 2012). Similarly, the correlation between relative peak power and lung function could be due to the patient doing more exercise and therefore

have greater MM or anaerobic power as a result of a training effect (Radtke *et al.,* 2017), which in turn aids improvement in lung function or a greater lung function facilitates the ability to exercise and improve peak power. However, it can be inferred due to the moderate correlations (r = 0.5), that higher fitness levels are related to better lung health in CF (and vice versa), synonymous with previous literature (the author failed to report the correlation coefficient; Pianosi *et al.,* 2005b).

5.3 Influence of age on exercise parameters

The median age of patients in the present analysis was 17 y, 3 years younger than the UK CF population (20 y) (UK Cystic Fibrosis Trust, 2017). Despite median age being similar, this analysis had an upper age range of 25 y therefore we cannot infer that this sample is representative of the whole CF population.

Pearson's correlation analysis revealed a weak positive correlation between age and $\dot{V}_{Epeak}$ (Fig. 4.2). Upon visual inspection of the data points in the correlation, it can be noted that $\dot{V}_{Epeak}$ appears to increase with age up to a point (~17 y) before then declining. When a bi lateral correlation approach was utilised, a strong positive relationship was noted up to the age of 17 y and a moderate negative correlation from 17 – 25 y. This observation can lead to the conclusion that simple linear regression is not suitable to identify the correlation in this example, and perhaps a more comprehensive quadratic regression approach or partial correlation may be more appropriate. Previous literature has not addressed the relationship between age and minute ventilation in CF. Longitudinal analyses in healthy children have noted increases in $\dot{V}_{Epeak}$ throughout childhood (Rowland and Cunningham, 1997). It is likely that the increase noted throughout childhood is related to the increases in body size. In

contrast to the present observation, in healthy populations it has been noted that there are greater ventilatory responses during exercise in elderly participants compared to young (Prioux *et al.,* 2000; Brischetto *et al.,* 1984), which suggests ventilatory responses in CF are altered with age compared to healthy populations. Additionally, age related declines in ventilatory parameters, throughout childhood, have been reported in CF populations (Pianosi and Wolstein, 1996). The novel bi-lateral correlation between $\dot{V}_{Epeak}$ and age noted in the present analyses warrants further investigation into the causality between the two variables in young people with CF.

Differences in $\dot{V}O_{2peak}$ ($L \cdot min^{1}$), $\dot{V}O_{2peak}$/Stature$^{*\beta}$, peak power (W), TTE (s), $\dot{V}CO_{2peak}$ ($L \cdot min^{1}$), RCP (% $\dot{V}O_{2peak}$), GET ($L \cdot min^{1}$), $\dot{V}_{Epeak}$ ($L \cdot min^{1}$), $\dot{V}_E/\dot{V}O_2$ ($L \cdot min^{-1}$), tidal volume (L) and RER_{peak} could be attributable to age as defined by tertiles. It is interesting to note that $\dot{V}O_{2peak}$ scaled to stature was significantly different between age tertiles, however $\dot{V}O_{2peak}$ scaled to BM or BSA was not. This is perhaps because in the present analysis stature is treated as a linear dimension and does not consider the change in volume with growth. Absolute $\dot{V}O_{2peak}$ was observed to increase between lowest and middle tertiles then decrease between middle and highest age tertiles, confirming the well documented findings in previous literature that exercise capacity declines with age in adults with CF (Weert-van Leeuwen *et al.,* 2012).

5.4 Applications and future research

The overriding observation from the results in the present analysis is that females with CF have a reduced exercise capacity than their male counterparts irrespective of the scaling method, body size variable used or clinical co variates. Therefore, females with CF may require a more tailored programme of exercise

than males to aid in managing the disease, with all programmes scaled to a parameter of capacity such as $\dot{V}O_{2peak}$. Future research should focus on identifying effective programs and individualising for males and females to enhance exercise capacity. Furthermore, literature has shown that children and adolescents with CF engage in less rigorous PA than their healthy counterparts, despite having healthy lung function (Nixon *et al.,* 2001); and females with CF engaged in less PA in general compared to males with CF (Schneiderman-Walker *et al.,* 2005). Therefore, the level of habitual PA should be considered as it is possible that females have a lower exercise capacity due to less participation in PA, rather than a direct result of a sex effect.

Many differences between sexes in CPET parameters are removed by accounting for body size or could be influenced by body size. In the clinical environment it would be more appropriate to compare group's parameters when scaled for body size variables. When age, lung function, body size and maturation are all taken into account, females still had a lower exercise capacity suggesting the possibility that differences in muscle size, efficiency or cellular variances could be the cause. Future research should aim to allometrically scale all exercise parameters to aid in comparisons and perhaps use of FFM or MM may be more appropriate than the body size variables used in the present analysis. It is likely that controlling for FFM or MM will explain additional variance in $\dot{V}O_{2peak}$ but still leaving some variance unexplained. Previous research has identified that mild to moderate CF participants experience changes in aerobic metabolism compared to healthy individuals that was intensity dependent (Saynor *et al.,* 2016). Therefore, these observations propose muscle oxygen extraction and utilisation is impaired in mild to moderate CF and future research should look to identify whether muscle oxygen utilisation and extraction are key to the differences in

maximal exercise capacity between the sexes in children with CF. Perhaps clinics around the UK should implement dual-energy X-ray absorptiometry scans, for measures of FFM or MM, into their annual review alongside basic measures of body size. This could help to provide a greater insight into body composition and enable more in-depth analyses of CPET results in the future. Although the small radiation risk, equivalent to less than two days exposure to natural background radiation (NHS, 2019), should be considered at an individualised level prior to any scan.

Additional correlational analyses may be required to further our understanding of relationships between clinical variables and CPET parameters. FEV_1 is correlated strongly to GET and relative peak power, but it may be similar relationships can be used to predict submaximal and maximal measures in clinics that cannot perform CPETs.

There is the scope that these results can be used as reference data for the south west region of the UK and specifically the RD&E hospital for ages 8 – 25 y. Furthermore, the systems in place to record data could be improved. At the present, clinical data is stored separately to CPET files and some CPET measures are in paper form making it difficult to combine all parameters for an overview of the patient. If the system is upgraded then it may help healthcare professionals e.g., physiotherapists and technicians see the 'whole picture' to better aid in the care of patients.

5.5 Limitations

The main limitation to this thesis is the lack of other body compositional measures such as FFM and MM that a prospective study could have been collected. With the addition of these parameters, the influence of body size could have been

entirely ruled out, leaving other qualitative parameters to explain any remaining variances. However, this retrospective analysis has made use of existing data from the hospital, which may not have been comprehensively analysed otherwise due to the busy schedules of clinical staff.

CPETs were completed in the clinical environment rather than the laboratory, therefore were subject to practical constraints such as appointment times, different experimenters, faulty equipment and annual review occurring on separate days to the exercise test. This meant in some cases the S_{max} was not completed or HR did not register, which may have been resolved if in the controlled scientific laboratory environment. Furthermore, CPETs used in this thesis were taken as the first CPET completed at the RD&E hospital, which may not have been the first CPET the patient had ever done, potentially meaning there may have been the issue of a learning effect between ramp and S_{max} or between patients. Although rigorous statistical analyses produced similar findings to previous literature, which confirms this may not have had such an impact on the results and conclusions.

Another limitation in the present analysis was that FEV_1 and other clinical parameters were not taken in conjunction with the exercise test due to practicalities of working within a clinical environment. However, this effect may be limited as studies have shown that the relationship between $FEV_{1\%predicted}$ and $\dot{V}O_{2peak}$ is not perfect, 50 % variance in $\dot{V}O_{2max}$ explained by $FEV_{1\%predicted}$ and r = 0.71 (Pastre *et al.,* 2014). Hence, the small variance in time between CPET and annual review may be negligible.

Additionally, a control group of healthy males and females would have provided further insight into whether the sex differences noted in the present analyses were

due to sex or an interaction of sex and CF. The differences observed here may be the same noted in healthy females and therefore not any different when in terms of CF. Furthermore, it is possible that this sample group is biased towards the fitter patients as taking part in the CPET is optional and often those with contraindications or very severe CF were not able to undertake the test.

5.6 Conclusion

In summary, this thesis demonstrates that in a young CF population, $\dot{V}O_{2peak}$ is significantly reduced in females compared to males. These observations were persistent throughout scaling, irrespective of the method or body size variable used, and after controlling for age, maturity and nutritional status. Previous literature has not yet comprehensively utilised these methods to determine sex differences in exercise capacity, therefore this thesis allows further interpretation of sex differences in aerobic fitness for CF populations.

REFERENCES

Abe, T., Kearns, C. F. and Fukunaga. T. (2003) Sex differences in whole body skeletal muscle mass measures by magnetic resonance imaging and its distribution in young Japanese adults, *British Journal of Sports Medicine*, 37(5), pp. 436-40.

Adegboye, A. R., Anderssen, S. A., Froberg, K., Sardinha, L. B., Heitmann, B. L. Steene-Johannessen, J., Kolle, E. and Andersen, L. B. (2011) Recommended aerobic fitness level for metabolic health in children and adolescents: a study of diagnostic accuracy, *British Journal of Sports Medicine*, 45(9), pp. 722-8.

Armstrong, N. (2006) Aerobic fitness of children and adolescents, *Journal of Pediatrics,* 82(6), pp. 406-8.

Armstrong, N., Kirby, B. J., McManus, A. M. and Welsman, J. R. (1997) Prepubescents' ventilatory responses to exercise with reference to sex and body size, *Chest*, 112(6), pp. 1554-60.

Baba, R., Nagashima, M., Goto, M., Nagano, Y., Yokota, M., Tauchi, N. and Nishibata, K. (1996) Oxygen uptake efficiency slope: a new index of cardiorespiratory functional reserve derived from the relation between oxygen uptake and minute ventilation during incremental exercise, *Journal of the American College of Cardiology*, 28(6), pp. 1567-72.

Barak, A., Wexler, I. D., Efrati, O., Bentur, L., Augarten, A., Mussaffi, H., Avital, A., Rivlin, J., Aviram. M., Yahav, Y. and Kerem, E. (2005) Trampoline use as physiotherapy for cystic fibrosis patients, *Pediatric Pulmonology*, 39(1), pp. 70-3.

Barker, A. R., Welsman, J. R., Fulford, J., Welford, D. and Armstrong, N. (2010) Quadriceps muscle energetics during incremental exercise in children and adults, *Medicine and Science in Sports and Exercise*, 42, pp. 1303-13.

Barker, A. R., Williams, C. A., Jones, A. M. and Armstrong, N. (2011) Establishing maximal oxygen uptake in young people during a ramp cycle test to exhaustion, *British Journal of Sports Medicine*, 45 (6), pp. 498-503.

Baxter-Jones, A. D. G., Eisenmann, J. C. and Sherar, L. B. (2005) Controlling for Maturation in Pediatric Exercise Science, *Pediatric Exercise Science*, 17(1), pp. 18-30.

Beaver, W. L., Wasserman, K. and Whipp, B. J. (1986) A new method for detecting anaerobic threshold by gas exchange, *Journal of Applied Physiology* (1985), 60(6), pp. 2020-7.

Benson, L. N., Newth, C. J. L., Desouza, M., Lobraico, R., Kartodihardjo, W., Corkey, C., Gilday, D. and Olley, P. M. (1984) Radionuclide assessment of right and left ventricular function during bicycle exercise in young patients with cystic fibrosis, *American Review of Respiratory Disease*, 130, pp. 987-92.

Berg, A. H. and Scherer, P. E. (2005) Adipose tissue, inflammation, and cardiovascular disease, *Circulation Research*, 96, pp. 939-49.

Bhatti, U., Rani, K. and Memon, M. Q. (2014) Variation in lung volumes and capacities among young males in relation to height, *Journal of Ayub Medical College Abbottabad*, 26(2), pp 200-2.

Blimkie, J. R. and Sale D. G. (1998) Strength development and trainability during childhood. In: Van Praagh E (ed.) *Pediatric anaerobic performance.*

Champaign, IL: Human Kinetics; p. 193– 224.

Boas, S.R., Joswiak, M,L., Nixon, P.A., Fulton, J.A. and Orenstein, D.M. (1996) Factors limiting anaerobic performance in adolescent males with cystic fibrosis, *Medicine and Science in Sports and Exercise*, 28 (3), pp. 291-8.

Bongers, B. C., De Vries, S. I., Helders, P. J. M. and Takken, T. (2013) The Steep Ramp Test in Healthy Children and Adolescents: Reliability and Validity, *Medicine and Science in Sports and Exercise*, 45(2), pp. 366-71.

Bongers, B. C., Werkman, M. S., Takken, T. and Hulzebos, E. H. J. (2014a) Ventilatory response to exercise in adolescents with cystic fibrosis and mild to moderate airway obstruction, *Springerplus*, doi: 10.1186/2193-1801-3-696.

Bongers, B. C., van Brussel, M., Hulzebos, E. H. J. and Takken, T. (2014b) *Pediatric norms for cardiopulmonary exercise testing: in relation to sex and age*. 2nd Edition. Uitgeverij BOXpress B.V.

Bongers, B. C., Werkman, M. S., Arets, H. G. M., Takken, T. and Hulzebos, E. H. J. (2015) A possible alternative exercise test for youths with cystic fibrosis: the steep ramp test, *Medicine and Science in Sport and Exercise*, 47 (3), pp. 485-92.

Borowitz, D., Baker, S. S., Duffy, L., Baker, R. D., Fitzpatrick, L., Gyamfi, J. and Jarembek, K. (2004) Use of fecal elastase-1 to classify pancreatic status in patients with cystic fibrosis, *Journal of Pediatrics,* 145(3), pp. 322-6.

Boyle, M. P. and De Boek, K. (2013). A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect, *The Lancet Respiratory Medicine*, 1, pp. 158-63.

Bradley, J. and Moran, F. (2008) Physical training for cystic fibrosis, *Cochrane Database of Systematic Reviews*, CD002768.

Bredella, M. A. (2017) Sex differences in body composition, *Advances in Experimental Medicine and Biology*, 1043, pp. 9-27.

Brischetto, M. J., Millman, R. P., Peterson, D. D., Silage, D. A. and Pack, A. I. (1984) Effect of aging on ventilatory response to exercise and CO2, *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 56(5), pp. 1143-50.

Buntain, H. M., Greer, R. M., Schluter, P. J., Wong, J. C. H., Batch, J. A., Potter, J. M., Lewindon, P. J., Powell, E., Wainwright, C. E. and Bell, S. C. (2004) Bone mineral density in Australian children, adolescents and adults with cystic fibrosis: a controlled cross sectional study, *Thorax*, 59(2), pp. 149-55.

Buys, R., Coekelberghs, E., Vanhees, L. and Cornelissen, V. A. (2015) The oxygen uptake efficiency slope in 1411 Caucasian healthy men and women ages 20-60 years: reference values, *European Journal of Preventative Cardiology*, 22(3), pp. 356-63.

Caspersen, C. J., Powell, K. E. and Christenson, G. M (1985) Physical activity, exercise and physical fitness: definitions and distinctions for health-related research, *Public Health Reports*, 100(2), pp. 126-3

Causer, A. J., Shute, J. K., Cummings, M. H., Shepherd, A. I., Bright, V., Connett, G., Allenby, M. I., Carroll, M. P., Daniels, T. and Saynor, Z. L. (2018) Cardiopulmonary exercise testing with supramaximal verification produces a safe and valid assessment of Vo2max in people with cystic fibrosis: a retrospective analysis, *Journal of Applied Physiology (1985),* 125(4), pp. 1277-83.

Cohen, J. (1988) *Statistical power analysis for the behavioural sciences*. 2nd Ed. Hillsdale: Lawrence Erlbaum.

Cole, T. J., Bellizzi, M. C., Flegal, K. M. andDietz, W. H. (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey, *British Medical Journal*, 320(7244), pp. 1240-3.

Cole, T. J., Flegal, K. M., Nicholls, D. and Jackson, A. A. (2007) Body mass index cut offs to define thinness in children and adolescents: international survey, *British Medical Journal*, 335(7612), pp. 194.

Conway, S. P., Morton, A., Oldroyd, B., Truscott, J., White, H., Smith, A. and Haigh, I. (2000) Osteoporosis and osteopenia in adults and adolescents with cystic fibrosis: prevalence and associated factors, *Thorax*, 55(9), pp. 798-804.

Corey, M., Edwards, L., Levison, H. and Knowles, M. (1997) Longitudinal analysis of pulmonary function decline in patients with cystic fibrosis, *Journal of Pediatrics*, 131(6), pp. 809-14.

Coutinho, T., Borlaug, B. A., Pellikka, P. A., Turner, S. T. and Kullo, I. J. (2013) Sex differences in arterial stiffness and ventricular arterial interactions, *Journal of the American College of Cardiology,* 61(1), pp. 96-103.

Cystic Fibrosis Foundation (2009) *Patient Registry: Annual Data Report 2009*.

Cystic Fibrosis Foundation (2017a) *Types of CFTR mutations*. Available: https://www.cff.org/What-is-CF/Genetics/Types-of-CFTR-Mutations/ [Accessed October 2017]

Cystic Fibrosis Foundation (2017b) *Cystic fibrosis related diabetes mellitus*. Available: https://www.cff.org/Life-With-CF/Daily-Life/Cystic-Fibrosis-Related-Diabetes/ [Accessed October 2017]

Cystic Fibrosis Foundation (2018) *Bronchodilators.* Available: https://www.cff.org/Life-With-CF/Treatments-and-Therapies/Medications/Bronchodilators/ [Accessed November 2018]

Cystic Fibrosis Foundation (2019) *Nutritional Basics.* Available: https://www.cff.org/Life-With-CF/Daily-Life/Fitness-and-Nutrition/Nutrition/Getting-Your-Nutrients/Nutritional-Basics/ [Accessed April 2019]

Cystic Fibrosis Trust (2007) *Bone Mineralisation in cystic fibrosis*. Factsheet.

Cystic Fibrosis Trust (2009) *Antibiotic Treatment for cystic fibrosis.* Factsheet.

Cystic Fibrosis Trust (2015a) *UK Cystic Fibrosis Trust Registry 2014 Annual Data Report*.

Cystic Fibrosis Trust (2015b) *Family genetic testing: the family cascade screening programme for cystic fibrosis*. Factsheet.

Cystic Fibrosis Trust (2016) *European Cystic Fibrosis Society annual data report 2013*

Cystic Fibrosis Trust (2017a) *Frequently asked questions about cystic fibrosis*. Available: https://www.cysticfibrosis.org.uk/what-is-cystic-fibrosis/faqs [Accessed November 2017]

Cystic Fibrosis Trust (2017b) *Cystic Fibrosis Related Liver Disease*. Factsheet.

Cystic Fibrosis Trust (2017c) *UK Cystic Fibrosis Registry annual data report 2016*

Cystic Fibrosis Trust (2018a) *Cystic fibrosis treatments and medications*. Available: https://www.cysticfibrosis.org.uk/what-is-cystic-fibrosis/cystic-fibrosis-care/treatments-and-medication [Accessed November 2018]

Cystic Fibrosis Trust (2018b) *Nutrition and cystic fibrosis.* Available: https://www.cysticfibrosis.org.uk/what-is-cystic-fibrosis/cystic-fibrosis-care/nutrition [Accessed November 2018]

Cystic Fibrosis Trust (2018c) *Cystic Fibrosis Physiotherapy*. Available: https://www.cysticfibrosis.org.uk/what-is-cystic-fibrosis/cystic-fibrosis-care/physiotherapy [Accessed November 2018]

Cystic Fibrosis Trust (2018d) *Cystic fibrosis and organ transplants.* Available: https://www.cysticfibrosis.org.uk/what-is-cystic-fibrosis/cystic-fibrosis-care/transplant-information-and-resources/cystic-fibrosis-and-organ-transplants [Accessed November 2018]

Cystic Fibrosis Trust (2018e) *Staying active*. Available: https://www.cysticfibrosis.org.uk/what-is-cystic-fibrosis/cystic-fibrosis-care/staying-active [Accessed November 2018]

Cystic Fibrosis Trust (2018f) *UK Cystic Fibrosis Registry annual data report 2017*

Dalleck, L. C., Astorino, T. A., Erickson, R. M., McCarthy, C. M., Beadell, A. A. and Botten, B. H. (2012) Suitability of verification testing to confirm attainment of VO2max in middle-aged and older adults, *Research in Sports Medicine*, 20 (2), 118-28.

Davies, C. T., Barnes, C. and Godfrey, S. (1972) Body composition and maximal exercise performance in children, *Human Biology*, 44(2), pp. 195-214.

de Jong, W., Kaptein, A. A., van der Schans, C. P., Mannes, G. P., van Aalderen, W. M., Grevink, R. G. and Koëter, G. H. (1997) Quality of life in patients with cystic fibrosis, *Pediatric Pulmonology*, 23, 95-100.

de Meer, K., Gulmans, V. A. and Van Der Laag, J. (1999) Peripheral muscle weakness and exercise capacity in children with cystic fibrosis, *American Journal of Respiratory and Critical Care Medicine*, 159, pp. 748-54.

de Meer, K., Jeneson, J.A.L., Gulmans, V.A.M., van der Laag, J. and Berger, R. (1995) Efficiency of oxidative work performance of skeletal muscle in patients with cystic fibrosis, *Thorax*, 50, pp. 980–3.

Dedoussis, G. V., Kapiri, A., Samara, A., Dimitriadis, D., Lambert, D., Pfister, M., Siest, G. and Visvikis-Seist, S. (2010) Expression of inflammatory molecules and associations with BMI in children, *European Journal of Clinical Investigation*, 40, pp. 388-92.

Demko, C. A., Byard, P. J. and Davis, P. B. (1995) Gender differences in cystic fibrosis: Pseudomonas aeruginosa infection, *Journal of Clinical Epidemiology*, 48(8), pp. 1041-9.

Dencker, M., Thorsson, O., Karlsson, M. K., Linden, C., Eiberg, S., Wollmer, P. and Anderson, L. B. (2007) Gender differences and determinants of aerobic fitness in children aged 8-11 years, *European Journal of Applied Physiology*, 99(1), pp. 19-26.

Deschenes, M. R., Hillard, M. N., Wilson, J. A., Dubina, M. I. and Eason, M. K. (2006), Effects of gender on physiological responses during submaximal exercise and recovery, *Medicine and Science in Sports and Exercise*, 38(7), pp. 1304-10.

Devries, M. C., Hamadeh, M. J., Phillips, S. M. and Tarnopolsky, M. A. (2006) Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise, *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 291(4), pp. 1120-8.

Divangahi, M., Balghi, H., Danialou, G., Comtois, A.S., Demoule, A., Ernest, S., Haston, C., Robert, R., Hanrahan, J.W., Radzioch, D. and Petrof, B.J. (2009) Lack of CFTR in skeletal muscle predisposes to muscle wasting and diaphragm muscle pump failure in cystic fibrosis mice, PLOS *Genetics*, 5(7):e1000586, doi: 10.1371/journal.pgen.1000586.

Dodge, J., Lewis, P., Stanton, M. and Wilsher, J. (2007) Cystic fibrosis mortality and survival in the UK: 1947-2003, *European Respiratory Journal*, 29(3), pp. 522-6.

Du Bois, D. and Du Bois, E. F. (1989) A formula to estimate the approximate surface area if height and weight be known 1916, *Nutrition*, 5(5), pp. 303-311.

Dunnink, M. A., Doeleman, W. R., Trappenburg, J. C. and De Vries, W. R. (2009) Respiratory muscle strength in stable adolescent and adult patients with cystic fibrosis*, Journal of Cystic Fibrosis*, 8, pp. 31-6.

Eason, J. M., Schwartz, G. A., Pavlath, G. K. and English, A. W. (2000) Sexually dimorphic expression of myosin heavy chains in the adult mouse masseter, *Journal of Applied Physiology*, 89(1), pp. 251-8.

Erickson, M.L., Seigler, N., McKie, K.T., McCully, K.K. and Ryan, A.H. (2015) Skeletal muscle oxidative capacity in patients with cystic fibrosis, *Experimental Physiology*, 100, pp. 545–52.

Fawkner, S. G. and Armstrong, N. (2004) Sex differences in the oxygen uptake kinetic response to heavy intensity exercise in pre-pubertal children, *European Journal of Applied Physiology*, 93, pp. 210-6.

Ferrazza, A. M., Martolini, D., Valli, G. and Palange, P. (2009) Cardiopulmonary exercise testing in the functional and prognostic evaluation of patients with pulmonary diseases, *Respiration*, 77, pp. 3-17.

Ferreira, R. G., Worthington, A., Huang, C., Aranki, S. F. and Muehlschlegel, J. D. (2015) Sex differences in prevalence of diastolic dysfunction in cardiac surgical patients, *Journal of Cardiac Surgery,* 30(3), pp. 238-45.

Fielding, J., Brantley, L., Seigler, N., McKie, K. T., Davison, G. W. and Harris, R. A. (2015) Oxygen uptake kinetics and exercise capacity in children with cystic fibrosis, *Pediatric Pulmonology*, 50(7), pp. 647-54.

Fitzsimmons, S. C. (1993) The changing epidemiology of cystic fibrosis, *Journal of Pediatrics*, 122(1), pp. 1-9.

Flume, P. A., Mogayzel, P. J. Jr., Robinson, K. A., Goss, C. H., Rosenblatt, R. L., Kuhn, R. J., Marshall, B. C. and Clinical practice guidelines for pulmonary therapies committee. (2009) Cystic Fibrosis Pulmonary Guidelines Treatment of Pulmonary Exacerbations, *American Journal of Respiratory and Critical Care Medicine*, 180, pp. 802-8.

Fogarty, A. W., Britton, J., Clayton, A. and Smyth, A. R. (2012) Are measures of body habitus associated with mortality in cystic fibrosis? *Chest*, 142(3), pp. 712-7.

Foster, K., Huang, G., Zhang, N., Crisalli, J., Chini, B., Amin, R. and Elder, D. (2018) Relationship between exercise capacity and glucose tolerance in cystic fibrosis, *Pediatric Pulmonology*, 53(2), pp. 154-61.

Gandevia, S. C. (2001) Spinal and Supraspinal Factors in Human Muscle Fatigue, *Physiological Reviews*, 81(4), pp. 1725-89.

Ghosh, A. K. (2004) Anaerobic threshold: its concept and role in endurance sport, *Malaysian Journal of Medical Sciences*, 11(1), pp. 24-36.

Giacchi, V., Rotolo, N., Amato, B., Di Dio, G., Betta, P., La Rosa, M., Leonardi, S. and Sciacca, P. (2015) Heart involvement in children and adults with cystic fibrosis: Correlation with pulmonary indexes and inflammation markers, *Heart, Lung and Circulation*, 24(10), pp. 1002-10.

Godfrey, S. and Mearns, M. (1971) Pulmonary function and response to exercise in cystic fibrosis, *Archives of Disease in Childhood*, 46(246), pp. 144-51.

Godfrey, S., Davies, C. T. M., Wozniak, E. and Barnes, C. A. (1971) Cardiorespiratory response to exercise in normal children*, Clinical Science*, 40, pp. 419-31.

Goedecke, J. H., Gibson, A., Grobler, L., Collins, M., Noakes, T. D. and Lambert, E. V. (2000) Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes*, American Journal of Physiology-Endocrinology and Metabolism*, 279(6), pp. E1325-E1334.

Gruber, W., Orenstein, D. M., Braumann, K. M., Paul, K. and Huls, G. (2011) Effects of an exercise program in children with cystic fibrosis: are there

differences between females and males? *Journal of Pediatrics*, 158(1), pp. 71-6.

Gruet, M., Brisswalter, J., Mely, L. and Vallier, J. M. (2010) Clinical utility of the oxygen uptake efficiency slope in cystic fibrosis patients, *Journal of Cystic Fibrosis*, 9(5), pp. 307-13.

Gruet, M., Decorte, N., Mely, L., Vallier, J. M., Camara, B., Quetant, S., Wuyam, B. and Verges, S. (2016) Skeletal muscle contractility and fatigability in adults with cystic fibrosis, *Journal of Cystic Fibrosis*, 15(1), pp. e1-8.

Gutiérrez, C. M. A., Olivo, M. H. and Moreno, R. M. G. (2016) Dehydration in Adult Cystic Fibrosis Patients, *Archivos de Bronconeumología (English Edition),* 52(7), pp. 398-9.

Haizlip, K. M., Harrison, B. C. and Leinwand, L. A. (2015) Sex-based differences in skeletal muscle kinetics and fiber-type composition, *Physiology*, 30(1), pp. 30-9.

Hameedullah, Khan, S. B., Khan, S. S., Khan, Z. A., Shah, I. and Hafizullah, M. (2015) Gender differences in left ventricular diastolic dysfunction in normotensive type 2 diabetic patients, *Pakistan Heart Journal*, 45(2), pp. 74-80.

Hankard, R., Munck, A. and Navarro, J. (2002) Nutrition and growth in cystic fibrosis, *Hormone Research*, 58, pp. 16-20.

Hanna, R. M. and Weiner, D. J. (2015) Overweight and obesity in patients with cystic fibrosis: A center-based analysis, *Pediatric Pulmonology*, 50, pp.35-41.

Harness-Brumley, C. L., Elliott, A. C., Rosenbluth, D. B., Raghavan, D. and Jain, R. (2014) Gender differences in outcomes of patients with cystic fibrosis, *Journal of Women's Health*, 23(12), pp. 1012-20.

Haworth, C. S., Selby, P. L., Horrocks, A. W., Mawer, E. B., Adams, J. E. and Webb, A. K. (2002) A prospective study of change in bone mineral density over one year in adults with cystic fibrosis, *Thorax*, 57(8), pp. 719-23.

Haycock, G. B., Schwartz, G. J. and Wisotsky, D. H. (1978) Geometric Method for Measuring Body Surface Area: A Height-Weight Formula Validated in Infants, Children and Adults, *Journal of Pediatrics*, 93(1), pp. 62-6.

Hebestreit, H., Arets, H. G. M., Aurora, P., Boas, S., Cerny, F., Hulzebos, E. H. J., Karila, C., Lands, L. C., Lowman, J. D., Swisher, A. and Urquhart, D. S. (2015) Statement on Exercise Testing in Cystic Fibrosis, *Respiration,* 90, pp. 332-51.

Huang, N. N., Schidlow, D. V., Szatrowski, T. H., Palmer, J., Laraya-Cuasay, L. R., Yeung, W., Hardy, K., Quitell, L. and Fiel, S. (1987) Clinical features, survival rate, and prognostic factors in young adults with cystic fibrosis, *American Journal of Medicine*, 82(5), 871-9.

Hulke, S. M., Phatack, M. S. and Vaidya, Y. P. (2012) Cardiorespiratory response to aerobic exercise programs with different intensity: 20 weeks longitudinal study, *Journal of Research in Medical Science,* 17(7), pp. 649-55.

Hulzebos, E. H., Bomhof-Roordink, H., Van De Weert-Van Leeuwen, P. B., Twisk, J. W., Arets, H. G., Van Der E, C. K. and Takken, T. (2014) Prediction of mortality in adolescents with cystic fibrosis, *Medicine and Science in Sports and Exercise*, 46, pp. 2047-52.

Hulzebos, H. J., Werkman, M. S., Van Brussel, M. and Takken, T. (2012) Towards an individualized protocol for workload increments in cardiopulmonary exercise testing in children and adolescents with cystic fibrosis, *Journal of Cystic Fibrosis,* 11, pp. 550-4.

Hussey, J., Gormley, J., Leen, G. and Greally, P. (2002) Peripheral muscle strength in young males with cystic fibrosis, *Journal of Cystic Fibrosis*, 1, pp. 116- 21.

Ionescu, A. A., IONESCU, A. A., Payne, N., Obieta-Fresnedo, I., Fraser, A. G. and Shale, D. J. (2001) Subclinical right ventricular dysfunction in cystic 61 fibrosis. A study using tissue Doppler echocardiography, *American Journal of Respiratory and Critical Care Medicine*, 163, pp. 1212-8.

Kerem, B., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M. and Tsui, L. (1989) Identification of the cystic fibrosis gene: Genetic analysis, *Science*, 245, pp. 1073-80.

Kerem, E., Reisman, J., Corey, M., Canny, G. J. and Levison, H. (1992) Prediction of mortality in patients with cystic fibrosis, *New England Journal of Medicine*, 326(18), pp. 1187-91.

Klijn, P. H. C., Van Der Net, J., Kimpen, J. L., Helders, P. J. M. and Van Der Ent, C. K. (2003) Longitudinal determinants of peak aerobic performance in children with cystic fibrosis, *Chest*, 124, pp. 2215-9.

Lamireau, T., Martin, S., Lallier, M., Marcotte, J. E. and Alvarez, F. (2006) Liver transplantation for cirrhosis in cystic fibrosis, *Canadian Journal of Gastroenterology*, 20(7), pp. 475-8.

Lamonwah, A.M., Bear, C.E., Huan, L.J., Chiaw, P.K., Ackerley, C.A. and Tein, I. (2010) Cystic fibrosis transmembrane conductance regulator in human muscle dysfunction causes abnormal metabolic recovery in exercise, *Annals of Neurology*, 67, pp. 802-8.

Laukkanen, J. A., Kurl, S., Salonen, J. T., Lakka, T. A. and Rauramaa, R. (2006) Peak oxygen pulse during exercise as a predictor for coronary heart disease and all cause death, *Heart (British Cardiac Society),* 92(9), pp. 1219-24.

Leroy, S., Perez, T., Neviere, R., Aguilaniu, B. and Wallaert, B. (2011) Determinants of dyspnea and alveolar hypoventilation during exercise in cystic fibrosis: impact of inspiratory muscle endurance, *Journal of Cystic Fibrosis*, 10, pp. 159-65.

Liou, T. G., Adler, F. R., Fitzsimmons, S. C., Cahill, B. C., Hibbs, J. R. and Marshall, B. C. (2001) Predictive 5-year survivorship model of cystic fibrosis, *American Journal of Epidemiology*, 153(4), pp. 345-52.

Lobelo, F., Stoutenberg, M. and Hutber, A. (2014) The Exercise is Medicine Global Health Initiative: 2014 update, *British Journal of Sports Medicine*, 48(22), pp.1-8.

Loomba-Albrecht, L. A. and Styne, D. M. (2009) Effect of puberty on body composition, *Current Opinion in Endocrinology, Diabetes, and Obesity,* 16(1), pp. 10-5.

Malina, R. M. (2011) Skeletal age and age verification in youth sport, *Sports Medicine*, 41(11), pp. 925-47.

https://doi.org/10.2165/11590300-000000000-00000 PMID: 21985214

Marinov, B., Mandadzhieva, S. and Kostianev, S. (2007) Oxygen-uptake efficiency slope in healthy 7- to 18-year-old children, *Pediatric Exercise Science*, 19(2), pp. 159-70.

Marshall, B. C., Butler, S. M., Stoddard, M., Moran, A. M., Liou, T. G. and Morgan, M. D. (2005) Epidemiology of cystic fibrosis-related diabetes, *The Journal of Pediatrics*, 146(5), pp. 681-7.

McIlwaine, M. (2007) Chest physical therapy, breathing techniques and exercise in children with CF, *Paediatric Respiratory Reviews*, 8(1), pp. 8-16.

McKone, E. F., Barry, S. C., FitzGerald, M. X. and Gallagher, C. G. (1999) Reproducibility of maximal exercise ergometer testing in patients with cystic fibrosis, *Chest*, 116, pp. 363-8.

McNarry, M. A., Farr, C., Middlebrooke, A., Welford, D., Breese, B., Armstrong, N. and Barker, A. R. (2015) Aerobic function and muscle deoxygenation dynamics during ramp exercise in children, *Medicine and Science in Sports and Exercise*, 47(9), pp. 1877-84.

Metro Health (2018) Pulmonary Function Test (PFT). Available: http://metrohealthgh.com/pulmonary-function-test-pft/ [Accessed November 2018]

Mirwald, R. L., Baxter-Jones, A. D. and Bailey, D. A. (2002) An assessment of maturity from anthropometric measurements, *Medicine and Science in Sport and Exercise*, 34(4), pp. 689-94.

Moorcroft, A. J., Dodd, M. E. and Webb, A. K. (1997) Exercise testing and prognosis in adult cystic fibrosis, *Thorax*, 52(3), pp. 291-3.

Moorcroft, A. J., Dodd, M. E., Morris, J. and Webb, A. K. (2005) Symptoms, lactate and exercise limitation at peak cycle ergometry in adults with cystic fibrosis, *European Respiratory Journal*, 25, pp. 1050-6.

Moore, S. A., McKay, H. A., Macdonald, H., Nettlefold, L., Baxter-Jones, A. D., Cameron, N. and Brasher, P. M. (2015) Enhancing a Somatic Maturity Prediction Model, *Medicine and Science in Sports and Exercise*, 47(8), pp. 1755-64.

Moran, A., Dunitz, J., Nathan, B., Saeed, A., Holme, B. and Thomas, W. (2009) Cystic fibrosis-related diabetes: Current trends in prevalence, incidence, and mortality, *Diabetes Care*, 32(9), pp. 1626-31.

Moser, C., Tirakitsoontorn, P., Nussbaum, E., Newcomb, R. and Cooper, D. M. (2000) Muscle size and cardiorespiratory response to exercise in cystic fibrosis, *American Journal of Respiratory and Critical Care Medicine*, 162, pp. 1823-7.

Mukaka, M. M. (2012) Statistics corner: A guide to appropriate use of correlation coefficient in medical research, *Malawi Medical Journal: the journal of Medical Association of Malawi,* 24(3), pp. 69-71.

National Health Service (2018) Faecal elastase. Available: https://www.southtees.nhs.uk/services/pathology/tests/faecal-elastase/ [Accessed November 2018]

National Health Service (2019) Bone density scan. Available: https://www.nhs.uk/conditions/DEXA-scan/ [Accessed October 2019]

National Institute for Health and Care Excellence (2018) Cystic Fibrosis. Available: https://www.nice.org.uk/guidance/qs168/chapter/quality-statement-3-treating-chronic-lung-infection [Accessed November 2018]

Neder, J. A., Nery, L. E. and Whipp, B. J. (2001) Reference values for dynamic responses to incremental cycle ergometry in males and females aged 20 to 80, *American Journal of Respiratory and Critical Care Medicine*, 164(8), pp. 1481-6.

Nixon, P. A., Orenstein, D. M., Kelsey, S. F. and Doershuk, C. F. (1992) The prognostic value of exercise testing in patients with cystic fibrosis, *New England Journal of Medicine*, 327, pp. 1785-8.

Pastre, J., Prevotat, A., Tardif, C., Langlois, C., Duhamel, A. and Wallaert, B. (2014) Determinants of exercise capacity in cystic fibrosis patients with mild to moderate lung disease, *BMC Pulmonary Medicine*, 14, pp. 74.

Perez, M., Groeneveld, I. F., Santana-Sosa, E., Fiuza-Luces, C., Gonzalez-Saiz, L., Villa-Asensi, J. R., Lopez-Mojares, L. M., Rubio, M. and Lucia, A. (2014) Aerobic fitness is associated with lower risk of hospitalization in children with cystic fibrosis, *Pediatric Pulmonology*, 49(7), pp. 641-9.

Pfaffenberger, S., Bartko, P., Graf, A., Pernicka, E., Babayev, J., Lolic, E., Bonderman, D., Baumgartner, H., Maurer, G. and Mascherbauer, J. (2013) Size Matters! Impact of Age, Sex, Height, and Weight on the Normal Heart Size, *Circulation: Cardiovascular Imaging*, 6(6), pp. 1073-9.

Pianosi, P., LeBlanc, J. and Almudevar, A. (2005a) Peak oxygen uptake and mortality in children with cystic fibrosis, *Thorax*, 60, pp.50-4.

Pianosi, P., LeBlanc, J. and Almudevar, A. (2005b) Relationship between FEV1 and peak oxygen uptake in children with cystic fibrosis, *Pediatric Pulmonology*, 40, pp. 324-9.

Pianosi, P. and Wolstein, R. (1996) Carbon dioxide chemosensitivity and exercise ventilation in healthy children and in children with cystic fibrosis, *Pediatric Research*, 40(3), pp. 508.

Poole, D. C., Wilkerson, D. P. and Jones, A. M. (2008) Validity of criteria for establishing maximal O_2 uptake during ramp exercise tests, *European Journal of Applied Physiology*, 102(4), pp. 403-10.

Prioux, J., Ramonatxo, M., Hayot, M., Mucci, P. and Prefaut, C. (2000) Effect of ageing on the ventilatory response and lactate kinetics during incremental exercise in man, *European Journal of Applied Physiology*, 81(1-2), pp. 100-7.

Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L., Culver, B. H., Enright, P. L., Hankinson, J. L., Ip, M. S., Zheng, J. and Stocks, J. (2012) Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations, *European Respiratory Journal*, 40(6), pp. 1324-43.

Radtke, T., Nevitt, S.J., Hebestreit, H. and Kriemler, S. (2017) Physical exercise training for cystic fibrosis, *Cochrane Database of Systematic Reviews*, 11, CD002768.

Ramsey, B. W. (2007) Use of Lung Imaging Studies as Outcome Measures for Development of New Therapies in Cystic Fibrosis*, Proceedings of the American Thoracic Society*, 4, pp. 359-63.

Rosenfeld, M., Davis, R., Fitzsimmons, S., Pepe, M. and Ramsey, B. (1997) Gender gap in cystic fibrosis mortality, *American Journal of Epidemiology*, 145(9), pp. 794-803.

Rosenthal, M., Narang, I., Edwards, L. and Bush, A. (2009) Non-invasive assessment of exercise performance in children with cystic fibrosis (CF) and non-cystic fibrosis bronchiectasis: Is there a CF specific muscle defect? *Pediatric Pulmonology*, 44, pp. 222-30.

Rowe, S. M., Miller, S. & Sorscher, E. J. (2005) Cystic fibrosis, *New England Journal of Medicine*, 352, pp. 1992-2001.

Rowland, T. W. and L. N. Cunningham (1997) Development of Ventilatory Responses to Exercise in Normal White Children: A Longitudinal Study, *Chest*, 111(2), pp. 327-32.

Sansum, K. M., Weston, M. E., Bond, B., Cockcroft, E. J., O'Connor, A., Tomlinson, O. W., Williams, C. A. and Barker, A. R. (2019) Validity of supramaximal test to verify maximal oxygen uptake in children and adolescents, *Pediatric Exercise Science*, 31(2), pp. 213-22.

Savi, D., Di Paolo, M., Simmonds, N., Onorati, P., Internullo, M., Quattrucci, S., Winston, B., Laveneziana, P. and Palange, P. (2015) Relationship between daily physical activity and aerobic fitness in adults with cystic fibrosis*, Pulmonary Medicine*, 15(59), doi: 10.1186/s12890-015-0036-9.

Saynor, Z. L., Barker, A. R., Oades, P. J. and Williams, C. A. (2013a) A protocol to determine valid VO2max in young cystic fibrosis patients, *Journal of Science and Medicine in Sport*, 16, pp. 539-44.

Saynor, Z. L., Barker, A. R., Oades, P. J. and Williams, C. A. (2013b) Reproducibility of maximal cardiopulmonary exercise testing for young cystic fibrosis patients, *Journal of Cystic Fibrosis,* 12, pp. 644-50.

Saynor, Z. L., Barker, A. R., Oades, P. J. and Williams, C. A. (2014) Impaired aerobic function in patients with cystic fibrosis during ramp exercise, *Medicine and Science in Sports and Exercise*, 46, pp. 2271-8.

Saynor, Z. L., Barker, A. R., Oades, P. J. and Williams, C. A. (2016) Impaired pulmonary VO2 kinetics in cystic fibrosis depend on exercise intensity*, Medicine and Science in Sports and Exercise*, 48(11), pp. 2090-9.

Saynor, Z. L., Causer, A. J., Allenby, M. I., Carroll, M., Connett, G., Daniels, T., Shute, J. K., Cummings, M. H. and Shepherd, A. I. (2018) Combined ramp and supramaximal cardiopulmonary exercise testing for individuals with cystic fibrosis, *Journal of Cystic Fibrosis*, 17(3), pp. S15.

Schneiderman-Walker, J., Wilkes, D. L., Strug, L., Lands, L. C., Pollock, S. L., Selvadurai, H. C., Hay, J., Coates, A. L. and Corey, M. (2005) Sex differences in habitual physical activity and lung function decline in children with cystic fibrosis, *Journal of Pediatrics*, 147(3), pp. 321-6.

Schorr, M., Dichtel, L. E., Gerweck, A. V., Valera, R. D., Torriani, M., Miller, K. K. and Bredella, M. A. (2018) Sex differences in body composition and association with cardiometabolic risk, *Biology of Sex Differences*, 9(1), pp. 28.

Seebauer, M., Sidler, M. A. and Kohl, J. (2003) Gender differences in workload effect on coordination between breathing and cycling, *Medicine and Science in Sports and Exercise*, 35(3), pp. 495-9.

Sellers, Z. M., Kovacs, A., Weinheimer, C. J. and Best, P. M. (2013) Left ventricular and aortic dysfunction in cystic fibrosis mice, *Journal of Cystic Fibrosis*, 12(5), pp. 517-24.

Selvadurai, H. C., Blimkie, C. J., Cooper, P. J., Mellis, C. M. and Van Asperen, P. P. (2004) Gender differences in habitual activity in children with cystic fibrosis, *Archives of Disease in Childhood*, 89(10), pp. 928-33.

Selvadurai, H. C., Blimkie, C. J., Meyers, N., Mellis, C. M., Cooper, P. J. and Van Asperen, P. P. (2002) Randomised controlled study of in-hospital exercise training programmes in children with cystic fibrosis, *Pediatric Pulmonology*, 33(3), pp. 194-200.

Singer, K., Eng, D. S., Lumeng, C. N., Gebremariam, A., and Lee, J. M. (2014) The relationship between body fat mass percentiles and inflammation in children, *Obesity*, 22(5), pp. 1332-6.

Smitt, C. P and Mehls, O. (2008) Disorders of bone mineral metabolism in chronic kidney disease, *Comprehensive Pediatric Nephrology*, pp. 773-92.

Southampton Children's Hospital (2016) *Meconium ileus*. Available: http://www.uhs.nhs.uk/OurServices/Childhealth/Neonatalsurgery/Conditionswetreat/MeconiumIleus.aspx [Accessed October 2017]

Staron, R. S., Hagerman, F. C., Hikida, R. S., Murray, T. F., Hostler, D. P., Crill, M. T., Ragg, K. E. and Toma, K. (2000) Fiber Type Composition of the Vastus Lateralis Muscle of Young Men and Women, *Journal of Histochemistry and Cytochemistry*, 48(5), pp. 623-9.

Stein, L., Pacht, C., Junge, S., Kaeding, T. S., Kuck, M., Maassen, N., Wittke, T. and Shuchakov, V. (2016) Skeletal Muscle Function in Young Patients With Cystic Fibrosis, *Pediatric Exercise Science*, 28(3), pp. 364-73.

Sue, D. Y., Wasserman, K., Moricca, R. B. and Casaburi, R. (1988) Metabolic acidosis during exercise in patients with chronic obstructive pulmonary disease: use of the V-slope method for anaerobic threshold determination, *Chest*, 94, pp. 931-8.

Sun, X., Hansen, J. E., Garatachea, N., Storer, T. W. and Wasserman, K. (2002) Ventilatory efficiency during exercise in healthy subjects, *American Journal of Respiratory and Critical Care Medicine*, 166(11), pp. 1443-8.

Tanner, J. M. (1949) Fallacy of per-weight and per-surface area standards, and their relation to spurious correlation, *Journal of Applied Physiology*, 2(1), pp. 1-15.

Thin, A. G., Linnane, S. J., McKone, E. F., Freaney, R., FitzGerald, M. X., Gallagher, C. G. and McLoughlin, P. (2002) Use of gas exchange threshold to noninvasively determine the lactate threshold in patients with cystic fibrosis, *Chest*, 121(6), pp. 1761-70.

Thomson, A.H. and Harris, A. (2008) *Cystic Fibrosis: the facts.* 4th Ed. New York, USA: Oxford University Press.

Tucker, M. A., Lee, N., Rodriguez-Miguelez, P., Looney, J., Crandall, R. H., Forseen, C., McKie, K. T. and Harris, R. A. (2019) Exercise testing in patients with cystic fibrosis – importance of ventilatory parameters, *European Journal of Applied Physiology*, 119, pp. 227-34.

Tucker, M.A., Berry, B., Seigler, N., Davison, G.W., Quindry, J.C., Eidson, D., McKie, K.T., and Harris, R.A. (2017) Blood flow regulation and oxidative stress during submaximal cycling exercise in patients with cystic fibrosis, *Journal of Cystic Fibrosis*, 17, pp. 1-8.

Van deWeert-van Leeuwen, P.B., Arets, H.G.M., van der Ent, C.K. and Beekman, J.M. (2013) Infection, inflammation and exercise in cystic fibrosis, *Respiratory Research*, 14, pp. 32.

Van deWeert-van Leeuwen, P.B., Slieker, M.G., Hulzebos, H.J., Kruitwagen, C.L., van der Ent, C.K. and Arets, H.G. (2012) Chronic infection and inflammation affect exercise capacity in cystic fibrosis, *European Respiratory Journal*, 39, pp. 893–8.

Vandekerckhove, K., Keyzer, M., Cornette, J., Coomans, I., Pyl, F., De Baets, F., Schelstraete, P., Haerynck, F., De Wolf, D., Van Daele, S. and Boone, J. (2017) Exercise performance and quality of life in children with cystic fibrosis and mildly impaired lung function: relation with antibiotic treatments and hospitalisation, *European Journal of Pediatrics,* doi: 10.1007/s00431-017-3024-7.

Vendrusculo, F. M., Heinzmann-Filho, J. P., da Silva, J. S., Perez Ruiz, M. and Donadio, M. V. F. (2019) Peak Oxygen Uptake and Mortality in Cystic Fibrosis: Systematic Review and Meta-Analysis, *Respiratory Care*, 64 (1), pp. 91-8.

Vieni, G., Faraci, S., Collura, M., Lombardo, M., Traverso, G., Cristadoro, S., Termini, L., Lucanto, M. C., Furnari, M. L., Trimarchi, G., Triglia, M. R., Costa, S., Pellegrino, S. and Magazzù, G. (2013) Stunting is an independent predictor of mortality in patients with cystic fibrosis, *Clinical Nutrition*, 32(3), 382-5.

Weert-van Leeuwen, P. B., Slieker, M. G., Hulzebos, H. J., Kruitwagen, C. L., van der Ent, C. K. and Arets, H. G. (2012) *Chronic infection and inflammation affect exercise capacity in cystic fibrosis, European Respiratory Journal*, 39(4), pp. 893-8.

Welsman, J. R. and Armstrong, N. (2000) Statistical techniques for interpreting body size related exercise performance during growth, *Pediatric Exercise Science*, 12, pp. 112-27.

Welsman, J. R., Armstrong, N., Kirby, B. J., Winsley, R. J., Parsons, G. and Sharpe, P. (1997) Exercise performance and magnetic resonance imaging determined thigh muscle volume in children, *European Journal of Applied Physiology and Occupational Physiology*, 76(1), pp. 92-7.

Werkman, M. S., Hulzebos, E. H., Helders, P. J., Arets, B. G. and Takken, T. (2014) Estimating peak oxygen uptake in adolescents with cystic fibrosis, *Archives of Disease in Childhood*, 99, pp. 21-5.

Werkman, M., Jeneson, J., Helders, P., Arets, B., van der Ent, K., Velthuis, B., Nievelstein, R., Takken, T. and Hulzebos, E. (2016) Exercise oxidative skeletal muscle metabolism in adolescents with cystic fibrosis, *Experimental Physiology*, 101(3), pp. 421-31.

Werkman, M.S. (2013) *Exercise testing, limitations and training in patients with cystic fibrosis: a personalised approach*. Zeist/Utrecht/Ridderkerk, Netherlands: Ridderprint BV.

Whipp, B. J. (2019) *The peak versus maximum oxygen uptake issue.* Available: https://www.researchgate.net/publication/264876075_the_peak_versus_maximum_oxygen_uptake_issue [Accessed June 2019].

Wiebe, C. G., Gledhill, N., Warburton, D. E., Jamnik, V. K. and Ferguson, S. (1998) Exercise cardiac function in endurance-trained males versus females, *Clinical Journal of Sport Medicine*, 8(4), pp. 272-9.

Wilkes, D. L., Schneiderman, J. E., Nguyen, T., Heale, L., Moola, F., Ratjen, F., Coates, A. L. and Wells, G. D. (2009) Exercise and physical activity in children with cystic fibrosis, *Paediatric Respiratory Reviews*, 10(3), pp. 105-9.

Williams, C. A., Benden, C., Stevens, D. and Radtke, T. (2010) Exercise training in children and adolescents with cystic fibrosis: Theory into practice*, Pediatrics*, 2010, doi: 10.1155/2010/670640.

Williams, C. A., Saynor, Z. L., Tomlinson, O. W. and Barker, A. R. (2014) Cystic fibrosis and physiological responses to exercise*, Expert Review of Respiratory Medicine*, 8, pp.751-62.

Williams, C. A., Tomlinson, O. W., Chubbock, L. V., Stevens, D., Saynor, Z. L., Oades, P. J. and Barker, A. R. (2018) The oxygen uptake efficiency slope is not a valid surrogate of aerobic fitness in cystic fibrosis, *Pediatric Pulmonology*, 53(1), pp. 36-42.

Wilmore, J. H., Stanforth, J. H., Gagnon, J., Rice, T., Mandel, S., Leon, A. S., Rao, D. C., Skinner, J. S. and Bouchard, C. (2001) Cardiac output and stroke volume changes with endurance training: the HERITAGE Family Study, *Medicine and Science in Sports and Exercise*, 33(1), pp. 99-106.

Wirth, A., TrAger, E., Scheele, K., Mayer, D., Dieham, K., Reischle, K. and Weicker, H. (1978) Cardiopulmonary adjustment and metabolic response to maximal and submaximal physical exercise of boys and girls at different stages of maturity, *European Journal of Applied Physiology and Occupational Physiology*, 39(4), pp. 229-40.

Yelling, M., Lamb, K. L. and Swaine, I. L. (2002) Validity of a pictorial perceived exertion scale for effort estimation and effort production during stepping exercise in adolescent children, *European Physical Education Review*, 8(2), pp. 157-75.

Zderic, T. W., Coggan, A. R. and Ruby, B. C. (2001) Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases, *Journal of Applied Physiology*, 90(2), pp. 447-53.

Zirbes, J. and Milla, C. (2009) Cystic fibrosis related diabetes, *Paediatric Respiratory Reviews*, 10(3), pp. 118-23.

Zhao, L., Peng, L., Wu, B., Bu, X. and Wang, C. (2016) Effects of dynamic hyperinflation on exercise capacity and quality of life in stable COPD patients, *The Clinical Respiratory Journal*, 10(5), pp. 579-88.

APPENDIX A

Health Research Authority Approval

NHS
Health Research Authority

Professor Craig Williams
Children's Health and Exercise Research Centre
University of Exeter
St. Luke's Campus
Heavitree Road
Exeter
EX1 2LU
C.A.Williams@exeter.ac.uk

Email: hra.approval@nhs.net

25 February 2016

Dear Professor Williams

Letter of HRA Approval

Study title:	**ANALYSIS OF CARDIOPULMONARY EXERCISE TESTING IN PATIENTS WITH CYSTIC FIBROSIS**
IRAS project ID:	**238996**
Protocol number:	**1718/25**
Sponsor	**University of Exeter**

I am pleased to confirm that HRA Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from the HRA website.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The attached document *'After HRA Approval – guidance for sponsors and investigators'* gives detailed guidance on reporting expectations for studies with HRA Approval, including:

- Working with organisations hosting the research
- Registration of Research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics and is updated in the light of changes in reporting expectations or procedures.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found through IRAS.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details on the HRA website.

Your IRAS project ID is **238996**. Please quote this on all correspondence.

IRAS project ID	238996

Yours sincerely

Gemma Oakes
Assessor

Email: hra.approval@nhs.net

Copy to: *Miss Chloe Bland, University of Exeter [Student]*
cb242@exeter.ac.uk
Ms Pam Baxter, University of Exeter [Sponsor Contact]
P.R.Baxter2@exeter.ac.uk
Mr Chris Gardner, Royal Devon and Exeter NHS Foundation Hospital
Professor Craig Williams [Lead NHS R&D Contact]
christopher.gardner@nhs.net

IRAS project ID	238996

Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

Document	Version	Date
Evidence of Sponsor Insurance or Indemnity (non NHS Sponsors only) [Insurance]		
HRA Schedule of Events	1	09 February 2018
HRA Statement of Activities	1	09 February 2018
IRAS Application Form [IRAS_Form_01022018]		01 February 2018
IRAS Application Form XML file [IRAS_Form_01022018]		01 February 2018
Letter from sponsor [Sponsor Letter]		15 January 2018
Other [Bland GCP]		
Referee's report or other scientific critique report [Supervisor report]		30 January 2018
Research protocol or project proposal [Protocol Overview]	1	15 January 2018
Summary CV for Chief Investigator (CI) [Craig Williams CV]		
Summary CV for student [Bland CV]		
Summary CV for supervisor (student research) [Alan Barker CV]		

IRAS project ID	238996

Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

For information on how the sponsor should be working with participating NHS organisations in England, please refer to the, *participating NHS organisations, capacity and capability* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections in this appendix.

The following person is the sponsor contact for the purpose of addressing participating organisation questions relating to the study:

Name: Ms Pam Baxter
Tel: 01392 723 588
Email: P.R.Baxter2@exeter.ac.uk

HRA assessment criteria

Section	HRA Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Not Applicable	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	The sponsor has submitted statement of activities and schedule of events for use as the agreement with the participating NHS site.
4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the activities expected of them for this

Section	HRA Assessment Criteria	Compliant with Standards	Comments
			research study.
4.3	Financial arrangements assessed	Yes	No external funding has been secured to run the study at site.
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	The applicant has confirmed research data will be retained for 5 years following completion of the study (and not 3 as originally stated in IRAS).
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Not Applicable	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
There is one site type participating in this study. The research activity taking place at this site is as follows: • A local member of staff (Dr Patrick Oades) will identify participant data (routinely collected data from routine clinical tests undertaken on children with cystic fibrosis) and provide in a pseudonymised format to the Chief Investigator. The information will be coded by the care team, a link to the data will be held at the participating NHS site only and the Chief Investigator will not access identifiable data.

The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website.

If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

Confirmation of Capacity and Capability

This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.

The HRA has determined that the participating NHS organisation in England **is not expected to formally confirm its capacity and capability to host this research**, because a local member of staff is required to extract routinely collected clinical data, anonymise it and provide it to the external research team to analyse on non-NHS premises only.

- The HRA has informed the relevant research management offices that you intend to undertake the research at their organisation. However, you should still support and liaise with these organisations as necessary.
- Following issue of the Letter of HRA Approval the sponsor may commence the study at these organisations when it is ready to do so.
- The document "Collaborative working between sponsors and NHS organisations in England for HRA Approval studies, where no formal confirmation of capacity and capability is expected" provides further information for the sponsor and NHS organisations on working with NHS organisations in England where no formal confirmation of capacity and capability is expected, and the processes involved in adding new organisations. Further study specific details are provided the *Participating NHS Organisations* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections of this Appendix.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).

Principal Investigators and Local Collaborators will not be required at the participating NHS site. Should additional NHS site types be added to the study then a new assessment of the need for Principal Investigators or Local Collaborators will be required.

Dr Patrick Oades (MD), Consultant Physician in Paediatrics, has been named as the local contact at the participating site to extract and provide the data to the external research team.

GCP training is not a generic training expectation, in line with the HRA/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken
As local members of staff (that already have contractual arrangements in place with the participating site) are extracting and providing anonymised data to the external research team to analyse on non-NHS premises, HR Good Practice arrangements are not required for this study.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.
The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

APPENDIX B

University of Exeter, Sport and Health Science Ethical Approval

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

St. Luke's Campus
University of Exeter
Heavitree Road
Exeter
EX1 2LU
United Kingdom

Certificate of Ethical Approval

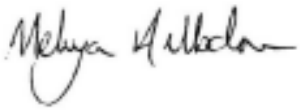
Proposal Ref No: 2017/M/06

Title: ANALYSIS OF CARDIOPULMONARY EXERCISE TESTING IN PATIENTS WITH CYSTIC FIBROSIS

Applicants: Chloe Bland, Prof Craig Williams, Owen Tomlinson, Dr Alan Barker

The proposal was reviewed by a Representative on the Committee.

Decision: This proposal has been approved until Feb 2019

Signature:  Date: 19/3/2018

Name/Title of Ethics Committee Reviewer: Dr Melvyn Hillsdon

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

APPENDIX C

Bongers Reference Values

Age	$\dot{V}O_{2peak}$	Reference Value	% Difference
Boys			
17.65	2.54	3.43	26
16.35	1.44	3.12	54
15.92	2.73	3.02	9
11.05	1.68	1.94	13
9.54	1.25	1.64	24
17.21	2.72	3.33	18
17.50	3.27	3.40	4
16.71	3.59	3.20	-12
9.30	1.08	1.59	32
17.74	2.20	3.46	36
12.07	1.24	2.15	42
10.92	1.30	1.91	32
17.05	3.08	3.29	6
13.22	2.16	2.40	10
8.82	1.19	1.50	21
13.49	2.25	2.46	9
13.75	1.46	2.52	42
13.97	3.29	2.57	-28
16.04	3.06	3.04	-1
Girls			
10.23	1.03	1.53	33
12.15	1.14	1.86	39
16.90	1.90	2.59	27
11.86	1.13	1.81	38
15.24	1.11	2.34	53

9.82	0.94	1.46	36
16.56	1.83	2.54	28
13.90	1.78	2.14	17
13.12	1.37	2.01	32
11.87	0.97	1.81	46
12.51	1.26	1.92	34
12.07	1.22	1.84	34