Cardiopulmonary exercise testing in the assessment and treatment of young people with cystic fibrosis

Submitted by Owen William Tomlinson to the University of Exeter
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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature:……

“It is easier to build strong children than to repair broken men”

- Frederick Douglass (1818 – 1895)

ABSTRACT

Cystic fibrosis (CF) is the most common, genetically inherited, life-shortening condition in the Caucasian population, with ~11,000 people in the United Kingdom having the disease. The genetic defect responsible for CF results in accumulation of thick, sticky mucus that blocks the airways and digestive systems. As there is currently no cure for CF, it is a disease that is managed using antibiotics, nutrition, physiotherapy and exercise. Exercise capacity, as measured by peak oxygen uptake ($\dot{V}O_{2peak}$), and where possible, maximal oxygen uptake ($\dot{V}O_{2max}$), is reduced in patients with CF and a low $\dot{V}O_{2peak}$ is associated with increased risk of hospitalisation, mortality and low quality of life. As a result, regular exercise testing is recommended, with cardiopulmonary exercise testing (CPET) considered the 'gold standard' procedure by leading international clinical organisations. The purpose of this thesis was to further our understanding surrounding the use of CPET in the assessment and treatment of children and adolescents with CF.

The first component of this thesis sought to identify and evaluate submaximal parameters of aerobic function derived from CPET, namely the oxygen uptake efficiency slope (OUES) and plateau (OUEP). Findings revealed that allometric scaling for body surface area (BSA) was necessary when evaluating OUES, and a power function of 1.40 (i.e. $OUES/BSA^{1.40}$) removed residual effects of body size (Chapter 4). Subsequently, results identified that the OUES was not a valid surrogate of aerobic fitness in CF, despite a significant correlation (r = 0.47, p = 0.004) with $\dot{V}O_{2max}$ when expressed relative to body mass, as it was unable to discriminate aerobic fitness within a CF group, nor against a control group (Chapter 5). As OUES was not a valid surrogate of aerobic fitness, the utility of OUEP as an independent marker of aerobic fitness was explored. Whilst the

OUEP was correlated with $\dot{V}O_{2peak}$ in CF, when expressed as an absolute value (r = 0.43, p = 0.010) and when allometrically scaled for body mass (r = 0.52, p = 0.001), it was unable to discriminate aerobic fitness to the same extent as $\dot{V}O_{2peak}$. However, the OUEP was associated with disease status and severity, being significantly ($p < 0.001$) lower in the CF group, but also significantly and positively correlated with lung function (forced expiratory volume in one-second [FEV_1]) in the CF group (r = 0.43, p = 0.010), a finding that warrants further, longitudinal investigation (Chapter 6).

The second component of this thesis utilised CPET to investigate musculoskeletal limitations to the reduced $\dot{V}O_{2max}$ that has previously been reported in CF. Parameters of muscle size (thigh cross-sectional area, muscle cross-sectional area and thigh muscle volume) were first quantified using magnetic resonance imaging, alongside the error associated with estimating muscle volume using alternative calculation techniques (Chapter 7). These parameters were then allometrically scaled for, which successfully removes residual effects of muscle size (i.e. muscle 'quantity') from $\dot{V}O_{2max}$. When this scaling is undertaken, $\dot{V}O_{2max}$ is lower in children with CF relative to age- and sex-matched controls, indicating that exercise capacity is not size-dependent in CF and that intrinsic muscular factors (i.e. muscle 'quality') are likely responsible for the reduced $\dot{V}O_{2max}$ observed in CF (Chapter 8).

Finally, the third component identified applications of CPET for both patients with CF and staff responsible for care. CPET, using a case-study approach, was utilised to describe exercise-related changes in an 11 year old female with CF following surgical insertion of a percutaneous endoscopic gastrostomy and overnight nutritional supplementation. This evaluation identified a maintenance of $\dot{V}O_{2max}$ over one year, in contrast to a fluctuation in FEV_1, and increase in body

mass index (BMI), therefore highlighting the independent prognostic information afforded by use of CPET (Chapter 9). Following this patient-centred application of CPET, two meetings were held with NHS staff, to provide a platform for exchange of ideas and best practice, but to also survey roles, responsibilities, prevalence of CPET and resources needed for effective implementation of exercise testing and training (Chapter 10).

In conclusion, this thesis has further highlighted the utility of CPET in the management of CF. Moreover, it has explored the prognostic and diagnostic properties of CPET, as well as its implementation for patients and staff alike.

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LIST OF ABBREVIATIONS

Δ	Difference
η^2	Partial eta squared (effect size)
6MWT	Six-minute walk test
^{31}P-MRS	^{31}Phosphorous-magnetic resonance spectroscopy
90% CI	90% confidence interval
95% CI	95% confidence interval
ACT	Airway clearance therapy
ADP	Adenosine diphosphate
ANOVA	Analysis of variance
aPHV	Age from peak height velocity
AT	Anaerobic threshold
ATP	Adenosine triphosphate
BIA	Bio-electrical impedance
BMI	Body mass index
$BMI_{\%}$	Body mass index percentile
BRI	Breathing reserve index
BSA	Body surface area
C_a	Oxygen concentration of arterial blood
CF	Cystic fibrosis
CFRD	Cystic fibrosis related diabetes
CFTR	Cystic fibrosis trans-membrane conductance regulator
CON	Control participants
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise testing
CSA	Cross sectional area

CSA_M	Cross sectional area of muscle
CSA_T	Cross sectional area of thigh
CV	Coefficient of variation
$C_{\bar{v}}$	Mean oxygen concentration of venous blood
ECFS	European Cystic Fibrosis Society
ERS	European Respiratory Society
ES	Effect size
FEV_1	Forced expiratory volume in one second
FFM	Fat-free mass
FVC	Forced vital capacity
GET	Gas exchange threshold
HR	Heart rate
HR_{max}	Maximal heart rate
ICC	Intraclass correlation coefficient
IMT	Inspiratory muscle training
LAS	Lung allocation score
LCI	Lung clearance index
LT	Lactate threshold
MBI	Magnitude based inference
mCSA	Muscle cross sectional area
MDT	Multi-disciplinary team
MRI	Magnetic resonance imaging
MST	Modified shuttle test
MV	Muscle volume
MVPA	Moderate to vigorous physical activity
MV_T	Thigh muscle volume

MVV	Maximal voluntary ventilation
NHS	National Health Service
NIRS	Near infra-red spectroscopy
OPEP	Oscillating positive expiratory pressure
OUE	Oxygen uptake efficiency
OUE_{GET}	Oxygen uptake efficiency at the gas exchange threshold
OUE_{RCP}	Oxygen uptake efficiency at the respiratory compensation point
OUEP	Oxygen uptake efficiency plateau
OUES	Oxygen uptake efficiency slope
PA	Physical activity
PCr	Phosphocreatine
PEG	Percutaneous endoscopic gastrostomy
PET-O_2	End-tidal tension of oxygen
PERT	Pancreatic enzyme replacement therapy
PEP	Positive expiratory pressure
PHV	Peak height velocity
P_i	Inorganic phosphate
$\dot{Q}$	Cardiac output
QoL	Quality of Life
RCP	Respiratory compensation point
RD&E	Royal Devon & Exeter NHS Foundation Trust Hospital
REC	Regional Ethics Committee
RER	Respiratory exchange ratio
rhDNase	Recombinant human deoxyribonuclease
SEE	Standard error of the estimate
S_{max}	Supramaximal (verification bout)

SpO_2	Blood oxygen saturation
SRT	Steep ramp test
SV	Stroke volume
tCSA	Thigh cross sectional area
TV	Thigh volume
$\dot{V}_E$	Minute ventilation
VentEq	Ventilatory equivalents
$\dot{V}_E/\dot{V}O_2$	Ventilatory equivalent for oxygen
$\dot{V}CO_2$	Volume of carbon dioxide production
$\dot{V}O_2$	Volume of oxygen uptake
$\dot{V}O_{2max}$	Maximal volume of oxygen uptake
$\dot{V}O_{2peak}$	Peak volume of oxygen uptake
VT	Ventilatory threshold
WR_{peak}	Peak work rate

PhD PUBLICATIONS AND PRESENTATIONS

The following publications and presentations are in direct relation to the published thesis and studies contained within. All other publications and presentations are listed in 'Additional Publications and Presentations'.

Journal Articles

Tomlinson, O. W., Barker, A. R., Oades, P. J., & Williams, C. A. (2016). Exercise capacity following a percutaneous endoscopic gastrostomy in a young female with cystic fibrosis: A case report. *Physiological Reports*. 4 (16), e12904. doi: 10.14814/phy2.12904

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Tomlinson, O. W., Barker, A. R., Fulford, J., Shelley, J., Wilson, P., Oades, P. J., & Williams, C. A. (2018). Scaling maximum oxygen uptake for thigh muscle volume in children with cystic fibrosis. *Pediatric Exercise Science* (Under Review).

Tomlinson, O. W., Barker, A. R., Fulford, J., Wilson, P., Oades, P. J., & Williams, C. A. (2018). Quantification of thigh muscle volume in children using magnetic resonance imaging. *European Journal of Sport Science* (Under Review).

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Tomlinson, O. W., Barker, A. R., Fulford, J., Wilson, P., Shelley, J., Oades, P. J., & Williams, C. A. (2017). Scaling maximal oxygen uptake for thigh muscle volume in children with cystic fibrosis. *Pediatric Exercise Science*: 29 (4 Suppl 1): 6.

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Williams, C. A., **Tomlinson, O. W.**, Chubbock, L., Stevens, D., Saynor, Z. L., Oades, P. J., & Barker, A. R. (2017) The oxygen uptake efficiency slope is not a valid measure of aerobic capacity in children with cystic fibrosis. *Pediatric Exercise Science*: 29 (4 Suppl 1): 4.

Tomlinson, O. W., Barker A. R., Chubbock, L. V., Stevens, D., Saynor, Z. L., Oades, P. J., & Williams, C. A. (2018). WS08.1 The utility of oxygen uptake efficiency as a marker of aerobic fitness in children with cystic fibrosis. *Journal of Cystic Fibrosis*, 17, S14.

Conference Presentations

Tomlinson, O. W., Barker, A. R., Oades, P. J., & Williams, C. A. *Clinical changes in cystic fibrosis after gastric surgery: A case study*. University of Exeter Sport and Health Science Postgraduate Research Day, 7 January 2016; University of Exeter, Exeter, UK.

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Tomlinson, O. W., Chubbock, L., Stevens, D., Saynor, Z. L., Oades, P. J., Barker, A. R., & Williams, C. A. *The oxygen uptake efficiency slope is not a valid measure of aerobic capacity in children with cystic fibrosis.* British Association of Sport & Exercise Sciences Student Conference, 12-13 April 2017; University of St Mark and St John, Plymouth, UK.

Tomlinson, O. W., Barker, A. R., Fulford, J., Wilson, P. Shelley, J., Oades, P. J., & Williams, C. A. *Scaling maximal oxygen uptake for thigh muscle volume in children with cystic fibrosis*. Pediatric Work Physiology Meeting XXX, 3-8 October 2017; Katerini, Greece.

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Poster Presentations

Chubbock, L. V., Barker, A. R., **Tomlinson, O. W.,** Saynor, Z. L., Stevens, D., Oades, P. J., & Williams, C. A. Oxygen uptake efficiency slope is not a valid submaximal measure of aerobic capacity in paediatric cystic fibrosis patients. Poster presented at: 'Fighting for a Life Unlimited'. UK Cystic Fibrosis Conference; 2015 September 22 – 23; Manchester, UK.

Chubbock, L. V., Barker, A. R., **Tomlinson, O. W.,** Saynor, Z. L., Stevens, D., Oades, P. J., & Williams, C. A. Oxygen uptake efficiency slope is not a valid submaximal measure of aerobic capacity in paediatric cystic fibrosis patients. Poster presented at: Exhibition of Clinical Research at the Royal Devon & Exeter; 2015 November 5; Exeter, UK

Tomlinson, O. W., Barker, A. R., Fulford, J., Wilson, P., Shelley, J., Oades, P. J., & Williams, C. A. Impact of muscle on exercise capacity in cystic fibrosis – quality or quantity? Poster presented at: University of Exeter Postgraduate Research Showcase; 2018 May 14 – 18; University of Exeter, Exeter, UK

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Tomlinson, O. W. (2014). Cardiopulmonary Exercise Testing. Presented at the Royal Devon and Exeter NHS Foundation Trust Hospital Bramble Seminar Series, October 28 2014; Exeter, UK.

Tomlinson, O. W. (2014). Cardiopulmonary Exercise Testing. Presented at the South West Cystic Fibrosis Meeting, November 14 2014; Taunton, UK.

Tomlinson, O. W., Barker, A. R., Williams, C. A., Shelley, J. (2017). Cardiopulmonary exercise testing in cystic fibrosis. Presented at UK Cystic Fibrosis Conference, September 7 2017; Nottingham, UK.

Tomlinson, O. W. (2017). The role of cardiopulmonary exercise testing in the assessment and treatment of young people with cystic fibrosis. Presented at: "CF Through The Looking Glass". All Wales National Cystic Fibrosis Conference, November 10 2017; Cardiff, UK.

Tomlinson, O. W., & Barker, A. R. (2018). Cardiopulmonary Exercise Testing – Making Sense of Data. Presented at 'Publication to Patient', Cystic Fibrosis and Exercise Network Meeting, February 23 2018; Liverpool John Moores University, Liverpool, UK.

ADDITIONAL PUBLICATIONS AND PRESENTATIONS

Journal Articles

Williams, C. A., Saynor, Z. L., **Tomlinson, O. W.,** & Barker, A. R. (2014). Cystic fibrosis and physiological responses to exercise. *Expert Review of Respiratory Medicine,* 8 (6), 751 – 762. doi: 10.1586/17476348.2014.966693

Tomlinson, O. W., Shelley, J., Trott, J., Bowhay, B., Chauhan, R., & Sheldon, C. (2018). The feasibility of online video calling to engage patients with cystic fibrosis in physical activity. *Journal of Telemedicine and Telecare* (Under Review).

Williams, C. A., Wedgwood, K. C. A., Mohammadi, H., Prouse, K., **Tomlinson, O. W.,** & Tsaneva-Atanasova, K. (2018). Cardiopulmonary responses to maximal aerobic exercise in patients with cystic fibrosis. *PLoS One* (Under Review).

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Trott, J., **Tomlinson, O.,** Bowhay, B., Williams, C., Withers, N. & Oades, P. (2018). P150 Reasons for non-compliance with cardiopulmonary exercise testing in cystic fibrosis. *Journal of Cystic Fibrosis*, 17, S101.

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Tomlinson, O. W., Barker, A. R., Oades, P. J., & Williams, C. A. *Exercise Testing in Children with Lung Disease*. CLESCon 2017: Frontiers in Life & Environmental Science, 22 June 2017; Living Systems Institute, University of Exeter, Exeter, UK.

Poster Presentations

Tomlinson, O. W., Barker, A. R., Oades, P. J., & Williams, C. A. The feasibility of a home-based exercise intervention for the improvement of aerobic function in young cystic fibrosis patients. Poster presented at: '40 Years of Sport and Exercise Science: A History in the Making'**.** 18th Annual British

Association of Sport and Exercise Sciences Student Conference; 2015 Mar 31 – Apr 1; Liverpool John Moores University, Liverpool, UK.

Tomlinson, O. W., Barker, A. R., Oades, P. J., & Williams, C. A. The feasibility of a home-based exercise intervention for the improvement of aerobic function in young cystic fibrosis patients. Poster presented at: University of Exeter Postgraduate Research Showcase; 2015 April 27 – 29; University of Exeter, Exeter, UK.

Tomlinson, O. W., Barker, A. R., Oades, P. J., & Williams, C. A. The feasibility of a home-based exercise intervention for the improvement of aerobic function in young cystic fibrosis patients. Poster presented at: Exhibition of Clinical Research at the Royal Devon & Exeter; 2015 November 5; Exeter, UK.

Tomlinson, O. W., Barker, A. R., Oades, P. J., & Williams, C. A. Exercise Testing in Cystic Fibrosis: Protocols, Patients & Practice. Poster presented at: University of Exeter Postgraduate Research Showcase; 2017 May 15 – 17; University of Exeter, Exeter, UK.

Tomlinson, O. W., Barker, A. R., Oades, P. J., & Williams, C. A. Exercise Testing in Cystic Fibrosis: Protocols, Patients & Practice. Poster presented at: University of Exeter CLESCon: Frontiers in Life & Environmental Science; 2017 June 22; Living Systems Institute, University of Exeter, Exeter, UK.

Bland, C. L., Barker, A. R., **Tomlinson, O. W.,** Stevens, D., Saynor, Z., Oades, P. J., & Williams, C. A. Sex Differences in Exercise Capacity in Children and Adolescents with Cystic Fibrosis. Poster Presented at: UK Cystic Fibrosis Conference; 2017 September 6 – 7; Nottingham, UK.

Bland, C. L., Barker, A. R., **Tomlinson, O. W.,** Stevens, D., Saynor, Z., Oades, P. J., & Williams, C. A. Sex Differences in Exercise Capacity in Children and

Adolescents with Cystic Fibrosis. Poster presented at: 'Posters in Parliament', British Conference of Undergraduate Research; 2018 February 20; Palace of Westminster, London, UK.

Day, A., Trott, J., **Tomlinson, O. W.**, Oades, P. J., & Withers, N. J. Associations between airway clearance techniques and exercise capacity in cystic fibrosis. Poster presented at: 'Facing the Challenges of Today', 2nd International Cystic Fibrosis Conference; 2018 April 26; Manchester, UK.

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Tomlinson, O. W., Trott, J., Bowhay, B., Shelley, J., Enderby, B., Chauhan, R., & Sheldon, C. Feasibility of using online video calling to engage patients in the management of cystic fibrosis. Poster presented at: 41st European Cystic Fibrosis Conference; 2018 June 6 – 9; Belgrade, Serbia.

Trott, J., **Tomlinson, O. W.,** Bowhay, B., Williams, C. A., Withers, N. J., & Oades, P. J. Reasons for non-compliance with cardiopulmonary exercise testing in cystic fibrosis. Poster presented at: 41st European Cystic Fibrosis Conference; 2018 June 6 – 9; Belgrade, Serbia.

Invited Presentations

Tomlinson, O. W. (2015). Exercise – Research in to Practice. Presented at the Cystic Fibrosis Nursing Association Meeting (South West & Wales), October 2 2015; Taunton, UK.

Tomlinson, O. W. (2016). Impact of diet, exercise and environment on health and well-being. Presented at Sidmouth Science Festival, October 9 2016; Sidmouth, UK.

Tomlinson, O. W. (2018). Exercise for Children with Respiratory Disease. Presented at Postgraduate MSc, Diploma and Certificate in Advanced Paediatric Physiotherapy, March 14 2018; Institute of Child Health (ICH), University College London, London, UK.

Tomlinson, O. W., & Shelley, J. (2018). 'By failing to prepare, you are preparing to fail' – Exercise prescription and remote monitoring. Presented at "Supporting Learning in Lung Transplantation" Association of Chartered Physiotherapists in Respiratory Care, June 5 2018; Royal Marsden Education Centre, London, UK.

1 INTRODUCTION

Cystic fibrosis (CF) is the most common, life-shortening, genetically inherited disease in the Caucasian population. It is caused by a mutation to the cystic fibrosis trans-membrane conductance regulator (CFTR) gene, which codes for the protein responsible for trans-epithelial chloride transportation (Kerem et al., 1989). This genetic mutation results in dysfunctional, or absent, chloride transporters on the cell membrane and subsequently results in an accumulation of a thick, sticky mucus in the lining of the airway and digestive tracts. This provides a platform for chronic bacterial infections, inflammation, bronchial obstructions, fibrosing of lung tissue and a decline in pulmonary function (represented by forced expiratory volume in one second [FEV_1]) (Elborn, 2016). Consequently, respiratory failure is the leading cause of mortality in CF, accounting for 85% of all patient deaths (Flume et al., 2009). Currently, there is no cure for CF. Therefore, it is disease that is managed using a combination of medication, nutrition, physiotherapy and exercise.

Currently, CF affects ~11,000 people in the United Kingdom (UK), with ~4,000 of these individuals being under the age of 16 (Cystic Fibrosis Trust, 2017b). The incidence of CF is currently ~1:2500 (Farrell, 2008), and 1 in 25 people of Caucasian ethnicity carry the recessive gene that results in manifestation of CF (Massie and Delatycki, 2013). The median age of death for patients with CF is currently 31 years, although advances in the understanding and management of the disease has increased predicted survival steadily over recent years, and now stands at 47 years of age for a new-born with CF (Cystic Fibrosis Trust, 2017b). This increase in projected life expectancy represents a fundamental shift in CF, having once been treated as a paediatric disease where survival was as low as 5 years in 1960 (Elborn et al., 1991), to now being treated as an adult disease,

with high quality paediatric care presenting a fundamental stage in improving longevity trough the adult years.

Despite promising advances being made recently with regard to a range of medications that can improve the functionality of the mutated CFTR protein that causes CF (Kuk and Taylor-Cousar, 2015), their widespread administration remains low, primarily for financial reasons and limited ability to target all genetic mutations (Balfour-Lynn, 2014). For example, Ivacaftor (Kalydeco®) acts as a CFTR potentiator by improving chloride transport (Davies et al., 2013), although is only suitable for individuals with the G551D mutation, which currently has a prevalence of 6% (Cystic Fibrosis Trust, 2017b). Therefore, the predominant medication given to patients with CF remain antibiotics to manage recurrent infection. However, nutrition, chest physiotherapy and exercise also form a cornerstone in the management of CF (Cystic Fibrosis Trust, 2016b, National Institute for Health and Care Excellence (NICE), 2017). Exercise training in particular, may be linked to numerous benefits, including increased lung function, health-related quality of life (QoL), exercise capacity, sputum expectoration and reduced breathlessness (Radtke et al., 2017b).

In addition to the benefits associated with exercise training, exercise capacity (represented by peak oxygen uptake [$\dot{V}O_{2peak}$]) is considered a clinically important variable, providing useful prognostic information independently of FEV_1. A lower $\dot{V}O_{2peak}$ is associated with increased risk of mortality (Nixon et al., 1992, Pianosi et al., 2005a) and hospitalisation (Pérez et al., 2014) as well as reduced QoL (Hebestreit et al., 2014). Therefore, it is imperative that exercise capacity is routinely monitored, with annual testing recommended to take place on at least an annual basis (Cystic Fibrosis Trust, 2017a, Hebestreit et al., 2015).

Exercise capacity can be measured using a variety of tests, protocols and equipment modalities. However cardiopulmonary exercise testing (CPET) is recognised as the 'gold standard' and is endorsed for use by the European Cystic Fibrosis Society (ECFS) and European Respiratory Society (ERS) (Hebestreit et al., 2015) and utilises pulmonary gas exchange to directly determine $\dot{V}O_{2peak}$. Furthermore, with the use of an additional supramaximal verification bout, maximal oxygen uptake ($\dot{V}O_{2max}$)[1] can be confirmed; a process that is valid and reliable in children and adolescents with CF (Saynor et al., 2013a, Saynor et al., 2013b). However, to obtain $\dot{V}O_{2max}$ requires a maximal effort from patients during CPET, which may not be feasible nor possible in some cases. Clinical status, motivation, dyspnoea and contraindications during testing may all factor into a patient's ability to perform maximal exercise, and therefore valid submaximal measures need to be identified for use in CF (Williams et al., 2014).

Measures of oxygen uptake efficiency (OUE) may provide submaximal alternatives to $\dot{V}O_{2max}$, although their validity in CF has yet to be robustly investigated. One derivative of OUE is the oxygen uptake efficiency slope (OUES), which has been investigated in patients (11.8 – 18.7 years) with CF (Bongers et al., 2012), although there are several methodological concerns with this evaluation such as the scaling procedures used and a failure to standardise submaximal parameters to submaximal thresholds (which would account for

[1] $\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$ are both parameters of oxygen uptake, and are representative of the integrated functioning of the cardiovascular, pulmonary and muscular systems to transport and utilise oxygen. However, the distinction between them is that $\dot{V}O_{2peak}$ represents the highest $\dot{V}O_2$ value obtained during an exercise test to exhaustion, whereas $\dot{V}O_{2max}$ is a verified and 'true' maximal value. The utilisation of supramaximal verification testing, and the invalidity of secondary criteria to determine $\dot{V}O_{2max}$ will be discussed and expanded upon in Chapter 2. Therefore, throughout this thesis, the terms $\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$ appear interchangeably, and do so deliberately, reflecting the protocols of cited studies and whether or not a 'true' maximum has been determined. For further insight into the 'peak vs. max' issue, please refer to Poole and Jones (2017).

variances in relative exercise intensity across patients). Furthermore, the oxygen uptake efficiency plateau (OUEP) has been identified as a significant predictor of mortality in patients with heart failure (Sun et al., 2012a), indicating clinical importance of this submaximal parameter. Whilst characterisation of parameters of OUE has been undertaken in children without CF (Bongers et al., 2015a), its profile in children and adolescents with CF remains unknown. Therefore, a re-analysis of OUES, and exploration of OUEP, in CF are needed.

Application of CPET in research and clinical practice has consistently identified that $\dot{V}O_{2peak}$ is reduced in children and adolescents with CF when expressed in absolute terms (Bongers et al., 2012), but also when controlled for body mass (Bongers et al., 2012, Bongers et al., 2014b, Keochkerian et al., 2008, Saynor et al., 2016b) and fat-free mass (Stevens et al., 2011, Tucker et al., 2018). However, the mechanisms by which exercise is impaired in this population are equivocal (Hulzebos et al., 2015). Whilst genetic (Radtke et al., 2017a), pulmonary (Pastre et al., 2014) and cardiovascular (Rosenthal et al., 2009) limitations to exercise have all been proposed, it is the musculoskeletal contributions towards exercise intolerance (i.e. reduced $\dot{V}O_{2peak}$) that provides the most recent evidence, with debate surrounding the relative contributions of muscle 'quality' vs. muscle 'quantity' (Hulzebos et al., 2017, Rodriguez-Miguelez et al., 2017).

Recent research has indicated that the CFTR gene is present, yet dysfunctional, in skeletal muscle in CF (Divangahi et al., 2009, Lamhonwah et al., 2010), indicating that muscle 'quality' may be responsible for reduced $\dot{V}O_{2peak}$. However, studies to date have not yet fully accounted for muscle 'quantity' when assessing $\dot{V}O_{2peak}$ in CF. One previous study controlled for muscle cross-sectional area (CSA) as a parameter of muscle size (Moser et al., 2000), finding $\dot{V}O_{2peak}$ (relative to CSA) to be significantly reduced in children with CF relative to non-CF controls

(CON). However, CSA is only a surrogate for the metabolically active muscle volume (MV) engaged in exercise and does not accurately predict MV (Morse et al., 2007). Therefore, a replication of the study undertaken by Moser et al. (2000) is warranted, utilising appropriately quantified MV (as opposed to CSA), to remove the influence of muscle size from $\dot{V}O_{2peak}$ in CF, and to further evidence for a 'qualitative' contribution to reduced exercise capacity.

Further to using CPET as a diagnostic and prognostic tool to identify prospective clinical risk and causes of exercise intolerance, exercise testing can be utilised to evaluate interventions and treatment regimens. Previous research has traditionally utilised CPET to evaluate exercise training interventions such as those delivered within hospitals (Selvadurai et al., 2002a) and at home (Schneiderman-Walker et al., 2000). Moreover, the clinical utility of $\dot{V}O_{2peak}$ as an independent prognostic marker has resulted in CPET evaluating antibiotic treatment (Alison et al., 1994), CFTR modulator therapy (Saynor et al., 2014a) and lung transplantation (Oelberg et al., 1998). Despite the advocacy for use of CPET to monitor interventions, no studies to date have utilised exercise testing to evaluate nutritional interventions, such as supplemental overnight feeding through a percutaneous endoscopic gastrostomy (PEG). Given that ~30% of patients with CF receive supplemental feeding (and ~6% via a PEG (Cystic Fibrosis Trust, 2017b)), evaluation of this treatment modality is required, and use of CPET can provide further clinical evaluation in addition to traditional parameters of FEV_1 and body mass index (BMI).

Implementation of CPET into clinical practice is dependent on a number of factors, such as personnel, knowledge, expertise, equipment and infrastructure. Only four previous surveys have sought to characterise the provision of exercise testing in the management of CF (Barker et al., 2004, Kaplan et al., 1991, Radtke et al.,

2011, Stevens et al., 2010), with all being in agreement that exercise testing is an under-utilised tool, particularly CPET. However, these surveys did identify that exercise testing was broadly perceived as 'very important' (Barker et al., 2004, Stevens et al., 2010) and therefore further work is required to identify the current status of CPET in the UK, as well as the needs of clinical teams in order to deliver effective exercise testing and training.

As described, the role of CPET in the management of CF is of utmost importance in children and adolescents, although several aspects surrounding its application warrant further investigation. Therefore, this thesis will:

- Provide a comprehensive review of the literature to date, with particular reference to the role of exercise and exercise testing in the management of CF (Chapter 2), as well as an overview of the methodology utilised in experimental chapters which contribute towards the thesis (Chapter 3);
- Provide a comprehensive examination of parameters of OUE and their suitability to act as submaximal surrogates of $\dot{V}O_{2max}$ in CF (Chapters 4-6);
- Utilise magnetic resonance imaging and CPET to establish relationships between MV and $\dot{V}O_{2max}$, to address the 'quality' vs. 'quantity' debate in muscular limitations to exercise in CF (Chapters 7 and 8);
- Explore the application and implementation of CPET in the clinical setting, by using CPET to evaluate a PEG-based intervention using a case-study approach (Chapter 9), as well as providing an update on the current provision of exercise in CF centres in the UK (Chapter 10).

Experimental hypotheses, where applicable, are presented in each chapter alongside findings and brief discussions. A summative discussion will be provided in Chapter 11, highlighting not only the novelty of each chapter and its

contribution to the literature base, but also to wider clinical implications for successful utilisation of CPET in the management of CF. Furthermore, limitations and prospective research avenues for future studies will be highlighted.

2 LITERATURE REVIEW

This literature review provides an overview of the pathophysiology of CF, its demographics and treatment, including the integral role exercise plays in the management of the disease. Furthermore, this review provides the basis for why assessment of exercise capacity is crucial, how this is undertaken and applied, and a summary of current understanding surrounding impaired exercise tolerance in individuals with CF.

2.1 Cystic fibrosis

Cystic fibrosis is a genetically inherited condition, primarily affecting the respiratory and digestive systems. It is the most common genetic life-shortening disease amongst the Caucasian population, with median age of survival being 31 years (Cystic Fibrosis Trust, 2017b). There is currently no cure for CF, and therefore it is a disease that is 'managed', rather than 'cured', using combinations of medicine, nutrition, physiotherapy and exercise.

2.1.1 Cause of cystic fibrosis

Cystic fibrosis, a Mendelian autosomal recessive disorder, was first identified by Dorothy Andersen in 1938, who made the pathological description of CF based upon development of cysts and fibrosis in the pancreas (Andersen, 1938). However it was not until 1989 that the genetic defect responsible for the disease was identified (Kerem et al., 1989). It is expressed as a mutation in the cystic fibrosis trans-membrane conductance regulator (CFTR) protein, located on the long arm of chromosome 7. The CFTR protein is an adenosine triphosphate (ATP)-gated chloride (Cl^-) channel that is found predominantly in ionoctyes that line the trachea (Montoro et al., 2018, Plasschaert et al., 2018). CFTR is also found in other epithelial cell membranes that line the lungs, pancreas, vas

deferens and skin, being regulated by cyclic adenosine monophosphate (cAMP) (Hull, 2012). When a CFTR mutation occurs, resulting in CF, trans-epithelial Cl^- transport is reduced, which results in increased trans-epithelial sodium (Na^+) absorption. This is due to the dependence of epithelial Na^+ channels (ENaC) upon CFTR, and when CFTR is dysfunctional, ENaC is not appropriately regulated (Berdiev et al., 2009). This increased Na^+ absorption in turn increases water absorption and therefore decreases the hydration status of the mucosal lining of the airways and digestive tracts (Figure 2.1).

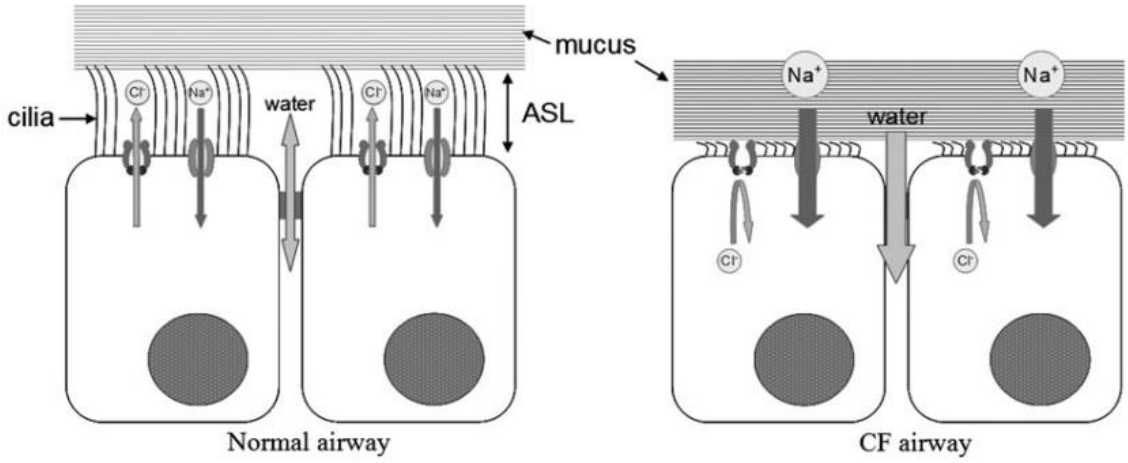


Figure 2.1 Differences in cystic fibrosis trans-membrane conductance regulator structure between normal (left), and cystic fibrosis (right), airways. Cl^-, chloride; Na^+, sodium; ASL, airway surface liquid. From Hull (2012, pS3) with permission.

This mucus is a pus that includes polymerised deoxyribonucleic acid (DNA) from degraded neutrophils, rather than mucin which is typically derived from mucus-producing cells (Henke and Ratjen, 2007), which has important consequences for medications used to clear mucus from the airways. This mucus becomes thick and viscous, covering the cilia of the airway (Figure 2.2). This also blocks the pancreatic ducts and exocrine secretions to the duodenum, which results in frequent infection and inflammation of the airways, pancreatic insufficiency, malnutrition and stunted growth.

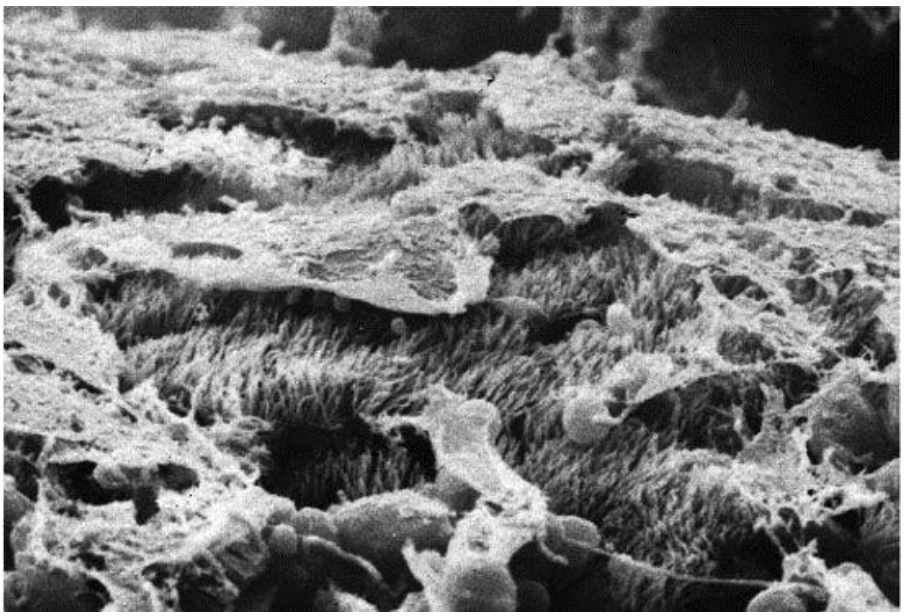


Figure 2.2 Scanning electron micrograph with cilia clearly visible. Layers of mucus are also evident on top of the cilia. In cystic fibrosis, the mucus forms a continuous layer over the cilia. Reprinted by permission from Springer Customer Service Centre GmbH: Springer, Gene Therapy, *Gene therapy progress and prospects: cystic fibrosis*, Griesenbach et al. © (2002, p1345).

Several types of mutation occur and consist of duplications, replications, deletions and shortenings of the gene, resulting in protein channels that do not function at all, function only poorly, are degraded quickly, or have an inadequate number (Rowe et al., 2005). These mutations are split into classification, dependent upon severity (Figure 2.3). These range from Class I (more severe) whereby no functional CFTR proteins are produced, to Class VI (less severe), whereby CFTR stability at the cell membrane is merely compromised. To date, nearly 2000 CFTR mutations have been reported (De Boeck et al., 2014), although not all mutations result in CF, with 159 variants accounting for 96% of CF alleles (Sosnay et al., 2013).

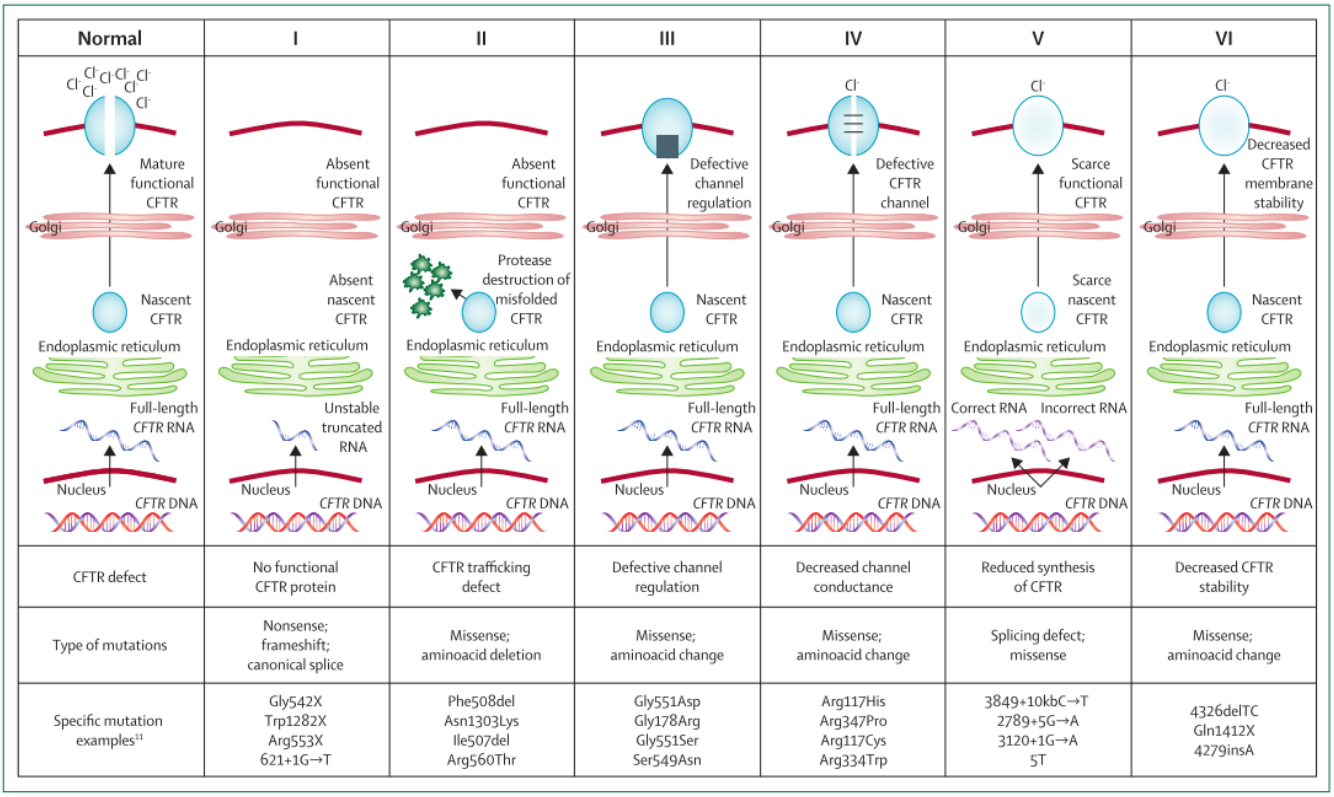


Figure 2.3 Classifications of cystic fibrosis transmembrane conductance regulator mutations, with functional defect and examples of mutations. Reprinted from The Lancet Respiratory Medicine, Vol. 1(2), Boyle & DeBoeck, *A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect*, p159, © (2012), with permission from Elsevier.

The most common CFTR mutation that results in CF is the 'ΔF508' allele, a three-nucleotide deletion of phenylalanine at the 508th codon, causing mis-folding of the CFTR protein (Bobadilla et al., 2002), and therefore CFTR remains in the sarcoplasmic reticulum and is not transported to the cell membrane. In the UK, 90.9 % of the population possess at least one 'ΔF508' allele, with 50.2 % of individuals with CF being homozygous for this Class II mutation (Cystic Fibrosis Trust, 2017b). The remaining common genotypes in the UK are listed in Table 2.1.

Table 2.1 The five most common CFTR genotype mutations in the United Kingdom, with patients who carry at least one of each mutation. Numbers are not mutually exclusive, and patients may occur twice. Data adapted from Cystic Fibrosis Trust (2017b).

Mutation	Class	Number	Percentage
ΔF508	2	8671	90.9
G551D	3	561	5.9
R117H	4	489	5.1
G542X	1	341	3.6
621+1G->T	1	244	2.6

Presently, new-born screening (Gonska and Ratjen, 2015) is utilised to detect CF and has been universally utilised across the UK since 2007. A heel-prick blood-spot test identifies levels of immunoreactive trypsinogen (Ranieri et al., 1991), with the highest 1% of values leading to further clinical investigations to confirm diagnosis of CF. Research has shown that individuals with CF that were diagnosed using new-born screening show improved nutritional outcomes and reduced morbidity, in comparison to patients who were diagnosed based upon clinical manifestations (Sims et al., 2005), which include meconium ileus, faltering growth and recurrent and chronic pulmonary distress (National Institute for Health and Care Excellence (NICE), 2017). Presence of CF is confirmed by a sweat test, with a sweat chloride value of >60 $mmol \cdot L^{-1}$ being considered diagnostic (Smyth, 2005). In cases where clinical manifestations and sweat chloride indicate CF, then genotyping would be undertaken to confirm diagnosis.

2.1.2 Pathophysiology of cystic fibrosis

Despite differences in mutation categories, the fundamental manifestation of CF in all patients remains the accumulation of mucus in the airway and digestive system. When mucus is not sufficiently cleared from the lungs, this results in declining pulmonary function due to a progressive fibrosing of lung parenchyma and bronchiectasis (Figure 2.4), resulting from consistent airway obstruction, infection and inflammation. This obstruction and restriction of the airways and

resultant progressive destruction of lung tissue results in respiratory failure, which is in turn responsible for 85% of patient deaths (Flume et al., 2009).

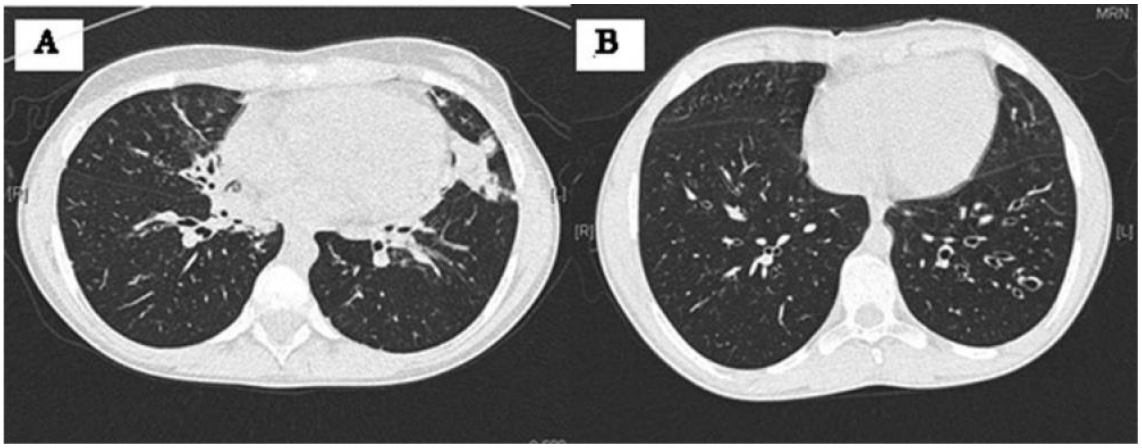


Figure 2.4 Computerised tomography images for two individual adolescents with cystic fibrosis (A: 14-year old female; B: 16-year old male). Scans display extensive bronchiectasis in the right middle and lingual lobes (A); and widespread bronchiectatic changes, but no significant swelling or hardening of tissue (B). Reprinted with permission: Saynor et al., (2014), *The Effect of Ivacaftor in Adolescents With Cystic Fibrosis (G551D Mutation): An Exercise Physiology Perspective*, Pediatric Physical Therapy, 26(4), p455, doi: 10.1097/PEP.0000000000000086.

The timeline of the progressive decline in pulmonary function (Figure 2.5) is variable between patients, but a noticeable decline in lung function is observed during pubertal years (Liou et al., 2010). Median FEV_1 has been shown to drop below the targeted 85% of predicted (a threshold indicative of normal lung health (Cystic Fibrosis Trust, 2015)) during this period. Whilst CF is a multi-organ disease, given that pulmonary dysfunction and failure remains the leading cause of death (Flume et al., 2009), maintaining lung function is the primary objective for many patients.

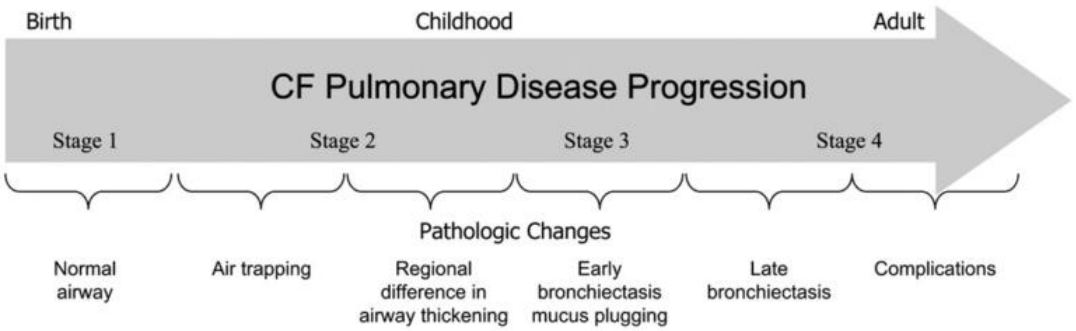


Figure 2.5 Stages of pathologic damage in the airways of patients with cystic fibrosis during growth. Reprinted with permission of the American Thoracic Society. Copyright © 2018 American Thoracic Society. Ramsey (2007), Use of Lung Imaging Studies as Outcome Measures for Development of New Therapies in Cystic Fibrosis, Proceedings of the American Thoracic Society, 4, p361. *Proceedings of the American Thoracic Society* is an official journal of the American Thoracic Society.

To categorise disease severity, percentages of predicted lung function (FEV_1) are commonly used: ≥70%, mild-to-moderate; 40-69%, moderate; <40% severe (Cystic Fibrosis Foundation, 2017). Prediction equations are available to quantify lung function as a percentage of a predicted value, with recent international collaboration resulting in the development of multi-ethnic reference values from the Global Lung Initiative, for patients aged between 3-95 years of age, which are endorsed by the ERS (Quanjer et al., 2012).

To further protect the lungs, patients will actively undertake therapies to prevent and eradicate accumulation of bacterial infections within the lung. The altered mucosal properties observed in the airways of patients make cross-infection between patients a serious concern (LiPuma et al., 1990), and subsequently stringent guidelines are in place to minimise such risks (Saiman et al., 2014). This results in patients being segregated in clinical environments and advised to avoid contact with one another (Jain et al., 2014).

Bacteria such as *Staphylococcus aureus* are common in childhood and cause

epithelial damage. In adults, *Pseudomonas aeruginosa* becomes the prevalent pathogen, with over 80% of patients being colonised (Coutinho et al., 2008), which is a significant negative predictor of lung function (Schaedel et al., 2002). With increases in life expectancy, exposure to environmental non-tuberculous Mycobacteria microorganisms such as *Mycobacterium abscessus,* bring increased burden to patients due to extensive treatment regimens (Hill et al., 2012) and contraindication to lung transplantation (Taylor and Palmer, 2006).

Beyond the respiratory manifestations of CF, gastrointestinal issues are also common in this group, with poor nutrition, failure to thrive and stunted growth being common in CF. These symptoms are due to the pancreatic insufficiency, that is caused in part by the pancreatitis (Elborn, 2016) reported by a large proportion (43%) of patients (Cystic Fibrosis Trust, 2017b). This is caused by blockages of the pancreatic duct by mucus, and therefore gastric enzymes required for digestion are prevented from acting on food in sufficient volume to appropriately digest food, and blockages of the intestine prevent adequate absorption of fat and fat-soluble vitamins. As a result, individuals with CF are recommended to increase their daily nutritional intake as part of their disease management (Ramsey et al., 1992). This malabsorption can also result in further complications such as vitamin deficiencies, osteoporosis, gastro-intestinal reflux and liver disease. When combined with increased nutritional intake, occurrence of CF related diabetes (CFRD) is likely (Cystic Fibrosis Trust, 2004), with ~7,000 patients in the UK aged ≥10 years currently receiving treatment for CFRD (Cystic Fibrosis Trust, 2017b). These nutritional complications can result in individuals with CF being either underweight, or overweight and obese, with an equal reporting of extremes of body size in one CF centre in the USA (Hanna and Weiner, 2015).

Further to the airways and digestive systems, the skin and vas deferens are impacted upon by CFTR dysfunction. This results in individuals with CF having salty sweat, a manifestation which aids diagnosis, and the majority (97-98%) of males being infertile (but not sterile), due to congenital absence of the vas deferens (Chen et al., 2012). In addition, *in vitro* studies have shown CFTR to be present in the vascular endothelium (Tousson et al., 1998), cardiac tissue (Davies et al., 2004) and skeletal muscle (Lamhonwah et al., 2010).

2.1.3 Demographics of cystic fibrosis

The demographics associated with CF are variable based upon nationality, sex and age. Therefore, an emphasis within this chapter is placed on the demographics of the CF population in the UK.

2.1.3.1 Prevalence and incidence

Cystic fibrosis currently affects ~11,000 people in the UK, with ~4,000 of these individuals under 16 years of age (Cystic Fibrosis Trust, 2017b). The CF population in the UK has increased steadily, as shown in Figure 2.6, and is predicted to increase to ~15,000 by 2025, a growth rate of 53.6% (Burgel et al., 2015), due to improvements in management of the disease.

This population equates to a prevalence (the proportion of actual cases) rate of approximately 1.37 per 10,000, which was the second highest in the European Union (EU) in the most recent analysis in 2008 (Farrell, 2008). This is behind the Republic of Ireland whose prevalence is the highest at 2.98 per 10,000. The prevalence rate of CF in the EU is 0.74 per 10,000, with rates of individual nations ranging from as low as 0.10 per 10,000 (Latvia), to above 1.00 per 10,000 (1.03 in Belgium, 1.37 in the UK, and 2.98 in Ireland) (Farrell, 2008). The prevalence of CF in the United States of America (USA) (Farrell, 2008), the only country in

the world with a higher total number of patients than the UK, is 0.80 per 10,000. In 2016, the CF population of the USA was ~30,000 (Cystic Fibrosis Foundation, 2017).

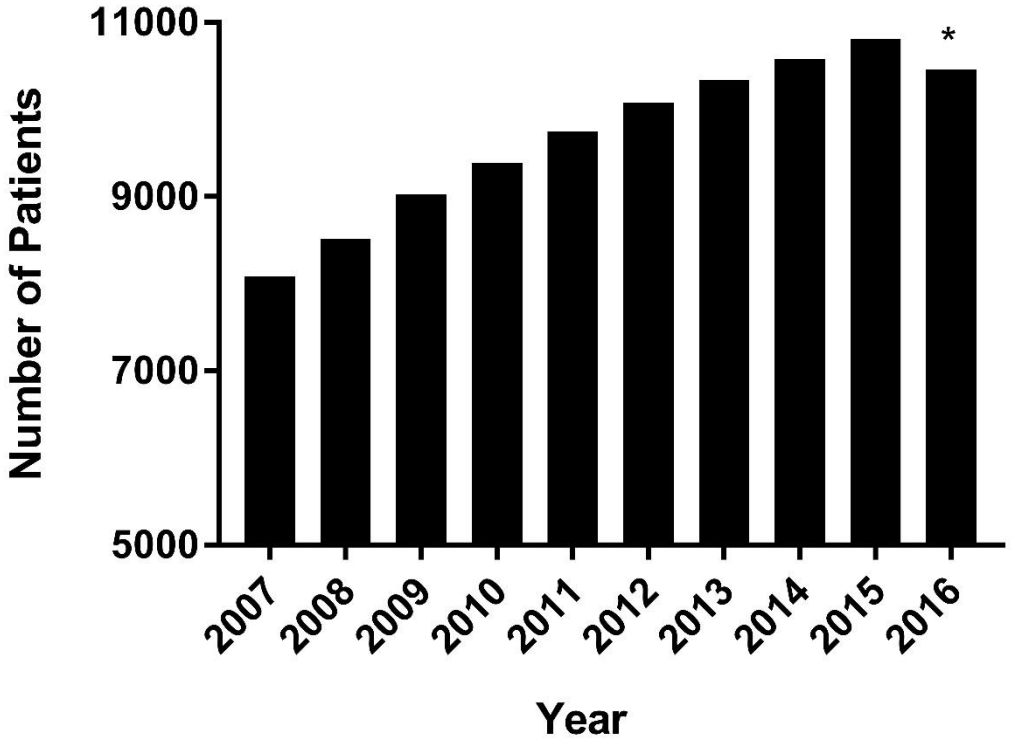


Figure 2.6 Increase in registered patients with cystic fibrosis in the United Kingdom from 2007-2016. Data adapted from Cystic Fibrosis Trust Registry (2009, 2013b, 2017b). *Decrease in registered patients due to 'data cleaning' exercise by Cystic Fibrosis Trust.

The most recent estimated incidence (the probability of occurrence) of CF in the UK is 1:2381 (Farrell, 2008), suggesting one in nearly 2,500 births will result in a child born with CF. This rate is again second only to the Republic of Ireland, with an incidence rate of 1:1353 (Farrell, 2008). This is stark contrast to Japan, whereby an incidence of 1:350,000 is reported (Yamashiro et al., 1997), reflecting the genetic difference between ethnic groups. Currently, 1 in 25 people of Caucasian ethnicity are carriers of the recessive genes responsible for CF (Massie and Delatycki, 2013), resulting in a 25% chance of two carriers giving

birth to a child with CF.

2.1.3.2 Age

Most patients with CF are diagnosed early in life, due in part to the aforementioned new-born screening. In 2016, 247 new patients were diagnosed with CF, with 180 patients being diagnosed via new-born screening, and the median age of diagnoses being two months. Of these new diagnoses, 26 were over the age of 16. Of the children (<16 years) in the UK with CF, the majority (79%) were diagnosed in the three months following birth, with this increasing to 93% in children <5 years of age. In contrast, of the adults (≥16 years) within the UK, a total of 16% were diagnosed over the age of 16, although the majority (57%) were diagnosed <1 year of age, and 78% being diagnosed ≤5 years of age (Cystic Fibrosis Trust, 2017b).

The age of diagnosis is clinically important, and despite the presence of new-born screening, patients can still be diagnosed with CF relatively late in life, as described above (Cystic Fibrosis Trust, 2017b), either due to a negative new-born screen (i.e. a negative immunoreactive trypsinogen, or no 'ΔF508' mutation), or a negative sweat test following a positive new-born screen. A recent retrospective review indicates that children who are diagnosed 'late' (mean age = 1.35 years), relative to those diagnosed immediately following birth (mean age = 0.12 years) have higher rates of respiratory illness, hospitalisation, chronic colonisation with *Pseudomonas aeruginosa* and worse lung function (Coffey et al., 2017). Furthermore, for adults (≥18 years) who are diagnosed later in life, an increased age at diagnosis is a significant predictor of reduced survival, with a 24% increase in risk of death or transplant with each 5-year increase in age at diagnosis (Desai et al., 2018).

As noted previously, of the ~11,000 patients in the UK with CF, ~4,000 are under the age of 16. This distribution of ages is categorised further in Figure 2.7. The age distribution of patients with CF has changed over the past decade, with now over 60% of the CF population of the UK being over 16 years of age, as shown in Figure 2.8. This distribution reached a significant landmark in 2002 (UKCF Database, 2006), when the proportion of adults (i.e. ≥16 years) equalled the proportion of children (<16 years), marking an evolution away from being a predominantly paediatric condition

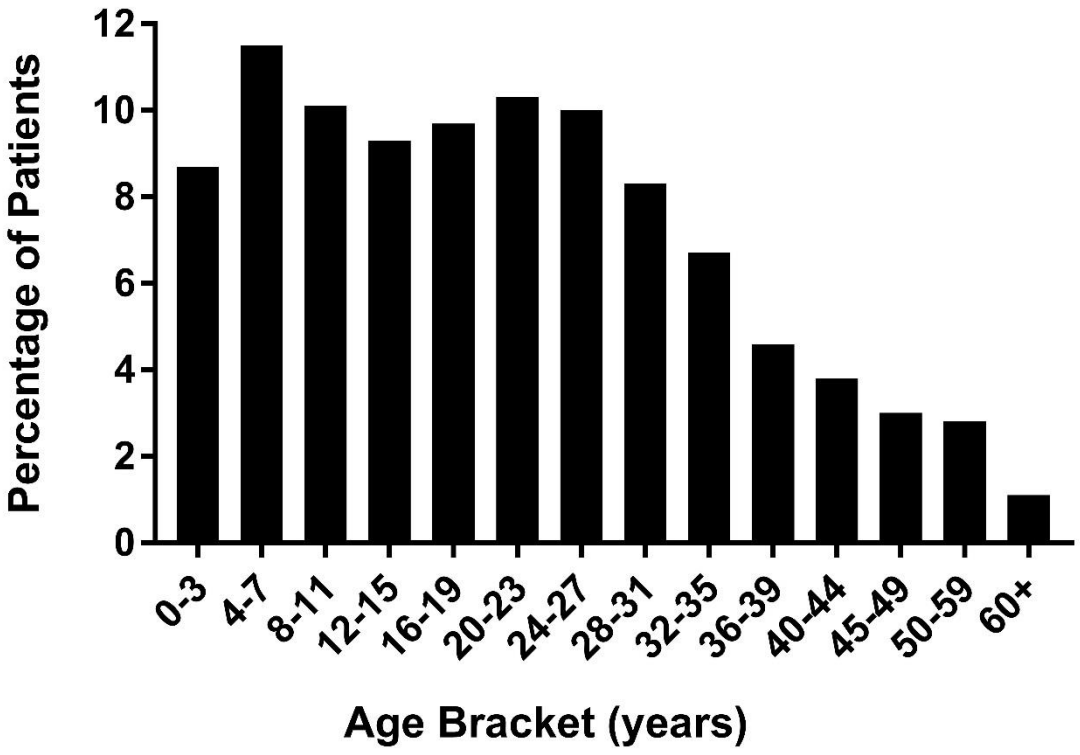


Figure 2.7 Age distribution of patients with cystic fibrosis in the United Kingdom in 2016. Data adapted from Cystic Fibrosis Trust Registry (2017b).

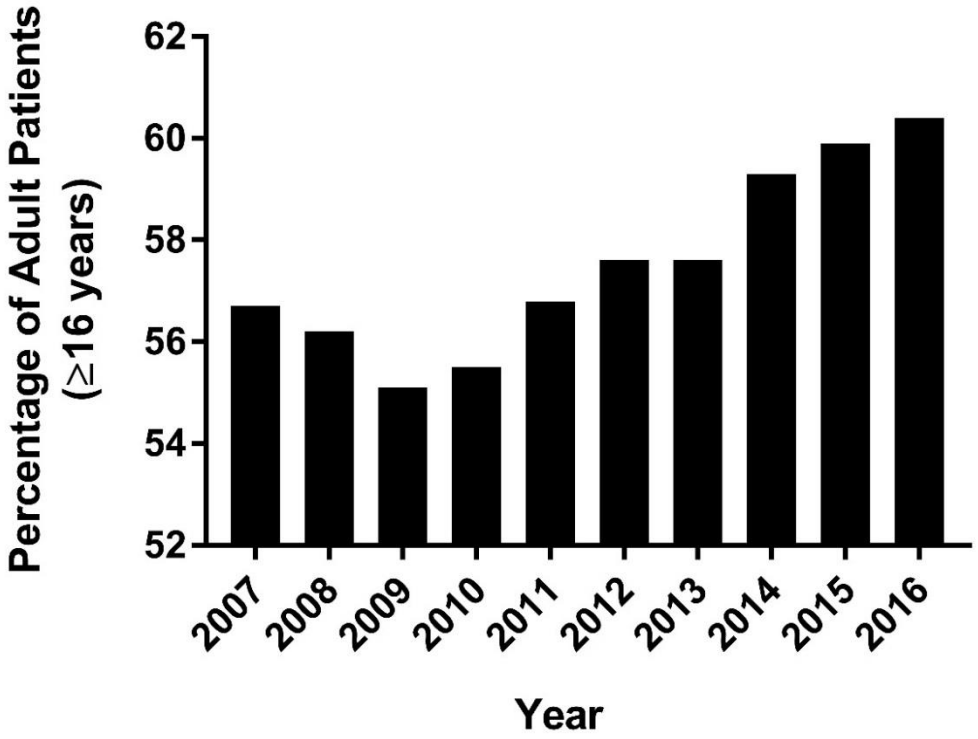


Figure 2.8 Change in proportion of patients with cystic fibrosis over the age of 16 in the United Kingdom from 2007-2016. Data adapted from Cystic Fibrosis Trust Registry (2009, 2013b, 2017b).

This change in age distribution is reflected by a change in the median age of patients with CF in the UK, with this now standing at 20 years of age (Cystic Fibrosis Trust, 2017b), and has steadily increased over recent years, having been as low as 16.1 in 2003 (CF Registry Cystic Fibrosis Trust, 2009). Consequently, care teams and medical services have changed from being solely paediatric in nature and now include adult services either as independent units, often at different hospitals, or as a combined adult/paediatric service under the remit of the same hospital. Patients with CF will 'transition' from paediatric to adult services, with this being recommended to occur between 14-18 years of age (Cystic Fibrosis Trust, 2013a), although discussions can begin as young as 11 years of age, with a view of completing the process by a patients 18th birthday

(Cystic Fibrosis Trust, 2016b).

This process moves away from a prescriptive atmosphere in the paediatric environment, and places greater autonomy on the patient as opposed to parents and/or carers (Nazareth and Walshaw, 2013). This transition period is associated with a decline in pulmonary function, although this rate of change is not statistically significant (Duguépéroux et al., 2008). There is further conflicting evidence on changes in BMI, outpatient attendance and requirement for antibiotic treatment (Coyne et al., 2017). Such changes may be attributed to this time being a period of 'flux', during which patients must acclimate to a new care team (Tierney et al., 2013), as well as personal changes in social situations (work, study, relocation) (Duguépéroux et al., 2008) and therefore highlights the importance of monitoring functional changes through childhood and adolescence.

2.1.3.3 Sex

In the UK, ~53% of the CF population are male (Cystic Fibrosis Trust, 2017b), with this marginally larger proportion having fluctuated between 52-54% since 2002 (CF Registry Cystic Fibrosis Trust, 2009, Cystic Fibrosis Trust, 2013b, 2017b). The relative proportion of males and females is currently equal between the ages of 0-3 years, with 8.7% of the population of each respective sex being within this age bracket. After this, the proportion of females is higher until 20-23 years, at which point the relative proportion of males is higher, as seen in Figure 2.9 (Cystic Fibrosis Trust, 2017b).

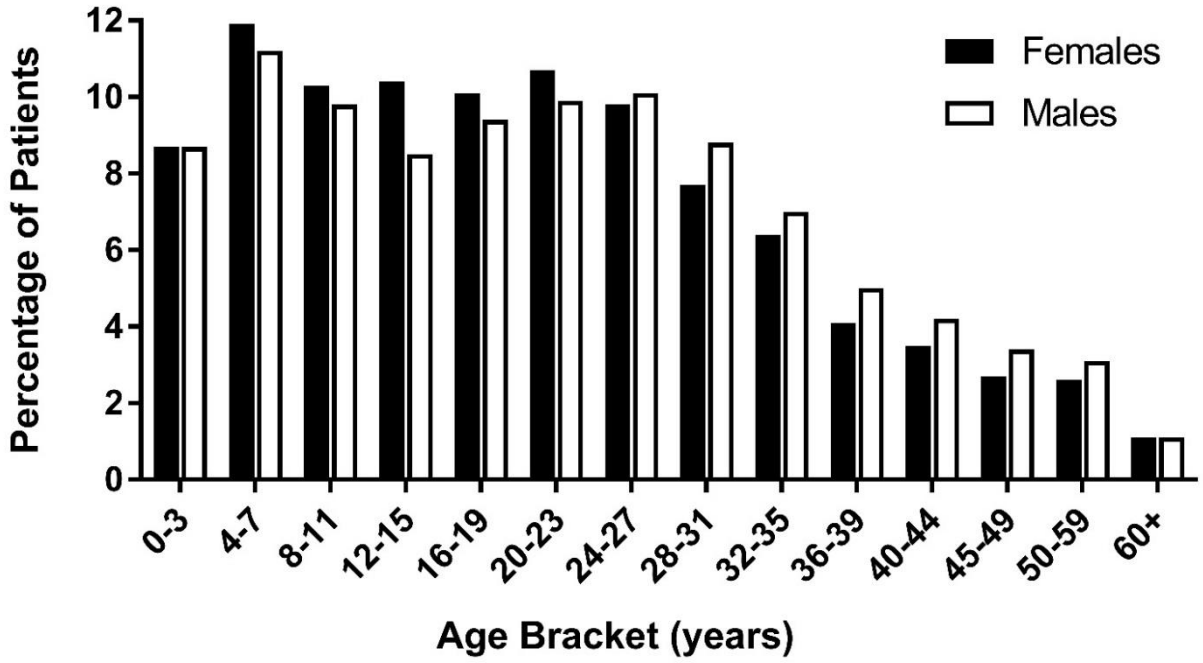


Figure 2.9 Relative distribution of patients with cystic fibrosis in the United Kingdom in 2016 when separated by sex. Data adapted from Cystic Fibrosis Trust Registry (2017b).

Females with CF have been reported to have reduced QoL (Arrington-Sanders et al., 2006), increased rates of lung function decline (Corey et al., 1997) and earlier *Pseudomonas aeruginosa* acquisition (Demko et al., 1995). Furthermore, females have been shown to have a greater mortality risk (hazard ratio = 2.22, 95% CI 1.79–2.77), even when controlling for lung function, BMI, genotype, pancreatic (in)sufficiency and microbiology (Harness-Brumley et al., 2014). Whilst precise mechanisms remain unclear, evidence suggests that female hormones may alter airway surface liquid (Coakley et al., 2008), cilia beat frequency (Jain et al., 2012) and mucoid conversion of *Pseudomonas aeruginosa* (Chotirmall et al., 2012).

2.1.3.4 Life expectancy

For patients today with CF, the median age of death is 31 years (Cystic Fibrosis Trust, 2017b), and this has steadily increased over the past decade (Figure 2.10).

In addition, there were 148 recorded deaths in 2017, totalling 1.5% of the national CF population. The age bracket with the highest number of deaths was 28-31 (*n* = 25). Encouragingly, there were fewer deaths in the 0-19 bracket than the 56+ group, with 8 and 15 respectively (Cystic Fibrosis Trust, 2017b).

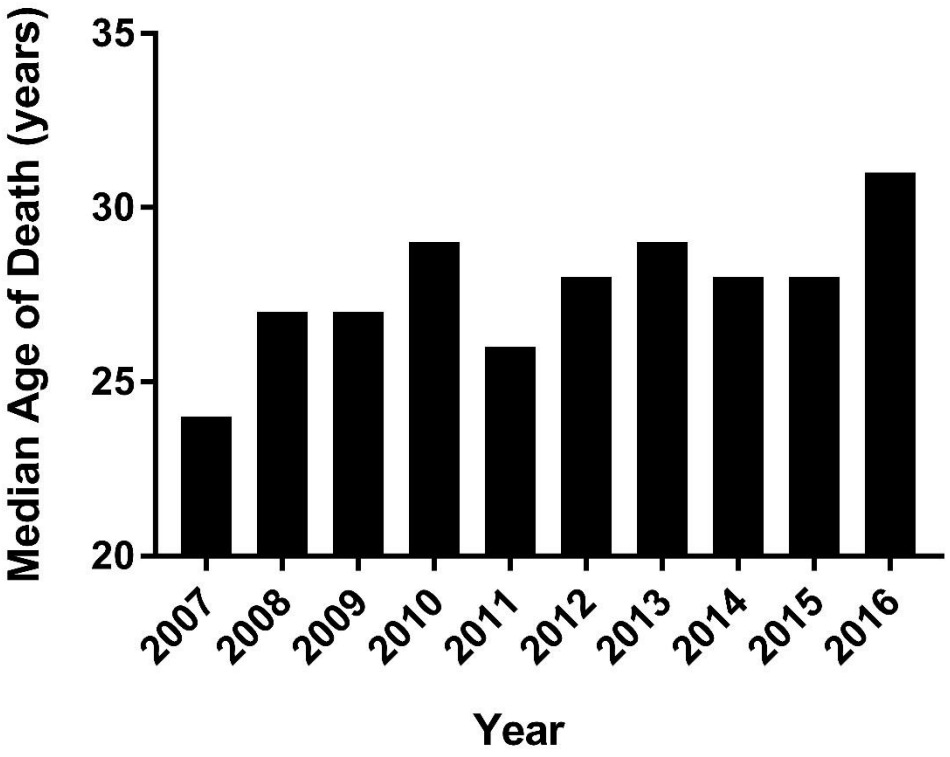


Figure 2.10 Change in median age of death for patients with cystic fibrosis in the United Kingdom, from 2007-2016. Data adapted from Cystic Fibrosis Trust Registry (2017b).

For new-born patients however, the median predicted survival age in the UK is higher than the current median age of death, and stands at 47 years (Cystic Fibrosis Trust, 2017b). Survival rates have increased significantly over the past 40 years (Dodge et al., 2007, Hurley et al., 2014), and are up from 34.4 years as recently as 2009 (Cystic Fibrosis Trust, 2013b), with this figure historically having been under 5 years of age in 1960 (Elborn et al., 1991).

With an increase in available data through national registries, a recent study conducted by Keogh et al. (2018) suggests that dependent on genotype, age of diagnosis and a present decline in annual mortality rates, life expectancy could increase up to 65 years for males and 56 years for females. This trend for an increase in life expectancy has also been reported for the EU as a whole, as well as constituent countries (Quintana-Gallego et al., 2016). Of interest, differing survival rates have recently been reported in North America. Canada has been reported to a have a median age of survival 10 years higher than the USA (50.9 vs. 40.6 years), with disparities in healthcare access and delivery having been suggested as potential reasons (Stephenson et al., 2017), therefore highlighting the importance of understanding and accounting for healthcare delivery methods and systems in the management of CF.

For these observed increases in life expectancy, factors such as early diagnosis, nutritional support, effective pulmonary medicines (Lopes-Pacheco, 2016), as well as the implementation of multiple, and increasingly personalised treatments, have all been acknowledged as contributors (see Figure 2.11 (Elborn, 2013)).

Traditional clinical factors such as FEV_1, BMI, genotype, pancreatic (in)sufficiency and pathogen acquisition have all been shown to be predictive of survival in CF (Harness-Brumley et al., 2014), as well as exercise derived factors such as $\dot{V}O_{2peak}$ (Nixon et al., 1992, Pianosi et al., 2005a) and ventilatory equivalents for oxygen (minute ventilation/oxygen uptake; $\dot{V}_E/\dot{V}O_2$) (Hulzebos et al., 2014), therefore highlighting the importance to monitor exercise alongside traditional clinical factors.

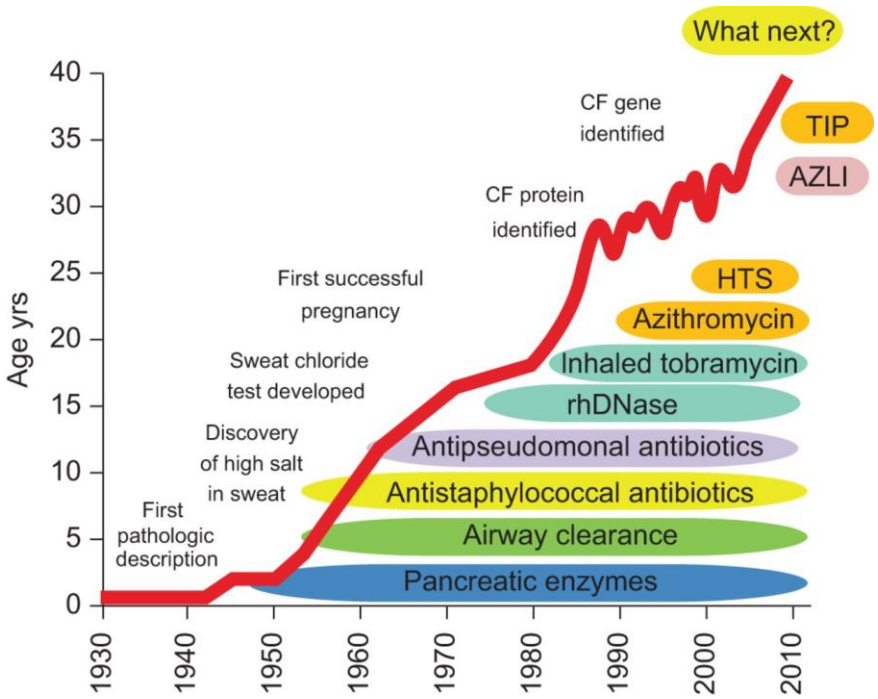


Figure 2.11 Increases in life expectancy in cystic fibrosis, attributed to introduction of novel therapies. AZLI, aztreonam for inhalation solution; CF, cystic fibrosis; HTS, high throughput screening; rhDNase, recombinant human deoxyribonuclease; TIP, tobramycin inhalation solution. Reproduced with permission of the © ERS 2018. *European Respiratory Review Mar 2013, 22 (127) 3-5; doi: 10.1183/09059180.00008112.*

2.1.4 Treatment and management of cystic fibrosis

Currently, there is no cure for CF, and whilst the identification of the genetic cause of CF has led to significant advances in the treatment of the disease, with resultant increases in life expectancy (Keogh et al., 2018), many new pharmacological products are: a) only targeted at a proportion of the CF population who possess a certain genotype (Ramsey et al., 2011); b) limited in their ability to physically correct for the structural defect (Kuk and Taylor-Cousar, 2015); or c) too expensive for patients or health services (Balfour-Lynn, 2014).

Therefore, CF is instead considered a disease that is managed, with a focus on alleviating the manifestations of the disease and associated symptoms. In mild-to-moderate cases of the disease, this is done with a combination of treatments, including pharmacological treatments (e.g. antibiotics), nutrition, physiotherapy

and exercise (National Institute for Health and Care Excellence (NICE), 2017). In severe cases of the disease, lung transplantation is used to increase prognosis (Adler et al., 2009). However, this is not routine for all patients, as only 46 adults (16 years+) and ≤5 children (<16 years) received a lung transplant in 2016 (Cystic Fibrosis Trust, 2017b).

2.1.4.1 Pharmacological treatment

Pharmacological treatment in CF consists of a range of products, with its respective regimen being dependent on genotype, and current clinical and infection status. Given the genetic cause of CF, gene therapy (Koehler et al., 2001) has presented a promising approach to disease management, however diffusion of vectors into the lung has proven more difficult than originally anticipated and has yet to demonstrate any clinical benefit (Griesenbach and Alton, 2013). Therefore, pharmacological treatment of CF currently includes antibiotics, inhaled mucolytics and more recently, CFTR modulators.

Antibiotics can be administered for prophylaxis, eradication and control of bacterial and fungal species that are commonly found in the lung (Cystic Fibrosis Trust, 2009). Various regimens are possible, dependent on infection and clinical status, with the possibility of them being administered orally (Remmington et al., 2016), intravenously (Hurley et al., 2015) or inhaled (Ryan et al., 2012a). Antibiotics can be delivered either as an in-patient, or within the home environment (Balaguer and Gonzalez de Dios, 2012) and either electively, or in response to symptoms (Breen and Aswani, 2012). However, optimal regimens and modalities remain variable between patients and are dependent on individual clinical profiles (Bhatt, 2013).

Inhaled mucolytics such as mannitol (Hurt and Bilton, 2012), dornase alfa

(rhDNase, recombinant human deoxyribonuclease [Pulmozyme®]) (Fuchs et al., 1994) and hypertonic saline (Enderby and Doull, 2007) work via differing mechanisms to reduce *in vivo* the viscosity of secretions, by either hydrolysing bonds within sputum, or increasing airway surface liquid and can augment airway clearance and facilitate expectoration (Rubin, 2015).

Recent developments have sought to correct the basic cellular defect that results in CF, by altering the structure and function of the mutated CFTR protein on the cell membrane and are termed 'modulators'. Dependent on a patient's mutation, only certain products will be suitable, and there are currently three modulators that are commercially available for use. Ivacaftor (Kalydeco®) is one such modulator, designed for patients with gating (Class III) mutations such as 'G551D', by acting as a potentiator and improving Cl^- transport through ion channels. Ivacaftor has been shown to improve lung function, QoL and sweat chloride (Davies et al., 2013). When ivacaftor is combined with the CFTR correctors lumacaftor and tezacaftor (to make Orkambi® and Symdeko™ respectively, and increase the volume of CFTR proteins at the cell surface), these resultant modulators have been shown to increase lung function and reduce the number of pulmonary exacerbations for patients homozygous for the 'ΔF508' mutation (Taylor-Cousar et al., 2017, Wainwright et al., 2015). However, as previously mentioned, limitations of only targeting certain genotypes (Ramsey et al., 2011), and price (Balfour-Lynn, 2014) of such modulators does limit their clinical impact.

2.1.4.2 Nutrition

Nutritional support in CF incorporates numerous strategies, dependent on an individual patients' maintenance of body mass. Targets for patients (which vary by age) are based upon BMI percentiles ($BMI_{\%}$), a screening tool which has been validated for use in CF (McDonald, 2008). For example, in patients with CF

between 2 and 18 years of age, a BMI% between the 25th and 75th percentiles would be considered as having 'normal' nutritional status and only require preventative nutritional counselling as a management tool (Cystic Fibrosis Trust, 2016a). However, a reliance on BMI% in children can mask stunted growth and therefore it should not be relied upon in isolation as a discrete score. Therefore changes in stature and body mass are considered alongside absolute BMI% scores in youth (Cystic Fibrosis Trust, 2016a). This is further compounded by differing timings and tempos of the onset of pubertal maturation in children, which can be delayed and slowed in children with CF (Zhang et al., 2013).

In CF, nutritional management is predominantly focused upon a) supplementing diets with enzymes, vitamins and minerals, and b) increasing caloric intake. The primary supplement used is pancreatic enzyme replacement therapy (PERT), a mixture of amylase, lipase and protease, collectively known as pancrelipase (Creon®), whose use has been shown to be clinically effective in maintaining and improving nutritional status (Somaraju and Solis-Moya, 2016). In addition, supplementation of fat-soluble vitamins (A, D, E, K, B12), as well as minerals such as zinc, may be required if blood-tests reveal deficiencies (Dodge and Turck, 2006). Alongside these supplements, the recommended energy intake for patients varies between 110-200% of that for an age- and sex-matched healthy population (Stallings et al., 2008).

For patients whose body mass falls below the 'normal' BMI% boundaries of the 25th-75th percentiles and have a sustained deviation below the 25th percentile, intensive nutritional support, such as a percutaneous endoscopic gastrostomy (PEG) may be required. This is a tube that is surgically placed through the abdominal wall, inserting directly into the stomach (Figure 2.12) and allows for additional calories to be digested overnight. Individual caloric requirements per

patient varying dependent on their current body mass status and target body mass (Cystic Fibrosis Trust, 2016a). Use of a PEG is clinically effective, improving nutritional status (i.e. BMI) and stabilising pulmonary function (FEV_1) (Woestenenk et al., 2013). Presently, ~6% of patients with CF currently have a PEG (Cystic Fibrosis Trust, 2017b).

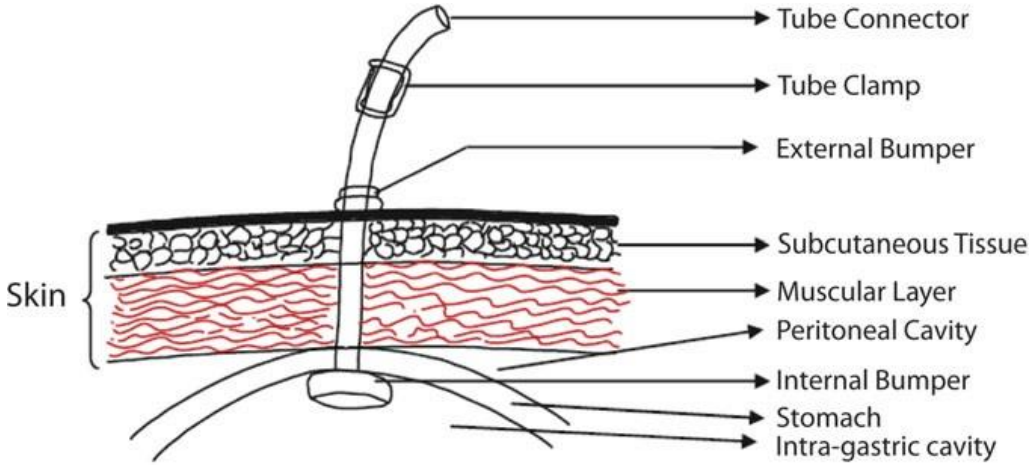


Figure 2.12 Schematic diagram of a percutaneous endoscopic gastrostomy, presented in a cross-sectional view. From Sohagia and Hertan (2012). Reprinted by permission from Springer Customer Service Centre GmbH: Springer, Geriatric Gastroenterology by C.S. Pitchumoni & T.S. Dharmarajan (Eds.) © (2012) doi: 10.1007/978-1-4419-1623-5_13.

A proportion of patients with CF are reported to be overweight (15%) and obese (8%) (Hanna and Weiner, 2015). Therefore nutritional management will focus on advice about healthy eating in order to facilitate loss of fat mass, whilst maintaining stature velocity for growing patients (i.e. children and adolescents) (Cystic Fibrosis Trust, 2016a).

Finally, for individuals with a diagnosis of CFRD, regular assessment of blood glucose is required, and administration of insulin may be required (Cystic Fibrosis Trust, 2004). For patients who achieve good glycaemic control, evidence shows an improvement in clinical outcomes for body mass, pulmonary function (FEV_1) and survival (Moran et al., 2010).

2.1.4.3 Physiotherapy

Physiotherapy for CF management predominantly consists of airway clearance therapy (ACT) to remove sputum from airways. Many different techniques are available and are broadly split into those that can be performed by a patient independently of a physiotherapist; forced expiratory breathing techniques and device-dependent techniques; and those that require assistance in the form of manual techniques (Main et al., 2015).

Breathing techniques, such as autogenic drainage and the active cycle of breathing, utilise breaths of varying depths and frequencies to facilitate movement of sputum from peripheral airways for clearance and are effective in producing clinically significant sputum yields (Morgan et al., 2015), although neither have been shown to be any more effective than other airway clearance techniques (McCormack et al., 2017, McKoy et al., 2016). Positive expiratory pressure (PEP) devices provide resistive pressure to the airways during exhalation and are of clinical benefit by significantly reducing the number of pulmonary exacerbations experienced (McIlwaine et al., 2015). Furthermore, oscillating PEP (OPEP) devices (such as the Flutter®, Acapella® and Aerobika®) generate intra- and extra-thoracic oscillations alongside PEP to mobilise mucus (Morrison and Innes, 2017). A retrospective review shows that ~25% of people with CF utilise OPEP devices as a modality of ACT (Figure 2.13) (Hoo et al., 2015), however their use has not been shown to be any more, or less, effective than any other form of chest physiotherapy for CF (Morrison and Innes, 2017). Manual techniques, such as postural drainage or percussion, have historically been used in the management of CF. However, data indicates that there is no clinical benefit over newer ACT devices (Main et al., 2005), and few patients (4%) opt for such modalities (Figure 2.13) (Hoo et al., 2015).

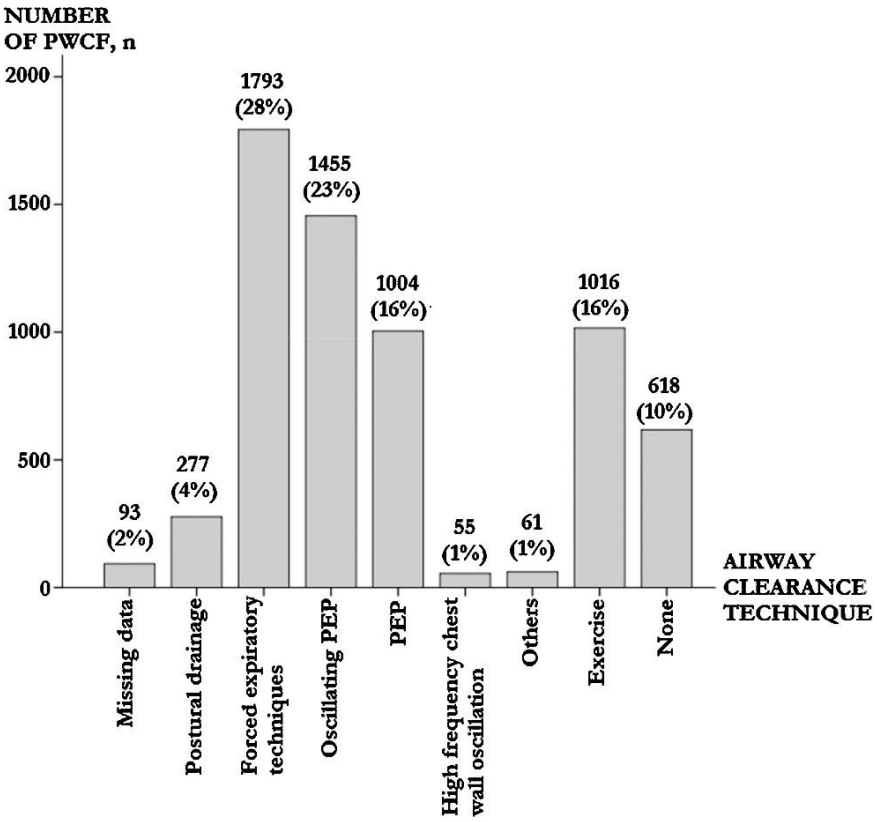


Figure 2.13 Airway clearance techniques used by patients with cystic fibrosis in the United Kingdom. PEP, positive expiratory pressure; PWCF, patients with cystic fibrosis. Reprinted from Physiotherapy, 101, Hoo et al., *Airway clearance techniques used by people with cystic fibrosis in the UK*, p342., © 2015, with permission from Elsevier.

Airway clearance therapies are combined with mucolytics to further facilitate sputum expectoration, and administration of rhDNase prior to ACT improves small airway patency in children with CF (van der Giessen et al., 2007). However, a lack of conclusive evidence on timing of mucolytics (Dentice and Elkins, 2016) has resulted in guidelines personalised to each patient (McIlwaine et al., 2017), and directed around by patient preference (Cystic Fibrosis Trust, 2017a).

In addition to ACT, use of inspiratory muscle training (IMT) has been proposed as a modality to improve lung function. Individual studies have shown improvements in maximal inspiratory pressure (Sawyer and Clanton, 1993) and inspiratory muscle endurance (de Jong et al., 2001), but not traditional pulmonary variables of FEV_1 or FVC (Houston et al., 2008), nor exercise endurance (Bieli et

al., 2017). Given differences in reported outcomes and discrepancies in study designs, a systematic review has failed to confirm, nor refute, the efficacy of IMT as a treatment option in CF (Houston et al., 2008).

Compliance with treatments varies between patients and their respective modalities of treatment. However, it has been reported that ACT and nebulisers have a lower adherence rate than CFTR modulators and antibiotics (Narayanan et al., 2017), with availability of time, and lack of enjoyment of ACT, being cited as primary reasons behind such non-compliance (Flores et al., 2013). Furthermore, the need to ensure airway clearance devices remain clean for infection prevention purposes (Manor et al., 2017), can only add to the daily treatment burden of patients, which averages over 100 minutes per day, of which airway clearance and nebulisers contribute the majority of this time (Sawicki et al., 2009). Given the notable burden associated with ACT, a recent 'Patient Setting Partnership' between patients, carers and clinicians has developed the research priority of *"Can exercise replace chest physiotherapy for people with cystic fibrosis?"* (Rowbotham et al., 2018) – thus highlighting the importance of utilising exercise in the management of CF.

2.1.4.4 Exercise

As previously noted, the role of exercise in the management of CF has been highlighted as a patient priority as an alternative form of ACT (Rowbotham et al., 2018) and currently 16% of patients in the UK use exercise as their primary mode of airway clearance (Hoo et al., 2015). In addition to being used for ACT, and improving and maintaining pulmonary function (as described below), exercise training is also utilised as a conditioning tool prior to, and following, lung transplantation in end-stage disease (Cystic Fibrosis Trust, 2017a, Hirche et al., 2014, Wickerson et al., 2010)

Acute bouts of exercise have been shown to aid sputum expectoration, both independently of (Dwyer et al., 2011), and in conjunction with (Kriemler et al., 2016) regular chest physiotherapy. This is potentially due to increased water content of mucus (Hebestreit et al., 2001) and resultant increases in airway surface hydration following exercise, as well as the oscillations of the trunk caused by exercise (Dwyer et al., 2011). In addition, an acute bout of maximal exercise significantly improves both FEV_1, and lung clearance index (LCI; a functional measure of peripheral airway obstruction), increasing within a 10 minute period following an exercise test to volitional exhaustion (Tucker et al., 2017). Whilst the mechanisms for increased FEV_1 are unclear, it has been suggested that the improvements in LCI are due to attenuation of dynamic hyperinflation (Tucker et al., 2017), but also improved mucociliary clearance, as previously described (Dwyer et al., 2011, Tucker et al., 2017).

Further to these clinically valuable effects of acute bouts of exercise, exercise training has multiple benefits for individuals with CF. Aerobic exercise training such as running and cycling has been shown to improve FEV_1 following both short-term (~3 weeks) (Selvadurai et al., 2002a) and long-term (3 months) (Kriemler et al., 2013) interventions, as well as offsetting the rate of annual decline in FEV_1 over a period of three years, relative to a control group of patients (Schneiderman-Walker et al., 2000). In addition to FEV_1, $\dot{V}O_{2max}$ has been shown to improve following aerobic training for children not only when supervised as an in-patient (Selvadurai et al., 2002a), but also when unsupervised as an out-patient (Hommerding et al., 2015). Furthermore, aerobic training has been shown to increase QoL (Selvadurai et al., 2002a), affect positive attitudes towards PA (Schneiderman-Walker et al., 2000), increase general self-worth and perceptions surrounding physical appearance (Gulmans et al., 1999), reduce breathlessness

(O'Neill et al., 1987) and increase sputum expectoration (Salh et al., 1989).

Increases in FEV_1 have been identified one-month and 18-months following anaerobic training by Selvadurai et al. (2002a) and Kriemler et al. (2013) respectively. In addition to FEV_1, anaerobic training significantly improved $\dot{V}O_{2peak}$ (Kriemler et al., 2013), physical functioning domains of QoL (Klijn et al., 2004) and strength and fat-free mass (FFM, (Selvadurai et al., 2002a)) – factors which are likely causatively related. Furthermore, when aerobic and anaerobic training are combined, glycaemic control is improved, with reductions in plasma glucose following an oral glucose tolerance test, and increases in insulin sensitivity being reported (Beaudoin et al., 2017).

Resistance training has been shown to improve FEV_1 (Selvadurai et al., 2002a, Shaw et al., 2016), $\dot{V}O_{2peak}$ (Sosa et al., 2012), as well as muscular strength when undertaken alongside aerobic training as a supervised inpatient (Sosa et al., 2012), as an independent mode of training (Selvadurai et al., 2002a), and when combined with daily nebuliser treatment (Shaw et al., 2016).

Responses of $\dot{V}O_{2peak}$ to exercise training in CF have been shown to be dependent on baseline fitness levels, independent of lung function, with larger increases seen in individuals with a lower baseline fitness (Gruber et al., 2011a), a response that is similarly seen in non-CF populations (Skinner et al., 2001). Further to baseline fitness, it has been shown that there is no effect of sex in responsiveness to training programmes, with males and females showing a 11.1% and 9.9% increase in $\dot{V}O_{2peak}$ respectively following a six-week mixed training programme, consisting of aerobic and resistance exercises (Gruber et al., 2011b).

With the breadth of exercise training programmes available, and variances in

improvements and maintenance of function, a recent systematic review concluded insufficient evidence to promote one modality as superior to another (Radtke et al., 2017b). As such, few guidelines have been published, with only a single consensus document from the European CF Society being produced (Swisher et al., 2015) in addition to generic recommendations from individual research groups (Boas, 1997, Williams and Stevens, 2013). Furthermore, the authors of the systematic review stated that there is no evidence to suggest that exercise should be discouraged (Radtke et al., 2017b). This is further supported by a number of exercise training studies that have reported no occurrence of adverse events (Kriemler et al., 2013, Santana-Sosa et al., 2014, Sosa et al., 2012). In addition, a survey of German CF centres and patients identified <1% of patients suffered an adverse event during in-patient exercise, but 22% of patients reported an asthma attack (or shortness of breath) whilst undertaking out-patient exercise (Ruf et al., 2010). Moreover, cases of haemoptysis (3%), hypoglycaemia (<1%) and loss of consciousness (<1%) are all low, thus affirming the safety of exercise training.

2.2 Exercise in cystic fibrosis

Exercise is a fundamental component of CF management and all patients are actively encouraged to undertake regular exercise trust (Cystic Fibrosis Trust, 2017a, National Institute for Health and Care Excellence (NICE), 2017), with many different exercise training interventions having been developed to further improve exercise capacity (Radtke et al., 2017b). As exercise intolerance (i.e. a failure to achieve exercise responses considered normal for age and gender (Owens and Gutin, 2000)) is a hallmark of disease progression (Orenstein and Higgins, 2005), regular assessment is required for both prognostic and diagnostic purposes, as well as prescription of personalised exercise regimens (Williams et

al., 2014). Moreover, clinicians in the UK identify exercise training, as well as exercise testing (to determine exercise capacity), as an important component of a patients' treatment regimen (Stevens et al., 2010) (Figure 2.14).

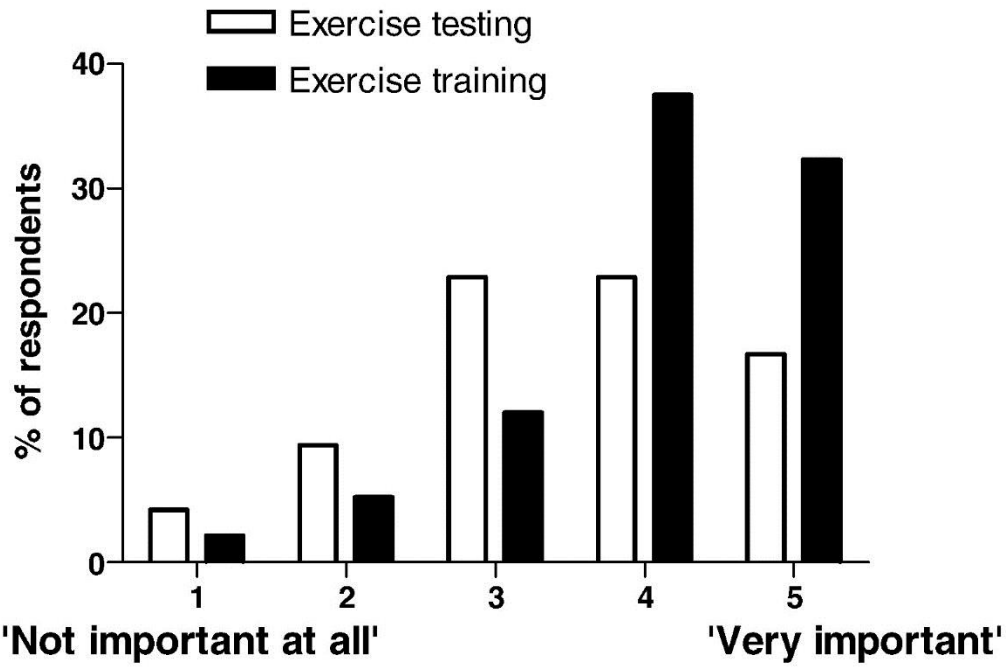


Figure 2.14 The relative importance assigned by clinicians of both exercise testing and exercise training in the management of cystic fibrosis in the United Kingdom. Reprinted from Journal of Cystic Fibrosis, 9, Stevens et al., *A survey of exercise testing and training in UK cystic fibrosis clinics*, p304., ©2010, with permission from Elsevier.

It is well established that CF adversely affects the cardiovascular, metabolic and muscular systems, in addition to the pulmonary system itself (Ferrazza et al., 2009). Given the integration of these systems through the oxygen transport and consumption pathway (Milani et al., 2004) (Figure 2.15), it is prudent to assess these systems simultaneously, both at rest and under metabolic stress. Exercise testing offers the simultaneous evaluation of all these systems and can allow clinicians and physiologists to determine underlying causes of exercise limitation in chronic diseases, such as CF (Palange et al., 2007).

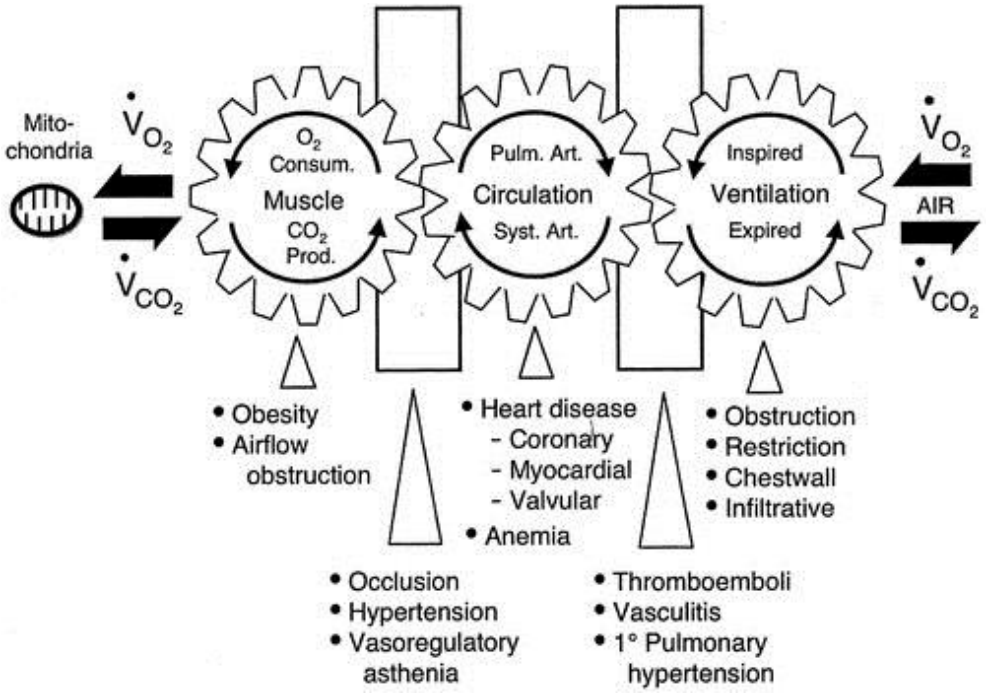


Figure 2.15 Schematic showing the integration of the musculoskeletal, cardiovascular and pulmonary systems in their contribution towards the oxygen consumption pathway and contributing factors to exercise intolerance for each system. Reprinted with permission of a) Milani et al., *Cardiopulmonary exercise testing: how do we differentiate the cause of dyspnoea?*, Circulation, 110(4), pe28, doi: 10.1161/01.CIR.0000136811.45524.2F, and b) Wasserman, Principles of Exercise Testing and Interpretation, 3rd Ed., ©Wolters Kluwer 2004.

When considering exercise testing to determine exercise capacity, there are multiple procedural factors to consider, including modality, protocols and outcome variables. Therefore, exercise capacity has multiple possible definitions, dependent on the way it is assessed (field tests vs. laboratory tests) and which variables are utilised (e.g. aerobic, aerobic and/or functional parameters). Direct measurement of aerobic power through the use of cardiopulmonary exercise testing (CPET; a 'gold standard' procedure), is now recommended by the ECFS and ERS (Hebestreit et al., 2015).

2.2.1 Exercise (dys)function in cystic fibrosis

Studies using CPET in CF have consistently shown reduced exercise capacity in adults (e.g. Moorcroft et al. (2005), Gruet et al. (2010), Rodriguez-Miguelez et al.

(2016)), for whom progressive disease severity with age will impact upon exercise capacity. Exercise capacity has been reported to be higher in children and adolescents than adults with CF (40.1 ± 6.3 vs. 32.0 ± 9.5 $mL \cdot kg^{-1} \cdot min^{-1}$, Causer et al. (2018)), given that disease status is typically less severe than their adult counterparts, although a longitudinal observation by Pianosi et al. (2005b) has shown an annualised decline of 2 $mL \cdot kg^{-1} \cdot min^{-1}$ per year over a five-year period in children and adolescents with CF.

Exercise capacity in children and adolescents with CF is still markedly reduced relative to non-CF peers (Table 2.2). All studies in Table 2.2 have utilised cycle ergometery to elicit maximal exercise, and exercise capacity is presented at $\dot{V}O_{2peak}$. However, a further two studies in this table (Saynor et al., 2014b, Saynor et al., 2016b) have utilised supramaximal (S_{max}) verification bouts and therefore data for these studies is presented as $\dot{V}O_{2max}$ (the conceptual issue difference between $\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$ is elaborated upon further in Chapter 2.2.3.2). Even though some studies do not reach statistical significance, the associated effect sizes (*ES*, Cohen (1992)) that indicate reduced $\dot{V}O_{2peak}$/$\dot{V}O_{2max}$ in CF are shown to be 'moderate' in size (≥ 0.5), which are likely to be deemed meaningful and would become statistically significant with increased sample sizes (e.g. Poore et al. (2013), Tucker et al. (2018)). Furthermore, the data in Table 2.2 highlights the importance of considering body size when interpreting exercise capacity. For example, in Saynor et al. (2016b), there is no statistically significant difference between CF and control participants when $\dot{V}O_{2max}$ is expressed in absolute terms (i.e. $L \cdot min^{-1}$), but there is when expressed relative to body mass ($mL \cdot kg^{-1} \cdot min^{-1}$), but not fat-free mass ($mL \cdot kgFFM^{-1} \cdot min^{-1}$). Similar differences between groups dependent upon body size are seen in further studies, where both *p* values (Tucker et al., 2018) and effect sizes (Bongers et al., 2012) change.

Table 2.2 Summary of studies examining differences in exercise capacity between children and adolescents with cystic fibrosis, and non-cystic fibrosis control participants**.**

Study	Group Characteristics		Group Exercise Capacity ($\dot{V}O_{2max}$ or $\dot{V}O_{2peak}$)		*p* Value	*ES*
	CF	CON	CF	CON		
Bongers et al.	*n* = 22, 13M/9F	*n* = 22, 13M/9F	2222.1 ± 547.5 mL·min^{-1}	2677.4 ± 698.6 mL·min^{-1}	**<0.05**	0.73
(2012)	15.7 ± 1.5 y	14.2 ± 1.5 y	40.9 ± 7.9 mL·kg^{-1}·min^{-1}	49.9 ± 7.9 mL·kg^{-1}·min^{-1}	**<0.001**	1. 14
			91.7 ± 18.1 %$_{Predicted}$ (kg^{-1})[a]	111.9 ± 18.9 %$_{Predicted}$ (kg^{-1})[a]	**<0.01**	1.09
Bongers et al.	*n* = 22, 12M/10F	*n* = 22, 12M/10F	2126 ± 516 mL·min^{-1}	2638 ± 685 mL·min^{-1}	**0.01**	0.84
(2014b)	14.3 ± 1.3 y	14.3 ± 1.4 y	42.4 ± 8.7 mL·kg^{-1}·min^{-1}	49.1 ± 7.2 mL·kg^{-1}·min^{-1}	**<0.01**	0.84
Fielding et al.	*n* = 16, 6M/10F	*n* = 15, 6M/9F	1.5 ± 0.7 L·min^{-1}	1.8 ± 0.7 L·min^{-1}	0.165	0.43
(2015)[$]	13.1 ± 3.9 y	13.6 ± 2.7 y	70.1 ± 14.3 %$_{Predicted}$ [b]	85.4 ± 16.0 %$_{Predicted}$ [b]	**0.009**	1.01
Keochkerian et	*n* = 9, 7M/2F	*n* = 9, 7M/2F	34.7 ± 8.4 mL·kg^{-1}·min^{-1}	49.2 ± 4.0 mL·kg^{-1}·min^{-1}	**<0.001**	2.20
al. (2008)	12.6 ± 1.3 y	13.3 ± 0.5 y				
Moser et al.	*n* = 22, 8M/14F	*n* = 54, 17M/37F	956 ± 380 mL·min^{-1}	1473 ± 397 mL·min^{-1}	**<0.001**	8.23
(2000)[$$]	10.3 ± 3.3 y	9.3 ± 0.7 y				
Nixon et al.	*n* = 30, 18M/12F	*n* = 30, 17M/13F	36.5 ± 8.3 mL·kg^{-1}·min^{-1}	41.4 ± 8.9 mL·kg^{-1}·min^{-1}	**0.036**	0.57
(2001)	10.8 ± 2.9 y	11.4 ± 2.2 y				
Poore et al.	*n* = 15, 5M/10F	*n* = 15, 6M/9F	1.44 ± 0.68 L·min^{-1}	1.81 ± 0.71 L·min^{-1}	0.156	0.53
(2013)	12.6 ± 3.4 y	13.6 ± 2.7 y	32.8 ± 6.2 mL·kg^{-1}·min^{-1}	36.6 ± 8.6 mL·kg^{-1}·min^{-1}	0.178	0.51
			71 ± 14 %$_{Predicted}$ [b]	85 ± 16 %$_{Predicted}$ [b]	**0.016**	0.93
Saynor et al.	*n* = 10, 9M/1F	*n* = 10, 9M/1F	1.93 ± 0.84 L·min^{-1}	2.21 ± 0.79 L·min^{-1}	0.45	0.34
(2014b)	12.7 ± 2.8 y	12.5 ± 2.8 y	36.3 ± 7.6 mL·kg^{-1}·min^{-1}	43.9 ± 5.2 mL·kg^{-1}·min^{-1}	0.01	1.11
			45.5 ± 9.1 mL·kgFFM^{-1}·min^{-1}	53.5 ± 6.4 mL·kgFFM^{-1}·min^{-1}	0.03	0.96

Saynor et al.	*n* = 7, 5M/2F	*n* = 7, 5M/2F	2.08 ± 0.74 L·min^{-1}	2.51 ± 0.91 L·min^{-1}	0.34	0.49
(2016b)	13.5 ± 2.8 y	13.6 ± 2.4 y	34.30 ± 8.88 mL·kg^{-1}·min^{-1}	47.75 ± 3.56 mL·kg^{-1}·min^{-1}	**<0.01**	1.79
			51.87 ± 34.90 mL·kgFFM^{-1}·min^{-1}	65.52 ± 24.65 mL·kgFFM^{-1}·min^{-1}	0.42	0.42
Stevens et al.	*n* = 19, 9M/10F	*n* = 19, 9M/10F	35 ± 8 mL·kg^{-1}·min^{-1}	44 ± 12 mL·kg^{-1}·min^{-1}	**0.01**	0.88
(2011)	13.4 ± 3.1 y	13.8 ± 3.5 y	43 ± 9 mL·kgFFM^{-1}·min^{-1}	54 ± 13 mL·kgFFM^{-1}·min^{-1}	**0.01**	0.98
Stevens et al.	*n* = 19, 9M/10F	*n* = 19, 9M/10F	79.8 ± 16.9 %$_{Predicted}$ [b]	101.3 ± 22.5 %$_{Predicted}$ [b]	**<0.01**	1.08
(2015)	13.4 ± 3.2 y	13.8 ± 3.5 y				
Tucker et al.	*n* = 14, 6M/8F	*n* = 14, 6M/8F	33.0 ± 6.2 mL·kg^{-1}·min^{-1}	36.4 ± 8.9 mL·kg^{-1}·min^{-1}	0.252	0.44
(2018)	14 ± 3 y	14 ± 3 y	43.7 ± 6.8 mL·kgFFM^{-1}·min^{-1}	50.4 ± 7.6 mL·kgFFM^{-1}·min^{-1}	**0.022**	0.93
			74 ± 12 %$_{Predicted}$ [b]	85 ± 16 %$_{Predicted}$ [b]	0.054	0.78

CF, cystic fibrosis; CON; control; *ES*; effect size (Cohen's *d*, (Cohen, 1992)), FFM = fat-free mass.
All valued presented as mean (± standard deviation). Significant ($p < 0.05$) differences highlighted in bold.
a. Reference values from Ten Harkel et al. (2011)
b. No reference provided for calculation of %$_{Predicted}$.
[$] $\dot{V}O_{2peak}$ data also expressed to mL·kg^{-1}·min^{-1} ($p = 0.136$) and mL·kgFFM^{-1}·min^{-1} ($p = 0.014$) in figures, but exact values not provided by authors.
[$$] $\dot{V}O_{2peak}$ data also expressed to mL·kg^{-1}·min^{-1} ($p < 0.001$) and mL·min^{-1}·cm^{-2} ($p < 0.001$) (thigh muscle cross-sectional area) in figures, but exact values not provided by authors.

2.2.2 Clinical importance of exercise capacity in cystic fibrosis

Measures of exercise capacity provide useful prognostic information within the management of CF, above and beyond traditional lung function parameters. Of greatest clinical importance in CF, exercise capacity ($\dot{V}O_{2peak}$) is predictive of mortality in both adults (Nixon et al., 1992) and children (Pianosi et al., 2005a). Independent of changes in lung function (FEV_1), both longitudinal studies have identified significant rates of mortality associated with lower fitness levels. When split into tertiles based upon baseline aerobic fitness, both studies identify an approximate 70% mortality rate in the groups with lowest fitness (≤58 $\%_{Predicted}$, (Nixon et al., 1992); ≤32 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, (Pianosi et al., 2005a), and higher survival rates in the groups with higher fitness; 83% in adults with $\dot{V}O_{2peak}$ ≥82 $\%_{Predicted}$ (Nixon et al., 1992), and 100% in children with a $\dot{V}O_{2peak}$ >45 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ (Pianosi et al., 2005a) (Figure 2.16).

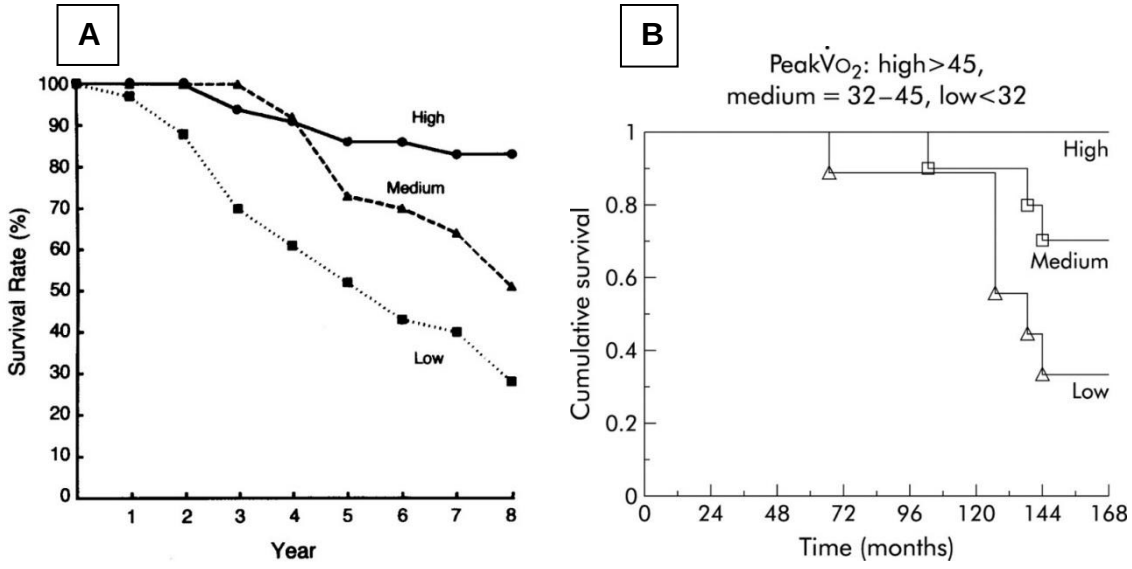


Figure 2.16 Survival among adults (A) and children (B) with cystic fibrosis, when divided by aerobic fitness ($\dot{V}O_{2peak}$). A: Reproduced with permission from Nixon et al. (1992) New England Journal of Medicine, 327(25), p1786. ©Massachusetts Medical Society. B: Reproduced from Thorax, Pianosi et al., 60 (1), p52, ©2005 with permission from BMJ Publishing Group Ltd.

Further to $\dot{V}O_{2peak}$, an elevated breathing reserve index (BRI), which represents the ratio between $\dot{V}_E$ and maximal voluntary ventilation (MVV), at the lactate threshold (LT) is a further predictor of mortality (Tantisira et al., 2002). When the BRI at the LT is elevated (≥0.7), a relative hazard risk of 17.5 (p = 0.004) is present. To contextualise this result, the hazard risk for FEV_1 at the same time (when BRI and FEV_1 were included in the same multivariate model) was 1.19 (p = 0.005). Whilst the magnitude of this result is exceptionally large, it should be noted that this study was undertaken solely in patients with CF awaiting lung transplantation, and therefore already have severe disease, as shown by baseline FEV_1 of the cohort who survived the length of the study (n = 30, FEV_1 = 27.3 ± 10.3 $\%_{Predicted}$).

An increased aerobic fitness is also associated with a reduced risk of hospitalisation (Pérez et al., 2014). When considered as independent factors (i.e. univariate modelling) contributing towards a risk of admission for an acute exacerbation requiring antibiotics, variables of FEV_1, FVC, blood oxygen saturation (SpO_2) and $\dot{V}O_{2peak}$ are all significant predictors. However, when considered in a multivariate model, only $\dot{V}O_{2peak}$ significantly predicts hospitalisation risk above and beyond age, sex, FEV_1, BMI and SpO_2.

In addition to offsetting major ‘endpoints’ of death and hospitalisation, a low $\dot{V}O_{2peak}$ is associated with poor glucose tolerance (Foster et al., 2017). Patients with CFRD have been identified as having a lower aerobic fitness than patients with normal glucose tolerance (33.0 ± 7.7 vs. 41.3 ± 9.4 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, p = 0.01), even though all patients had a preserved FEV_1. A lower $\dot{V}O_{2peak}$ is also significantly correlated with a higher number of antibiotic treatments over a prior three-year period (Vandekerckhove et al., 2017). An increased $\dot{V}O_{2peak}$ is additionally associated with higher levels of QoL. Numerous domains of QoL are

positively correlated with exercise capacity, including physical functioning (r = 0.37, p < 0.01), role limitations (r = 0.32, p < 0.05), health perception (r = 0.40, p < 0.001) and respiratory symptoms (r = 0.42, p < 0.001) (Hebestreit et al., 2014).

Exercise capacity (as determined by distance walked in the 6 Minute Walk Test [6MWT]) is also used in the calculation of a 'Lung Allocation Score' (LAS), to prioritise patients awaiting lung transplantation. A walking distance < 150 feet in a 6MWT contributes towards a higher LAS, which in turn increases their priority for surgery (Egan et al., 2006). An analysis of ~1,400 transplants over a seven year period in the USA found that of patients with a higher LAS (≥50), 26.7% had a 6MWT score <150 feet, in contrast to 8.5% of patients with a LAS <50 at the time of surgery (Braun et al., 2015). The 6MWT score is considered alongside traditional factors of FVC, BMI and age, but also factors such as the need for supplemental O_2 at rest and continuous mechanical ventilation (Braun et al., 2015), highlighting the importance of exercise testing in screening for functional capacity in CF.

Beyond maximal variables (such $\dot{V}O_{2peak}$), submaximal parameters derived from exercise testing have clinical importance in CF. The gas exchange threshold (GET) is related to disease severity (Thin et al., 2002) and the peak ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) is also predictive of mortality in adolescents with CF (Hulzebos et al., 2014).

2.2.3 Assessment of exercise capacity in cystic fibrosis

Given the clinical importance of exercise in the assessment and treatment of CF, national and international guidelines stipulate that exercise testing should occur on at least an annual basis (Cystic Fibrosis Trust, 2017a, Hebestreit et al., 2015, National Institute for Health and Care Excellence (NICE), 2017). The use of CPET

is identified as the ‘gold standard’ technique, and has recently been advocated by the ECFS ‘Exercise Working Group’ as the preferred method of assessing exercise tolerance in CF youth, a position which is further endorsed by the ERS (Hebestreit et al., 2015). However, the protocol of choice within the position statement from Godfrey et al. (1971) has a number of methodological issues pertaining to validity and reliability which have been raised by Saynor et al. (2016a). However, the concept that CPET should be utilised wherever possible is a welcome one, leading the way for the phasing out of previously used field tests – a number of which are summarised below.

2.2.3.1 Field tests

Prior to the widespread endorsement and advocacy of CPET, exercise capacity has previously been assessed using field tests. A survey by Stevens et al. (2010) identified that 37% of clinics utilise a modified shuttle test (MST), and 24% a 6MWT to assess exercise capacity in their patients with CF. The MST, developed by Singh et al. (1992) has been shown to be valid (Bradley et al., 1999) and reliable (Bradley et al., 2000) in adults with CF, based upon the high correlation between MST distance covered and body-mass relative $\dot{V}O_{2peak}$ from a treadmill test (r = 0.95, p < 0.01). Furthermore, the MST is apparently valid and reproducible in children with CF, again due to a high correlation (r = 0.91) between MST distance covered and body-mass relative $\dot{V}O_{2peak}$ (Selvadurai et al., 2003). However, a consistent underestimation of $\dot{V}O_{2peak}$ is present (-5.30 ± 4.63 $mL \cdot kg^{-1} \cdot min^{-1}$) when compared to a measured $\dot{V}O_{2peak}$ using a treadmill protocol. In addition to the MST, the 6MWT is deemed valid and reliable in children and adolescents, due to high correlations between walking distance and absolute $\dot{V}O_{2peak}$ (r = 0.76, p < 0.001) as well as between two tests when performed one-week apart (r = 0.90, p < 0.001) (Gulmans et al., 1996). However,

these analyses assessing the MST and 6MWT have largely inferred validity on the basis of high correlation coefficients, which fail to account for systematic bias between measures. Therefore, the suitability of these field tests as suitable alternatives to CPET is called into question.

Further to the MST and 6MWT, the one-minute sit-to-stand (Gruet et al., 2016) and 3-minute step (Narang et al., 2003) tests have been developed. However, they have not been found to be suitable replacements for CPET as they fail to elicit similar physiological responses (i.e. HR_{max}, SaO_2) as maximal exercise tests, and hold no clinical prognostic relevance (unlike CPET).

The advantage of field testing procedures is that they typically have minimal costs associated with them, can be easily implemented with few time, facility, or staffing constraints, and provide an opportunity for patients with CF to undertake a physical assessment of their exercise tolerance in lieu of CPET. However, results hold limited clinical value, protocols are limited in their ability to directly identify causes of fatigue and underlying dysfunction (Hebestreit et al., 2015) and there is a risk of over-, or under-reporting, performance and function. This in turn can have clinical consequences, such as an exaggerated risk of hospitalisation and therefore it is clear that 'gold-standard' CPET procedures are preferred to determine exercise capacity.

2.2.3.2 Cardiopulmonary exercise testing (CPET)

Cardiopulmonary exercise testing, in contrast to field tests, makes a direct measurement of exhaled gases at the mouth (i.e. O_2, CO_2) and based upon the respective volume of these gases (i.e. $\dot{V}O_2$ and $\dot{V}CO_2$) utilised and generated during exercise, researchers and clinicians can identify the energy cost of exercise, as well as contributing fuel sources (i.e. carbohydrate, lipids).

It has been long established that the oxygen cost of exercise increases with the amount of work done (Hill and Lupton, 1923), and that there is a physiological upper limit to oxygen consumption ($\dot{V}O_{2max}$) (Astrand and Saltin, 1961). It is the process of eliciting this upper limit to exercise tolerance that forms the basis of CPET, with differing modalities (e.g. cycling, running) and protocols (e.g. step, ramp) available which increase the amount of work the human body is required to undertake.

Treadmill based protocols utilise increases in speed and incline to increase the workload upon the body, with the Bruce protocol (Bruce et al., 1973) being the most popular for use (Hebestreit et al., 2015). Whilst the reliability of this protocol has been established in a clinical group of children (those with heart murmurs; Cumming et al. (1978)), no such data are available for children with CF.

Primary outcomes of the Bruce protocol (as with all CPET protocols) do include $\dot{V}O_{2peak}$ and exercise duration, factors which are in turn associated with traditional markers of pulmonary function of FEV_1 (Klijn et al., 2003, Pouliou et al., 2001). When analysis of pulmonary gas exchange is not available for directly determining $\dot{V}O_{2peak}$, it may be estimated using existing equations (Foster et al., 1984, Pollock et al., 1982), although the validity of such equations in CF is unknown. Furthermore, calculation of WR_{peak} is possible, although, this requires a function of the patient's body mass, gravitational constant (*g*; 9.81 $m \cdot s^{-2}$), final incline grade and velocity, as well as time spent on the final stage; a calculation that is less accurate than cycle ergometry (Hebestreit et al., 2015).

Finally, the Bruce protocol has been used to longitudinally monitor exercise capacity (Klijn et al., 2003), as well as responses to training (Selvadurai et al., 2002a) in children with CF. Therefore, this highlights the clinical utility of treadmill

testing as a feasible option when undertaking CPET, however there are several practical considerations that favour the use of cycle ergometry instead, which are elaborated upon below.

The results of a survey by Ruf et al. (2010) identified no adverse reactions associated with exercise testing, although a small number of adverse events were recorded by patients with regards to general exercise and activity. These include shortness of breath, arthritis and hypoglycaemia, although their relative incidence was each <1% per 1,000 patient years. Whilst the survey of Ruf et al. (2010) did not identify testing modalities in their survey, it can be argued that a cycle ergometer poses fewer risks than a treadmill. Patients who are deconditioned have no risk of falling off the cycle ergometer (unlike the risk of falling off a treadmill due to a failure to maintain speed); it is easier to terminate exercise safely in the case of adverse events; and provides patients with greater control of their test, as they can cease exercising immediately if they wish, without the need for a treadmill belt to slow, or stop completely.

The cycle ergometer protocol that is currently endorsed for use by the ECFS is the Godfrey protocol (Godfrey et al., 1971), a step incremental test that increases work rate based upon a patients stature; either 10 $W \cdot min^{-1}$ (<120 cm), 15 $W \cdot min^{-1}$ (120-150 cm), or 20 $W \cdot min^{-1}$ (>150 cm). A number of methodological issues have been associated with this protocol, notably a lack of validity and reliability data in CF (Saynor et al., 2016a). Furthermore, to determine a maximal effort, this protocol is reliant upon a plateau in $\dot{V}O_2$ and secondary criteria to verify whether a maximal test has been achieved, whereas S_{max} verification should be utilised. In paediatric studies, the secondary criteria include reaching a maximal heart rate (HR_{max}) at or above age-predicted maximum (i.e. 220 – age), a HR_{max} of 95% age-predicted maximum or a HR of 180 $beats \cdot min^{-1}$; a respiratory

exchange ratio (RER) of >1.00, 1.03, 1.05, or 1.10; subjective ratings of perceived exertion (RPE) of 9-10 on a 0-10 scale, or ≥17 on a 6-20 scale; and blood lactate ≥6 $mmol \cdot L^{-1}$ (Hebestreit et al., 2015, Saynor et al., 2013a). As noted from the list of secondary criteria, there are numerous cut-offs to be chosen from, which itself poses methodological discrepancies between studies. However, it has also been observed that such cut points typically occur at submaximal points of incremental exercise in children and adolescents with CF (Saynor et al., 2013a). Furthermore, during incremental exercise to exhaustion, only ~1/3 of children without CF will exhibit a plateau in $\dot{V}O_2$ (Armstrong et al., 1996, Barker et al., 2011). These factors can therefore lead to an under-reporting of $\dot{V}O_{2max}$, and this subsequently explains why the term '$\dot{V}O_{2peak}$' is utilised instead in the absence of a plateau.

To circumvent the reliance upon inadequate secondary criteria and truly ascertain whether a '$\dot{V}O_{2max}$' has been reached (as opposed to '$\dot{V}O_{2peak}$'), a two-stage exercise test on a cycle ergometer is used. This protocol utilises a combined ramp incremental phase and S_{max} verification bout, as shown in Figure 2.17. The utility of S_{max} verification testing has been widely researched (Barker et al., 2011, Day et al., 2003, Rossiter et al., 2006) and reviewed (Schaun, 2017), with a recent *'Cores of Reproducibility in Physiology'* (CORP) statement advocating its use in CPET as a 'gold standard' (Poole and Jones, 2017).

The work rate increments for the ramp phase are individualised to each patient and guided by existing equations to estimate peak power, based upon anthropometric and lung function data (Hulzebos et al., 2012), instead of arbitrary values for stature as per Godfrey et al. (1971). Following completion of a ramp stage to volitional exhaustion, and a brief break off the bike of ~10-15 minutes, the patient is asked to cycle again to exhaustion, using a 'square-wave' S_{max} stage at 110% of the peak power obtained during the ramp, to verify attainment of

$\dot{V}O_{2max}$. As this secondary bout of exercise is performed in the higher regions of the severe intensity domain (Xu and Rhodes, 1999), this stage is typically much shorter in duration (~1 – 4 minutes) than the first ramp stage (which is designed to typically last 8 – 12 minutes; Buchfuhrer et al. (1983)). If the $\dot{V}O_{2peak}$ obtained during the S_{max} phase is less than a 9% increase, then this indicates that $\dot{V}O_{2max}$ has been achieved as there has not been a 'meaningful' increase in $\dot{V}O_2$ between the bouts. The value of 9% is based upon day to day reliability data obtained in children with CF (Saynor et al., 2013b)

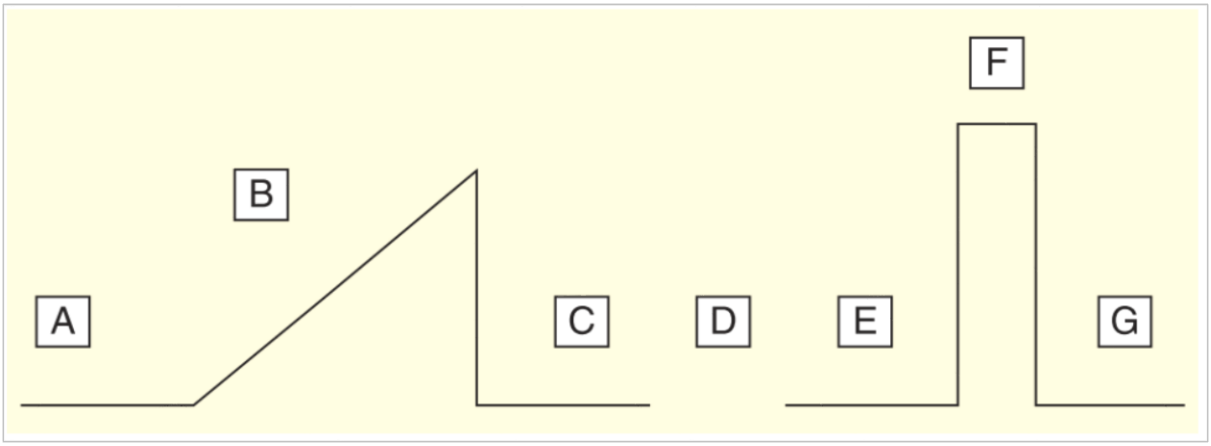


Figure 2.17 Two-stage ramp and supramaximal exercise protocol. A: 3-minute warm up at 20 W. B: Incremental ramp exercise at a predetermined rate (dependent upon individual data). C: 5-minute active recovery (unloaded pedalling). D: 10-minute seated recovery off the cycle ergometer. E: 3-minute warm-up at 20 W. F: Supramaximal confirmation bout at 110% of peak power output produced during ramp exercise. G: 3-minute recovery (unloaded pedalling). Reproduced with permission from Williams et al. (2014), *Expert Review of Respiratory Medicine*, 8(6), p753. doi: 10.1586/17476348.2014.96669.

This protocol has previously been demonstrated to verify $\dot{V}O_{2max}$ in healthy children (Barker et al., 2011), and has been validated for use in children with CF (Saynor et al., 2013a). Furthermore, reliability data are available with regards to the short-term (48 hours) and medium-term (4-6 weeks) reproducibility of $\dot{V}O_{2max}$ (Saynor et al., 2013b), and has also been shown to be safe and feasible in adults

and children with CF (Causer et al., 2018). Therefore, such data indicates the combined ramp and S_{max} protocol is likely more suitable for clinical practice than the Godfrey protocol as it shown to identify $\dot{V}O_{2max}$.

2.2.3.3 Maximal oxygen uptake ($\dot{V}O_{2max}$)

$\dot{V}O_{2max}$ represents the maximal aerobic power of the integrated pulmonary, cardiac and musculoskeletal systems (as per Milani et al. (2004), Figure 2.15) to utilise oxygen during exercise for movement. The $\dot{V}O_2$ can be calculated with the Fick equation:

Equation 2.1: $\dot{V}O_2 = \dot{Q} \times (C_a - C_{\bar{v}})$

whereby $\dot{Q}$ is cardiac output, and C_a and $C_{\bar{v}}$ are the oxygen concentrations of arterial and venous blood respectively. Numerous factors contribute towards the respective calculation of cardiac output (e.g. heart rate, stroke volume, ventricular capacity, pre-load and after-load) and the arterio-venous oxygen difference (e.g. haemoglobin concentration and saturation, capillary density and blood flow, alveolar O_2 pressure).

Ideally, aerobic fitness is measured directly using measures of gaseous exchange ($\dot{V}O_2$, $\dot{V}CO_2$). However, this may not always be possible in CF, either due to restrictions of staff, equipment and/or facilities, or due to risk of cross-infection. Therefore, when direct measurement of $\dot{V}O_2$ through pulmonary gas exchange is not available, estimates can be made based upon sex, stature and age (Jones et al., 1985). However, these existing equations do not account for exercise performance in terms of completed work, and therefore an equation from (Werkman et al., 2014) has been validated for use in children and adolescents with CF, which accounts for sex and WR_{peak}:

Equation 2.2: $\dot{V}O_{2peak}$ (mL·min^{-1}) = 216.3 – 138.7*Sex (0 = female, 1 = male) + 11.5*WR_{peak}
R^2 = 0.909, SEE = 172.57

This equation was developed in a group of 363 children with a wide range of disease statuses (FEV_1 = 37 – 147 $\%_{Predicted}$), and can prognostically assign patients to categories of high, medium, or low fitness (based upon divisions of Pianosi et al. (2005a)), although the success rate of prognostic assignment was only 50% in patients of high aerobic fitness. Furthermore, this equation has yet to be independently validated (although it was internally validated in a group of 60 children with CF) and therefore the suitability of applying such this formula in further groups of people with CF in unknown.

An alternative to this equation is the use of the steep ramp test (SRT). Originally developed for use in patients with chronic heart failure (Meyer et al., 1997), it uses work-rate increments between 10-20 W·s^{-1} (as opposed to 10-20 W·min^{-1} as per traditional CPETs) to elicit fatigue in ~2 minutes and produces peak work rate (WR_{peak}) as its primary outcome. It has since been validated for use in healthy children, with development of an equation from which to predict $\dot{V}O_{2peak,}$ using WR_{peak} as a predictor (Bongers et al., 2013):

Equation 2.3: $\dot{V}O_{2peak}$ (mL·min^{-1}) = (8.262WR_{peak}) + 177.096
R^2 = 0.917, SEE = 237.4

The SRT has subsequently been utilised in children with CF, as a S_{max} verification bout (Werkman et al., 2011), and as a predictor of $\dot{V}O_{2peak}$ using the equation above from Bongers et al. (2013). When 40 patients with CF (17 boys, 23 girls; 14.7 ± 1.7 years) undertook both a CPET and SRT, a significant correlation

between WR_{peak} from the SRT and $\dot{V}O_{2peak}$ from a CPET was identified (r = 0.82, p < 0.001, Bongers et al. (2015b)). However, use of correlation coefficients does not necessarily indicate agreement between values and agreement between measured and estimated $\dot{V}O_{2peak}$ was found to over-estimate aerobic power by a mean of 0.18 ± 0.31 $L \cdot min^{-1}$.

2.2.3.4 Submaximal measures

Whilst the value of maximal testing is clearly of prognostic relevance in CF, it is not always possible to obtain maximal parameters in a clinical population as patients may be unwilling, or unable, to exercise to volitional exhaustion. Therefore submaximal measures could provide a suitable alternative in the assessment of function (Williams et al., 2014), with these broadly split into the following 'thresholds' and measures of 'efficiency'.

The GET is a submaximal threshold during incremental exercise whereby $\dot{V}CO_2$ increases disproportionately to $\dot{V}O_2$, indicating increase in metabolic acidosis due the production and accumulation of lactic acid. This threshold is therefore reflective of the anaerobic threshold (AT), lactate threshold (LT) and ventilatory threshold (VT) (Beaver et al., 1986). Whilst the AT, LT and VT are separate physiological thresholds, they all fundamentally represent the point at which exercise enters the 'heavy' intensity domain (Xu and Rhodes, 1999). This is where a sustained anaerobic contribution towards exercise metabolism is present, but a non-exhaustive, $\dot{V}O_2$ steady-state of can be achieved. Individuals undertaking exercise in the heavy domain will sense a notable increase in effort and breathlessness.

The GET and LT are significantly correlated in CF, both for patients with mild (r = 0.86, p < 0.05) and moderate/severe (r = 0.82, p < 0.05) disease (Thin et al.,

2002). The GET has also been associated with disease severity in CF, with the absolute value of the GET decreasing with increasing severity (mild: 1202 ± 562, moderate: 995 ± 216, severe: 742 ± 245 $mL \cdot min^{-1}$) (Thin et al., 2002). However, these values are not normalised for body size, nor as a percentage of $\dot{V}O_{2peak}$, therefore limiting their interpretation and comparison with other populations.

Further evidence in the same study from Thin et al. (2002) suggests that the GET is reduced in CF relative to a non-CF CON group (1787 ± 512 $mL \cdot min^{-1}$), although the aforementioned issues with normalisation for body size and exercise intensity make this claim difficult to verify. Furthermore, studies which have normalised the GET as a percentage of $\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$, have found no difference between groups of children and adolescents with, and without CF, when using the validated combined ramp/S_{max} CPET protocol (Saynor et al., 2014b, Saynor et al., 2016b), thus ensuring a 'true' $\dot{V}O_{2max}$ has been achieved and utilised, and those that have used the Godfrey protocol (Bongers et al., 2014b) and therefore reporting GET relative to $\dot{V}O_{2peak}$.

The detection rates for the GET are variable: 82% in children (Hebestreit et al., 2000), 85% in adults with CF (Thin et al., 2002) and 92% in children with CF (Saynor et al., 2013b). This detection can vary dependent on the methodology used, as multiple approaches are available. Whilst the V-Slope method proposed by Beaver et al. (1986) has been used predominantly to date (Hebestreit et al., 2000, Saynor et al., 2013b, Saynor et al., 2014b, Thin et al., 2002), techniques including use of ventilatory equivalents (VentEq) for O_2 and CO_2, the end-tidal tension of oxygen (PET-O_2) method and when the RER = 1.0 (Visschers et al., 2015) have all been used to infer the threshold whereby exercise enters the heavy domain. When examined in CF, the intraclass correlation coefficient (ICC; of observed $\dot{V}O_2$) for the V-Slope (0.916), VentEq (0.839) and PET-O_2 (0.873)

methods are higher and produce comparable values for the GET, whereas the RER = 1.0 method has a lower ICC (0.747) and produces a significantly different values for the GET (as a percentage of $\dot{V}O_{2peak}$) relative to the other three methods (Visschers et al., 2015).

When the GET is identified in a CPET, the reproducibility can vary between tests and the observers (i.e. researchers, clinicians) performing the evaluation. The ICC over a short-term period (48 hours) is 0.8, whilst over a medium-term period (4-6 weeks), is 0.74 (Saynor et al., 2013b). When multiple observers identify the VT, the ICC is high between independent observers (0.94) and also when the same data are re-analysed following a period of time (6 weeks; ICC = 0.92, Hebestreit et al. (2000)).

Whilst the evidence for the use of the GET as a submaximal parameter of aerobic fitness is robust in terms of its association with disease severity and construct validity, normalisation relative to $\dot{V}O_{2peak}$ (for comparisons within and between individuals) requires an individual to reach volitional exhaustion itself, thus negating its potential as a submaximal replacement for $\dot{V}O_{2peak}$, as $\dot{V}O_{2peak}$ would have been obtained during the process regardless.

This same argument exists for the use of the respiratory compensation point (RCP), a further submaximal threshold between the GET and $\dot{V}O_{2peak}$, reflective of the disproportionate increase in minute ventilation ($\dot{V}_E$), relative to $\dot{V}CO_2$ (Beaver et al., 1986). As this threshold is normalised to a percentage of $\dot{V}O_{2peak}$ and occurs up to ~85% $\dot{V}O_{2peak}$ (Beaver et al., 1986), a peak effort is first required and thus rendering such a submaximal value redundant in comparison.

Furthermore, data surrounding the occurrence and reliability of assessing the RCP is unclear as it is rarely reported by studies, with some instead reporting an

alternative measure up to the point of the RCP, such as the $\dot{V}_E/\dot{V}CO_2$ slope (Bongers et al., 2014b). Furthermore, as the RCP is a variable that is predominantly driven by ventilation ($\dot{V}_E$), and as breathing patterns during incremental exercise are altered relative to non-CF controls and associated with disease severity (FEV_1) (Keochkerian et al., 2008), it is unclear as to whether there is clinical merit in routinely measuring and reporting the RCP in CF.

Further to use of thresholds, parameters of aerobic efficiency that characterise $\dot{V}O_2$ relative to changes in $\dot{V}_E$ can account for the impaired pulmonary function observed in CF and are relatively unexplored in CF. These include parameters of oxygen uptake efficiency (OUE; $\dot{V}O_2/\dot{V}_E$), and the oxygen uptake efficiency slope (OUES). The OUES has been developed as a submaximal parameter of aerobic fitness and is a derivation of the logarithmic relationship between $\dot{V}O_2$ and minute ventilation ($\dot{V}_E$), therefore removing the curvilinear component of $\dot{V}_E$ observed during exercise, allowing for direct comparison with other CPETs through the use of the subsequent linear regressions (Baba et al. (1996), Figure 2.18).

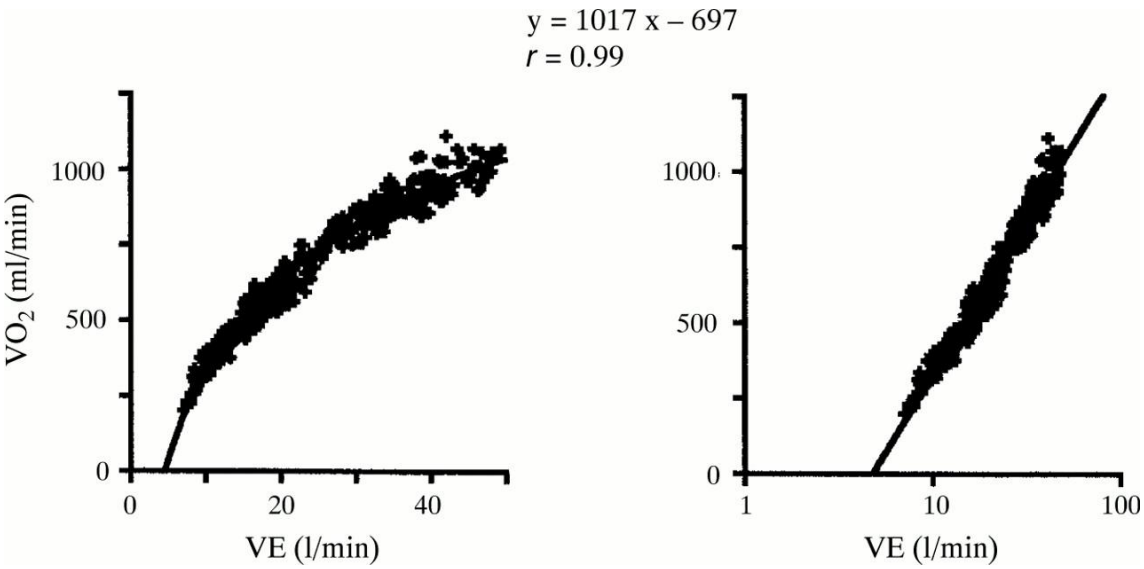


Figure 2.18 The relationship between $\dot{V}O_2$ and $\dot{V}_E$ during incremental exercise (left), and when $\dot{V}_E$ has been logarithmically transformed (right). The subsequent regression equation produces the value for the oxygen uptake efficiency slope (in this instance, 1017 $mL \cdot min^{-1} \cdot logL^{-1}$). Reproduced from Archives of Disease in Childhood, Baba et al., 81 (1), p73, ©1999 with permission from BMJ Publishing Group Ltd

The clinical utility of the OUES has been established in adults with CF, having been shown to be a significant predictor ($R^2 = 0.83$, $p < 0.01$) of absolute $\dot{V}O_{2peak}$ (Gruet et al., 2010), however its suitability for use in children, and as an independent parameter of aerobic fitness, is unclear. Previous research has characterised OUES at differing time points during exercise (50%, 75% and 100% of exercise duration; Bongers et al. (2012)) using a group of 22 children and adolescents with CF, and 22 non-CF controls. This study identified that absolute values for the OUES were lower than CON participants at each time point, although not statistically significant (100%, 2598.7 ± 642.9 vs. 2703.9 ± 637.2; 75%, 2487.1 ± 610.5 vs. 2664.1 ± 695.1; 50% 2201.1 ± 546.1 vs. 2547.2 ± 685.6 $mL \cdot min^{-1} \cdot logL^{-1}$). In addition, this study further normalised OUES by scaling relative to body surface area (BSA), subsequently finding OUES/BSA at 50% of exercise duration to be significantly lower in CF relative to the non-CF controls. Moreover, the OUES/BSA values were found to hold medium-to-large

correlations with body-mass relative $\dot{V}O_{2peak}$ in the CF (r = 0.41 – 0.54) and CON (r = 0.55 – 0.78) groups, with both statistically, and non-statistically, significant values for such correlations. Subsequently, the authors of this study concluded that the OUES was of limited value in children and adolescents with CF given the limited correlations with $\dot{V}O_{2peak}$ and inability to routinely distinguish between individuals with, and without, CF. However, there are methodological concerns with this study: a) the approach of using time to exhaustion to normalise exercise effort fails to account for individual differences in relative exercise intensity as a percentage of $\dot{V}O_{2max}$; and b) the scaling of OUES relative BSA was undertaken without an appropriate assessment of whether this scaling procedure effectively removed the residual effects of body size. As a result, replication of this study is warranted with a robust methodology to clarify the clinical utility of OUES as a submaximal parameter of aerobic fitness in children and adolescents with CF.

Further to the potential use of the OUES, OUE is as another submaximal parameter of aerobic efficiency that warrants investigation. In contrast to the OUES, the OUE incorporates the curvilinear ventilatory response to exercise (Sun et al., 2012b), as seen in Figure 2.19. It has been assessed in chronic diseases such as heart failure (Sun et al., 2012a), pulmonary hypertension (Tan et al., 2014) and chronic obstructive pulmonary disease (COPD) (Barron et al., 2016), however, it has yet to be investigated for use in CF. Parameters of OUE have been characterised in in a group of 214 Dutch children (without CF), using the highest 90-second average (oxygen uptake efficiency plateau [OUEP]), and the 60-second OUE average prior to the VT (Bongers et al., 2015a). When analysed, no significant differences were found between boys and girls for the OUEP (42.6 ± 4.7 vs. 42.3 ± 4.6) and OUE at the VT (42.0 ± 4.6 vs. 41.9 ± 4.7) respectively. Furthermore, the OUEP was found to be significantly correlated with

$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$) in this group (r = 0.65, p < 0.001), indicating potential surrogacy as a variable of aerobic fitness when $\dot{V}O_{2peak}$ is not available.

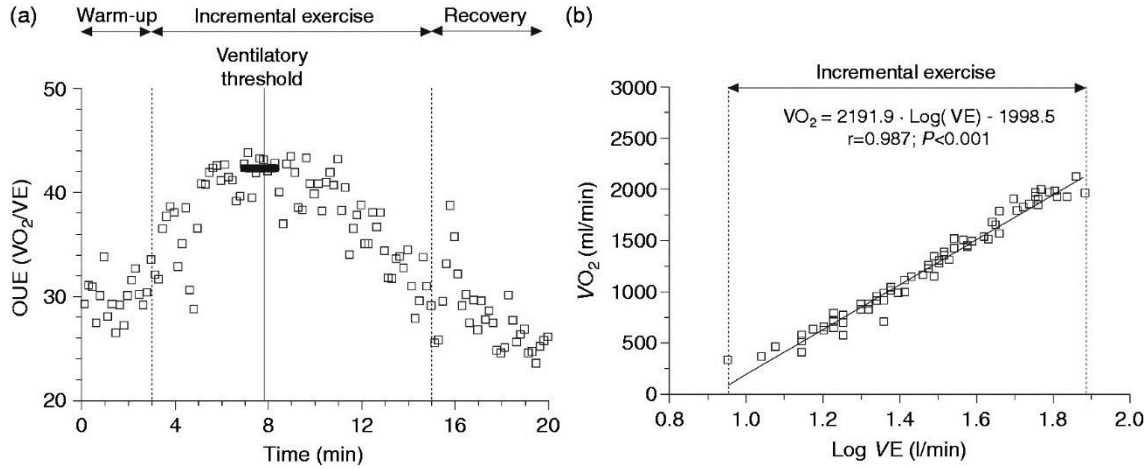


Figure 2.19 Comparative profiles of a) oxygen uptake efficiency, with the oxygen uptake efficiency plateau (42.3) represented by solid black horizontal line, and b) the oxygen uptake efficiency slope (2919.9), for a cardiopulmonary exercise test in a healthy, 13-year old girl. Reprinted with permission from Bongers et al. (2015a).

OUE has several technical advantages over OUES, having been shown to have a lower level of test-retest variability than OUES (Bongers et al., 2015a, Sun et al., 2012b), lower variation between participants (Bongers et al., 2015a) and a lower correlation with body mass (r = 0.57 vs. r = 0.83; Bongers et al. (2015a), indicating that scaling for body size may not be necessary. Furthermore, as parameters of OUE do not require a logarithmic transformation (like the OUES), this could make OUE an appealing variable of use for clinicians given the minimal technical requirements for its calculation.

Moreover, unlike submaximal thresholds such as GET and RCP which require a maximal effort from a CPET (in order to express values relative to $\dot{V}O_{2peak}$), the OUEP only requires the highest $\dot{V}O_2/\dot{V}_E$ ratio during exercise (which occurs close to the GET and VT; Bongers et al. (2015a)). The truly submaximal nature of this parameter could characterise aerobic function in patients with CF who are unable,

or unwilling, to perform maximal exercise and therefore warrants investigation as a prospective surrogate for $\dot{V}O_{2peak}$ in this disease group.

2.2.3.5 Relationship of parameters with body size

Parameters of physiological function (including exercise capacity) are often expressed relative to parameters of body size (e.g. body mass) in order to facilitate comparisons between individuals of differing sizes, track individual changes with growth and maturation (Nevill et al., 1992) and associate with disease outcomes (e.g. FEV_1, QoL). This process of normalising for body size is referred to as 'scaling'.

With regards to $\dot{V}O_{2peak}$, this process of normalisation for body size has been widely undertaken in CF, as can be seen in Table 2.2. Studies have normalised exercise capacity relative to parameters of body mass (Bongers et al., 2012, Bongers et al., 2014b, Saynor et al., 2014b), fat-free mass (Saynor et al., 2014b, Saynor et al., 2016b, Stevens et al., 2011, Tucker et al., 2018) as well as muscle cross-sectional area (mCSA; Moser et al. (2000)). In addition to $\dot{V}O_{2peak}$ being scaled for body size, the OUES has also been expressed relative to BSA (Bongers et al., 2012). Moreover, as noted in Chapter 2.2.1, this process of normalisation can result in statistically significant differences being found between groups (i.e. CF and non-CF controls) for $\dot{V}O_{2peak}$, where previously there where none based upon absolute values (e.g. Saynor et al. (2016b)). This therefore highlights the importance of scaling exercise data in CF, particularly when large variance in body size are reported within this disease group (Hanna and Weiner, 2015).

However, it has been shown that the use of ratio-standard scaling – the simple division of a numerator (i.e. $\dot{V}O_{2peak}$) by a denominator (i.e. body mass) – does

not effectively remove the residual effects of body size from physiological parameters (Armstrong and Welsman, 1994) and results in heavier individuals being penalised, as this approach can over-control for body size. The fallacy that ratio-standard scaling is sufficient is well known (Tanner, 1949), and consequently, allometric scaling (Welsman and Armstrong, 2000) has been identified as a robust statistical technique that effectively removes the residual effects of body size from physiological variables.

Simply, allometric scaling utilises a power function ratio (Y/X^{b}), whereby an exponent 'b' is derived from a regression between logarithmically transformed variables (i.e. $\dot{V}O_{2peak}$ and body mass) and this process is elaborated upon further in Chapter 3.5. Consequently, this has been successfully utilised in studies involving analysis of $\dot{V}O_{2peak}$ in adults and children relative to body mass (Welsman et al., 1996), lean mass (Graves et al., 2013), BSA (Rogers et al., 1995) and muscle cross-sectional area (Zanconato et al., 1994) and volume (Tolfrey et al., 2006). Given the wide variances in body sizes between and within groups of children, adults and disease populations, scaling exponents have been reported to vary widely, from 0.24 to 1.02, with a recent meta-analysis identifying a pooled scaling exponent of 0.70 when scaling $\dot{V}O_{2peak}$ relative to body-mass (Lolli et al., 2017).

However, despite the aforementioned variance in body size in CF (Hanna and Weiner, 2015), and the evidence in support of allometric scaling, it appears to be an underutilised statistical tool when exploring CPET derived parameters (e.g. $\dot{V}O_{2peak}$,OUES) in this population. Therefore, future studies should consider this approach where applicable. Furthermore, whilst the majority of studies have scaled for body-mass, additional parameters of body-size such as stature and BSA must be considered for scaling if they hold significant correlations with CPET

derived parameters as they can be reflective of further physiological differences between individuals that can contribute towards differing exercise-related results. For example, BSA has been suggested to control for pulmonary volume (Hollenberg and Tager, 2000), a parameter of significant interest and clinical importance in CF.

2.3 Exercise limitation in children with cystic fibrosis

Based upon the results of a CPET, such as $\dot{V}O_{2peak}$ and associated submaximal variables, schematics such as the one presented in Figure 2.20 can guide clinicians in the interpretation of outcomes and provide an indication as the cause of exercise limitation for individual participants and patients, particularly when a disease diagnosis may not be known. However, in CF, causes of exercise intolerance (including $\dot{V}O_{2peak}$), as described above in Table 2.2, are widely debated. Each organ system (cardiac, pulmonary and musculoskeletal) along the oxygen transportation and consumption pathway (i.e. from O_2 inhalation in the lung, to O_2 utilisation in the muscle; (Figure 2.15, Milani et al. (2004)), as well as the genetic root of the disease itself, have been proposed as contributors towards this exercise intolerance (Hulzebos et al., 2015). As such, a multifactorial approach must be considered when discussing exercise intolerance in young CF patients (Gruet and Saynor, 2017). The evidence for each of these potential contributing factors are briefly reviewed in the following sections.

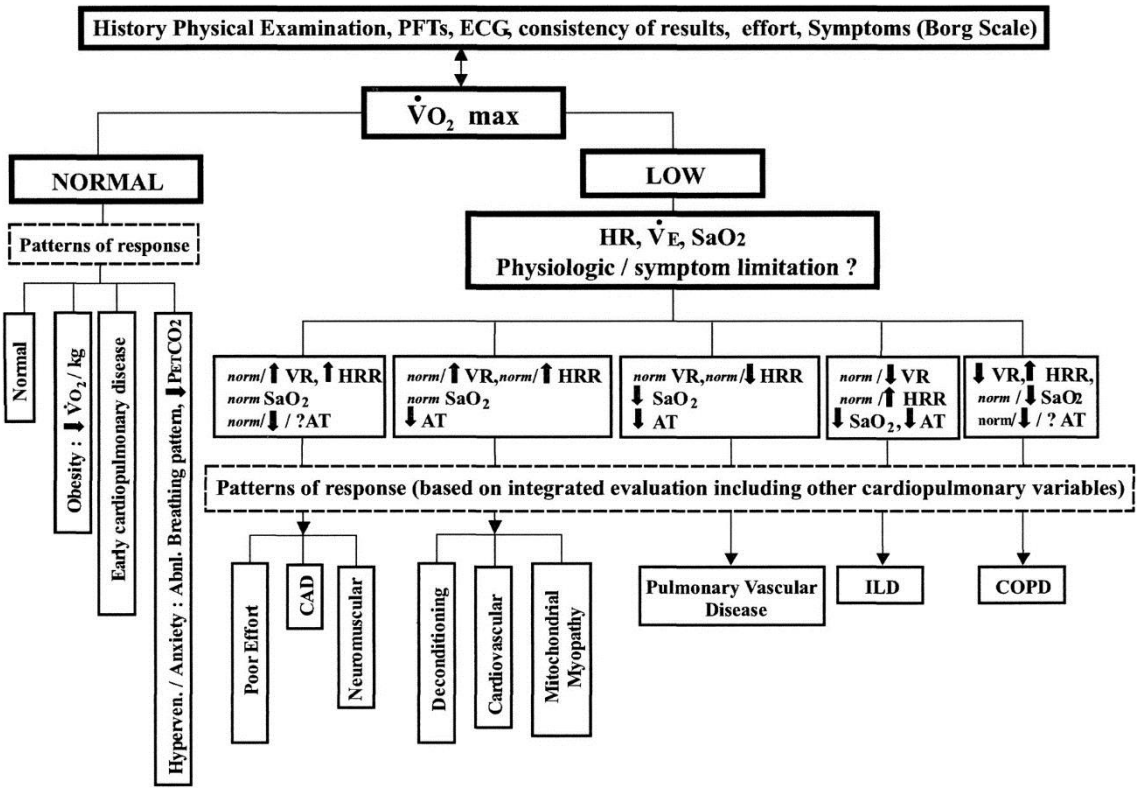


Figure 2.20 A basic schematic for interpretation of cardiopulmonary exercise test results, with likely causes of exercise limitation. AT, anaerobic threshold; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; ECG, electrocardiogram; HR, heart rate; HRR, heart rate reserve; ILD, interstitial lung disease; SaO_2, arterial blood oxygenation; PET-CO_2, end-tidal tension for carbon dioxide; PFT, pulmonary function test; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, volume of oxygen consumption; $\dot{V}O_{2max}$, maximal volume of oxygen consumption; VR, ventilatory reserve. Reprinted with permission of the American Thoracic Society. Copyright © 2018 American Thoracic Society. ATS/ACCP Statement on Cardiopulmonary Exercise Testing (2003), 16, p251. The *American Journal of Respiratory and Critical Care Medicine* is an official journal of the American Thoracic Society.

2.3.1 Genetic factors

Previous research in healthy populations has identified a genetic component of exercise trainability (Bouchard et al., 1999) and therefore given the genetic nature of CF, it is reasonable to assess genetic components to exercise (in)tolerance in this patient group. However, the evidence for a genetic basis to exercise limitation is divided, and compounded by the existence of different classifications (as seen

in Figure 2.3), and the frequency of individual CFTR mutations (De Boeck et al., 2014).

The association between genotype and $\dot{V}O_{2peak}$ was first conducted by Kaplan et al. (1996), finding a minimal effect size ($\eta^2 = 0.08$) between two groups of patients, based upon presence of the ΔF508 mutation (homozygous ΔF508, n = 10, $\dot{V}O_{2peak}$ = 37.0 ± 10.1 mL·kg^{-1}·min^{-1} vs. heterozygous ΔF508, n = 20, $\dot{V}O_{2peak}$ = 36.3 ± 12.6 mL·kg^{-1}·min^{-1}). However, only 75% of mutations (including ΔF508) were identifiable in this study. A similar stratification approach based upon ΔF508 was also utilised by McBride et al. (2010), finding no significant differences (p = 0.99) between three groups of children with CF (homozygous ΔF508, n = 36, $\dot{V}O_{2peak}$ = 38.1 ± 8.3 mL·kg^{-1}·min^{-1} vs. heterozygous ΔF508, n = 19, $\dot{V}O_{2peak}$ = 38.2 ± 8.8 mL·kg^{-1}·min^{-1} vs. 'Other/Other' [i.e. no ΔF508], n = 9, $\dot{V}O_{2peak}$ = 38.1 ± 9.9 mL·kg^{-1}·min^{-1}).

A further study by Selvadurai et al. (2002b) only assessed patients who were heterozygous for ΔF508 and further separated patients based upon their secondary mutation class (as per Figure 2.3). This study identified significant differences ($p < 0.05$) in $\dot{V}O_{2peak}$ between those with a second mutation in Class I and II compared to those with a Class III, IV or V mutation (Class I, n = 15, $\dot{V}O_{2peak}$ = 29.8 ± 4.2 mL·kg^{-1}·min^{-1} vs. Class II, n = 38, $\dot{V}O_{2peak}$ = 32.1 ± 4.9 mL·kg^{-1}·min^{-1} vs. Class III, n = 17, $\dot{V}O_{2peak}$ = 44.3 ± 6.4 mL·kg^{-1}·min^{-1} vs. Class IV, n = 17, $\dot{V}O_{2peak}$ = 54.0 ± 7.2 ml·kg^{-1}·min^{-1} vs. Class V, n = 10, $\dot{V}O_{2peak}$ = 54.3 ± 6.8 mL·kg^{-1}·min^{-1}). However, no control of covariates occurred despite there being a significant difference in BMI between groups, thus failing to isolate the sole effect of genotype upon exercise capacity.

The most comprehensive study to date, an international, multicentre study analysed CPETs from 726 patients with CF (18.7 ± 8.5 years), from 17 centres

across 14 countries (Radtke et al., 2017a). When separated by the milder of the two CFTR mutations, patients per group were as follows: Class I/I, *n* = 32; Class ≤II/II, *n* = 550; Class ≤III/III, *n* = 39; Class ≤IV/IV, *n* = 63; Class ≤V/V, *n* = 42. No significant differences were found between CFTR classes when $\dot{V}O_{2peak}$ was expressed as a percent of predicted using prediction equations of Orenstein (1993): Class I/I, 74 ± 17; Class ≤II/II, 79 ± 19; Class ≤III/III, 78 ± 24; Class ≤IV/IV, 83 ± 18; Class ≤V/V, 74 ± 19 $\%_{Predicted}$. Furthermore, when merged into two groups based upon CFTR classification (one group with two CFTR mutations in class I-III vs. one group with at least one mutation in class IV or V; *n* = 621 vs. 105), no difference was again found in $\dot{V}O_{2peak}$ (79 ± 19 vs. 80 ± 19 $\%_{Predicted}$). Finally, univariate prediction models failed to identify an association between CFTR functional class and $\dot{V}O_{2peak}$, whereas age, FEV1 ($\%_{Predicted}$), BMI *z*-score, CFRD and chronic *Pseudomonas aeruginosa* infection were all associated with $\dot{V}O_{2peak}$. Therefore, the increased sample size (and subsequent power) relative to previous studies, as well as robust statistical analyses led the authors to conclude there is no role of CFTR genotype upon $\dot{V}O_{2peak}$ in CF.

2.3.2 Pulmonary factors

As the primary manifestation of CF is both an obstructive and restrictive pulmonary disease, which in turn accounts for the predominant cause of death in CF (Flume et al., 2009), a mechanical constraint on the pulmonary contribution to exercise is to be expected.

The structural defects associated with CF (bronchiectasis, bronchial thickening, mucus plugging) that cause such chronic obstruction have been shown to be associated with a decreased exercise tolerance (Sovtic et al., 2014). This study assessed Modified Chrispin-Norman scores – chest radiographs that evaluate

over-inflation, and bronchial line, nodular, ring and large shadows in each of the four quadrants of the posteroanterior view – in 42 children with CF, aged 8-17 years. A higher score indicates greater disease severity, and was found be significantly and negatively correlated with $\dot{V}O_{2peak}$ (r = -0.41, p < 0.05).

Furthermore, $\dot{V}O_{2peak}$ has been shown to be more sensitive to structural change than pulmonary function. In the absence of significant changes in FEV_1, body-mass relative $\dot{V}O_{2peak}$ decreases when Bhalla scores (a further radiographic score of bronchiectasis and peri-bronchial thickening) increase – indicating a worsening of structural defects in the lung (Hatziagorou et al., 2016). Furthermore, within this study, $\dot{V}O_{2peak}$ was significantly and negatively correlated with the extent of bronchiectasis (r = -0.38, p = 0.0495), mucus plugging (r = -0.53, p = 0.004), and total Bhalla score (r = -0.48, p = 0.010), whereas FEV_1 was not significantly correlated with any of the radiographic components that contribute towards the Bhalla score.

Ventilatory parameters that may impair exercise capacity, such as BRI are only shown to be significantly compromised ($\dot{V}_E$/MVV > 70%; (American Thoracic Society, 2003)) in patients with severe CF (FEV_1 < 40 $\%_{Predicted}$), thus showing that it is only in severe disease that ventilatory factors are responsible for a diminished exercise capacity (Moorcroft et al., 2005). Furthermore, upon the onset of exercise, whilst minute ventilation will increase to match metabolic demand, the ventilatory pattern with which this increase occurs is variable between patients (Keochkerian et al., 2008), therefore making it difficult to identify a limiting ventilatory parameter that could potentially be targeted and corrected for (e.g. physiotherapy or pharmacological intervention) to improve exercise capacity.

Finally, both a number of cross-sectional (De Jong et al., 1997, Pianosi et al., 2005b) and longitudinal (Klijn et al., 2003) studies have identified significant positive correlations between FEV_1 and $\dot{V}O_{2peak}$. However, these correlation values vary greatly between studies, e.g. $r = 0.37$ (McBride et al., 2010), $r = 0.44$ (Sovtic et al., 2014), $r = 0.58$ (Perpati et al., 2010), $r = 0.60$ (Gruber et al., 2011a), $r = 0.62$ (Klijn et al., 2003), $r = 0.65$ (Kaplan et al., 1996), $r = 0.70$ (Moorcroft et al., 2005). Identifying the true nature of this relationship is further compounded by different studies reporting both $\dot{V}O_{2peak}$ and FEV_1 in differing formats, with absolute, relative and $\%_{Predicted}$ values all being utilised in numerous studies, therefore making a pooled analysis and evaluation difficult. In addition, values such as FEV_1 are only indicative of central airflow obstruction within the trachea and proximal bronchi and subsequently only account for approximately one-third of the reduction in $\dot{V}O_{2peak}$ patients with CF with and FEV_1 <50 $\%_{Predicted}$ (R^2 = 0.31), and only one-fifth ($R^2 = 0.18$) in patients with an FEV_1 ≥50 $\%_{Predicted}$ (Pastre et al., 2014), thus effectively leaving 82% unexplained variance in $\dot{V}O_{2peak}$ in patients with mild-to-moderate disease status.

Given the shared variance between FEV_1 and $\dot{V}O_{2peak}$, a measure of peripheral airway obstruction in the form of the LCI (Chapter 2.1.4.4) has been developed and is obtained using a multiple breath washout manoeuvre using an inert gas such as N_2 or SF_6 (Kent et al., 2014), with an increased LCI being associated with a worse disease severity. Data examining the association between $\dot{V}O_{2peak}$ and LCI are limited, although one study to research this association has identified that LCI is a weak, but significant, predictor of $\dot{V}O_{2peak}$ ($R^2 = 0.19$, $p < 0.001$) in a group of 97 Greek children (14.9 ± 4.6 y) with CF of a wide range of disease states (FEV_1 = 34 – 120 $\%_{Predicted}$) (Avramidou et al., 2018). However, in this study it is unclear as to whether this shared variance (R^2) between $\dot{V}O_{2peak}$ and LCI is

independent of FEV_1, and therefore how much this 19% variance explains above and beyond traditional pulmonary markers, given that the correlation between FEV_1 and LCI was moderately negative and significant (r = -0.71, p < 0.001).

2.3.3 Cardiac factors

At rest, cardiac output ($\dot{Q}$) is similar between individuals with CF and non-CF controls, but during exercise this is significantly reduced in CF (Rosenthal et al., 2009). This is primarily caused by a reduced stroke volume (SV) (Marcotte et al., 1986), and whilst compensated for by an increased HR during exercise (Pianosi and Pelech, 1996), it fails to sufficiently increase $\dot{Q}$ and therefore oxygen delivery is reduced during maximal and submaximal exercise (Rosenthal et al., 2009). Whilst these studies have estimated $\dot{Q}$ indirectly using the Fick equation, a reduced SV is still to be found when echocardiography is used, with a significant level of right ventricular (RV) systolic dysfunction (expressed as amplitude of long-axis excursion of the RV free-wall; Florea et al. (2000)), which subsequently correlates with disease severity (Ionescu et al., 2001). In addition, post-mortem studies have identified RV hypertrophy in 70% of children with CF (Royce, 1951).

Patients with CF have been shown to have an impaired ability to raise RV ejection fraction during exercise (Benson et al., 1984), which results in hypoxaemia (Matthay et al., 1980), and subsequently increases the risk of developing pulmonary hypertension and further right ventricular dysfunction (Fraser et al., 1999). It is also feasible that such hypoxemia may be exacerbated by ventilation-perfusion abnormalities and alveolar hypoventilation (Keochkerian et al., 2008). In chronic disease, such as COPD, development of pulmonary hypertension is characterised by chronic remodelling of the pulmonary vasculature, and when accompanied by dysregulation of the pulmonary vascular response to exercise a

substantial intolerance towards exercise is observed (Lewis et al., 2013).

It has recently been reported that ~51% and ~11% of adults with severe CF (FEV_1 <40 $\%_{Predicted}$) have underlying mild pulmonary hypertension (mean arterial pressure [MAP] ≥ 25 mmHg) and severe pulmonary hypertension (MAP ≥ 35 mmHg, Hayes et al. (2014)). However, despite this prevalence, it is unknown whether young people with CF exhibit pulmonary hypertension during exercise and whether such pulmonary vascular dysfunction contributes towards impaired exercise capacity in young people.

2.3.4 Musculoskeletal factors

Muscular strength positively correlates with aerobic fitness ($\dot{V}O_{2peak}$) in CF (de Meer et al., 1999). Given that both muscular strength (Hussey et al., 2002) and size are significantly smaller in children with CF when compared to non-CF controls (de Meer et al., 1999, Moser et al., 2000), it could be proposed that a simple size differential is contributing to the reduced indices of exercise capacity (i.e. strength, $\dot{V}O_{2peak}$) in CF. However, a number of intrinsic factors have also been proposed, including mitochondrial (Valdivieso et al., 2012) and vascular (Poore et al., 2013, Rodriguez-Miguelez et al., 2016) dysfunction. This has led to the development of a muscle 'quantity' vs. 'quality' debate within the literature (Hulzebos et al., 2017, Rodriguez-Miguelez et al., 2017).

When considering a 'quantitative' cause, $\dot{V}O_{2peak}$ remains lower in CF relative to a healthy non-CF control group after normalisation for mCSA ($\dot{V}O_2$/CSA; $mL \cdot min^{-1} \cdot cm^{-2}$), which is obtained using magnetic resonance imaging (MRI). This suggests an intrinsic muscular defect may be present (Moser et al. (2000) as a 'quantitative' defect has been statistically controlled for. However, despite this unique insight into a potential muscular defect, mCSA is a poor indicator of total

leg muscle volume, as previous research in adults has identified a 27% error rate in predicting MV when using a CSA slice at 40% of the length from the distal end of the femur. Whilst this error decreases when using a CSA slice from 50% of femur length (13% error), and 60% femur length (10% error), such estimations still result in systematic under- and over-estimations of MV (Morse et al., 2007). Furthermore, it is unclear as to how the use of single CSA slices obtained via MRI can be used to predict MV in a paediatric population, given differences in muscle volume (Tolfrey et al., 2006) and mass (Barker et al., 2008) between children and adults.

In addition to estimating MV from a single CSA (Morse et al., 2007), it can also be estimated by summing multiple CSA slices to infer a total MV. However, previous studies have all used differing CSA slice thicknesses, and number of total CSA slices to calculate total MV. Studies in heathy populations have used as few as seven slices (Walton et al., 1997), with disease groups (COPD) using 17 slices (Mathur et al., 2008). However, evidence has repeatedly shown that the error rate of MV estimation increases as the number of CSA slices decreases (Barnouin et al., 2015, Nordez et al., 2009, Tracy et al., 2003) and data pertaining to this error rate in paediatrics in lacking. Therefore, an appropriate quantification of the error associated with MV estimation in relation to the slicing strategy is required in children and adolescents before any analysis can be undertaken to precisely define the association between MV and $\dot{V}O_{2peak}$ in CF.

Previous studies in healthy children have examined the relationship between thigh (Welsman et al., 1997), calf (Tolfrey et al., 2006) and total lower leg (Graves et al., 2013) MV and aerobic fitness, although no such muscle volume-related data exists in a CF cohort. It is appropriate to control $\dot{V}O_{2peak}$ for MV as opposed to CSA alone, as it better reflects the amount of metabolically active muscle

during exercise and therefore normalising for MV may shed further insight into whether aerobic fitness is truly impaired in this patient group once muscle size has been accounted for. In addition, previous work has normalised aerobic fitness for mCSA using a ratio standard procedure (Moser et al., 2000), whereby allometric scaling procedures may be more appropriate (Tolfrey et al., 2006) to differentiate between CF and non-CF youth with regards to MV and aerobic fitness. Therefore, future studies should control for MV using allometric techniques to appropriately remove a 'quantitative' defect being responsible for reduced $\dot{V}O_{2peak}$ in CF.

Aside from a 'quantitative' element to the musculoskeletal reasons for exercise limitation, there are arguments to be made for a 'qualitative' component to exercise dysfunction in CF. Studies undertaken *in vitro* have identified that CFTR is presented in skeletal muscle, which may cause Ca^{2+} dysregulation, and in turn exercise intolerance (Divangahi et al., 2009, Lamhonwah et al., 2010). Furthermore, studies undertaken *in vivo* have utilised ^{31}Phosphorous-magnetic resonance spectroscopy (^{31}P-MRS) to non-invasively monitor muscle metabolism at rest and during exercise. Use of ^{31}P-MRS assesses turnover of muscle phosphates, finding decreased efficiency in oxidative ATP synthesis (de Meer et al., 1995) and delayed post-exercise phosphocreatine (PCr) recovery (Wells et al., 2011), thus suggesting impaired muscle aerobic oxidative metabolism in patients with CF. However, contrasting work has since yielded no significant differences between children with, and without, CF (Werkman et al., 2015). These discrepancies may be due to limited (and therefore underpowered) sample sizes (n = 6, Werkman et al. (2015)) or infection status, as chronic *Pseudomonas aeruginosa* infection has been associated with a decline in exercise capacity (van de Weert-van Leeuwen et al., 2012).

In addition to ^{31}P-MRS, near-infrared spectroscopy (NIRS) has been utilised as an alternative, non-invasive, approach to assessing skeletal muscle oxidative metabolism. Use of NIRS is cheaper and more practical, due to their small size and portable nature, and has been shown to produce reliable signals to assess *in vivo* oxidative capacity (Ryan et al., 2012b). When this technique has been applied in CF, evidence has been found for impaired skeletal muscle metabolism, as evidenced by a reduced recovery rate of oxygen consumption of the vastus lateralis (Erickson et al., 2015). However, this study isolated muscle contractions at rest using externally controlled electrical stimulation and arterial occlusions (Erickson et al., 2015), but when children with CF underwent whole body exercise, no clear difference in oxygen extraction could be identified between CF and non-CF children (Saynor et al., 2014b, Saynor et al., 2016b). This unaltered O_2 *extraction* supports previous research that reduced O_2 *delivery* during exercise is primarily responsible for exercise limitation in children with bronchiectasis (Rosenthal et al., 2009). When multiple approaches are utilised (^{31}P-MRS and NIRS), there is no suggestion of intrinsic metabolic abnormalities in oxygenation or oxidative metabolism during exercise in children with CF (Werkman et al., 2015). However, as patients in this study were of a similar fitness ($\dot{V}O_{2peak}$) to non-CF controls, evidence for impaired metabolic function would be difficult to identify if exercise capacity is not in fact impaired in such a small sample.

Therefore, there is evidence to show an intrinsic defect in skeletal muscle metabolism, although there is significant debate surrounding this argument (Hulzebos et al., 2017, Rodriguez-Miguelez et al., 2017). However, studies must first rule out the possibility of a quantitative defect (i.e. size-dependence) through appropriate establishment of MV and allometric scaling, prior to inferences being made on qualitative defects.

2.4 Exercise testing and the cystic fibrosis clinic

As described in Chapter 2.1.4.4, markers of exercise function (and in particular $\dot{V}O_{2peak}$) are utilised as outcome variables to assess the efficacy of exercise training regimens. However, the use of $\dot{V}O_{2peak}$ should be considered for use as an end-point for non-exercise intervention studies given its clinical importance. As CF treatment options are rapidly advancing (Armstrong et al., 2014), comprehensive and accurate outcomes are needed to evaluate such therapies and interventions to determine their efficacy. Furthermore, as $\dot{V}O_{2max}$ has been shown to be a reliable measure over the short (48 hours) and medium (4-6 weeks) term (Saynor et al., 2013b), this allows for a clinicians and researchers to identify whether clinically meaningful changes have occurred, allowing for accurate evaluation of non-exercise therapeutic interventions. However, for the use of CPET (and therefore $\dot{V}O_{2max}$) to become standard practice, clinical teams are required to adopt CPET and implement it in their own clinics.

With regards to antibiotic treatment, both shuttle tests (Cox et al., 2006, Cox et al., 2011) and CPET (Alison et al., 1994, Cerny et al., 1984, Selvadurai et al., 2002a) have been used to evaluate functional changes in exercise capacity. When using the MST as a marker of exercise performance, performance significantly ($p < 0.001$) increased by a mean distance of 102 m (18%) following a hospital admission lasting a mean of 15 days in a group of 28 children and adolescents, totalling 40 admissions (Cox et al., 2006). In contrast, no difference was found in MST distance following a home-based regimen of antibiotics (Cox et al., 2011). Interestingly, FEV_1 increased by similar magnitudes in both studies; by 15% ($p \leq 0.001$; Cox et al. (2006)) and 12% ($p < 0.05$; Cox et al. (2011)). These contrasting findings therefore query the sensitivity and utility of the MST as an outcome variable, thus increasing the scope for CPET as the primary exercise

test of choice for clinicians. When CPET has been used to assess efficacy of antibiotics, an incremental exercise test to exhaustion (without pulmonary gas exchange) found increases in WR_{peak} (relative to body-mass) of 23% ($p < 0.001$) and peak HR by 6% ($p < 0.001$; Cerny et al. (1984)). When studies have been able to utilise pulmonary gas exchange, $\dot{V}O_{2peak}$ increased from 1.11 ± 0.36 to 1.35 ± 0.46 $L{\cdot}min^{-1}$ (22%; $p < 0.005$) in adults performing exercise on a cycle ergometer (Alison et al., 1994). Furthermore, in Selvadurai et al. (2002a), the CON group of a three-arm training study also performed CPET, using the Bruce protocol on a treadmill, with $\dot{V}O_{2peak}$ declining by 1.22 ± 6.15 $mL{\cdot}kg^{-1}min^{-1}$ (4%) after a mean 18.6 ± 3.8 days of treatment. Whilst this initial value decreased upon discharge, $\dot{V}O_{2peak}$ improved by 2.65 ± 6.02 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ (8%) above baseline at a one month follow up; although both these observations were non-significant ($p > 0.05$) relative to baseline values.

When considering the role of CPET in assessing the efficacy of CFTR modulators, the evidence base is sparse given the relative novelty of this therapeutic treatment option, and the limited number of patients enrolled on open trials. A case report examining the role of Ivacaftor upon two adolescents with the G551D mutation found differing directions, and magnitudes, for changes in $\dot{V}O_{2max}$. One patient (14 y old female) improved $\dot{V}O_{2max}$ from 29.4 $mL{\cdot}kg^{-1}min^{-1}$ to 31.3 $mL{\cdot}kg^{-1}min^{-1}$ after six weeks of treatment, and further again to 38.3 $mL{\cdot}kg^{-1}min^{-1}$ following a further six weeks. In contrast, a second patient (16 y old male), saw a small increase in $\dot{V}O_{2max}$, from 44.2 $mL{\cdot}kg^{-1}min^{-1}$, to 45.7 $mL{\cdot}kg^{-1}min^{-1}$ at six weeks, before declining to 41.9 $mL{\cdot}kg^{-1}min^{-1}$ after a further six weeks (Saynor et al., 2014a). These findings for $\dot{V}O_{2max}$ contrasted to changes in FEV_1, whereby both patients increased over the 12-week period, by 4.7% and 11.0% respectively. Further to this, a 28-day crossover trial in 20 patients with CF, found no significant

differences in the magnitude of improvement in body-mass relative $\dot{V}O_{2peak}$ (obtained using cycle ergometry and the Godfrey protocol) relative to the placebo group. Both groups displayed a 4-5% increase in $\dot{V}O_{2peak}$, whereas FEV_1 increased significantly in the Ivacaftor group relative to placebo (+0.4 vs. +14.1 $\%_{Predicted}$), highlighting the independence between the two variables (Edgeworth et al., 2017). These studies further highlight the utility of CPET to evaluate therapeutic outcomes, by providing information that complements standard pulmonary measures to enhance the evaluation of therapeutic treatment regimens.

When considering the role of CPET in evaluating surgical procedures, a retrospective review of 153 patients who underwent lung transplantation (which included 35 with CF), identified a mean increase in $\dot{V}O_{2peak}$ of 21% up to 30 months post-transplant (Bartels et al., 2011). In contrast, a greater magnitude of improvement for FEV_1 was observed over the same period, improving by 147%. This highlights the discrepancy between pulmonary and exercise related outcomes in evaluating such treatments. The relatively small increase in exercise capacity is likely related to reduced muscle size (Pinet et al., 2004), and subsequent delayed recovery of muscular strength (Walsh et al., 2013). When $\dot{V}O_{2peak}$ is assessed in a sole group of 10 patients with end-stage CF (unlike Bartels et al. (2011) who pooled data for analyses), exercise capacity is observed to increase from 31 ± 3 to 45 ± 4 $\%_{Predicted}$, however clearly remained far below a normal result (i.e. 100%, Oelberg et al. (1998)).

Of interest, there is little data on the effect of nutritional interventions on exercise parameters in CF. Given that BMI, alongside $\dot{V}O_{2peak}$, is a significant predictor of mortality in adolescents with CF (Hulzebos et al., 2014), it would seem prudent to assess this relationship following nutritional intervention. Furthermore, 6% of

people with CF <16 years currently have a PEG in place for supplemental feeding (Cystic Fibrosis Trust, 2017b), although the impact of such procedures upon $\dot{V}O_{2peak}$ has yet to be reported upon in CF.

For the successful integration of CPET into the CF clinic, there must be advocacy from senior management within organisations, as well as acceptability from staff who undertake day-to-day duties within the healthcare system. The recent ECFS/ERS statement on exercise testing in CF (Hebestreit et al., 2015) has indicated a professional advocacy for the implementation of CPET in clinics across Europe. As previously mentioned, a survey by Stevens et al. (2010) of UK CF centres showed that clinical teams value exercise testing. However, 18% of centres that responded to the survey did not have any equipment available for testing (e.g. treadmill, cycle ergometer, pulse oximeter), and only 5% had access to a metabolic cart with which to undertake analysis of pulmonary gas exchange. It was also reported that physiotherapists perform the majority of exercise testing and training. This equipment availability in the UK is in stark contrast to Barker et al. (2004), whose survey of 62 German CF centres, identified that 53% of centres had access to a metabolic cart. Within the centres surveyed, 18% of tests were incremental cycle tests using the Godfrey protocol, and 15% were treadmill tests using the Bruce protocol. Further to these surveys, an additional survey of 22 global centres within the International Pediatric Lung Transplant Collaborative found that all centres undertake exercise testing, although only 6/22 centres used CPET to evaluate patients as part of transplant assessment and annual monitoring (Radtke et al., 2011). Where exercise testing was undertaken, a mixture of staff, including physiotherapists, technicians, exercise physiologists and specialist nurses were responsible.

Given that these surveys have highlighted the relatively low rate of CPET

implementation and identified the allied health professional responsible for testing (i.e. physiotherapists), there is clearly a need to ensure education and training surrounding CPET and exercise testing in general is sufficient to support current professionals. A recent survey of general practitioners (GPs) in the UK found that the majority (80%) are unfamiliar with national physical activity (PA) guidelines, and only 43% were confident in discussing PA with patients (Chatterjee et al., 2017). This therefore further highlights the need for sufficient education and training, particularly with regards to CF.

2.5 Summary

Exercise capacity ($\dot{V}O_{2peak}$, $\dot{V}O_{2max}$) has been shown to be reduced in young people with CF (Saynor et al., 2014b) and has several clinical implications, notably with regards to an increased risk of hospitalisation (Pérez et al., 2014) and mortality (Pianosi et al., 2005a). Given these risks, it is important for patients with CF to maintain, or augment, exercise capacity. This is especially pertinent in youth, as lung function has been shown to decline in adolescence (Liou et al., 2010) and the percentage of patients with chronic infections peaks a few years later in early adulthood (20-27 years) (Cystic Fibrosis Trust, 2017b). In addition, patients with CF will transition from paediatric care to adult care during adolescence (Nazareth and Walshaw, 2013), and with adults surviving for longer, and in greater numbers (Cystic Fibrosis Trust, 2017b, Dodge et al., 2007), it is imperative that this transition process in youth is tailored to individuals (Hewer and Tyrrell, 2008) in order to maintain health throughout adolescence and into young adulthood (Tuchman and Schwartz, 2013).

The prognostic value of maximal exercise testing is clear in this population (Nixon et al., 1992, Pianosi et al., 2005a), however further work is needed to clarify the validity of alternative submaximal measures for patients who are unable and/or

unwilling to exercise to a maximal level. Therefore, parameters such as the OUES (Baba et al., 1996, Bongers et al., 2012) and OUE (Bongers et al., 2015a) may have utility as alternative parameters of aerobic fitness and must be assessed for their ability to act as submaximal surrogates for $\dot{V}O_{2peak}$.

In establishing factors related to impaired exercise capacity observed in CF, there is still no clear consensus as why such limitations occur (Hulzebos et al., 2015), particularly with regards to musculoskeletal contributions, where the 'quality' vs. 'quantity' debate is contested at present (Hulzebos et al., 2017, Rodriguez-Miguelez et al., 2017). Therefore, appropriate determination of $\dot{V}O_{2max}$ and muscle size, as well as accurate scaling procedures, are required to comprehensively evaluate the relationship between muscle size and $\dot{V}O_{2max}$ in CF. Resultant findings could subsequently support or contrast the evidence for an intrinsic muscular defect contributing towards exercise intolerance.

Furthermore, despite CPET being advocated as an important outcome measure, it is still under-utilised in evaluating interventional strategies, particularly strategies aimed at improving body mass and therefore, changes in exercise related outcomes associated with insertion of a PEG and supplemental feeding need characterising.

Finally, there is still an apparent dearth of exercise testing taking place in CF centres. Therefore, education and training are required for clinical staff (such as physiotherapists and technicians) that undertake front line duties to increase understanding and confidence when discussing exercise with patients, as well as promoting the utilisation of CPET in those centres not currently offering the service.

2.6 Aims and objectives

As has been described throughout this review, the role of CPET in the management of CF is of utmost importance in children and adolescents. However, several aspects surrounding CPET and its application warrant further investigation: evaluation of submaximal alternatives to $\dot{V}O_{2max}$; identifying causes for reduced $\dot{V}O_{2max}$; and furthering the practical applications of CPET for both patients with CF and staff alike. Specifically, this thesis will:

- Determine relationships between parameters of body size (stature, body mass and body surface area) with OUES, and identify appropriate scaling procedures to adjust for these body size parameters when analysing the OUES in CF (Chapter 4);
- Provide an evaluation of the validity of the OUES as a submaximal surrogate for $\dot{V}O_{2max}$ in a group of children and adolescents with CF (Chapter 5);
- Evaluate the utility of OUE as a submaximal surrogate for $\dot{V}O_{2peak}$ in CF, with particular reference to OUEP (Chapter 6);
- Determine the error rate associated with using differing CSA slicing strategies (derived from MRI) to quantify MV in children and adolescents with and without CF (Chapter 7);
- Statistically control for MV to evaluate differences in $\dot{V}O_{2max}$ between children with and without CF, to determine dependence of exercise capacity upon body size (Chapter 8);
- Using a case-study approach, utilise CPET to evaluate changes in exercise capacity following insertion of a PEG and supplemental feeding in a young girl with CF (Chapter 9);

- Summarise two national meetings held with CF clinical staff across the United Kingdom, reporting on the current provision of exercise in CF centres (Chapter 10).
- Provide a summary of key findings, synthesise key themes within this thesis, as well as highlighting practical applications of CPET for future researchers and clinicians.

3 GENERAL METHODS

This chapter outlines the general, and common methods, utilised in subsequent experimental chapters. Further detail, where necessary, is provided in the relevant experimental chapters (Chapters 4 to 10).

3.1 Study designs

Throughout this thesis, differing study designs are utilised. Chapters 4 to 6 are retrospective analyses of existing datasets; Chapters 7 and 8 are observational case-control investigations; Chapter 9 is a case study; and Chapter 10 is a cross-sectional study.

3.1.1 Ethics approval

Due to the differing experimental designs utilised, alternative ethics approval processes were required, dependent on the nature of the study.

Chapters 4 to 6 utilise existing data, collated from previous experimental studies – all of which were approved by respective ethics committees prior to original data collection. Therefore, due to the retrospective, and anonymised, nature of the database, additional ethics approval was not required for these chapters.

For experimental chapters (Chapters 7 and 8), ethics approval was obtained from the local NHS Research Ethics Service Committee (14/SW/0061; Appendix A), with subsequent approval from Research and Development at the Royal Devon and Exeter NHS Foundation Trust Hospital (Appendix B) and the Chair of the University of Exeter Sport and Health Science Ethics Committee (Appendix C).

Ethics approval for the case study in Chapter 9 was obtained due to the prior involvement of the patient in an existing study which had obtained approval from the local NHS Research Ethics Service Committee (13/SW/0166; Appendix D) and Research and Development at the Royal Devon and Exeter NHS Foundation

Trust Hospital (Appendix E). In addition, the patient and parent signed journal-specific assent and consent forms prior to submission for publication, authorising release of data (example of consent form in Appendix F).

Throughout all studies, all personal information pertaining to participant was solely restricted to members of the study team. Furthermore, all procedures were undertaken, and measures obtained by, researchers with relevant Disclosure and Barring Service (DBS) checks to certify their ability with work with young people, and Good Clinical Practice (GCP) certificates where necessary.

3.1.2 Funding

The primary funding to report is from the Royal Devon & Exeter NHS Foundation Trust and the Cystic Fibrosis Trust. Additional funding for Chapters 7 and 8 was provided by the Department of Sport & Health Science for facility requirements, and salaries for staff were supported via an NIHR grant. Further funding for Chapter 10 was provided by the University of Exeter Open Innovation Link Fund. All funding to report for studies related to this thesis are stated in the appropriate sections of the respective manuscripts published as a result of the research.

3.1.3 Participants

Throughout this thesis, data from both children and adolescents with and without CF has been utilised retrospectively and generated prospectively. Dependent upon the study design, differing inclusion and/or exclusion criteria were applied prior to recruitment for studies, which are outlined below.

3.1.3.1 Patients with cystic fibrosis

All individuals with CF were recruited from outpatient appointments at the Royal Devon & Exeter NHS Foundation Trust Hospital. Patients were approached

alongside parent(s)/guardian(s) for inclusion in studies based on clinician's advice and objective, pre-approved criteria:

- CF diagnosis based on clinical features, supported by an abnormal sweat test (sweat chloride > 60 mmol·L^{-1} > 100 mg sweat), and where possible, diagnostic genotyping.
- Lung function considered stable and within 10 % of their best in the preceding 6 months.
- No increase in symptoms or loss of body mass in the preceding 2 weeks.

In addition to the above physiological inclusion criteria, additional study-specific inclusion criteria were identified, and ethics approved, for Chapters 7 and 8:

- Child is regularly participating in physical activity
- Child presents with no contraindications to performing exhaustive exercise within an MR scanner
- Child can understand and cooperate with the study protocol

Furthermore, for individuals with CF, the following exclusion criteria were employed at the screening stage to ensure patient safety and enjoyment during studies:

- Any non-pulmonary conditions that may impair exercise ability, such as musculoskeletal disorders (active arthritis, joint or muscle disease) and cardiovascular disease (congenital heart disease or cardiomyopathy).
- Unstable co-morbid asthma (daily pulmonary function variability of >20 %).
- Child presents with co-morbidities to performing exhaustive exercise within an MR scanner.
- Unable to understand or cooperate with the study protocol due to learning difficulties or otherwise.
- < 10 years of age.

- > 18 years of age.

Furthermore, in addition to the exclusion criteria above used during screening, the following exclusion criteria were also utilised at the onset of the study described in Chapters 7 and 8:

- Onset of acute infection.
- Unable to understand or cooperate with study protocol.
- Child and/or parent guardian to not wish to participate further.
- Child presents with co-morbidity which will deem it unsafe for them to perform exhaustive exercise within the MR scanner.
- Child is not happy being within the MR scanner environment.

3.1.3.2 Non-CF controls

All non-CF controls were recruited from local schools and sports clubs. After initial contact with teachers and coaches, investigators spoke to prospective children. Where children identified interest with the project, they were provided with participant information sheets and asked to discuss participation with parent(s)/guardian(s). Investigators then contacted parent(s)/guardian(s) to confirm interest and organise familiarisation visits.

For experimental processes described in Chapters 7 and 8, the following inclusion criteria for CON children were established:

- Healthy males and females aged 10-18 years who are age- and gender-matched to patients with CF.
- No diagnosis of chest disease or asthma.
- Child is regularly participating in physical activity.
- Child presents with no contraindications to performing exhaustive exercise within an MR scanner.

- Child can understand and cooperate with the study protocol.

Furthermore, the following exclusion criteria were also utilised at the recruitment stage:

- Any pulmonary conditions.
- Any non-pulmonary conditions that may impair exercise ability, such as musculoskeletal disorders (active arthritis, joint or muscle disease) and cardiovascular disease (congenital heart disease or cardiomyopathy).
- Child presents with co-morbidities to performing exhaustive exercise within an MR scanner.
- Unable to understand or cooperate with the study protocol due to learning difficulties or otherwise.
- Not an age- or gender-match for the chest diseased participants.
- > 18 years of age.

Furthermore, the following exclusion criteria were applied at the onset of the study:

- Onset of acute infection.
- Unable to understand or cooperate with study protocol.
- Child and/or parent guardian does not wish to participate further.
- Child presents with co-morbidity which will deem it unsafe for them to perform exhaustive exercise within the MR scanner.
- Child is not happy being within the MR scanner environment.

3.1.4 Participant information and consent/assent

All participants (CF and CON) and their parent(s)/guardian(s) were provided with ethics approved, written documentation (i.e. participant information sheets), detailing the purpose of the study in question, as well as the procedures involved and expected time commitments. Once prospective participants and

parent(s)/guardian(s) had asked any questions, written informed consent and assent was obtained from adults and children respectively. Examples of participant information sheets, alongside respective informed consent for parent(s)/guardian(s) and assent for children for Chapters 7 and 8 are provided in Appendices G to L (CF forms only provided for reference).

3.1.5 Inclusion in multiple studies

Participants were involved in multiple studies throughout this thesis for pragmatic reasons (i.e. reducing participant burden). Chapters 4 to 6 each describe a sample size of *n* = 72 (36 CF, 36 CON). These are the same 72 individuals used throughout each of the three chapters and subsequent analyses. Chapters 7 (*n* = 15, 8 CF, 7 CON) and 8 (*n* = 14, 7 CF, 7 CON) have utilised the same participants. For Chapter 8, one less individual with CF is included in analyses due to the requirement to age- and gender-match participants for analyses. The individual who is the participant of the case-study in Chapter 9 also contributed exercise data to Chapters 4 to 6. For these chapters, only their baseline data (i.e. pre-intervention) was included.

3.2 Participant information

Numerous anthropometric and exercise related variables were collated throughout the course of this thesis. These are described in further detail below.

3.2.1 Age and maturation

Age was determined in two different ways – chronological age and biological age.

3.2.1.1 Chronological age

Chronological age was calculated, where possible, as a decimal to the nearest 0.1 year between date of birth and date of testing. Where participants were

involved in multiple test visits within a short space of time (as per Chapters 7 and 8), reported age is age taken at the first visit (i.e. familiarisation).

3.2.1.2 Biological age

Given that maturation occurs independently of chronological age in adolescents, studies within this thesis also determine biological age. In Chapters 5 and 6, due to limitations imposed by the retrospective nature of analyses, age from peak height velocity (aPHV) was estimated using published equations (Moore et al., 2015) that only require the participants age and standing height (measured in cm).

Maturity offset for boys:

Equation 3.1: -7.999994 + (0.0036124 * (age * height))
R^2 = 0.896, SEE = 0.542

Maturity offset for girls:

Equation 3.2: -7.709133 + (0.0042232 * (age * height))
R^2 = 0.898, SEE = 0.528

In addition, pubertal status was obtained using a validated self-assessment (Morris and Udry, 1980) according to the five stages of pubic hair development (Marshall and Tanner, 1969, Marshall and Tanner, 1970, Tanner and Whitehouse, 1976). The scales for boys and girls are presented in Appendices M and N respectively. After an explanation of these stages of pubertal development by an investigator of the same sex, participants were requested to take the form home and circle the stage that best reflect their own development, before returning the questionnaire in a sealed envelope on their next visit to the laboratory. These envelopes were opened following the participants final visit to the laboratory.

Both aPHV and pubertal staging were utilised to assess maturity in this thesis, as issues do surround the use of aPHV as a marker of pubertal status, particularly

in late-maturing individuals (Malina and Koziel, 2014a, Malina and Koziel, 2014b), and as equations are not valid in boys >18 years, and girls >16 years. However, compliance with pubertal staging is not always guaranteed due to participants either forgetting to return questionnaires, having uncertainty regarding answers, or being unwilling to respond, hence the use of both methods.

3.2.2 Anthropometric measures

The range of anthropometric measures taken are described in detail below.

3.2.2.1 Stature

Stature was assessed using a wall-mounted stadiometer. The make and model of stadiometer differed between the exercise laboratory (Holtain; Crymych, UK) and outpatient clinic in the hospital (Seca; Birmingham, UK). Participants removed footwear and placed their heels against the base of the stadiometer, with feet together, and stood upright whilst looking forward. With the head in the Frankfort plane, stature was taken to the nearest 0.1 cm.

3.2.2.2 Seated stature

Seated stature was assessed using a seated stadiometer (Holtain; Crymych, UK). Participants sat on the stadiometer, in such a position that no body mass was supported by the ground, with participants legs laying over the edge of the stadiometer at an approximate 90-degree angle. The participant was instructed to sit upright, looking ahead, and the stadiometer was then placed against the participants back. With the head in the Frankfort plane, seated stature was taken to the nearest 0.1 cm.

3.2.2.3 Body mass

Body mass was taken to the nearest 0.1 kg using an electronic scale (Seca, Birmingham, UK). Participants were instructed to remove shoes and heavy clothing prior to assessment of mass.

3.2.2.4 Body mass index

Body mass index (BMI) was determined using the following equation:

Equation 3.3: BMI = body mass (kg) / stature (m^2)

BMI was presented as an absolute value, and for Chapter 9, also as a percentile using free to download, specialist software (WHO AnthroPlus; World Health Organization, Geneva, Switzerland) based upon international growth reference data (de Onis et al., 2007)).

3.2.2.5 Body surface area

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

Equation 3.4: $BSA\ (m^2) = mass^{0.5378} * height^{0.3964} * 0.024265$

3.2.2.6 Body fat estimation

Skinfold measurements were made using skin fold callipers (Harpenden; Baty International, Burgess Hill, UK), in line with recommended guidelines (Eston et al., 2009). Four sites were assessed - triceps, biceps, subscapular and suprailiac – with each measure taken three times, and the median utilised to determine the sum of skinfolds (SSkF). Estimates of body fat percentage (BF%) were made using published equations (Slaughter et al., 1988) to estimate body fat percentage, from which fat-free mass can be calculated.

Body fat percentage for pre-pubertal males:

Equation 3.5: $BF\% = 1.21 * (triceps + subscapular) – 0.008 (triceps + subscapular)^2 – 1.7$

Body fat percentage for circum-pubertal males:

Equation 3.6: $BF\% = 1.21 * (triceps + subscapular) – 0.008 (triceps + subscapular)^2 – 3.4$

Body fat percentage for post-pubertal males:

Equation 3.7: $BF\% = 1.21 * (triceps + subscapular) – 0.008 (triceps + subscapular)^2 – 5.5$

Body fat percentage for all females:

Equation 3.8: $BF\% = 1.33 * (triceps + subscapular) – 0.013 (triceps + subscapular)^2 – 2.5$

For the above equations, pubertal stages (Marshall and Tanner, 1969, Marshall and Tanner, 1970) were utilised to define pre- (stage 1 and 2), circum- (stage 3), and post-pubertal (stage 4 and 5) status, in line with original methodology (Slaughter et al., 1988). Where pubertal staging was not available, aPHV in years was used to define pre- (aPHV > -2 years), circum- (-2 years > aPHV < +2 years), and post-pubertal (+2 years < aPHV) status (Karlberg et al., 2003).

Once body fat percentage was estimated, fat free mass (FFM; kg) was determined using the following equation:

Equation 3.9: $FFM\ (kg) = body\ mass\ (kg) – (body\ mass * (\%BF / 100))$

This method has been shown to be valid and reliable in children and adolescents (Silva et al., 2013), and has subsequently been utilised in studies involving children with CF (Saynor et al., 2014b), and without CF (Cockcroft et al., 2015).

3.3 Testing procedures

Further to the age-related and anthropometric measures described above, additional testing procedures to obtain measures of pulmonary function, physical activity, exercise capacity and muscle volume are described below.

3.3.1 Pulmonary function

Pulmonary function was measured throughout according to standardised guidelines (British Thoracic Society, 1994, Miller et al., 2005a, Miller et al., 2005b, Pellegrino et al., 2005, Wanger et al., 2005), using a hand-held spirometer (MicroPlus, Micro Medical Ltd., Rochester, UK). Verbal encouragement was given with each manoeuvre, and measures of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were taken, with the best of three manoeuvres recorded.

The Tiffeneau Index (FEV_1/FVC) was subsequently calculated using the highest FEV_1 and FVC obtained, regardless of if two variables were from the same manoeuvre (Miller et al., 2010).

Volumes were calculated as a percentage of predicted values (based upon age and height), using a free-to-download desktop calculator from the Global Lung Initiative (GLI) (GLI-2012 Desktop Software for Individual Calculations, v.3.3.1, European Respiratory Society, Lausanne, Switzerland) and multi-ethnic reference values (Quanjer et al., 2012).

For Chapters 4 to 6, additional reference values (Quanjer et al., 1993, Zapletal et al., 1987) were utilised to calculate FEV_1 and FVC as a percent of predicted due to the retrospective nature of the database. Recent research suggest that prior results based on different equations should be accepted as reliable when compared to the GLI equations (Konstan et al., 2017).

Finally, maximal voluntary ventilation (MVV) was calculated using the following formula:

Equation 3.10: $MVV = FEV_1 (L) * 35$

3.3.2 Physical activity

In Chapter 8, participants wore a triaxial, wrist-mounted, accelerometer (GeneaActiv; ActivInsights, Kimbolton, UK) to objectively measure habitual PA. This was worn on the participant's non-dominant wrist for seven consecutive days, with data collected at a frequency of 100Hz. An activity diary (Appendix O) was utilised to qualitatively describe the activity undertaken by each participant during the seven days, broken down into 1-hour slots over the seven days. This was also used in conjunction with an 'on/off' log (Appendix P) to identify the time the accelerometer was worn each day from waking up, to going to bed, as well as any non-wear time (e.g. showering, water sports). Data collected by the accelerometer was subsequently exported in 60 second epochs, and using validated paediatric cut-points (Phillips et al., 2013), the time spent in each PA domain (sedentary, light, moderate and vigorous) was determined in both absolute number of minutes and as a percentage of wear time. Minimum wear time for analyses was set at a reliability coefficient of 0.86, which can be achieved with a minimum of 10 hours for two days (Rich et al., 2013).

3.3.3 Cardiopulmonary exercise testing

Throughout all chapters, participants have undertaken exercise tests to determine $\dot{V}O_{2max}$. For consistency, the same ergometers and gas analysers have been used where possible. If this has not been possible, the same make/models have been used to maintain the error within a certain manufacturer.

3.3.3.1 Equipment

For the CPETs used within this thesis, all exercise has been undertaken on upright cycle ergometers (Lode Excalibur; Lode, Groningen, the Netherlands). To observe outcomes of the CPET, participants wore a rubber oro-nasal facemask (Hans Rudolph, Shawnee, KS, USA), connected to a turbine and gas sampling line, which in turn were connected to a metabolic cart (Cortex Metalyzer 3B; Cortex Biophysik, Leipzig, Germany). This procedure permitted breath-by-breath collection of exhaled $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$, which allows for subsequent calculation of derivatives such as respiratory exchange ratio (RER). The metabolic cart was calibrated for pressure, gas and volume prior to each CPET. Participants also wore a Bluetooth heart rate monitor (Polar Electro; Polar, Kempele, Finland) and fingertip pulse oximetry (Nellcor N-20; Medtronic, Minneapolis, MN, USA) throughout.

3.3.3.2 Protocol

A two-stage incremental exercise test to volitional exhaustion was used, utilising a ramp phase and S_{max} verification bout. This has been validated for use in children with CF (Saynor et al., 2013a), and healthy controls (Barker et al., 2011). Both the ramp and S_{max} are described in detail below, with a schematic previously provided in Figure 2.17. For data utilised in Chapters 4 to 6, several CPETs (*n* = 39) were conducted without the use of S_{max} phase, as it was developed and validated following these initial studies from which data was obtained. This number of CPETs utilised a single incremental test to volitional exhaustion (i.e. no supramaximal verification phase as described below), and therefore some $\dot{V}O_2$ values are described as 'peak' $\dot{V}O_2$, as opposed to $\dot{V}O_{2max}$.

3.3.3.2.1 Ramp incremental phase

This protocol starts with a three-minute period of pedalling at a low resistance (10-20 W), before an incremental ramp is used to progressively increase the resistance against which a participant is cycling. The intensity of this ramp-rate differed between children as factors such as age, gender and stature can influence peak power. Therefore, an estimate of peak power was made, where possible, using the following equation from Hulzebos et al. (2012):

Equation 3.11: WR_{peak} (W) = -142.865 + 2.998 * Age (years) – 19.206 * Sex (0 = male, 1 = female) + 1.328 * Height (cm) + 23.362 * FEV_1 (L)

R^2 = 0.79, SEE = 21.0

The estimated peak power derived from this equation was divided by 10 as so to elicit VO_{2max} in approximately 10 minutes (i.e. within 8-12 minutes as per recommendations (Buchfuhrer et al., 1983)), and rounded to the nearest 5 W for ease of calculations.

The child was required to maintain a constant cadence between 60-80 revolutions per minute (rpm) throughout the test. The test was terminated when cadence fell <10 rpm below the self-selected cadence (i.e. <65 rpm if pedalling at 75 rpm) for five consecutive seconds despite strong verbal encouragement. After a five-minute cool-down at 10-20 W, the participant was given a 10-minute period of seated rest prior to the commencement of the S_{max} phase.

3.3.3.2.2 Supramaximal verification phase

After seated rest of at least 10-minutes, the participant returned to the bike, again warming up at 10-20 W for three minutes, maintaining the same cadence from the first phase. After the three minutes, participants undertook a square-wave ‘step’ transition to 110% of the peak power achieved in the first bout. Again, the

test was terminated when cadence fell <10 rpm below the self-selected cadence for five consecutive seconds despite strong verbal encouragement. At exhaustion, participants cooled down at 10-20 W for a further three minutes. An example of the $\dot{V}O_2$ response from the ramp incremental and S_{max} phase is given in Figure 3.1.

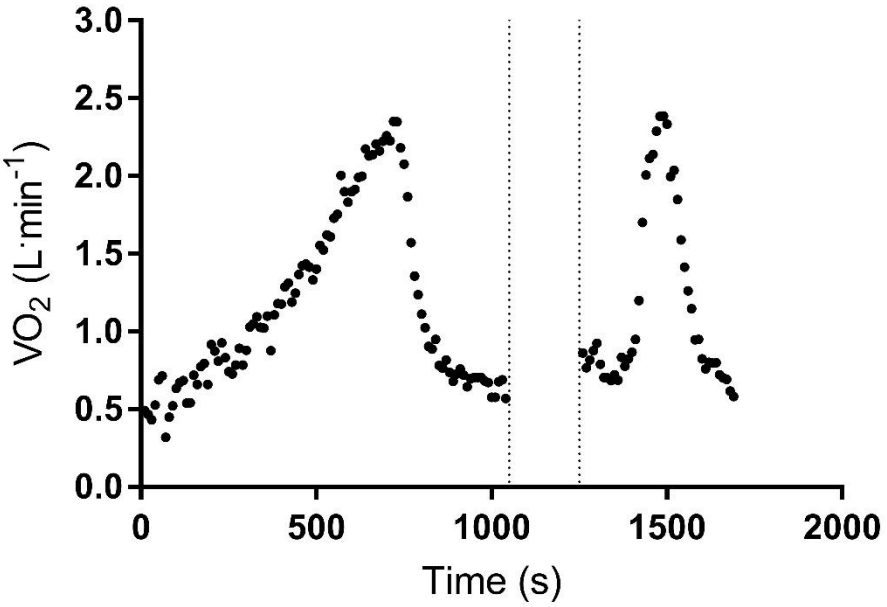


Figure 3.1 An example of the $\dot{V}O_2$ response to the ramp incremental and S_{max} exercise bouts, used to determine $\dot{V}O_{2max}$, for a healthy 16-year old female. Dashed vertical lines indicate end of ramp incremental phase and start of S_{max} phase respectively.

3.3.3.3 Outcome variables

Following the CPET, data was exported in a breath-by-breath format before being smoothed into 10-second averages for analysis.

3.3.3.3.1 Maximal oxygen uptake ($\dot{V}O_{2max}$)

For all studies utilising $\dot{V}O_{2max}$ as an outcome variable, this was determined using methodology previously described for use in adults (Day et al., 2003), and

subsequently applied in healthy children (Barker et al., 2011), and children with CF (Saynor et al., 2013a). Briefly, a linear regression was plotted over the 'linear' portion of the ramp-incremental phase, with data from the first two minutes, and the three-minutes prior to exhaustion excluded, to exclude the influence of $\dot{V}O_2$ kinetics, and deviations from linearity (i.e. plateaus) (Figure 3.2a). The $\dot{V}O_2$ from this linear portion was then extrapolated over the remainder of the test (Figure 3.2b), and the residuals from final 60-seconds isolated and examined against the extrapolated portion. A negative residual indicated a deceleration in $\dot{V}O_2$ against power output and was defined as a plateau when the magnitude of residuals was ≥5% of projected $\dot{V}O_2$ (Figure 3.3a). Either a positive or negative residual <5% of projected $\dot{V}O_2$ indicated a linear response (Figure 3.3b). Finally, a positive residual ≥5% indicated an acceleration in $\dot{V}O_2$ against power output (Figure 3.3c) (Barker et al., 2011, Saynor et al., 2013a).

Furthermore, the highest 10-second average $\dot{V}O_2$ value was obtained from the S_{max} verification bout. Where the highest $\dot{V}O_2$ value from the S_{max} phase increased by more than 9% above the peak $\dot{V}O_2$ from the ramp phase – a value based upon reliability data (Saynor et al., 2013b) – this was considered a 'meaningful' change between the two peak $\dot{V}O_2$ values and therefore the highest $\dot{V}O_2$ value obtained during the CPET was considered a 'peak $\dot{V}O_2$' rather than a true $\dot{V}O_{2max}$.

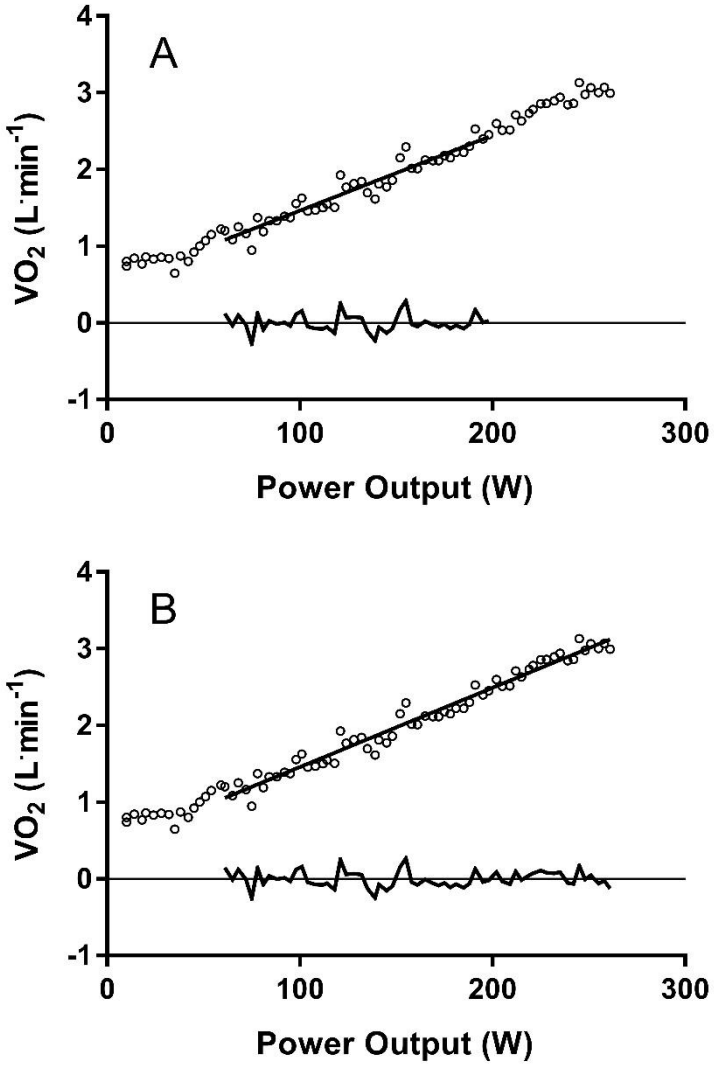


Figure 3.2 Example $\dot{V}O_2$ responses to increasing work-rate during the ramp phase of a cardiopulmonary exercise test, with linear regression plotted to establish $\dot{V}O_{2max}$. A: Linear regression plotted through 'linear' portion of the ramp incremental phase, excluding the first two minutes, and excluding the last three minutes. B: Linear regression is extrapolated to cover final three minutes of test. Profile depicts linear response of a 14-year old male with cystic fibrosis.

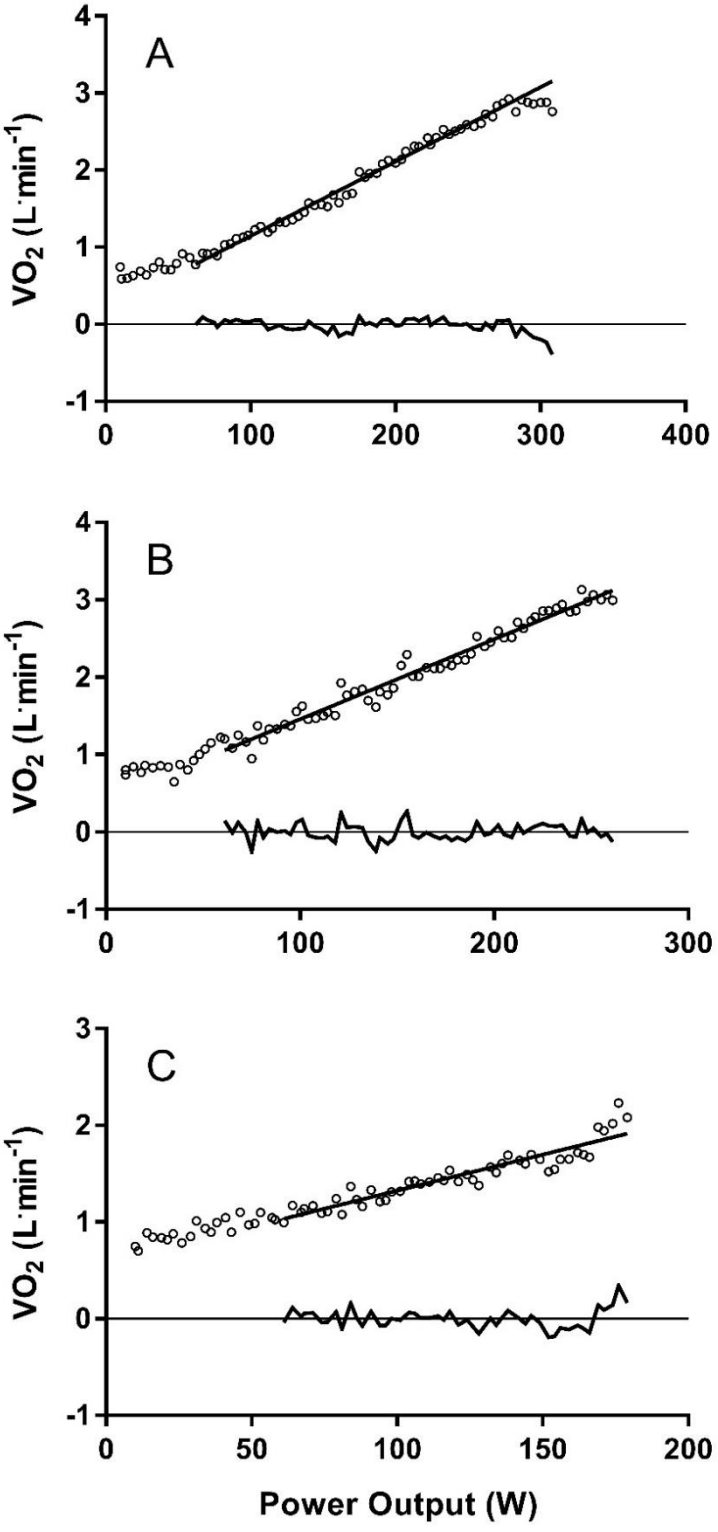


Figure 3.3 Example $\dot{V}O_2$ responses to increasing work-rate during the ramp phase of a cardiopulmonary exercise test. A: Deceleration of $\dot{V}O_2$, producing a plateau (17-year old male with cystic fibrosis); B: Linear response (14-year old male with cystic fibrosis); C: Acceleration of $\dot{V}O_2$ against power (14-year old male with cystic fibrosis). For all cases, the extrapolated regression line is fitted from 120 seconds, through to volitional exhaustion.

3.3.3.3.2 Gas exchange threshold

The gas exchange threshold (GET) was identified using the V-slope method, described by Beaver et al. (1986). Simply, a plot of $\dot{V}CO_2$ (*y*-axis) against $\dot{V}O_2$ (*x*-axis) is made, with data excluded from the initial 3-minute warm-up phase, and between the RCP (described in section 3.3.3.3.3) and volitional exhaustion. Subsequently, the first disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$ is denoted as the GET (Figure 3.4). This is then visually confirmed using the ventilatory equivalents of $\dot{V}O_2$ and $\dot{V}CO_2$ (i.e. $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$), whereby $\dot{V}_E/\dot{V}O_2$ beings to increase, having been stationary or decreasing, whilst no equivalent increase in $\dot{V}_E/\dot{V}CO_2$ occurs (Figure 3.4). To facilitate determination of GET, purpose-built software (LabVIEW; National Instruments, Newbury, UK) was utilised (Figure 3.5). The use of the V-Slope methods has been shown to be a reliable method of determining GET in children with CF (CV = 11.2% (Saynor et al., 2013b)) and healthy control children (CV = 7.5% (Fawkner et al., 2002)).

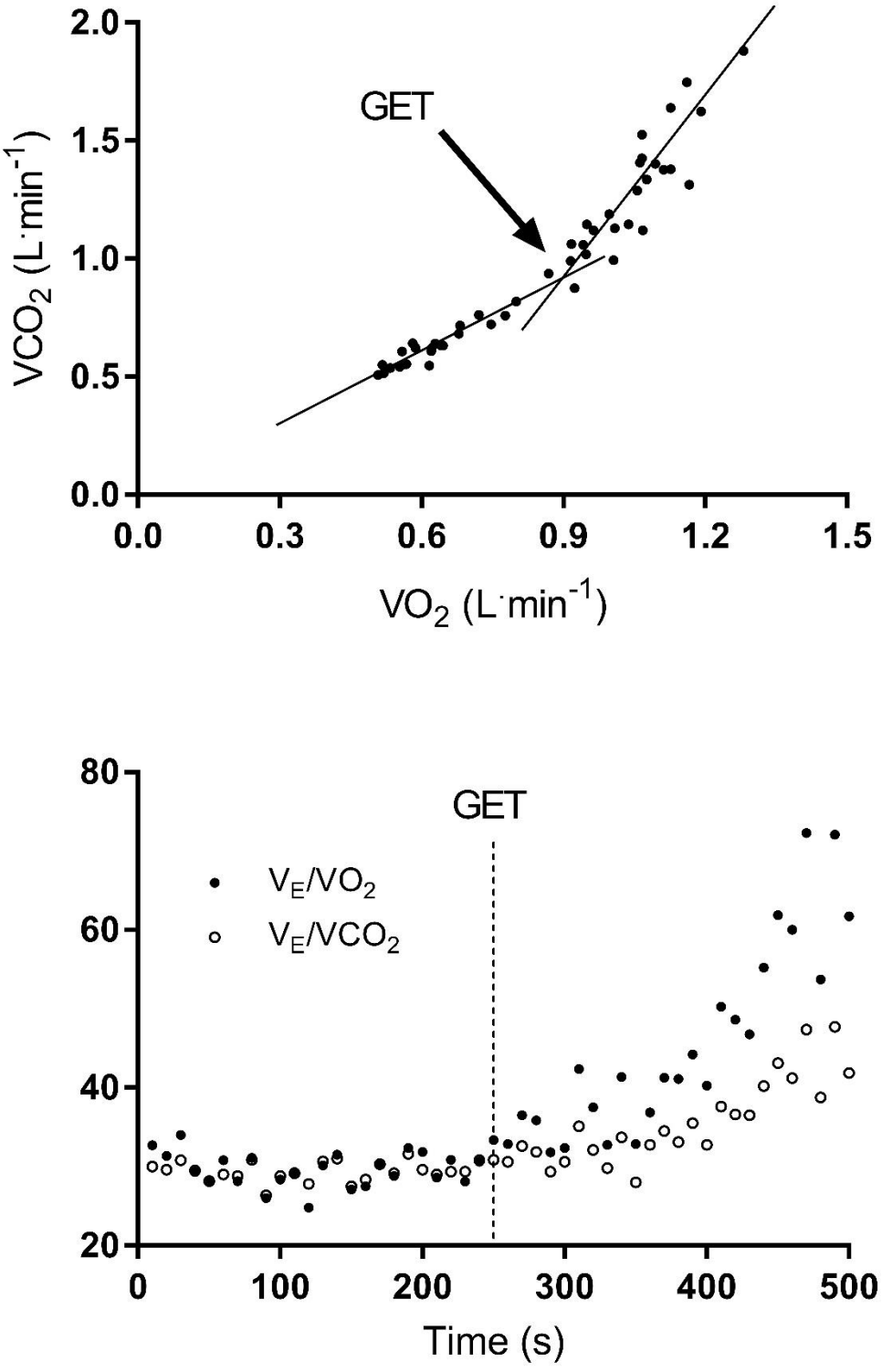
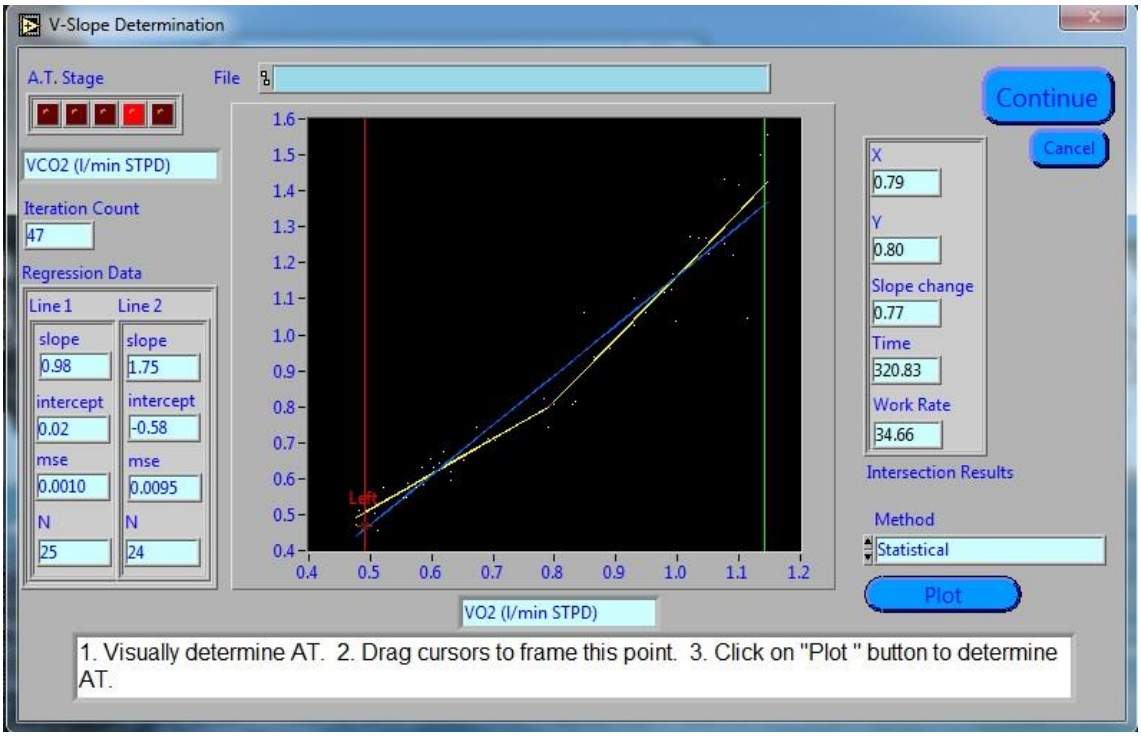


Figure 3.4 Example of establishment of gas exchange threshold, using V-slope method (above) and ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$ (below). Both examples display the same cardiopulmonary exercise test data for a 12-year old female with cystic fibrosis.

A



B

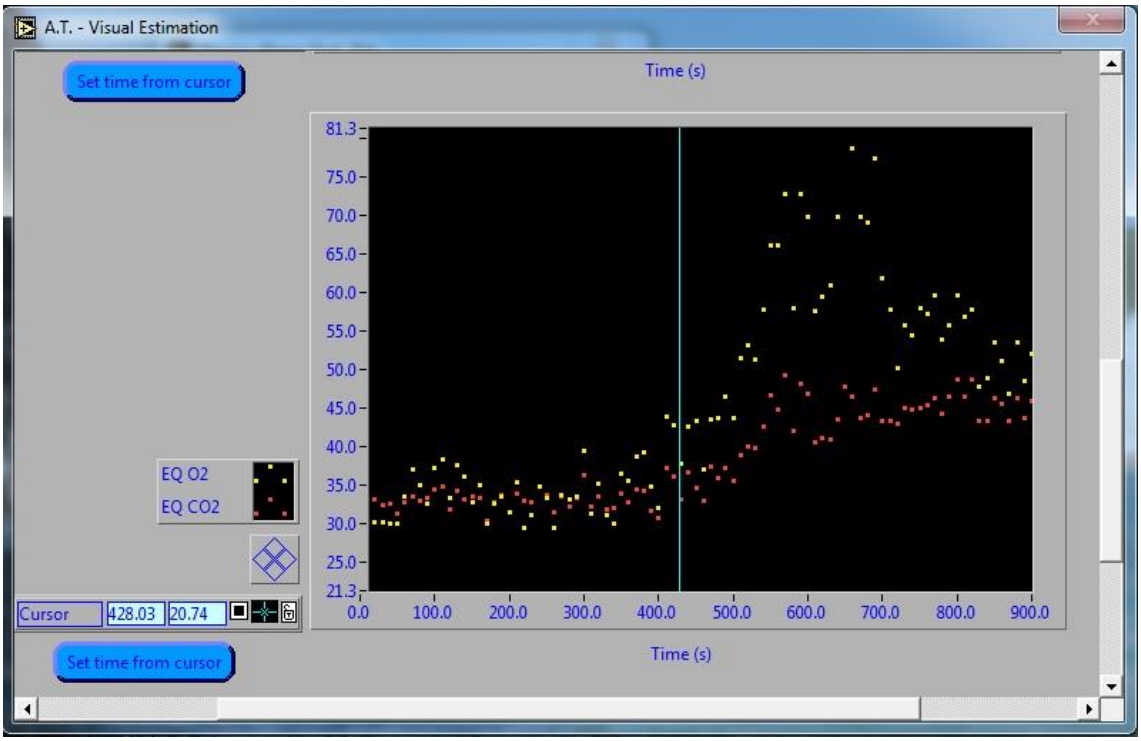


Figure 3.5 Worked example of establishing the gas exchange threshold using V-slope method (A), and ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$ (B), using purpose-built software (LabVIEW; National Instruments, Newbury, UK). Data displayed is the same at that in Figure 3.4.

3.3.3.3.3 Respiratory compensation point

In addition to the GET, the RCP was also identified using methods described by Beaver et al. (1986). This is identified on a plot of $\dot{V}_E$ (*y*-axis) against $\dot{V}CO_2$ (*x*-axis), and the rapid increase observed in $\dot{V}_E$ relative to $\dot{V}CO_2$ indicates hyperventilation due to increased metabolic acidosis in the tissue and is the RCP (Figure 3.6).

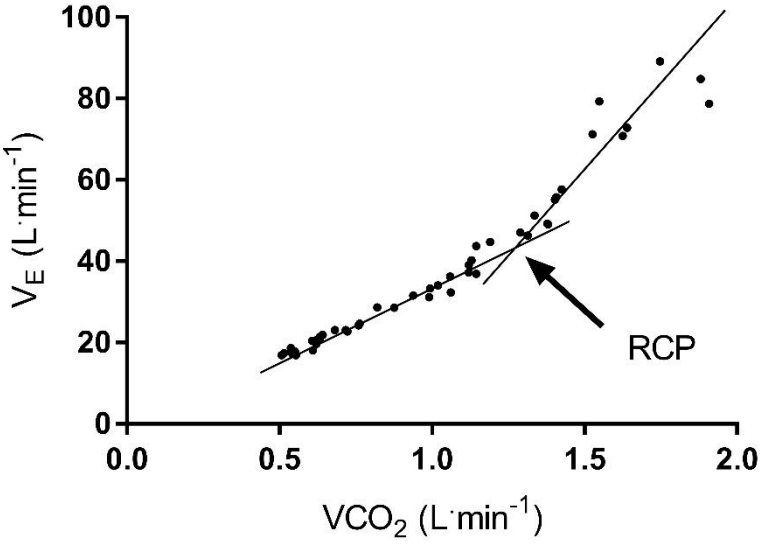


Figure 3.6 Establishment of respiratory compensation point using methods of Beaver et al. (1986) by examining relationship between $\dot{V}_E$ and $\dot{V}CO_2$.

3.3.3.3.4 Oxygen uptake efficiency slope

In accordance with previous research (Baba et al., 1996), the OUES was calculated throughout the linear portion of the CPET (i.e. excluding warm-up and cool-down). To derive OUES, $\dot{V}O_2$ is plotted against the common logarithm of $\dot{V}_E$. This removes the curvilinear profile of ventilation that can be observed during a CPET, and provides a regression coefficient in the following form:

Equation 3.12: $\dot{V}O_2 = a * \log\dot{V}_E + b$

In this equation, ‘a’ equals the OUES. This value is then carried forward for analysis. An example of the logarithmic transformation of minute ventilation is provided in Figure 3.7.

The OUES was calculated at several points during the incremental phase of the CPET, utilising data up to, and including the following thresholds: 100% $\dot{V}O_{2max}$, 75% $\dot{V}O_{2max}$, 50% $\dot{V}O_{2max}$, 100% time to exhaustion (TTE), 75% TTE, 50% TTE, GET and RCP. The OUES value for 100% $\dot{V}O_{2max}$ also describes 100% TTE, therefore giving seven OUES variables per participant. OUES was subsequently scaled against $BSA^{1.40}$ (an exponent derived from Chapter 4) for Chapter 5.

Reliability of the OUES has previously been reported in healthy children and adolescents (CV = 33% (Bongers et al., 2015a)) , and those with CF (CV = 12% (Saynor et al., 2013b)).

3.3.3.3.5 Oxygen uptake efficiency

Three parameters of OUE were collected for Chapter 6. To obtain OUE, each $\dot{V}O_2$ ($mL \cdot min^{-1}$) measure is divided by the corresponding $\dot{V}_E$ ($L \cdot min^{-1}$) throughout the linear portion of the CPET. The highest 90-second average of OUE is taken to be the oxygen uptake efficiency plateau (OUEP). The average of the 60-seconds prior to the GET and RCP are taken as the OUE_{GET} and OUE_{RCP} respectively. Reliability of the OUEP in healthy children has been reported previously (CV = 10.9% (Bongers et al., 2015a)). An example of the profiles of OUEP, OUE_{GET} and OUE_{RCP} in relation to CPET duration for an individual with CF is provided in Figure 3.8.

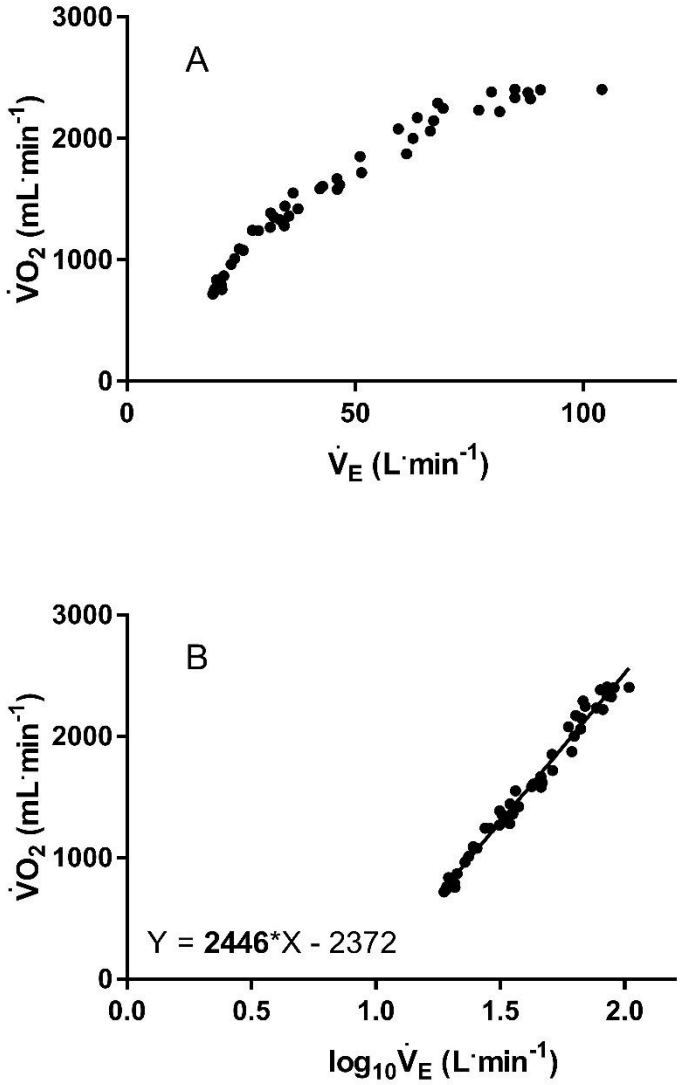


Figure 3.7 An example of the logarithmic transformation of the curvilinear ventilatory response to incremental exercise during a cardiopulmonary exercise test in a healthy 13-year old male. A: Curvilinear ventilatory response to incremental exercise, from the start of the incremental ramp phase to volitional exhaustion (i.e. $\dot{V}O_{2peak}$). B: The same response profile as (A), however ventilation has been log-transformed (base 10). The resultant linear regression for (B) in the example above produces a value of 2446 for 'a' in Equation 3.12. This value is the oxygen uptake efficiency slope, which is subsequently carried forward for analysis.

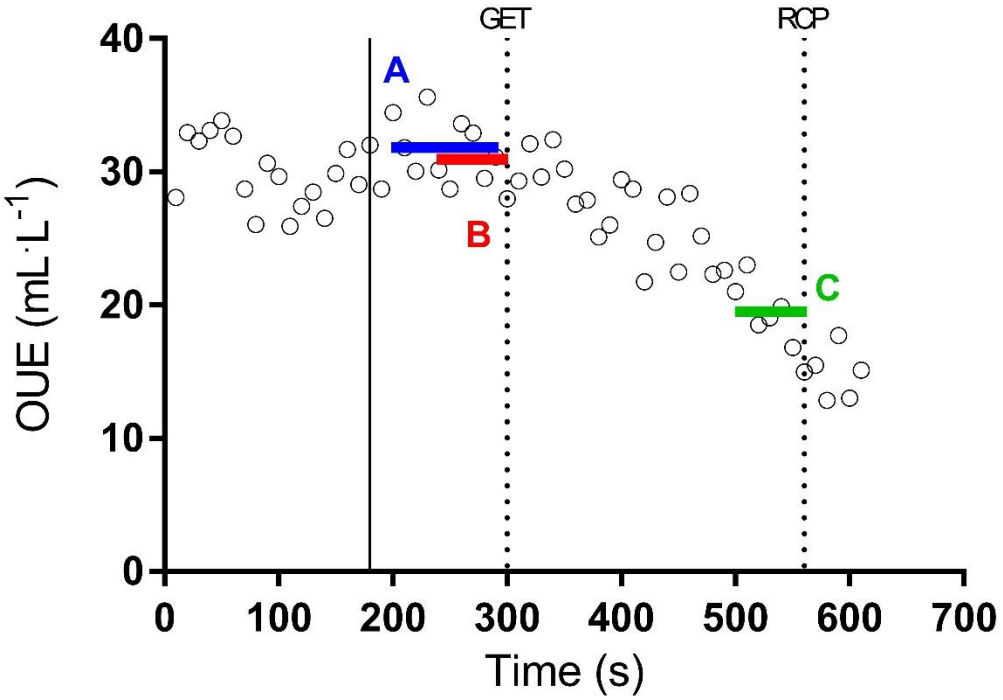


Figure 3.8 An example of the three parameters of oxygen uptake efficiency analysed throughout this thesis, and their position within a cardiopulmonary exercise test (from warm-up, to volitional exhaustion) undertaken by a 12-year old female with cystic fibrosis. The solid, vertical line at 180 seconds indicates the beginning of the ramp incremental phase of the cardiopulmonary exercise test. The two dashed, vertical lines entitled 'GET' and 'RCP' indicate the gas exchange threshold and respiratory compensation point, respectively. A: oxygen uptake efficiency plateau (OUEP); B: oxygen uptake efficiency at the GET (OUE_{GET}); C: oxygen uptake efficiency at the RCP (OUE_{RCP}).

3.3.3.3.6 Rating of perceived exertion and dyspnoea

Ratings of perceived exertion (RPE) were obtained at one minute intervals using the Pictorial Children's Effort Rating Table (P-CERT), validated by Yelling et al. (2002) (Appendix Q). Ratings of perceived dyspnoea (RPD) were also obtained at one minute intervals, using the modified Borg 0-10 scale (Borg, 1982) (Appendix R).

3.3.3.3.7 Oxygen saturation

Blood oxygen saturation during exercise was estimated via non-invasive via fingertip pulse oximetry (SpO_2), with nadir SpO_2 during the CPET recorded. In

addition to a measure of desaturation, SpO_2 also provides an objective termination point of the CPET, for safety reasons. Initial guidelines from the American Thoracic Society (ATS) and American College of Chest Physicians (ACCP) suggested a test be terminated at SpO_2 < 80% (American Thoracic Society, 2003). This cut-off has since been corroborated by disease specific guidelines published by the ECFS (Hebestreit et al., 2015).

3.3.4 Magnetic resonance based variables

For collection of magnetic resonance (MR) based variables, participants lay prone in the MR scanner. Participants' legs were strapped down to minimise movement within the scanner in order to improve clarity of images. CSA, thigh volume (TV) and MV were calculated using specialist inbuilt MRI software. Each CSA slice was traced around to produce an area for both TV and MV, to isolate fat-free mass of the thigh. Each CSA value (in mm^2) was multiplied by 5.5 to reflect the 5 mm slice thickness and 0.5 mm gap between slices, to produce a slice volume in cm^3. Each slice volume was then summed, to produce a total volume for both TV and MV. An example of the MRI software, with the slices around which an area was traced is provided in Figure 3.9. A fuller explanation of calculation of MV is provided in Chapter 7.

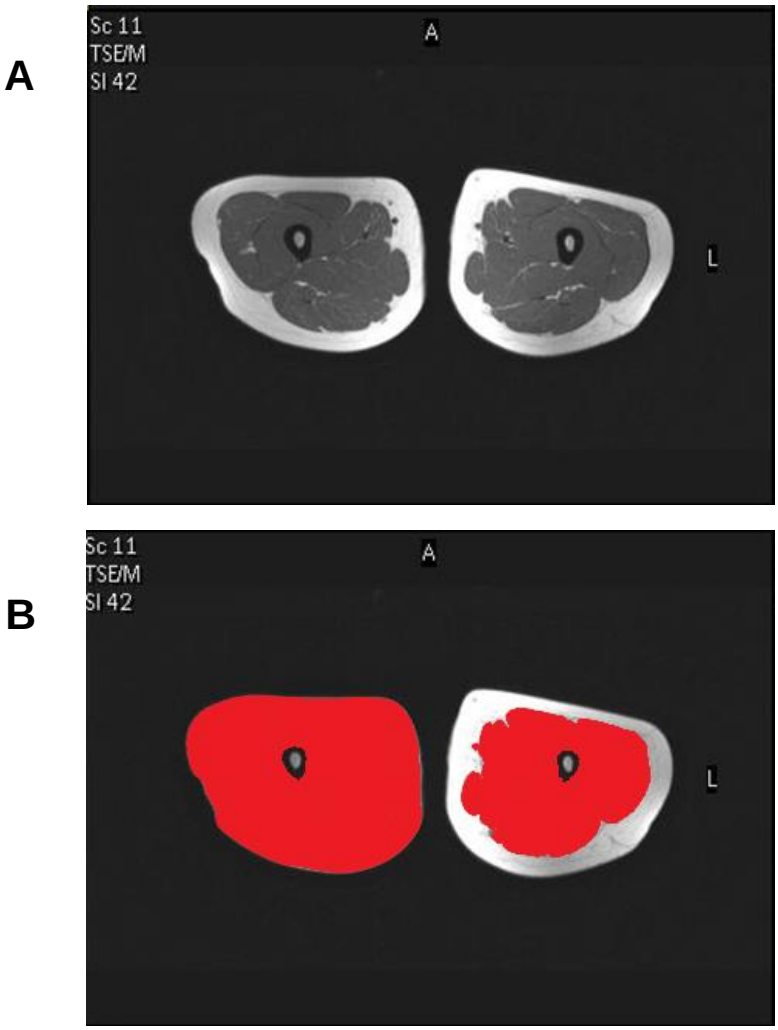


Figure 3.9 Example magnetic resonance images of the mid-thigh of a 14-year old female with cystic fibrosis. A: Cross-sectional area of mid-thigh as seen using magnetic resonance imaging software. B: The same image as (A), but with the shaded area in red on the left indicates the cross-sectional area used to calculate thigh volume (muscle and subcutaneous fat), with the shaded area on the right indicating the cross-sectional area used to calculate muscle volume. For both volumes, bone is excluded from cross-sectional area and subsequent calculation of thigh volume and muscle volume.

3.3.5 Scaling of outcome variables

Throughout this thesis, $\dot{V}O_{2max}$ was scaled for body-mass. However, other parameters including muscle cross-sectional area and thigh muscle volume, derived from MR imaging, were also used in Chapter 8. In addition to scaling for $\dot{V}O_{2max}$, OUES was also scaled against BSA using an exponent of 1.40 (i.e.

OUES/BSA$^{1.40}$) in Chapter 5. The process of obtaining this exponent is described in greater detail explained in Chapter 4.

Numerous studies have described the process of scaling $\dot{V}O_{2max}$ for body size in children (Armstrong and Welsman, 1994, Tolfrey et al., 2006, Welsman et al., 1997), and a brief outline of the process is described below.

To identify if an outcome variable (such as $\dot{V}O_{2max}$) required scaling for body size, an initial correlation coefficient is established between $\dot{V}O_{2max}$ and body-size parameters of interest (e.g. mass, FFM). If significant correlation coefficients exist between variables, then these variables are related, and scaling is required to remove residual effects of body size. For example, in Figure 3.10a, absolute $\dot{V}O_{2max}$ is correlated with body-mass in both boys and girls, and therefore scaling of $\dot{V}O_{2max}$ is required.

Ratio standard scaling divides the dependent variable (e.g. $\dot{V}O_{2max}$) against the independent body-size variable (e.g. mass, FFM) in the format Y/X, attempting to produce a size-free variable (i.e. $\dot{V}O_{2max}$/mass). The scaled variable is then correlated against the original body-size variable to identify if a relationship with body-size still exists (i.e. $\dot{V}O_{2max}$/mass vs. mass). If a significant correlation coefficient remains between the scaled variable and independent body-size parameter, then this shows ratio-standard scaling is ineffective in removing residual effects of body size. For example, in Figure 3.10b, ratio-standard $\dot{V}O_{2max}$ is still correlated with body-mass in both boys and girls (now in a negative direction), indicating a residual effect of body size.

If ratio-standard scaling is not sufficient in removing residual effects of body size, allometric scaling is utilised (Welsman and Armstrong, 2000). Briefly, this process utilises the natural logarithm of both the dependent and independent variable against which it is scaled. The independent variable is inserted into a linear

regression model alongside any grouping variable (e.g. disease status, gender). The subsequent 'b' (β) value that is produced (alongside 95% confidence intervals (CIs)) is then carried forward for use as a power function ratio in the format Y/X^{b}. The scaled dependent variable is then correlated against the original independent variable to identify if any residual relationship continues to exist. In the example provided by Figure 3.10c, a 'b' exponent of 0.72 has been derived by linear regression and applied to body mass as a scaling factor (i.e. $\dot{V}O_{2max}/mass^{0.72}$), and subsequently produces non-significant correlations, indicating that residual effects of body size have been removed.

Whilst numerous exponents for body-mass have been proposed when scaling $\dot{V}O_{2max}$ (0.24 – 1.02; pooled β = 0.70) (Lolli et al., 2017), each study within this thesis has derived its own scaling exponents to ensure they remain reflective the spectrum of body-sizes reported by participants within these studies and CF in general (Hanna and Weiner, 2015).

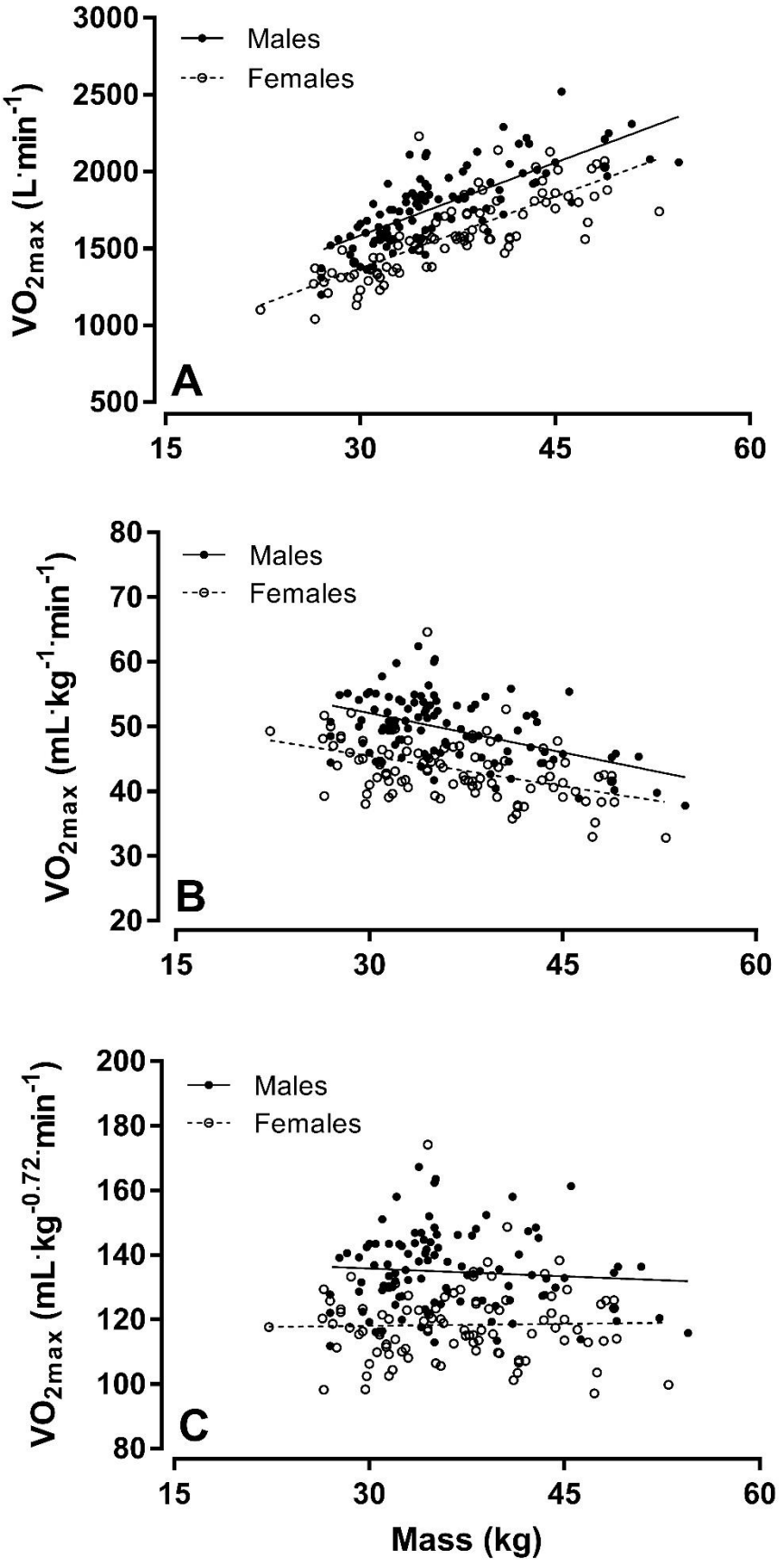

Males
Females
VO_{2max} ($L \cdot min^{-1}$)
3000
2500
2000
1500
1000
500
A
15
30
45
60
Males
Females
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)
80
70
60
50
40
30
20
B
15
30
45
60
Males
Females
VO_{2max} ($mL \cdot kg^{-0.72} \cdot min^{-1}$)
200
180
160
140
120
100
80
C
15
30
45
60
Mass (kg)

Figure 3.10 (overleaf) A: Relationship between body mass (kg) and absolute $\dot{V}O_{2peak}$ ($L \cdot min^{-1}$) in boys (black circles) and girls (white circles). The relationship between variables is statistically significant for boys (r = 0.76, p < 0.001) and girls (r = 0.80, p < 0.001). B: Relationship between body mass (kg) and ratio-standard $\dot{V}O_{2peak}$ ($mL \cdot kg \cdot min^{-1}$) in boys (black circles) and girls (white circles). The relationship between variables is statistically significant for boys (r = -0.49, p < 0.001) and girls (r = -0.44, p < 0.001). C: Relationship between body mass (kg) and allometrically scaled $\dot{V}O_{2peak}$ ($mL \cdot kg^{-0.72} \cdot min^{-1}$) in boys (black circles) and girls (white circles). The relationship between variables is no longer statistically significant for boys (r = -0.08, p = 0.429) and girls (r = 0.02, p = 0.815).

3.3.6 Statistical analyses

Throughout this thesis, various statistical techniques have been utilised, dependent on the study design and objectives. For all analyses, normality of data were assessed using standard ranges for skewness (-1 to +1) and kurtosis (-1 to +2). All data were found to be normally distributed throughout.

All null-hypothesis significance tests were conducted using IBM SPSS (IBM, Corp., Armonk NY, USA), using an initial p-value of 0.05 throughout to indicate statistical significance, although further post-hoc corrections were undertaken where necessary, and therefore adjusting the associated p-value threshold required to achieve statistical significance. In addition, effect sizes (*ES*) for between-group comparisons were calculated using the following equation:

Equation 3.13: $$d = M_1 – M_2 / S_{pooled}$$

Whereby d = effect size; M_1 = mean of group 1; M_2 = mean of group 2; S_{pooled} = pooled standard deviation for the two groups (S_1 and S_2 respectively), with this being calculated using:

***Equation 3.14*:** $$\sqrt{[(S_1^2 + S_2^2) / 2]}$$

Subsequently, thresholds of Cohen (1992) were used to describe the magnitude of the effect (small = 0.2, medium = 0.5, large = 0.8). Effects sizes of Cohen (1992)

were also utilised to describe the magnitude of correlation coefficients (small = 0.1, medium = 0.3, large = 0.5).

Furthermore, in Chapter 8, magnitude-based inferences (MBI) (Hopkins et al., 2009) were utilised. This process uses 90% confidence intervals (90% CI) and the smallest worthwhile *ES* change of 0.2 (Cohen, 1992), to identify the likelihood that an observed effect was substantially positive, trivial, or substantially negative and reported using quantitative chances (%) and the following qualitative terminology: <0.5%, ‘‘most unlikely’’; 0.5%–5%, ‘‘very unlikely’’; 5%–25%, ‘‘unlikely’’; 25%–75%, ‘‘possibly’’; 75%–95%, ‘‘likely’’; 95%–99.5%, ‘‘very likely’’; >99.5%, ‘‘most likely’’. This statistical methodology has previously been utilised previously in assessing clinical differences in exercise capacity between children with, and without, CF (Saynor et al., 2014b).

3.4 Practical considerations

Undertaking exercise testing in individuals with CF poses unique challenges above and beyond working with children without a chronic disease. Whilst considerations for patient safety are always a necessity when undertaking CPETs in clinical groups, infection control is an additional area of concern within CF (Saiman et al., 2014). Additional steps to ensure patient safety are described below.

3.4.1 Patient safety during exercise

Prior to undertaking exercise, all participants (CF and CON) undertook a medical history questionnaire (filled out by parent(s)/guardian(s)) to identify any contraindications to exercise (Appendix S). Individuals with CF had been recommended for inclusion in studies by their physician, and no patient deemed

unsuitable for participation and maximal exercise was recommended, nor approached.

For safety and logistical reasons, all individuals with CF undertook exercise testing in a hospital gymnasium. All CON participants undertook tests in the paediatric laboratory at the University of Exeter.

As previously noted, SpO_2 was recorded throughout exercise to monitor oxygen saturation in individuals with CF. If SpO_2 fell below 80%, exercise was terminated, in line with current recommendations (American Thoracic Society, 2003, Hebestreit et al., 2015). Supplemental O_2 was available if patients presented with severe hypoxemia, with medical personnel trained in basic life support in adjacent wards if needed. Throughout the course of all experimental work, no individuals presented with any adverse reactions to exercise.

Furthermore, patients reported to have cystic fibrosis related diabetes (CFRD) were advised to bring sugary snacks/drinks with them for exercise sessions. Patients were also advised to monitor blood sugar levels prior to, and following, maximal exercise and take corrective responses in the event of adverse readings. No adverse glycaemic reactions to exercise were reported across any testing session.

3.4.2 Infection control

To minimise risk of cross-infection between patients with CF, strict protocols were followed as per clinical practice guidelines. This included the following:

- No two individuals with CF were scheduled for tests at the same time (i.e. to avoid face-to-face contact).
- All rooms and content were cleaned with disinfectant wipes following exercise testing and any other meetings/testing periods. Contents

included, but was not limited to, exercise bikes, computers, tables and chairs.

- Following cleaning, any room was ventilated for a minimum of a full hour and an individual with CF was not permitted into the room for that period.
- Any shared equipment (e. g. facemasks) underwent a full cleaning procedure following use. This included washing with specialised detergent (Neutracon; Decon Laboratories, Hove, UK) and sterilising fluid (Milton Sterilising Fluid; Milton Pharmaceuticals, Gloucester, UK), before drying in a heated cabinet. This cleaning method was approved by the Infection Control team at the Royal Devon & Exeter NHS Foundation Trust Hospital.
- If a patient was positively identified as culturing a non-tuberculosis mycobacterium (e.g. *Mycobacterium abcessus*) during the course of their involvement in a study, they were withdrawn to minimise risk to other patients. This occurred with one patient in Chapters 7 and 8.

3.4.3 Contraindications to MR scanner environment

Prior to participation in the MR scanner in Chapters 7 and 8, participants were required to fill out a questionnaire disclosing whether they had any implants, devices or objects that may be hazardous in the MR environment (e.g. pacemakers, infusion pumps, internal or external metallic objects). No participants reported any contraindications to the MR environment. A copy of the questionnaire is provided in Appendix T. No participants reported any contraindications to being in the scanner environment, and all continued their participation in the study without issue.

4 SCALING THE OXGEN UPTAKE EFFICIENCY SLOPE FOR BODY SIZE IN CYSTIC FIBROSIS

4.1 Abstract

Purpose:

The aim of this study was to describe the relationship between body size and the oxygen uptake efficiency slope (OUES) in paediatric patients with cystic fibrosis (CF) and healthy controls (CON), in order to identify appropriate scaling procedures to adjust the influence of body size upon OUES.

Methods:

The OUES was derived using maximal and submaximal points from cardiopulmonary exercise testing in 72 children (36 CF and 36 CON). OUES was subsequently scaled for stature, body mass (BM) and body surface area (BSA) using ratio-standard (Y/X) and allometric (Y/X^b) methods. Pearson's correlation coefficients were utilised to determine the relationship between body size and the OUES.

Results:

When scaled using the ratio-standard method, OUES had a significant positive relationship with stature (r = 0.54, P < 0.001) and BSA (r = 0.25, P = 0.031) and significant negative relationship with BM (r = -0.38, P = 0.016) in the CF group. Combined allometric exponents (b) for CF and CON were: stature 3.00, BM 0.86, BSA 1.40. A significant negative correlation was found between OUES and stature in the CF group when scaled allometrically (r = -0.37, P = 0.027). Non-significant (P > 0.05) correlations for the whole group were found between OUES and allometrically scaled BM (CF: r = -0.25, CON: r = 0.15) and BSA (CF r = -0.27, CON r = 0.13).

Conclusions:

Only allometric scaling of either BM or BSA, and not ratio-standard scaling, successfully eliminates the influence of body size upon OUES. Therefore, this enables a more direct comparison of the oxygen uptake slope between patients with CF and healthy controls.

4.2 Introduction

It has been established that a high cardiopulmonary fitness (as represented by maximal oxygen uptake [$\dot{V}O_{2max}$]) is of benefit to young patients with cystic fibrosis (CF), being associated with an increased quality of life (Hebestreit et al., 2014) and reduced risk of hospitalisation (Pérez et al., 2014) and mortality (Pianosi et al., 2005a). As such, regular, maximal, exercise testing is recommended to provide clinically relevant prognostic information for clinicians and patients (Cystic Fibrosis Trust, 2011), with cardiopulmonary exercise testing (CPET) endorsed as method of choice by the European Cystic Fibrosis Society and European Respiratory Society (Hebestreit et al., 2015). However, measuring $\dot{V}O_{2max}$, by definition, requires a maximal effort and some patients may be unable or unwilling to reach a volitional maximum. Therefore, the oxygen uptake efficiency slope (OUES) (Baba et al., 1996), a reliable (Saynor et al., 2013b) and effort-independent measure of ventilatory efficiency, may be a viable submaximal alternative to $\dot{V}O_{2max}$ in this patient group (Gruet et al., 2010).

Previous research in healthy adults has shown that OUES is strongly related to body size variables including stature, body mass (BM) and body surface area (BSA) (Buys et al., 2015), and has subsequently been applied to clinical settings including cardiac (Van Laethem et al., 2005), neurological (Heine et al., 2014) and respiratory (Barron et al., 2016) populations, including a single study of adults with CF (Gruet et al., 2010). This strong dependency on body size confounds

interpretation of OUES and requires the use of scaling techniques to ensure appropriate interpretation within and between groups. However, scaling procedures have been performed by most (Barron et al., 2016, Buys et al., 2015, Gruet et al., 2010, Van Laethem et al., 2005) but not all (Heine et al., 2014) studies to date.

The strong positive relationship between OUES and body size has further been observed in paediatric studies using stature (Marinov et al., 2007), BM (Breithaupt et al., 2012) and BSA (Akkerman et al., 2010). Whilst such paediatric studies have attempted to control for body size (Akkerman et al., 2010, Bongers et al., 2012, Bongers et al., 2011, Drinkard et al., 2007), it has been assumed that the ratio standard scaling method (OUES/body size [Y/X]) is an effective approach at removing the influence of body size. However, there are validity concerns associated with the ratio-scaling procedure that have been utilised to date (Nevill et al., 1992). This issue may have greater implications in children (Armstrong and Welsman, 1994), whose body size is rapidly changing with age, and furthermore in children with CF, who are characterised by malnutrition and inadequate growth (Culhane et al., 2013).

Previous research has identified allometric scaling (Y/X^b, where 'b' represents a power function to which X is raised) as a superior technique to the ratio-standard methods for controlling for body size when assessing $\dot{V}O_{2max}$ in both adults (Batterham et al., 1999) and children (Graves et al., 2013). However, its applicability for scaling OUES in contrast to the currently employed ratio standard method remains unknown

Although the use of OUES in children with CF has been proposed (Bongers et al., 2012), there are currently no studies that critically examine the validity of scaling methods to adjust for body size. Furthermore, the one previous study to

have examined the role of OUES in children with CF (Bongers et al., 2012) scaled for BSA using a ratio-standard approach. However, the utility of other body size variables that are frequently collected by clinical teams (stature, body mass) were not systematically considered.

Therefore, the aim of this study was twofold: Firstly, to characterise the relationship between body size and OUES in children with CF; and secondly, to identify the most appropriate procedure (ratio standard or allometric) for scaling OUES against different body size variables (stature, BM and BSA) in paediatric patients with CF and a matched control (CON) group. It is hypothesised that the allometric scaling procedure will remove the residual effects of body size on OUES compared to ratio standard procedures.

4.3 Methods

4.3.1 Study participants

Data were extracted from existing databases of valid CPET data, with 45 children and adolescents with CF being considered for inclusion in the current analysis. A total of 9 participants were excluded due to inadequate data (insufficient, or missing data, n = 7; insufficient test length, n = 2), resulting in a final sample of 36 children and adolescents with CF. Data were then age- and gender-matched against existing CON CPETs, resulting in a total sample of 72 participants (36 CF, 36 CON; mean age 13.3 ± 2.8 years).

For original data collection, ethics approval was granted by institutional and NHS Research Ethics committees. Written informed consent and assent were obtained from parents/guardians and children respectively.

4.3.2 Experimental measures

Stature was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain Ltd., Crymych, UK) and BM to the nearest 0.1 kg using a digital scale (Seca, Birmingham, UK). Body surface area (BSA) was estimated using the Haycock equation (Haycock et al., 1978). Pulmonary function was assessed using a hand-held spirometer, with values for forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) being determined.

4.3.3 Experimental protocol

All participants undertook an incremental CPET to volitional exhaustion on an electronically braked cycle ergometer (Lode, Groningen, the Netherlands). Breath-by-breath gas exchange data were collected using an online Cortex gas analysis system (Cranlea, Birmingham, UK) and exported in 10-second averages. Within the sample, 33 children (20 CF, 13 CON) undertook an additional supramaximal verification bout to determine $\dot{V}O_{2max}$ (Barker et al., 2011, Saynor et al., 2013a). However, as not all children undertook the verification bout the highest $\dot{V}O_2$ observed is described as peak $\dot{V}O_2$.

Peak $\dot{V}O_2$ was obtained from the highest 10-second average from either the ramp or supramaximal bout (where applicable) and the gas exchange threshold (GET) was identified using the V-slope method (Beaver et al., 1986) and confirmed through visual inspection of ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$. OUES was ascertained at three different intensities (100%, 75% and 50% of peak $\dot{V}O_2$), using data from the whole test up to, and including, the intensity of interest, in line with previous research (Bongers et al., 2012). Simple, linear regressions between $\dot{V}O_2$ ($mL \cdot min^{-1}$) and $log\dot{V}_E$ were calculated in the form using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA):

Equation 4.1: $\dot{V}O_2 = a\ (\log \dot{V}_E) + b$

Where the constant 'a' the slope is defined as the OUES, and 'b' the intercept with the *y*-axis (Baba et al., 1996). Regression constants were subsequently produced, as per Figure 4.1, to allow comparisons between groups.

4.3.4 Scaling approaches

Each body size variable (stature, BM and BSA) was used to scale OUES at peak $\dot{V}O_2$, and at the GET, using the ratio-standard (Y/X) and allometric (Y/X^b) scaling methods. Allometric scaling of OUES was performed using log-linear regression models (Tolfrey et al., 2006) with disease status (CF or CON) and the anthropometric variable in question (stature, BM, BSA) entered as predictor variables. Age and gender were not entered into the model due to the prior matching of patients. The log-linear regression models produced scaling exponents (b) and associated 95% confidence intervals (CIs) that were used to scale the OUES using a power function ratio (Y/X^b). All regression models assumptions (multicollinearity and independence, homoscedasticity, linearity and normal distribution of residuals) were checked and satisfied. The log-linear regression model was conducted for each group (CF and CON separately) and as a combined whole (CF and CON combined) for each OUES parameter (peak $\dot{V}O_2$, 75% peak $\dot{V}O_2$, 50% peak $\dot{V}O_2$).

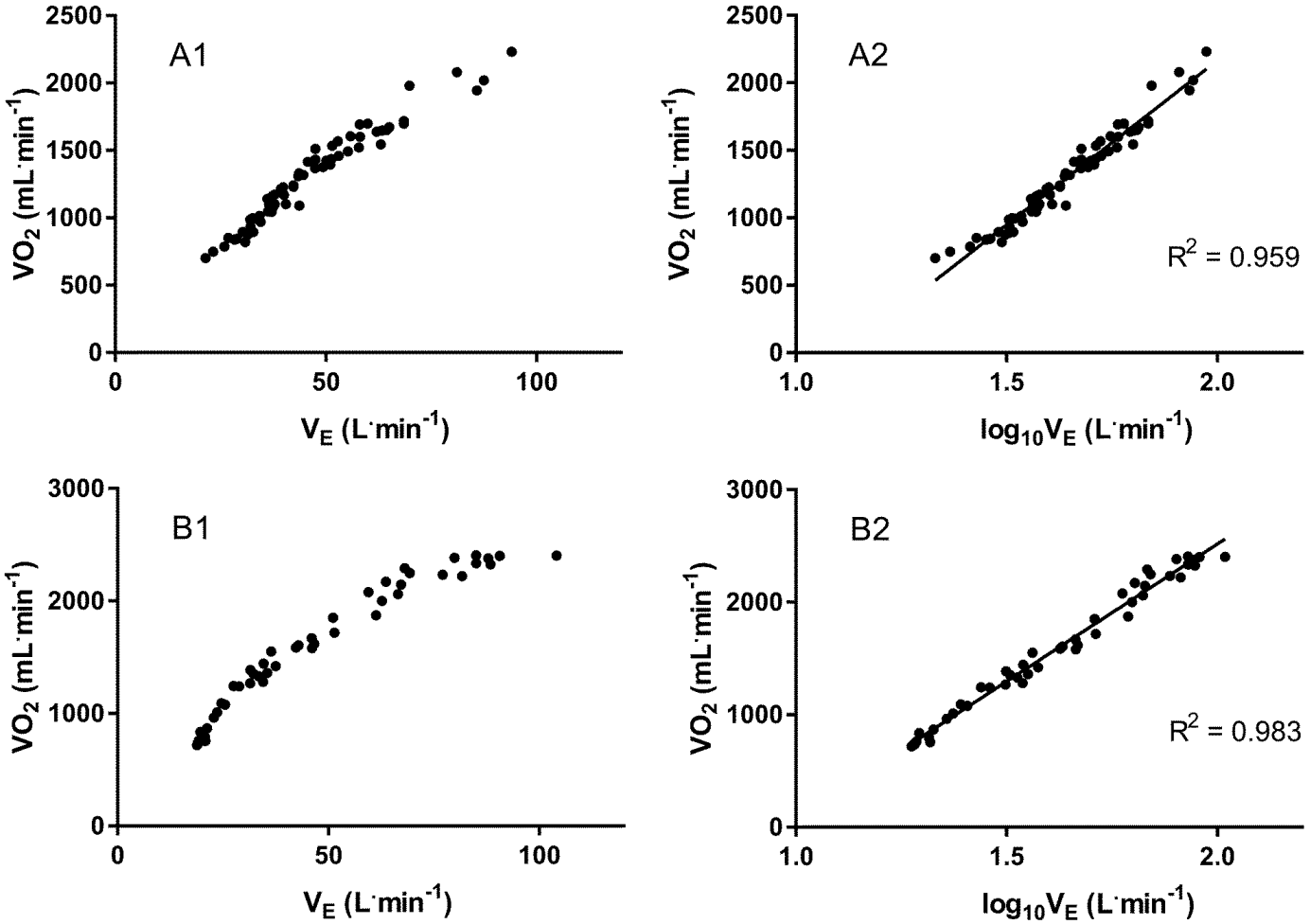


Figure 4.1 Relationship between oxygen uptake ($\dot{V}O_2$; mL·min^{-1}) and minute ventilation (V_E; L·min^{-1}) [1]; and $\dot{V}O_2$ (mL·min^{-1}) and $log_{10}V_E$ (L·min^{-1}) [2] during incremental exercise in representative 13-year old boys – one with CF [A] and one without [B]. Differences in ventilation are clear between participants (i.e. linear vs. curvilinear response), however normalisation of ventilation through log transformation (thus producing OUES) allows for direct comparison between individuals.

4.3.5 Statistical analyses

Statistical analyses were conducted using SPSS v.23 (IBM, Armonk, NY, USA). Independent *t*-tests identified mean differences in the anthropometric and CPET outcomes between CF and CON. Pearson's correlation coefficients were run to examine the relationship between each body size variable and the absolute, ratio-standard scaled and allometrically-scaled OUES to assess size dependence of OUES. Fisher's z-transformations identified group differences between correlations. The alpha level was set at 0.05 for all analyses.

4.4 Results

All descriptive data are presented as mean and standard deviation. Differences between group means with regards to the anthropometric, pulmonary and CPET outcomes are presented in Table 4.1. No significant differences ($P > 0.05$) were observed between groups for anthropometric or CPET variables. A significantly ($P < 0.05$) lower FEV_1 ($\%_{Predicted}$) was observed in the CF group, but no other pulmonary variables.

Each body size variable was significantly ($P < 0.001$) and positively correlated with OUES (Figure 4.2; A1, B1, C1). This finding is consistent across CF, CON, and as a combined group (Table 4.2), with the magnitude of the correlation consistently lower in CF when compared against CON. However, this was only statistically significant ($P < 0.05$) for absolute OUES when plotted against stature (Figure 4.2; A1).

Table 4.1. Anthropometric, pulmonary and exercise-related differences between children with CF and age- and gender-matched controls.

Variable	CF	CON	*P* Value
Stature (cm)	155.6 ± 13.6	159.1 ± 15.2	0.32
Body Mass (kg)	50.2 ± 15.5	51.2 ± 14.5	0.78
Body Surface Area (m^2)	1.46 ± 0.28	1.49 ± 0.28	0.65
FEV_1 ($L \cdot min^{-1}$)*	2.46 ± 0.97	2.96 ± 0.86	0.07
FEV_1 ($\%_{Predicted}$)*	88.0 ± 19.6	101.9 ± 12.2	**0.002**
FVC ($L \cdot min^{-1}$)*	3.10 ± 1.14	3.44 ± 1.02	0.30
FVC ($\%_{Predicted}$)*	94.8 ± 15.9	100.2 ± 12.5	0.21
Peak $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.74 ± 0.57	2.03 ± 0.88	0.09
Peak $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	38 ± 8	40 ± 11	0.32
GET (% p$\dot{V}O_2$)	53.3 ± 9.3	55.0 ± 8.0	0.42
Peak Power Output (W)	146 ± 57	175 ± 72	0.06
OUES (at 100% peak $\dot{V}O_2$)	1927.58 ± 583.49	2148.77 ± 846.55	0.20
OUES (at 75% peak $\dot{V}O_2$)	1842.81 ± 541.13	2066.11 ± 892.96	0.20
OUES (at 50% peak $\dot{V}O_2$)	1604.87 ± 661.75	1815.92 ± 852.51	0.27

Values are presented as mean ± standard deviation. *P* value, independent samples *t*-test significance level. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; peak $\dot{V}O_2$, peak oxygen uptake; GET, gas exchange threshold; OUES, oxygen efficiency uptake slope. * Unequal groups for pulmonary volumes (CF, *n* = 36; CON, *n* = 18).

When the ratio-standard scaling (Y/X) method was used, significant and positive correlations were present between the scaled maximal OUES and both stature and BSA for the combined group (Table 4.2) and CON group (Figure 4.2; A2, C2), but not the CF group. Whilst OUES scaled for BM did not retain a significant relationship with BM itself at the combined level (Table 4.2), it approached significance (P = 0.073). When split into sub-groups, a significant negative relationship was observed between scaled OUES and BM in CF (Figure 4.2; B2).

The output from the log-linear regression models is displayed in Table 4.3. Smaller ‘b’ exponents were observed for the CF group, when compared to CON, for each anthropometric factor. The exponents for the combined group were as follows: at 50% peak $\dot{V}O_2$ (stature = 3.60, BM = 1.06, BSA = 1.72); at 75% peak $\dot{V}O_2$ (stature = 2.93, BM = 0.80, BSA = 1.31), and at 100% peak $\dot{V}O_2$ (stature = 2.59, BM = 0.77, BSA = 1.24). A greater difference was evident between the scaling exponents (*Δb*) of CF and CON groups for stature (1.39) relative to those for body mass (0.16) and BSA (0.36). When the exponents were averaged across groups and OUES parameters, the scaling factors were stature = 3.00, BM = 0.86, and BSA = 1.40.

Table 4.2. Pearson's correlation coefficients for OUES at peak $\dot{V}O_2$ when scaled for body size using differing scaling procedures for whole-group (CF + CON)

	CF	CON	Combined
Absolute			
Stature vs. OUES	***r* = 0.545, *P* < 0.001**	***r* = 0.800, *P* < 0.001**	***r* = 0.703, *P* < 0.001**
Mass vs. OUES	***r* = 0.536, *P* < 0.001**	***r* = 0.747, *P* < 0.001**	***r* = 0.640, *P* < 0.001**
BSA vs. OUES	***r* = 0.578, *P* < 0.001**	***r* = 0.783, *P* < 0.001**	***r* = 0.685, *P* < 0.001**
Ratio Standard			
Stature vs. OUES/Stature	*r* = 0.296, *P* = 0.079	***r* = 0.704, *P* < 0.001**	***r* = 0.543, *P* < 0.001**
Mass vs. OUES/Mass	***r* = -0.379, *P* = 0.016**	*r* = -0.042, *P* = 0.806	*r* = -0.212, *P* = 0.073
BSA vs. OUES/BSA	*r* = 0.021, *P* = 0.905	***r* = 0.447, *P* = 0.006**	***r* = 0.254, *P* = 0.031**
Allometric			
Stature vs. OUES/Stature$^{3.00}$	***r* = -0.369, *P* = 0.027**	*r* = 0.111, *P* = 0.520	*r* = -0.139, *P* = 0.245
Mass vs. OUES/Mass$^{0.86}$	*r* = -0.253, *P* = 0.136	*r* = 0.150, *P* = 0.383	*r* = -0.041, *P* = 0.730
BSA vs. OUES/BSA$^{1.40}$	*r* = -0.272, *P* = 0.108	*r* = 0.129, *P* = 0.453	*r* = -0.062, *P* = 0.606

Bold text indicates a significant ($P < 0.05$) correlation. Bivariate plots are shown in Figure 4.2.

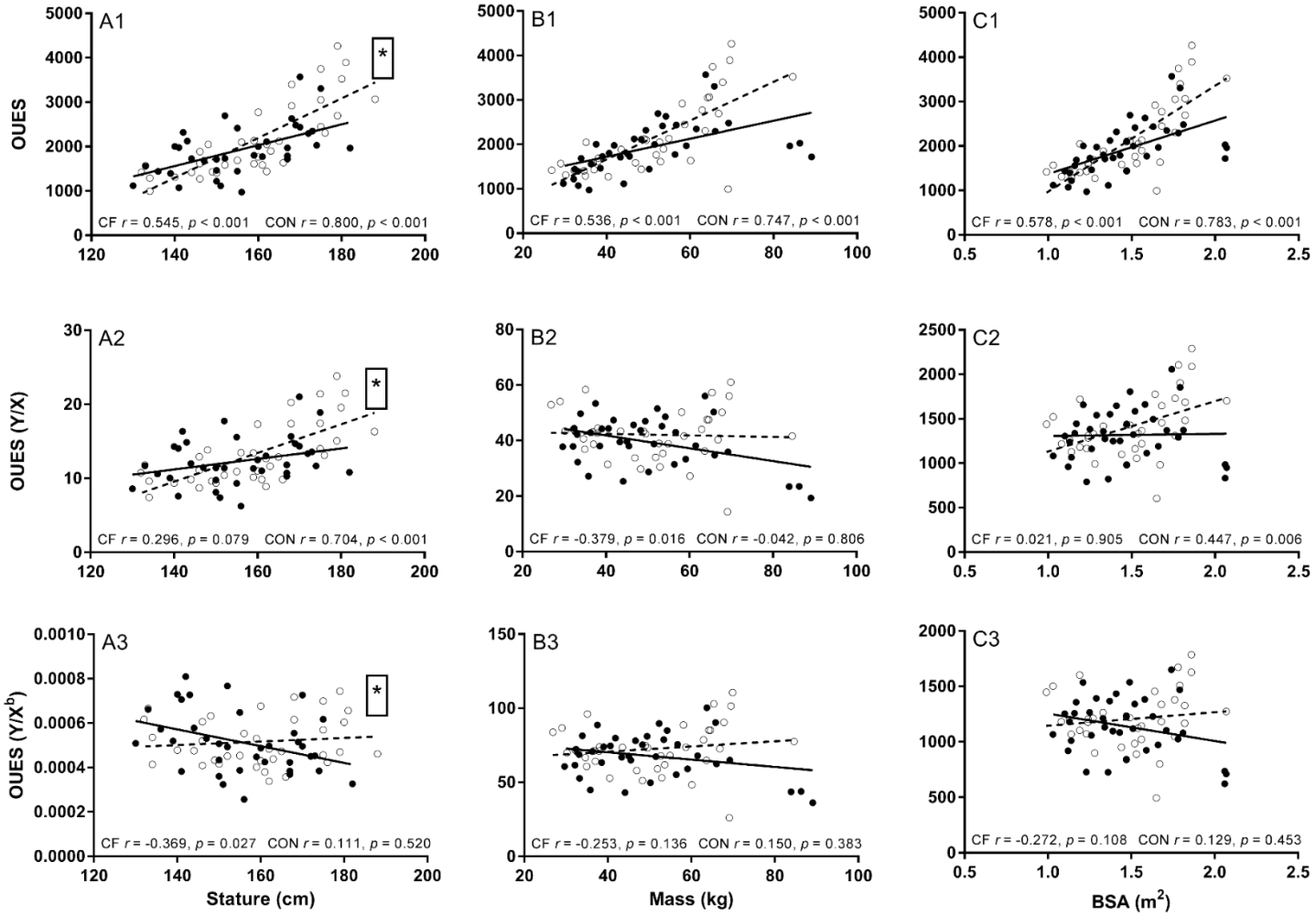


Figure 4.2 Scatter plots with Pearson's correlation coefficients for CF (●, solid line) and CON (○, dashed line) groups for OUES from peak exercise when scaled utilising each variable (stature [A], body mass [B] and body surface area [C]) and procedure (absolute [1], ratio-standard [2] and allometric [3]). * Significant difference ($P < 0.05$) between the magnitude of the correlation coefficients between CF and CON.

When OUES was scaled allometrically (Y/X^b) using the averaged exponents from Table 4.3, no significant correlations were present against BM or BSA at either the group (Figure 4.2; B3, C3) or combined (Table 4.2) level. However, a significant ($P < 0.05$) negative relationship was evident within the CF group between allometrically-scaled OUES and stature (Figure 4.2; A3). Furthermore, allometric scaling of OUES at submaximal intensities (50% peak $\dot{V}O_2$ and 75% peak $\dot{V}O_2$), using the exponents identified in Table 4.3 for BM and BSA produced non-significant correlations ($P > 0.05$; data not reported).

4.5 Discussion

The aims of this study were to initially describe the relationship between OUES and body size in children with CF and to identify appropriate procedures for scaling OUES against different body size variables. The main results have shown both significant relationships between OUES and body size; and that ratio-standard scaling is ineffective in controlling for body size, whereas allometric scaling does remove residual influences.

Table 4.3. Allometric exponents for the OUES measures and body size in young patients with CF and healthy age- and gender-matched controls.

		CF		CON			COMBINED	
OUES measure	Body size variable	b	95% CI	b	95% CI	*Δb*	b	95% CI
50% peak $\dot{V}O_2$	Stature (cm)	2.77	1.19 - 4.36	4.17	3.23 - 5.10	*1.40*	3.60	2.72 - 4.47
	Body Mass (kg)	1.06	0.62 - 1.51	1.06	0.61 - 1.51	*0.00*	1.06	0.75 - 1.37
	BSA (m^2)	1.63	0.94 - 2.33	1.78	1.14 - 2.42	*0.15*	1.72	1.26 - 2.17
75% peak $\dot{V}O_2$	Stature (cm)	2.17	1.26 - 3.08	3.56	2.74 - 4.37	*1.39*	2.93	2.31 - 3.55
	Body Mass (kg)	0.68	0.42 - 0.95	0.91	0.55 - 1.27	*0.23*	0.80	0.58 - 1.02
	BSA (m^2)	1.09	0.68 - 1.49	1.52	1.00 - 2.04	*0.43*	1.31	0.98 - 1.64
Peak $\dot{V}O_2$	Stature (cm)	1.88	0.87 - 2.89	3.17	2.41 - 3.92	*1.29*	2.59	1.96 - 3.21
	Body Mass (kg)	0.66	0.39 - 0.94	0.88	0.57 - 1.18	*0.22*	0.77	0.57 - 0.98
	BSA (m^2)	1.03	0.60 - 1.47	1.44	1.00 - 1.88	*0.41*	1.24	0.94 - 1.55
AVERAGE	Stature (cm)	2.23	1.54 - 2.91	3.62	3.11 – 4.12	***1.39***	**3.00**	**2.58 - 3.43**
	Body Mass (kg)	0.78	0.58 - 0.97	0.94	0.72 - 1.15	***0.16***	**0.86**	**0.72 – 1.01**
	BSA (m^2)	1.21	0.91 - 1.51	1.57	1.26 - 1.88	***0.36***	**1.40**	**1.18 - 1.62**

b: scaling exponent; 95% CI: 95% confidence interval for b. Averaged exponents are highlighted in bold. *Δb* indicated difference in exponents between CF and CON groups.

The relationships between body size and OUES for the present study are shown in Table 4.2 and Figure 4.2. These analyses identified large correlations for the CON group, with the magnitude closely resembling previous OUES research in healthy 7-18 year olds (Marinov et al., 2007). No previous study has detailed the magnitude of the relationship between OUES and body size in children with CF. The magnitude of the correlation in the CF group is lower than the CON group and reached statistical significance for the relationship between OUES and stature (Table 4.2, Figure 4.2). This could be due to the shorter stature typically observed in children with CF (Cystic Fibrosis Trust, 2016c) – a consequence of the chronic malnutrition associated with the disease (Culhane et al., 2013). However, the reported non-significant difference in body size, including stature, and OUES at peak exercise between CF and CON groups is similar to previous studies, despite decreased mean OUES values at peak exercise for both CF and CON groups in relation to previous research – a difference potentially accounted for by differences in aerobic fitness (Bongers et al., 2012). This suggests additional body size independent factors affect the OUES in CF and therefore may account for the smaller correlation coefficients observed in the present study. When ratio-standard scaling is utilised to adjust OUES, significant correlations exist against all the body size variables (Table 4.2, Figure 4.2; A2, B2, C2), with the magnitude, and significance, of coefficients being different for each body size variable and group. These significant positive coefficients result in biasing against individuals with a smaller stature or BSA. Whilst the combined correlation coefficient for BM is non-significant, it remains significant and negative within the CF group, thus biasing against heavier individuals, and removing its potential to be uniformly utilised across both groups. Furthermore, evidence against the use of the ratio-standard method to scale OUES is provided by the b values obtained

in the log-linear regression. For the ratio-standard method to be effective, the b values would be required to equal, or at least be very close to, 1 (Tanner, 1949). As is shown in Table 4.3, the obtained values do not equal 1, nor do the 95% CI, which represent the uncertainty of the point estimate, span 1 consistently across both groups. Therefore, the ratio-standard procedure does not uniformly control for size in children with, and without CF, for each body size variable.

Previous research has advocated scaling of OUES in children using a ratio-standard approach, controlling for fat-free mass (FFM) or BSA (Akkerman et al., 2010). However, the authors did not verify the assumption that this technique appropriately removes the influence of body size. As a result, subsequent studies have cited this study as reason for scaling OUES in such a manner when making comparisons between groups in paediatric populations with chronic disease (Bongers et al., 2012, Bongers et al., 2011, Tsai et al., 2016). However, the results of the current study have shown the ratio standard approach to be invalid and is likely to result in incorrect conclusions in previous OUES research due to the inaccurate expression of data (Bongers et al., 2012).

Upon utilising allometric scaling, non-significant relationships ($P > 0.05$) were found between the corrected OUES from peak exercise and both BM and BSA for CF and CON groups, as well as the combined group values. However, the magnitude of coefficient is between -0.25 and -0.30 for BM and BSA in the CF group, indicating that this method does not fully control for size, but remains an improvement on the ratio-standard method. Unlike BM and BSA, stature retained a significant relationship with allometrically corrected OUES within the CF group ($P < 0.05$; Figure 4.2; A3). A non-significant mean difference between CF and CON for stature was found, therefore suggesting it is not stature itself, but the interaction of the two (stature and OUES) that is different between groups. This

difference in the relationship between stature and OUES is further evidenced by 'b' values between groups (Table 4.3), with the *Δb* between CF and CON of 1.39 being over three times greater than that of BSA (*Δb* = 0.36). Therefore, our data suggest stature is an unsuitable variable for scaling OUES, regardless of which scaling procedure is used. In contrast, the more homogenous 'b' values between CF and CON groups for both BM and BSA (Table 4.3) indicate these body size variables should be used for future allometric scaling of OUES, as the exponents can be uniformly applied to both groups. The same results were found for OUES at submaximal intensities (50% peak $\dot{V}O_2$ and 75% peak $\dot{V}O_2$), with allometric scaling proving to be the optimal methodology for removing residual effects of body size. This is a notable finding, as it highlights the importance of scaling, even for submaximal parameters of exercise, given that many patients may be unable, or unwilling, to perform maximal exercise.

The results shown above indicate that either BM or BSA is an appropriate body size variable against which to scale OUES, provided an allometric approach is used. However, previous research is equivocal on which body size variable to use, with both BM (Baba et al., 1996, Breithaupt et al., 2012, Marinov et al., 2007, Rogowski et al., 2012) and BSA (Bongers et al., 2012, Bongers et al., 2011, Breithaupt et al., 2012, Tsai et al., 2016) being frequently used. BSA has been suggested for use, due to its ability to normalise for pulmonary volume (Hollenberg and Tager, 2000). However due to progressive declining of lung function observed in individuals with CF (Harun et al., 2016), it is unclear whether BSA appropriately normalises for pulmonary volume, a point further supported by the significant differences in lung function between groups in the current study. In addition, whilst BM remains a suitable anthropometric scaling variable, ideally, FFM should be used as it better reflects the metabolic cost of exercise (Akkerman

et al., 2010). However, this measure is not routinely collected by CF clinics, and body composition data, as estimated from skinfold and bioelectrical impedance methods, have poor accuracy at the individual level (Alicandro et al., 2015). As such, there is no evidence to suggest superiority of either BM or BSA for use in scaling OUES. Therefore, the suitability of each anthropometric variable needs to be investigated further to ensure future standardisation of research.

Clinicians involved in the management of CF perceive CPET as a useful tool (Stevens et al., 2010), with regular exercise testing recommended for individuals with CF (Hebestreit et al., 2015). Given the clinical importance of exercise testing, it is therefore essential that appropriate measures and methodologies are being utilised to analyse outcomes. In order to streamline analyses for clinical teams, the ‘b’ exponent values for BM and BSA provided here may be utilised, provided patient characteristics are in line with current study. However, the purpose of this study was not to create a universal scaling exponent for OUES, as it is likely that scaling exponents may change between patient cohorts, and therefore future studies should utilise these described methodologies to derive their own exponents to ensure a size-free expression of OUES.

4.6 Conclusion

This study has identified that ratio-standard scaling of the OUES is an invalid scaling method when using stature, BSA or BM as a significant relationship still exists with body size. In contrast, allometric scaling of BM and BSA was better able to control for body size in young people with CF and age and sex matched controls and should be used in future research investigating the clinical utility of OUES in this patient group. Therefore, this study recommends that allometrically scaled BM or BSA should be promoted for use in future research and/or clinics where OUES is sought as an outcome measure from a CPET.

5 THE OXYGEN UPTAKE EFFICIENCY SLOPE IS NOT A VALID SURROGATE OF AEROBIC FITNESS IN CYSTIC FIBROSIS

5.1 Abstract

Background:

Maximal cardiopulmonary exercise testing is recommended on an annual basis for children with cystic fibrosis (CF), due to a clinically useful prognostic information provided by maximal oxygen uptake ($\dot{V}O_{2max}$). However, not all patients are able, or willing, to reach $\dot{V}O_{2max}$, and therefore submaximal alternatives are required. This study explored the validity of the oxygen uptake efficiency slope (OUES) as a submaximal measure of $\dot{V}O_{2max}$ in children and adolescents with CF.

Methods:

Data were collated from 72 cardiopulmonary exercise tests (36 CF, 36 controls), with OUES determined relative to maximal and submaximal parameters of exercise intensity, time and individual metabolic thresholds. Pearson's correlation coefficients, independent t-tests and factorial ANOVAs were used to determine validity.

Results: Significant ($p < 0.05$) correlations with $\dot{V}O_{2max}$ were observed for most expressions of OUES, but were consistently weaker in CF ($r = 0.30 – 0.47$) when compared to CON ($r = 0.58 – 0.89$). Mean differences for all OUES parameters between groups were not significant ($p > 0.05$). When split by $\dot{V}O_{2max}$ tertiles, minimal significant differences were found between, and within, groups for OUES, indicating poor discrimination of $\dot{V}O_{2max}$.

Conclusions:

The OUES is not a valid (sub)maximal measure of $\dot{V}O_{2max}$ in children and adolescents with mild-to-moderate CF. Clinicians should continue to use maximal markers (i.e. $\dot{V}O_{2max}$) of exercise capacity.

5.2 Introduction

Previous research indicates the benefit of high levels of cardiorespiratory fitness, as characterised by maximal oxygen uptake ($\dot{V}O_{2max}$), for young people with cystic fibrosis (CF). A high $\dot{V}O_{2max}$ is associated with an improved quality of life (Hebestreit et al., 2014), reduced risk of hospitalisation for pulmonary exacerbations (Pérez et al., 2014) and reduced mortality risk (Pianosi et al., 2005a). Consequently, individuals with CF are advised to increase their exercise and habitual physical activity levels, with regular maximal cardiopulmonary exercise testing (CPET) also recommended and endorsed by the European CF Society (Hebestreit et al., 2015) and European Respiratory Society, to monitor changes in their aerobic fitness status.

However, assessing $\dot{V}O_{2max}$ requires patients to provide a maximal physical effort and is thus considered an 'effort dependent' test. Motivation, discomfort, excessive dyspnoea, chronic fatigue and naivety towards protocols may make patients with CF more unwilling or unable to reach volitional exhaustion and their $\dot{V}O_{2max}$. Therefore, physiological markers of aerobic fitness that can be attained during submaximal regions of a CPET can be particularly useful (Williams et al., 2014).

One such marker is the oxygen uptake efficiency slope (OUES), a submaximal, effort-independent parameter describing the relationship between $\dot{V}O_2$ and the common logarithm of minute ventilation ($\dot{V}_E$) (Baba et al., 1996). Given the curvilinear relationship between ventilation and oxygen uptake during

incremental exercise, it is difficult to model and therefore normalisation of ventilation (i.e. log$\dot{V}_E$) allows for direct comparison between tests (and groups). A higher value for the OUES indicates a greater ventilatory efficiency. The OUES has been shown to significantly and positively correlate with $\dot{V}O_{2max}$ in healthy children (Marinov et al., 2007) and children with heart disease (Baba et al., 1996), indicating its potential as a submaximal surrogate of aerobic fitness in paediatric groups.

Despite OUES appearing to be a valid determinant of exercise tolerance in adults with CF (Gruet et al., 2010), evidence for its use in children and adolescents with CF requires further verification. Only one study has previously sought to validate the OUES as an effort-independent marker of $\dot{V}O_{2max}$ in a paediatric population with mild-to-moderate CF (Bongers et al., 2012). This study calculated OUES at 100%, 75% and 50% of the test duration and concluded it invalid, due to the observed moderate positive correlations between the OUES and $\dot{V}O_{2max}$ (r = 0.41 – 0.54). Furthermore, despite decreased $\dot{V}O_{2max}$ in children with CF, the OUES was unable to differentiate fitness status between children with, and without CF; leading authors to conclude the invalidity of OUES in this patient group. However, there are multiple methodological weaknesses to this study. Firstly, utilising CPET time to exhaustion (TTE) as a measure of intensity may be flawed, as it does not account for variances in individual metabolic thresholds. As the presence of reduced maximal capacity (Saynor et al., 2014b) and an altered oxygen cost of exercise (Moser et al., 2000) have been demonstrated in individuals with CF, it is conceivable that patients in this previous study (Bongers et al., 2012) may be exercising at differing relative exercise intensities (i.e. as a percentage of $\dot{V}O_{2max}$), and even within differing intensity domains, despite being matched for exercise duration. Secondly, there was a lack of appropriate

normalisation for the influence of body size, with authors utilising ratio-standard scaling, whereas previous research has shown this to be insufficient at removing residual effects of body size from OUES (Tomlinson et al., 2017).

Given aforementioned issues associated with previous research (Bongers et al., 2012), OUES should instead be assessed at individually determined parameters of relative exercise intensity (%$\dot{V}O_{2max}$) and domain thresholds, such as the gas exchange threshold (GET) and respiratory compensation point (RCP) (Beaver et al., 1986), alongside utilising allometric scaling protocols to ensure a size-free analysis of OUES (Tomlinson et al., 2017).

Therefore, the purpose of this study was to examine correlates of allometrically-scaled OUES with $\dot{V}O_{2max}$, and to systematically investigate differences in the OUES between children with CF and healthy controls (CON) at appropriately matched parameters of relative exercise intensity (%$\dot{V}O_{2max}$), TTE and individual metabolic boundaries (GET and RCP). In addition, the study will examine whether the OUES can differentiate between patients of differing aerobic fitness statuses vs. healthy matched controls and, therefore, its suitability as a submaximal surrogate for $\dot{V}O_{2max}$.

5.3 Materials and methods

5.3.1 Participants

Data from 45 children and adolescents with CF were considered for inclusion in the current retrospective analysis. Nine children were excluded due to inadequate data (insufficient, or missing data, n = 7; insufficient test length, n = 2). Remaining data were subsequently age- and gender-matched from existing exercise databases of healthy children, resulting in a final sample of n = 72 (36 CF, 36 CON; 21 males per group; mean age 13.3 ± 2.8 years). All CON children were

screened for contraindications to exercise prior to CPET participation, including pulmonary disorders and unstable co-morbid asthma.

As the study was a retrospective analysis of existing data, additional ethics approval was not required. Ethics approval for data collected was originally approved by South West NHS Research Ethics and local institutional ethics committees, whereby fully informed written consent and assent were obtained from parents/guardians and paediatric participants, respectively.

5.3.2 Data collection

All participants undertook a CPET to volitional exhaustion on an electronically braked cycle ergometer, to determine $\dot{V}O_{2max}$ and submaximal measures of cardiorespiratory fitness. If required by patients with CF, bronchodilators were administered prior to CPET. Pulmonary function was assessed using a hand-held spirometer, with maximal values of forced expiratory volume in one-second (FEV_1) and forced vital capacity (FVC) compared to normative values (Quanjer et al., 2012, Quanjer et al., 1993, Zapletal et al., 1987). Pubertal status of children was determined as age from peak height velocity (aPHV), using published equations (Moore et al., 2015).

5.3.3 Data analysis

Pulmonary gas exchange and ventilation data were collected breath-by-breath, and subsequently averaged to 10 second time intervals. Previously described techniques were utilised to ascertain $\dot{V}O_{2max}$ (Barker et al., 2011), GET and RCP (Beaver et al., 1986). To ascertain OUES values, linear regressions were obtained between $\dot{V}O_2$ and the logarithmic transformation of $\dot{V}_E$ ($\log\dot{V}_E$), using data up to the following boundaries: 100%, 75% and 50% of TTE (100_{TTE}, 75_{TTE}, 50_{TTE}), 100%, 75% and 50% of $\dot{V}O_{2max}$ ($100_{\dot{V}O2max}$, $75_{\dot{V}O2max}$, $50_{\dot{V}O2max}$), GET and

RCP. The time point of 100%$\dot{V}O_{2max}$ also describes 100%$_{TTE}$ – providing eight OUES parameters per participant.

5.3.4 Scaling of data

All OUES values were allometrically scaled to BSA (Haycock et al., 1978), in line with recent recommendations (Tomlinson et al., 2017). An allometric model was applied to remove residual effects of body size, with OUES scaled to $BSA^{1.40}$. $\dot{V}O_{2max}$ was not scaled using allometric procedures as ratio-standard scaling sufficiently removed residual effects of body size.

5.3.5 Statistical analyses

Descriptive data are reported as mean (± standard deviation (SD)) unless otherwise stated. Pearson's correlation coefficients were calculated between $\dot{V}O_{2max}$ and each of the eight normalised OUES values, to identify if the two variables are significantly related. Independent samples *t*-tests were also performed to identify differences between CF and CON for all variables, and identify the impact of disease status upon OUES. Finally, factorial ANOVAs were conducted to identify the interaction between $\dot{V}O_{2max}$ status, split by tertile (Pianosi et al., 2005a), and disease status upon $\dot{V}O_{2max}$ and $OUES/BSA^{1.40}$. Where main or interaction effects were found, pairwise comparisons using Bonferroni corrections were applied to identify where relationships existed. Statistical significance was set at an alpha of 0.05 and Cohen's thresholds are used to report effect sizes (ES) and illustrate the magnitudes of the mean difference (Cohen, 1992).

5.4 Results

5.4.1 Participant characteristics

Participant characteristics and mean differences between groups are presented in Table 5.1. Significant differences were observed between CF and CON for pulmonary function and the absolute $\dot{V}O_2$ at the GET.

Table 5.1. Anthropometric, pulmonary function and exercise-related differences between CF and CON groups.

Variable	CF	CON	*p* value	Effect Size
Stature (cm)	155.6 (13.5)	159.1 (15.2)	0.32	0.24
Body mass (kg)	50.2 (15.5)	51.2 (14.5)	0.78	0.07
BMI ($kg \cdot m^{-2}$)	20.28 (3.67)	19.91 (4.18)	0.70	0.09
BSA (m^2)	1.46 (0.28)	1.49 (0.28)	0.65	0.11
aPHV	0.27 (2.70)	0.65 (2.44)	0.89	0.15
FEV_1 (L)*	2.46 (0.97)	2.96 (0.86)	0.07	0.53
FEV_1 ($\%_{Predicted}$)*	88.0 (19.6)	101.9 (12.2)	**0.002**	0.79
FVC (L)*	3.10 (1.14)	3.44 (1.02)	0.30	0.31
FVC ($\%_{Predicted}$)*	94.8 (15.9)	100.2 (12.5)	0.21	0.36
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	1.74 (0.57)	2.03 (0.88)	0.093	0.39
$\dot{V}O_{2max}$ ($mL.kg^{-1} \cdot min^{-1}$)	38 (8)	40 (11)	0.32	0.23
GET ($L \cdot min^{-1}$)	0.91 (0.28)	1.12 (0.54)	**0.035**	0.49
GET (% $\dot{V}O_{2max}$)	53.4 (9.3)	55.0 (8.0)	0.42	0.18
HR_{max} ($beats \cdot min^{-1}$)	182 (8)	185 (14)	0.30	0.26
$\dot{V}_{Emax}$ ($L \cdot min^{-1}$)	74.66 (35.62)	69.18 (33.45)	0.50	0.16
RER_{max}	1.27 (0.23)	1.21 (0.13)	0.22	0.32

Measures are presented as mean (± SD). Significant mean diffeences are denoted by a bolded p vlaue. * Unequal groups for pulmonary volumes (CF, *n* = 36; CON, *n* = 18). BMI: body mass index; BSA, body surface area; aPHV, age from peak height velocity; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; $\dot{V}O_{2max}$, maximal oxygen uptake; GET, gas exchange threshold; HR, heart rate; $\dot{V}_E$, minute ventilation; RER, respiratory exchange ratio.

5.4.2 Correlation between OUES and $\dot{V}O_{2max}$

All OUES/BSA$^{1.40}$ variables significantly correlated with body mass relative $\dot{V}O_{2max}$, apart from 50%$_{TTE}$ within the CF group (Table 5.2).

Table 5.2. Correlations at different thresholds between parameters of oxygen uptake and ventilatory efficiency and $\dot{V}O_{2max}$ relative to body mass.

Oxygen Uptake Parameter	CF	CON
OUES/BSA$^{1.40}$ @ 50% $\dot{V}O_{2max}$	0.36 (0.040)	0.75 (< 0.001)
OUES/BSA$^{1.40}$ @ 50%$_{TTE}$	0.30 (0.071)	0.76 (< 0.001)
OUES/BSA$^{1.40}$ @ 75%$\dot{V}O_{2max}$	0.33 (0.049)	0.85 (< 0.001)
OUES/BSA$^{1.40}$ @ 75%$_{TTE}$	0.38 (0.023)	0.87 (< 0.001)
OUES/BSA$^{1.40}$ @ 100%$\dot{V}O_{2max}$ & $_{TTE}$	0.47 (0.004)	0.89 (< 0.001)
OUES/BSA$^{1.40}$ @ GET	0.35 (0.042)	0.58 (< 0.001)
OUES/BSA$^{1.40}$ @ RCP	0.45 (0.007)	0.88 (< 0.001)

Values are presented as correlation coefficients (*r*) with *p* values in parentheses.

5.4.3 Difference in OUES between CF and CON

Mean values for BSA corrected OUES values were lower, but not significantly, in CF compared to CON at each threshold (50$_{\dot{V}O2max}$: 923 ± 273 vs. 992 ± 290; 75$_{\dot{V}O2max}$: 1088 ± 224 vs. 1153 ± 293; 50$_{TTE}$: 1019 ± 219 vs. 1091 ± 273; 75$_{TTE}$: 1101 ± 225 vs. 1182 ± 284; 100$_{\dot{V}O2max}$ and 100$_{TTE}$: 1141 ± 257 vs. 1206 ± 267; GET: 958 ± 296 vs. 996 ± 361; RCP: 1148 ± 251 vs. 1189 ± 297; $p > 0.05$ for all comparisons (range = 0.18 – 0.63); units for all parameters: $mL \cdot min^{-1} \cdot logL^{-1} \cdot m^{-2.8}$). Figure 5.1 represents the data for OUES relative to BSA, according to categories of duration, intensity and the metabolic thresholds.

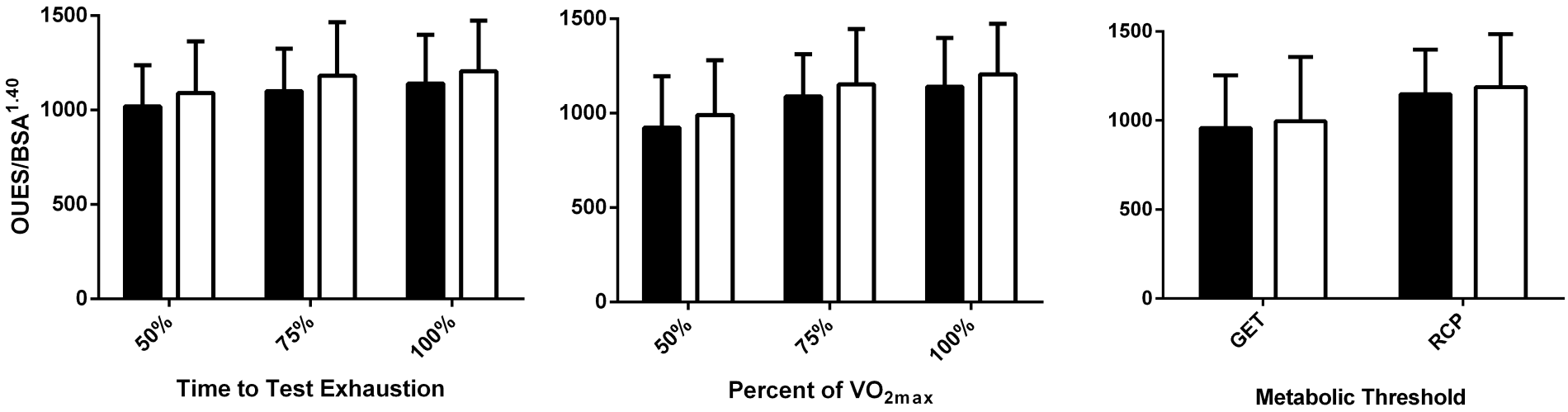


Figure 5.1 Comparison of OUES/BSA$^{1.40}$ values between children and adolescents with CF (black bars) and healthy age- and gender-matched controls (white bars) at different exercise thresholds.

5.4.4 OUES and fitness tertiles

When the data were split by tertiles according to $\dot{V}O_{2max}$ (Figure 5.2), a significant difference was observed between tertiles within both CF (46 ± 5 vs. 38 ± 2 vs. 30 ± 5 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively) and CON (52 ± 6 vs. 39 ± 3 vs. 29 ± 6 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively) groups with regards to aerobic fitness ($p < 0.001$ for all pairwise comparisons, $ES = 2.07 – 3.84$). However, there was only a significant difference in $\dot{V}O_{2max}$ between CF and CON in the highest aerobic fitness tertile ($p < 0.001$, $ES = 1.19$).

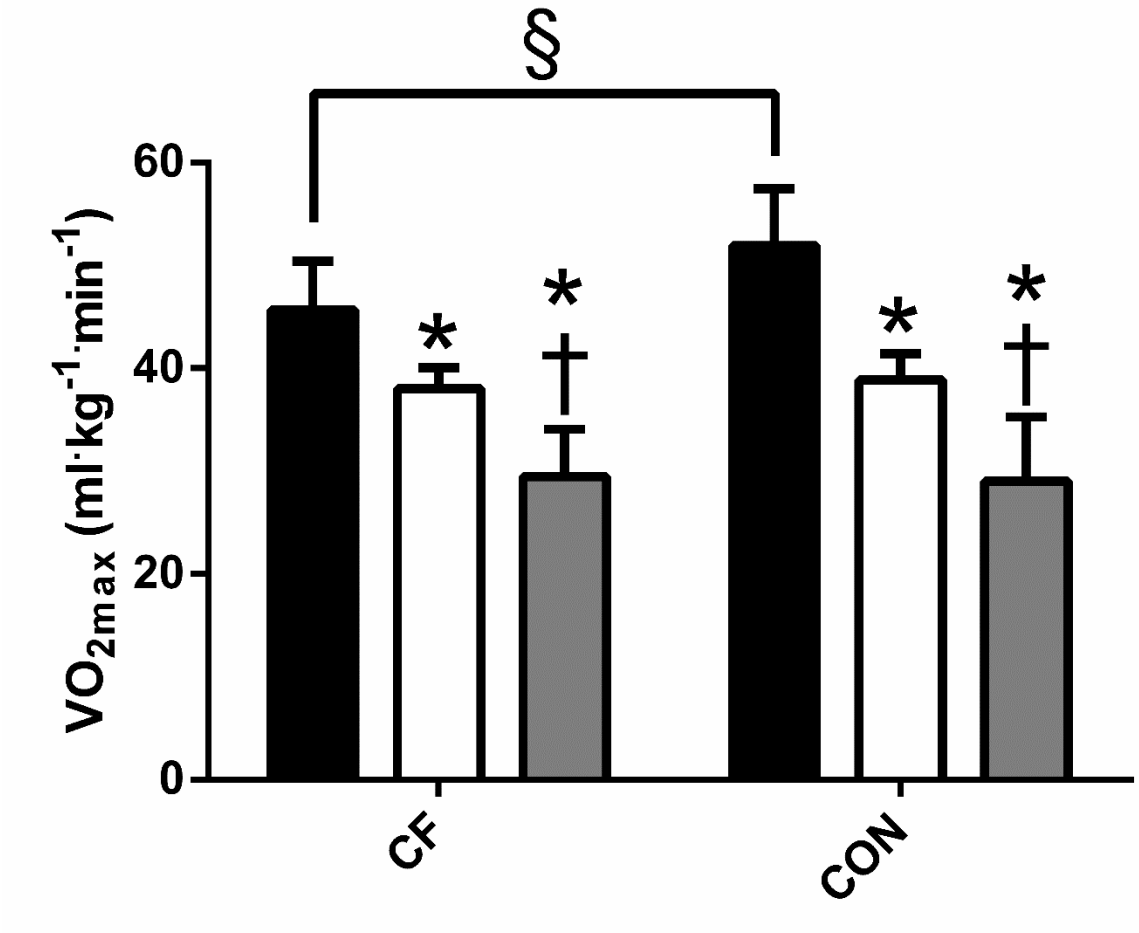


Figure 5.2 Comparison of $\dot{V}O_{2max}$, split by $\dot{V}O_{2max}$ tertile (black bars = highest tertile, white bars = middle tertile, grey bars = lowest tertile), within the CF and healthy control groups. * Significant ($p < 0.01$) difference from highest tertile. † Significant ($p < 0.01$) difference from middle tertile. § Significant ($p < 0.05$) difference between groups.

When split by $\dot{V}O_{2max}$ tertiles, there was no significant difference in $OUES/BSA^{1.40}$ at $100\%_{TTE}$ ($p > 0.05$). In CF, at $100\%_{TTE}$, $OUES/BSA^{1.40}$ was significantly higher in the highest (1271 ± 241) relative to the lowest (1020 ± 281) fitness tertile ($p = 0.016$, $ES = 0.96$). The middle tertile (1131 ± 198) was not significantly different between either the highest ($p = 0.34$, $ES = 0.63$) or lowest tertile ($p = 0.62$, $ES = 0.46$). By comparison, in the CON group significant differences were found between the highest (1441 ± 211) and lowest (957 ± 206; $p < 0.001$, $ES = 2.32$), between the middle (1219 ± 108) and the lowest ($p = 0.011$, $ES = 1.59$) and middle and highest ($p = 0.041$, $ES = 1.32$; Figure 5.3) tertiles.

There was no significant difference in $OUES_{GET}/BSA^{1.40}$ between the groups ($p > 0.05$). When $OUES_{GET}/BSA^{1.40}$ was split by aerobic fitness tertiles, a significant difference was only found within the CON group between the highest (1221 ± 336) and lowest tertiles (798 ± 273, $p = 0.005$, $ES = 1.38$). The middle tertile (952 ± 356) was not significantly different to either the highest ($p = 0.114$, $ES = 0.78$) or lowest tertile ($p = 0.712$, $ES = 0.49$). In the CF group, no significant differences were found between any tertiles (highest: 1017 ± 273; middle: 1006 ± 324; lowest: 854 ± 290, all $p > 0.61$, $ES = 0.04 – 0.58$). No significant differences between groups were observed for each tertile (all $p > 0.11$, $ES = 0.16 – 0.64$; Figure 5.3).

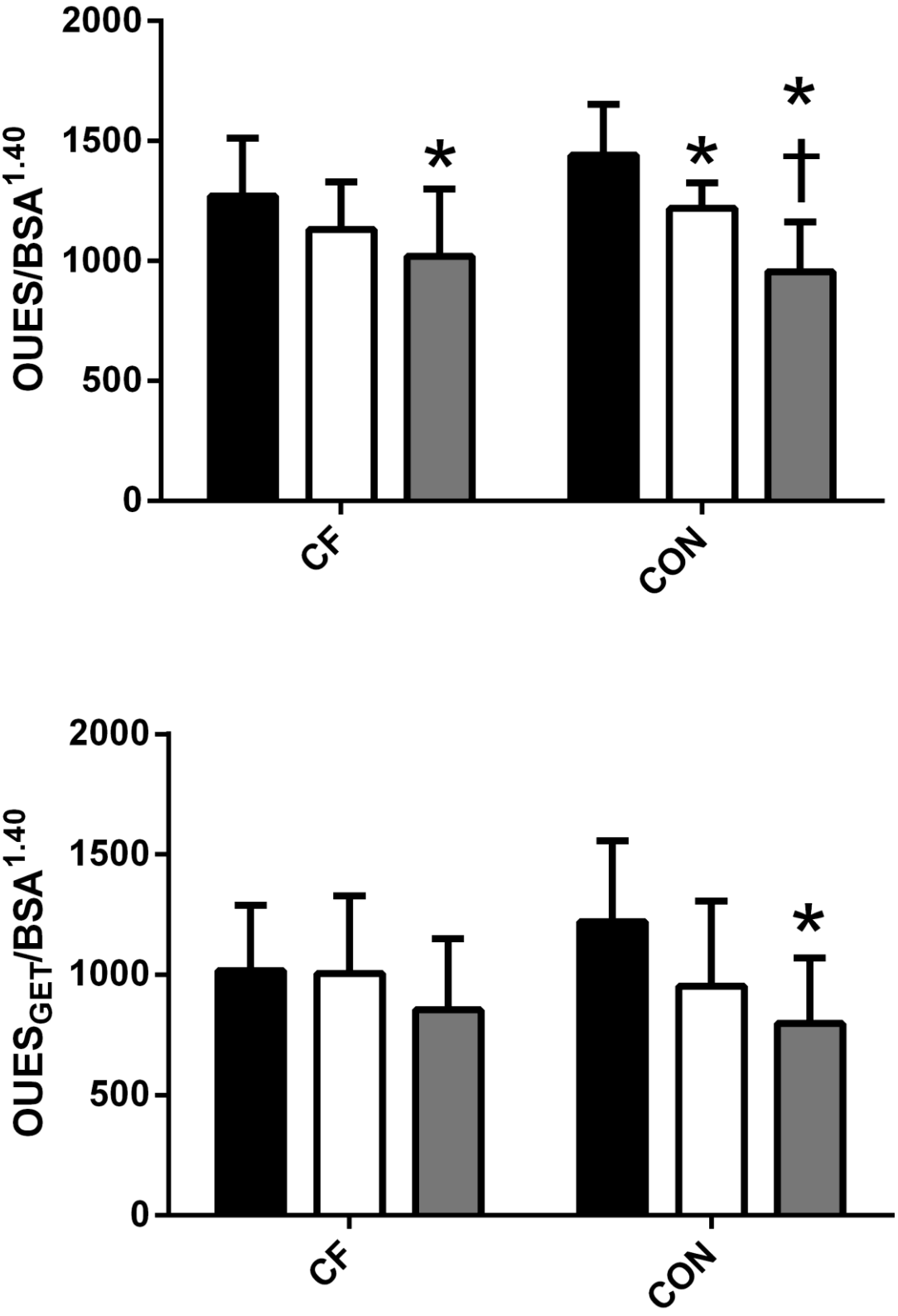


Figure 5.3 Comparison of OUES/BSA$^{1.40}$ at 100%$_{TTE}$ and OUES$_{GET}$/BSA$^{1.40}$ split by $\dot{V}O_{2max}$ tertile (black bars = highest tertile, white bars = middle tertile, grey bars = lowest tertile), within the CF and healthy control groups. * Significant ($p < 0.05$) difference from highest tertile. † Significant ($p < 0.05$) difference from middle tertiles.

5.5 Discussion

The primary purpose of this study was to investigate the validity of the OUES as a submaximal alternative to $\dot{V}O_{2max}$ in young people with CF – utilising a larger CF cohort than previous research (Bongers et al., 2012, Bongers et al., 2014b). Specifically, we comprehensively compared differences in the OUES, when appropriately normalised for BSA (Tomlinson et al., 2017), between children and adolescents with mild-to-moderate CF and their healthy peers, at parameters of time and relative exercise intensity. Although OUES was associated with $\dot{V}O_{2max}$ in both CF and CON groups, coefficients were consistently smaller in CF. Despite differences in these correlations, statistically significant differences in OUES could not be found between groups, regardless of whether it was standardised to percentage of $\dot{V}O_{2max}$, test duration or submaximal metabolic thresholds. Furthermore, OUES could not discriminate fitness status within, and between, groups. Taken collectively, these observations suggest OUES does not provide a valid surrogate of $\dot{V}O_{2max}$ in children and adolescents with CF, supporting previous findings (Bongers et al., 2012).

In this present study, significant correlations were observed between body-mass relative $\dot{V}O_{2max}$ and the majority of BSA corrected OUES thresholds, except at $50\%_{TTE}$ in the CF group. The locations of significance are identical to the only previous OUES study in children with a similar severity of CF during incremental cycling exercise, with magnitudes of correlations in the CF and CON groups corroborating previous work (Bongers et al., 2012) as CON shows larger effect sizes (r = 0.58 – 0.89) in comparison to the medium effect sizes (r = 0.30 – 0.47) of the CF cohort. As the correlation coefficients in the CF groups suggest a shared variance (R^2) of between 9 and 22% (unlike 34 – 79% in CON), these

results suggest that despite their association, OUES may not be a viable surrogate for $\dot{V}O_{2max}$.

Despite positive correlations with $\dot{V}O_{2max}$, no mean differences in OUES were observed between CF and CON at each parameter (of intensity, time and metabolic thresholds) – a finding contrasting previous adult and paediatric studies assessing OUES in independent groups (Akkerman et al., 2010, Baba et al., 1996, Drinkard et al., 2007, Gruet et al., 2010, Marinov et al., 2007). However, it could be argued that since a significantly lower $\dot{V}O_{2max}$ was not observed in CF versus CON in the present study, in contrast to previous findings (Bongers et al., 2014b, Saynor et al., 2014b), a recruitment bias may be present. The lack of differences between groups may be due to deconditioning of control participants (as opposed to increased fitness in CF), with $\dot{V}O_{2max}$ being 10 $mL \cdot kg^{-1} \cdot min^{-1}$ lower in the current study, when compared to previous research (Bongers et al., 2012). Consequently, it would also be expected that no differences in OUES would be observed. However, factorial ANOVAs sought to identify the sensitivity of the OUES measurement in discriminating between children of differing fitness. As the OUES supposedly represents $\dot{V}O_{2max}$ when maximal exercise efforts cannot be reached (Baba et al., 1996), it is assumed that the OUES should follow a similar profiling pattern to $\dot{V}O_{2max}$ and differentiate between patients of differing clinical and aerobic fitness states.

When data were categorised into fitness based upon aerobic fitness tertiles, a division shown to predict mortality in CF (Pianosi et al., 2005a), a significant difference in $\dot{V}O_{2max}$ was clearly evident both within and between the groups, but the former was only seen at the highest fitness level. This observation identifies that differences in aerobic fitness ($\dot{V}O_{2max}$) can be isolated within children with CF. However, when represented as aerobic fitness tertiles, differences in the OUES

and $OUES_{GET}$ (Figure 5.3) were not clearly defined, with a difference only evident between high-fit and low-fit children and adolescents with CF for OUES at $100\%_{TTE}$. In contrast, better discriminatory sensitivity was evident in the CON group, showing differences in OUES between all tertiles for aerobic fitness. Thus, even though some discriminatory power may be evident between children and adolescents with CF for high and low aerobic fitness, this was only found for OUES at $100\%_{TTE}$. This suggests that to isolate individuals of differing fitness status, a measurement of OUES would need to be taken at maximal exercise, as opposed to a submaximal parameter which can be identified in real-time during a CPET, such as the GET (characterised by a disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$). However, if participants would be required to reach volitional maximum to produce a maximal OUES value, clinicians would benefit from utilising $\dot{V}O_{2max}$ as opposed to OUES from peak exercise.

Since the purpose of the OUES is to provide a measure that is useful in lower functioning patients, i.e. those unable/unwilling to reach volitional exhaustion, differentiation between these patients is a key requisite of this CPET parameter, especially at submaximal thresholds. Unfortunately, this study demonstrates that the OUES does not provide such sensitivity in children and adolescents with CF. Therefore, despite the OUES showing potential as a clinical outcome in other paediatric cohorts (Baba et al., 1996, Drinkard et al., 2007), its use as a surrogate of $\dot{V}O_{2max}$ in children and adolescents with CF is doubtful.

Previous studies have assessed the validity of the OUES in clinical populations, such as congestive heart failure (Hollenberg and Tager, 2000) and congenital heart disease (Bongers et al., 2011), finding it, to an extent, to be a suitable, effort-independent, parameter of aerobic fitness. Moreover, two previous studies have assessed the applicability of the OUES in individuals with CF. One,

conducted in 31 adults and 34 healthy controls, concluded that OUES at 80% of test duration is a valid predictor of maximal aerobic fitness, due to high correlation (r = 0.91) with $\dot{V}O_{2peak}$ – and therefore may be a clinically useful submaximal exercise parameter (Gruet et al., 2010). In addition, Bongers et al. (2012) sought to validate the OUES at 50%, 75% and 100% of test duration in 22 children and adolescents with CF and 22 healthy controls. In contrast to earlier findings in adults, it was concluded to be an invalid measure, due to limited distinguishing properties and moderate correlations with $\dot{V}O_{2max}$. However, previous studies have analysed OUES at submaximal parameters of time, without attempts to standardise and individualise exercise intensity, meaning participants may be exercising in differing metabolic domains, despite matching for exercise duration. Hence, the current study accounted for these factors, by analysing OUES at submaximal parameters of intensity, time and individual metabolic thresholds. Furthermore, the groups in the existing paediatric study (Bongers et al., 2012) were poorly matched, with a significant difference in age evident between children with CF and healthy counterparts. As previous work has identified age- and sex-related differences in the OUES (Marinov et al., 2007), this may have inadvertently affected results. In addition, inappropriate ratio-standard scaling methods were utilised, whereas previous research has shown that allometric procedures are required to remove residual effects of body size from OUES (Tomlinson et al., 2017). In order to solely isolate the effects of disease status, the current study deliberately age- and gender-matched participants, utilising allometric scaling to ensure all influencing factors were controlled for.

Given that the OUES is physiologically dependent on metabolic CO_2 production ($\dot{V}CO_2$) and the ratio of pulmonary dead space to tidal volume (V_D/V_T) (Baba et al., 1996), it is prudent to examine which factors are altered in CF which may

account for its weaker relationship with $\dot{V}O_{2max}$ compared to their healthy counterparts. Whilst a reduced $\dot{V}O_{2max}$ has been reported in children with CF (Bongers et al., 2014b, Saynor et al., 2014b), no differences exist between CF and CON for the percentage of $\dot{V}O_{2max}$ at which GET (an indication of the onset of metabolic acidosis (Beaver et al., 1986)) occurs (Bongers et al., 2012, Bongers et al., 2014b, Saynor et al., 2014b, Saynor et al., 2016b), suggesting metabolic development of CO_2 is not impaired in CF, and it may be the V_D/V_T ratio responsible for reduced OUES – a suggestion proposed, and supported by, previous research (Bongers et al., 2012). Given the progressive decline in lung function with age in CF, due to bronchiectasis and airway obstruction (Elborn, 2016), such pulmonary impairments may contribute towards elevated dead space ventilation in CF (Thin et al., 2004), thus impacting upon OUES. As this decline in lung function is observed with age (Harun et al., 2016), this may account for the discrepancy observed between the current research and previous OUES analyses in adults with CF (Gruet et al., 2010). Furthermore, given that the majority of patients in this study had mild-to-moderate CF (FEV_1 > 70 $\%_{Predicted}$ in 31/36 patients), it is unclear if the OUES will display a differing profile in patients with severe CF (FEV_1 < 40 $\%_{Predicted}$).

In conclusion, the OUES is not a valid submaximal surrogate of aerobic fitness in children and adolescents with CF. This research subsequently provides clinical teams with the clear evidence that only maximal markers of prognostic value (i.e. $\dot{V}O_{2max}$) should continue to be measured in patients with CF. Furthermore, continued research is required to identify submaximal variables that may hold clinical utility in this patient population when unable or unwilling to exercise to volitional exhaustion.

6 ANALYSIS OF OXYGEN UPTAKE EFFICIENCY PARAMETERS IN YOUNG PEOPLE WITH CYSTIC FIBROSIS

6.1 Abstract

Purpose:

This study characterised oxygen uptake efficiency (OUE) in children with mild-to-moderate cystic fibrosis (CF). Specifically, it investigated *1)* the utility of OUE parameters as potential submaximal surrogates of peak oxygen uptake ($\dot{V}O_{2peak}$), and *2)* the relationship between OUE and disease severity.

Methods:

Cardiopulmonary exercise test (CPET) data were collated from 72 children (36 CF, 36 age- and sex-matched controls [CON]), with OUE assessed as its highest 90-s average (plateau; OUEP), the gas exchange threshold (OUE_{GET}) and respiratory compensation point (OUE_{RCP}). Pearson's correlation coefficients, independent *t*-tests and factorial ANOVAs assessed differences between groups and the use of OUE measures as surrogates for $\dot{V}O_{2peak}$.

Results:

A significant ($p < 0.05$) reduction in allometrically scaled $\dot{V}O_{2peak}$ and all OUE parameters was found in CF. Significant ($p < 0.05$) correlations between measurements of OUE and allometrically scaled $\dot{V}O_{2peak}$, were observed in CF ($r = 0.49 – 0.52$) and CON ($r = 0.46 – 0.52$). Furthermore, measures of OUE were significantly ($p < 0.05$) correlated with pulmonary function ($FEV_{1\%Predicted}$) in CF ($r = 0.38 – 0.46$), but not CON ($r = -0.20 – 0.14$). OUEP was able to differentiate between different aerobic fitness tertiles in CON but not CF.

Conclusions:

OUE parameters were reduced in CF, but were not a suitable surrogate for $\dot{V}O_{2peak}$. Clinical teams should, where possible, continue to utilise maximal CPET

parameters to measure aerobic fitness in children and adolescents with CF. Future research should assess the prognostic utility of OUEP as it does appear sensitive to disease status and severity.

6.2 Introduction

It is well established that a high level of aerobic fitness, typically characterised by peak oxygen uptake ($\dot{V}O_{2peak}$), is of benefit for young people with cystic fibrosis (CF). A higher $\dot{V}O_{2peak}$ is associated with an improved quality of life (Hebestreit et al., 2014), reduced risk of hospitalisation for pulmonary exacerbations (Pérez et al., 2014) and reduced mortality risk (Nixon et al., 1992, Pianosi et al., 2005a). As a result, regular cardiopulmonary exercise testing (CPET) is recommended by the European CF Society and endorsed by the European Respiratory Society (Hebestreit et al., 2015), to monitor changes in aerobic fitness and guide decisions concerning clinical status and therapeutic interventions.

CPET is considered the gold standard method to assess aerobic fitness, with assessment of $\dot{V}O_{2peak}$ requiring the individual to provide a maximal physical effort. Factors such as excessive dyspnoea and/or a lack of motivation may cause individuals with CF to be unwilling or unable to reach volitional exhaustion and thus $\dot{V}O_{2peak}$. It has, therefore, been proposed that submaximal markers of aerobic fitness should be investigated as viable alternatives that can provide clinically useful information in such circumstances (Williams et al., 2014).

Previous research has shown the oxygen uptake efficiency slope (OUES) (Baba et al., 1996) to be a potentially useful submaximal parameter of aerobic fitness due to its high correlation with $\dot{V}O_{2peak}$ in clinical populations, including adults with CF (Gruet et al., 2010). However, there are several issues that preclude the use of OUES as an alternative marker of aerobic fitness in CF. Firstly, OUES is dependent on body size and requires allometric scaling to normalise data

(Tomlinson et al., 2017) – a process that may be time consuming in clinical practice. Secondly, the OUES has a high level of variability (as measured by coefficients of variation [CV]), both between participants, and in terms of test-retest reproducibility in healthy adults (Sun et al., 2012b) and children (Bongers et al., 2015a). Finally, the OUES is unable to discriminate aerobic fitness within children and adolescents with mild-to-moderate CF (Williams et al., 2018).

The utility of other submaximal CPET parameters in children with CF, such as oxygen uptake efficiency (OUE) – the ratio between oxygen uptake ($\dot{V}O_2$) and ventilation ($\dot{V}_E$) ($\dot{V}O_2/\dot{V}_E$ (Sun et al., 2012b)), therefore warrants consideration. Unlike the OUES, which utilises a log-transformation of $\dot{V}_E$ (Baba et al., 1996) to linearise the non-linear ventilatory profile often observed during incremental exercise, the OUE parameter accommodates this curvilinear relationship between $\dot{V}_E$ and $\dot{V}O_2$ (Bongers et al., 2015a). Furthermore, OUE has been shown to have less variability (CV) than OUES within groups of adults (39.5% vs. 14.6%) (Sun et al., 2012b) and children (32.9% vs. 10.9%) (Bongers et al., 2015a) and is not dependent on body size (Sun et al., 2012b). This independence of body size therefore removes potential bias due to growth and the subsequent need to scale data, which may be of further benefit in a clinical setting.

Practically, OUE can be measured at any point during an incremental exercise test. However the highest 90 second (s) plateau (oxygen uptake efficiency plateau; OUEP), which typically occurs prior to, or at, the ventilatory threshold (VT) (Bongers et al., 2015a) or gas exchange threshold (GET) (Sun et al., 2012b), has been shown to be a predictor of mortality in heart failure (Sun et al., 2012a). Despite demonstrated clinical utility in cardiac populations, its role in chronic respiratory disease remains unknown. Furthermore, given that the ratio of $\dot{V}_E$ to $\dot{V}O_2$ (ventilatory equivalent for oxygen) at peak exercise has been shown to be a

more significant predictor of mortality in children and adolescents with CF than body-mass relative $\dot{V}O_{2peak}$ (Hulzebos et al., 2014), it is clear that the relationship between $\dot{V}_E$ and $\dot{V}O_2$ is of clinical significance, and warrants further investigation, particularly when it is not feasible nor possible to assess $\dot{V}O_{2peak}$, e.g., due to pathophysiological or motivational reasons. Therefore, the OUE (and in particular the OUEP) has the potential to be considered submaximal measures of aerobic fitness that could be used to quantify pathophysiological and/or therapeutically-induced changes. However, evidence for this utilisation of OUE is required, with recent research calling for further investigation into the prognostic properties of other OUE parameters in children and adolescents with chronic health conditions, such as CF (Bongers et al., 2015a).

Therefore, the aim of this study was to explore the utility of OUE parameters, in children and adolescents with mild-to-moderate CF, as potential submaximal surrogates for $\dot{V}O_{2peak}$. This is conducted firstly by characterising the OUE responses during CPET in children and adolescents with mild-to-moderate CF, compared with age- and sex-matched controls. Secondly, by assessing the utility of OUE as an objective, submaximal surrogate for $\dot{V}O_{2peak}$ in this population. Thirdly, identifying the relationship between OUE parameters and disease status and severity in individuals with CF.

6.3 Methods

6.3.1 Participants

Data from 72 children and adolescents (36 with mild-to-moderate CF and 36 age- and sex-matched CON; 21 males per group; mean age 13.3 ± 2.8 years) were included in this study. Participant characteristics are presented in Table 6.1.

6.3.2 Ethics approval

This study was a retrospective analysis of existing data, and therefore did not require additional ethics approval. Ethics approval for original data collected was approved by the South West NHS Research Ethics Committee and the University of Exeter Sport and Health Sciences Ethics Committee. Fully informed written consent and assent were obtained from parents/guardians and paediatric participants, respectively.

6.3.3 Anthropometric variables

Stature was measured to the nearest 0.1 cm using a stadiometer (Holtain Ltd., Crymych, UK) and body mass to the nearest 0.1 kg using digital scales (Seca, Birmingham, UK). Body mass index (BMI) was subsequently calculated, and body surface area (BSA) was estimated using the Haycock equation (Haycock et al., 1978).

6.3.4 Pulmonary function

Pulmonary function was assessed using flow-volume loop spirometry, with the maximal values from three acceptable manoeuvres for forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) expressed relative to normative reference values from the Global Lung Function Initiative (Quanjer et al., 2012). Maximal voluntary ventilation (MVV) was calculated by multiplying FEV_1 (L) by 35 (Wasserman et al., 2005).

6.3.5 Exercise variables

All participants undertook a CPET to volitional exhaustion on an electronically braked cycle ergometer (Lode, the Netherlands) to determine maximal and submaximal measures of aerobic fitness. Breath-by-breath changes in pulmonary gas exchange and ventilation were measured, and subsequently averaged to 10

s time intervals. Of the 72 participants within the study, 33 children (20 CF, 13 CON) undertook a previously described supramaximal verification bout to determine a 'true' $\dot{V}O_{2max}$ (Barker et al., 2011, Saynor et al., 2013a). However, as not all participants underwent this verification testing, the highest $\dot{V}O_2$ obtained during the course of testing procedures is referred to as '$\dot{V}O_{2peak}$'. Following determination of $\dot{V}O_{2peak}$, the GET and respiratory compensation point (RCP) were independently verified by two researchers using methods described by Beaver et al. (1986) – the disproportionate increases in $\dot{V}CO_2$ relative to $\dot{V}O_2$ (i.e. V-slope method for GET) and $\dot{V}_E$ relative to $\dot{V}CO_2$ for RCP. This process is reliable in children with CF (CV = 11.2%, Saynor et al. (2013b)), and those without CF (CV = 7.5%, Fawkner et al. (2002)). $\dot{V}O_{2peak}$ was compared to normative reference values, chosen due their similar participant characteristics and methodology, whilst also accounting for age and sex (Bongers et al., 2014a), and split into aerobic fitness tertiles (a division shown to predict mortality in CF (Pianosi et al., 2005a)) for each group. Reliability of all gaseous exchange variables for children and adolescents with CF (Saynor et al., 2013b), and without CF (Bongers et al., 2015a, Welsman et al., 2005), have previously been reported. OUE values were calculated in line with previous work (Sun et al., 2012b), and were obtained by averaging $\dot{V}O_2/\dot{V}_E$ in the 60 s prior to the GET (OUE_{GET}) and RCP (OUE_{RCP}). The OUEP was taken as the highest 90 s $\dot{V}O_2/\dot{V}_E$ average. Warm-up and cool-down data during exercise were omitted from data analysis in order to isolate the incremental profile of the CPET. OUEP was also compared to normative values (Bongers et al., 2015a, Bongers et al., 2014a).

6.3.6 Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics v23 (IBM Corp., Armonk NY, USA). Allometric scaling was utilised to remove the influence of body

mass from $\dot{V}O_{2peak}$ in both CF and CON groups (Welsman et al., 1996). Scaling of OUE variables was not required as there were no significant relationships with body size, thereby indicating size-independence, as previously reported in adults (Sun et al., 2012b).

Pearson's correlation coefficients determined relationships between all OUE parameters and $\dot{V}O_{2peak,}$ as well as, the traditional clinical marker of disease severity, FEV_1 (expressed as a percentage of predicted). Independent samples *t*-tests established mean differences in anthropometric, pulmonary function and CPET parameters between groups. Factorial analyses of variance (ANOVAs) were used to establish interaction effects between disease status and aerobic fitness tertiles (as described in '*Exercise Variables*') upon $\dot{V}O_{2peak}$ and OUE parameters. For ANOVAs, the tertiles for $\dot{V}O_{2peak}$ to which participants were categorised (i.e., high, middle, low) remained the same throughout all ANOVAs, regardless of OUE value. Where significant effects occurred, planned pairwise comparisons with a Sidak correction factor were applied, chosen for its correction of multiple comparisons (reducing Type 1 error), whilst simultaneously being less conservative than Bonferroni corrections (thus reducing Type 2 error) (Abdi, 2007). Statistical significance was set at an alpha level of 0.05, and effect sizes (*ES*) for mean comparisons were described using Cohen's thresholds (small = 0.2, medium = 0.5, large = 0.8) (Cohen, 1992).

6.4 Results

6.4.1 Differences in OUE between groups

All OUE outcomes were detected in 68/72 participants (94%). Both OUE_{GET} and OUE_{RCP} were identified in 35/36 (97%) of children and adolescents in the CF group. In the CON group, OUE_{GET} was detected in all participants (36/36, 100%),

and OUE_{RCP} was detected in 34/36 (94%) of participants. The profile of OUEP, OUE_{GET} and OUE_{RCP} during a CPET for a representative individual with CF are shown in Figure 6.1. A representative comparison of OUEP for one participant with CF against CON is shown in Figure 6.2.

Differences between groups were observed for pulmonary function, absolute GET, OUE_{GET}, OUE_{RCP} and OUEP, with CON being significantly higher than CF. No significant difference was observed between CF and CON groups for $\dot{V}O_{2peak}$, when expressed as an absolute value. However, the CON group was revealed to have a significantly ($p < 0.05$) greater $\dot{V}O_{2peak}$ when allometric scaling had removed residual effects of body size (Table 6.1). Individual differences between age- and sex-matched pairs for $\dot{V}O_{2peak}$ and OUEP are displayed in Figure 6.3.

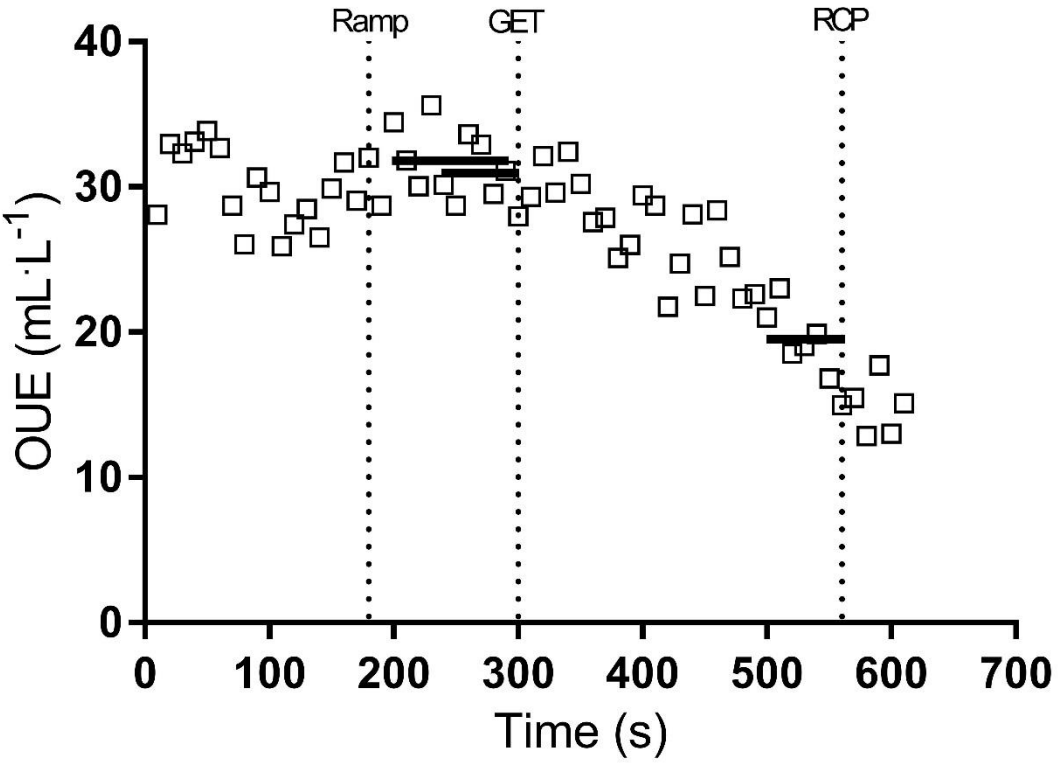


Figure 6.1 Profiles of OUEP, OUE_{GET} and OUE_{RCP} in a representative CPET from an individual child with CF (female, 12 years, homozygous ΔF508, FEV_1 82.0 $\%_{Predicted}$, $\dot{V}O_{2peak}$ 37 $mL \cdot kg^{-1} \cdot min^{-1}$, 73 $mL \cdot kg^{-0.86} \cdot min^{-1}$). Vertical line at 180 s indicates end of warm-up, and beginning of ramp phase. Vertical lines also indicate point of GET and RCP. Horizontal lines between 200 – 290 s = OUEP (31.9 $mL \cdot L^{-1}$), 240 – 300 seconds = OUE_{GET} (31.0 $mL \cdot L^{-1}$), 500 – 560 seconds = OUE_{RCP} (19.7 $mL \cdot L^{-1}$).

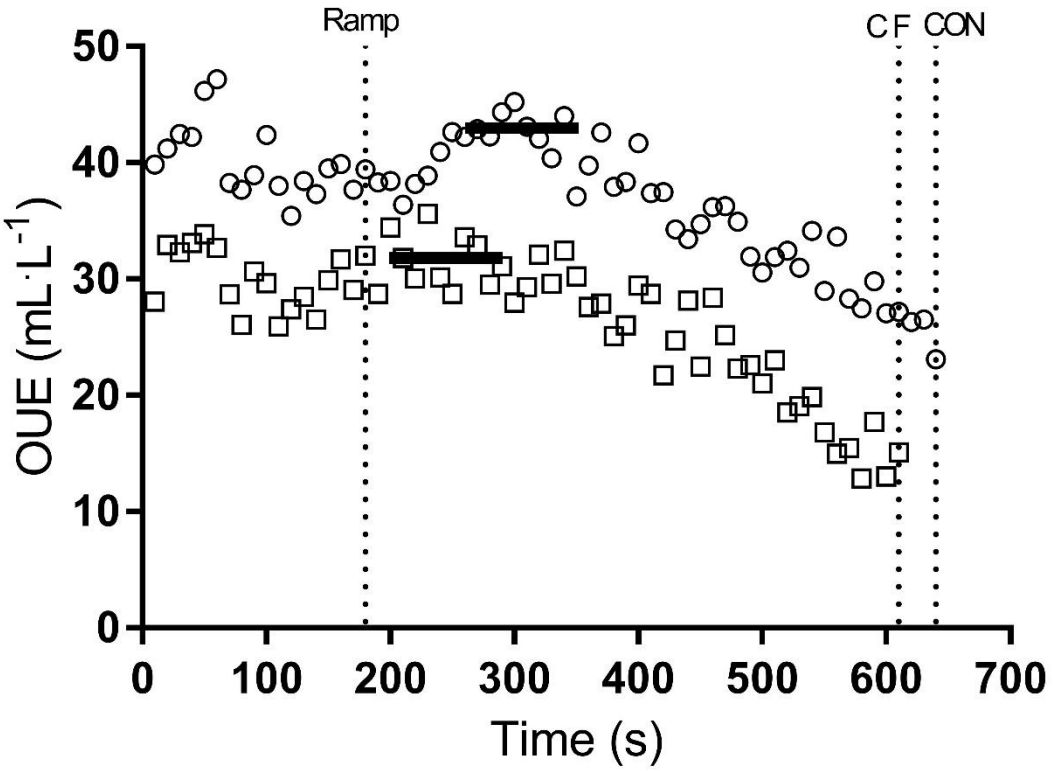


Figure 6.2 Differences in OUE ($\dot{V}O_2/\dot{V}_E$) between two representative children, CF (□) and CON (○), throughout a ramp incremental CPET. Vertical line at 180 s indicates the end of the warm-up and beginning of ramp phase of the test. Vertical lines at 610 s and 640 s indicate exhaustion for CF, and CON participant respectively. Solid horizontal lines at 31.9 $mL \cdot L^{-1}$ (CF) and 43.0 $mL \cdot L^{-1}$ (CON) indicate OUEP (highest 90 s average) for each group, respectively.

Table 6.1. Anthropometric, pulmonary function and exercise-related differences between CF and CON groups.

Variable	CF	CON	*p*-value	*ES*
Age (years)	13.4 (2.7)	13.2 (2.9)	0.77	0.08
Stature (cm)	155.6 (13.5)	159.1 (15.2)	0.32	0.24
Body mass (kg)	50.2 (15.5)	51.2 (14.5)	0.78	0.07
BMI ($kg \cdot m^{-2}$)	20.28 (3.67)	19.91 (4.18)	0.70	0.09
BSA (m^2)	1.46 (0.28)	1.49 (0.28)	0.65	0.11
FEV_1 (L)*	2.46 (0.97)	2.96 (0.86)	0.07	0.55
FEV_1 ($\%_{Predicted}$)*	85.0 (20.0)	97.5 (10.6)	**0.004**	0.71
FVC (L)*	3.10 (1.14)	3.44 (1.02)	0.30	0.31
FVC ($\%_{Predicted}$)*	92.7 (16.6)	98.6 (11.0)	0.18	0.39
MVV ($L \cdot min^{-1}$)*	86.2 (34.0)	103.6 (30.0)	0.07	0.53
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	1.74 (0.57)	2.03 (0.88)	0.09	0.39
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	38 (8)	40 (11)	0.32	0.23
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-0.86} \cdot min^{-1}$)	75 (15)	85 (24)	**0.031**	0.52
Relative $\dot{V}O_{2peak}$ ($\%_{Predicted}$)	83.3 (16.8)	87.8 (20.8)	0.32	0.24
GET ($L \cdot min^{-1}$)	0.91 (0.28)	1.12 (0.54)	**0.035**	0.49
GET (% $\dot{V}O_{2peak}$)	53.4 (9.3)	55.0 (8.0)	0.42	0.18
HR_{max} ($beats \cdot min^{-1}$)	182 (8)	185 (14)	0.30	0.26
$\dot{V}_{Emax}$ ($L \cdot min^{-1}$)	74.66 (35.62)	69.18 (33.45)	0.50	0.16
$\dot{V}_{Emax}$ (% MVV)*	88.3 (30.4)	60.9 (23.3)	**0.001**	0.97
OUEP ($mL \cdot L^{-1}$)	35.58 (5.40)	45.09 (5.78)	**<0.001**	1.70
OUE_{GET} ($mL \cdot L^{-1}$)	34.08 (5.40)	43.24 (5.08)	**<0.001**	1.75
OUE_{RCP} ($mL \cdot L^{-1}$)	29.49 (4.95)	35.15 (4.52)	**<0.001**	1.19
OUEP ($\%_{Predicted}$)	83.2 (13.9)	105.7 (13.0)	**<0.001**	1.68

Measures are presented as mean (± SD). Significant mean differences are denoted by a bold *p*-value. * Unequal groups for pulmonary variables: CF = 36, CON = 18. BMI, body mass index; BSA, body surface area; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MVV, maximal voluntary ventilation; $\dot{V}O_2$, volume of oxygen uptake; GET, gas exchange threshold; HR, heart rate; $\dot{V}_E$, minute ventilation; RER, respiratory exchange ratio; OUEP, oxygen uptake efficiency plateau; OUE_{GET}, oxygen uptake efficiency at the gas exchange threshold; OUE_{RCP}, oxygen uptake efficiency at the respiratory compensation point; *ES*, effect size.

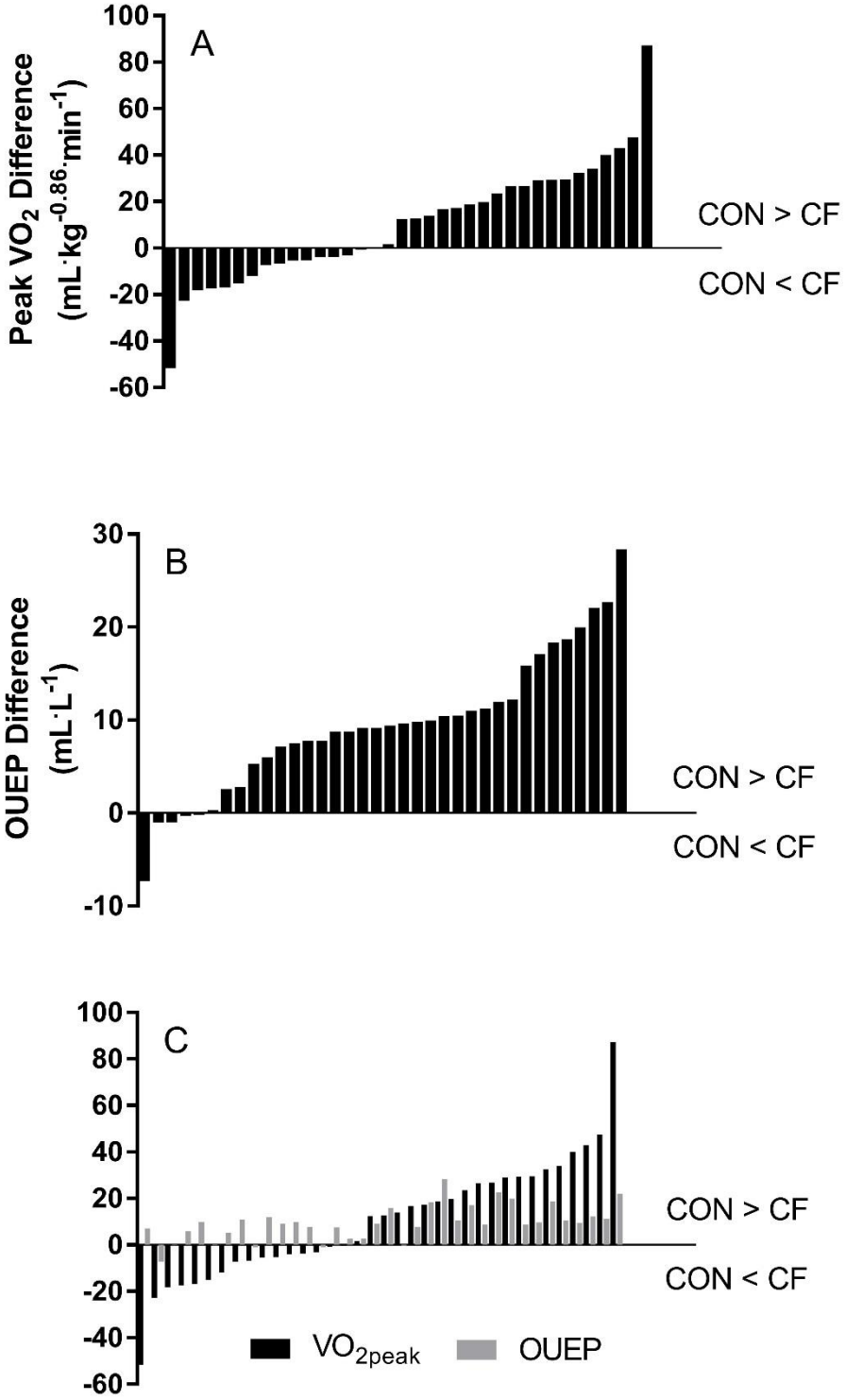


Figure 6.3 Individual differences between age- and sex-matched CON and CF pairs for CPET derived variables. All plots are calculated as CON minus CF, i.e. bars underneath $y = 0$ on x-axis indicate participant with CF has a greater value than CON counterpart. *A*: Differences in allometrically scaled $\dot{V}O_{2peak}$ between pairs. *B*: Differences in OUEP between pairs, independent of differences in $\dot{V}O_{2peak}$. *C*: Differences in $\dot{V}O_{2peak}$ ($mL \cdot kg^{-0.86} \cdot min^{-1}$) between pairs, plotted alongside within-pair differences in OUEP ($mL \cdot L^{-1}$). Black bars represent $\dot{V}O_{2peak}$, and grey bars indicate OUEP.

6.4.2 Correlation of with OUE with $\dot{V}O_{2peak}$

OUEP and OUE_{GET} were significantly and positively correlated with absolute $\dot{V}O_{2peak}$ in the CF and CON groups, however OUE_{RCP} was not correlated with absolute $\dot{V}O_{2peak}$ in either CF or CON groups. OUEP and OUE_{GET}, but not OUE_{RCP}, were correlated with allometrically scaled $\dot{V}O_{2peak}$ in both CF and CON (Table 6.2).

Table 6.2. Correlations between OUE parameters and $\dot{V}O_{2peak}$, and FEV_1.

	CF	CON	Combined
Absolute $\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)			
OUE_{GET}	**0.36 (0.036)**	**0.40 (0.017)**	**0.41 (<0.001)**
OUE_{RCP}	0.12 (0.50)	0.29 (0.09)	**0.28 (0.022)**
OUEP	**0.43 (0.010)**	**0.42 (0.010)**	**0.44 (<0.001)**
OUEP ($\%_{Predicted}$)	0.22 (0.20)	0.12 (0.51)	**0.24 (0.040)**
Allometrically Scaled $\dot{V}O_{2peak}$ ($mL \cdot kg^{-0.86} \cdot min^{-1}$)			
OUE_{GET}	**0.49 (0.003)**	**0.46 (0.005)**	**0.51 (<0.001)**
OUE_{RCP}	0.31 (0.08)	0.24 (0.17)	**0.35 (0.003)**
OUEP	**0.52 (0.001)**	**0.52 (0.002)**	**0.54 (<0.001)**
OUEP ($\%_{Predicted}$)	**0.49 (0.003)**	**0.38 (0.021)**	**0.47 (<0.001)**
*FEV_1 ($\%_{Predicted}$)**			
OUE_{GET}	**0.38 (0.026)**	-0.06 (0.83)	**0.44 (0.001)**
OUE_{RCP}	0.07 (0.68)	0.14 (0.61)	0.24 (0.08)
OUEP	**0.43 (0.010)**	-0.20 (0.43)	**0.43 (0.001)**
OUEP ($\%_{Predicted}$)	**0.46 (0.005)**	-0.19 (0.45)	**0.44 (0.001)**

Values are presented as correlation coefficients (*r*) with *p*-values in parentheses. Bold text indicates a significant ($p < 0.05$) coefficient. $\dot{V}O_2$, oxygen uptake; FEV_1, forced expiratory volume in 1 s. * Unequal samples for pulmonary variables: CF = 36, CON = 18.

6.4.3 Differences between aerobic fitness groups

When the data were split by tertiles according to allometrically scaled $\dot{V}O_{2peak}$, a significant difference in aerobic fitness was observed between tertiles within both CF (high: 91 ± 8 vs. mid: 75 ± 5 vs. low: 58 ± 8 $mL \cdot kg^{-0.86} \cdot min^{-1}$) and CON (110 ± 16 vs. 86 ± 7 vs. 62 ± 13 $mL \cdot kg^{-0.86} \cdot min^{-1}$) groups ($p < 0.05$ for all pairwise comparisons, ES = 1.91 – 4.13). However, when comparisons were made between groups, a significant difference in allometrically scaled $\dot{V}O_{2peak}$ between CF and CON was only evident in the high ($p < 0.001$, ES = 1.47) and middle (p = 0.50, ES = 1.85) aerobic fitness tertiles, not for the lowest (p = 0.39, ES = 0.38).

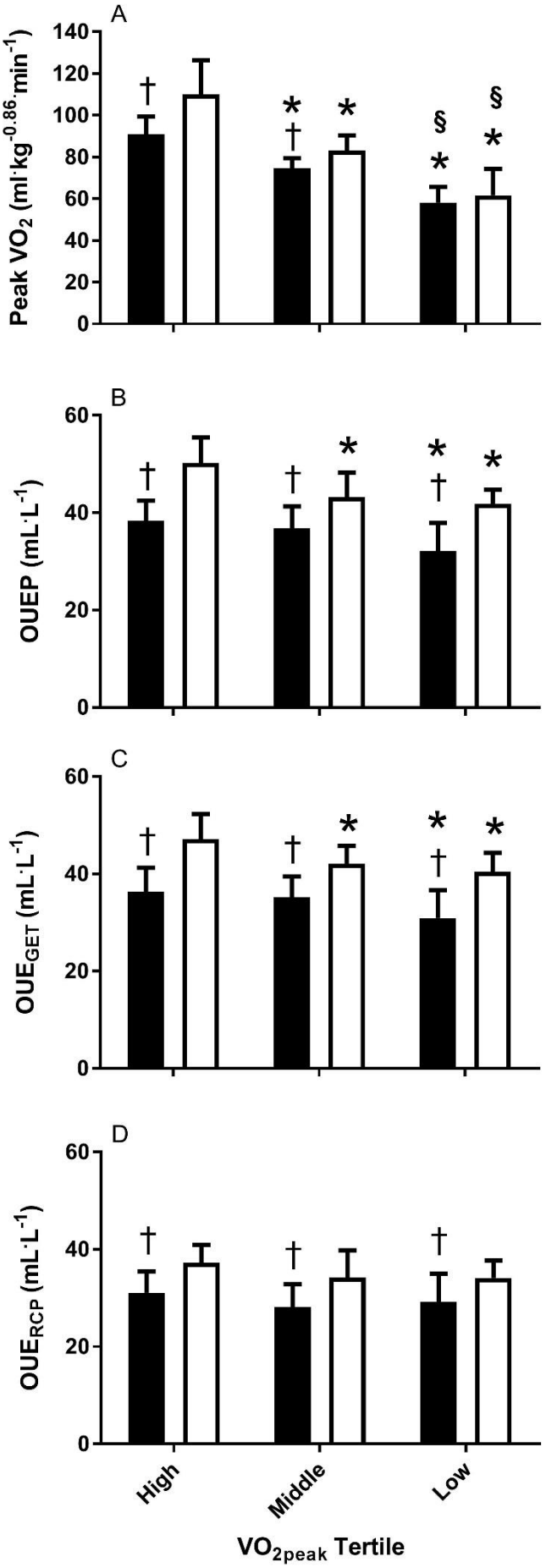
When assessing OUEP by fitness tertile and disease group, significant main effects were seen for group ($p < 0.001$) and fitness tertile ($p < 0.001$), but no significant fitness tertile by group interactions were evident (p = 0.20; Figure 6.4). Pairwise comparisons identified mean differences between CF and CON for OUEP at each level of fitness respectively (high; 38.32 ± 4.21 vs. 50.26 ± 5.22, $p < 0.001$, ES = 2.52, middle; 36.22 ± 4.57 vs. 43.19 ± 5.06, p = 0.001, ES = 1.45, low; 32.19 ± 5.73 vs. 41.81 ± 2.93, $p < 0.001$, ES = 2.11).

Mean differences for OUEP were found between the highest and lowest between aerobic fitness tertiles within CF (p = 0.006, ES = 1.22), but not between high and middle (p = 0.62, ES = 0.48) nor middle and low (p = 0.11, ES = 0.78). Further pairwise comparisons revealed differences within the CON group, as the tertile with the highest aerobic fitness had a significantly higher OUEP than the middle (p = 0.001, ES = 1.38) and lowest ($p < 0.001$, ES = 2.00) tertiles. No significant difference was evident between the middle and lowest tertiles with regards to OUEP for CON (p = 0.85, ES = 0.33; Figure 6.4).

When ANOVAs were repeated for OUE_{GET} and OUE_{RCP}, significant main effects for group were found ($p < 0.001$) for both parameters. Further significance ($p < 0.05$) between groups was identified when split by fitness tertile. A main effect for fitness tertile was present for OUE_{GET} ($p < 0.001$), but not OUE_{RCP} ($p = 0.08$). Main interaction effects between group and aerobic fitness were not present for either OUE_{GET} ($p = 0.34$) or OUE_{RCP} ($p = 0.88$; Figure 6.4). However, for OUE_{GET}, pairwise comparisons revealed significant differences within CF between high and low fitness tertiles ($p = 0.021$, $ES = 1.01$). For CON, differences were found between high and low ($p = 0.002$, $ES = 1.48$), and high and middle fitness tertiles ($p = 0.026$, $ES = 1.15$). For OUE_{RCP}, no differences between tertiles within groups ($p = 0.37 – 1.00$, $ES = 0.01 – 0.86$). In addition, pairwise comparisons revealed significant differences ($p < 0.05$) between groups at each tertile for both OUE_{GET} and OUE_{RCP} (Figure 6.4).

6.4.4 Relationship with disease severity (FEV_1)

OUEP and OUE_{GET} were significantly correlated with FEV_1 within the CF group, but OUE_{RCP} was not. None of the OUE parameters were significantly correlated with FEV_1 within CON (Table 6.2). Allometrically scaled $\dot{V}O_{2peak}$ was significantly correlated with FEV_1 ($\%_{Predicted}$) in CF ($r = 0.46$, $p = 0.004$), but not CON ($r = -0.20$, $p = 0.43$).


A
Peak VO2 (ml·kg-0.86·min-1)
140
120
100
80
60
40
20
0
†
*
†
*
*
§
*
§
B
OUEP (mL·L-1)
60
40
20
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OUEGET (mL·L-1)
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OUERCP (mL·L-1)
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High
Middle
Low
VO2peak Tertile

Figure 6.4 (overleaf) Comparison of $\dot{V}O_{2peak}$ (*A*) and OUE parameters (*B*: OUEP; *C*: OUE_{GET}; *D*: OUE_{RCP}) between CF (black) and CON (white), split by $\dot{V}O_{2peak}$ tertile. * = Significant ($p < 0.05$) difference from highest tertile (within group). § = Significant ($p < 0.05$) difference from middle tertile (within group). † = Significant ($p < 0.05$) difference between groups (within tertile).

6.5 Discussion

In this study, whilst all OUE parameters were significantly reduced in children and adolescents with CF in the current study, results show that OUE does not provide a viable surrogate for $\dot{V}O_{2peak}$ in this group. However, the novel finding of this study is that OUE appears to hold clinical utility as an independent marker of aerobic fitness, since it can differentiate between CF and CON, and holds a significant relationship with disease severity (as shown by FEV_1) in the CF group. An example is shown in Figure 6.3, whereby allometrically scaled $\dot{V}O_{2peak}$ was greater in individuals with CF in 16/36 (44 %) age- and sex-matched pairs, but OUEP was only greater in individuals with CF in 5/36 (14 %) matched pairs (and $\dot{V}O_{2peak}$ and OUEP were only greater in CF in 4/36 (11%) of cases), thus indicating reduced OUE in CF, regardless of fitness status. This is further corroborated by the significant relationship between OUE (OUEP, OUE_{GET}) and FEV_1 ($\%_{Predicted}$) within the CF cohort, showing that OUE is associated with traditional clinical markers of disease severity.

For individuals with CF, a reduced aerobic fitness is a hallmark of disease progression (Orenstein and Higgins, 2005) and assessment of $\dot{V}O_{2peak}$ is therefore recommended on at least an annual basis (Hebestreit et al., 2015). However, as maximal testing may not always be possible in this patient group (due to pathophysiological and/or motivation related factors), viable submaximal measures are needed to assess aerobic fitness. Whilst submaximal physiological thresholds such as the GET are related to disease severity (Thin et al., 2002),

detection rates are variable in CF (12/13; 92% (Saynor et al., 2013b)), and non-CF (45/55; 82% (Hebestreit et al., 2000) groups and are typically dependent on knowledge of $\dot{V}O_{2peak}$ in order to be expressed as a percentage of maximal capacity. In the present study, all OUE values were identified in the majority (94%) of participants, with OUEP identified in 100% of participants. The identification of OUEP is related to the averaging of 90-s of data and is not dependent on prior detection of the GET or RCP (to produce OUE_{GET} and OUE_{RCP}). The OUEP occurs at a submaximal point near the VT (Bongers et al., 2015a) and/or GET (Sun et al., 2012b), a threshold that reportedly occurs at 50-60% of $\dot{V}O_{2peak}$ in children and adolescents with CF (Bongers et al., 2014b, Saynor et al., 2014b, Saynor et al., 2016b). Therefore, the exercise intensity required to generate a value for OUEP should be feasible for most children to achieve despite being unable or unwilling to exercise to exhaustion, such as those with advanced pulmonary disease, or more prone to increased levels of dyspnoea and desaturation upon exertion. The simplicity of the OUEP measure highlights how feasible a measure it may be to implement in busy clinical environments, suiting patients, researchers and clinicians alike.

In the current study, OUE variables were significantly correlated with $\dot{V}O_{2peak}$ in the CON and CF groups, indicating the two variables have a medium (as defined by Cohen (1992)) relationship (R^2 = 27% between OUEP and allometrically scaled $\dot{V}O_{2peak}$ in both CF and CON). Given previous research (Williams et al., 2018) has identified differences in $\dot{V}O_{2peak}$ within, and between, CF and CON groups when split by aerobic fitness tertile, a division shown to predict for mortality (Pianosi et al., 2005a), it would therefore be anticipated that parameters of OUE would follow a similar pattern in discriminating between individuals' of differing aerobic fitness statuses. Differences are seen within the CON group for

OUEP, with the highest fitness tertile having significantly greater OUEP relative to children in the middle and lowest fitness tertiles. Thus, showing OUEP can discriminate between individuals on different fitness status. However, the same discriminatory ability is not seen for the CF group as it is only the group with the lowest aerobic fitness that is different to the group with the highest fitness (Figure 6.4). Therefore, despite a relationship with $\dot{V}O_{2peak}$, the inability to discriminate between the fitness groups shows that the OUEP cannot act as a surrogate for $\dot{V}O_{2peak}$.

Of the limited research to have characterised the OUEP in youth, a large cross-sectional study of 214 healthy Dutch children identified similar mean values for OUEP (boys, 42.6 ± 4.7; girls, 42.3 ± 4.6 $mL \cdot L^{-1}$) and OUE at the VT (boys, 42.0 ± 4.6; girls, 41.9 ± 4.7 $mL \cdot L^{-1}$) to those of the CON group in the current study (Bongers et al., 2015a). They also identified a stronger relationship ($r = 0.65$, $p < 0.01$) between the OUEP and absolute $\dot{V}O_{2peak}$ than the CON group in the current study, potentially due to the higher $\dot{V}_{Emax}$ observed in both boys and girls (80 ± 25; 71 ± 21 $L \cdot min^{-1}$ respectively) relative to the current CON group (69.2 ± 33.5 $L \cdot min^{-1}$), which may therefore bias the relationship between $\dot{V}O_2$ and $\dot{V}O_2/\dot{V}_E$ (OUE). However, as the current study builds upon this previous work and is the first to comprehensively examine OUE at multiple metabolic thresholds in children and adolescents with CF, only limited comparisons can be made, as no previous research has provided values against which to compare our novel data. Furthermore, the only application of OUE in clinical groups has been in adults with heart failure (Sun et al., 2012a), pulmonary hypertension (Tan et al., 2014), chronic obstructive pulomary disease (Barron et al., 2016) and pulmonary embolism (Guo et al., 2016). However, minimal comparisons and inferences can

be made against children with CF and these adult-onset, and predominantly vascular conditions.

As the current study has shown that OUEP (nor any OUE parameter) is not able to act as a surrogate measure of aerobic fitness, alternative submaximal factors must be considered. Ventilatory drive ($\dot{V}_E/\dot{V}CO_2$) has received recent attention in predictive models of mortality (Hulzebos et al., 2014), and may be a viable candidate, given its low variability compared to $\dot{V}_E/\dot{V}O_2$ (Sun et al., 2002) and superior prognostic value relative to OUES in patients with heart failure (Arena et al., 2007). As such, further research should continue to explore the potential utility of this variable in individuals with CF, either as an alternative for $\dot{V}O_{2peak}$, or an independent prognostic variable. However, it is unclear whether any parameter of OUE may be of use in individuals with a more severe form of CF, or have longitudinal relevance in mild-to-moderate CF, and therefore further research is warranted.

A number of limitations associated with the present study are worthy of comment. Primarily, this study is focused in children and adolescents with mild-to-moderate CF (FEV_1 > 40 $\%_{Predicted}$). However, defining severity on FEV_1 alone does not account for the nutritional measures, number of exacerbations, inflammatory markers and infection statuses that also contribute towards a patient profile and definition of severity. Consequently, these results may not be applicable to those with lower lung function, a cohort for whom FEV_1 has a greater influence upon $\dot{V}O_{2peak}$ (Pastre et al., 2014). Furthermore, the CON group in the current study display a reduced level of aerobic fitness relative to previous studies investigating OUE (Bongers et al., 2015a), which may explain the number of individuals with CF having a higher $\dot{V}O_{2peak}$ within age- and gender-matched pairs (Figure 6.3). In addition, the lack of all participants undertaking supramaximal verification

bouts (Barker et al., 2011) within CPETs could potentially influence detection of a 'true' $\dot{V}O_{2max}$ (hence our use of $\dot{V}O_{2peak}$). This is likely to have minimal effect, as previous work has shown that the ramp only test elicits a 'true' $\dot{V}O_{2max}$ in ~90% of healthy children (Barker et al., 2011) and ~80% of children with CF (Saynor et al., 2013a). Finally, when these methodological issues are considered in conjunction with our sample size, true effects may be obscured regarding the ability for OUEP to discriminate aerobic fitness. For example, the difference between middle- and low-fitness tertiles in CF revealed a *p*-value of 0.11, yet an *ES* of 0.78, thus indicating an effect is likely present but cannot be statistically confirmed. We have utilised the Sidak correction factor in this study as opposed to the more conservative Bonferroni in an attempt to alleviate the potential for Type 2 errors, yet statistical significance was not found in some comparisons and a statistical error might still have occurred. Larger clinical sample sizes would be advantageous but are not always feasible in young people who are sick.

6.6 Conclusion

The current study is the first to comprehensively characterise parameters of OUE in children and adolescents with mild-to-moderate CF, and assess the utility of such parameters as submaximal surrogates for $\dot{V}O_{2peak}$. Despite promising findings in other clinical populations, and a significant relationship between OUE and allometrically scaled $\dot{V}O_{2peak}$ in the present study, the inability to differentiate between aerobic fitness statuses indicates that OUE is unable to provide a viable surrogate for $\dot{V}O_{2peak}$ in this population. Further research is therefore warranted to identify suitable submaximal variables to characterise aerobic fitness status in children and adolescents with CF when determination of $\dot{V}O_{2peak}$ is not possible. Moreover, the prognostic utility of OUE in CF when $\dot{V}O_{2peak}$ cannot be determined also warrants investigation.

7 QUANTIFICATION OF THIGH MUSCLE VOLUME IN CHILDREN USING MAGNETIC RESONANCE IMAGING

7.1 Abstract

Quantifying muscle volume (MV) using magnetic resonance imaging (MRI) requires a time-consuming process of summating multiple cross-sectional area (CSA) slices. Estimation of MV using a reduced number of CSA slices introduces error that is known in adults but not in children, where body size can differ greatly due to growth and maturation. This study sought to identify error in estimating leg MV in children when using fewer CSA slices. Fifteen children (11 males, 14.8 ± 2.1 years) underwent MRI scans of the right thigh using a 1.5 T scanner. A criterion MV was determined by tracing around and summing all CSAs, with MV subsequently estimated using every second, third, fourth and fifth CSA slice. Bland-Altman plots displayed mean error and limits of agreement (LoA) between methods for calculating MV. CSA measures at 50% thigh length were also used to predict MV. Pearson's correlation coefficients determined relationships between error and measures of body size/composition. Criterion MV was positively correlated with estimated MVs (r = 1.0, p < 0.001 for all). LoA increased as CSA slice count decreased, to a maximum of ±2.0%. CSA at mid-thigh predicted MV (R^2 = 0.94, 0.53; SEE = 199, 570 cm^3). All body size/composition measures were correlated (r = -0.78 – 0.86, p < 0.05) with the error between criterion and estimated MV. It was concluded that MV can be accurately estimated using fewer CSA slices. However, the associated error must be considered when calculating MV in studies involving children and adolescents, as body size biases estimates.

7.2 Introduction

Accurate quantification and interpretation of muscle size is important in physiological studies, such as those measuring hypertrophy following training (Tracy et al., 1999), muscle atrophy following immobilisation (Wall et al., 2014) or aging (Ogawa et al., 2012), and examining the consequences of chronic disease (Godi et al., 2016).

To quantify muscle volume (MV), magnetic resonance imaging (MRI) is considered the preferred technique due to use of non-ionising radiation, while producing high resolution images (Narici et al., 1992), and consists of the measurement and summation of multiple sequential cross-sectional areas (CSA) (Barnouin et al., 2015, Tracy et al., 2003). As this is time consuming, studies have sought to identify the measurement error associated with increasing the distance between measured CSAs with the objective of reducing the number of CSAs required and the time taken for analysis (Barnouin et al., 2015, Tracy et al., 2003, Walton et al., 1997). However, as the number of CSA slices decreases, the associated error with estimated MV increases. For example, Tracy et al. (2003) reports the limits of agreement (LoA) increasing from ±0.7% to ±6.4% of total MV when 11 mm and 91 mm gaps between slices are used.

A further consideration is the direction in which CSA slices are sequentially summed to estimate MV. Previous studies have estimated MV from the knee, working towards the hip (i.e. distal to proximal [D-P]) (Nordez et al., 2009, Tracy et al., 2003), a process that may under-estimate thigh volume. However, no study has systematically evaluated whether the direction of measurement (i.e. D-P, or a proximal-to-distal [P-D] direction) has a bearing on final MV estimates.

To our knowledge, studies examining the errors associated with determining MV have only been undertaken in adults. Therefore, the measurement strategies

applied may not be suitable for groups involving children and adolescents. Compared to adults, children have a different body geometry (Feber and Krásničanová, 2012) and the process of maturation (timing and tempo of maturity stages) leads to children of equal chronological age, but different body size (Mirwald et al., 2002) and MV (Pitcher et al., 2012). These factors are likely to influence the error when determining MV using MRI, and warrant further investigation.

The recognition of measurement errors are vital in clinical populations. For example, Duchenne muscular dystrophy, where progressive MV decline can arise and has previously been quantified using MRI (Godi et al., 2016). Similarly, nutritional complications in cystic fibrosis (CF) lead to considerable variations in body-size (Culhane et al., 2013), and recent debate has queried whether a qualitative or quantitative muscular defect is predominantly responsible for impaired oxidative metabolism (Hulzebos et al., 2017, Rodriguez-Miguelez et al., 2017). Within CF, some studies have utilised only muscle CSA from a single slice (e.g. at 50% of limb length) to reflect muscle size, most likely due to wanting to minimise time and cost. However, whilst single site CSA is a poor surrogate for total MV in healthy adults (Morse et al., 2007), this has yet to be examined in clinical and non-clinical groups of children and adolescents.

The primary aim of this study was to identify the error associated with estimating MV from MRI using a differing number of CSA slices in two groups of children and adolescents; healthy controls (CON), and a group with CF. Secondary aims included: a) identifying the difference in estimated MV when employing a P-D or D-P approach to analysing CSA slices; b) identify the relationship between body size and the error in quantifying MV; and c) identify the utility of mid-thigh CSA to predict MV.

7.3 Methods

7.3.1 Study population

Fifteen children (8 CF [2 female, 6 male], 7 healthy controls [CON; 2 female, 5 male], 14.8 ± 2.1 years) volunteered for the study, with descriptive characteristics presented in Table 7.1. Children with CF were age- and sex-matched against non-CF controls. Children were recruited from a hospital CF clinic, local schools and sports clubs. Ethical approval was obtained from NHS Regional Ethics Committee (14/SW/0061), and children and parents/guardians provided written informed assent and consent respectively.

Table 7.1. Mean differences between CF and CON groups for anthropometric and MRI derived variables.

Variable	Combined (*n* = 15)	CF (*n* = 8)	CON (*n* = 7)	*p* Value	*ES*
Age (years)	14.8 (2.1)	15.10 (2.14)	14.43 (2.21)	0.57	0.31
Stature (m)	1.62 (0.11)	1.63 (0.11)	1.62 (0.11)	0.84	0.09
Sitting stature (m)	0.85 (0.56)	0.85 (0.06)	0.84 (0.06)	0.63	0.33
Leg length (m)	0.78 (0.52)	0.77 (0.05)	0.78 (0.06)	0.89	0.18
Body mass (kg)	57.2 (15.5)	61.7 (18.0)	52.1 (11.0)	0.25	0.63
Body fat (%)	17.8 (6.1)	18.6 (6.3)	16.7 (6.1)	0.61	0.31
FFM (kg)	46.5 (11.5)	49.3 (12.5)	43.3 (10.1)	0.33	0.52
Fat mass (kg)	10.8 (6.5)	12.4 (8.1)	8.8 (3.7)	0.30	0.56
Criterion MV (cm^3)	2778 (801)	2823 (763)	2726 (901)	0.83	0.12
CSA_M (cm^2)	59.67 (15.57)	62.75 (13.99)	56.15 (17.61)	0.43	0.42
CSA_T (cm^2)	93.17 (24.16)	98.86 (25.64)	86.66 (22.41)	0.35	0.50
CSA_M (% of CSA_T)	64.7 (10.1)	64.6 (11.4)	64.7 (9.5)	0.98	0.01

Data are presented as mean ± standard deviation. FFM, fat-free mass; MV, muscle volume; CSA_M, muscle cross-sectional of area at mid-thigh; CSA_T, cross-sectional area of muscle and subcutaneous fat at mid-thigh; *ES*, effect size.

7.3.2 Anthropometric measures

Stature, seated stature and leg length (i.e. stature – seated stature) were obtained using wall-mounted and seated stadiometers (Holtain, Crymych, Wales) to the nearest 0.1 cm. Body mass (BM) was measured to the nearest 0.1 kg (Seca, Birmingham, UK). Skinfold callipers and published equations (Slaughter et al., 1988) were used to estimate body fat percentage, which was used to determine fat mass and fat-free mass (FFM).

7.3.3 Estimation of volume

MV of the right thigh was determined using a 1.5 T superconducting whole-body scanner (Gyroscan Intera, Philips, the Netherlands), utilising a T1 weighted image sequence to obtain a series of transverse slices covering the whole upper leg with optimal fat/muscle signal contrast. Participants lay in the prone position within the scanner, with the hips and upper legs extended and secured to avoid unnecessary movement, but not to cause compression of muscle tissue.

Slices were acquired with 5 mm thickness and 0.5 mm slice gap, similar to previous research (Barnouin et al., 2015, Nordez et al., 2009). CSA was determined using Philips software, by manually tracing around the muscle within each slice. The CSA value for each individual slice, apart from the first and last was multiplied by 5.5 mm (5 mm slice thickness + 0.5 mm slice gap) to produce individual slice volumes. The slices at the distal and proximal ends were multiplied by 5.25 mm to reflect the absence of the 0.25 mm contribution from the adjacent slice gap. All individual volumes were then summed over all slices to calculate a criterion measure of MV. This method has been used previously (Lund et al., 2002, Walton et al., 1997) and is favourable in comparison to the truncated cone formula (Ross et al., 1996), which and has been shown to produce a higher level of error (Barnouin et al., 2015, Nordez et al., 2009).

In accordance with previous research (Tracy et al., 2003), estimated MV was calculated by increasing the interval between CSA slices using every second (MV2), third (MV3), fourth (MV4) and fifth (MV5) slice. For MV2 – MV5, each slice CSA, apart from for the first and last slice, was multiplied by 11, 16.5, 22 or 27.5 mm respectively, prior to summing over all slices to calculate MV. When the number of CSA slices covering the upper leg did not exactly fit the slice sampling frequency, a reduced number of slices were examined (Tracey et al. 2003). For example, where 30 CSA slices covered the leg, with the MV5 strategy, slices 1, 6, 11, 16, 21 and 26 were examined. Under such circumstances a new criterion measure was established to ensure the distance covered on the thigh remained equal between measures. In this example, CSA slices 1-26 were used to create a criterion measure specific to this individual and slicing strategy. This procedure was completed for both the D-P and P-D directions, and therefore for the described example, in the P-D direction slices 30, 25, 20, 15, 10 and 5 would be used to estimate MV5, and would be compared against further criterion measure calculated using each slice from 30-5.

In addition, the CSA of the slice obtained that lay nearest to 50% of the length of the measured femur (i.e. midpoint between femoral head and medial epicondyle) was assessed to determine the predictive ability of a single slice in estimating MV. The CSA of the whole thigh (muscle and subcutaneous fat; CSA_T) and muscle-only CSA (CSA_M) were recorded. Bone was excluded from all CSA slices.

CSA analyses were undertaken by two investigators, with a within-observer coefficient of variation (CV) < 1.5% and a between-investigator CV of 1.2%. The number of CSA slices required to cover the whole thigh ranged from 40 to 56, dependent on thigh length.

7.3.4 Statistical analyses

Pearson's correlation coefficients and paired samples t-tests were conducted to identify relationships and mean differences, respectively, between the criterion MV, and each of the MV estimates (i.e. MV2 to MV5). Bland-Altman plots (Bland and Altman, 1986) were produced to identify the mean bias between measures of MV (MV and MV2, MV3, MV4, MV5 for both D-P and P-D directions), and associated 95% LoA. Furthermore, Bland-Altman analyses were conducted between predicted MV from both CSA_T and CSA_M. The MV predicted from CSA_T and CSA_M was obtained using simple bi-linear regression. For each Bland-Altman plot, Pearson's correlations between means (*x*-axis) and differences (*y*-axis) were conducted to identify whether the size and direction of the error is associated with estimated MV itself.

To identify if the over- or under-estimation of MV is associated with anthropometric variables, the absolute difference between each MV estimate and the criterion MV was calculated and correlated against chronological and biological age, as well as body size parameters (stature, leg length, body mass, FFM, fat mass) using Pearson's correlation coefficients. All statistical analyses were performed using SPSS v.23 (IBM Corp., Armonk, NY, USA), with statistical significance taken at an alpha value of 0.05. Effect sizes (*ES*) were described for mean comparisons (small = 0.2, medium = 0.5, large = 0.8) and correlation coefficients (small = 0.1, medium = 0.3, large = 0.5) (Cohen, 1992).

7.4 Results

No differences were observed between children with, and without, CF for any anthropometric, MV or CSA variables ($p > 0.05$, Table 7.1). Therefore, all variables are pooled (i.e. $n = 15$) for subsequent analyses.

Mean values (with associated LoA) for the difference from each respective criterion MV for all estimation methods and directions are displayed in Figure 7.1. The mean criterion MV was significantly greater than each estimated volume using the D-P slicing direction, and significantly lower than each estimated volume for the P-D direction (all $p < 0.001$, $ES = 0.03 – 0.12$). All estimated MV variables were significantly, and positively correlated with their respective criterion MV (all $r = 1.0$, all $p < 0.001$). Furthermore, the mean bias and LoA associated with each estimation method increased as the interval between slices increases (see Figure 7.1 using Bland-Altman plots). When using the D-P direction, a significant and positive correlation was evident between the mean difference between criterion and estimated MV, and the respective means for MV3 ($r = 0.77$, $p = 0.001$), MV4 ($r = 0.78$, $p = 0.001$) and MV5 ($r = 0.82$, $p < 0.001$), but not MV2 ($r = 0.29$, $p = 0.30$). For P-D, significant and negative correlations are observed between the means and differences of criterion and estimated MV for all slicing strategies (MV2, $r = -0.69$, $p = 0.004$; MV3, $r = -0.83$, $p < 0.001$; MV4, $r = 0.83$, $p < 0.001$; MV5, $r = -0.70$, $p = 0.004$).

Both CSA_M and CSA_T were significant predictors of MV (Figure 7.2). When each predictive equation was used to estimate MV, mean bias was equal to zero for both CSA parameters, with LoA for CSA_M (384 cm^3, 13.8%) being smaller than CSA_T (1099 cm^3, 39.6%; Figure 7.1). The correlations between the mean and difference of the criterion and estimated MV for CSA_M ($r = 0.12$, $p = 0.66$) and CSA_T ($r = 0.43$, $p = 0.13$) were positive, but not statistically significant.

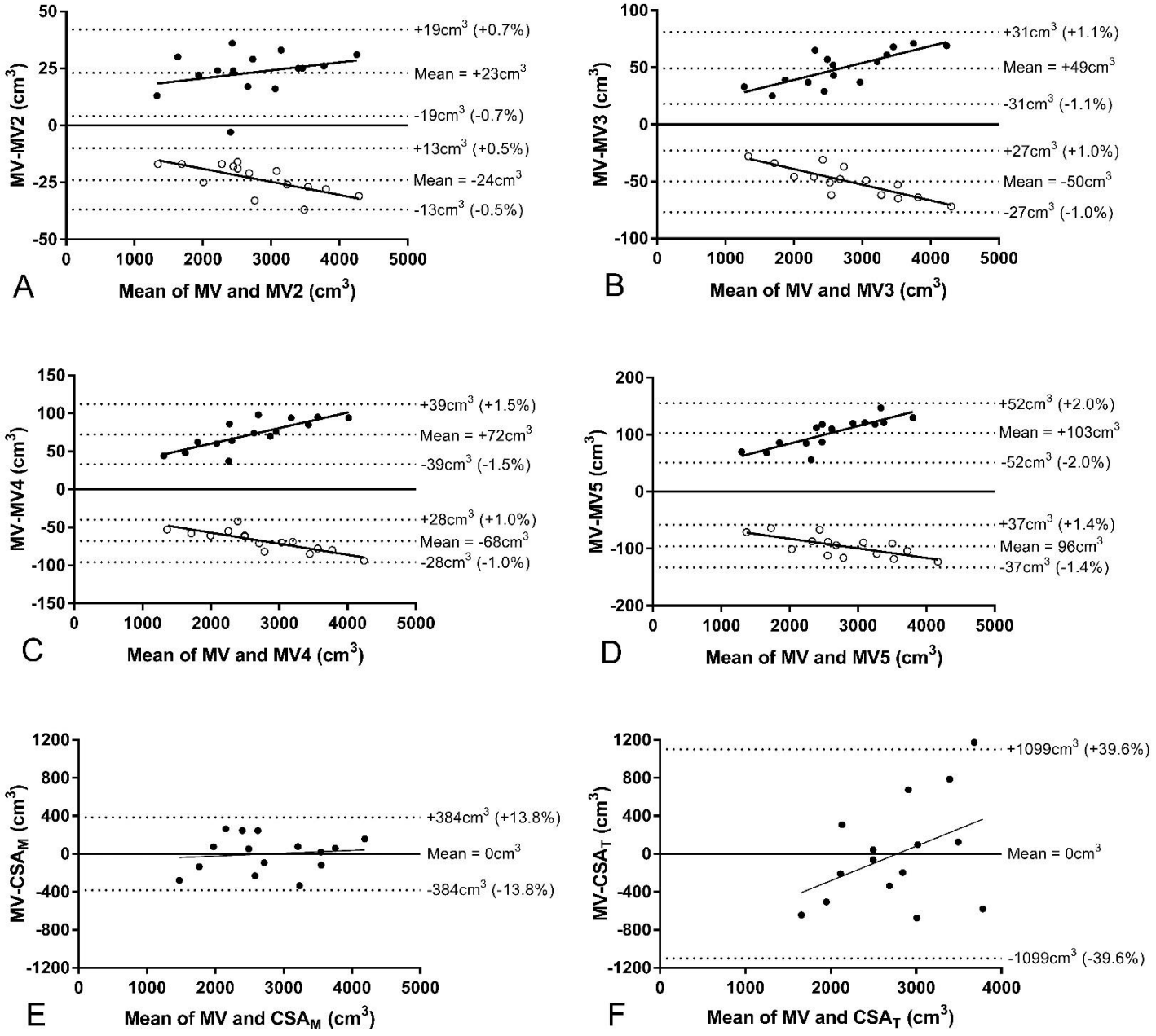

Figure 7.1 Bland-Altman plots identifying relationships between the differences between (*y*–axis) and mean of (*x*–axis) estimated and criterion muscle volume (MV). Plots display use of different slicing strategies (A = MV2, B = MV3, C = MV4, D = MV5) and directions (D-P = black circles, P-D = white circles). Predicted MV from CSA_M and CSA_T are in plots E and F respectively. All plots show: mean bias (central dashed horizontal line); 95% limits of agreement limits (±2 standard deviations; upper and lower dashed horizontal lines) presented as absolute values (cm^3) and as a % of MV; correlation between means and differences (solid diagonal lines) for each MV estimate. MV*n*, estimated muscle volume using every nth CSA slice; CSA_M, muscle only cross-sectional area of mid-thigh; CSA_T, whole thigh cross-sectional area of mid-thigh

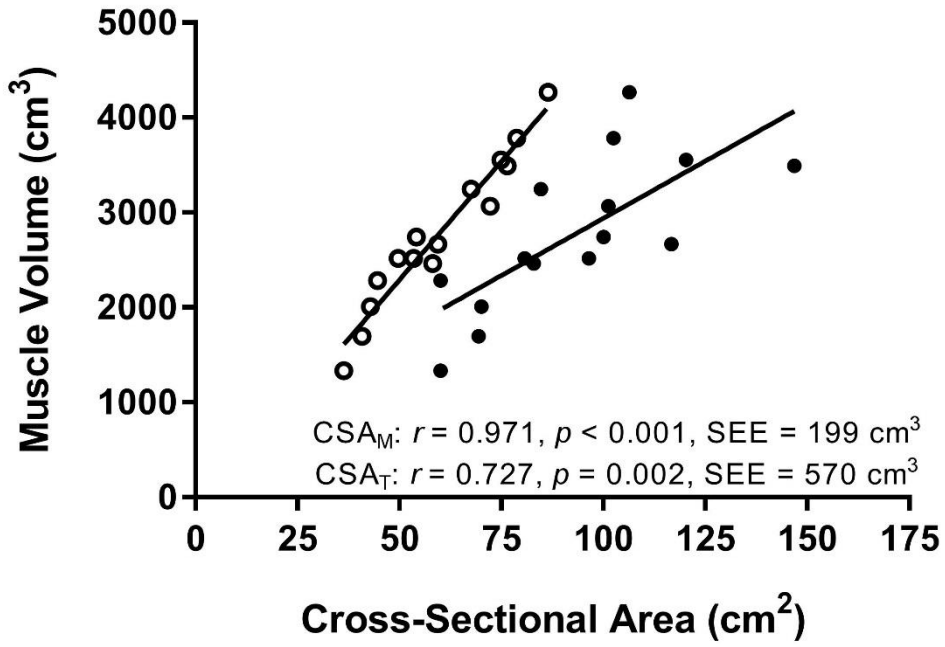

Figure 7.2 The relationship between criterion muscle volume and CSA_M (white circles) and CSA_T (black circles). CSA_M, muscle only cross-sectional area of mid-thigh; CSA_T, whole thigh cross-sectional area of mid-thigh; *r*, Pearson's correlation coefficient; *p*, significance value; SEE = standard error of the estimate.

Significant correlations were found between age and body size values, and both the absolute and percentage difference between criterion and estimated MV (Table 7.2) for both the D-P (r = 0.13 – 0.86, r = -0.04 – -0.51) and P-D (r = -0.20 – -0.78, r = -0.29 – 0.75) directions. Differences from criterion MV estimated using CSA_M were associated with leg length, and estimates using CSA_T were associated with stature, leg length, body fat percentage, FFM and fat mass. The only slicing strategy to not hold any significant correlations (in either absolute or percentage terms) was MV2 in the D-P direction. Leg length significantly correlated with the greatest number of slicing estimate differences.

Table 7.2 Pearson's correlation coefficients between differences of each estimation method and criterion MV, and body size variables.

	Age (years)	Stature (m)	Leg Length (m)	Body Mass (kg)	Body Fat (%)	FFM (kg)	Fat Mass (kg)
Distal to Proximal							
$MV2_a$	r = 0.33, p = 0.23	r = 0.19, p = 0.50	r = 0.14, p = 0.62	r = 0.13, p = 0.66	r = 0.16, p = 0.57	r = 0.13, p = 0.64	r = 0.62, p = 0.83
$MV2_{\%}$	r = -0.25, p = 0.37	r = -0.46, p = 0.08	r = -0.47, p = 0.08	r = -0.39, p = 0.15	r = 0.22, p = 0.43	r = -0.48, p = 0.07	r = -0.07, p = 0.80
$MV3_a$	**r = 0.73, p = 0.002**	**r = 0.64, p = 0.011**	**r = 0.55, p = 0.035**	**r = 0.65, p = 0.009**	r = 0.03, p = 0.92	**r = 0.70, p = 0.004**	r = 0.31, p = 0.26
$MV3_{\%}$	r = -0.08, p = 0.78	r = -0.37, p = 0.18	r = -0.36, p = 0.18	r = -0.15, p = 0.60	r = 0.17, p = 0.55	r = -0.28, p = 0.31	r = -0.14, p = 0.62
$MV4_a$	**r = 0.86, p < 0.001**	**r = 0.70, p = 0.004**	**r = 0.56, p = 0.031**	**r = 0.71, p = 0.003**	r = 0.31, p = 0.26	**r = 0.70, p = 0.004**	r = 0.47, p = 0.08
$MV4_{\%}$	r = -0.04, p = 0.90	r = -0.40, p = 0.14	r = -0.43, p = 0.11	r = -0.15, p = 0.59	**r = 0.56, p = 0.029**	r = -0.39, p = 0.15	r = -0.32, p = 0.24
$MV5_a$	**r = 0.80, p < 0.001**	**r = 0.78, p = 0.001**	**r = 0.61, p = 0.016**	**r = 0.83, p < 0.001**	r = 0.32, p = 0.25	**r = 0.82, p < 0.001**	**r = 0.53, p = 0.045**
$MV5_{\%}$	r = -0.09, p = 0.76	r = -0.40, p = 0.14	**r = -0.51, p = 0.005**	r = -0.07, p = 0.81	**r = 0.56, p = 0.031**	r = -0.30, p = 0.27	r = -0.37, p = 0.18
Proximal to Distal							
$MV2_a$	**r = -0.64, p = 0.011**	**r = -0.67, p = 0.006**	r = -0.49, p = 0.067	**r = -0.74, p = 0.001**	r = -0.43, p = 0.11	**r = -0.69, p = 0.004**	**r = -0.55, p = 0.045**
$MV2_{\%}$	r = 0.32, p = 0.24	r = 0.46, p = 0.08	**r = 0.57, p = 0.027**	r = 0.18, p = 0.52	**r = -0.55, p = 0.036**	r = 0.41, p = 0.13	r = -0.29, p = 0.30
$MV3_a$	**r = -0.70, p = 0.003**	**r = -0.76, p = 0.001**	**r = -0.72, p = 0.002**	**r = -0.65, p = 0.009**	r = 0.07, p = 0.80	**r = -0.76, p = 0.001**	r = -0.20, p = 0.48
$MV3_{\%}$	r = 0.27, p = 0.33	r = 0.42, p = 0.11	r = 0.33, p = 0.23	r = 0.31, p = 0.27	r = -0.09, p = 0.75	r = 0.40, p = 0.14	r = 0.03, p = 0.93
$MV4_a$	**r = -0.78, p = 0.001**	**r = -0.76, p = 0.001**	**r = -0.57, p = 0.026**	**r = -0.74, p = 0.002**	r = -0.24, p = 0.39	**r = -0.76, p = 0.001**	r = -0.41, p = 0.13
$MV4_{\%}$	r = 0.47, p = 0.08	**r = 0.68, p = 0.005**	**r = 0.75, p = 0.001**	r = 0.44, p = 0.10	r = -0.34, p = 0.22	**r = 0.63, p = 0.012**	r = -0.06, p = 0.84
$MV5_a$	**r = -0.69, p = 0.005**	**r = -0.71, p = 0.003**	**r = -0.63, p = 0.012**	**r = -0.65, p = 0.009**	r = -0.30, p = 0.28	**r = -0.64, p = 0.011**	r = -0.42, p = 0.12
$MV5_{\%}$	r = 0.42, p = 0.09	**r = 0.65, p = 0.009**	**r = 0.61, p = 0.016**	r = 0.47, p = 0.08	r = -0.35, p = 0.21	**r = 0.66, p = 0.007**	**r = -0.06, p = 0.040**
Mid-point Cross Sectional Areas							
CSA_{Ma}	r = 0.21, p = 0.45	r = 0.27, p = 0.33	**r = 0.73, p = 0.002**	r = -0.05, p = 0.85	r = -0.16, p = 0.57	r = 0.03, p = 0.92	r = 0.23, p = 0.42
$CSA_{M\%}$	r = 0.28, p = 0.32	r = 0.40, p = 0.14	**r = 0.55, p = 0.035**	r = 0.09, p = 0.74	r = -0.09, p = 0.74	r = 0.17, p = 0.55	r = -0.07, p = 0.78
CSA_{Ta}	r = 0.25, p = 0.37	**r = 0.56, p = 0.034**	r = 0.50, p = 0.06	r = 0.10, p = 0.73	**r = -0.71, p = 0.003**	r = 0.44, p = 0.10	**r = 0.76, p = 0.001**
$CSA_{T\%}$	r = 0.32, p = 0.24	**r = 0.67, p = 0.007**	**r = 0.79, p = 0.001**	r = 0.23, p = 0.40	**r = -0.57, p = 0.026**	**r = 0.53, p = 0.041**	r = -0.39, p = 0.16

MVn, estimated muscle volume using every n^{th} CSA slice; CSA_M, muscle only cross-sectional area of mid-thigh; CSA_T, whole thigh cross-sectional area of mid-thigh; FFM, fat-free mass. Significant results (p < 0.05) are highlighted in bold. Subscript 'a' and '%' indicate whether error is expressed as an absolute value or percentage.

7.5 Discussion

This study confirms, in children and adolescents, that as the interval between slices increases (and therefore the number of CSA slices decreases), the mean bias and 95% LoA associated with the error also increases. In addition, it has been shown that the direction of slicing affects the magnitude of the mean bias and associated LoA, although for both P-D and D-P directions, as MV increases, as does the error associated with their respective over- and under-estimation. Furthermore, our results have established the error associated with using a single CSA slice to predict MV, and the relationships between measures of body size and differences between criterion MV and estimated MV. These findings are novel for children and adolescents, adding to previous work conducted in adults (Barnouin et al., 2015, Tracy et al., 2003), and therefore have implications for the accurate determination of MV in individuals that present different morphology to adults, including children with chronic disease.

In the current study, as the number of slices decreased (i.e. from MV2 to MV5), the error associated with estimating MV increased – in agreement with research in adults (Barnouin et al., 2015, Nordez et al., 2009, Tracy et al., 2003, Walton et al., 1997). Within this finding, the greatest bias was evident at MV5 (27.5 mm gap; LoA = ±2.0% of MV), a finding that is similar to Tracy et al. (2003), who utilised a slice gap of 31 mm (Tracy et al., 2003), and resulted in a LoA of ±1.7% of MV. Previous studies in adults, however, have only sought to identify the MV of the quadriceps femoris (QF) group, whereas the present study used the MV of the whole thigh. Studies investigating structure and functional relationships should consider utilising whole-thigh MV (as the current study has done), as research has identified equal recruitment of both quadriceps and hamstrings during cycling exercise (Richardson et al., 1998).

Whilst the error established in this study could be considered small, the acceptability of such error is dependent on the research question being addressed. In cross-sectional studies assessing differences in MV between groups with disease (Mathur et al., 2008), or age differences in healthy participants (Maden-Wilkinson et al., 2014, Tolfrey et al., 2006), such error would be consistently applied across both groups and therefore such estimation methods could be acceptably utilised. However, interventional studies investigating temporal changes in MV (i.e. atrophy, hypertrophy) may be required to detect changes that may fall inside the margins of error established in the current study. For example, a bed-rest study in adults from Belavy et al. (2009), identified reductions in MV in individual thigh muscles ranging from 9 cm^3 (7.3%; biceps femoris) to 34 cm^3 (12.3%; semimembranosus) following 56 days of immobilisation; such changes in MV may not be detected when a larger inter-slice distance is used. Therefore, the acceptable slice interval will be dependent on the level of precision needed in any outcome variables.

A new outcome in this study is that the measurement error is dependent upon the direction of measurement (i.e. D-P vs. P-D), which has not previously been assessed in children and adolescents. In previous work (Nordez et al., 2009, Tracy et al., 2003), measures from the knee towards the hip (D-P), have under-estimated MV. The findings of the current study identified a reduced LoA observed in the P-D direction compared to D-P. This difference was greatest between estimates using MV5, where a LoA of 15 cm^3 (0.6%) was reported. Whilst the mean bias and LoA do show a difference for the slicing directions, given the magnitude of previously described MV changes following interventions (Belavy et al., 2009), the direction of measurement is unlikely to have a clinically meaningful impact upon final MV estimates. However, as shown in Figure 7.1,

the significant correlation between the means and differences of each criterion and estimated MV measure using the P-D direction suggests that use of this direction may be biased. The only MV measure that did not identify a significant correlation is MV2 using the D-P direction, indicating this option may be the most suitable MV estimate in the current study.

A further noteworthy finding within this study is the association between body size and the absolute and percentage differences between criterion and estimated MV (Table 7.2). These findings suggest that a child's age and body size can impact the final MV estimates, and have implications when heterogeneous groups of children are being assessed (e.g. those with variances in age, stature and mass). Results show that when the error is presented as a percentage of criterion MV (to minimise further bias by muscle size), the error associated with estimates using MV2 and MV3 in the D-P direction do not provide significant correlations with body size and may therefore be suitable for future use as they are not biased by the range of different body sizes within children and adolescents. Of note, leg length held medium correlations ($r > 0.3$) with all MV errors when expressed as a percentage, further confirming our concerns regarding biasing of estimates due to body size. This association was shown to be significant when greater inter-slice distances are used (i.e. MV5), and therefore this may result in an upper-limit to the value of the inter-slice distance used when estimating MV. This is of further concern when studies utilise a fixed number of CSA slices to calculate MV (Mathur et al., 2008, Nordez et al., 2009) as this can result in a varying inter-slice distance for each participant dependent on the size of the limb being investigated. This is of concern in studies involving children and adolescents, where body size is heterogeneous, as evidenced by the use of between 40 and 56 CSA slices per participant to calculate the criterion MV in the current study. Therefore, when the

relationship between error and body size, and the possible evidence for an upper limit between slices is considered, use of a fixed number of slices may not provide a uniform amount of bias across participants in studies calculating MV. Therefore, this approach cannot be recommended for use without an appropriate comparison of the respective methodologies (i.e. fixed inter-slice distance vs. fixed number of slices).

The number of studies using MRI to undertake MV calculations in disease groups is limited, with Duchenne muscular dystrophy (Godi et al., 2016), chronic obstructive pulmonary disorder (Mathur et al., 2008) and CF (Moser et al., 2000) utilising this methodology. In individuals with CF, we are aware of only one previous study that has utilised MRI to identify muscle size, which used mid-thigh CSA to infer reduced exercise capacity (Moser et al., 2000). However, the use of a single CSA slice has been shown to be a poor predictor (R^2 = 0.79, SEE = 27%) of MV in adults (Morse et al., 2007). The current study agreed with Morse et al. (2007) in identifying a significant relationship between CSA and MV (Figure 7.2). Whilst the shared variance between these variables (R^2 = 0.53, 0.94) would initially indicate a predictive ability, a large SEE was also identified, with nearly 40% error being reported as the LoA for MV predicted from CSA_T and over 13% for CSA_M. These errors are over twenty-times, and seven-times, the size of the largest LoA for MV5 reported in the present study, respectively. Therefore, whilst the use of a single CSA slice is a time-efficient method in comparison to summation of multiple CSA slices, the magnitude of error observed suggests estimation from a single CSA slice is not a valid method for determining MV and should be discouraged.

In summary, when calculating MV in children and adolescents using CSA slices obtained from MRI scans, this study has identified: a) an increased error when

the intervals between slices is increased: b) an influence of the direction in which MV is estimated: c) the poor predictive ability of a single CSA slice to estimate MV, and d) when a slice interval of MV3 and above is used, the resultant differences are related to body size. These findings lead to a practical recommendation that use of MV2 in the D-P direction may be suitable for quantification of MV in children as: a) it halves the time required for analysis whilst, b) the resultant error does not hold a relationship with body size parameters, nor is systematically biased by the mean of the criterion and estimated MV itself.

8 SCALING MAXIMUM OXYGEN UPTAKE FOR THIGH MUSCLE VOLUME IN CHILDREN WITH CYSTIC FIBROSIS

8.1 Abstract

Purpose:

Maximal oxygen uptake ($\dot{V}O_{2max}$) is reduced in children with cystic fibrosis (CF) with intrinsic metabolic deficiencies (muscle 'quality') and skeletal muscle size (muscle 'quantity') proposed as potential causes. This study utilises allometric scaling to remove residual effects of muscle size from $\dot{V}O_{2max}$ to address this 'quality' vs. 'quantity' debate.

Methods:

Fourteen children (7 CF vs. 7 age- and sex-matched controls) participated in this study. $\dot{V}O_{2max}$ was allometrically scaled for muscle cross-sectional area (mCSA) and thigh muscle volume (MV_T), derived from magnetic resonance imaging. Effect sizes (*ES*) and magnitude-based inferences identified differences between groups and associations between $\dot{V}O_{2max}$, mCSA and MV_T.

Results:

Differences between groups for mCSA and MV_T were 'unclear' and absolute $\dot{V}O_{2max}$ was 'possibly lower' in CF. mCSA and MV_T had 'likely' positive relationships with absolute $\dot{V}O_{2max}$. These became 'unclear' when allometrically scaled for mCSA and MV_T. Allometrically scaled $\dot{V}O_{2max}$ was 'likely' lower in CF when controlled for mCSA (*ES* = 1.82) and MV_T (*ES* = 0.91).

Conclusions:

Allometric scaling of mCSA and MV_T removes residual effects of muscle size from $\dot{V}O_{2max}$. A lower $\dot{V}O_{2max}$ is found in children with CF after scaling for mCSA and MV_T, suggesting reduced muscle 'quality' in CF, likely reflecting intrinsic metabolic defects.

8.2 Introduction

A high aerobic fitness (as represented by peak oxygen uptake [$\dot{V}O_{2peak}$]) in people with cystic fibrosis (CF) is associated with increased quality of life (Hebestreit et al., 2014) and reduced risk of hospitalisation (Pérez et al., 2014) and mortality (Pianosi et al., 2005a). It has further been shown that $\dot{V}O_{2peak}$ is reduced in children with CF compared to healthy peers (Bongers et al., 2014b, Saynor et al., 2014b), although the mechanisms have yet to be resolved (Hulzebos et al., 2015). Recent debate has surrounded the contributions of skeletal muscle metabolism towards reduced $\dot{V}O_{2peak}$ (Hulzebos et al., 2017, Rodriguez-Miguelez et al., 2017), and whether muscle size or its intrinsic function (muscle 'quantity' or 'quality' respectively) is predominantly responsible.

Previous research has shown that muscular force positively correlates with $\dot{V}O_{2peak}$ (de Meer et al., 1999) and that muscle size is significantly smaller in children with CF (de Meer et al., 1999, Moser et al., 2000). Consequently, it could be proposed that a muscle-size deficit contributes towards the reduced $\dot{V}O_{2peak}$ in CF. This observation may be explained by the nutritional compromise that occurs in individuals with CF (Culhane et al., 2013), alongside delayed and attenuated pubertal growth (Zhang et al., 2013) and catabolism during pulmonary exacerbations (Bhatt, 2013). Previous studies have expressed $\dot{V}O_{2peak}$ relative to both body mass (BM) and fat-free mass (FFM) (Saynor et al., 2014b, Stevens et al., 2011, Tucker et al., 2018), in order to account for differences in body size. However, expressing $\dot{V}O_{2peak}$ relative to BM and FFM may not be appropriate, as they: a) only provide surrogates for the metabolically active muscle during exercise, and b) the ratio between FFM and leg muscle volume (MV) is not stable during periods of growth, with MV increasing per unit of FFM as FFM itself increases (Tolfrey et al., 2006). Therefore, FFM may be an inferior surrogate of

MV during growth and therefore accounting for leg MV when assessing $\dot{V}O_{2peak}$ in CF may be more appropriate than BM and/or FFM as a parameter of body size. Presently, only one study has accounted for muscle size when examining $\dot{V}O_{2peak}$ in CF, scaling for muscle cross-sectional area (CSA), in an attempt to create a size-free expression of aerobic fitness ($\dot{V}O_2$/CSA; $mL \cdot min^{-1} \cdot cm^{-2}$). This study subsequently identified a reduced $\dot{V}O_{2peak}$ in children with CF relative to healthy control (CON) participants when muscle CSA was controlled for, with the authors proposing an intrinsic muscular defect may be present (Moser et al., 2000). However, participants in this previous study were not age- nor sex-matched, with analyses based on uneven samples (CF = 22, CON = 54). Furthermore, muscle CSA may be a poor indicator of total leg MV, as previous research has reported a standard error of the estimate of up to 27% when CSA is used to estimate MV (Morse et al., 2007).

Previous studies in healthy children have established positive correlations between thigh (Welsman et al., 1997), calf (Tolfrey et al., 2006) and total lower leg (Graves et al., 2013) MV, and $\dot{V}O_{2peak}$, as opposed to using CSA alone. However, no MV-related data exists in children with CF. Quantifying MV data in CF could therefore establish relationships with $\dot{V}O_{2peak}$ and provide novel insight into the 'quantity' vs. 'quality' debate as to why $\dot{V}O_{2peak}$ is impaired compared to healthy peers. In addition, previous work has normalised $\dot{V}O_{2peak}$ relative to muscle CSA using a ratio standard procedure (Moser et al., 2000), whereas allometric scaling procedures may be more appropriate (Tolfrey et al., 2006). Use of allometric scaling has been shown to be effective in removing the residual effects of body size from alternative parameters of aerobic power (the oxygen uptake efficiency slope) when comparing children and adolescents with CF to a control group (Tomlinson et al., 2017).

Therefore, the purpose of this study was to: a) establish the relationship between thigh MV (MV_T) and CSA, measured using magnetic resonance imaging (MRI), and $\dot{V}O_{2peak}$ in children with CF and age- and sex-matched controls, and b) extend the research of Moser et al. (2000) by using allometric scaling procedures to explore whether $\dot{V}O_{2peak}$ is impaired in CF patients compared to CON after normalising for parameters of volume (thigh volume [TV], MV_T) and CSA (thigh CSA [tCSA], muscle CSA [mCSA]). Findings could provide further insight into the muscle 'quantity' vs. 'quality' debate; once muscle size is allometrically controlled for, should $\dot{V}O_{2peak}$ remain lower in CF, this would support an intrinsic muscular defect in this disease group.

8.3 Methods

8.3.1 Study population

Ten children with mild-to-moderate CF (i.e. forced expiratory volume in 1 second [FEV_1] >40 $\%_{Predicted}$) were recruited from outpatient clinics at a local CF centre and 15 healthy children were recruited from local sports clubs and schools to act as an age- and sex-matched CON group. Ethics approval was provided an NHS Research Ethics Committee (14/SW/0061), and written informed consent and assent were obtained from both parents/guardians and participating children prior to any procedures being undertaken.

Inclusion criteria for patients with CF included a positive diagnosis of CF based upon clinical features, supported by an abnormal sweat test and where possible, diagnostic genotyping; a stable lung function (i.e. within 10 % of their best in the preceding 6 months); and no increase in symptoms or loss of body mass in the preceding 2 weeks. Furthermore, no contraindications towards exhaustive

exercise, nor presence inside an MR scanner (e.g. metallic implants) were required for the CF and CON groups alike.

8.3.2 Anthropometry and pulmonary function

Stature was measured to the nearest 0.1 cm (Holtain stadiometer, Crymych, Wales) and BM to the nearest 0.1 kg (Seca, Birmingham, UK). Body fat percentage was estimated using skinfold callipers and validated equations for use in healthy children (Slaughter et al., 1988), with fat-mass (FM) and FFM subsequently calculated. Pubertal status was determined by self-assessment of the five stages of pubic hair development (Morris and Udry, 1980).

Pulmonary function was obtained using a hand-held spirometer (MicroPlus, Micro Medical Ltd, Rochester, UK), with values of forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio being recorded. The best of three attempts was taken as the maximal value, with results expressed relative to normative values (Quanjer et al., 2012).

8.3.3 Physical activity

Physical activity (PA) was objectively assessed using accelerometers (GENEActiv; ActivInsights, Kimbolton, UK) worn on the non-dominant wrist for one week. PA was analysed in 60-second epochs, using cut-points validated for use in healthy children and adolescents (Phillips et al., 2013). Data from at least two days with 10 hours each (Rich et al., 2013) was included for analyses. Number of minutes, and percentage of wear time, for sedentary and moderate-to-vigorous PA (MVPA) were reported.

8.3.4 Cardiopulmonary exercise testing

All participants undertook a combined ramp incremental and supramaximal cardiopulmonary exercise test (CPET) to volitional exhaustion on an

electronically braked cycle ergometer (Lode, Groningen, the Netherlands). Breath-by-breath gas exchange data were collected using an online Cortex gas analysis system (Cranlea, Birmingham, UK) and exported in 10-second averages, with $\dot{V}O_{2max}$ taken as the highest 10-second average, and the gas exchange threshold (GET) obtained by previously validated methods (Beaver et al., 1986). This CPET employed an initial ramp phase with a supramaximal verification bout, to establish a 'true' $\dot{V}O_{2max}$. This protocol has been validated for use in children with (Saynor et al., 2013a) and without (Barker et al., 2011) CF. $\dot{V}O_{2max}$ ($L \cdot min^{-1}$) was expressed to a percent of predicted using age and sex reference data (Bongers et al., 2014a).

8.3.5 Determination of cross-sectional area and muscle volume

Parameters of TV, MV_T, tCSA and mCSA were determined using a 1.5 T superconducting whole-body MR scanner (Gyroscan Intera, Philips, the Netherlands). A T1 weighted image sequence was used to optimise fat/muscle signal contrast to obtain a stack of axial images from below the knee to above the hip. Participants were positioned in a prone position within the scanner, with the hips and upper legs secured to the bed with straps to avoid unnecessary movement. Parameters of TV and MV_T were calculated from MRI scans, using the sum of each anatomical CSA multiplied by 5.5 mm (5 mm slice thickness + 0.5 mm slice gap). The slices at the distal and proximal ends were multiplied by 5.25 mm to reflect the absence of the 0.25 mm contribution from the adjacent slice gap. Starting from the medial epicondyle and terminating at the head of the femur, TV (muscle and adipose) and MV_T (muscle only, no adipose) were determined by manually tracing around each CSA compartment using inbuilt Philips software. Parameters of tCSA and mCSA were established at mid-thigh (i.e. 50% of thigh length between medial epicondyle and femoral head) (Moser et

al., 2000). Bone was excluded from all analyses. Analyses were undertaken by two investigators with an intra-investigator coefficient of variation (CV) < 1.5%. Between-investigator CV was 1.2%. For consistency of analyses, the largest tCSA and mCSA (whether from right or left legs), and total TV and MV_T (i.e. right plus left leg) were utilised to control for prospective leg dominance.

8.3.6 Scaling procedures

Where absolute $\dot{V}O_{2max}$ was significantly correlated with parameters of muscle size, these were carried forward for allometric scaling to remove residual effects of body size. Allometric scaling utilised log-linear regressions (Tolfrey et al., 2006, Tomlinson et al., 2017), with disease status (i.e. CF, CON) and body size variables entered as predictors to obtain a subsequent scaling exponent (*b*), with its associated 95 % confidence intervals (CIs), to use as a power function to which body size variables were raised (i.e. Y/X^b). All regression assumptions (multicollinearity of independent variables and independence, homoscedasticity, linearity and normal distribution of residuals) were checked and satisfied. Age and sex were not entered into the models due to the prior matching of groups.

8.3.7 Statistical analyses

Data are reported as mean (± standard deviation) unless stated otherwise. Independent samples *t*-test (SPSS v.24; IBM, Armonk, NY, USA) derived *p* values were used for subsequent inferential analyses of mean differences. Facilitated by published spreadsheets (Hopkins, 2007), 90% CIs and effect sizes (*ES*) were utilised to derive magnitude-based inferences (MBI) on the observed effect statistic (Hopkins et al., 2009) and identify any influence of CF upon anthropometric, pulmonary, exercise, MRI-derived muscular variables and PA; as well as scaled differences in $\dot{V}O_{2max}$.

Using 90% CI and the smallest worthwhile *ES* change of 0.2 for mean differences (Cohen, 1992), the likelihood that the observed effect was substantially greater (e.g. higher $\dot{V}O_{2max}$), trivial, or substantially lower (e.g. lower $\dot{V}O_{2max}$) was reported using the following mechanistic inferences: <0.5%, ‘‘most unlikely’’; 0.5%–5%, ‘‘very unlikely’’; 5%–25%, ‘‘unlikely’’; 25%–75%, ‘‘possibly’’; 75%–95%, ‘‘likely’’; 95%–99.5%, ‘‘very likely’’; >99.5%, ‘‘most likely’’. This statistical methodology has been utilised previously in assessing clinical differences in exercise capacity in children with CF (Saynor et al., 2014b).

Furthermore, following prior research (Tomlinson et al., 2017), Pearson’s correlation coefficients were run to assess relationships between body-size, $\dot{V}O_{2max}$ and subsequent allometrically-scaled $\dot{V}O_{2max}$. Cohen thresholds (Cohen, 1992) for small (0.1), moderate (0.3), large (0.5), and very large (0.7) relationships describe magnitudes of correlations. The smallest worthwhile *ES* of 0.1 (Cohen, 1992) was used to determine magnitude based inferences for correlation coefficients.

8.4 Results

Of the 10 children with CF recruited, *n* = 8 undertook all required procedures for this study. Two patients withdrew from this study, one due to increasing pulmonary instability, and one due to a positive culture of non-tuberculosis mycobacteria. Of the 15 CON children recruited, 12 completed all measurements. Two did not undertake CPET, and one did not undertake MRI scans to determine MV. When age- and sex-matching are considered, a total of seven suitable pairs were identified, resulting in a final *n* = 14 (7 CF, 7 CON) carried forward for analyses. Genotypes of patients with CF included in the current study include: ΔF508/ΔF508 (*n* = 3), ΔF508/Unknown (*n* = 1), ΔF508/E585X (*n* = 1), ΔF508/711+1G->T (*n* = 1) and 18G->T/1-8G->C (*n* = 1).

Mean differences between CF and CON for anthropometric, pulmonary, exercise, MRI-derived muscle and PA variables are listed in Table 8.1. Mechanistic inferences show that the CF group 'likely' had a higher GET (%$\dot{V}O_{2max}$), and a 'very likely' higher FEV_1/FVC ratio relative to CON, but also a 'possibly' lower $\dot{V}O_{2max}$ ($L \cdot min^{-1}$) and GET ($L \cdot min^{-1}$), as well as a 'very likely' lower HR_{max} and time spent in MVPA (mins). Further to differences in group means, individual differences between each respective CF-CON pair is presented in Table 8.2.

All body size variables had a 'likely' positive relationship with $\dot{V}O_{2max}$ at the combined group level (Table 8.3). When categorised by groups, relationships between $\dot{V}O_{2max}$ and MRI-derived muscular variables varied from 'unclear' (tCSA, TV) to 'most likely' positive (mCSA, MV_T).

For the allometric scaling procedures, only tCSA, mCSA and MV_T were significant predictors of $\dot{V}O_{2max}$. The derived exponents for each of these MRI-derived variables are displayed in Table 8.4. Application of these power functions (tCSA, β = 0.76; mCSA, β = 1.02; MV_T, β = 0.78) to scale $\dot{V}O_{2max}$ resulted in 'unclear' size-residual correlations (Table 8.3).

When mean differences in scaled $\dot{V}O_{2max}$ (Table 8.5) are assessed, $\dot{V}O_{2max}$ in CF was 'likely' lower than the CON group when MV_T was controlled for (*ES* = -0.91), and 'most likely' lower when mCSA was controlled for (*ES* = -1.82). Use of tCSA resulted in an 'unclear' difference between groups, although a medium effect size was present (*ES* = -0.77).

Table 8.1. Anthropometric, pulmonary, exercise and MRI-derived muscle-related differences between CF and CON groups.

Variable	CF (*n* = 7)	CON (*n* = 7)	Mean difference, 90% CI	Inference in CF	Effect size
Age (years)	14.8 ± 2.1	14.6 ± 2.4	0.2 ± 1.7	Unclear	0.09
Stature (cm)	161.9 ± 10.1	160.1 ± 8.3	1.8 ± 27.8	Unclear	0.19
Body mass (kg)	56.7 ± 12.1	51.0 ± 9.0	5.7 ± 10.2	Unclear	0.54
Body Fat (%)	17.0 ± 4.7	17.7 ± 5.5	-0.7 ± 5.0	Unclear	-0.14
FM (kg)	9.7 ± 3.8	8.7 ± 3.8	1.0 ± 3.6	Unclear	0.26
FFM (kg)	47.0 ± 10.2	42.3 ± 7.9	4.7 ± 8.7	Unclear	0.52
FEV_1 (L)	3.32 ± 0.88	2.98 ± 0.67	0.64 ± 0.74	Unclear	0.43
FEV_1 ($\%_{Predicted}$)	104.7 ± 11.3	98.1 ± 21.8	6.6 ± 16.5	Unclear	0.38
FVC (L)	3.80 ± 0.98	3.74 ± 0.69	0.06 ± 0.83	Unclear	0.07
FVC ($\%_{Predicted}$)	103.9 ± 9.5	101.7 ± 11.4	2.2 ± 10.3	Unclear	0.21
FEV_1/FVC (%)	87.61 ± 4.05	79.52 ± 7.93	8.1 ± 5.9	Very likely higher	1.28
$\dot{V}O_{2max}$ ($L{\cdot}min^{-1}$)	2.28 ± 0.76	2.57 ± 0.69	-0.29 ± 0.69	Possibly lower	-0.40
$\dot{V}O_{2max}$ ($\%_{Predicted}$)	87.2 ± 22.8	101.6 ± 12.5	-14.4 ± 17.5	Unclear	-0.78
GET ($L{\cdot}min^{-1}$)	1.18 ± 0.45	1.20 ± 0.32	-0.02 ± 0.46	Possibly lower	-0.05
GET (%$\dot{V}O_{2max}$)	54.4 ± 9.1	48.5 ± 4.8	5.9 ± 6.8	Likely higher	0.81
HR_{max} ($beats{\cdot}min^{-1}$)	182 ± 7	196 ± 9	-14 ± 13	Very likely lower	-1.74
tCSA (cm^2)	92.1 ± 18.2	87.1 ± 22.0	5.0 ± 19.3	Unclear	0.25
mCSA (cm^2)	61.3 ± 14.2	57.0 ± 17.1	4.3 ± 15.0	Unclear	0.27
TV (cm^3)	8921 ± 2410	9139 ± 2554	-218 ± 2365	Unclear	-0.09
MV_T (cm^3)	5426 ± 1532	5450 ± 1828	-25 ± 1606	Unclear	-0.01
MV (%TV)	61.5 ± 12.0	59.7 ± 10.3	1.8 ± 10.7	Unclear	0.16
Sedentary time (mins)	396 ± 107	444 ± 62	48 ± 83	Unclear	-0.55
Sedentary time (%)*	54.2 ± 10.1	56.4 ± 5.4	2.2 ± 7.7	Unclear	-0.27
MVPA (mins)	93 ± 33	128 ± 41	35 ± 35	Very likely lower	-0.94
MVPA (%)*	14.0 ± 6.7	16.4 ± 5.7	2.4 ± 5.9	Unclear	-0.39

Measures are presented as mean ± SD. 90% CI, 90% confidence interval. CF, cystic fibrosis; CON, control; FM, fat mass; FFM, fat free mass; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; $\dot{V}O_{2max}$, maximal oxygen uptake; GET, gas exchange threshold; HR, heart rate; tCSA, thigh cross-sectional area; mCSA, muscle cross-sectional area; TV, thigh volume; MV_T, muscle volume of the thigh; MVPA, moderate to vigorous physical activity. *Sedentary time and MVPA are measured as a percentage of daily wear-time.

Table 8.2. Differences between matched pairs for primary anthropometric and MRI-derived muscle variables utilised for scaling procedures.

	Pair/Sex	1/M	2/M	3/M	4/M	5/F	6/F	7/M
CF	Age (years)	12.1	13.2	13.5	14	16.6	16.9	17.5
	Pubertal Stage*	-	3	-	4	4	5	5
	Body mass (kg)	35.5	51.5	51.8	64	55.2	69.6	69.2
	$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	1.24	2.21	2.23	3.29	1.82	1.9	3.27
	Total MV_T (cm^3)	2663	4843	6394	6190	5014	5352	7525
CON	Age (years)	11.9	12.4	12.9	13.9	15.6	17	17.4
	Pubertal Stage*	2	4	-	3	4	4	-
	Body mass (kg)	39.4	46.7	41.1	50.7	59.3	57.2	62.3
	$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	2.22	2.02	2.34	2.41	2.06	2.98	4.04
	Total MV_T (cm^3)	3384	4033	4546	5036	5413	7138	8601
Difference (Δ)	Age (years)	0.2	0.8	0.6	0.1	1.0	-0.1	0.1
	Pubertal Stage*	-	1	-	1	0	1	-
	Body mass (kg)	-3.9	4.8	10.7	13.3	-4.1	12.4	6.9
	$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	-0.98	0.19	-0.11	0.88	-0.24	-1.08	-0.77
	Total MV_T (cm^3)	-721	810	1848	1154	-399	-1786	-1076
Difference (%)	Age (years)	1.7	6.5	4.7	0.7	6.4	-0.6	0.6
	Body mass (kg)	-9.9	10.3	26.0	26.2	-6.9	21.7	11.1
	$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	-44.1	9.4	-4.7	36.5	-11.7	-36.2	-19.1
	Total MV_T (cm^3)	-21.3	20.1	40.7	22.9	-7.4	-25.0	-12.5

Measures are presented as individual values with matched pairs, ordered from youngest (pair 1) to oldest (pair 7). CF, cystic fibrosis; CON, control; M, male; F, female; $\dot{V}O_{2max}$, maximal oxygen uptake; MV_T, muscle volume of the thigh; Δ, absolute difference (CF – CON); % difference indicates CF relative to CON. *Pubertal stage information only available for 4 age- and sex-matched pairs due to participants declining to return self-assessment forms.

Table 8.3. Pearson's correlation coefficients for $\dot{V}O_{2max}$ when scaled for body size parameters using differing scaling procedures.

	CF	CON	Combined
Absolute			
BM vs. $\dot{V}O_{2max}$	*r* = 0.69 ± 0.45, Likely positive	*r* = 0.61 ± 0.51, Likely positive	*r* = 0.56 ± 0.34, Very likely positive
FFM vs. $\dot{V}O_{2max}$	*r* = 0.84 ± 0.29, Very likely positive	*r* = 0.79 ± 0.36, Very likely positive	*r* = 0.69 ± 0.27, Very likely positive
tCSA vs. $\dot{V}O_{2max}$	*r* = 0.52 ± 0.56, Unclear	*r* = 0.60 ± 0.52, Unclear	*r* = 0.51 ± 0.36, Likely positive
mCSA vs. $\dot{V}O_{2max}$	*r* = 0.94 ± 0.13, Most likely positive	*r* = 0.92 ± 0.17, Most likely positive	*r* = 0.86 ± 0.15, Most likely positive
TV vs. $\dot{V}O_{2max}$	*r* = 0.41 ± 0.61, Unclear	*r* = 0.50 ± 0.58, Unclear	*r* = 0.45 ± 0.38, Likely positive
MV_T vs. $\dot{V}O_{2max}$	*r* = 0.85 ± 0.28, Very likely positive	*r* = 0.90 ± 0.20, Most likely positive	*r* = 0.86 ± 0.15, Most likely positive
Allometric			
tCSA vs. $\dot{V}O_{2max}/tCSA^{0.76}$	*r* = 0.18 ± 0.66, Unclear	*r* = -0.15 ± 0.67, Unclear	*r* = -0.05 ± 0.46, Unclear
mCSA vs. $\dot{V}O_{2max}/mCSA^{1.02}$	*r* = 0.62 ± 0.51, Unclear	*r* = -0.44 ± 0.60, Unclear	*r* = -0.12 ± 0.45, Unclear
MV_T vs. $\dot{V}O_{2max}/MV_T^{0.78}$	*r* = 0.32 ± 0.64, Unclear	*r* = -0.23 ± 0.66, Unclear	*r* = 0.03 ± 0.46, Unclear

Correlations are presented as coefficients ± 90% confidence interval, with mechanistic inference. CF, cystic fibrosis; CON, control; BM, body mass; FFM, fat-free mass; tCSA, thigh cross-sectional area at 50% thigh length; mCSA, muscle cross-sectional area at 50% thigh length; TV, thigh volume; MV_T, muscle volume of the thigh; $\dot{V}O_{2max}$, maximal oxygen uptake.

Table 8.4. Allometric exponents for $\dot{V}O_{2max}$ and parameters of muscle size in young patients with CF and healthy age- and sex-matched controls.

Body size variable	*b*	95% CI	R^2	SEE
tCSA	0.76	0.08 – 1.44	0.40	0.25
mCSA	1.02	0.71 – 1.33	0.84	0.13
MV_T	0.78	0.48 – 1.09	0.76	0.16

b, scaling exponent; 95% CI, 95% confidence interval for *b*; R^2, shared variance between $\dot{V}O_{2max}$ and body size parameter; SEE, standard error of the estimate. tCSA, thigh cross-sectional area at 50% thigh length; mCSA, muscle cross-sectional area at 50% thigh length; MV_T, muscle volume of the thigh.

Table 8.5. Mean differences in scaled $\dot{V}O_{2max}$ between children in CF and CON groups.

$\dot{V}O_{2max}$/Body Size	CF	CON	Mean difference, 90% CI	Inference (in CF)	Effect Size
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	*2.28 ± 0.76*	*2.57 ± 0.69*	*-0.29 ± 0.69*	*Unclear*	*-0.40*
/$tCSA^{0.76}$ ($mL \cdot min^{-1} \cdot cm^{-1.52}$)	73.04 ± 19.59	87.48 ± 17.80	-14.44 ± 21.80	Unclear	-0.77
/$mCSA^{1.02}$ ($mL \cdot min^{-1} \cdot cm^{-2.04}$)	33.85 ± 4.18	42.48 ± 5.25	-8.62 ± 5.52	Most likely lower	-1.82
/$MV_T^{0.78}$ ($mL \cdot min^{-1} \cdot cm^{-2.34}$)	2.68 ± 0.64	3.09 ± 0.42	-0.40 ± 0.51	Likely lower	-0.91

Measures are presented as mean ± SD. 90% CI, 90% confidence interval. CF, cystic fibrosis; CON, control; tCSA, thigh cross-sectional area at 50% thigh length; mCSA, muscle cross-sectional area at 50% thigh length; MV_T, muscle volume of the thigh; $\dot{V}O_{2max}$, maximal oxygen uptake. Absolute $\dot{V}O_{2max}$ values provided for reference.

8.5 Discussion

The main results of this study have shown that absolute $\dot{V}O_{2max}$ has positive relationships with MRI-derived variables of tCSA, mCSA, TV and MV_T. Furthermore, allometric scaling successfully removed the residual effects of muscle size (mCSA, MV_T) from $\dot{V}O_{2max}$. Use of this scaling method subsequently reveals that $\dot{V}O_{2max}$ is 'likely' lower in CF. Thus, after accounting for muscle 'quantity', these data support the case for muscle 'quality' being responsible for reduced $\dot{V}O_{2max}$ in CF.

Large correlation coefficients have been reported before in healthy children between absolute $\dot{V}O_{2peak}$ and BM (Welsman and Armstrong, 2000), FFM (Tolfrey et al., 2006), CSA (Moser et al., 2000) and MV (Welsman et al., 1997). For children with CF, a similar correlation between mCSA and $\dot{V}O_{2max}$ (r = 0.89) has been previously reported (Moser et al., 2000), indicating a strong positive relationship between the two variables. However, no study has previously detailed the relationship between MV_T and $\dot{V}O_{2max}$ in this disease group. The correlation coefficients for the combined (r = 0.86) and CF (r = 0.85) groups in the current study are similar to previous research in healthy children (r = 0.80-0.81), whereas the magnitude of the correlation between $\dot{V}O_{2max}$ and MV_T is higher in the current CON group (r = 0.90) than the study from Welsman et al. (1997). This small difference in the magnitude of coefficients between studies may be accounted for by differences in the size and heterogeneity of samples, or the increased mean ages and $\dot{V}O_{2max}$ in the present study. Due to the larger coefficients established (and therefore shared variance) between $\dot{V}O_{2max}$, and mCSA and MV_T (relative to BM and FFM), these results suggest that these MRI-derived parameters of muscle size are superior variables against which to scale

$\dot{V}O_{2max}$, likely because they better reflect the musculature activated during exercise.

This study has identified that allometric scaling is effective in removing the residual effects of body size from $\dot{V}O_{2max}$. As shown in Table 8.3, when allometric procedures were utilised (using coefficients from Table 8.4), residual effects of tCSA, mCSA and MV_T appear to be removed from $\dot{V}O_{2max}$. The resultant correlations between scaled $\dot{V}O_{2max}$ and parameters of muscle size at the group level were 'unclear', and an increased sample size would be required to change this to 'trivial'. Therefore, a dependence on muscle-size cannot be fully excluded, although the reported *ES* are 'small' (r = -0.12 – 0.03) suggesting that allometric scaling successfully partitioned the residual effects of body size from $\dot{V}O_{2max}$ in this study.

The point estimate for the MV_T scaling coefficient (β = 0.78) in the current study is larger than a previous finding in healthy children (β = 0.62) (Tolfrey et al., 2006), with this difference potentially due to the inclusion of the CF group, but also use of a treadmill protocol and scaling for calf volume (as opposed to a cycling protocol and use of thigh volume as per the present study). The previous study by Tolfrey et al. (2006) had a similar sample size (n = 15) to the present study (n = 14), and the current study presents a consistently greater magnitude of difference in both the mean values for BM (+17%) and $\dot{V}O_{2max}$ (+15%). Whilst no reference data are available for MV_T coefficients in individuals with CF, the exponent derived for mCSA (β = 1.02) is strikingly similar to that established for the CF group of Moser *et al.* (β = 1.03 ± 0.12), but not their CON group (β = 0.80 ± 0.16) (Moser et al., 2000), suggesting differing relationships between mCSA and $\dot{V}O_{2peak}$ between CF and CON in previous work. Unfortunately, no combined exponent from Moser et al. (2000) was presented with which to draw comparisons

to the current study. Moreover, within the previous study of Moser et al. (2000), the authors utilised ratio-standard scaling, despite the derivation of different scaling exponents for each group as mentioned previously. Furthermore, no analyses were undertaken to identify if ratio-standard scaling sufficiently removed residual effects of muscle size.

When allometric scaling was undertaken, $\dot{V}O_{2max}$ was reduced in CF, with large effect sizes between groups for $\dot{V}O_{2max}$ found for both mCSA (1.82) and MV_T (0.91) and a medium effect size for tCSA (0.77). These indicate that $\dot{V}O_{2max}$ in children with CF is 'likely' lower when scaled for MV_T and 'most likely' lower when scaled for mCSA. These finding are in agreement with previous research that identifies a reduced $\dot{V}O_{2peak}$ in children with CF, particularly when BM (Bongers et al., 2014b, Saynor et al., 2014b) and FFM (Saynor et al., 2014b, Stevens et al., 2011, Tucker et al., 2018) are accounted for. However, as previously mentioned, BM and FFM are poor surrogates for the metabolically active muscle and therefore quantification of muscle size has been advocated in previous studies (Graves et al., 2013, Tolfrey et al., 2006), with statistical control of mCSA and MV_T undertaken in this study. The conclusions of the current study are akin to those of Moser et al. (2000), despite the aforementioned concerns related to the scaling methods used, and use of mCSA as opposed to MV_T.

Collectively, the findings of the current study and previous work by Moser et al. (2000) indicate a 'qualitative' defect in skeletal muscle function in CF. Studies conducted *in vitro* have identified the protein responsible for manifestation of CF (CFTR) is normally expressed in the sarcoplasmic reticulum (Divangahi et al., 2009), lower resting ATP and Ca^{2+} dysregulation in CF muscle (Lamhonwah et al., 2010) and mitochondrial dysfunction (Valdivieso et al., 2012). Furthermore, *in vivo* studies in patients with mild-to-moderate CF have also identified vascular

dysfunction, which is significantly associated with $\dot{V}O_{2peak}$ (Poore et al., 2013, Rodriguez-Miguelez et al., 2016), as well as prolonged phosphocreatine recovery following exercise, as identified using magnetic resonance spectroscopy, inferring impaired aerobic oxidative metabolism (Wells et al., 2011). Whilst the current study was not designed to identify which of these factors is responsible for the reduced $\dot{V}O_{2max}$ in CF, it is likely that a combination of these factors is responsible.

A strength to the current study is the age- and sex-matching of participants, which strives to ensure that disease status remains the discerning characteristic between groups. Furthermore, the children and adolescents with CF in the current study were relatively healthy. They had preserved pulmonary function (FEV_1 = 104.7 ± 11.3 $\%_{Predicted}$) and were physically active, with both groups presenting a mean MVPA above the recommended daily guidelines of 60 minutes per day (Janssen, 2007). This therefore indicates that despite increased FEV_1 and PA, factors associated with increased $\dot{V}O_{2peak}$ in this group (Hebestreit et al., 2006), exercise capacity was still reduced in the CF group relative to age- and sex-matched peers.

It is acknowledged that this study has a limited sample size – an unfortunate consequence of research in clinical populations and deliberate age- and sex-matching, which has resulted in a limited number of pairs. As a result, inferences were made using MBI, based upon *ES* (Batterham and Hopkins, 2006). The simultaneous presentation of both MBI and *ES* provides several perspectives on the data, and complement one another in lieu of traditional *p* values, derived from null hypothesis significance testing. However, further research is warranted to corroborate these findings in a larger sample of children with CF.

Finally, in accord with previous research (Tomlinson et al., 2017), the body size exponents derived in the current study are not intended for immediate use by clinicians or researchers, as patient groups will inevitably vary in their clinical status, but highlight the requirement for allometric scaling when analysing variables (such as $\dot{V}O_{2max}$) may be influenced by body size. As exponents can vary greatly between studies (Lolli et al., 2017), future studies will be required to derive their own scaling exponents for use with the presented statistical methodology.

8.6 Conclusion

In addition to identifying that allometric scaling successfully removed residual effects of muscle size from $\dot{V}O_{2max}$, this study has identified that once muscle 'quantity' is controlled for, a difference in $\dot{V}O_{2max}$ is still evident between individuals with and without CF. This therefore suggests that an intrinsic muscular defect is likely responsible for reduced exercise capacity in children with CF, with evidence for a skeletal muscle defect (i.e. muscle 'quality') being strengthened by the current study.

9 EXERCISE CAPACITY FOLLOWING A PERCUTANEOUS ENDOSCOPIC GASTROSTOMY IN A YOUNG FEMALE WITH CYSTIC FIBROSIS: A CASE REPORT

9.1 Abstract

Cystic fibrosis (CF) is a genetic condition affecting the respiratory and gastrointestinal systems, with patients experiencing problems maintaining weight, especially during rapid growth periods such as puberty. The aim of this case report was to monitor the effect of gastrostomy insertion and implementation of overnight supplemental feeding upon clinical outcomes, including body mass index (BMI), lung function (FEV_1) and exercise related variables (maximal oxygen uptake [VO_{2max}] and ventilatory efficiency [V_E/VO_2]) in an 11-year-old female with CF. Combined incremental and supra-maximal exercise testing to exhaustion was performed at four time-points: three-months prior to the procedure (T1), 2-days prior to (T2), four-months (T3), and one-year following the procedure (T4). Improvements following gastrostomy insertion were observed at the one year follow up with regards to BMI (+ 20%); whilst absolute VO_{2max} remained stable and lung function fluctuated throughout the period of observation. Declines in function with regards to body weight relative VO_{2max} (- 16.3%) and oxygen uptake efficiency (+ 7.5%) were observed during this period. This case report is the first to consider exercise-related clinical outcomes in assessing the effect of implementing gastrostomy feeding in CF. The varied direction and magnitude of the associations between variables shows that further investigations are required.

9.2 Introduction

Cystic fibrosis (CF) is a genetically inherited, life-shortening disease characterised by respiratory and digestive problems which manifests in a

decreased exercise capacity (Saynor et al., 2014b) and malnutrition (Panagopoulou et al., 2014). Increased mortality risk is reported when patients exhibit decreased lung function and poor nutritional status (Liou et al., 2001). However, exercise related predictors of mortality, including maximal oxygen uptake (VO_{2max}) (Pianosi et al., 2005a) and peak ventilatory equivalent ratio for oxygen (a measure of ventilatory efficiency; V_E/VO_2) (Hulzebos et al., 2014) are also reported in this patient group.

Patients with CF are encouraged to increase their exercise levels (Swisher et al., 2015) and daily caloric intake (Stallings et al., 2008) to improve clinical outcomes, in line with clinical practice guidelines (Cystic Fibrosis Trust, 2011). However, nutritional targets are not always met despite a high level of calorie intake relative to non-CF controls (Woestenenk et al., 2014). When patients fail to gain weight as predicted and conservative dietary interventions fail, invasive support through the insertion of a percutaneous endoscopic gastrostomy (PEG) may be required. This procedure has been shown to improve nutritional status (Williams et al., 1999) and stabilise lung function (Bradley et al., 2012).

Exercise testing is a valuable tool for evaluating interventions and profiling the clinical status of patients with CF (Cystic Fibrosis Trust, 2011), but has yet to be utilised to assess the effectiveness of this procedure. Therefore, this case report is the first to describe exercise-related changes alongside nutritional status and lung function following a PEG implant, and supplemental feeding, in a paediatric patient with CF, over a 15-month period.

9.3 Patient information

The subject of this case report, an 11-year-old female, presented at birth with meconium ileus requiring surgery and was subsequently confirmed to have CF (sweat chloride > 60 $mmol \cdot L^{-1}$ and homozygous for the ΔF508 mutation).

Her clinical course through childhood was complicated by *Pseudomonal* and *Staphylococcal* chest infections as well as relapsing Allergic Broncho-Pulmonary Aspergillosis, treated with recurrent courses of antibiotics, inhaled mucoactives (DNase and hypertonic saline), corticosteroids, antifungals and chest physiotherapy (autogenic drainage and oscillating PEP). She developed impaired glucose tolerance at age 9, then CF-related diabetes requiring insulin treatment at 10 years of age (as shown in Figure 9.1).

During the 15-month period reported in the current paper, the patient was unstable (as shown by FEV_1 in Figure 9.2) and underwent 22 days of intravenous antibiotic treatment.

9.4 Timeline

Changes in clinically important measures of exercise performance over a 15-month period were assessed for this report, with anthropometric and lung function data provided for the three years prior to the procedure and one year following. Exercise testing was conducted at scheduled clinical appointments, corresponding with four time points: three-months pre-procedure (T1); two-days pre-procedure (T2); four-months post-procedure (T3) and one-year post-procedure (T4).

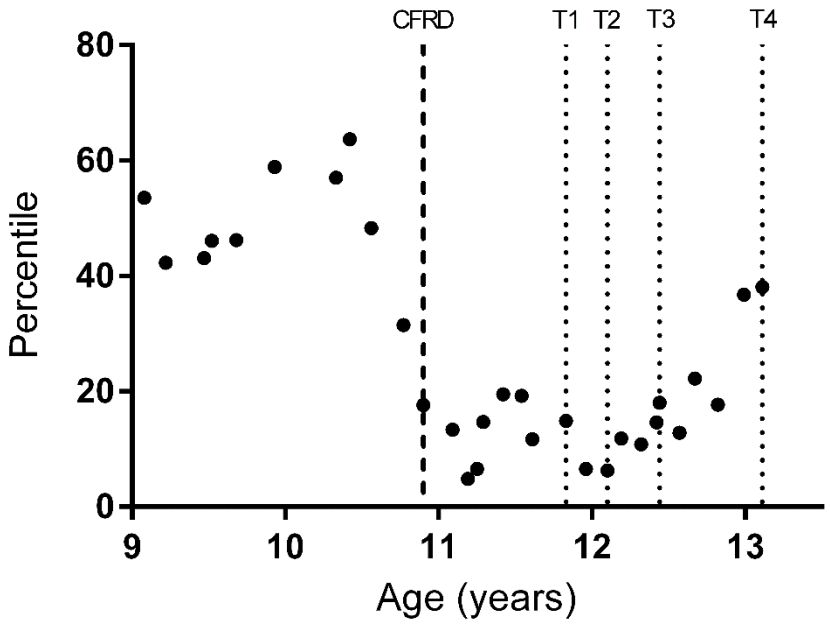

Figure 9.1 Changes in BMI as measured by percentile in the three-year period preceding the procedure and one-year following. Dashed line at 10.9 years indicates diagnosis of CFRD. Dotted lines at 11.8, 12.1, 12.4 and 13.1 years indicate T1, T2, T3 and T4 respectively. PEG inserted two-days after T2.

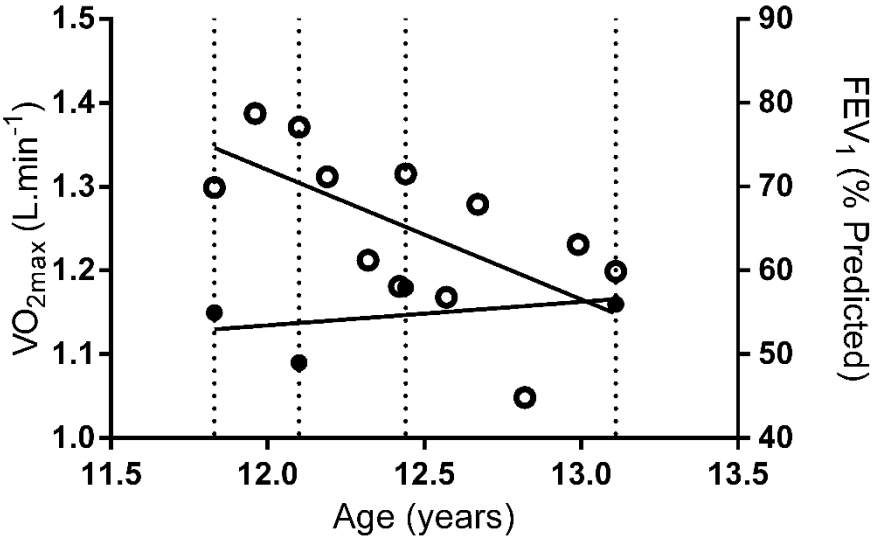

Figure 9.2 Changes in predicted FEV_1 (○) (r = -0.64) and absolute VO_{2max} (●) (r = 0.40) over the 15-month observation window of this case report. Four vertical lines indicate T1-T4. PEG inserted two-days after T2.

A fall in body mass index (BMI) from the 64th to 5th percentile in 10 months (Figure 9.1) prompted the need to investigate weight-gain methods, after conservative dietary changes (visiting the patient's school, meetings with parents and introduction of twice daily Enshake® drinks) failed. Her growth failed to respond to these non-invasive nutritional supplements, leading to consideration of overnight supplemental feeding via a PEG.

9.5 Diagnostic assessment

9.5.1 Anthropometric measures

Stature was measured to 0.1 cm (Holtain wall-mounted stadiometer; Crymych, Wales) and body weight to 0.1 kg (Seca electronic column scale; Birmingham, England), with BMI compared to normative percentiles (de Onis et al., 2007).

9.5.2 Lung function

Lung function was assessed using a hand-held spirometer (MicroPlus, Micro Medical Ltd; Rochester, UK), with maximal (best of three) values of forced expiratory volume in one-second (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio being recorded and compared to normative values (Quanjer et al., 2012).

9.5.3 Exercise parameters

The patient exercised on an electronically-braked cycle ergometer (Lode Excalibur Sport, Lode; Groningen; The Netherlands), completing a validated (Saynor et al., 2013a) combined ramp-incremental and supra-maximal test to exhaustion to determine VO_{2max} and gas exchange threshold (GET) (Saynor et al., 2014b). Measures of VO_{2max} were normalised to a percentage of predicted maximum (Bongers et al., 2014a). The same work-rate (15 $W \cdot min^{-1}$), warm-up and recovery timings were used across all tests. Pulmonary gas exchange was

assessed with a calibrated metabolic cart (Cortex Metalyzer 3B, Cortex Medical; Leipzig, Germany). Blood oxygen saturation (SpO_2) was measured throughout the test (Nellcor N-20, Medtronic; Minneapolis, USA) and subjective ratings of perceived effort (RPE) and dyspnoea (RPD) on a 1-10 scale were assessed.

9.6 Therapeutic intervention

A PEG tube was inserted under general anaesthetic into the stomach, as described previously (Russell et al., 1984). Overnight supplemental feeding with 500 mL of Fresubin® HP Energy (630 kJ/150 kcal) was subsequently introduced. Composition of the feed (per 100 mL) was: 7.5 g protein (20% total energy); 17 g carbohydrate (45%); 5.8 g fat (35%). This volume avoided interference with morning appetite and minimised vomiting risk with physiotherapy.

9.7 Follow-up and outcomes

9.7.1 Anthropometric outcomes

Anthropometric and pulmonary outcomes are shown in Table 9.1. From T1 to T2, increases in stature (+ 1.7 cm), but a fall in body weight (0.6 kg), resulted in a decrease in BMI by 0.63 $kg{\cdot}m^{-2}$ (- 8.6 percentile points). Following the PEG procedure (T3), increases in stature (+ 1.5 cm) and body weight (+ 3.5 kg), resulted in a gain of 11.7 BMI percentile points (+ 1.26 $kg{\cdot}m^{-2}$). At the one-year (T4) follow up, stature had increased by 3.2 cm relative to T2, as had body weight (+ 9.2 kg) and BMI (+ 3.02 $kg{\cdot}m^{-2}$), resulting in an increase to the 38^{th} percentile for BMI.

9.7.2 Pulmonary outcomes

Changes in lung function (Figure 9.2) showed large variation across the 15-month observation period, although an overall trend for a decline in function was evident (r = – 0.64). There was an increase in FEV_1 from T1 (69.9%) in the lead up to the

procedure (T2; 77.1%), before declining at the subsequent observations, T3 (71.5%) and T4 (59.9%).

9.7.3 Exercise outcomes

Exercise related measures are listed in Table 9.2. Time to exhaustion increased by 49% across all trials (T1 to T4), with an 18% increase observed at the one-year follow up (T2 to T4). Absolute VO_{2max} fluctuated over the 15-month period, decreasing from T1 (1.15 $L \cdot min^{-1}$) to T2 (1.09 $L \cdot min^{-1}$), before increasing at T3 (1.18 $L \cdot min^{-1}$) at T4 (1.15 $L \cdot min^{-1}$). When VO_2 was expressed relative to body mass a decrease was observed over the one-year follow up period (-16.3% from T2 to T4), a change associated with the observed weight gain. When normalised for body weight VO_{2max} decreased as a percentage of predicted from 79.3% (T2) to 66.0% (T4).

Changes were observed in relation to V_E/VO_2, with a large increase seen between T1 (34.83) and T2 (51.17). Further, but smaller, increases were then observed at T3 (53.14, +3.8%) and T4 (55.00, +7.5%), relative to T2. V_E/VCO_2 decreased over the one year follow up (T2 = 44.62, T4 = 42.53; -4.7%), although the magnitude of change was not as large as that of V_E/VO_2. Ventilation (V_E), increased from T2 (55.78 $L \cdot min^{-1}$) to T4 (63.80 $L \cdot min^{-1}$; + 14.4%).

Table 9.1 Changes in anthropometric and lung function measures over the 15-month observation period.

Variable	**T1 (3M-Pre)**	**T2 (2D-Pre)**	**T3 (4M-Post)**	***% Change from T2-T3***	**T4 (1Y-Post)**	***% Change from T2-T4***
Date	11.06.2014	19.09.2014	19.01.2015		21.09.2015	
Age (years)	11.83	12.10	12.44	*2.8*	13.11	*8.4*
Stature (cm)	146.6	148.3	149.8	*1.0*	153.0	*3.2*
Stature (Percentile)	29.7	30.2	28.6	*-5.3*	29.0	*-4.0*
Weight (kg)	33.9	33.3	36.8	*10.5*	42.5	*27.6*
BMI ($kg \cdot m^{-2}$)	15.77	15.14	16.40	*8.3*	18.16	*20.0*
BMI (Percentile)	14.9	6.3	18.0	*185.7*	38.1	*504.8*
FVC (L)	1.67	2.06	2.17	*5.3*	2.23	*8.3*
FVC ($\%_{Predicted}$)	64.3	76.5	78.3	*2.4*	75.2	*-1.7*
FEV_1 (L)	1.61	1.84	1.76	*-4.4*	1.58	*-14.1*
FEV_1 ($\%_{Predicted}$)	69.9	77.1	71.5	*-7.3*	59.9	*-22.3*
FEV_1/FVC (%)	96.41	89.32	81.11	*-9.2*	70.85	*-20.7*

Time points: 3M-Pre (3 months prior to the procedure); 2D-Pre (2 days prior to the procedure); 4M-Post (4 months following the procedure); 1Y-Post (1 year following the procedure).
BMI (body mass index); FVC (forced vital capacity); FEV_1 (forced expiratory volume in one second).

Table 9.2. Changes in exercise-related parameters over the 15-month observation period.

Variable	T1 (3M-Pre)	T2 (2D-Pre)	T3 (4M-Post)	*% Change from T2-T3*	T4 (1Y-Post)	*% Change from T2-T4*
Peak Power (W)	84	98	101	*3.1*	115	*17.4*
Exercise Duration (min)	4m 43s	5m 58s	6m 17s	*5*	7m 3s	*18*
VO_{2max} ($L \cdot min^{-1}$)	1.15	1.09	1.18	*8.3*	1.16	*6.4*
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	34	33	32	*-1.2*	27	*-16.3*
VO_{2max} ($L \cdot min^{-1}$; $\%_{Predicted}$)	63.8	59.0	62.0	*5.1*	57.6	*-2.4*
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$; $\%_{Predicted}$)	82.4	79.3	78.1	*-1.5*	66.0	*-16.8*
VCO_2 ($L \cdot min^{-1}$)	1.16	1.25	1.43	*14.4*	1.50	*20.0*
RER	1.01	1.15	1.21	*5.2*	1.29	*12.2*
V_E ($L \cdot min^{-1}$)	40.06	55.78	62.70	*12.4*	63.80	*14.4*
V_E/VO_2	34.83	51.17	53.14	*3.8*	55.00	*7.5*
V_E/VCO_2	34.53	44.62	43.85	*-1.7*	42.53	*-4.7*
HR_{max} ($beats \cdot min^{-1}$) *	196	-	175		-	
GET ($L \cdot min^{-1}$)	0.77	0.71	0.73	*2.8*	0.65	*-8.5*
GET (% VO_{2max})	67	65	62	*-4.6*	56	*-13.9*
SpO_2	96	98	94	*-4.1*	96	*-2.0*
RPE	5	6	6	*0*	4	*-33.3*
RPD	4	3	4	*33.3*	4	*33.3*

* HR_{max} only available for two tests due to equipment malfunction.

VO_{2max}: maximal oxygen uptake; VCO_2: maximal carbon dioxide production; RER: respiratory exchange ratio (VCO_2/VO_2); V_E: minute ventilation; V_E/VO_2: peak ventilatory equivalent ratio for oxygen; V_E/VCO_2: ventilatory equivalent for carbon dioxide; HR_{max}: maximal heart rate; GET: gas exchange threshold; SpO_2: arterial oxygen saturation; RPE: rating of perceived effort; RPD: rating of perceived dyspnoea

9.8 Discussion

This case report shows the inclusion of exercise-related factors amongst short term fluctuations in clinical measures, following the insertion of a gastrostomy and implementation of overnight feeding in a young CF patient.

For this patient to have been considered a 'normal' BMI (i.e. 50th percentile), she was required to weight 39.75 kg at T2. At T4, this requirement was 44.25 kg. The difference in required and achieved weight at T4 (1.75 kg) relative to T2 (6.45 kg) has justified the requirement of the PEG and supplemental feeding. Such gains are in accordance with prior gastrostomy feeding studies, which have shown similar increases in body weight (Levy et al., 1985) and BMI (Truby et al., 2009). Whilst lung function has not been shown to increase following a gastrostomy, studies have shown stabilising of function (Bradley et al., 2012, Williams et al., 1999). However, these studies only present lung function data at distant time points following such interventions (e.g. one year) and do not provide serial measurements, which may bias assessment of intervention efficacy, dependent on the patients function at the time of clinical review. This case reports all clinical visits (averaging every 41 days; range 7 – 62 days) over the 15 months follow-up period and show large fluctuation and a trend for decline in lung function.

As see in Figure 9.2, absolute VO_{2max} remained stable over the 15 months, despite the fluctuating FEV_1, highlighting the independence between the two outcomes. As exercise related factors can be predictors of mortality (Pianosi et al., 2005a) and indicators of disease severity (Thin et al., 2002) when they are very low, it is therefore important to incorporate such factors in assessing progression of disease alongside FEV_1 and BMI.

Changes in absolute VO_{2max} were minimal and fall within the typical error associated with the CPET over the medium term (4-6 weeks; (Saynor et al.,

2013b)) and as such, a minimal change in predicted absolute VO_{2max} at the one-year follow up (+0.07 $L.min^{-1}$; 59.0 to 57.6 %; Table 9.2) was observed. However, as VO_{2max} is highly dependent on body size, changes are routinely expressed relative to body weight, thus resulting in a decrease in body weight relative VO_{2max}, from T2 to T4 (-6 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; 79.3 % to 66.0 $\%_{Predicted}$; Table 9.2). This change is greater than previously observed annualised declines (Pianosi et al., 2005b) and the decline in predicted relative VO_{2max} has a greater magnitude of change than the predicted absolute VO_{2max} value. This decline is of particular relevance given its clinical implication (i.e. risk of mortality (Pianosi et al., 2005a) and hospitalisation (Pérez et al., 2014)). Whilst it would normally suggest a deconditioning effect, it could be proposed that the rapid increase in weight (+9.2 kg from T2-T4, resulting in an increase of 31.8 BMI percentile points) is driving this change and deconditioning is not in fact occurring. However, to appropriately determine and interpret such changes, an accurate assessment of body composition (e.g. skinfolds) is required. However, clinical constraints prevented such measures in the current report.

Increases in V_E without a corresponding increase in VO_{2max} (Table 9.2) indicates a reduction in the efficiency of gaseous exchange. However, given the increase in VCO_2 alongside the increase in V_E and the stability of V_E/VCO_2, it can be suggested that an increase in CO_2 release may be driving the change in ventilation. This is supported by the rise in RER from T1 to T4, suggesting an increased 'non-metabolic' increase in CO_2 at maximal exercise perhaps due to increased anaerobic metabolism, carbohydrate metabolism and/or CO_2 storage during exercise. The increase in peak power (exercise performance) indicates an increase in muscle power, but as no increase in absolute VO_{2max} was observed,

this suggests oxidative capacity of the muscle was not enhanced and a greater contribution likely originated from anaerobic metabolism.

9.9 Conclusion

This case report has provided novel data combining clinical and exercise measures in a young patient with CF following the implementation of gastrostomy feeding. Of the key measures described, BMI increased whilst relative VO_{2max} showed a decline due to body weight changes, amid a fluctuating FEV_1. Furthermore, absolute VO_{2max} remained stable against a decreased function of V_E/VO_2. The direct impact of the feeding protocol upon exercise capacity cannot be directly obtained due to the patients' clinical instability and lack of a control patient. This case report does highlight the utility of exercise and body composition testing in assessing the outcome profile of individual patients following interventions, warranting its further use in the assessment and treatment of CF.

10 PROMOTION OF EXERCISE IN THE MANAGEMENT OF CYSTIC FIBROSIS – SUMMARY OF NATIONAL MEETINGS

10.1 Abstract

Rationale, aims and objectives:

Physical activity (PA) and exercise are important in maintaining and improving health and wellbeing in people with cystic fibrosis (CF), and measures of exercise capacity are useful outcomes in monitoring disease progression. The roles and responsibilities of CF multi-disciplinary team (MDT) members in supporting PA and exercise have yet to be fully defined. This communication reports on national meetings of CF MDT staff whose interest is to improve and standardise exercise provision and testing as part of routine CF care. We also introduce the role of the physiotherapy technician in supporting PA interventions.

Meetings:

The two meetings covered a range of presentations, discussions and workshops, focusing on the role of exercise and PA in CF management. Forty people from 15 NHS Hospital Trusts and 3 universities were asked to provide feedback via a questionnaire.

Results:

The common roles and responsibilities of clinical staff involved in exercise testing and prescription are described, with a wide range of duties identified. In addition, physiotherapists were reported as the main MDT member responsible for exercise provision. The majority of teams reported discussing exercise at every clinical visit (57%) and felt confident in discussing exercise with patients (67%).

Conclusions:

Whilst this report highlights the current provision of exercise in CF MDTs, it also gives insight into the resources MDTs may require in order to enhance the profile

of exercise within CF services, including enhanced training, guidelines and standardised clinical roles.

10.2 Introduction

It is well established that physical activity (PA) (Hebestreit et al., 2014) and exercise (Bradley and Moran, 2008) are of benefit to individuals with cystic fibrosis (CF). However, exercise testing and training are currently underutilised in CF clinics due to limited resources such as time, personnel, facilities and equipment (Stevens et al., 2010), despite patients identifying the role of exercise as a top priority in the management of their condition (Rowbotham et al., 2018). This potential lack of external support can contribute to adherence issues experienced by patients with CF (Prasad and Cerny, 2002).

Whilst all members of the CF Multi-Disciplinary Team (MDT) have a role in promoting PA, a survey of CF clinics in the United Kingdom (UK) has shown that physiotherapists are the main MDT member responsible for exercise advice, testing and prescription (Stevens et al., 2010). There are a number of recommendations for the physiotherapy management of CF, which pertain to exercise testing and prescription. It is recommended that patients should have access to prescribed exercise programmes and should receive education and verbal and written support with exercise, as well as having the opportunity to exercise daily during hospital admissions (Cystic Fibrosis Trust, 2017a). In addition to this, patients should undergo an annual exercise test, with a cardiopulmonary exercise test (CPET) currently considered the gold standard (Hebestreit et al., 2015), with further PA assessment using motion sensors and questionnaires being recommended (Cystic Fibrosis Trust, 2017a).

Meeting such recommendations is only one component of the physiotherapy management of CF which also includes; CF clinic provision, musculo-skeletal

review, incontinence assessment, nasal airway treatments, airway clearance treatments and inhalation therapies. Furthermore, it is acknowledged that the assessment of PA and exercise capacity, using motion sensors and CPET respectively, can be technical and require specific expertise (Cystic Fibrosis Trust, 2017a). In supporting the role of physiotherapists, psychologists will help motivate patients, and dieticians support the nutritional requirements of physical activity and optimal body composition, therefore highlighting the potential for an exercise professional to support duties relating to the provision of exercise.

The purpose, and use, of exercise professionals within general clinical practice has been discussed previously (Franklin et al., 2009). However, unlike other clinical staff members (Brown et al., 2013, Cottrell and Burrows, 2009) the role of such exercise-based clinical staff within the CF MDT has yet to be fully defined. Such definition will best be achieved by the sharing of good practice and the standardisation of roles and procedures.

Therefore, to further understand and enhance the role of exercise provision within the CF MDT, this document reports on two meetings held in August 2016 and February 2017, of health care professionals with an interest in the importance of exercise in CF care in the UK. This document aims to report on the outcomes of these meetings, specifically; current exercise provision within the CF MDT, identifying staff members responsible for exercise promotion in the CF MDT (and their respective roles and responsibilities), reporting the requirements in terms of exercise provision of all staff involved in exercise provision within the CF MDT.

10.3 Meetings

10.3.1 Meeting 1 – August 2016

Seven delegates from five National Health Service (NHS) CF Centres and one university from across the UK attended a free one-day meeting at the Royal

Devon and Exeter NHS Foundation Trust Hospital, with the purpose of exchanging best-practice ideas and establishing a continuing network of non-physiotherapist professionals involved in utilising and promoting exercise and PA, in CF management.

Given the small number of non-physiotherapist staff involved in exercise provision in the UK, attendees were invited to this meeting based upon word of mouth and personal communications. Themes and topics discussed included; the development of a uniform job description for those in a similar, but non-affiliated position, the potential to seek affiliation to a recognised body (to set standards and govern practice), the development of a continuing network and the exchange of clinical practices, including virtual clinics (use of *Skype*), CPET and the use of technology in engaging patients in exercise. Furthermore, it was verbally agreed to advertise the network broadly and invite additional attendees to a further meeting.

10.3.2 Meeting 2 – February 2017

Following the initial meeting, it was agreed to host a second and to invite further members of the CF MDT to discuss exercise provision. Forty delegates from 15 NHS CF Centres (regional centres and networked clinics) and three universities, from across the UK, attended a free one-day event at the Children's Health and Exercise Research Centre, University of Exeter. This meeting was open to all health care professionals (30/40 attendees) and researchers (10/40 attendees) with an interest in CF and exercise and was again advertised through word of mouth and personal contacts as well as details being circulated via the Association of Chartered Physiotherapists in Cystic Fibrosis group.

The content of this meeting was discussed among attendees of the previous meeting and consisted of sessions deemed important/useful by members of the

network, including; presentations on the clinical benefits associated with exercise, exercise testing and infection control. There were also interactive workshops on exercise testing, physical activity monitoring, behavioural change and patient engagement. There was also an open discussion on the roles of staff in exercise promotion and testing. Throughout, collaboration and sharing of best practice was encouraged to allow individuals to identify where their own clinical practice and resources differed from that of others.

As part of the feedback process, attendees completed two questionnaires. The first questionnaire (Table 10.1) related to current clinical practices within their own MDT. Where multiple representatives were in attendance from the same CF centre, attendees were asked to complete one survey per centre to avoid duplication. In addition, all clinical attendees were asked to complete a further questionnaire (Table 10.2) with a non-clinical focus, relating to the running of the meeting itself.

Questions (from Table 10.1) pertained to staff members responsible for exercise testing and prescription, as well as what resources would assist with exercise provision. Questions were presented on a 5-point Likert scale (with five as the maximum score), categorical responses produced quantitative feedback and open answers allowed for qualitative feedback. Questions from a prior survey (Stevens et al., 2010) were used to provide an overview of current provision amongst CF centres represented at the meeting. Descriptive statistics, and thematic summaries of the free text qualitative responses are presented.

Table 10.1 Questionnaire relating to clinical practice.

Cystic Fibrosis and Exercise Questionnaire		
1. **Is your centre paediatric/adult/combined?**		
ADULT	PAEDIATRIC	COMBINED
2. **Who is primarily responsible for exercise provision in your MDT? Circle more than one if necessary.**		
CLINICIAN/DOCTOR	NURSE	PHYSIOLOGIST
PHYSIOTHERAPIST	EXERCISE TECHNICIAN	MULTIPLE STAFF
OTHER (PLEASE SPECIFY)		
3. **What exercise testing (if any) is currently undertaken? And by whom?**		
WALKING TEST (6 MIN)	WALKING TEST (12 MIN)	SHUTTLE TEST (INCREMENTAL)
STEP TEST	TREADMILL TEST (MAX)	CYCLE ERGOMETRY (MAX)
NONE	OTHER (PLEASE SPECIFY)	PERFORMED BY:
4. **Who is primarily responsible for exercise training/prescription in your MDT?**		
CLINICIAN/DOCTOR	NURSE	PHYSIOLOGIST
PHYSIOTHERAPIST	EXERCISE TECHNICIAN	MULTIPLE STAFF
OTHER (PLEASE SPECIFY)		
5. **How often do you currently discuss exercise prescription at clinics?**		
EVERY VISIT	REGULARLY (1 IN 2 VISITS)	RARELY (MORE THAN 1 IN 2)
ANNUAL REVIEW	ONLY WHEN PATIENT REQUESTS	NEVER
6. **What exercise advice is given? (e.g. discussions, written programmes, booklets etc.)**		

7. **How confident do you/your team feel in discussing exercise with your patients?**				
NOT CONFIDENT AT ALL				VERY CONFIDENT
1	2	3	4	5
8. **Do you feel you would benefit from additional exercise resources/training in exercise provision?**				
YES			NO	
9. **If yes, what would you like/find useful? (e.g. guidelines, video resources, apps, meetings, training, qualifications etc.)**				

Table 10.2. Questionnaire relating to study day feedback.

Study Day Feedback					
1.	**How useful was today at enhancing exercise knowledge for CF?**				
	NOT USEFUL AT ALL				VERY USEFUL
	1	2	3	4	5
2.	**How useful was the advance information (agenda, transport, communication etc.)?**				
	NOT USEFUL AT ALL				VERY USEFUL
	1	2	3	4	5
3.	**Will this help inform future practice in your own clinic?**				
	YES		NO		
4.	**If Yes – How? If No – Why not?**				
5.	**What did you find useful today?**				
6.	**What could be improved?**				
7.	**Which afternoon session did you attend?**				
	CARDIOPULMONARY EXERCISE TESTING	PHYSICAL ACTIVITY		BEHAVIOUR CHANGE	
8.	**How useful was this?**				
	NOT USEFUL AT ALL				VERY USEFUL
	1	2	3	4	5
9.	**What was useful?**				
10.	**What could be improved?**				
11.	**Would you attend a future meeting?**				
	YES		NO		
12.	**If Yes – How frequently? If No – Why not?**				

10.4 Results

10.4.1 Meeting 1

The attendees of this first meeting held different job titles, and subsequently had different responsibilities within their own MDTs, despite having an overall duty to cater for the exercise and PA needs of patients. The titles of attendees were as follows: *Therapy Practitioner in CF, Respiratory Technician, Physiotherapy Technical Instructor, Physiotherapy Technician, Therapy Assistant* and *Exercise Practitioner*. Attending staff members were from centres that were collectively responsible for 614 paediatric, and 1200 adult patients, representing 14% of the paediatric and 21% of the adult CF populations of the UK respectively (Cystic Fibrosis Trust, 2016c).

The common, and differing, roles and responsibilities of these staff members in terms of exercise provision are provided in Figure 10.1. Further discussion led to consensus among attendees that an established network of such professionals, with appropriate schemes for accreditation, training and affiliation was required. Several organisations, such as the Health and Care Professions Council, Chartered Society of Physiotherapists, the Registration Council for Clinical Physiologists and the British Association of Sport and Exercise Scientists were suggested to provide a basis for such demands.

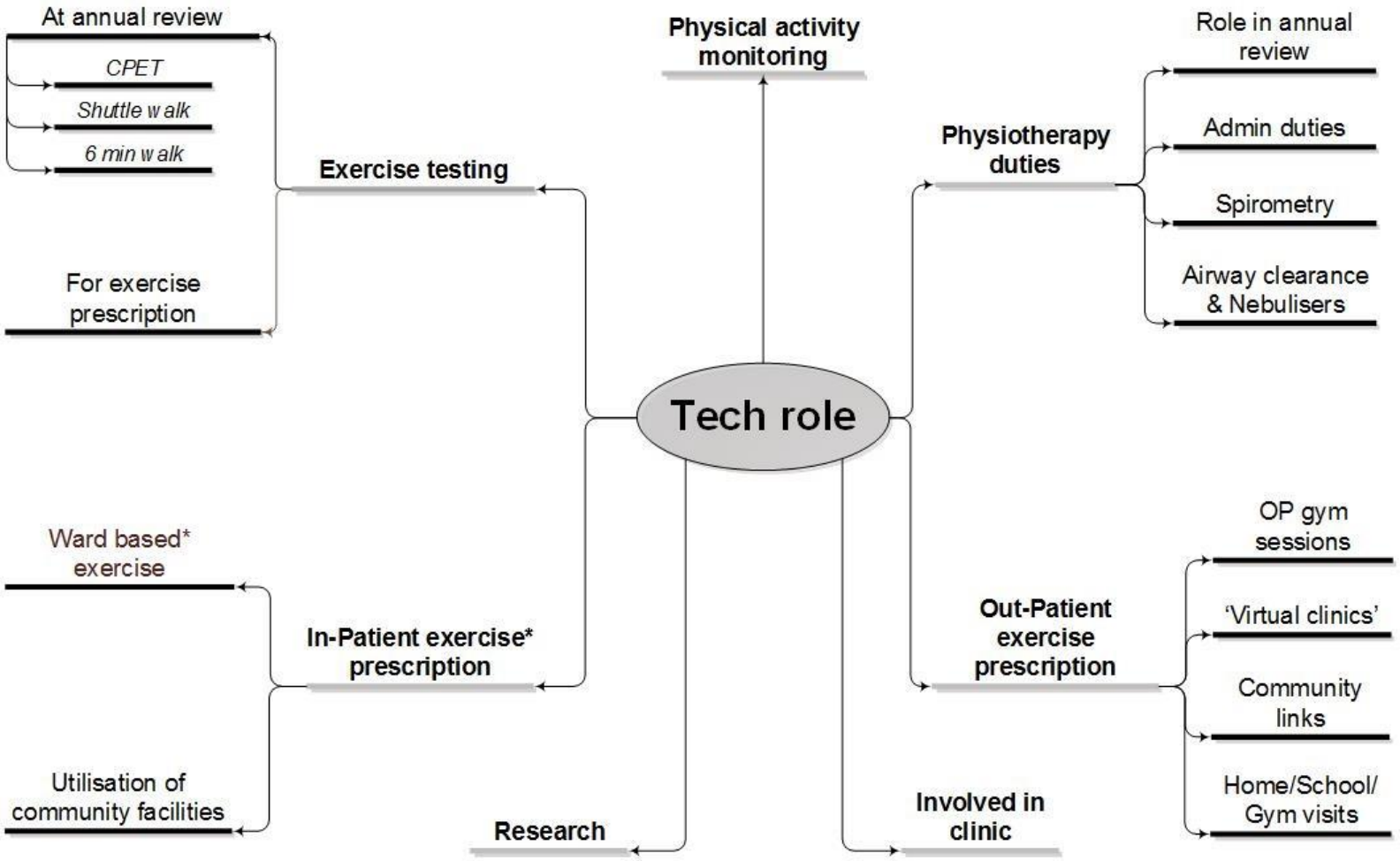

Figure 10.1 Schematic representation of the technician role within a multi-disciplinary cystic fibrosis team across multiple NHS trusts. *Common duties included in the job description of all technicians attending first meeting

10.4.2 Meeting 2

Attending clinical staff were from five adult centres (33%), seven paediatric centres (47%), and three combined centres (20%), collectively responsible for 1336 paediatric and 2153 adult patients, representing 31% of the paediatric and 38% of the adult CF population of the UK respectively (Cystic Fibrosis Trust, 2016c). Attendees represented major and networked centres from across England, Scotland and Wales were represented, with a variety of clinical roles attending, including: *Physiotherapist, Physiotherapy Assistant, Physiotherapy Technician, Therapy Technician, Physiotherapy Technical Instructor, Research Physiotherapist, Exercise Practitioner, Therapy Support Practitioner, Respiratory Clinical Physiologist* and *Consultant Paediatrician*.

Questionnaires (from Table 10.1) were returned from attendees from all 15 CF centres. Furthermore, 23/30 clinical attendees completed the questionnaire presented in Table 10.2. Ninety one percent of respondents rated the day as useful (4/5 or 5/5). Furthermore, all respondents stated that the meeting would inform future practice in their own clinics, as well as stating that they would attend a similar day in the future.

The majority of centres stated that physiotherapists were responsible for exercise testing (79%) and prescription of exercise training (75%; Figure 10.2). Fifty seven percent of MDTs discuss exercise prescription at every clinical visit; with another 29% discussing it regularly (at least alternate visits) and 14% rarely discuss it (less than alternate visits).

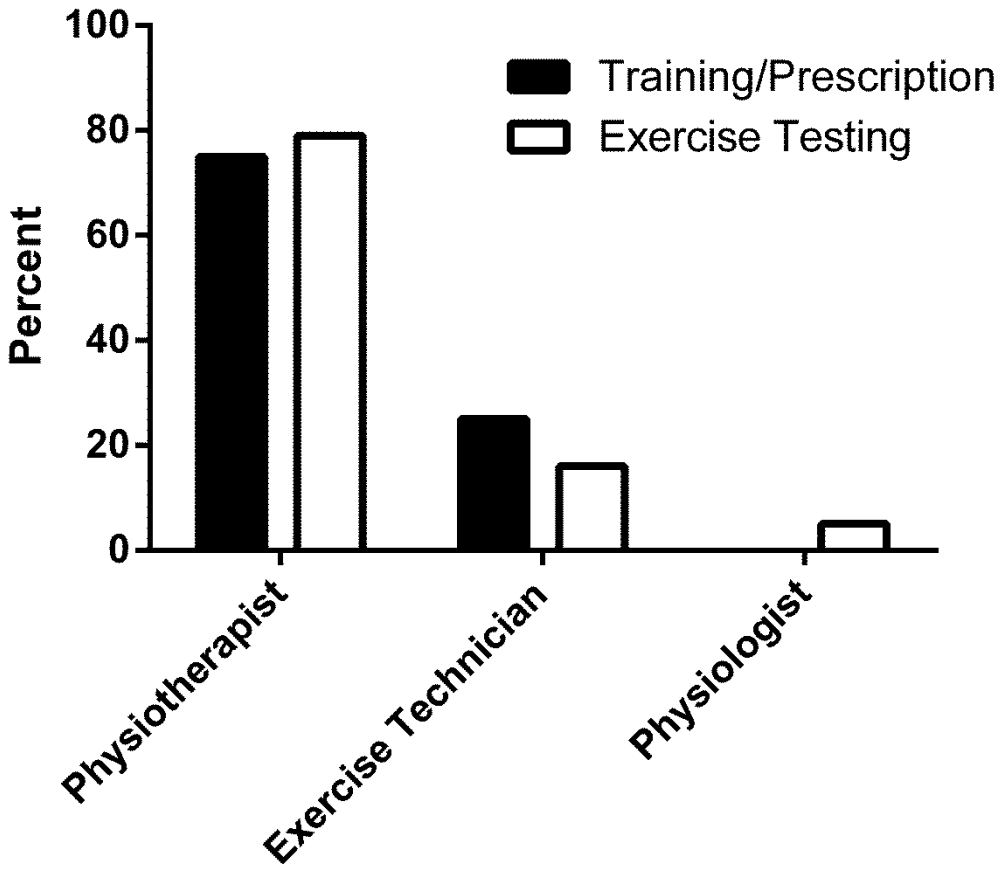

Figure 10.2 Responses to question surrounding staff members responsible for exercise within the CF MDT (Table 10.1, Q3 and Q4). More than one response was permitted if applicable.

When asked to describe what exercise advice is given to their patients, delegates reported that advice included; general discussions about exercise (*n* = 7), general education (2), information about the benefits of exercise (2), information about how to exercise (types of exercise, frequency and intensity) (2), information about guidelines (2) and information about available applications (apps) or technology (4). Two delegates mentioned encouragement and motivation, and eight delegates provided patients with written or verbal exercise programs.

Clinics performed a range of exercise tests at annual review, including gold-standard CPET (Hebestreit et al., 2015), with 4 centres (27%) using cycle ergometery and 2

centres (13%) using a treadmill. Additionally, 73% of centres performed an incremental shuttle test, 53% performed the 6 minute walk test (6MWT), and 40% performed a step test. Tests are performed by various members of staff, including physiotherapists, physiotherapy technicians (exercise technicians) and physiologists which are external to the CF MDT (i.e. respiratory clinical physiologists).

When rating confidence in discussing exercise ("How confident do you/your team feel in discussing exercise with your patients?"), 67% of respondents felt confident in discussing exercise (rating 4 or 5 out of 5; Figure 10.3). Of respondents, 100% answered 'yes' to the question "Do you feel you would benefit from additional exercise resources/training in exercise provision?".

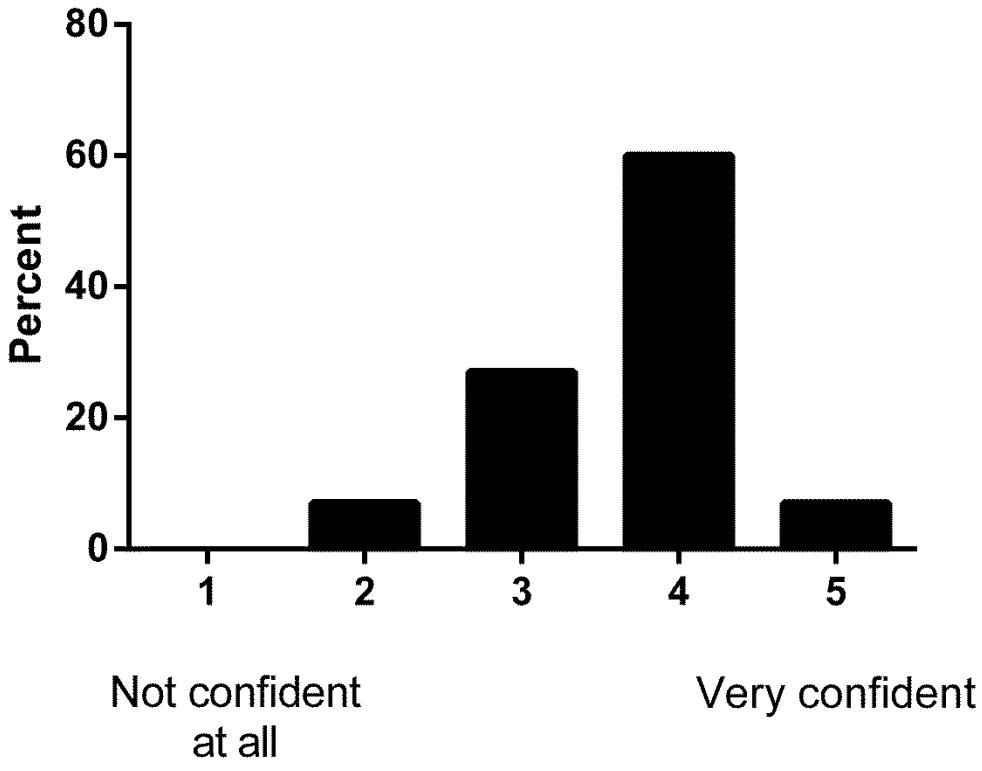

Figure 10.3 Responses to question "How confident do you/your team feel in discussing exercise with your patients?"

To elaborate on the previous question, delegates were then asked to discuss what resources would be useful with regards to exercise prescription. Six delegates noted that training courses and practical sessions would be beneficial. Videos, resources and applications were also mentioned (5). Delegates stated that the following would be useful; more meetings and other opportunities for collaborations (3), CF specific exercise guidelines (3), training on the interpretation of CPET results (1), information on how to engage patients (1), and four delegates highlighted the need for accreditation, qualifications, or standardisation of technicians roles.

10.5 Discussion

The purpose of this report was to discuss the roles and responsibilities of exercise professionals within the CF MDT; and provide quantitative and qualitative feedback from meetings of interested personnel regarding the provision of exercise within the CF MDT.

10.5.1 Roles and responsibilities

A number of NHS CF Centres in the UK now employ additional health care professionals to complement MDTs, relieve the workload of physiotherapists, and assist with exercise provision. The roles of these professionals vary in title (personal trainers, physiotherapy technicians, and physiology technicians amongst others) and responsibility, with specific duties differing depending on individual skills, the patient cohort, funding, capital infrastructure or equipment available. The first meeting of such professionals not only provided impetus for establishment of a network, but also provided the first categorical description of the different roles within the CF MDT that are responsible for exercise delivery, detailing key responsibilities, with results displayed in Figure 10.1. The only common duty amongst attending staff was the provision of in-patient and ward-based exercise. In contrast, there were a number of

tasks that were not mutually undertaken by all – involvement in clinics, research, physical activity monitoring, exercise testing, out-patient exercise and physiotherapy duties. This represents a wide array of skills that can require specialised education and training.

Given the advent of international guidance on exercise prescription (Swisher et al., 2015), refinement and implementation of defined roles within the CF MDT require further work. The physiotherapy technician/physiologist/exercise technician is potentially an important additional team member who could enable this. However, given the lack of uniformity in job descriptions, necessary qualifications, accreditation, roles, responsibilities and expectations, further discussions are warranted at both the local and national level. The practicalities of standardised service development and provision could be recognised by organisational impetus from national or international bodies (e.g. European Cystic Fibrosis Society), or by peer liaison and support – which was subsequently enabled by the secondary meeting of interested health care professionals.

Delegates at this second meeting found the day useful, citing that they would attend again. As exercise is considered a key requirement of CF management (Cystic Fibrosis Trust, 2017a), it is prudent that clinical staff are given access to courses and educational resources to enhance knowledge, and improve clinical care as well as contributing to their own continued professional development.

It is noted that physiotherapists are currently the key staff members responsible for exercise testing and prescription, which is consistent with previous findings (Stevens et al., 2010). However, whilst physiotherapists have traditionally held this role, it is worthy to note that additional health care professionals (exercise technicians and physiologists) appear to have an increasingly important role within the MDT.

Unlike Australia (Smart et al., 2016) and Canada (Warburton et al., 2013), two countries with similar prevalence of CF to the UK (Cystic Fibrosis Australia, 2016, Cystic Fibrosis Canada, 2016), there are no formal guidelines in the UK regarding ancillary exercise staff in the NHS. As the role is not a protected title (like physiotherapists), there are no formal qualification criteria, or professional affiliations required to attain such a position. Whilst advances have been made in CF Trust Standards of Care (Cystic Fibrosis Trust, 2017a) with definitions of Therapy Practitioners, recommendations stop short of detailing fully-qualified exercise professionals. Furthermore, as National CF Service Specifications (Specialised Respiratory Clinical Reference Group, Specialised Respiratory Clinical Reference Group) do not mention these roles, it is subsequently desirable for there to be a clear 'top-down' (i.e. NHS) definition of roles and responsibilities of CF MDT members in relation to their support of PA and exercise provision, and for this to include exercise technicians. The roles and responsibilities exemplified in Figure 10.1 make clear the independent nature of the role of the exercise technician (i.e. not being physiotherapists), and their unique skill set they can provide to the CF MDT.

In addition, there would be a requirement for further support from physicians, hospital management teams and policy makers to actively value, recruit and efficiently utilise such exercise technicians. However, this will only be feasible if CF centres continue to value the role of exercise testing and training and have adequate resources available to them.

10.5.2 Exercise provision

The results from meeting two provided updated evidence on the role exercise testing plays in the CF clinic. Whilst there is an increase in the utilisation of CPET since a previous survey (Stevens et al., 2010), this again may be biased by the nature of the

attending centres. However, it is encouraging to note that all centres were adhering to recommendations (Cystic Fibrosis Trust, 2017a) and performing some form of exercise test annually. Furthermore, results of this meeting also revealed the frequency with which exercise prescription is discussed with patients. Of the attending centres, 86% stated that they discuss exercise prescription with patients at least every one in two visits, if not every visit. This is an encouraging statistic, but may be biased by the fact that attending delegates may have already had an increased interest in exercise and are therefore more likely to discuss this with patients – especially if their role was that of an 'exercise technician' (or similar non-physiotherapist allied health professional). Details of what is discussed ranged from a generic "exercise is recommended", to an increased level of detail that may involve use of individualised programmes, applications, websites, diaries and even further referrals. This variety in responses provides scope for further development of standardised checklists, or a pro-forma, to guide practice and patient progress. Such a tool could be utilised by an exercise technician to prescribe individualised CF care, and would align with recent calls for 'personalised' medicine, but fundamentally remain affordable (Balfour-Lynn, 2014). However, the process required for such development and standardisation requires further investigation and collaboration.

Furthermore, it is worthy to highlight the confidence with which MDT members have in discussing exercise with patients. Of the respondents, 67% reported feeling confident in discussing exercise with patients, which is a positive finding. Contrastingly a considerable number of respondents (33%) were either neutral, or not fully confident in discussing exercise with patients – a statistic that may in turn contribute towards the fact that exercise is not always discussed with patients, as previously discussed. This results in a number of individuals that are not confident in discussing exercise, with

this number potentially being higher for individuals/MDTs that did not attend and may not place as a high a priority on exercise. Consequently, this is reflected in the fact that 100% of respondents felt they would benefit from additional, specific, training, resources, and accreditation. This is a similar response to a previous survey in German CF centres (Barker et al., 2004).

These results provide a unique insight into the current provision of exercise within CF MDTs in the UK. However, they represent an opportunistic, cross-sectional view of a limited number of NHS Trusts, and may be biased by answering questions following the study day as opposed to prior to it, and the nature of attendees themselves – already being interested in the role of exercise management of CF. A further challenge will be to engage clinicians and CF MDTs that do not place an emphasis on exercise provision; whether by choice, or necessity (i.e. funding, infrastructure).

However, the views and requests of CF MDT staff clearly suggest that more work is required to increase resources and knowledge, to ensure an increased level of confidence and ability in prescribing and discussing exercise with patients. Furthermore, it identifies the need to define and standardise roles, including new, and complementary ones.

10.6 Conclusion

The meetings discussed here have highlighted the roles and responsibilities that allied health professionals have in using exercise to manage CF in UK MDTs. Furthermore, the role exercise plays in managing CF appears to be growing, successfully heeding the advice national and international recommendations.

11 GENERAL DISCUSSION

This thesis focused on the role of CPET within the management of CF, with an emphasis on evaluating submaximal parameters as surrogates for $\dot{V}O_{2max}$; quantifying the relationship between MV and $\dot{V}O_{2max}$; and applying CPET in clinical practice for both patients and staff alike. The experimental chapters outlined within this thesis have made a distinct contribution towards the existing literature and are summarised within the broad themes outlined below. Furthermore, implications for clinical practice, strengths and limitations of the work, and topics for future investigation are also discussed.

11.1 Submaximal alternatives to $\dot{V}O_{2max}$

Whilst $\dot{V}O_{2max}$ has been established as a parameter of interest in patients with CF, given its clinical associations with mortality (Pianosi et al., 2005a) and hospitalisation (Pérez et al., 2014), there is a requirement for valid, submaximal parameters of aerobic fitness for patients unable or unwilling to perform exhaustive exercise to obtain $\dot{V}O_{2max}$ (Williams et al., 2014). A recent service evaluation identified that 14% of patients within a single, combined (adult and paediatric) CF centre, were unable to complete an annual CPET for clinical reasons, including clinical instability (requiring antibiotics), musculoskeletal problems, obesity, pregnancy/maternity and being pre/post lung transplantation (Trott et al., 2018), and therefore these submaximal parameters could be of use in this subset of patients.

This thesis sought to assess whether parameters of OUE, particularly the OUES and OUEP, could be utilised as submaximal, alternative, measures of aerobic power (as opposed to $\dot{V}O_{2max}$). The advantage of using the OUES is that it removes the curvilinear ventilatory response observed during incremental exercise, allowing for a direct comparison between individuals over the course of an entire test, or identify

changes within an individual over time. Furthermore, its submaximal nature ensures the OUES is an effort independent test as it does not require the patient to work to volitional maximum, as the ventilatory efficiency can be measured up to any point during an incremental test, such as the metabolic boundaries of the GET or RCP. As a result, the OUES has been widely characterised in clinical populations, having originally been developed as an objective and independent measure of aerobic fitness for use in patients with cardiac disease (Baba et al., 1996), before being further applied in heart failure (Van Laethem et al., 2005), multiple sclerosis (Heine et al., 2014), COPD (Barron et al., 2016) and adults with CF (Gruet et al., 2010). The only previous study to evaluate OUES in children with CF concluded that it was OUES was an invalid measure of cardiopulmonary exercise capacity (Bongers et al., 2012). However, there were a number of aforementioned methodological issues with the study of Bongers et al. (2012), pertaining to scaling of OUES, and use of time to exhaustion (as opposed to metabolically matched thresholds) to characterise OUES. These limitations were addressed in the current thesis.

Results from Chapter 4 indicated that: a) OUES was significantly correlated with stature, body mass and BSA; b) ratio-standard scaling was ineffective in removing residual effects of body-size, and that allometric scaling was successful; and c) allometric scaling for body mass and/or BSA best controlled for body size when OUES was utilised as a parameter of interest in CF. When OUES was assessed (in Chapter 5) using appropriately scaled variables (i.e. $OUES/BSA^{1.40}$) and at metabolic boundaries of the GET and RCP, it was not different between: a) CF and CON groups at each metabolic threshold and duration marker; b) between groups when split into tertiles of $\dot{V}O_{2max}$; nor c) within groups when split into tertiles of $\dot{V}O_{2max}$. Therefore, it was concluded that the OUES cannot be used as a submaximal surrogate of $\dot{V}O_{2max}$

in children and adolescents with CF. However, because this study was only undertaken in children and adolescents with mild-to-moderate CF and it is unclear as to whether the same results would be observed in patients with severe disease. Given the relationship between $\dot{V}O_{2peak}$ and FEV_1 has been shown to be greater when $FEV_1 \leq 50\ \%_{Predicted}$ (R^2 = 31%), as opposed to when $FEV_1 > 50\ \%_{Predicted}$ (R^2 = 18% Pastre et al. (2014)), it is feasible the relationship between alternative forms of aerobic power (such as OUES) may be influenced by disease severity.

The requirement to perform a logarithmic transformation of $\dot{V}_E$ in order to obtain a value for OUES requires additional analysis time from clinical staff, which can therefore reduce the appeal for using the OUES. In contrast, the simplicity of calculating the OUEP by using a direct ratio between $\dot{V}_E$ and $\dot{V}O_2$ enhances the appeal of this measure for clinical teams. However, there are no reference data in disease groups for this parameter in contrast to the OUES, having only been described in healthy children (Bongers et al., 2015a). Therefore, this thesis explored whether parameters of OUE could be used as a submaximal surrogate for $\dot{V}O_{2peak}$ (Chapter 6). All parameters of OUE were significantly ($p < 0.001$) reduced in CF, indicating a sensitivity to disease status that did not occur with parameters of OUES. Furthermore, even though $\dot{V}O_{2peak}$ was marginally different between groups within this thesis, OUEP was consistently lower in the CF group, with only 11% of matched pairs having both a greater $\dot{V}O_{2peak}$ and OUEP within the individual with CF, showing that regardless of fitness status, OUEP is reduced in CF, thus suggesting a sensitivity to aerobic fitness independent of $\dot{V}O_{2peak}$ that warrants further investigation. In addition, absolute $\dot{V}O_{2peak}$ was also significantly and positively correlated with OUEP (r = 0.43, p = 0.010). However, when split by tertiles of aerobic fitness, the ability to discriminate aerobic fitness within groups was limited and therefore it was concluded that parameters of

OUE were not valid submaximal surrogates for $\dot{V}O_{2peak}$. However, as per Chapter 5, these findings are only applicable to children and adolescents with mild-to-moderate CF, and it is unknown as to whether the same result would be found in patients with severe CF.

As CF is characterised by pulmonary dysfunction and a progressive decline in lung function, it is feasible that the variation in lung function within the CF group may impact upon parameters of ventilation during exercise. Previous research has shown that children with lower lung function (50-60 $\%_{Predicted}$) augment increases in $\dot{V}_E$ by increasing breathing frequency, with minimal increases in tidal volume (Keochkerian et al., 2008). This indicates a mechanical restriction to exercise performance and can in turn suggest the presence of dynamic hyperinflation. Whilst a rapid and shallow breathing pattern has a lower energetic cost relative to deep breathing (Younes and Burks, 1985), the associated alveolar ventilation is less efficient, due to the increased dead space ventilation that occurs during exercise (Thin et al., 2004). Therefore, as the OUES is a direct function of $\dot{V}_E$, the disease status of individuals with CF and compromised airways (including increased ventilatory dead space) may unduly bias the $\dot{V}_E$ observed during exercise as less physiological space is available for gaseous exchange within the lung. This, therefore, may account for the conclusion that OUES, a useful parameter in cardiac conditions, is not valid in CF. In support of this explanation, the study undertaken by Bongers et al. (2012), identified that the mean ratio of residual volume relative to total lung capacity was elevated in CF (153 ± 43 $\%_{Predicted}$; range = 93 – 276 $\%_{Predicted}$). However, dead space ventilation was not measured in the studies that compromise this thesis, and so these possible pathophysiological explanations cannot be completely confirmed within the context of the present results.

In addition to issues surrounding disease severity that may account for the invalidity of OUES as an alternative for aerobic power, it is also possible that the relatively poor level of repeatability (relative to OUEP) may further account for the observed invalidity. The CV, as determined by Bongers et al. (2015a), for OUES has been shown to be 32.9%, whereas it is 10.9% for OUEP. Even when controlled for BSA (using ratio-standard scaling), the CV of the OUES reduces, but remains higher than OUEP, at 18.3%. It is unclear whether using allometric scaling has further reduced the CV of the OUES in the current thesis, however it is feasible that the reduced CV for OUEP – whose magnitude is in line with other parameters of aerobic power (Saynor et al., 2013b) – may account for its greater potential as a submaximal surrogate for aerobic power relative to the OUES.

Whilst this thesis has concluded that OUES is not valid as submaximal surrogates for $\dot{V}O_{2peak}$ in CF, the association between FEV_1 and OUEP in Chapter 6 indicates this parameter is sensitive to disease status and severity. Therefore, future research is warranted to identify any clinical utility of OUEP beyond acting as a surrogate for $\dot{V}O_{2peak}$, for example longitudinal changes and any independence from simultaneous changes in FEV_1 with disease progression. Additionally, alternative submaximal parameters such as ventilatory drive warrant further investigation in this population given its association with mortality (Hulzebos et al., 2014), low variability (Sun et al., 2002) and superior prognostic value relative to OUES in a cardiac population (Arena et al., 2007).

11.2 Using CPET to identify musculoskeletal limitations to exercise

The findings of these initial chapters clearly identified that $\dot{V}O_{2max}$ remains the primary variable of interest from CPET with which to measure maximal exercise capacity. This is due to the validity and reliability of obtaining this parameter (Saynor et al., 2013a,

Saynor et al., 2013b) and lack of suitable submaximal alternatives (Tomlinson et al., 2018, Williams et al., 2018) – although there appears to be some detail that could be added by use of OUE given its association with disease status and severity. Therefore, attention must also be given to CPET and $\dot{V}O_{2max}$ as a diagnostic too, in order to identify limitations to exercise capacity and enhance and improve patient care and outcomes.

CPET is acknowledged for its use as a differential tool for evaluating undiagnosed exercise intolerance (American Thoracic Society, 2003) and can aid in the identification of cardiac, pulmonary and musculoskeletal limitations to exercise in individuals with chronic disease. Identifying these symptom limitations therefore allow for appropriate, personalised, exercise prescription to counter the dysfunction in the identified system. Whilst this has clear applications for individuals, use of CPET on a wider, population level allows for broader causes of general exercise intolerance.

As noted in Chapter 2.3, there are several factors associated with exercise limitation in CF (Hulzebos et al., 2015). As CF is predominantly a pulmonary disease, the influence of lung function cannot be discounted. However, as previously discussed, the shared variance (R^2) between FEV_1 and $\dot{V}O_{2peak}$ in patients with mild-to-moderate CF ($FEV_1 \geq 50\ \%_{Predicted}$) is 18% (in a positive direction), and therefore leaves a further ~80% of this variance unaccounted for (Pastre et al., 2014). Therefore, this thesis has utilised group level data of $\dot{V}O_{2max}$ and MV to ascertain the musculoskeletal contributions towards reduced $\dot{V}O_{2max}$ in CF in an attempt to address the 'quality' vs. 'quantity' debate within the literature (Hulzebos et al., 2017, Rodriguez-Miguelez et al., 2017). The strengths and novelty of the methods employed within this thesis surround the use of accurately determined $\dot{V}O_{2max}$ and MV, identified using gold-standard CPET (with supramaximal verification bouts) and MRI.

To calculate MV, this thesis expanded upon previous work in adults that identified large SEE when using mCSA to estimate MV (27%; Morse et al., 2007), by identifying a lower SEE of 14% in children (Chapter 7). However, to use mCSA as a parameter to estimate MV necessitates acquisition of such measures via MRI which is a costly and lengthy process. In contrast, tCSA can be obtained easily by clinical teams through surface anthropometry, although this thesis identified an even greater error associated with using TV (~40%) and therefore, summation of multiple mCSA slices is required to accurately quantify MV. The error associated with quantifying MV in Chapter 7 was broadly in line with previous studies in adults (Barnouin et al., 2015, Nordez et al., 2009, Tracy et al., 2003) and this was the first time such error was quantified in children and adolescents, as well as those with CF. Use of this volume data is more reflective of metabolically active muscle during exercise and therefore, when $\dot{V}O_{2max}$ is allometrically scaled using these parameters of MV, the resultant reduced $\dot{V}O_{2max}$ can be attributed to a 'qualitative' defect with greater confidence than the study of Moser et al. (2000) whose use of ratio-standard scaling (and mCSA) may not have accurately reflected, and controlled for, effects of muscle size (Chapter 8).

The results from the present thesis support aforementioned *in vitro* studies showing lower resting ATP and Ca^{2+} dysregulation (Lamhonwah et al., 2010) and mitochondrial dysfunction (Valdivieso et al., 2012) in CF muscle, as well as *in vivo* studies showing vascular dysfunction (Poore et al., 2013, Rodriguez-Miguelez et al., 2016). However, not all studies have shown a difference between CF and non-CF controls with regards to a 'qualitative' defect in CF muscle. A study conducted by Werkman et al. (2015) utilised ^{31}P-MRS to non-invasively assess changes in muscle metabolism during incremental exercise in a group of adolescents with CF and age-matched CON. This study reported no difference between groups in end-exercise PCr or inorganic

phosphate (P_i) following incremental exercise, nor a difference in the time constants of PCr recovery. Furthermore, the patients with CF in the Werkman et al. (2015) study were similar to those in the current thesis, with preserved pulmonary function (FEV_1: 92.8 ± 14.6 $\%_{Predicted}$) and matched PA, albeit with a reduced amount spent in higher intensities relative to CON, using a self-assessed subjective questionnaire validated for use in CF (Wells et al., 2008b). However, whilst this study does appear to conflict with the present thesis by providing *in vivo* data during exercise on metabolic markers, it could be proposed that the patients with CF, as well as CON, failed to reach a maximal $\dot{V}O_2$ and were therefore exercising at a lower relative intensity. No supramaximal verification was undertaken to confirm $\dot{V}O_{2peak}$, and the HR_{max} reached in both groups (CF, 162 ± 12; CON, 164 ± 9 beats·min^{-1}) was lower than the secondary criteria typically used to confirm a maximal effort (Hebestreit et al., 2015); although it is acknowledged that secondary criteria cannot be wholly relied upon for such verification (Saynor et al., 2013a). Therefore, given this lower relative exercise intensity within Werkman et al. (2015), it may be proposed that exercise dysfunction observed in CF is intensity-dependent, and that muscle 'quality' may only be defective in heavier domains. This is supported by slowed oxygen uptake kinetics in adolescents with mild-to-moderate CF during very-heavy intensity (60%Δ), but not moderate intensity (90% GET), exercise (Saynor et al., 2016b). Furthermore, patients with CF have been reported to have slower PCr recovery following 90-seconds of high intensity exercise relative to CON participants, but no differences in PCr recover following a five-minute moderate intensity bout (Wells et al., 2011). These studies therefore provide evidence that qualitative defects in skeletal muscle become evident at higher metabolic rates in CF.

Whilst the present thesis has not confirmed these findings, the diagnostic use of CPET has furthered the evidence for such a 'qualitative' defect in CF. To further investigate the cause of exercise intolerance in CF, multi-modal assessments are required (Gruet and Saynor, 2017) and therefore, inclusion of modern techniques such as aforementioned MRI and ^{31}P-MRS, as well as blood-oxygen level dependent imaging, NIRS, echocardiography and LCI can be used in conjunction with one another to provide greater detail on univariate and multivariate causes of exercise intolerance and move this area of investigation forward.

11.3 Applying CPET in clinical practice

This thesis has successfully shown that CPET can be used in clinical practice to monitor individual patient treatment, but that its implementation as a standard prognostic and diagnostic test is still not universally applied across the NHS. CPET has been previously utilised to assess outcomes associated with antibiotic therapy (Alison et al., 1994, Selvadurai et al., 2002a), CFTR modulator therapy (Edgeworth et al., 2017, Saynor et al., 2014a) and lung transplantation (Oelberg et al., 1998), and this thesis provides the first reported application of CPET in evaluating the efficacy of insertion of a PEG and a supplemental feeding regimen (Chapter 9). The results clearly indicate a gain in body mass, although it is unclear as whether this is driven by gains in muscle mass, fat mass, or a more likely combination of both.

Assessing body composition in CF is complicated by the variances in body size seen within the patient population (Hanna and Weiner, 2015). Studies have found conflicting results with regards to the validity of different methods. Studies suggest skin-fold equations of Slaughter et al. (1988) may be suitable, although there are wide 95% limits of agreement for percentage body-fat (-0.7 ± 6.9 %) and lean body mass (0.4 ± 3.2 kg) when compared against dual energy x-ray absorptiometry (DEXA) (Wells et

al., 2008a). Furthermore, use of bio-electrical impedance (BIA) has been shown to have poor agreement with DEXA (Ziai et al., 2014) and skin-fold equations (Alicandro et al., 2015), with a mean bias of 10% and up to 19% respectively, and therefore body composition is not routinely undertaken within the CF clinic, nor is it recommended because of the lack of validated techniques and low-cost equipment.(Cystic Fibrosis Trust, 2016a). Therefore, in the absence of these techniques, a stable $\dot{V}O_{2max}$ can be used to infer no change in aerobic contributions to exercise, and the increased WR_{peak} – alongside changes in $\dot{V}_E$, $\dot{V}_E/\dot{V}CO_2$ and RER – indicate an increased anaerobic contribution to exercise and are likely due to increased muscle mass as a result of the supplemental feeding. This case has shown that CPET can be utilised to infer mechanisms of change following interventions.

For similar applications of CPET to be undertaken in clinical practice, staff within the NHS must be adequately resourced and trained to implement exercise testing. This thesis has identified that CPET is undertaken in CF centres, in agreement with Stevens et al. (2010), although the self-selective nature of centres attending the training days in Chapter 10 means that this finding is not necessarily representative of the wider NHS. Therefore, a wider survey is required, preferably with an international response in light of international recommendations (Hebestreit et al., 2015) to gauge prevalence of CPET across CF centres.

The findings of Chapter 10 further identified that additional resources and training are required to improve knowledge and understanding. Whilst the 'Exercise is Medicine'® initiative has been present for a number of years (Lobelo et al., 2014), it would appear education pertaining to exercise and PA is lacking, with over 50% of medical schools in the USA not providing courses on PA (Cardinal et al., 2015). This is reflective of GP's understanding of PA in the UK, whereby 55% report not having any training with

regards to encouraging PA, and 80% being unfamiliar with current guidelines (Chatterjee et al., 2017). Of medical students currently undergoing training in the UK, a survey has shown that 74% believe they have received sufficient teaching about the benefits of PA, although only 52% stat they feel adequately trained to provide PA advice (Dunlop and Murray, 2013). To combat this dearth of knowledge, the 'Exercise Works!' initiative has developed in order to develop training resources surrounding PA and exercise for medical schools in the UK (Gates, 2015, Gates, 2016), although it is unclear how much detail this programme will provide on the topics of exercise testing and exercise prescription. In physiotherapy, curricula have a greater provision of exercise testing and prescription, with 100% of courses in Ireland providing some form of exercise prescription content (O'Donoghue et al., 2011), although 66% of practitioners are unhappy with their knowledge and require further training (O'Donoghue et al., 2012). Unfortunately, data is not available for UK physiotherapy curricula, and therefore future research is required. However, a survey of UK physiotherapists reveals that 77% routinely discuss PA with patients and 68% routinely deliver brief interventions (Lowe et al., 2017), further indicating that front-line staff can, and do, discuss PA with patients. However, such discussions are largely focused on short-term restoration of function (Lowe et al., 2018) and barriers such as time restraints and a focus on discharge (Walkeden and Walker, 2015) can limit the effectiveness of discussions and interventions. Therefore, whilst physiotherapists have historically been responsible for exercise testing (Stevens et al., 2010), the role of exercise therapists, therapy practitioners and therapy assistants, as per Chapter 10, have an increasingly valuable role to play in exercise for provision for CF and training and continuing professional development is needed for this specialist sub-group of NHS staff. In addition, the NHS has developed a national 'Scientist Training

Programme' to train individuals as clinical scientists, with respiratory and sleep science being a sub-speciality that seeks to employ use of CPET as a diagnostic and prognostic tool (National School of Healthcare Science, 2018).

11.4 Strengths and limitations

The methodological strengths associated with the studies are related to statistical control for body size, and use of 'gold-standard' CPET. As with any studies involving paediatric participants, a wide range in body size can bias results, which is further compounded by the range of body sizes observed in CF (Hanna and Weiner, 2015). Therefore, use of allometric scaling within this thesis has allowed for removal of residual effects of body size from parameters of aerobic function (OUES, Chapter 4; $\dot{V}O_{2max}$, Chapter 8) and removes potential sources of bias in the results. Furthermore, in contrast to international recommendations that advocate use of the Godfrey (1971) protocol (Hebestreit et al., 2015), this thesis has utilised, where possible, a valid and reliable ramp-S_{max} protocol to verify $\dot{V}O_{2max}$ had been achieved (Saynor et al., 2013a, Saynor et al., 2013b). This verification of $\dot{V}O_{2max}$ ensures that a 'true' maximal value is obtained and there is no risk of a submaximal $\dot{V}O_{2peak}$ being carried forward for analysis. Furthermore, this thesis has used MRI as a gold-standard technique that to ensure accurate measures of MV have been obtained and carried forward for analysis, as opposed to a reliance on CSA, which has previously been shown to have a high level of error (Morse et al., 2007) and may not appropriately reflect the metabolically active muscle during exercise.

Of the limitations associated with this thesis, the first to be addressed is that the majority of participants presented with mild-to-moderate disease only ($FEV_1 \geq 40\ \%_{Predicted}$). This therefore means that findings may not necessarily be applied to those with severe CF ($FEV_1 < 40\ \%_{Predicted}$) as the shared variance between exercise

capacity and FEV_1 is increased when FEV_1 <50 $\%_{Predicted}$ in CF, relative to those with an FEV_1 >50 $\%_{Predicted}$ (Pastre et al., 2014). Furthermore, these participants were recruited from a single CF centre, with a history of a high median FEV_1, high median BMI and low rates of chronic *Pseudomonas aeruginosa* colonisation (Figure 11.1). Whilst this ensures that patients recruited for the current thesis all received similar care and advice, it also partially limits the external validity of findings to the wider UK CF community.

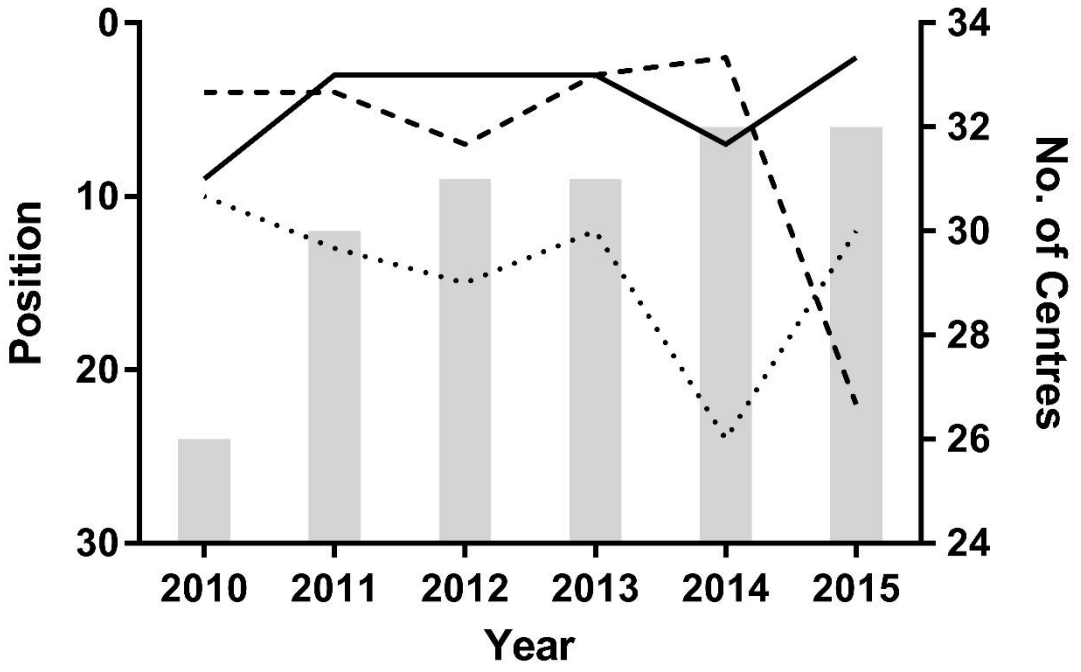

Figure 11.1 Annual rankings for paediatric (<16 years) patients with cystic fibrosis under the care of the Royal Devon & Exeter NHS Foundation Trust Hospital, and associated networked clinics (North Devon District Hospital, Barnstaple and Torbay District General Hospital, Torquay). Median FEV_1 ($\%_{Predicted}$) amongst patients ≥ 6 years, with no history of lung transplantation using Global Lung Index 2012 equations (Quanjer et al., 2012) (solid line). Median body mass index percentile among patients 2-15 years (dashed line). Proportion of patients with chronic *Pseudomonas aeruginosa* (dotted line). Number of centres reporting data represented by grey bars. Data only available until 2015 as ranking ceased by Cystic Fibrosis Trust in favour of funnel plots, which account for centre size.

The second limitation to be addressed is the validity associated with measures of somatic maturation used within this thesis. Due to the retrospective nature of analyses in Chapters 4-6, pubertal stage data based upon secondary sex characteristics (Marshall and Tanner, 1969, Marshall and Tanner, 1970) were not available and therefore aPHV has been presented as a measure of somatic maturation in lieu where applicable. However, the association between secondary sexual characteristics and aPHV differs between sexes, with the majority of girls (69.1%) having reached PHV by the time they are pubertal stage 3, whereas the majority of boys (58.9%) reach PHV by pubertal stage 4 (Granados et al., 2015). The variability in timing of PHV suggest that it is part of a dynamic process and that reliance upon a single measure of maturation may not be appropriate, especially when researching mixed groups of children (i.e. boys and girls). Furthermore, use of aPHV as a continuous variable may be invalid (Malina and Koziel, 2014a, Malina and Koziel, 2014b), and therefore it may only provide categorical information about the cohort of children being studied (i.e. pre-, or post-aPHV). Finally, common equations to establish aPHV (e.g. Mirwald et al. (2002), Moore et al. (2015)) have been developed and validated in groups of healthy children and therefore their validity in children with CF remains unknown, especially given that pubertal maturation can be delayed and slowed in children with CF (Zhang et al., 2013).

The final limitation to be acknowledged is the amalgamation of data for male and female aerobic power (Chapters 4, 5, 6 and 8). Whilst $\dot{V}O_{2peak}$ has been shown to greater in boys than girls (e.g. Armstrong et al. (1991)), the same difference has yet to be shown in children and adolescents with mild-to-moderate CF. A training study in adults with CF by Gruber et al. (2011b) identified a greater $\dot{V}O_{2peak}$ in men compared to women (32.1 ± 8.9 vs. 29.5 ± 7.9 $mL \cdot kg^{-1} \cdot min^{-1}$, $p < 0.001$), although this difference

is only partially explained by clinical factors as males had significantly higher BMI, yet females had significantly FEV_1 ($\%_{Predicted}$). It is unclear whether the same difference would be observed in children and adolescents, especially as a number of clinical factors may bias the analyses (e.g. FEV_1, BMI, pancreatic (in)sufficiency) and the need to be statistically controlled for. Whilst an optimal approach to analyses within the current thesis would involve separating male and female data, the sample sizes (e.g. male = 21, female = 15; Chapters 4-6) per group would reduce statistical power and require a greater number of comparisons between groups to identify mean differences; a process whereby statistical corrections can falsely inflate Type 2 error. In lieu of further separating CF and CON groups by sex, the studies within the thesis have sought to deliberately age- and sex-match children and adolescents with CF against health controls to prospectively remove these confounding effects. Future research is warranted to examine the difference between boys and girls with CF whilst correcting for aforementioned clinical factors.

11.5 Recommendations for clinical practice

The novel findings from this thesis have several implications for clinical practice. Because of the findings of Chapters 4-6, $\dot{V}O_{2max}$ remains the primary clinical parameter that clinical team should strive to identify in patients with CF; although future research is warranted with regards to OUE, given its sensitivity to disease status and severity. Use of $\dot{V}O_{2max}$ does therefore require a maximal CPET to be conducted, in line with international recommendation (Hebestreit et al., 2015). Whilst there is debate on the exact protocol to be utilised to elicit such a maximal response (Saynor et al., 2016a), it is still acknowledged that $\dot{V}O_{2peak}$ (and where possible, $\dot{V}O_{2max}$) is the primary outcome. How $\dot{V}O_{2peak}$ should be presented however, is still variable between studies, as it has been presented in absolute terms, and using ratio-standard scaling relative

to body mass and fat-free mass. Furthermore, standardisation of predicted values is required, to ensure uniform reporting across centres and research studies, as studies to date have utilised differing equations, whilst some studies have failed to reference which equations have been used (e.g. Table 2.2). Although numerous prediction equations are available (Paap and Takken, 2014), a comprehensive evaluation and validation has yet to occur in CF. Until such standardisation is set, research studies and clinicians should utilise reference values that are most appropriate for the study and/or patient group at hand. The present thesis utilised reference values of Bongers et al. (2014a) for this very reason, as equations had been developed in both young boys and girls, using cycle ergometry to elicit $\dot{V}O_{2peak}$.

The results of Chapters 7 and 8, identifying a probable 'qualitative' defect in skeletal muscle function has implications for exercise training in CF (Gruet et al., 2017), and places an emphasis upon aerobic training and intensity-specific exercise regimens (i.e. to a set percentage of $\dot{V}O_{2max}$). This prescription of exercise intensity (using $\dot{V}O_{2max}$) has only been undertaken by a small number of training studies to date (e.g. Beaudoin et al. (2017), Kriemler et al. (2013)). Otherwise, training studies have utilised percentages of HR_{max} (e.g. Elbasan et al. (2012), Orenstein et al. (2004), Schmidt et al. (2011)), or percentage of WR_{peak} (Gulmans et al., 1999), although these approaches do not ensure that training is occurring at the same metabolic intensity for all patients. These discrepancies in study design may provide an explanation as to why exercise training programmes have failed to find an optimal modality or intensity for patients with CF, particularly with reference to aerobic training (Radtke et al., 2017b).

However, resistance training to increase muscle volume must not be fully discounted. Whilst muscular hypertrophy may not directly increase $\dot{V}O_{2max}$, muscle size remains a

clinically important parameter. Increased muscle size is associated with favourable post-operative outcomes following lung transplantation such as length of time on mechanical ventilation and time in intensive care (Weig et al., 2016), as well as recovery of exercise capacity (Walsh et al., 2013).

The application of patient-centred CPET and its implementation in CF clinics indicates that this is a useful tool to take forward and implement at annual reviews for patients, to monitor longitudinal changes in function and evaluate responses to therapeutic treatments such as pharmacological, nutritional, or surgical procedures. Importantly, the outcomes associated with CPET (such as $\dot{V}O_{2max}$) can be used independently of traditional factors, such as BMI and FEV_1, to provide a holistic analysis of a patient's condition and disease trajectory.

11.6 Conclusion

This thesis has provided a number of experimental chapters, encompassing prognostic, diagnostic and practical applications of CPET in the management of CF. Specifically, it has: a) provided novel insight into submaximal markers obtained from CPET; b) furthered evidence for a defective 'qualitative' musculoskeletal contribution towards exercise (in)tolerance; and c) provided a novel patient-centred application of CPET, as well as updated information regarding the role of CPET in the UK CF clinic and the staffing requirements for implementation of this testing modality.

Exercise testing is essential for successful monitoring of disease status and progression in CF (Cystic Fibrosis Trust, 2017a, Hebestreit et al., 2015), and the endorsement of CPET as the method of choice by the ECFS and ERS is a welcome one (Hebestreit et al., 2015). However, further research is still warranted on the utility of submaximal measures obtained from CPET, and their clinical associations and significance. As the CF population grows and ages (Keogh et al., 2018), the demand

for exercise testing, and CPET, is also set to increase. This will be particularly pertinent amongst patients of greater disease severity, who are typically excluded from small-scale observational studies, but stand to benefit from clinical decisions made from CPET results. These could include listing for transplantation, change of medication to include CFTR modulators, or even re-classification of their disease status and treatment if $\dot{V}O_{2max}$ is shown to be independent of FEV_1 in some individuals. Furthermore, to facilitate the incorporation of CPET into the UK CF clinic, reference data must be developed to ensure accurate interpretation in patients of all disease status and severity. In addition, multimodal assessments of aerobic function are required (Gruet and Saynor, 2017), to truly identify causes of exercise intolerance in CF and provide therapeutic treatment options for future patients.

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APPENDICES

Appendix A

NHS
Health Research Authority
NRES Committee South West - Exeter
Bristol Research Ethics Committee Centre
Whitefriars
Level 3
Block B
Lewins Mead
Bristol
BS1 2NT

Telephone: 01173421380
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02 May 2014

Miss Jamille Paschoal-Vicente
Children's Health and Exercise Research Centre
University of Exeter, St. Luke's Campus, Heavitree Road,
Exeter, Devon
EX1 2LU

Dear Miss Paschoal-Vicente

Study title:	**Direct investigation of skeletal muscle metabolic, pH and blood oxygenation exercise response of young cystic fibrosis and non-cystic fibrosis bronchiectatic patients**
REC reference:	**14/SW/0061**
IRAS project ID:	**147576**

Thank you for your letter of 22 April 2014, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC at a meeting held on 30th April 2014. A list of the sub-committee members is attached.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager Mrs Kirsten Peck, nrescommittee.southwest-cornwall-plymouth@nhs.net.

Confirmation of ethical opinion

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

as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

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

Approved documents

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

Document	*Version*	*Date*
Covering Letter		22 April 2014
Evidence of insurance or indemnity		
Investigator CV		
Letter of invitation to participant	Control - 2 - Clean & Tracked	15 April 2014
Letter of invitation to participant	Patient - 2 - Clean & Tracked	15 April 2014
Other: Letter from funder		16 January 2014
Other: Maturation assessment letter	1	06 January 2014
Other: Maturation assessment proceedures (females) for Parent/Guardian	1	06 January 2014
Other: Maturation assessment proceedures (males) for Parent/Guardian	1	06 January 2014
Other: Activity Log Book	1	06 January 2014
Other: Parent On-Off Log	1	06 January 2014
Other: Participant interest form	1	16 December 2013
Other: Photo permission form	1	16 December 2013
Other: Patient Recruitment Flyer	1	16 December 2013
Other: Healthy control participant recruitment flyer	1	16 December 2013
Other: CV for Dr Patrick Oades		
Other: CV for Dr Alan Barker		
Other: CV for Jonathan Fulford		
Other: CV for Zoe Saynor		
Other: non-NHS SSI Form		
Other: Physical activity assessment instructions	2 - Clean & Tracked	15 April 2014
Participant Consent Form: Assent Patient 10-15y	3	30 April 2014

Participant Consent Form: Assent Healthy Controls 10-15y	3	30 April 2014
Participant Consent Form: Healthy Control 16-18y	2	30 April 2014
Participant Consent Form: Patient-Parent/Guardian	2	30 April 2014
Participant Consent Form: Patient 16-18y	2	30 April 2014
Participant Consent Form: Control-Parent/Guardian	2	30 April 2014
Participant Information Sheet: Control 10-15y	2 - Clean & Tracked	15 April 2014
Participant Information Sheet: Control 16-18y	2 - Clean & Tracked	15 April 2014
Participant Information Sheet: Control Parent & Guardian	2 - Clean & Tracked	15 April 2014
Participant Information Sheet: Patient 10-15y	2 - Clean & Tracked	15 April 2014
Participant Information Sheet: Patient 16-18y	2 - Clean & Tracked	15 April 2014
Participant Information Sheet: Patient Parent & Guardian	2 - Clean & Tracked	15 April 2014
Protocol	2 - Clean & Tracked	15 April 2014
REC application		03 March 2014
Referees or other scientific critique report		27 February 2014
Response to Request for Further Information		22 April 2014

Statement of compliance

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

After ethical review

Reporting requirements

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

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

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

Feedback

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

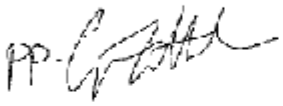
Further information is available at National Research Ethics Service website > After Review

14/SW/0061	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely



Dr Denise Sheehan
Chair

Email: nrescommittee.southwest-exeter@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

"After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Mrs Gail Seymour*
Professor Craig A. Williams, University of Exeter

NRES Committee South West - Exeter

Attendance at Sub-Committee of the REC meeting on 29 April 2014

Committee Members:

Name	*Profession*	*Present*	*Notes*
Joan Ramsay	Retired Associate Director of Nursing (Women and Children) Locum Safeguarding Children Nurse	Yes	
Dr Nicole Dorey	Consultant Clinical Oncologist	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Georgina Castledine	REC Assistant

Appendix B

Royal Devon and Exeter NHS

NHS Foundation Trust

Professor Craig Williams
Children's Health and Exercise Research Centre
University of Exeter
Heavitree Road
Exeter
EX1 2LU

Royal Devon and Exeter
Hospital (Wonford)
Barrack Road
Exeter
EX2 5DW

Tel: 01392 411611
RESEARCH AND DEVELOPMENT DIRECTORATE
Direct Dial: 01392 406933
Direct Fax: 01392 403012
Email: rde-tr.Research@nhs.net

Ref: CG/JL

04 December 2015

Dear Professor Williams

Study Title: Direct investigation of skeletal muscle metabolic, pH and blood oxygenation exercise response of young cystic fibrosis and non-cystic fibrosis bronchiectatic patients

R&D Number: 1409990 **REC Ref:** 14/SW/0061

I have reviewed the Trust R&D file for the above named study, which has received approval from the appropriate regulatory bodies, and I am happy to give approval on behalf of the Royal Devon & Exeter NHS Foundation Trust (RD&E). I can confirm from your SSI form that you aim to recruit up to 20 patients plus 10 healthy volunteers in the required time

The documents approved for use in this study are those approved by Ethics. These are detailed on a separate sheet.

As named Investigator for this research that is being undertaken at the RD&E, it is your responsibility to manage and conduct this study in accordance with:

- The requirements of the **Research Governance Framework for Health and Social Care (2005)** and **Medicines for Human Use (Clinical Trials) Regulations 2004** (if applicable).
- **ICH-GCP** (Good Clinical Practice) – It is mandatory for those staff who will be consenting participants into this study to have undertaken GCP and to ensure it is updated every 2 years.
- The **Data Protection Act 1998** which details the eight principles of 'good information handling'.
- **R&D Standard Operating Procedures** (SOPs) and **Trust policies** which are available on the Trust intranet site

As Lead Investigator for this research, you are required to ensure study specific duties are appropriately delegated and clearly documented on the study Delegation Log. This guarantees clarity of roles and must be signed and dated by each individual on the study and yourself as Lead Investigator.

Safety Reporting
Guidance on the classification of Adverse Events/Reactions (AEs/ARs) / Serious Adverse Events/Reactions (SAEs/SARs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) and the requirements for reporting to the sponsor can be found in the study protocol.

All safety events that involve RD&E patients, which require reporting to the Sponsor, must also be reported to the R&D Office within 24 hours of becoming aware of the event, using the appropriate Trust R&D fax template which can be found on the Adverse Event Reporting pages of the R&D intranet site (http://ian.exe.nhs.uk/weknow/directorates/research-and-development/rd_administration/adverse-event_reporting/).

Progress Reporting
The sponsor is required to submit regular recruitment updates to the R&D Office, as well as annual progress reports to Ethics, MHRA (where applicable) and R&D. Please note that current government and Trust targets require you to have recruited your **first patient within 30 days of the date of Trust Approval** and to have recruited your target number of participants within the time frame stipulated on your SSI form (Time to Target)

Monitoring and Audit
Your study may be monitored by the Sponsor and selected for audit by the R&D Office (where RD&E is not the Sponsor) and Regulatory Authorities at any time. The team involved in conducting this research must ensure full co-operation with any requests from any of these bodies. Action may be taken to suspend research if it is found to not be conducted in accordance with the protocol and all applicable regulations.

Archiving
Upon completion of this research all studies must be archived appropriately and in accordance with the applicable Law.

Any publications arising from the Research conducted at this site must be sent to the R&D Office as part of the on-going Research Governance Process.

You should be aware that the Trust accepts no responsibility for the provision of any study drug outside of Clinical Trials and specifically would not fund the continuing prescription of any therapy once the trial has concluded unless there is a written agreement.

Trust Approval is for the duration of the study, as specified in your SSI form. Research must **commence** within **six months** of Trust Approval. If you have received an Honorary Contract or Letter of Access in order to conduct the above research at this Trust, it is important that you check the termination date on these documents and if applicable contact the R&D Office to extend the document end date. We wish you every success with your study.

Yours sincerely

C M Gardner

Mr Chris Gardner
R&D Directorate Manager

cc: Gail Seymour

Enc: Document Log

Study Short Title: Investigation of skeletal muscle metabolic response Study Number: 1409090

DOCUMENT LOG

Document Name	Version (Trust Approval)	Document Date	Ethics Approval Date	MHRA Approval Date
Protocol	V3	25/06/2014	22/08/2014	n/a
Assent Form (10-15yr)	V4	26/06/2014	22/08/2014	n/a
Consent Form (16-18yr)	V3	26/06/2014	22/08/2014	n/a
Consent Form (Parent)	V3	26/06/2014	22/08/2014	n/a
Participant Information Sheet (10-15yrs)	V3	18/08/2015	22/08/2014	n/a
Participant Information Sheet (16-18yr)	V4	18/08/2014	22/08/2014	n/a
Parent Information Sheet	V3	18/08/2014	22/08/2014	n/a
Healthy control Assent Form (10-15yr)	V4	26/06/2014	22/08/2014	n/a
Healthy control Consent Form (16-18yr)	V3	26/06/2014	22/08/2014	n/a
Healthy control Consent Form (Parent)	V3	26/06/2015	22/08/2014	n/a
Healthy Volunteer Letter (10-15yrs)	V4	18/08/2014	22/08/2014	n/a
Healthy Volunteer Information Sheet (16-18yr)	V4	18/08/2014	22/08/2014	n/a
Healthy Volunteer Parent Information Sheet	V4	18/08/2014	22/08/2015	n/a
Patient recruitment flyer	V1	16/12/2013	22/05/2014	n/a

Appendix C

Owen Tomlinson
Post Graduate Research student

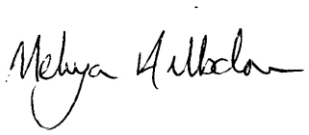
14th December 2016

Dear Owen

Re: Direct investigation of skeletal muscle metabolic, pH and blood oxygenation exercise response of young cystic fibrosis and non-cystic fibrosis bronchiectatic patients

The SHS REC is satisfied with the approval you received from the South West - Exeter Research Ethics Committee on the 14th October 2016. In addition, the relevant paperwork has been reviewed by David Childs, a member of the SHS ethics committee and the health and safety advisor to the committee. He is satisfied that all procedures meet the required standards.

Kind regards



Melvyn Hillsdon
Chair, SHS research ethics committee
m.hillsdon@exeter.ac.uk
01392 7222868

Appendix D

Health Research Authority

NRES Committee South West - Exeter
Bristol Research Ethics Committee Centre
Whitefriars
Level 3
Block B
Lewins Mead
Bristol
BS1 2NT

Telephone: 0117 342 1552
Facsimile: 0117 342 0445

06 August 2013

Professor Craig Williams
Director: Children's Health & Exercise Research Centre (CHERC)
University of Exeter
St. Luke's Campus
Heavitree Road
Exeter
EX1 2LU

Dear Professor Williams

Study title:	**An Integrated Approach to Exercise Prescription and Management in Young Cystic Fibrosis Patients – A Feasibility Study**
REC reference:	**13/SW/0166**
IRAS project ID:	**131690**

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

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Kirsten Peck, nrescommittee.southwest-exeter@nhs.net.

Confirmation of ethical opinion

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

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.
Management permission or approval must be obtained from each host organisation prior to the

start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

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

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

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

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

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

Approved documents

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

Document	Version	Date
Covering Letter		24 July 2013
GP/Consultant Information Sheets	1	24 July 2013
Investigator CV		03 June 2013
Letter from Sponsor		06 May 2013
Letter of invitation to participant	1	29 April 2013
Other: CV - Zoe Saynor		03 June 2013
Other: Letter from Pender		27 March 2013
Other: Photo Permission form	1	29 April 2013
Other: CV - Dr Alan Barker		
Other: CV - Dr Patrick Oades		
Participant Consent Form: Parent Guardian	2	22 July 2013
Participant Consent Form: Patient 16y	2	22 July 2013
Participant Information Sheet: parent guardian Information Sheet	2	22 July 2013
Participant Information Sheet: Patient: 16y	2	22 July 2013
Participant Information Sheet: Patient 10y	2	23 July 2013
Protocol	1	15 May 2013
REC application	3.5	20 May 2013
Referees or other scientific critique report		25 March 2013

Statement of compliance

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

After ethical review

Reporting requirements

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

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

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

Feedback

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

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

13/SW/0166	Please quote this number on all correspondence

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Kirsten Peck

pp **Dr Denise Sheehan**
Chair

e-mail:nrescommittee.southwest-exeter@nhs.net

Enclosures: "After ethical review – guidance for researchers" (SL-AR2)

Copy to: Mr Christopher Gardner
Miss Lynda Garcia, Royal Devon & Exeter NHS Foundation Trust

Appendix E

Professor Craig Williams
Children's Health & Exercise Research Centre
University of Exeter
St Luke's Campus
Heavitree Road
Exeter
EX1 2LU

Royal Devon and Exeter
Hospital (Wonford)
Barrack Road
Exeter
EX2 5DW

Tel: 01392 411611
RESEARCH AND DEVELOPMENT
DIRECTORATE
Direct Dial: 01392 406933
Direct Fax: 01392 409012
Email: rde-tr.Research@nhs.net
Ref: CB/R&D/CG

9 September 2013

Dear Craig

Study Title: **An Integrated Approach to Exercise Prescription and Management in Young Cystic Fibrosis Patients – A Feasibility Study**

R&D No: 1402887 **MREC Ref: 13/SW/0166** **EudraCT No: n/a**

I have reviewed the Trust R&D file for the above named study, which has received approval from the appropriate regulatory bodies, and I am happy to give approval on behalf of the Royal Devon & Exeter NHS Foundation Trust (RD&E).

The documents approved for use in this study are those approved by ethics, these are detailed on a separate sheet.

As named Investigator for this research that is being undertaken at the RD&E, it is your responsibility to manage and conduct this study in accordance with:

- The requirements of the **Research Governance Framework for Health and Social Care (2005)** and **Medicines for Human Use (Clinical Trials) Regulations 2004** (if applicable).
- **ICH-GCP** (Good Clinical Practice) – It is mandatory for those staff who will be consenting participants into this study to have undertaken GCP and to ensure it is updated every 2 years.
- The **Human Tissue Act 2004** and the **EU Tissue and Cells Directive (2006)** for research involving human tissue.
- The **Data Protection Act 1998** which details the eight principles of 'good information handling'.
- **R&D Standard Operating Procedures** (SOPs) and **Trust policies** which are available on the Trust intranet site

As Lead Investigator for this research, you are required to ensure study specific duties are appropriately delegated and clearly documented on the study Delegation Log. This guarantees clarity of roles and must be signed and dated by each individual on the study and yourself as Lead Investigator.

Safety Reporting
Guidance on the classification of Adverse Events/Reactions (AEs/ARs) / Serious Adverse Events/Reactions (SAEs/SARs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) and the requirements for reporting to the sponsor can be found in the study protocol. For RD&E

sponsored studies this is also detailed in the sponsorship letter. All safety events that involve RD&E patients, that require reporting to the Sponsor, must also be reported by fax to the R&D Office within 24 hours of becoming aware of the event, using the appropriate Trust R&D fax template which can be found on the Adverse Event Reporting pages of the R&D intranet site (http://lan.exe.nhs.uk/welcome/directorates/research_and_development/rd-administration/adverse-event-reporting).

Progress Reporting
You are required to submit regular recruitment updates to the R&D Office, as well as annual progress reports to Ethics, MHRA (where applicable) and R&D. Please note that new government and Trust targets require you to have recruited your **first patient within 30 days of the date of Trust Approval** and to have recruited your target number of participants within the time frame stipulated on your SSI form (Time to Target).

Monitoring and Audit
Your study may be monitored by the Sponsor and selected for audit by the R&D Office (where RD&E is not the Sponsor) and Regulatory Authorities at any time. The team involved in conducting this research must ensure full co-operation with any requests from any of these bodies. Action may be taken to suspend research if it is found to not be conducted in accordance with the protocol and all applicable regulations.

Archiving
Upon completion of this Research an **End of Study Report** must be submitted to the Regulatory Authorities (this will be done by the CI) and a copy submitted to the R&D Office. All studies must be archived appropriately and in accordance with the applicable Law. Where RD&E is the Sponsor or where the Sponsor has delegated archiving to the Investigator team, it is your responsibility to contact the R&D Office to discuss appropriate archiving arrangements.

Any publications arising from the Research conducted at this site must be sent to the R&D Office as part of the on-going Research Governance Process.

You should be aware that the Trust accepts no responsibility for the provision of any study drug outside of Clinical Trials and specifically would not fund the continuing prescription of any therapy once the trial has concluded unless there is a written agreement.

Trust Approval is for the duration of the study, as specified in your SSI form. If you have received an Honorary Contract or Letter of Access in order to conduct the above research at this Trust, it is important that you check the termination date on these documents and if applicable contact the R&D Office to extend the document end date.

We wish you every success with your study.

Yours sincerely

Dr **Colin Berry**
Medical Director

MARTIN COOKE
ACTING MEDICAL DIRECTOR

CC: R&D Study File
Zoe Saynor

Enc: Approved Documents

Study No. 1402887 List a documents on f e
Study Short Title: Exercise Prescription and Management in Young CF Patients

Doc Name	Version	Date	Date of letter from ethics	Notes
Protocol	1	16/05/2013	06/08/2013	
GP/Consultant Information Sheets	1	24/07/2013	06/08/2013	
Letter of Invitation to Participant	1	29/04/2013	06/08/2013	
Photo Permission Form	1	29/04/2013	06/08/2013	
Participant Consent Form: Parent/Guardian	2	22/07/2013	06/08/2013	
Participant Consent Form: Patient 16y	2	22/07/2013	06/08/2013	
Participant Consent Form: Patient 10y	2	22/07/2013	06/08/2013	
Participant Information Sheet: Parent/Guardian	2	22/07/2013	06/08/2013	
Participant Information Sheet: Patient 16y	2	22/07/2013	06/08/2013	
Participant Information Sheet: 10y	1	29/04/2013	06/08/2013	

Appendix F

Physiological Reports Open Access

Patient Consent Form

To record a patient's consent to publication of information about them or their relative in *Physiological Reports*.

NAME OF PATIENT: _____

TITLE OF CASE REPORT: _____
_____

CORRESPONDING AUTHOR: _____

CORRESPONDING AUTHOR'S ADDRESS: _____
_____

MANUSCRIPT NUMBER, IF KNOWN: _____
_____

I, [.....NAME OF PATIENT / PARENT / GUARDIAN / RELATIVE***], give my permission to [.....NAME OF HEALTH PROFESSIONAL] to use information (including photographs) about [.....NAME AND RELATIONSHIP***] in *Physiological Reports* published by John Wiley & Sons, Ltd. ("Wiley") such permission to extend to publication of the information by Wiley and its licensees in all media and languages throughout the world.

***In cases where the patient has died or is incapable of giving consent, consent may be given by the next of kin. If the patient is under the age of 16, consent should be given by a parent or guardian.

I have seen and read the material to be submitted to the journal.

I understand that:
(1) My name will not be published and *Physiological Reports* will endeavour to ensure I remain anonymous, other than in relation to identifiable photographs for which I have given consent. However I also understand that it is possible somebody may recognise me from the case report.

(2) I understand that *Physiological Reports* is an open access publication, and content is made available under the terms of the Creative Commons, primarily the Creative Commons Attribution Non-Commercial License which permits use, distribution and reproduction in any medium, provided that the content is properly cited and is not used for commercial purposes. This means that my information can be read and used by anyone around the world for free.

(3) I can change my decision to give consent to publish information about me at any time before final approval for publication by Wiley, but once the case report has been approved for publication in its final form it will not be possible to change my decision to give consent.

*****SIGNATURE OF PATIENT / PARENT / GUARDIAN / NEXT OF KIN:**

_____

*****IF PARENT / GUARDIAN / NEXT OF KIN, STATE RELATIONSHIP TO PATIENT:**

_____

ADDRESS:

_____

_____

DATE: _____

SIGNATURE OF HEALTH PROFESSIONAL:

_____

ADDRESS:

_____

_____

DATE: _____

Note to corresponding author: The original signed consent form should be retained by the corresponding author. You do not need to upload the consent documentation when submitting your manuscript. However, we may request copies of the documentation at any time.

Note to health professional: In addition to the consent form, please ensure that any other necessary permissions are cleared for use of the information, including any permissions required for use of information contained in medical records.

Appendix G

Royal Devon and Exeter NHS Foundation Trust

Exercise Study for Young People with Chest Disease

Study Number: 14/SW/0061
Version 3
Date 18-08-2014

Patient's Letter (10-15 years of age)

Dear Patient,

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

What is the study about?

- The study will help us find out how your leg muscles respond during exercise to help us understand why you may become more tired during exercise than somebody who does not have a chest disease.

What will happen?

- You will visit the university in Exeter 3 times to carry out some leg exercise
- During the exercise tests you will be within a scanner (you may have seen one of these before if you have ever has an MRI scan)
- You will not feel anything when in the scanner, but it may be a bit noisy.
- You will be given clear instructions about everything
- The first visit will be to make sure you understand what we need you to do and that you are happy with exercising in the scanner
- The people who will be testing you will be different to your usual direct care team at the hospital, but they are trained in exercise testing like this and regularly exercise test young people.

Are the exercise tests safe?

- The exercise tests are very safe.
- Your doctor will also look at your health records before you exercise to check that it is safe for you to take part.
- The research team will be watching you during all exercise tests just in case there are any problems.
- Although the exercise is safe, it is exhausting and this means that it is likely that you will have tired heavy legs after exercising. This feeling may continue for 1-2 days.

What else will happen on each visit?

Visit 1:

- On your first visit to our exercise centre we will measure how tall you are and how much you weigh.
- We will then measure the amount of fat on your arm, back and hip. We do this by very gently pinching the skin and using an instrument to measure the fold of skin. This does not hurt but may tickle.
- You will then be shown the equipment we will use
- Finally, you will practice lying inside a pretend scanner so that you can get used to this and then you will practice the exercise test a number of times
- **You will need to be at the exercise centre for about 2 hours for this 1st visit.**

Visits 2 and 3:

- The next 2 times that you come to the exercise centre you will be doing the same exercise test you have practiced in a real scanner.
- This scanner is not different however it may make some noise – you do not need to worry about this
- Before you have to do any exercise you will be asked to rest
- During the exercise test you will be doing leg exercise when we tell you and will be encouraged to keep going for as long as possible until your legs are so tired that you must stop
- We will talk you through all of the details of what we will be asking you to do.
- We will then give you a drink and you can rest before going home.

After you have finished taking part in the study

- We also need to know how developed your body is. To do this we will ask you to go look at yourself at home and answer a question about how far your body has developed. The question is an easy choice out of 5. We would also like you to wear a device like a watch for 1 week after you have finished your testing, to see how much exercise/play you usually do and how hard this is. You and your parent/guardian will also keep a record of what type of activities you are doing and when you take off the watch.

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

1. For 2 – 3 days before each visit you must not do any really hard exercise (if possible).
2. You can eat and drink as normal but do not drink or eat foods which contain caffeine such as tea, coffee, Coca-Cola for 3 hours before coming to see us. Speak to your parent or carer if you aren't sure what caffeine is.
3. You will also be asked if you would like to have your photograph taken during testing, for us to use when talking about the findings from this study in the future. This is completely up to you and you don't have to say yes. If you do agree that you would like your photograph taken, your face will be covered so that nobody can see it.

Do you have to take part?

- It is up to you if you take part.
- If you take part you can drop out at any time and don't have to give us a reason.
- It is important that you understand that you will need to attend all 3 visits which will require some of your time.

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

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

Thank you for reading this letter.

Details to Contact the Research Team:

Primary contact: Mr Owen Tomlinson

(Children's Health & Exercise Research Centre, University of Exeter)
Tel (work): (01392) 264721
E-mail: o.w.tomlinson@exeter.ac.uk

Miss Zoe Louise Saynor
(Children's Health & Exercise Research Centre, University of Exeter)
Tel (work): (01392) 724759
E-mail: z.l.saynor@exeter.ac.uk

Dr. Jon Fulford
(Exeter Medical School MRI Unit, University of Exeter)
Email: j.fulford@exeter.ac.uk

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

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

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

Miss Jamille Paschoal-Vicente
(Children's Health & Exercise Research Centre, University of Exeter)
E-mail: jp446@exeter.ac.uk

Appendix H

CHILDREN'S HEALTH AND EXERCISE RESEARCH CENTRE

Sport and Health Sciences
St. Luke's Campus
Heavitree Road
Exeter
EX1 2LU

Tel: +44 (0) 1392 264721
Email: o.w.tomlinson@exeter.ac.uk
Web: www.sshs.ex.ac.uk

PATIENT (16-18 y) INFORMATION SHEET FOR

THE EFFECTS OF CHRONIC CHEST DISEASE ON THE LEG MUSCLE RESPONSE DURING EXERCISE

Version number: 4
Date: 18/08/14

1. Study Title

Direct investigation of skeletal muscle metabolic, pH and blood oxygenation exercise response of young cystic fibrosis and non-cystic fibrosis patients.

2. Invitation paragraph

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

Thank you for reading this information sheet.

3. What is the purpose of the study?

It is recommended that children and teenagers with cystic fibrosis (CF) take part in sports and games to stay fit and healthy. Exercise not only helps improve fitness and enhances their quality of life through socialising with peers and friends, but it may also help them to cope better with aspects of their disease. We know that people with chronic chest diseases often have lower aerobic fitness than those who are healthy. However, we still need to find out more information about what causes this.

This study will look at whether there are any differences between how your leg muscles respond during exercise compared with young people who don't have chest disease. We are interested in whether there are differences in how much blood and oxygen is delivered to the muscles which need it and any differences in the size and response of your muscle itself to exercise tasks.

Although the main aim of this study is to gather the above information, it will also provide you with useful information about how well you tolerate exercise. Additionally, we know that participating in

regular exercise is beneficial to your health and, as such, this research project will also serve as 3 sessions of supervised leg exercise.

4. Why have I been chosen?

We are interested in young people with CF and non-CF bronchiectasis, aged between 10-18 years old, who have stable disease and regularly take part in physical activity.

5. Do I have to take part?

Taking part is entirely voluntary and it is up to you to decide whether or not you want to take part. If you do want to be involved, you will be given this information sheet to keep, and be asked to sign a consent form giving your permission (your parent/guardian will do the same). Even if you say yes, you will be free to withdraw from the study at any time and there is no need to give a reason. Any decision to withdraw at any time or a decision not to take part in the first place will not affect the standard clinical care you receive.

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

The study will involve 4 trips to the St. Luke's campus (University of Exeter), separated by at least 48 h and ideally completed over a maximum 2 week period (to minimise the chance of any significant change in your health and/or fitness). All testing will take place at times which are suitable for you and your parent(s)/guardian(s) and this can be at weekends or after school if preferred. Visit 1 will take place in an exercise laboratory within Sport and Health Sciences, where you will be familiarised with a replica scanner (like an MRI scanner). Visits 2 and 3 will take place within the Exeter Medical School MRI Unit.

Visit 1 – Baseline assessments and familiarisation session

You and your parent(s)/guardian(s) will be given the opportunity to discuss any queries you may have, give your informed consent and be familiarised with the equipment and testing procedures that we will use during the next 3 testing visits.

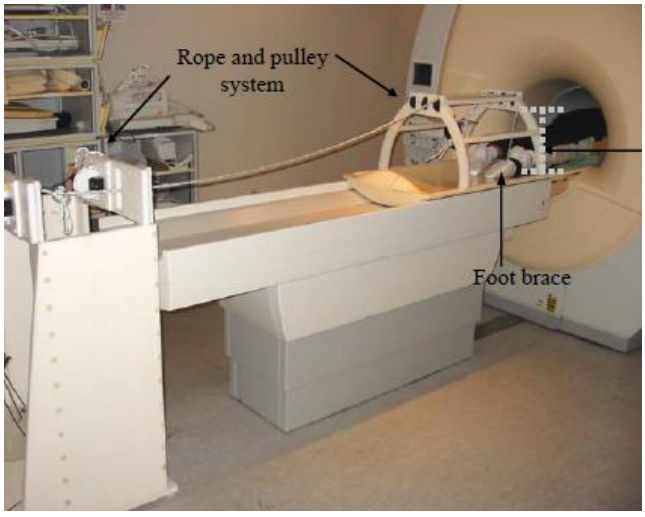


Figure 1. A child performing exercise testing inside the MR scanner.

Baseline assessments: We will first obtain some information about your body composition. Your height, sitting height, and weight will be measured. We will then measure a number of 'skin folds' at the front and back of your arm, just below your shoulder blade and at the front of your hip. This is done by very gently pinching the skin and measuring the width of the fold of skin between the thumb and index finger. We will then measure your lung function at rest, this will involve you taking a big breath and breathing into a plastic tube – I am sure you have probably done this a number of times in clinic.

Familiarisation session: We will then get you used to exercising within an MR scanner environment. We will use a pretend scanner for this. You will perform the identical tasks that you will be asked to do when you come back to the next real testing sessions. The exercise test involves you carrying out single-legged (right leg) exercise whilst lying on your front inside the scanner. Your right foot will be fastened to a Velcro strap, which is connected to a basket using a rope and pulley system (see figure 1). This system means that there will be some resistance when you move your leg up and down.

Your first task will be to practice exercising at different intensities whilst keeping up with instructions you will be given. You will then complete at least 3 practice tests to exhaustion. We will separate these with rest periods. The test will then be changed slightly to one with more rest periods, which will be a modified test used during visits 3 and 4. These protocols will be exactly the same as what we will ask you to perform in the real scanner. How many practice tests you will need to do will depend on how long it takes you to be comfortable with what we need you to do. This will typically be at least 3 practice tests.

You and your parent(s)/guardian(s) can ask any questions to the research staff at any time during the session.

This session (visit 1) will usually last ~2 hours.

<u>Visits 2-4 – Exercise testing within the MR scanner</u>

During visits 2-4 you will carry out the same exercise test that you learnt during the familiarisation session, however we will be collecting various different measurements during these sessions using the scanner. However, this will not mean anything different to you and you will either be laying down resting or carrying out the same exercise test. ***Visits 2 and 3 will each usually last ~1 hour.***

Visit 2:

Overview: At the start of visit 2, we will measure the size of your thigh muscles. Whilst we are measuring these things, you will simply need to relax and lie still within the scanner. As the scanner is a little noisy, you can wear headphones if you like. The exercise test itself will simply require you to move your leg (attached to the pulley system) up and down in time with the instructions for 24 seconds and, following enough rest, you will then complete and incremental exercise test until you are too tired to continue. You will then perform the 24 second test again immediately. The amount of oxygen present in your blood (oxygen sats.) will also be monitored throughout all exercise via the fingertip and a box on your leg will be measuring oxygen in the blood in the leg muscles. The research team will always be keeping an eye on you to make sure that you are ok.

Visits 3 and 4:

Overview: At the start of visits 3 and 4, we will measure the size of your thigh at rest. This should not take as long as the previous visit. During these 2 visits you will not need to complete the 24 second exercise test with rest, you will just come in and complete an intermittent test until you are exhausted.

These visits will be shorter than visit 2. Again, the amount of oxygen present in your child's blood (oxygen sats.) will also be monitored throughout all exercise via the fingertip and on the thigh.

Post-testing

Maturation: As well as your age, we also need to know how mature your body is (growth and development). To do this we will need you to self-assess something called your pubertal stage, by simply selecting from 5 options on a picture chart which shows different pictures of pubic hair through the stages of maturation. You will do this at home after your last visit with us and your parent/guardian will be asked to return the paperwork to us in a sealed envelope.

Physical activity: We are also interested in getting an idea of how active you usually are. To measure this you will wear a small device (like a watch) on your wrist for 7 days, whilst also completing a simple diary about what physical activities you have been doing between waking up and going to bed. Your parent/guardian will also complete an on-off log in consultation with you to keep a record of when you are wearing the monitor.

7. What else will I have to do?

If you do choose to take part and you have permission from your parent(s)/guardian(s), we would need you to attend all 3 visits. We ask that you are rested when you arrive, more specifically that you have not performed any exhaustive exercise in the 2-3 days before you come (other than involvement in this study). Any specific questions regarding any exercise training or sport you may be doing prior to testing sessions can be discussed with the research team on an individual basis.

Each time you come to the laboratory we also ask that you have had enough to eat and drink and that you wear the same or similar clothes for each exercise test (i.e., sports kit, usually shorts, t-shirt and trainers). We also ask that you don't consume any caffeine 3 hours prior to each visit (ask your parents about this if you are not sure what contains caffeine). However, it is important that you do eat a light meal or snack before each visit (i.e. sandwich, cereal etc.).

We will ask you and your parent(s)/guardian(s) if we can take photographs during your visits. The purpose of these photographs is to possibly be used within University of Exeter publicity materials, in Owen Tomlinson's and Zoe Saynor's PhD theses and in conference or teaching presentations, such as posters and PowerPoint presentation. This is completely voluntary and will in no way affect any subsequent involvement in the study if you and/or your parent(s)/guardian(s) do not want these to be taken. Permission to take any photographs will be obtained in writing before involvement in the study and if you say no then no photographs will be taken. Your face will not be identifiable from any photographs used in future presentation materials.

8. What are the possible risks of me taking part?

The exercise testing is very safe. An assessment of your health will be made before you start any exercise tests to see if it is safe for them to take part. During the exercise tests you will be carefully monitored and observed by members of the research team to make sure that you are safe. Due to the fact that the leg exercise in this study is exhaustive, you will experience tired heavy legs following exercise and this may continue for the next 24-48 hours. This study will also require a certain time commitment from you and your parent guardian, as you will need to attend 3 testing visits.

Measures will be taken to minimise the possible risk of cross-infection. Segregation of patients will be observed in testing places. In addition, both equipment and the testing environment will be thoroughly cleaned using appropriate bacteriocidal wipes and solutions between patients and at least 2 hours will separate testing sessions during which time period the laboratory will be ventilated. N.B. It will be rare that 2 patients are tested within the same 12 hour period.

9. What are the potential benefits of me taking part?

This research is intended to help us understand why children with chronic chest disease may find exercise more difficult than healthy children and to help us inform interventions to improve fitness. Hopefully, you will also find involvement to be a positive and enjoyable experience. Sessions will also act as supervised leg exercise sessions for you, using hi-tech equipment. You will be given the results of your exercise tests and talked through them by a member of the research team. This will hopefully prove an interesting and constructive exercise, particularly if you are interested and/or involved in sport or exercise.

10. What happens if I do not want to continue in the study?

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

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

11. What if something goes wrong?

If taking part in this study harms you, compensation arrangements are under the directive of the University of Exeter. Your rights are the same as any person undergoing research, i.e. if you are harmed due to someone's negligence, then you may have grounds for legal action, but you may have to pay for it. If you or your parent(s)/guardian(s) wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University complaints procedures will be available to you. In any such case, complaints should be directed to Professor Craig Williams, Director of Research for Sport and Health Sciences, University of Exeter [+44 (0) 1392 724890; c.a.williams@exeter.ac.uk].

12. Will my involvement in this study be kept confidential?

We have a responsibility to inform you of how we will collect, store and use any of the information gathered about you during this study. The primary concern is that any information that we collect about you will be confidential. All information, such as your name, date of birth, contact details, details of health and test results will be transferred to a paper study file, which will be kept in a secure room in a locked filing cabinet. Members of the research team will be outside your direct standard care team and will have access to their data. However, your information will be kept anonymous by assigning you a unique study code and participant number. Only your date of birth will be used to identify the results. The results from the exercise tests are collected on paper and stored in a locked filing cabinet. The exercise results are then transferred from paper and stored on a computer. The

only personal information stored on computer will be your date of birth and participant number. Their exercise results will be password protected, as will the computer used to store the information. All the paper and computer files will be stored for 15 years, after this period paper files will be destroyed and computer files erased. Only the researchers directly involved in the study will have access to your medical records and exercise results.

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

Once the study is completed, which is targeted to be September 2014, the results will be analysed and interpreted and your parent(s)/guardian(s) will subsequently be sent a summary of our research findings. It is the intention to submit the results of the research to relevant journals for publication, and to inform our colleagues of the findings at scientific meetings. In publishing and talking about the study you will not be identifiable. Some of the data will also be used to form Miss Paschoal-Vicente's MSc degree dissertation.

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

14. Who is organising and funding the research?

The study is a collaboration between the Paediatric Unit of the Royal Devon and Exeter (RD&E) NHS Foundation Trust Hospital, Children's Health and Exercise Research Centre and Medical School (Dr. Jon Fulford) at the University of Exeter. The study is sponsored by the RD&E NHS Foundation Trust and has been supported financially by internal funding from the University of Exeter. A monetary contribution will be provided to go some way towards covering your travel expenses and/or parking costs, bus or rail fares when attending testing sessions.

15. Who has reviewed the study?

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

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

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

17. What if I have a question?

If you or your parent(s)/guardian(s) have any questions please do not hesitate to get in touch with a member of the research team using the details provided on page 7 and at the top of page 1.

18. Contact for further information

If you need further information please contact the research time using the details presented on page 7 and at the top of page 1.

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

More specialised information regarding participation in clinical research is published by the UK Clinical Research Collaboration (UKCRC). For further information visit www.ukcrc.org or request a printed copy from: UKCRC, 20 Park Crescent, London, W1B 1AL.

The Research Team

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Miss Jamille Paschoal-Vicente
(Children's Health & Exercise Research Centre, University of Exeter)
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N.B. Contact outside of office hours should be made to Mr Owen Tomlinson.

Thank you for considering taking part in this study. Please read this information carefully, before you sign the consent form. Please take this leaflet home for reference.

CHILDREN'S HEALTH AND EXERCISE RESEARCH CENTRE

Sport and Health Sciences
St. Luke's Campus
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EX1 2LU

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Appendix I

PARENT(S)/GUARDIAN(S) INFORMATION SHEET FOR

THE EFFECTS OF CHRONIC CHEST DISEASE ON THE LEG MUSCLE RESPONSE DURING EXERCISE

Version number: 3
Date: 18/08/14

1. Study Title

The effects of chronic chest disease on the leg muscle response during exercise.

2. Invitation paragraph

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

Thank you for reading this information sheet.

3. What is the purpose of the study?

It is recommended that children and teenagers with cystic fibrosis (CF) take part in sports and games to stay fit and healthy. Exercise not only helps improve fitness and enhances their quality of life through socialising with peers and friends, but it may also help them to cope better with aspects of their disease. We know that people with chronic chest diseases often have lower aerobic fitness than those who are healthy. However, we still need to find out more information about what causes this.

This study will look at whether there are any differences between how your child's leg muscles respond during exercise compared with young people who don't have chest disease. We are interested in whether there are differences in how much blood and oxygen is delivered to the muscles which need it and any differences in the size and response of your child's muscle itself to exercise tasks.

Although the main aim of this study is to gather the above information, it will also provide your child with useful information about how well they tolerate exercise. Additionally, we know that participating in regular exercise is beneficial to their health and, as such, this research project will also serve as 3 sessions of supervised leg exercise.

4. Why has my child been chosen?

We are interested in young people with CF and non-CF bronchiectasis, aged between 10-18 years old, who have stable disease and regularly take part in physical activity.

5. Does my child have to take part?

Taking part is entirely voluntary and it is up to you and your child to decide whether or not you want to take part. If you do want to be involved, you will be given this information sheet to keep, and be asked to sign a consent form giving your permission (your child will do the same). Even if you say yes, you will be free to withdraw your child from the study at any time and there is no need to give a reason. Any decision to withdraw at any time or a decision not to take part in the first place will not affect the standard clinical care your child receives in any way.

6. What will happen to my child if they do want to take part?

The study will involve 4 trips to the St. Luke's campus (University of Exeter), separated by at least 48 h and ideally completed over a maximum 2 week period (to minimise the chance of any significant change in your child's health and/or fitness). All testing will take place at times which are suitable for you and your child and this can be at weekends or after school if preferred. Visit 1 will take place in an exercise laboratory within Sport and Health Sciences, where you will be familiarised with a replica scanner (like an MRI scanner). Visits 2 and 3 will take place within the Exeter Medical School MRI Unit.

Visit 1 – Baseline assessments and familiarisation session

You and your child will be given the opportunity to discuss any queries you may have, give your informed consent/assent (depending on their age) and be familiarised with the equipment and testing procedures that we will use during the next 3 testing visits.

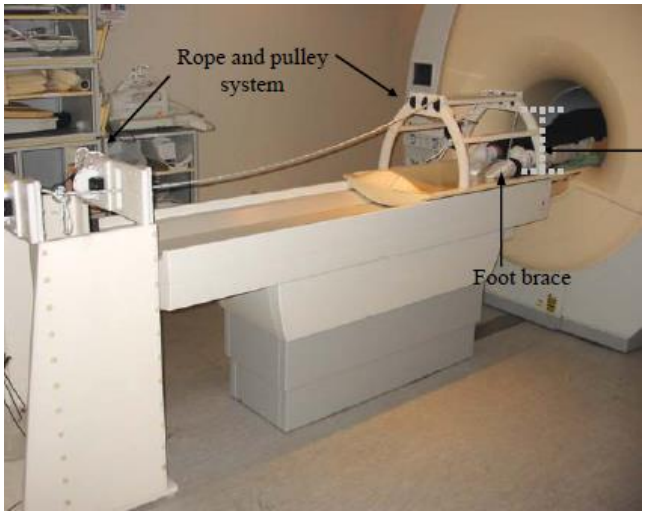

Figure 1. A child performing exercise testing inside the MR scanner.

Baseline assessments: We will first obtain some information regarding your child's body composition. Their height, sitting height, and weight will be measured. They will also have their skin folds measured at the front and back of their arm, just below their shoulder blade and at the front of their hip. This is done by very gently pinching the skin and measuring the width of the fold of skin between the thumb and index finger. They will then have their resting lung function measured. This will involve your child taking a big breath and breathing into a plastic tube and is no different to the lung function tests they routinely perform in clinics.

Familiarisation session: Your child will then be familiarised with exercising within an MR scanner environment, using a purpose build replica of the real machine. They will perform the identical task that they will undergo during the next 2 sessions. Exercise testing involves them performing single-legged (right leg), leg exercise whilst lying on their front inside the scanner. Their right foot will be fastened to a Velcro rest, which is connected to the ergometer load basket via a rope and pulley system (see Figure 1). This system means that there will be some resistance when your child moves their leg up and down. They will do this in time with some instructions.

Your child's first task will be to practice exercising at different intensities at the required speed. They will then complete at least 3 practice tests to exhaustion, separated with sufficient rest. This protocol will be exactly the same as the one they will perform in the real MR scanner during visit 2. The test will then be changed slightly to one with more rest periods, which will be a modified test used during visits 3 and 4. The amount of familiarisation required will depend on how fast your child becomes used to the exercise test and is happy, but will typically be at least 3 trials.

The aim of this first session is to make sure that *1)* your child is happy exercising within the scanner environment, *2)* your child can competently complete the testing under our instruction and, *3)* to provide you and your child with an opportunity to ask any questions to the research staff regarding the requirements of the next exercise testing sessions.

This session (visit 1) will typically last ~2 hours.

<u>Visits 2-4 – Exercise testing within the MR scanner</u>

During visits 2-4 your child will perform the same exercise tests they learnt during the familiarisation session, however we will be collecting measurements this time. ***Visits 2 and 3 will each typically last ~1 hour.***

Visit 2:

Overview: At the start of visit 2, we will obtain baseline measurements relating to the size of your child's thigh muscles. Whilst we are measuring these things, your child will simply need to relax and lie still within the scanner. As the scanner is a little noisy, they can wear headphones if they like. The exercise test itself will simply require your child to move their leg (attached to the pulley system) up and down in time with the instructions for 24 seconds and, following enough rest, they will do this undergo the incremental test practiced during familiarisation until they are too tired to continue. Your child will not feel anything when we take the measurements in their legs. The amount of oxygen present in your child's blood (oxygen sats.) will also be monitored throughout all exercise via the fingertip as will muscle oxygenation via near-infrared spectroscopy.

Visits 3 and 4:

Overview: At the start of visits 3 and 4, we will obtain baseline measurements relating your child's thigh muscles at rest. This should not take as long as during visit 2. During these visits your child will not need to perform the 24 second exercise with rest, they will simply complete an intermittent test to exhaustion. These visits will therefore be shorter than visit 2. Again, the amount of oxygen present in your child's blood (oxygen sats.) will also be monitored throughout all exercise via the fingertip and on the thigh.

Post-testing

We also need to know your child's maturation (growth and development). This will require them to self-assess their pubertal stage by selecting from 5 options on a chart which shows different pictures of pubic hair through the stages of maturation. Self-assessment will take place at home and you will be asked to return the scale in a sealed envelope you will be provided with. We are also interested in assessing how physically active your child is during a typical week, if you and they agree to do so. This will be assessed by wearing a small wrist-mounted plastic box (like a watch) for 7 days, whilst completing a brief diary of the physical activities they perform from the time they wake up to the time they go to bed. The monitor would be removed if they swim or take a bath/shower and when in bed. You will also complete an on-off log in consultation with your child to log the wear time of the monitor.

7. What else will my child have to do?

If your child wishes to take part in the study and he/she has your permission we would like them to attend all 3 visits at the exercise laboratory. We ask that they be in a rested state on arrival, and have performed no exhaustive exercise in the 2-3 days prior to visits (other than involvement in this study). Any specific questions regarding any exercise training or sport your child may be doing prior to testing sessions can be discussed with the research team prior to any involvement in this research study.

On each visit to the laboratory we also ask that your child has had sufficient food and drink before he/she arrives, and that they wear the same or similar clothes for each exercise test (that is sports kit, usually shorts, t-shirt and trainers). We also ask that they don't consume any caffeine 3 hours prior to each visit (ask a member of the research team if you are unsure at all what they can and cannot have). However, it is important that they do eat a light meal or snack before each visit (i.e. sandwich, cereal etc.).

We may ask your child if we can take photographs during their exercise tests. The purpose of these photographs is to possibly be used within University of Exeter publicity materials, in Miss Saynor's and Mr Tomlinson's PhD theses and in conference or teaching presentations, such as posters and PowerPoint presentation. This is entirely voluntary and will in no way affect any subsequent involvement in the study if you and/or your child should decline. Permission to take any photographs will be obtained in writing prior to involvement in the study. Your child's face will not be identifiable from any photographs used in future presentation materials.

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

The exercise testing is very safe. An assessment of your child's health will be made before they start the exercise tests to see if it is safe for them to take part. During the exercise tests your child will be carefully monitored and observed to ensure their safety and well-being. Due to the fact that the leg exercise in this study is exhaustive, your child will experience tired heavy legs following exercise and this may continue for the next 24-48 hours. This study will also require a certain time commitment from you and your child, as they will need to attend 3 testing visits.

Measures will be taken to minimise the possible risk of cross-infection. Segregation of patients will be observed in testing places. In addition, both equipment and the testing environment will be thoroughly cleaned using appropriate bacteriocidal wipes and solutions between patients and at least 2 hours will separate testing sessions during which time period the laboratory will be ventilated. N.B. It will be rare that 2 patients are tested within the same 12 hour period.

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

This research is intended to enhance our understanding of how children with chronic chest disease tolerate exercise and, more specifically, why they may find it more tiring that their healthy counterparts.

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

10. What happens if my child does not want to continue in the study?

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

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

11. What if something goes wrong?

If taking part in this study harms your child, compensation arrangements are under the directive of the University of Exeter. Your rights are the same as any person undergoing research, i.e. if your child is harmed due to someone's negligence, then you may have grounds for legal action, but you may have to pay for it. If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University complaints procedures will be available to you. In any such case, complaints should be directed to Professor Craig Williams, Director of Research for Sport and Health Sciences, University of Exeter [+44 (0) 1392 724890; c.a.williams@exeter.ac.uk].

12. Will my child's involvement in this study be kept confidential?

We have a responsibility to inform you of how we will collect, store and use your child's information gathered during this study. The primary concern is that any information that we collect about your child will be confidential. All information collected, such as their name, date of birth, contact details, details of their health and test results will be transferred to a paper study file, which will be kept in a secure room in a locked filing cabinet. Members of the research team will be outside your child's direct standard care team and will have access to their data. However, your child's exercise results will be kept anonymous by assigning them a unique study code and participant number. Only your child's date of birth will be used to identify the results. The results from the exercise tests are collected on paper and stored in a locked filing cabinet. The exercise results are then transferred from paper and stored on a computer. The only personal information stored on computer will be your child's date of birth and participant number. Their exercise results will be password protected, as will the computer used to store the information. All the paper and computer files will be stored for 15 years, after this period paper files will be destroyed and computer files erased. Only the researchers directly involved in the study will have access to your child's medical records and exercise results.

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

Once the study is completed, which is targeted to be September 2014, the results will be analysed and interpreted and you will subsequently be sent a summary of our research findings. It is the intention to submit the results of the research to relevant journals for publication, and to inform our colleagues of the findings at scientific meetings. In publishing and talking about the study your child will not be identifiable. Some of the data will also be used to form Miss Paschoal-Vicente's MSc degree dissertation.

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

14. Who is organising and funding the research?

The study is a collaboration between the Paediatric Unit of the Royal Devon and Exeter (RD&E) NHS Foundation Trust Hospital, Children's Health and Exercise Research Centre and Medical School (Dr. Jon Fulford) at the University of Exeter. The study is sponsored by the RD&E NHS Foundation Trust and has been supported financially by internal funding from the University of Exeter. A monetary contribution will be provided to go some way towards covering your travel expenses and/or parking costs, bus or rail fares when attending testing sessions.

15. Who has reviewed the study?

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

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

If your child would like to take part in the study you must give your permission by completing the consent form and your child must also complete their assent (<16 y) / consent (16-18 y) form (depending on their age). You should then return the two forms to a member of the research team.

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

If you or your child have any questions please do not hesitate to get in touch with a member of the research team using the details provided on page 7 and at the top of page 1.

18. Contact for further information

If you need further information please contact the research time using the details presented on page 7 and at the top of page 1. For more information regarding participation in research you can access the public information pack published by INVOLVE (a non-profit organization promoting public

involvement in the NHS, public health and social care research). Visit their website www.involve.org.uk/ or obtain a paper copy by writing to: Involve, Royal London House, 22-25 Finsbury Square, London, EC2A 1DX.

More specialised information regarding participation in clinical research is published by the UK Clinical Research Collaboration (UKCRC). For further information visit www.ukcrc.org or request a printed copy from: UKCRC, 20 Park Crescent, London, W1B 1AL.

The Research Team

Primary contact:
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(Children's Health & Exercise Research Centre, University of Exeter)
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N.B. Contact outside of office hours should be made to Mr Owen Tomlinson.

Thank you for considering taking part in this study. Please read this information carefully, before

Appendix J

Study Number: 14/SW/0061
Patient Identification Number for this trial:

ASSENT FORM FOR PATIENTS (10-15 y)

(To be completed by the patient and their parent/guardian)

The effects of chronic chest disease on the leg muscle response to exercise

Child (or if unable, parent on their behalf) /young person to circle all they agree with please:

Have you read (or had read to you) about this project [Version 3 dated 26/06/14] Yes/No

Has somebody else explained this project to you? Yes/No

Do you understand what this project is about? Yes/No

Have you asked all the questions you want? Yes/No

Have you had your questions answered in a way you understand? Yes/No

Do you understand that you will be working with people different to your usual care team at the hospital?

Yes/No

Do you understand that people different to your usual care team at the hospital will have access to parts of your medical notes to get information needed for the study and give permission for this to happen?

Yes/No

Do you understand that people from your usual care team at the hospital and people in the study team will have access to your study data and give permission for this to happen?

Yes/No

Do you understand it's OK to stop taking part at any time? Yes/No

Are you happy to take part? Yes/No

If any answers are 'no' or you **don't** want to take part, **don't** sign your name!

If you do want to take part, please write your name and today's date

Your name _____

Date _____

Your parent or guardian must write their name here too if they are happy for you to do the project

Print Name _____

Sign _____

Date _____

The person from the research team who explained this project to you needs to sign too:

Print Name _____

Sign _____

Date _____

Thank you for your help.

CHILDREN'S HEALTH AND EXERCISE RESEARCH CENTRE

Sport and Health Sciences
St. Luke's Campus
Heavitree Road
Exeter
EX1 2LU

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Web: www.sshs.ex.ac.uk

Appendix K

Study Number: 14/SW/0061
Patient Identification Number for this trial:

PATIENT (16-18 y) CONSENT FORM

Title of Project: **The effects of chronic chest disease on the leg muscle response during exercise**

Name of Researchers: **Miss Zoe L. Saynor, Dr. Jon Fulford, Dr. Alan R. Barker, Dr. Patrick J. Oades, Miss Jamille Paschoal-Vicente and Prof. Craig A. Williams**

Please initial box

1	I have read and understand the information sheet [Version 3 dated 26/06/14] for the above study and have had the opportunity to ask questions.	☐
2	I understand that taking part is voluntary and I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.	☐
3	I understand that sections of my medical records may be looked at by the researchers (who are outside my usual direct clinical care team at the hospital) in the study where it is relevant to my participation, and that this information will be kept secure.	☐
4	I understand that during the period of my participation that hard exercise is to be avoided but that I should eat and drink normally.	☐
5	I am aware that my exercise results will be kept anonymous by giving them a unique study code and participant number, only my date of birth will be used to identify the results. I give consent for my medical records and exercise results to be used as indicated in the patient (16-18 y) information sheet [Version 3 dated 26/06/14]. I understand that my exercise results will be stored for 15 years after which they will be destroyed.	☐
6	I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my child's records and data.	☐
7	I give my consent to take part in the above study.	☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of parent/guardian	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Appendix L

CHILDREN'S HEALTH AND EXERCISE RESEARCH CENTRE

Sport and Health Sciences
St. Luke's Campus
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Exeter
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Email: z.l.saynor@exeter.ac.uk
Web: www.sshs.ex.ac.uk

Study Number: 14/SW/0061
Patient Identification Number for this trial:

PARENT/GUARDIAN CONSENT FORM

Title of Project: **The effects of chronic chest disease on the leg muscle response during exercise**

Name of Researchers: **Miss Zoe L. Saynor, Dr. Jon Fulford, Dr. Alan. R. Barker, Dr. Patrick J. Oades, Miss Jamille Paschoal-Vicente and Prof. Craig A. Williams**

Please initial box

1	I have read and understand the information sheet [Version 3 dated 26/06/14] for the above study and have had the opportunity to ask questions.	☐
2	I understand that my child's taking part is voluntary and they are free to withdraw at any time, without giving any reason and without his/her medical care or legal rights being affected.	☐
3	I understand that sections of any of my child's medical records may be looked at by the researchers (who are outside their usual direct clinical care team at the hospital) in the study where it is relevant to his/her participation, and that this information will be kept secure.	☐
4	I understand that during the period of his/her participation that hard exercise is to be avoided but that he/she should eat and drink normally.	☐
5	I am aware that my child's exercise results will be kept anonymous by giving them a unique study code and participant number, only my child's date of birth will be used to identify the results. I give consent for his/her medical records and exercise results to be used as indicated in the parent/guardian information sheet [Version 3 dated 26/06/14]. I understand that my child's exercise results will be stored for 15 years after which they will be destroyed.	☐
6	I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my child's records and data.	☐
7	I give my consent for my child to take part in the above study.	☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of parent/guardian	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Appendix M

MATURATION ASSESSMENT

PROCEDURES (MALES) – PARENT/GUARDIAN

The assessment of physiological and physical change during growth is essential for the valid interpretation of human performance.

Pubertal maturity will be self-assessed at home following completion of the exercise testing protocol.

Drawings of male and female pubic hair developed by Morris and Udry (1980) made from photographs by Tanner (1975) will be used. A written description of each of the 5 stages of male and female pubic hair development will be given on the chart as how to select the correct pubertal stage.

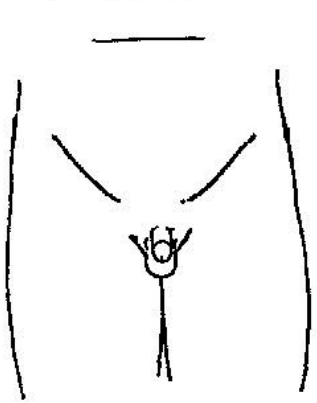
The most practical approach to assess maturation was developed by Tanner (1962). Tanner developed a 5-point scale to assess 'biological maturity' through observation of secondary sexual characteristics. The scales depicted five or more stages of breast and pubic hair (girls), pubic hair and genitalia development (boys). A limitation is that trained health professionals such as paediatricians and school nurses are typically employed to assess the scales. Subsequently Morris and Udry (1980) developed a self-assessment scale based on Tanner stages and found that children were able to accurately assess their own stage of maturation with correlation coefficients in the range of 60-70% (Matsudo & Matsudo, 1993).

The secondary sex characteristics described by Tanner (1962) will be used to assess pubertal maturity. Pubertal stage 1 will be identified as pre-pubertal, stages 2, 3 and 4 circum-pubertal, and stage 5 post-pubertal.

(Please find these below and circle the drawing which you feel best represents you)

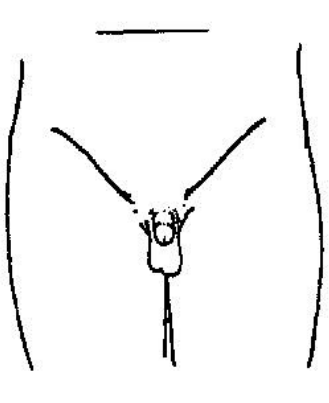
THE DRAWINGS ON THIS PAGE SHOW DIFFERENT AMOUNTS OF MALE PUBIC HAIR. A BOY PASSES THROUGH EACH OF THE FIVE STAGES SHOWN BY THESE DRAWINGS. PLEASE LOOK AT EACH DRAWING AND READ THE SENTENCES UNDER THE DRAWING. THEN CHOOSE THE DRAWING CLOSEST TO YOUR STAGE OF YOUR HAIR DEVELOPMENT. MARK A 1 ON THE LINE ABOVE THAT DRAWING. THEN CHOOSE THE DRAWING THAT IS NEXT CLOSEST TO YOUR STAGE OF HAIR DEVELOPMENT AND MARK IT A 2. IN CHOOSING THE RIGHT PICTURE, LOOK ONLY AT THE PUBIC HAIR, AND NOT AT THE SIZE OF THE TESTES, SCROTUM, AND PENIS.

1. DRAWING A



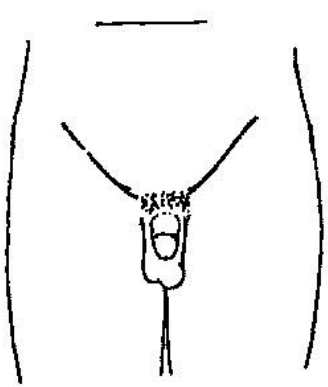
THERE IS NO PUBIC HAIR AT ALL.

2. DRAWING B



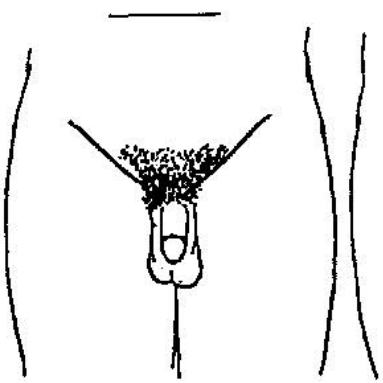
THERE IS A LITTLE SOFT, LONG, LIGHTLY COLORED HAIR, MOST OF THE HAIR IS AT THE BASE OF THE PENIS. THIS HAIR MAY BE STRAIGHT OR A LITTLE CURLY.

3. DRAWING C



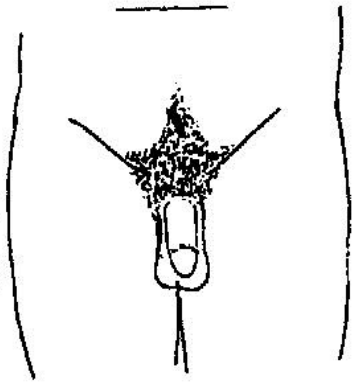
THE HAIR IS DARKER IN THIS STAGE. IT IS COARSER AND MORE CURLED. IT HAS SPREAD OUT AND THINLY COVERS A SOMEWHAT LARGER AREA.

4. DRAWING D



THE HAIR IS NOW AS DARK, CURLY, AND COARSE AS THAT OF AN ADULT MALE. HOWEVER, THE AREA THAT THE HAIR COVERS IS NOT AS LARGE AS THAT OF AN ADULT MALE. THE HAIR HAS NOT SPREAD OUT TO THE THIGHS.

5. DRAWING E



THE HAIR HAS SPREAD OUT TO THE THIGHS. THE HAIR IS NOW LIKE THAT OF AN ADULT MALE. IT COVERS THE SAME AREA AS THAT OF AN ADULT MALE.

Appendix N

MATURATION ASSESSMENT PROCEDURES (FEMALES) – PARENT/GUARDIAN

The assessment of physiological and physical change during growth is essential for the valid interpretation of human performance.

Pubertal maturity will be self-assessed at home following completion of the exercise testing protocol.

Drawings of male and female pubic hair developed by Morris and Udry (1980) made from photographs by Tanner (1975) will be used. A written description of each of the 5 stages of male and female pubic hair development will be given on the chart as how to select the correct pubertal stage.

The most practical approach to assess maturation was developed by Tanner (1962). Tanner developed a 5-point scale to assess 'biological maturity' through observation of secondary sexual characteristics. The scales depicted five or more stages of breast and pubic hair (girls), pubic hair and genitalia development (boys). A limitation is that trained health professionals such as paediatricians and school nurses are typically employed to assess the scales. Subsequently Morris and Udry (1980) developed a self-assessment scale based on Tanner stages and found that children were able to accurately assess their own stage of maturation with correlation coefficients in the range of 60-70% (Matsudo & Matsudo, 1993).

The secondary sex characteristics described by Tanner (1962) will be used to assess pubertal maturity. Pubertal stage 1 will be identified as pre-pubertal, stages 2, 3 and 4 circum-pubertal, and stage 5 post-pubertal.

(Please find these below and circle the drawing which you feel best represents you)

THE DRAWINGS ON THIS PAGE SHOW DIFFERENT AMOUNTS OF FEMALE PUBIC HAIR. A GIRL PASSES THROUGH EACH OF THE FIVE STAGES SHOWN BY THESE DRAWINGS. PLEASE LOOK AT EACH DRAWING AND READ THE SENTENCES UNDER THE DRAWINGS. THEN CHOOSE THE DRAWING CLOSEST TO YOUR STAGE OF HAIR DEVELOPMENT AND MARK IT 1. THEN CHOOSE THE DRAWING THAT IS NEXT CLOSEST AND MARK IT 2.

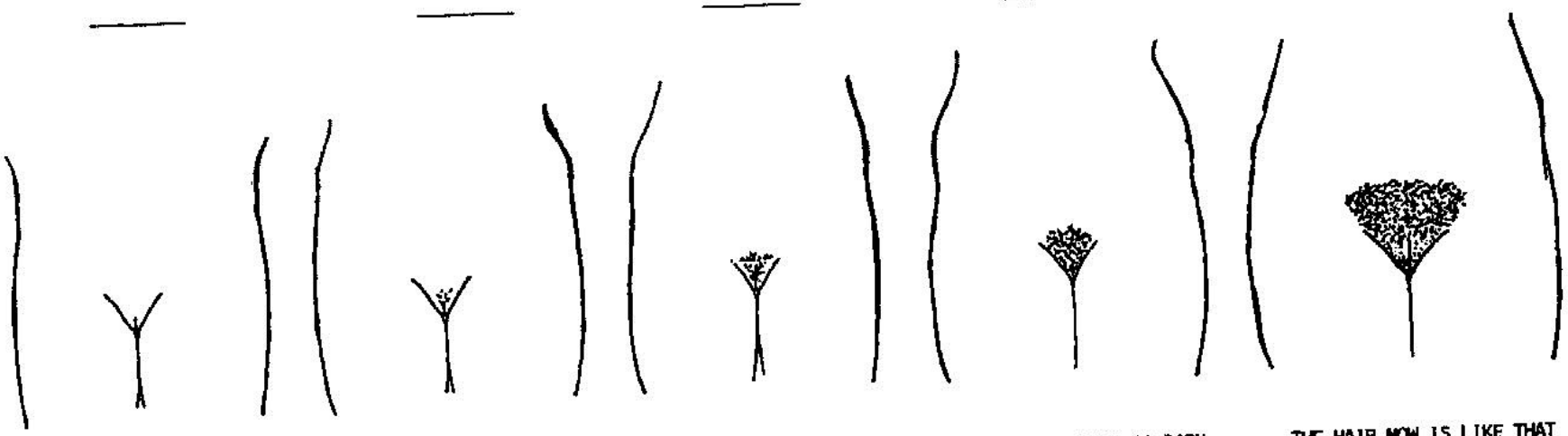
1. DRAWING A

2. DRAWING B

3. DRAWING C

4. DRAWING D

5. DRAWING E



THERE IS NO PUBIC HAIR.

THERE IS A LITTLE LONG, LIGHTLY COLORED HAIR. THIS HAIR MAY BE STRAIGHT OR A LITTLE CURLY.

THE HAIR IS DARKER IN THIS STAGE. IT IS COARSER AND MORE CURLED. IT HAS SPREAD OUT AND THINLY COLVERS A LARGER AREA.

THE HAIR IS NOW AS DARK, CURLY, AND COARSE AS THAT OF AN ADULT FEMALE. HOWEVER, THE AREA THAT THE HAIR COVERS IS NOT AS LARGE AS THAT OF AN ADULT FEMALE. THE HAIR HAS NOT SPREAD OUT TO THE THIGHS.

THE HAIR NOW IS LIKE THAT OF AN ADULT FEMALE. IT ALSO COVERS THE SAME AREA AS THAT OF THE ADULT FEMALE. THE HAIR USUALLY FORMS A TRIANGULAR (▽) PATTERN AS IT SPREADS OUT TO THE THIGHS.

Appendix O

Activity Log Book

DAY 1:

Time	Activity
06:00 – 07:00	
07:00 – 08:00	
08:00 – 09:00	
09:00 – 10:00	
10:00 – 11:00	
11:00 – 12:00	
12:00 – 13:00	
13:00 – 14:00	
14:00 – 15:00	
15:00 – 16:00	
16:00 – 17:00	
17:00 – 18:00	
18:00 – 19:00	
19:00 – 20:00	
20:00 – 21:00	
21:00 – 22:00	
22:00 – 23:00	
23:00 – 00:00	

Appendix P

Physical Activity Assessment – Parent/Guardian On-Off Log

Dear Parent,

Your child is currently wearing a physical activity monitor. Please ensure that monitors are removed prior to swimming, bathing, showering and before bedtime. Could you please make a note of the time your child puts the monitor on each morning and the time it is taken off at night. It would also be helpful if any times the monitor is removed during the day could be recorded. Thank you.

Day	Time put on in morning	Time taken off at night	Times removed during the day
Day 1			
Day 2			
Day 3			
Day 4			
Day 5			
Day 6			
Day 7			

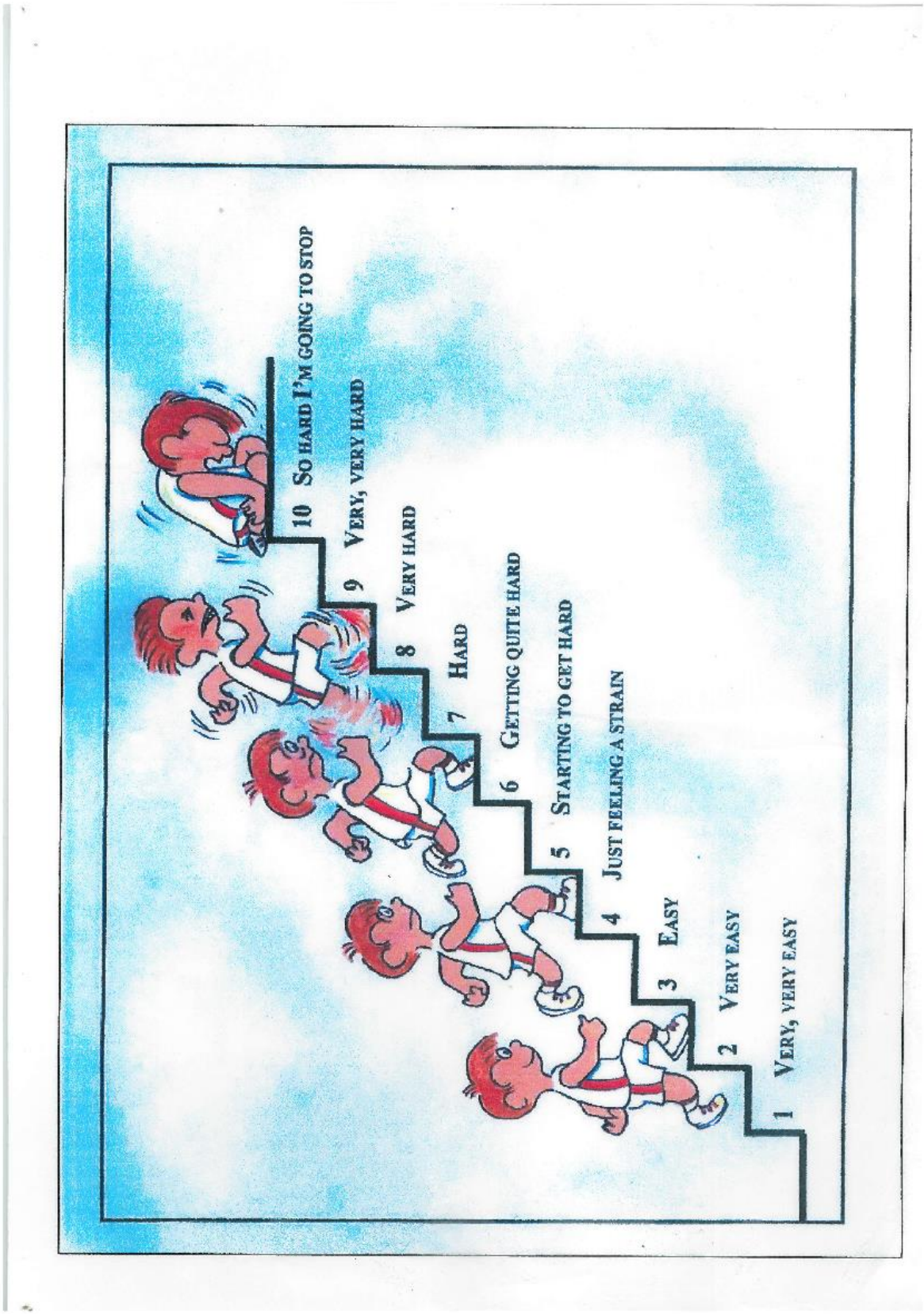
Please return the monitor in the envelope provided following the 7-day assessment.

PLEASE ENSURE THE MONITOR IS RETURNED ON: **ASAP FOLLOWING 7-DAY ASSESSMENT**

Thank you

Owen Tomlinson
01392 264721
o.w.tomlinson@exeter.ac.uk

Appendix Q


1 VERY, VERY EASY
2 VERY EASY
3 EASY
4 JUST FEELING A STRAIN
5 STARTING TO GET HARD
6 GETTING QUITE HARD
7 HARD
8 VERY HARD
9 VERY, VERY HARD
10 SO HARD I'M GOING TO STOP

Appendix R

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	
7	Very severe
8	
9	
10	Very, very severe (almost maximum)

Appendix S

UNIVERSITY OF EXETER

HEALTH SCREEN FOR CHILD VOLUNTEERS (PARENTAL FORM) – March 2009

Name: .....

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is:

(i) To ensure their own continuing well-being
(ii) To avoid the possibility of individual health issues confounding study outcomes.

Your answers to the questions in this questionnaire, on behalf of your child, are **strictly confidential**.

Please complete this brief questionnaire to confirm your child's fitness to participate:

1. **At present,** does your child have any health problem for which they are:
   - (a) On medication, prescribed or otherwise ..... Yes ☐ No ☐
   - (b) Attending a general practitioner ..... Yes ☐ No ☐
   - (c) On a hospital waiting list ..... Yes ☐ No ☐

2. **In the past two years,** has your child had any illness that required them to:
   - (a) Consult your family GP ..... Yes ☐ No ☐
   - (b) Attend a hospital outpatient department ..... Yes ☐ No ☐
   - (c) Be admitted to hospital ..... Yes ☐ No ☐

3. **Has your child ever** had any of the following:
   - (a) Convulsions/epilepsy ..... Yes ☐ No ☐
   - (b) Asthma ..... Yes ☐ No ☐
   - (c) Eczema ..... Yes ☐ No ☐
   - (d) Diabetes ..... Yes ☐ No ☐
   - (e) A blood disorder ..... Yes ☐ No ☐
   - (f) Head injury ..... Yes ☐ No ☐
   - (g) Digestive problems ..... Yes ☐ No ☐
   - (h) Heart problems ..... Yes ☐ No ☐
   - (i) Lung problems ..... Yes ☐ No ☐
   - (j) Problems with bones or joints ..... Yes ☐ No ☐
   - (k) Disturbance of balance/coordination ..... Yes ☐ No ☐
   - (l) Numbness in hands or feet ..... Yes ☐ No ☐
   - (m) Disturbance of vision ..... Yes ☐ No ☐
   - (n) Ear / hearing problems ..... Yes ☐ No ☐

(o) Thyroid problems..... Yes ☐ No ☐

(p) Kidney or liver problems..... Yes ☐ No ☐

(q) Allergy to nuts..... Yes ☐ No ☐

(r) Eating disorder ..... Yes ☐ No ☐

4. Do you know of any other reason why your child should not engage in physical activity?

Yes ☐ No ☐

If YES to any question, please describe briefly (for example, to confirm problem was/is short-lived, insignificant or well controlled.)

A member of our research team may contact you if we have any further questions.

Thank you for your cooperation!

UNIVERSITY OF EXETER

Comment by Head of Research Project:-

Signed: Date:

Appendix T

Magnetic environment screening form for Non-scanned Individuals

The MR system has a very strong magnetic field that may be hazardous to individuals entering the MR environment if they have certain metallic, electronic, magnetic or mechanical implants, devices or objects. Therefore all individuals are required to fill out this form before entering the scanner room. Be advised, the magnet is **always ON.**

Date:..... **Name:**.....
Contact Address:.....

1. Have you had prior surgery or an operation (e.g., arthroscopy, endoscopy, etc.) of any kind?

☐ No ☐ Yes

If yes, please indicate date and type of surgery:.....

2. Have you had an injury to the eye involving a metallic object (e.g., slivers, foreign body)?

☐ No ☐ Yes

If yes, please describe:.....

3. Have you ever been injured by a metallic object or foreign body (e.g. shrapnel, bullet etc.)?

☐ No ☐ Yes

If yes, please describe:.....

4. Are you pregnant, or suspect that you may be pregnant? ☐ No ☐ Yes

WARNING: Certain implants, devices, or objects may be hazardous to you in the MR environment. Do not enter the scanning room if you have any question or concern regarding an implant, device or object.

5. Please indicate if you have any of the following:

☐ No ☐ Yes Aneurysm clip(s)
☐ No ☐ Yes Cardiac pacemaker
☐ No ☐ Yes Implanted cardioverter defibrillator (ICD)
☐ No ☐ Yes Electronic implant or device
☐ No ☐ Yes Magnetically-activated implant or device
☐ No ☐ Yes Neurostimulation system
☐ No ☐ Yes Spinal cord stimulator
☐ No ☐ Yes Cochlear implant or implanted hearing aid
☐ No ☐ Yes Insulin or infusion pump
☐ No ☐ Yes Implanted drug infusion pump
☐ No ☐ Yes Any type of prosthesis or implant
☐ No ☐ Yes Artificial or prosthetic limb
☐ No ☐ Yes Any metallic fragment or foreign body
☐ No ☐ Yes Any external or internal metallic object
☐ No ☐ Yes Hearing aid
☐ No ☐ Yes Other implant .....

Important Instructions

Remove all metallic objects before entering the scanner room including hearing aids, mobile phone, keys, glasses, hair pins, jewellery, watches, safety pins, paperclips, credit cards, magnetic strip cards, coins, pens, pocket knives, nail clippers, steel-toed boots/shoes and all tools. Loose metallic objects are especially prohibited within the MR environment.

Please consult a member of staff if you have any questions or concerns before entering the scanner room.

I confirm the above information is correct to the best of my knowledge. I have read and understood the entire contents of this form and have had the opportunity to discuss its contents to my satisfaction.

Signature (person completing the form):..... Reviewed by:..... Signature:.....