Cardiopulmonary interactions and exercise capacity in patients with a Fontan circulation

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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STATEMENT OF ORIGINALITY

This is to certify that to the best of my knowledge; the content of this thesis is my own work and that all the assistance received in preparing this thesis and sources have been acknowledged. This thesis has not been submitted for any degree or other purposes.

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AUTHORSHIP ATTRIBUTION STATEMENT

For all manuscripts arising from this thesis, I significantly contributed to the conceptualisation of the project, methodology, data curation and formal analyses, drafting and revision of the manuscripts. In cases where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author.

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ABSTRACT

Children born with a functionally single ventricle who undergo a Fontan completion have altered haemodynamics associated with increased morbidity. They have significantly impaired lung function and reduced exercise capacity. This thesis aims to provide a more in-depth understanding of the altered cardiopulmonary interaction, abnormal pulmonary function and exercise intolerance in children and adults with a Fontan circulation.

Chapter 1 provides a summary of the evolution of the Fontan circulation, and critically reviews the current literature on cardiopulmonary interaction in the normal and Fontan circulations. Due to the altered haemodynamics of the Fontan circulation, the normal effects of respiration on the cardiovascular system are more pronounced. Inspiration alters intrathoracic and intra-abdominal pressures thereby influencing SVC and IVC return, and augmenting antegrade pulmonary blood flow. Due to this close interaction, abnormal lung function and respiratory and skeletal muscle weakness have been linked to impaired exercise capacity. For these reasons, a more comprehensive assessment of lung function and exercise capacity are being explored, and treatments directed at improving lung function and muscle strength, both before and after Fontan completion, are of great interest.

In Chapter 3 we sought to further characterise the abnormalities of lung function in the Fontan cohort and gain a better understanding of underlying aetiology. Through detailed lung function testing we showed significantly impaired diffusing capacity of carbon monoxide and nitric oxide, alveolar volume, and lung stiffness (measured by X5); but resistance, capillary volume and capillary membrane function were preserved. We went on to demonstrate that the low diffusing capacity of carbon monoxide in Fontan patients was largely driven by a low alveolar volume. Our results suggests that an earlier Fontan completion may have a beneficial effect on lung function.

In Chapter 4 we assessed the benefits of inspiratory muscle training on exercise capacity. Patients with a Fontan circulation have been shown to have reduced respiratory muscle strength. Using a 6-week home based program, we found significant improvement in inspiratory muscle strength, and an associated improvement in the ventilatory efficiency of

exercise (based on VE/VCO_2 slope). Through exercise cardiac magnetic resonance imaging we were also able to demonstrate improvement in resting cardiac output and ejection fraction, suggestive of improved haemodynamics. These results support the use of a simple therapy, namely inspiratory muscle training, that can be added to current management of Fontan patients.

In Chapter 5 we explore other parameters of exercise capacity in patients with a Fontan circulation. It is well-established that Fontan patients have reduced exercise capacity, and many fail to reach a maximal cardiopulmonary exercise test. Hence, sub-maximal exercise parameters may be more helpful in the interpretation of exercise in Fontan patients. In the healthy population, peak oxygen pulse is used as a non-invasive measure of stroke volume. However, in the latter part of exercise, oxygen pulse is predominantly determined by oxygen extraction, which can be impaired in the Fontan circulation, making peak oxygen pulse a poor marker of stroke volume. We explored using oxygen pulse measurements across the whole of exercise, and examined alternate oxygen pulse kinetics, namely oxygen pulse slope. We demonstrated that peak oxygen pulse slope correlated with peak aortic flow measured during exercise cardiac magnetic resonance imaging. We propose that peak oxygen pulse slope is more reflective of peak stroke volume than peak oxygen pulse in the Fontan population and may be a useful submaximal exercise parameter.

In Chapter 6 we established Fontan-specific normative data for maximal workload, and oxygen uptake at anaerobic threshold and peak exercise. We derived this normative data from asymptomatic patients with a New York Heart Association class I, establishing normative values for well-functioning Fontan patients. We utilised the publicly available data from the Paediatric Heart Network. We propose that this is potentially more useful normative data than that derived from healthy controls, given the known inherent circulatory limitations of the Fontan circulation, and will assist in management decisions and prognostication.

This thesis characterises the impaired lung function and abnormal exercise capacity in patients with a Fontan circulation. It provides a better understanding of the impaired respiratory function, haemodynamic responses to exercise, and altered cardiopulmonary interactions, in Fontan patients.

MANUSCRIPTS AND PRESENTATIONS

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Abstract: Older age of Fontan completion is associated with impaired lung function

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Laohachai K and Ayer J. Impairments in pulmonary function in Fontan patients: Their causes and consequences. Front. Pediatr. 2022 Apr 15. Published online.

Laohachai K, Badal T, Thamrin C, Robinson PD, Kennedy B, Rice K, Selvadurai H, Weintraub R, Cordina R, d'Udekem Y, Ayer J. Older age at Fontan completion is associated with reduced lung volumes and increased lung reactance. Int J Cardiol. 2022 Oct 1; 364:38-43

Manuscripts in Preparation

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Other Publications Utilising data from this Thesis

(Data derived from this thesis was utilised for these publication but does not constitute part of the current submission)

Wadey CA, Barker AR, Stuart G, Tran DL, ***Laohachai K***, Ayer J, Cordina R, Williams CA. Scaling Peak Oxygen Consumption for Body Size and Composition in People With a Fontan Circulation. J Am Heart Assoc. 2022 Dec 20;11(24):e026181

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Oral Presentations from work in this thesis

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2017 7th World Congress of Paediatric Cardiology & Cardiac Surgery, Barcelona, Spain
Laohachai K, Winlaw D, Selvadurai H, Gnanappa GK, d'Udekem Y, Celermajer D, Ayer J. Inspiratory muscle training improves inspiratory muscle strength, resting cardiac output and the ventilatory efficiency of exercise in patients with a Fontan circulation.

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ABBREVIATIONS

ANZFR	Australia and New Zealand Fontan Registry
AP	Atriopulmonary
AT	Anaerobic threshold
ATS	American Thoracic Society
AV	Atrioventricular
BDG	Bidirectional Glenn
BiPAP	Bi-level positive airway pressure
BMI	Body mass index
BPV	Biphasic ventilation
BR	Breathing reserve
$C(a\text{-}v)O_2$	Arteriovenous oxygen content difference
CHAT	Congenital Heart Adolescent and Teenager
CHD	Congenital heart disease
CHF	Chronic heart failure
CHW	The Children's Hospital at Westmead
CMR	Cardiac magnetic resonance
CO	Carbon monoxide
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise test
CVP	Central venous pressure
DLCO	Diffusing capacity of carbon monoxide
DLNO	Diffusing capacity of nitric oxide
DmCO	Alveolar-capillary membrane diffusing capacity for carbon monoxide
ECC	Extracardiac conduit
ECG	Electrocardiogram
EF	Ejection fraction
EOV	Exercise oscillatory ventilation
ERS	European Respiratory Society
FEV1	Forced expiratory volume in 1 second

FRC	Functional residual capacity
FSV	Functional single ventricle
FVC	Forced vital capacity
HLHS	Hypoplastic left heart syndrome
HR	Heart rate
HRQOL	Health related quality of life
I:E	Inspiratory: expiratory ratio
IMT	Inspiratory muscle training
IPAQ	International Physical Activity Questionnaire
IQ	Intelligence quotient
IVC	Inferior vena cava
KCO	Rate of uptake of carbon monoxide
LCI	Lung clearance index
LT	Lateral tunnel
LV	Left ventricle
MBNW	Multiple breath nitrogen washout
MEP	Mean expiratory pressure
MIP	Mean inspiratory pressure
MRI	Magnetic resonance imaging
NO	Nitric oxide
NPV	Negative pressure ventilation
NYHA	New York Heart Association
O_2	Oxygen
OUES	Oxygen uptake efficiency slope
PA	Pulmonary artery
PCO_2	Partial pressure of carbon dioxide
PEEP	Positive end-expiratory pressure
PFT	Pulmonary function test
PLE	Protein-losing enteropathy
PPV	Positive pressure ventilation
PVR	Pulmonary vascular resistance
R5	Resistance at 5Hz
RER	Respiratory exchange ratio

RMT	Respiratory muscle training
RPA	Right pulmonary artery
RPAH	The Royal Prince Alfred Hospital
RV	Right ventricle
SNIP	Sniff nasal inspiratory pressure
SV	Stroke volume
SVC	Superior vena cava
TCPC	Total cavopulmonary connection
TLC	Total lung capacity
VA	Alveolar volume
VC	Capillary volume
VCO_2	Carbon dioxide production
Vd/Vt	Dead space ventilation
VE	Minute ventilation
VE/VCO_2	Ventilatory equivalence of carbon dioxide
VE/VO_2	Ventilatory equivalence of oxygen
VO_2	Oxygen consumption
V/Q	Ventilation / Perfusion
WIMR	Woolcock Institute of Medical Research
X5	Reactance at 5Hz

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CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

Parts of this chapter is based on the publication:

Laohachai K and Ayer J. Impairments in pulmonary function in Fontan patients: Their causes and consequences. Front. Pediatr. 2022 Apr 15. Published online.

1.1 Introduction and Aims

Patients with a functionally single ventricle anatomy who have undergone Fontan completion have impaired lung function and reduced exercise capacity. The aetiology behind this has been explored, however a more in-depth understanding of the pathophysiology may shed light on potential future management to improve morbidity in the growing and ageing Fontan population. The goal of this thesis is to provide a better understanding of respiratory function, haemodynamic responses to exercise, and altered cardiopulmonary interactions, in patients with a Fontan circulation. This knowledge will provide insight into ways of improving the long-term management of these patients, reducing morbidity. The specific aims of this thesis are to: i) explore the mechanisms for and determinants of altered lung function, ii) investigate a specific intervention targeting respiratory function, namely inspiratory muscle training and, iii) explore new measures of exercise performance.

1.2 Congenital Heart Disease

Congenital heart disease (CHD) represents the most common birth defect, with its incidence estimated to be around 6-10 per 1,000 live births [1,2]. Since the 20th century there has been an increasing prevalence of CHD, with ninety-seven percent of children with CHD now surviving into adulthood [3,4]. There has been a significant fall in mortality rate, and the median age of adults with CHD is now in the forties [5].

Despite these improvements in survival, both children and adults with “repaired” CHD face important long-term health challenges. Due to their complex anatomy, multiple previous surgical procedures, and altered haemodynamics, these patients are prone to cardiac and non-cardiac complications including arrhythmias, coronary artery disease, heart failure, pulmonary hypertension, restrictive lung disease, renal and hepatic failure, psychiatric disorders, and developmental disabilities [6-9]. Patients with CHD are known to have abnormal lung function and impaired exercise capacity compared to their healthy peers, with significant morbidity [10-12]. Although advances in medical and surgical management have improved the life expectancy for these patients, their complex anatomy and multi-system co-morbidities requires life-long specialised care. There is now more focus on preventing and managing the longer-

term complications of these conditions including improving exercise capacity and quality of life.

1.3 Functional Single Ventricle

1.3.1 Epidemiology

The incidence of severe congenital heart defects requiring specialist care is reported to be 2.5-3 per 1,000 live births [1] and of these, an estimated 4-8 per 10,000 live births have a functional single ventricle (FSV) [13]. A FSV anatomy occurs when two well-developed ventricles fail to form, and one or both ventricular chambers are unable to support either the pulmonary or systemic circulation independently. In some patients, despite having two well-formed ventricles, a biventricular repair is unable to be achieved due to the spatial relationship of the cardiac chambers and great vessels. These underling cardiac anatomies result in only a solitary ventricle acting as a pump to sustain both the systemic and pulmonary circulations (figure 1.1).

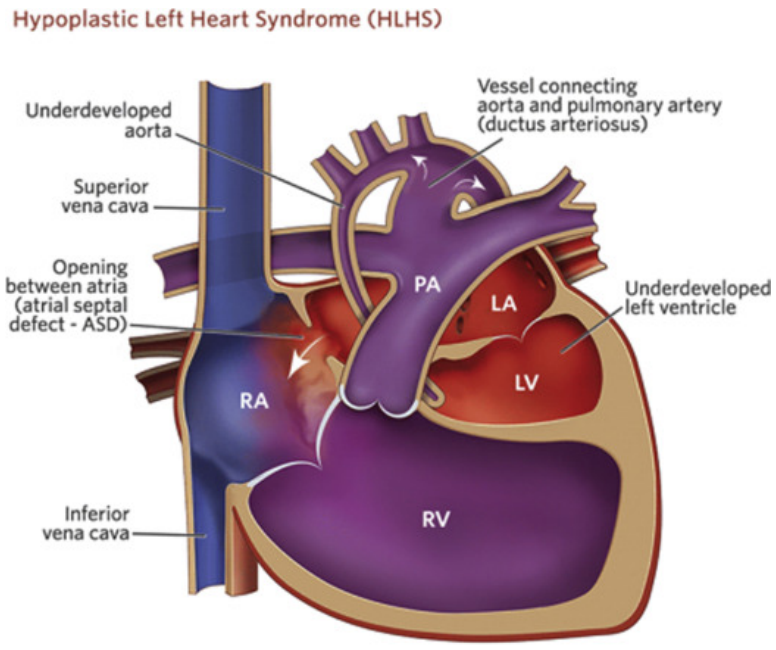


Figure 1.1. Example of a functional single ventricle anatomy

Reproduced from Metcalf and Rychik, 2020 [14]

Single ventricle physiology occurs in a diverse spectrum of anatomical cardiac defects. The commonest cardiac defects with a FSV are tricuspid atresia (24% of FSV), double inlet left ventricle (18%), double outlet right ventricle (14%) and hypoplastic left heart syndrome

(HLHS) (8%) [15]. Children with a FSV are now typically diagnosed antenatally or early during the neonatal period after presenting with cyanosis, tachypnoea, or low cardiac output. The natural course of these pathologies varies substantially depending on the balance between systemic and pulmonary blood flow.

1.3.2 Surgical Pathway Evolution

Surgical options for patients with a FSV have been explored since the late 1940s and have evolved over subsequent years, developing into the current era of staged palliative procedures. Surgical strategies to deal with FSV were initially developed in patients with a hypoplastic right ventricle (RV), for example, in patients with tricuspid atresia. In the absence of a RV to pump blood to the pulmonary circulation, several approaches evolved to bypass the hypoplastic ventricle and divert blood to the pulmonary circulation.

In 1945 Blalock and Taussig described the creation of an anastomosis between a branch of the head and neck vessels arising from the aorta (innominate or subclavian artery) and a pulmonary artery (PA) branch [16]. This allowed blood pumped systemically from the single ventricle to reach the pulmonary arteries and be oxygenated. In 1949 Rodbard and Wagner reported the first attempt to bypass the RV completely in a canine, by attaching the tip of the right atrial appendage to the main pulmonary artery, with closure of the atrial communication and oversewing of the tricuspid valve annulus [17]. In 1950, Carlon, Mondini and de Marchi connected the superior vena cava (SVC) to the right pulmonary artery (RPA) as the source of pulmonary blood flow in those with right heart obstruction [18].

This procedure was revisited by Glenn and Patino in 1954, confirming that a pumping chamber was not required to push blood forward into the pulmonary circulation [19]. They published their results of performing an anastomosis between the SVC and RPA in 75 animals, and an inferior vena cava (IVC) to RPA anastomosis in 46 animals. These experiments demonstrated that an SVC to RPA anastomosis was more favourable than an IVC to RPA anastomosis due to splanchnic venous congestion occurring after the latter. Hence, the use of an IVC to RPA connection was temporarily abandoned and the SVC to RPA anastomosis was further explored. The first successful SVC to PA anastomosis in a human was undertaken about 4 years later, in a 7-year old boy with transposition of great arteries and pulmonary valvar stenosis [20].

Due to persistent mixing of desaturated systemic venous blood from the IVC and oxygenated pulmonary venous blood from the SVC to RPA anastomosis, patients undergoing this procedure remained desaturated. To overcome this, Kreutzer et al. performed an operation in 1971 to route all systemic venous return to the pulmonary circulation without a driving pumping chamber ("Classical Fontan", figure 1.2A) [21,22]. This was achieved by connecting the right atrium directly to the PA (atriopulmonary (AP) connection) and closure of the atrial communication. This allowed all desaturated systemic venous blood to drain directly to the lungs, and oxygenated pulmonary venous blood to drain into the single ventricle before being pumped systemically, eliminating complete mixing of oxygenated and deoxygenated blood. Incorporation of the right atrium into the circulation was with the intent that the right atrium could act as a pump to push blood forward into the lungs. This procedure was performed in 3 patients who were "sufficiently well developed, without pulmonary arterial hypertension" [22]. All three patients had good outcomes at their 30 months follow-up. In their description of the procedure, they highlighted that for the procedure to be successful, the pulmonary arteries had to be large enough to allow a cavopulmonary anastomosis and the pressures in the lungs had to be low to allow forward flow from the vena cavae into the branch pulmonary arteries [22].

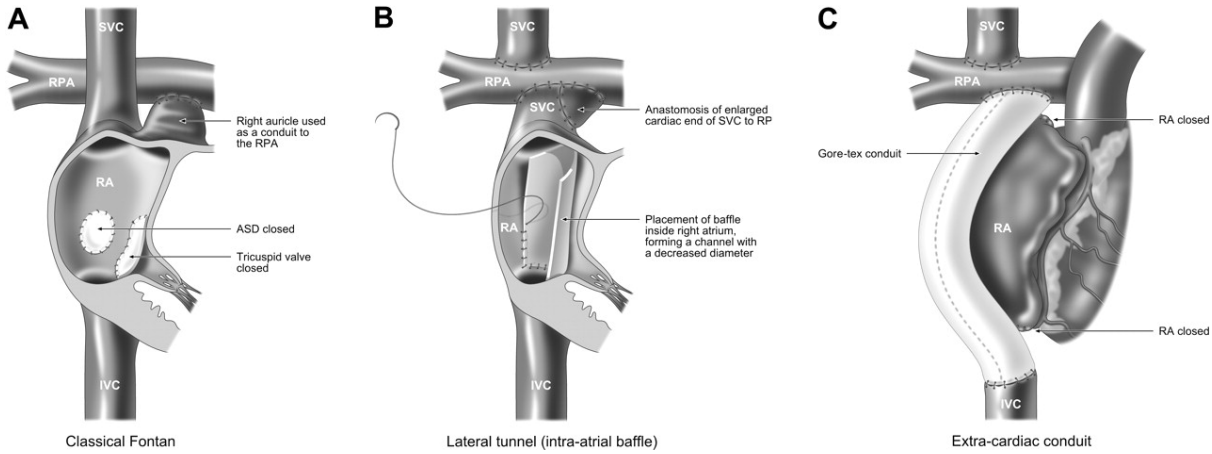


Figure 1.2. Evolution of Fontan techniques.

A) Classical atriopulmonary connection, B) Lateral tunnel, and C) extracardiac conduit. Reproduced from d'Udekem et al., 2007 [23]

Although their initial reports of the operation were only in patients with tricuspid atresia, Fontan et al. were hopeful that the operation could be applied to many types of FSV. This procedure, which has come to be known as the "Fontan" operation, was originally performed as a single stage operation but has subsequently undergone several modifications to become a multi-staged procedure, taking place over the first few years of life.

1.4 The Fontan Procedure

Over time, the original Fontan procedure proved to be problematic with low pulmonary blood flow, pulmonary venous obstruction from the atrial septum protruding into the left atrium, and significant arrhythmias from severe atrial dilatation. Early re-operation rates were higher than 40% and late arrhythmias were reported to occur in 69% of subjects [24,25]. To improve the circulation's efficiency and overall outcomes, the procedure and its techniques have undergone numerous revisions, with different systemic to pulmonary connections and use of valves within the circuit. A variety of materials for the circuit have also been trialled including valved conduits, homografts and polytetrafluorethylene grafts [26]. Along with these changes, there has been expansion of patient selection and a widening of the indications for the Fontan procedure.

1.4.1 Indications for Fontan

When Fontan and Baudet first described the Fontan operation, it was aimed as a surgical treatment for tricuspid atresia. They described selection criteria for patients most suited for this operation, commonly referred to as the "10 Commandments" [27]. These "commandments" stated that the patient selected for a Fontan procedure should have: normal caval drainage, normal right atrial volume, a mean pulmonary arterial pressure ≤15mmHg, a pulmonary arterial resistance <4U/m^2, a PA to aorta diameter ratio ≥0.75, normal ventricular function with an ejection fraction >60%, a competent left atrioventricular valve and no impairing effects from previous shunts. They also concluded that patients should be a minimum of 4 years and be in sinus rhythm. However, with advances in neonatal management and surgical technique, the selection criteria has been simplified to include patients who meet essentially two criteria: low transpulmonary gradient and left atrial pressures [24]. It is now used as a surgical treatment for a wide range of complex congenital cardiac lesions that are not suitable for biventricular repair, including HLHS.

1.4.2 Evolution of the Total Cavopulmonary Connection

De Leval et al. tested the haemodynamics of the Fontan circulation and assessed ways to improve the efficiency of the circulation [28]. They described a modified total cavopulmonary (TCPC) Fontan procedure with end-to-end anastomosis of the SVC to the RPA, insertion of an

intra-atrial tunnel incorporating the posterior right atrial wall, and attachment of the IVC to the main pulmonary artery – now known as a lateral tunnel (LT) (figure 1.2B) [29]. They initially performed this procedure in 20 patients with tricuspid atresia, double inlet left ventricle, and hypoplastic right or left ventricles. They had three deaths related to ventricular failure and raised pulmonary vascular resistance. Through excluding most of the right atrium from the circuit, De Leval et al. reported reduction in turbulent flow and low pressure gradients throughout the circuit on post-operative cardiac catheterisation [28]. Due to the finding of lower right atrial pressures, they predicted a lower risk of longer-term atrial tachyarrhythmias. As the pathway in a LT involves part of the native right atrium, there is growth potential, allowing the procedure to be performed at a younger age.

In 1990, Marcelletti et al. published a new technique, eliminating the right atrium completely from the Fontan circuit – an extracardiac conduit (ECC) connection (figure 1.2C) [30]. In this procedure, the IVC flow is routed to the PA through a conduit placed external to the heart. This procedure was developed as an alternative surgical strategy in patients with hypoplastic atrial chambers or abnormal systemic and pulmonary venous drainage [30].

The TCPC procedure (LT and ECC connections) is now the surgical procedure of choice for patients with a FSV circulation. The main advantages of a cavopulmonary connection over an AP connection is less risk of progressive atrial dilatation, atrial arrhythmias, and atrial thrombi formation.

1.4.3 Staged Procedures

The original single-stage “Fontan” procedure has evolved into a series of staged operations (figure 1.3). The need for staging relates to the presence of important physiological changes in the circulation that occur over the first few years of life. The neonatal period and early infancy are characterised by high pulmonary vascular resistance which precludes effective “passive” blood flow (without a subpulmonary pump) to the lungs via a cavopulmonary connection.

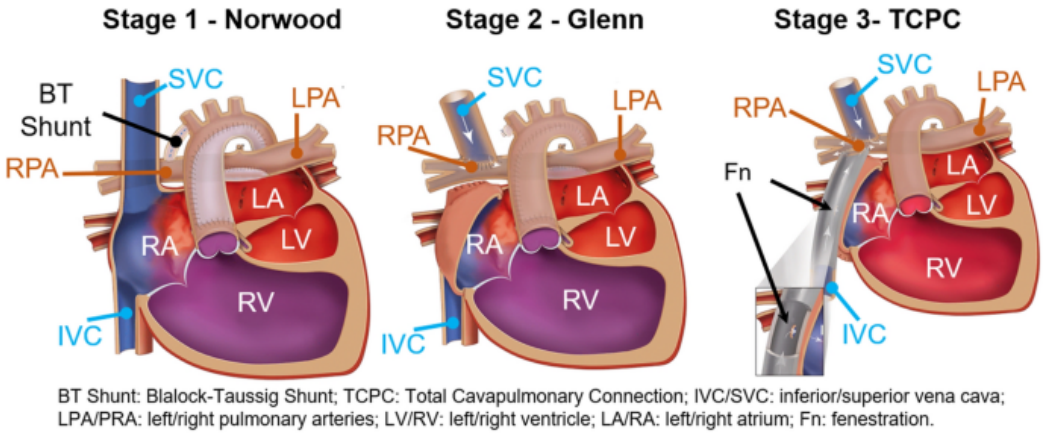


Figure 1.3. Fontan stages.

Reproduced from Wei and Fogel, 2021 [31]

1.4.3.1 The First Stage

The aim in the neonatal and early infancy period is to balance the maldistribution of blood flow through the pulmonary and systemic circulations. This can be with either a systemic arterial shunt (figure 1.3, stage 1) to augment inadequate pulmonary blood flow or with a pulmonary artery band to restrict excessive pulmonary blood flow. This allows the single ventricle to pump blood both systemically and to the pulmonary circulation simultaneously. The goals for the first stage of palliation are to provide unobstructed systemic blood flow, reliable pulmonary blood flow and unobstructed mixing of pulmonary and systemic venous return. Different types of systemic arterial shunts exist, depending on the underlying anatomy and surgical preference. These include a modified Blalock-Taussig shunt (connecting the right subclavian artery to PA), a Sano shunt (connecting the RV to PA), a central shunt (connecting aorta to PA) or a hybrid procedure involving stenting of the ductus arteriosus (figure 1.4) [32].

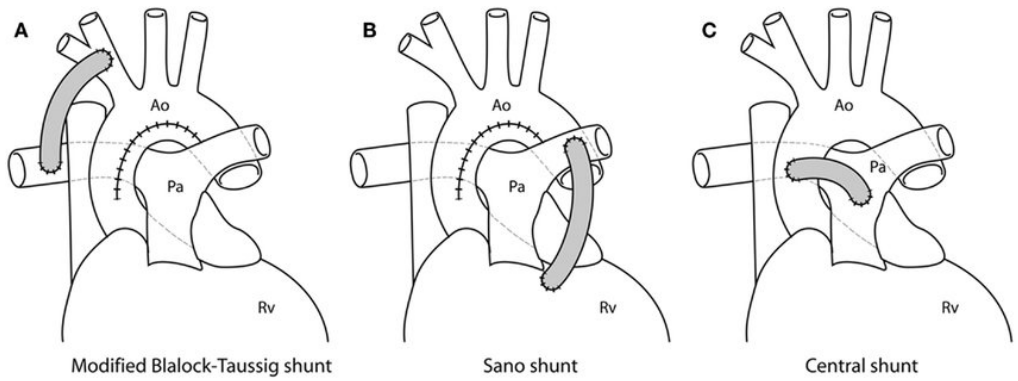


Figure 1.4. Arterial shunts

Reproduced from Biglino et al. [33]

1.4.3.2 The Second Stage

The second stage of palliation for patients with a FSV involves anastomosing the SVC to the RPA and disconnecting the systemic arterial shunt [18]. This results in systemic venous return from the upper body to drain into the pulmonary arteries. The second stage has two modifications, the hemi-Fontan and the bidirectional Glenn (BDG) [34]. The hemi-Fontan is a side-to-side anastomosis from the back of the SVC and right atrial junction to the anterior surface of the RPA, with insertion of an interatrial patch between the atrium and PA. In the BDG, the SVC is transected from the right atrium and attached to superior surface of the RPA via an end-to-side anastomosis (figure 1.3, stage 2). The procedure used during the second stage determines the surgical procedure for the third stage, with the hemi-Fontan typically resulting in the intracardiac lateral tunnel and then BDG resulting in an ECC circulation [34]. This interstage procedure has been shown to reduce morbidity and mortality following Fontan completion [35].

1.4.3.3 The Third (Final) Stage

The final stage, the Fontan completion, separates the systemic and pulmonary circulations by redirecting IVC flow to the pulmonary arteries and bypassing the single ventricle (figure 1.3, stage 3). This is achieved with either the LT or ECC connection (see section 1.4.2 - Evolution of the Total Cavopulmonary Connection). This results in all systemic venous return draining passively into the pulmonary arteries and pulmonary venous return draining into the common atrium. Blood is the ejected only to the systemic circulation from the single ventricle.

1.4.3.4 Single versus Staged Procedures

Introduction of the staged procedures has improved mortality following Fontan completion [35,36]. Pridjian et al. demonstrated that the BDG procedure can be safely performed in patients deemed as a high risk for a Fontan operation [35]. Wolff et al. analysed differences in mortality between the single- or staged- Fontan procedures in 203 patients undergoing a Fontan procedure [36]. Timing of the Fontan operation was dependent on clinical signs and symptoms of desaturation and exercise intolerance. The Fontan type was determined on an individual basis, with utilisation of either the LT, right atrial auricle tunnel or ECC. For those who underwent a staged procedure, the timing for a BDG was initially based on symptoms but over the study period evolved to be performed routinely at 3-6 months of age. Tricuspid atresia was the most common underlying anatomy (39%). Fifty-eight percent of their studied patients had

a single-stage procedure and 42% were staged. They found a significant reduction in mortality rate over the last decade, predominantly in early mortality, with multivariate analyses showing single stage Fontan as an independent risk factor for early mortality [36].

1.4.4 Fenestrated versus Non-fenestrated Fontan

The concept of a fenestrated Fontan circulation (figure 1.5) was introduced in the 1990s to improve early post-operative mortality. A fenestration (a hole between the Fontan circulation and the right atrium) can limit high central venous pressure, increase preload, and therefore improve cardiac output [37]. Bridges et al. described this procedure by punching a hole in the LT baffle creating a communication between the LT and atrium allowing right to left shunting and reducing systemic venous pressure [37]. It has become a routine part of the Fontan completion in many centres. However, a fenestration is at the expense of systemic desaturation and increased risk of thromboembolic events; and therefore, there remains controversy around the benefits of fenestration in non-high-risk patients and beyond the early post-operative period.

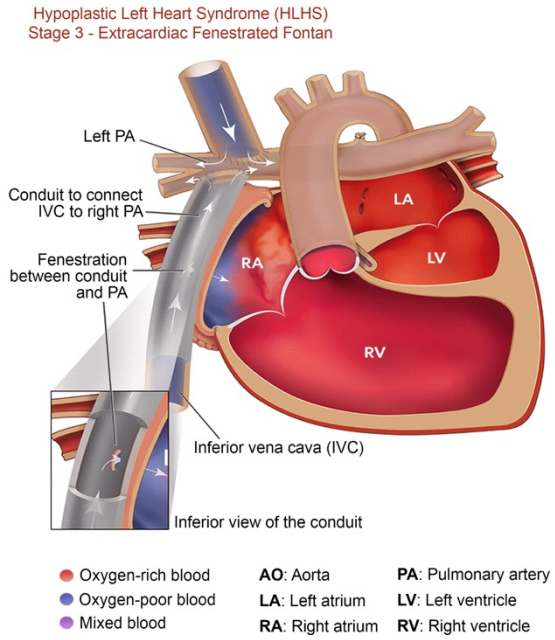


Figure 1.5. Fenestration in a Fontan circulation.

Reproduced from Files et al., 2018 [38]

Many studies have shown that fenestration is beneficial in high-risk patients with raised pulmonary vascular resistance (PVR) and impaired ventricular function. The benefits have included shorter hospital stays immediately post operatively, reduced pleural drainage and

lower morbidity [37,39-44]. Airan et al. found absence of fenestration to be the only significant predictor of Fontan failure, and a risk factor for significant prolonged pleural drainage [39]. In keeping with this, Lemler et al. found a 55% reduction in total chest tube drainage, 41% shorter length of hospitalisation and 67% fewer additional procedures in the post-operative period in the fenestrated subjects compared to the non-fenestrated subjects [44]. In a larger study of 500 patients, Gentles et al. also found an increased likelihood of Fontan failure in the non-fenestrated cohort [40], and that the major benefit was in the early post-operative period. Additionally reduced incidence of tachyarrhythmias in those with a fenestration, and increased incidence after fenestration closure, have been reported [45,46]. In patients with a failing Fontan, creation of a fenestration may be useful through improvement in cardiac output and a reduction in venous congestion [47].

In contrast, right to left shunting across the fenestration allows desaturated systemic venous blood to mix with the oxygenated blood being pumped systemically therefore causing systemic desaturation and mild cyanosis. This in turn has potential to cause pulmonary vasoconstriction and increased PVR. Additionally, it introduces an increased risk of thromboembolic events and consequent cerebrovascular accident due to the right to left shunting. In a study comparing subjects with and without fenestration, Atik et al. found lower central venous pressure in the fenestrated patients, but not rates of pericardial and pleural effusions nor length of hospital stay. Fan et al. assessed the benefits of fenestration in high versus low-risk patients [42]. They defined high-risk patients as those with high PA pressure, high ventricular end-diastolic pressure, PA distortion, single RV, and moderate-severe atrioventricular valve regurgitation. In 183 subjects they found the volume and duration of pleural effusion drainage to be significantly lower with fenestration in the high-risk group, but similar in the low-risk group [42]. In a meta-analysis of 19 studies with 4,806 subjects, Bouhout et al. found fenestration was associated with lower pulmonary pressures and less need for pleural effusion drainage, however, length of hospital stay, incidence of thromboembolic events, risk of Fontan failure, and early mortality were not different in the fenestrated subjects [41]. As expected, the fenestrated cohort also had lower systemic saturations. Incidence of arrhythmia, neurological events, Fontan failure and mortality are similar between the two groups [41-43].

Studies have further evaluated the effects of fenestration by looking at outcomes following fenestration closure. In 154 paediatric patients with a median follow-up of 3.4 years, Goff et

al. demonstrated improvement in saturations and growth, and rationalisation of heart failure medications following device closure of the fenestration [45]. Momenah et al. and Mays et al. also demonstrated improvement in exercise parameters including peak oxygen (O_2) saturations, peak oxygen consumption (VO_2) and exercise duration [48,49]. Atz et al. looked at 536 patients with a mean age of 11.9 years, of which 361 (67%) were fenestrated [46]. At 8 ± 3 years, after Fontan completion, they found only 19% had a patent fenestration. The majority were closed via catheterisation (59%), 1% closed surgically and 40% had spontaneous closure. They also found that those with a patent fenestration had lower systemic saturations and were on more medications. However, unlike Goff et al. they found no difference in growth, functional health as assessed by the Child Health Questionnaire, or exercise variables [46]. Additionally, there was no difference in long-term complication rates. Other studies have also found no difference in exercise parameters (VO_2 or peak oxygen pulse) after fenestration closure [50]. VE/VCO_2 (ventilatory equivalence of carbon dioxide) slope, a marker of the ventilatory response to exercise, was better in the non-fenestrated subjects likely secondary to reduced right to left shunting.

Fenestration is likely beneficial in patients at high-risk of Fontan failure during the early post-operative period but probably does not change outcome in standard-risk patients. Longer-term there are limited benefits of maintaining the fenestration, with potential for more complications. Accordingly, many centres no longer perform fenestration routinely as part of their Fontan completion but are selected on a case-by-case basis, and fenestration closure is considered longer-term.

1.4.5 Outcomes of Fontan

With these advances, the prognosis for children with these conditions has improved significantly over the last 50 years. Ten-year survival rates now range from 85-97% in Australia and New Zealand, depending on the type of Fontan procedure [15,23,51]. However, patients post Fontan completion are still at risk of late mortality and multi-system morbidity. Late complications include ventricular dysfunction, atrioventricular valve regurgitation, arrhythmias, conduit stenosis, arteriovenous malformations, plastic bronchitis, liver dysfunction and cirrhosis, protein-losing enteropathy, thromboses, and renal and neurological impairment [52-55]. Pulmonary hypertension may occur secondary to multiple aetiologies

including ventricular dysfunction, increased pulmonary vascular resistance or severe atrioventricular valve regurgitation.

1.4.5.1 Mortality

Survival rates following Fontan completion have improved substantially. Perioperative mortality rates have improved likely secondary to improvements in surgical technique (e.g., introduction of staging, extracardiac conduit, and fenestration) and medical peri-operative and long-term care. Early post-operative mortality has reduced significantly from nearly 20% in the early era of the Fontan operation, to 2% in the late 1990s, and is now close to 1% [56,57]. Risk factors for perioperative mortality can include prolonged cardiopulmonary bypass, high PVR, a systemic RV, heterotaxia, significant common atrioventricular valve regurgitation, impaired pre-operative ventricular function, and protein-losing enteropathy [58-62]. Across all Fontan-type circulations, 10-year survival is now at least 90% [23,51,59,60,62-66]. More recently, 10-year survival has been reported at 89% after AP connections and 97% after LT and ECC connections with a 30-year survival of 85% [60,67,68]. Mortality rates have differed between underlying anatomy, with HLHS patients having an increased morbidity and mortality [69]. Mortality may also differ between types of Fontan circulation [70].

Khairy et al. looked at causes of death in the Fontan population, finding that 9% were caused by ‘sudden death’, which they attributed to arrhythmias, 8% due to a thromboembolic cause, 7% were heart failure related, and 3% secondary to sepsis [62]. Lack of antiplatelet or anticoagulant therapy and presence of intracardiac thrombi were independent risk factors for thromboembolism. A meta-analysis by Poh and d’Udekem identified Fontan failure as the primary cause of late death [67]. They found prolonged chest tube drainage following Fontan operation, presence of protein losing enteropathy, increased ventricular end-diastolic volume, and a permanent pacemaker, as the most significant predictors of death [67].

1.4.5.2 Morbidity

Patients with a FSV are prone to multi-system complications, including neurological complications secondary to altered brain development, unstable haemodynamics in the neonatal period, multiple surgical procedures including circulatory arrest as a neonate, and thromboembolic events. The altered haemodynamics in FSV patients from the fetal period, and later following the Fontan operation, leads to risk of neurodevelopmental disabilities and multi-

system end organ damage. These morbidities are mediated by a combination of reduced cardiac output, raised central venous pressure, splanchnic venous congestion and deranged lymphatic return [71]. Freedom from Fontan failure (mortality, heart transplantation, Fontan takedown or conversion, protein-losing enteropathy, plastic bronchitis, or symptoms with New York Heart Association III or IV) is 95% at 5 years, 90% at 10 years and 70% at 20 years [60,62,72].

1.4.5.3 Health-related Quality of Life and Neuropsychological Outcomes

Children and adults with a Fontan circulation have the highest risk of neurodevelopmental disabilities compared to other forms of CHD [73,74]. Reduced oxygen and nutrient delivery to the developing fetal brain, secondary to altered haemodynamics, may also alter brain growth [75]. Neuroimaging has shown reduced brain volume from second trimester of pregnancy, which persists into adolescence [76,77]. Whole exome sequencing has revealed an overlap between de novo mutations in children with CHD and those associated with neurodevelopmental disorders, however further studies are required to fully understand the implications of these genetic mutations [78]. Other factors such as prematurity and early-term birth, low birth weight and low socioeconomic status can further increase risk [73,79,80]. Multiple procedures and surgeries during the neonatal and early childhood period may provide further insult to the developing brain. These factors affect longer-term quality of life, neurocognitive development, and mental health. Neurodevelopmental outcomes performance scores are lower in the Fontan population compared to their healthy peers, but with a normal intelligence quotient (IQ) score [81].

Assessment of health-related quality of life (HRQOL) looking at the impact of an illness on daily life, has been studied in Fontan patients across childhood, adolescence, and adulthood [82]. A meta-analysis and review of 50 articles found both self-reported and proxy reported HRQOL were reduced in patient of all ages with a Fontan circulation compared to their healthy counterparts across all domains (physical, psychosocial emotional, social and school/work) [82]. Physical functioning was the most affected domain. Marshall et al. identified older age of Fontan completion to be associated with poorer emotional function and a diagnosis of HLHS to be associated with poorer social functioning [82].

Reduced HRQOL and neurodevelopmental disability can have a significant impact on children with FSV transitioning to adulthood, and their ability to undertake normal essential daily

activities and maintain employment. Current international management guidelines recognise and emphasise the increased risk of neurodevelopmental, psychological, and behavioural disorders [57,81,83].

1.4.5.4 Ventricular Dysfunction

Ventricular dysfunction is a common finding in FSV patients with a Fontan circulation and contributes to early mortality. Many Fontan patients have a morphological RV as the systemic pumping chamber (e.g., HLHS), which is required to adapt to systemic pressures. Initial neonatal palliation with an arterial shunt or PA band can result in volume or pressure overload. Due to complete mixing of blood, systemic arterial oxygen saturations are low, including blood supplying the myocardium. Presence of significant valvar regurgitation further increases stress on the myocardium, in addition to multiple cardiac surgeries. Prolonged arrhythmia can further impair ventricular function.

In the short-term, systolic function is usually preserved [84] but systolic dysfunction can develop longer-term [85]. In a cross-sectional study of 546 children with a Fontan circulation, Anderson et al. found that ejection fraction (EF), a marker of systolic function, was normal in the majority (73%) of the subjects [84]. Systolic dysfunction was more likely in those with a morphological RV as their systemic ventricle [84,86]. Additionally, they noted low end-diastolic volumes with greater ventricular mass [84]. The systemic single ventricle in a Fontan circulation is exposed to a chronically reduced preload, which can result in ventricular hypertrophy with impaired ventricular relaxation and diastolic dysfunction [84,87]. Abnormal echocardiographic diastolic doppler parameters have been shown, suggestive of reduced ventricular compliance and abnormal relaxation. Anderson et al. found a large proportion (72%) of children with a Fontan circulation having diastolic dysfunction [84] and Cheung et al. demonstrated progressive decline in diastolic function through serial assessments, with impaired early relaxation and reduced ventricular compliance [88]. Abnormal ventriculo-vascular coupling may further contribute to the development of diastolic dysfunction [89,90].

1.4.5.5 Atrioventricular Valve Regurgitation

Severe atrioventricular (AV) valve regurgitation was identified early as a contra-indication to Fontan completion [27], adversely affecting the haemodynamics of the FSV circulation. Significant AV valve regurgitation can lead to volume overload, atrial and ventricular dilation,

increased pulmonary venous pressures and reduced ventricular contractility. There is usually a slow decline in AV valve function over time. The mechanism is multifactorial and is affected by chronic volume overload leading to annular dilation, AV valve abnormalities, and ventricular dysfunction [91]. Anderson et al. reported a very high incidence of any AV valve regurgitation at 74%, however the incidence of moderate-severe regurgitation was 19% [84]. In a meta-analysis involving 7,536 patients, Poh and d'Udekem found a similar incidence of 10% with significant AV valve regurgitation [67]. This is high when compared to an estimated prevalence of ~3% of at least moderate mitral or tricuspid regurgitation in the general elderly adult (≥65 years of age) population [92], with a likely lower incidence in the younger population.

1.4.5.6 Arrhythmia

In the original description of a vena-cava to pulmonary artery anastomosis, Fontan et al. discussed the potential complications of atrial arrhythmia secondary to the altered haemodynamics [22]. Sinus node dysfunction, atrial, junctional, and ventricular tachyarrhythmias have all been reported in Fontan patients. Underlying abnormal cardiac anatomy, surgical interventions with atrial suture lines, altered volume load, and AV valve regurgitation, likely contribute to the development of arrythmias. Presence of tachyarrhythmias can have detrimental effects on the Fontan circulation.

Sinus node dysfunction can occur following the Fontan operation [93-97]. It may be secondary to inherent underlying cardiac anatomical changes or to altered myocardial blood supply [95], and can predispose patients to atrial flutter or fibrillation. Atrial tachycardias are the most common arrhythmia experienced in Fontan patients, with an incidence of up to nearly 50% [98,99]. A large multi-centre cross-sectional study through the Pediatric Heart Network involving 520 paediatric patients, found a prevalence of atrial tachyarrhythmia of 9.6% [100]. Of these, 78% were intra-atrial re-entrant tachycardia, 14% were re-entrant atrioventricular tachycardia and 8% were ectopic atrial tachycardia [100]. They found that the prevalence of intra-atrial re-entrant tachycardia increased with age, with a mean age of presentation at 14.4 years (compared to 11.7 years for other types of tachyarrhythmias). A lower functional status based on Child Health Questionnaire [101], paced rhythm, and AP connection, but not underlying cardiac anatomy, were associated with development of intra-atrial re-entrant tachycardia [100]. Ghai et al. reviewed 94 patients following Fontan completion and found

34% had sustained atrial tachyarrhythmia [102]. They found that those who developed atrial tachyarrhythmia were more likely to develop heart failure, right atrial thrombus, atrial enlargement and have moderate-severe AV valve regurgitation. There was no difference in survival demonstrated in their study. Idorn et al. studied 235 paediatric and adult Fontan patients and found an increased incidence of arrhythmia in the older age groups [103]. However, long term follow-up has shown a continued risk for atrial arrhythmias after LT Fontan completion, with a prevalence as high as 44% [104], potentially related to the atrial suture line for attaching the lateral tunnel [105].

Ventricular arrhythmias are less common and have been noted in only 3.5-5% of patients [100,106]. Tan et al. performed a retrospective study looking at Fontan patients with documented ventricular tachyarrhythmia, ventricular fibrillation, resuscitated cardiac arrest or sudden cardiac death >30 days after any surgical intervention [107]. They found 21 out of 1611 patients (1.3%) had ventricular arrhythmias, of which 6 had sudden cardiac death at time of diagnosis. Older age at Fontan operation, early era of surgery, and moderate to severe ventricular dysfunction were associated with risk of ventricular arrhythmia and/or sudden cardiac death.

Blaufox et al. analysed data from the Pediatric Heart Network and assessed the association between heart rate and arrhythmia, with functional outcome [108]. Of 521 paediatric patients, 10% had a history of atrial tachycardia and 13% had a permanent pacemaker. They found that bradycardia was not associated with poor functional outcome and speculated that in fact a high resting heart rate may be secondary to low cardiac output and sympathetic stimulation. Additionally, a lower heart rate prolongs diastolic filling times, improving preload, which is limited in the Fontan circulation. At peak exercise, a higher peak heart rate was associated with a higher oxygen consumption at anaerobic threshold (a measure of exercise capacity) and higher Child Health Questionnaire scores [108].

1.4.5.7 Thrombosis

Patients with a Fontan circulation are at increased risk of thromboembolic formation secondary to the low flow through the Fontan circulation, and presence of foreign material including stents, conduits, and devices from fenestration closure. A wide prevalence of thrombosis is reported, ranging from 3 to 33% [109-113]. These studies differ in the method of identifying

thrombosis (e.g., transthoracic versus transoesophageal echocardiogram), duration of follow-up, type of Fontan circulation and selection of anticoagulation.

Patients are at most risk of thrombus formation during the first year following Fontan operation [110,114]. Risk factors for thrombosis are abnormal coagulation, use of prosthetic material, conduit stenosis, atrial arrhythmia, chronic systemic venous hypertension, and protein-losing enteropathy [113,115]. McCrindle et al. conducted a multi-centre randomised control trial to assess the efficacy of aspirin versus warfarin post Fontan completion [116]. They identified distortion of the PA, use of central venous lines for longer than 10 days or until hospital discharge, lower O_2 saturations 24 hours after the operation, lower pre-operative unconjugated bilirubin and those with an underlying diagnosis of pulmonary atresia / intact septum, to have higher risk of thrombosis [116]. Mortality from a thromboembolic event is ~25% [117].

Thromboembolic complications can occur in two main sites: 1) Formation of thrombosis within the Fontan circulation causing conduit obstruction. These thrombi can extend into the pulmonary arteries, or embolise into the pulmonary vasculature or systemically across a fenestration or systemic venous collateral. 2) Within the common atrium or pulmonary venous pathway. These are at risk of embolising systemically to the brain or into a coronary artery. Coon et al. described a cohort of 592 patients post Fontan operation, of which 52 (8.8%) had thrombosis [110]. At the time of identifying the thrombus, 24 (46%) were treated with aspirin, 6 (12%) were on warfarin, and 1 was on heparin therapy for protein losing enteropathy. Of these, they reported 48% in the systemic venous atrium, 44% in the pulmonary venous atrium, 2% in both atria, 8% in the hypoplastic ventricular cavity and 2% in the ligated pulmonary stump. At the time of detecting an intracardiac thrombus, 15% had a cerebrovascular event [110]. Other studies report an incidence of a stroke ranging from 3 to 19% [112].

Patients with a Fontan circulation are known to be predisposed to thromboembolic events. Guidelines have been established for use of anticoagulation in this population. However, several algorithms for anticoagulation have been developed, with significant variability between sites.

1.4.5.8 Collateral Formation and Arteriovenous Malformations

Although the Fontan operation is aimed at separating the systemic and pulmonary circulations, majority of patients remain mildly desaturated. Even though the main sources of systemic venous return, being SVC and IVC, are routed to the PAs, the coronary sinus still usually drains into the right side of the common atrium, allowing desaturated blood to mix with the oxygenated systemic circulation. Additionally, many centres will routinely create a fenestration (see section 1.4.4 – Fenestrated versus non-fenestrated Fontan) allowing right to left shunting. Ventilation-perfusion mismatch is also commonly present [57]. Additionally, veno-venous collaterals can develop secondary to high systemic venous and pulmonary artery pressures, resulting in a further reduction systemic desaturation [118]. Sugiyama found the most common site of collateral origin to be the brachiocephalic vein. A higher mean pulmonary arterial pressure and longer duration from surgery was associated with larger collateral formation. Pulmonary arteriovenous malformations can also develop, particularly in those with heterotaxy syndrome, resulting in intrapulmonary shunting and increased cyanosis [119]. This is thought to be related to the lack of pulsatile forward blood flow to the lungs, lack of "hepatic factor" reaching the pulmonary vascular bed and abnormalities in angiogenesis [120,121].

1.4.5.9 Hepatic Cirrhosis

Fontan-associated liver disease is known complication of the Fontan circulation. Patients with a Fontan circulation develop hepatic venous congestion secondary to elevated systemic venous pressures and low cardiac output, potentially leading to hepatic fibrosis, cirrhosis, and hepatocellular carcinoma [122]. The liver's venous drainage is solely through the hepatic veins, which drain directly into the IVC. Following the Fontan completion, the IVC drains directly into the pulmonary arterial system. Increased liver stiffness and congestion occurs immediately following the Fontan operation [123]. The progression of liver disease in this population begins with liver congestion and sinusoidal dilation, before the development of fibrosis, cirrhosis, and portal hypertension. In the advanced stages, patients are at risk of ascites, varices, and hepatocellular carcinoma [124].

In a study of 13 patients undergoing a liver biopsy, Schwartz et al. found that 92% had portal fibrosis including 1 patient with cirrhosis [125]. All had sinusoidal fibrosis. Johnson et al. performed a post-mortem study to further characterise liver dysfunction in Fontan patients [126]. They found 60% had significant sinusoidal fibrosis and 52% had portal fibrosis. The

degree of fibrosis correlated with the time from Fontan completion to death. They additionally found a significant association between age of Fontan operation and sinusoidal fibrosis [126]. The incidence of hepatic cirrhosis in the Fontan population varies widely between studies ranging from <10% to 55%, likely due to differences in imaging modality used for diagnosis, Fontan types and length of follow-up [127,128]. Goldberg et al. performed elective liver biopsies in 67 asymptomatic children and young adults, 10 years or more after Fontan completion. They found all subjects had collagen deposition in the liver, indicative of hepatic fibrosis, with time from Fontan completion being associated with degree of fibrosis [129].

Advanced hepatic fibrosis and liver cirrhosis are the single most important risk factors for developing hepatocellular carcinoma and is now recognised as rare but fatal complication of the Fontan circulation [130]. It can occur at a relatively young age, with Possner et al. finding a mean age of diagnosis at 30 ± 9.4 years, and mean duration from Fontan surgery at 21.6 ± 7.4 years [131]. One-year survival rates were low at only 50%. This stresses the importance of regular liver surveillance to ensure early detection.

1.4.5.10 Protein-losing Enteropathy

Protein-losing enteropathy (PLE) is a relatively uncommon complication following a Fontan operation, with an estimated incidence of 3-15% [24,67,132]. It however, is associated with significant morbidity and mortality [133,134]. PLE causes significant loss of proteins, including immunoglobulins and coagulation factors, through the gastrointestinal tract, causing diarrhoea, abdominal distension and discomfort and peripheral oedema. Patients are predisposed to fluid retention, thrombosis, and infection.

The mechanism behind PLE is not yet understood but is thought to be related to i) increased lymph production secondary to raised systemic venous pressure, and a relative reduction in chyle drainage from the thoracic duct [132]; and ii) leakage of lymph into the duodenum resulting in enteric protein loss [135]. Chronic systemic inflammation may also be a contributing factor, with Miranda et al. demonstrating elevated faecal calprotectin levels (a marker of intestinal inflammation) in Fontan patients with PLE [136]. Clinical factors that predispose patients to PLE have been found to include right ventricular morphology, AV valve regurgitation prior to Fontan operation, longer intra-operative cross-clamp time, prolonged pleural effusions post-operatively, bidirectional Glenn at an earlier age, and HLHS [137,138].

John et al. reported a five-year survival following a diagnosis of PLE of 88% in a cohort of 42 patients aged 18.9 ± 11.0 years [139]. Patients with high mean Fontan pressures, reduced ventricular systolic function and a New York Heart Association functional class of >2 at diagnosis had reduced survival. Treatment of PLE remains challenging and further insight into the mechanism of the disease process is vital to improved management.

1.4.5.11 Plastic Bronchitis

Plastic bronchitis is a rare complication, with incidence of ~1-4% following the Fontan operation [132]. It is a severe respiratory disorder characterised by mucous plugs within the airway. Like PLE, it is associated with significant morbidity and mortality, resulting in respiratory symptoms and desaturation [140]. The pathophysiology of this condition is not well understood but is likely related to chronic elevation of systemic venous pressures, lymphatic stasis and chronic inflammation, resulting in leakage of lymph through lymphatic to bronchial communications [140]. Using immunofluorescence microscopy, Racz et al. found these airway casts in plastic bronchitis to be composed of fibrin and are inflammatory in nature [141]. They proposed that abnormalities in inflammation resolution contributes to cast formation. These casts can partially or completely block the airway, causes severe hypoxaemia, asphyxiation, and death [57].

1.5 Normal Cardiopulmonary Interaction

The respiratory and cardiac systems are linked anatomically and physiologically, working together to ensure adequate delivery of oxygen to bodily organs and tissue. Dysfunction of one system significantly impacts the other. For example, pulmonary dysfunction and ventilation/ perfusion (V/Q) mismatch are frequently seen in patients with chronic heart failure (CHF) [142,143].

The effects of respiration on the circulatory system have been published since 1733 [144]. Absence of the radial pulse during inspiration in patients with tuberculous pericarditis was described and termed pulses paradoxus [145]. In 1947, Cournand et al. reported the effects of ventilation on cardiac output, noting reduced RV filling pressure and cardiac output with increasing ventilation pressure [146]. Due to this finding, they proposed that the reduction in cardiac output during inspiration is compensated for during the expiratory phase. They went

on to describe a ventilatory mechanism which will have minimal effects on cardiac output, consistent with bi-level positive airway pressure (BiPAP) [146]. It is now known that inspiration causes a fall in left ventricular (LV) stroke volume and subsequently blood pressure.

The close interaction between the heart and lung exists because of two main mechanisms: 1) Spontaneous breathing is a form of exercise. Respiratory muscles require perfusion and oxygen during spontaneous breathing, adding to the demand on cardiac output and producing carbon dioxide (CO_2) which needs to be cleared by the respiratory system. 2) The activation of respiratory muscles during inspiration, causes chest wall expansion thereby increasing lung volume and causing negative intrathoracic pressure. As the heart is located within an enclosed thoracic cage, changes in intrathoracic pressure affects venous return and cardiac output [147].

Respiration induces changes in intrathoracic pressures and thereby lung volumes, which in turn affects preload, afterload, and stroke volume. Lauson et al. described the influence of respiration on changes in circulatory pressure [148]. They found only small variations in pressures during tidal breathing consistent with minimal changes in heart rate or stroke volume. However, during deep inspiration, there was an increase in systemic venous return and RV stroke volume, and a decrease in LV stroke volume. In the normal circulation, the majority of LV filling occurs during the first portion of ventricular diastole with atrial systole providing a small contribution at end diastole. Riggs and Snider demonstrated that the reduction in LV stroke volume during inspiration is secondary to a reduction in early LV filling and not during atrial systole, through electrocardiographic and respiratory gated echocardiography [149,150]. Heart rate also increases with inspiration [149]. Several mechanisms for these effects of inspiration have been proposed: (i) an increase in pulmonary venous capacitance, (ii) reduced diastolic filling time secondary to the accelerated heart rate, (iii) an increase in systemic afterload secondary to increase in intrathoracic pressure, and (iv) alterations in the interventricular septal shape secondary to RV filling resulting in increased LV diastolic pressure [149,151].

1.5.1 Right Ventricular Preload

Preload refers to the force that stretches myocytes prior to contraction and is represented by end-diastolic volume. Systemic venous return from the superior and inferior vena cava is dependent on the existence of a pressure gradient between the extra-thoracic venous system

and the right atrium. The venous system is a low resistance, low pressure, and high compliance circulation. In normal respiration, during inspiration a decrease in intrapleural pressure occurs from contraction of the external intercostal and diaphragmatic muscles, causing an increase in right atrial transmural pressure (pressure exerted across the wall). This results in the right atrial chamber distending causing a reduction in right atrial pressure and increasing systemic venous return to right atrium and RV [149]. With contraction of the diaphragmatic muscles during inspiration, intra-abdominal pressure increases, and transmural pressure of the abdominal vessels decrease. This effectively causes constriction of the vessels, increasing IVC return to the right atrium, thereby increasing RV preload.

1.5.2 Right Ventricular Afterload

Afterload represents the impediment to ventricular output. PVR is the main determinant of RV afterload and is altered by respiration through changes in lung volume, blood pH levels and alveolar oxygen levels. Alveolar hypoxia causes vasoconstriction to maintain ventilation-perfusion balance by diverting blood away from under ventilated areas to well ventilated areas. Since 1961, PVR was found to rise with extreme changes (increases or decreases) in lung volume, outside normal respiration [152]. The pulmonary vasculature consists of intra-alveolar (within the septa) and extra-alveolar vessels (within the interstitium and exposed to intrapleural pressure) [153]. When lung volume falls below functional residual capacity (FRC), the volume of air remaining in the lungs at the end of normal expiration, both extra-alveolar vessels and alveoli collapse, causing hypoxia and inducing hypoxic pulmonary vasoconstriction [154]. This results in a rise in PVR. PVR can also increase when lung volumes increase above FRC due to compression of intra-alveolar vessels. High RV afterload result in reduced pulmonary arterial forward flow.

1.5.3 Left Ventricular Preload

During inspiration, RV volume increases with the increase in systemic venous return, causing flattening of the interventricular septum and reducing LV compliance and filling. The degree of LV filling (preload) is determined by pulmonary venous return, LV diastolic pressure and LV compliance, which in turn can be affect by ventricular-ventricular interactions [155]. As the RV is thin-walled and less contractile than the LV, elevation in RV afterload (namely PVR) can result in reduced pulmonary arterial flow and pulmonary venous return. The increase in RV diastolic pressure causes a shift in the interventricular curvature, bowing into the LV and

reducing LV capacity and compliance. All these changes result in a reduction in LV preload with inspiration.

1.5.4 Left Ventricular Afterload

In a normal circulation, LV afterload is determined by aortic pressure, arterial elastance and systemic arterial resistance [156]. Elevated aortic elastance results in increased LV afterload. Changes in intrathoracic pressure alters LV afterload through changes in transmural pressure (aortic systolic pressure – intrathoracic pressure), LV end-diastolic volume and ejection pressure [155]. Therefore, during inspiration, transmural pressure increases resulting in increased LV afterload and increased LV end-systolic volume.

1.6 Altered cardiopulmonary interaction in Fontan patients

The Fontan circulation functions on a single pumping chamber, with systemic veins draining passively into the pulmonary arteries. There is absence of a subpulmonic pumping chamber to overcome resistance within the pulmonary circulation, and chronically elevated systemic venous pressure. The efficacy of the circulation relies heavily on a balance between systemic and pulmonary vascular resistance. Small changes in intrathoracic pressure can have a significant impact on pulmonary arterial blood flow, and the effects of respiration on the Fontan circulation can have important short and long-term implications in these patients.

1.6.1 Effects of respiration

The importance of spontaneous respiration in the Fontan circulation was highlighted by Fontan and Baudet in 1971, stating that “respiratory assistance should be stopped early because positive pressure prevents central venous return” [22]. Spontaneous breathing has been shown to be the main determinant of cardiac output in Fontan patients, with subsequent studies demonstrating that inspiration augments antegrade pulmonary artery flow, through inducing negative intrathoracic pressure [157-159]. Both systemic venous return and pulmonary blood flow are augmented with inspiration [159-162]. Penny et al. demonstrated that antegrade pulmonary blood flow and peak velocity increased during atrial systole, and was further augmented with inspiration [157]. In an AP Fontan circulation, there was an increase in pulmonary forward flow of nearly 64% during inspiration compared to expiration [157]. Redington et al. also highlighted the significant effects of intrathoracic pressure in the Fontan

circulation through Doppler echocardiography [158]. They demonstrated augmentation of pulmonary blood flow with inspiration, and further augmentation with the Mueller manoeuvre (inspiration against close mouth and nares). They speculated that this was likely secondary to increased systemic venous return from the negative intrathoracic pressure created by inspiration. During a Valsalva manoeuvre they noted the opposite effect with blood flowing away from the lungs, with no spontaneous forward flow when the manoeuvre was sustained. They also noted a reduction in LV size, consistent with reduced preload. During normal tidal breathing, the cardiac cycle had no significant effect on pulmonary blood flow [158]. Shafer et al. demonstrated augmentation of ventricular preload, secondary to skeletal muscle contraction [163]. Through cardiac magnetic resonance (CMR) imaging, an increase in indexed end-diastolic volume of the single ventricle during inspiration has been shown [162]. The effect of respiration during exercise has also been studied. The effects of inspiration of ventricular filling was maintained during exercise [162], however there was a reduction in stroke volume during exercise during an expiratory load [163]. These study highlights the impact of changes in intrathoracic pressure alone, without changes in lung volume or PVR.

1.6.2 Pulmonary Vascular Resistance

The cavopulmonary connection limits blood flow into the lungs, and subsequently pulmonary venous return and ventricular preload. This results in chronically elevated systemic venous pressures and low cardiac output. Increases in pulmonary arterial pressures and pulmonary vascular resistance significantly impairs pulmonary arterial forward flow, pulmonary venous return, and consequently cardiac output. Potential augmentation of pulmonary blood flow is also limited, impairing the ability to recruit and distend pulmonary vasculature during exercise and induce a fall in pulmonary vascular resistance [164]. Small increases in PVR further impairs pulmonary blood flow. This was highlighted in a study by Williams et al. looking at the haemodynamic response to positive end-expiratory pressure (PEEP) in the post-operative Fontan patients [165]. They demonstrated a reduction in cardiac index with increasing PEEP, with associated increases in PVR. There were no changes in heart rate, atrial pressure or mean arterial blood pressure. Elevated PVR is often seen in Fontan patients and is likely related to a combination of altered pulmonary vasculature, non-pulsatile flow causing endothelial dysfunction and adverse remodelling [166,167]. Egbe et al. demonstrated that Fontan patients with low cardiac index and high PVR were at highest risk of Fontan failure, highlighting the significance of pulmonary vascular disease in this cohort [168].

1.6.3 Systemic Venous Return

Following Fontan completion, pulmonary flow is dependent on flow within the SVC, IVC and hepatic veins, and therefore factors controlling systemic venous return now determine pulmonary venous return, ventricular preload, and ultimately cardiac output [169]. Hsia et al. demonstrated increase in venous return through the hepatic veins and IVC to the RV during inspiration in normal and Fontan subjects [159]. However, the pattern of flow differed between the two cohorts. In normal subjects, there was biphasic forward flow within the hepatic veins and IVC, with a small amount of reversal during atrial systole. This pattern was absent in Fontan subjects where the atrium was excluded from the circulation. Thirty percent of flow through the Fontan conduit was dependent on respiration, compared to only 15% of IVC flow in normal subjects. A higher percentage of hepatic flow (55%) was also respiratory dependent in the Fontan patients. These authors postulated that this was secondary to hepatic venous congestion and reduced compressibility. They also found that gravity had a more significant hemodynamic effect on Fontan than normal subjects, with reduced net forward flow and increased flow reversal when erect [159].

1.6.4 Ventricular Function

Significant changes in ventricular volume load occur in FSV patients, starting with volume overload prior to the Glenn operation, to having preload insufficiency after the Fontan operation [170]. Sluysmans et al. found ventricular volumes obtained from echocardiography were 2-3 times higher than normal prior to the Fontan procedure, and ventricular afterload became abnormal after 2 years of age [171]. This can lead to ventricular hypertrophy, systolic and diastolic dysfunction [88]. Systolic ventricular function is generally preserved; however a degree of diastolic dysfunction is usually present (see section 1.4.5.4 - Ventricular Dysfunction), further impeding ventricular filling.

In summary, these studies demonstrate **altered cardiopulmonary interactions in the Fontan circulation** compared to normal (figure 1.6). The effects of respiration are more pronounced, with inspiration augmenting antegrade pulmonary flow. These changes are induced by changes in intrathoracic and intra-abdominal pressures thereby influencing SVC and IVC return.

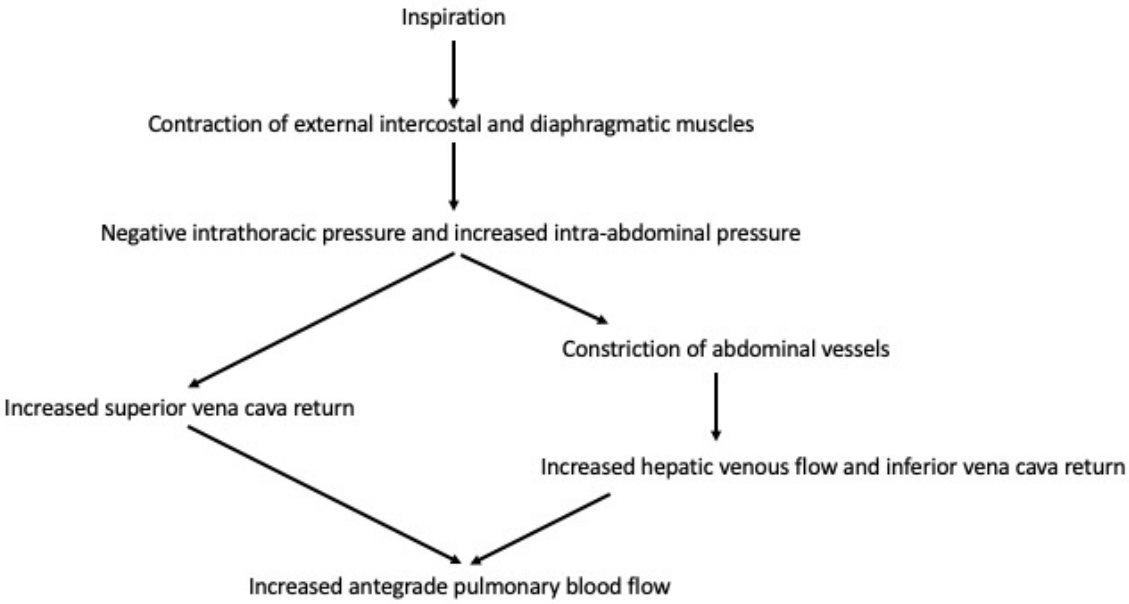


Figure 1.6. Cardiopulmonary interactions in the Fontan circulation
Reproduced from Laohachai and Ayer, 2022 [172]

1.7 Impairments of Respiratory Function

Lung development continues postnatally, and alveolar number and size continues to increase significantly over the first two years of life, with a slower increase into adolescence [173,174]. Lung volume, alveolar surface area and number increases from 29-weeks gestation to at least 12-weeks postnatally, with a close association to body weight[175]. During the first three years of life, increases in lung volume are predominantly due to increases in alveolar number rather than size. Subsequently, alveoli increase in both number and size, continuing through childhood and into adolescence, although at a reduced rate, with ninety-five percent of alveolar surface area in adults being formed after birth [176]. Lung development is altered, with abnormal lung parenchyma and pulmonary vasculature, in subjects with CHD, even in the absence of medical procedures and surgical intervention [174]. Lung pathologies and alterations in haemodynamics can impair this development resulting in abnormal lung function.

1.7.1 Lung Function Abnormalities in Congenital Heart Disease

All forms of CHD are frequently associated with abnormal lung function, and lung function is an independent predictor of survival in adult CHD patients [10,12,177-180]. For example, in

475 patients with a range of congenital heart defects, Fredriksen et al. found lower than normal forced vital capacity (FVC), a marker of lung volume. This was found in all types of CHD apart from those with an atrial septal defect [179]. Other studies have shown the prevalence of abnormal spirometry to be highest in those with a Fontan circulation (89%) and Tetralogy of Fallot (76%) [181]. In a larger cohort of 1,188 adults with CHD, nearly 30% had moderately to severely reduced FVC, and 17% had mildly reduced FVC [10]. The severity of CHD was related to the degree of impaired lung function. Medium-term mortality was 1.6 times higher in the group with moderately to severely impaired lung function. Predictors of moderately to severely reduced FVC included previous thoracotomy, moderate to severe scoliosis, cardiomegaly on chest X-ray, and complex congenital heart disease in all CHD patients; and diaphragmatic palsy in the repaired CHD cohort [10]. These results highlight the impact of surgical procedures, musculoskeletal deformities, and impaired cardiac function on lung function.

De Troyer et al. demonstrated that patients with isolated congenital pulmonary valve stenosis had smaller than normal lung volumes [182], suggesting the importance of pulmonary blood flow on lung development. In this study, more detailed lung function testing (body plethysmography and lung diffusing capacity for carbon monoxide) suggested that the basis for this was alterations in the pulmonary vasculature, rather than parenchymal abnormalities [182].

Spirometry testing has documented a high prevalence of restrictive lung disease in patients with CHD [12,177,180,183]. For example, Ginde et al. found that 44% of adults with CHD had restrictive lung disease, with a higher incidence in those with Tetralogy of Fallot or a FSV anatomy [12]. In a cohort of 1,163 paediatric patients, Hawkins et al. found a smaller but still significant prevalence of restrictive lung disease in those who had undergone cardiac surgery for CHD (25.5%) [177]. They identified that those who had a surgical intervention at a younger age had a greater risk of restrictive lung disease; and those who had multiple previous surgeries had a further increased risk. Other studies have also demonstrated an increased prevalence of restrictive lung disease in those with CHD who have not had previous cardiothoracic surgical procedure [180].

Reduced lung volumes in patients with CHD may also be related to the degree of participation in physical exercise from a young age. In view of their underlying heart condition, many patients are overly restricted from exercise and activity, resulting in a sedentary lifestyle. However, there is a known association between lung function and degree of aerobic fitness [184-186], promoting studies to assess the impact of lung function of exercise prescription on children and adults with CHD.

1.7.2 Lung Function Abnormalities in Patients with a Fontan Circulation

Pulmonary blood flow is altered in patients with a FSV from the fetal period and persists throughout life. Prior to Fontan completion, neonatal palliation of pulmonary blood flow may result in abnormalities of pulmonary vascular development. After Fontan completion pulmonary blood flow is non-pulsatile with altered wall shear- stress and reduced pulmonary endothelial function [187], The degree to which alveolar development is impacted by abnormal pulmonary vascular development in patients with a Fontan circulation remains to be determined.

Lung development may also be adversely impacted by other factors commonly seen in patients with a FSV circulation: desaturation, mechanical ventilation, lymphatic dysfunction, multiple sternotomies and thoracotomies, scoliosis or pectus deformity, and postoperative complications such as pleural adhesions and diaphragmatic palsy. Diaphragmatic palsy, for example, can reduce ventilatory function by ~25%, particularly in the setting of generalized respiratory muscle weakness [188]. Flow distribution to different segments of the lung may not be uniform and can result in collateral formation. Surgical procedures and interventions may result in pulmonary artery stenoses. Thoracotomies, pleural effusions, prolonged ventilation, diaphragmatic hernia, respiratory muscle weakness, previous thoracic surgeries, chest wall deformities and pulmonary arterial hypertension are amongst other factors that can impact lung function in Fontan patients [10,177].

Animal studies have demonstrated abnormal lung parenchyma after Fontan completion [189]. Kanakis et al. demonstrated normal lung parenchyma in 8 pigs at baseline, with rapid development of a mononuclear infiltration, in keeping with bronchiolitis, within 2 hours of Fontan completion. Pulmonary capillary recruitment has also been demonstrated in isolated dog lungs, with rises in pulmonary arterial pressure and pulsatile flow [190]. This finding is

suggestive that long-term non-pulsatile flow, in the setting of Fontan circulation, can cause adverse parenchyma lung remodelling and increased PVR. However, these findings may not extend to changes in human parenchyma.

1.7.2.1 Lung Volumes and Restrictive Lung Disease

Numerous studies have shown reduced total lung capacity and vital capacity in Fontan patients, suggestive of small lungs [164,179,191-196]. The prevalence of restrictive lung disease is high at 58-60%, with all studies finding reduced forced expiratory volume in 1 second (FEV_1), FVC and a normal or high FEV_1/FVC ratio [179,197,198].

In a large cohort of Fontan subjects aged 6-18 years from the Pediatric Heart Network Fontan Cross-Sectional study, Opotowsky found a high percentage (45.8%) of subjects with low FVC [164]. In 2001, Fredriksen et al. studied lung function in 52 Fontan patients and found a reduced mean FVC (77 +/- 16%) and FEV_1 (80 +/- 16%), with a high prevalence (58%) of restrictive lung disease [179]. They found no difference in values between age groups. Similarly, Paridon et al. found Fontan patients to have both a FVC and FEV_1 of 76% predicted [199]. Callegari et al. later performed a retrospective cross-sectional study on 232 Fontan patients with similar results [197]. A large proportion (59.5%) had a reduced FEV_1 with a mean of 74.7 +/- 17.8%, and FVC at 71.1 +/- 16.9% of predicted normal. Almost 60% (59.5%) had FEV_1/FVC ratio >80%, indicative of a restrictive ventilatory pattern. Similar results were seen by Guenette et al., with 65% having a restrictive pattern [200].

Ohuchi et al. found reduced total lung capacity (TLC), vital capacity and functional residual capacity in Fontan subjects compared to controls [193]. Although their Fontan cohort had normal residual volume, residual volume / TLC ratio was increased indicative of air trapping. These authors speculated that repeated surgical interventions lead to reduced mechanical mobility of the lungs, causing air trapping [193]. Matthews et al. also found an increased residual volume / TLC ratio but an increased residual volume when measured by plethysmography (Z-score 2.46±1.87) [192]. This difference in residual volumes may be related to method of testing, with Matthews et al. performing a helium dilution single breath test which measures all the gas within the thoracic cavity, rather than only the gas communicating with large airways.

1.7.2.2 Diffusing Capacity of Carbon Monoxide

Diffusing capacity of carbon monoxide (DLCO) is a measure of lung gas transfer from alveolar gas to haemoglobin within the pulmonary capillaries. It is affected by diffusion across the alveolar-capillary membrane, haemoglobin levels and capillary blood volume. Therefore, reduced DLCO may be secondary to reduced capillary blood volume available for gas transfer, reduced alveolar volume and/or abnormal alveolar membrane conductance. DLCO is strongly associated with aerobic capacity, measured by peak VO_2 [201].

Fontan patients have reduced DLCO, ranging from Z-scores of -2.85 to -3.1 [191-193,195,198]. Matthews et al. proposed that the reduction in DLCO may be due to two mechanisms: i) the non-pulsatile nature of the pulmonary blood flow inducing changes within the pulmonary bed and causing thickening of the alveolar capillary membrane; and ii) recurrent microembolism [192]. Larsson et al. also proposed that the non-pulsatile nature of pulmonary blood flow impairs gas exchange within the lungs [191]. Idorn et al. examined the aetiology of reduced DLCO in more detail through assessing different components of DLCO [195]. In their cohort, they found reduced pulmonary capillary blood volume but normal diffusing capacity across the alveolar membrane. Their data suggested preserved alveolar membrane function and reduced pulmonary perfusion. These authors also found an increase in DLCO and increased capillary blood volume in the supine compared with sitting position. They speculated that this may be secondary to improved perfusion of the upper lobes, secondary to blood flow being more gravity dependent in the absence of a subpulmonary pump. In a small cohort of 19 Fontan patients, del Torso et al. also found abnormalities in lung perfusion in 8 of their 19 patients, with the majority being localized perfusion defects [202]. Matsushita et al. went on to demonstrate normal ventilation in Fontan patients [203], and like others showed gravity-dependent blood redistribution [204,205]. Further studies, potentially utilizing double diffusion (DLCO and the diffusing capacity of nitric oxide, DLNO) are required to determine the exact aetiology of low DLCO in the Fontan population.

1.7.2.3 Association of Lung Function with Outcomes in Patients with a Fontan Circulation

Ohuchi et al. found that vital capacity was associated with the number of other previous surgical procedures performed (average of 1 to 2.4 procedures) in children and young adults with a Fontan circulation [193]. They demonstrated that over time (follow-up of 0.7 to 17.5

years) vital capacity reduced significantly. Opotowsky et al. however did not find an association of low FVC with any demographic or clinical variables in children with a Fontan circulation [164]. However, the presence of restrictive lung disease has been associated with clinical, and potentially modifiable, factors. Moderate restrictive lung disease was identified in 44% of subjects studied by Turquetto et al., which was associated with presence of postural deviations (e.g., kyphosis and scoliosis) and previous thoracotomies [198]. Callegari et al. also found restrictive lung disease to be associated scoliosis, in addition to the number of interventions, low body mass index, and diaphragmatic paralysis [197]. They also highlighted the significance of restrictive lung disease in the Fontan cohort, finding a correlation between FEV_1 percent predicted and self-reported quality of life scores, which relates to physical functioning [197].

These studies demonstrate that **Fontan patients have abnormal lung function, in particular restrictive lung disease, with abnormal gas transfer.** The presence of restrictive lung disease has been associated with other clinical factors, and impacts quality of life.

1.8 Determinants of CPET Parameters in Health

Cardiopulmonary exercise testing (CPET) is the gold standard to measure cardiac and respiratory response to exercise [206], and provides a reliable objective measure of exercise capacity. It involves a patient being connected to electrocardiogram and blood pressure monitoring, as well as continuous breath-by-breath sampling of O_2 and CO_2. Parameters obtained during CPET have been identified as a useful tool in risk stratification and are strong predictors of mortality in patients with CHD [11,207].

1.8.1 Maximal Parameters

Ensuring patients achieve a maximal test is important in the interpretation of the CPET results. A maximal test is achieved when: i) a plateau in VO_2 is observed, ii) peak minute ventilation is >85% of maximal voluntary ventilation, iii) evidence of ventilatory limitation; iv) respiratory exchange ratio (RER) of >1.05, v) the patient achieves >80% of predicted workload, and vi) a heart rate of >80% of predicted maximum is achieved [208].

1.8.1.2 Maximal Work Rate

Exercise testing can be performed using a variety of ergometers, with the most common being a treadmill and stationary cycle ergometer. Treadmills allow subjects to walk, jog and run at different speeds and inclines, creating different estimated work rates. On a treadmill, a higher VO_2 can be achieved, ~5-10% more than on a cycle ergometer [209]. However, due to the nature of the exercise, it is more likely to produce artefacts in measurement of the electrocardiogram, blood pressure, ventilation, and gas exchange. Cycle ergometers allow more accurate estimation of work rate and can be performed sitting or reclining. This form of testing allows variation in work rate to be implemented as a step, incremental or ramp protocol, and has less measurement artefact. Force is determined by the product of mass (kg) and acceleration (m/s^2). Work is performed when force is applied over a distance (force ($kg.m/s^2$) x distance (m)) [210]. Cycle ergometer work rate is expressed as Watts (W). Normally VO_2 increases in a linear fashion with work rate. The VO_2 / work slope reflects the metabolic efficiency of the musculoskeletal system [211].

1.8.1.3 Peak Oxygen Uptake

Oxygen uptake is determined by the cellular demand for O_2 and maximal rate of O_2 transportation. Factors influencing O_2 availability includes haemoglobin levels, arterial oxygen saturations, the haemoglobin dissociation curve, cardiac output, and peripheral tissue blood flow and oxygen extraction [211]. VO_2 is limited by the amount of O_2 the cardiopulmonary system can deliver to exercising muscles, namely the ability to increase cardiac output. The maximum VO_2 (VO_2 max) is the gold standard for assessing aerobic fitness and represents the point when there is a plateau in oxygen uptake (failure to increase by 150ml/min) [212]. This plateau occurs secondary to various factors that limit oxygen uptake despite increasing workload, for example maximal heart rate, stroke volume or oxygen tissue extraction [211]. However, this plateau is not always seen and as such the highest VO_2 achieved is frequently reported (peak VO_2). Peak VO_2 establishes whether maximal aerobic function is obtainable with the patient's physiology [210]. VO_2 is typically normalised to body weight, but in obese subjects, other measures of body size such as lean body mass may be more reliable [213].

1.8.1.4 Respiratory Exchange Ratio

The ratio between O_2 uptake and CO_2 production (VCO_2) is known as the gas exchange ratio or the respiratory exchange ratio (RER). RER is representative of tissue metabolism measured

through gas exchange at the mouth. It is calculated as VCO_2/ VO_2 throughout exercise testing and generally reflects the subjects' effort. At rest, RER is normally around 1.0 and reflects the fuels used for metabolic processes i.e. carbohydrates or protein [211]. A maximal RER of ≥1.05 is considered to be reflective of maximal effort [208].

1.8.1.5 Heart Rate

In healthy subjects, heart rate increases linearly with work rate and VO_2. The initial increase is mediated by a reduction in parasympathetic activity and later an increase in sympathetic activity [211]. At maximal exercise, heart rate reserve, being the difference between age-predicted maximal heart rate and maximal heart rate achieve, is minimal.

1.8.1.6 Cardiac Output

Cardiac output is determined by the product of stroke volume and heart rate. In the normal circulation, during exercise, heart rate increases with a reduction in vagal tone and subsequently sympathetic stimulation. Due to increased venous return from contracting skeletal muscles, reduced intrathoracic pressure with breathing and increased myocardial contractility, stroke volume increases. However, stroke volume plateaus at mid-range exercise and further increases in cardiac output are achieved through increases in heart rate [214].

With exercise, cardiac output increases to support the rising metabolic demand of tissues and is the best index of cardiac function. It can be indirectly calculated using the Fick equation where cardiac output = VCO_2 / arteriovenous O_2 content difference ($C(a\text{-}v)O_2$). The arteriovenous O_2 content difference is derived through measurements of mixed venous partial pressure of carbon dioxide (PCO_2) and arterial PCO_2 estimated from end-tidal PCO_2 [210]. However, these measures can be unreliable particularly in those with cardiac and pulmonary abnormalities. Direct measurements can be obtained through sampling the pulmonary artery and systemic artery throughout testing. This invasive method is the gold standard, but not applicable to daily practice. Stringer et al. proposed a non-invasive measure of cardiac output [214]. They demonstrated that $C(a\text{-}v)O_2$ was linearly related to VO_2 during incremental exercise, concluding that cardiac output and stroke volume can be accurately estimated from VO_2.

Oxygen pulse (O_2 pulse) reflects the amount of O_2 extraction per heart beat and is the product of stroke volume and $C(a\text{-}v)O_2$ [210]. O_2 extraction is equal to the arterial content of O_2 minus the mixed venous O_2 content ($C(a\text{-}v)O_2$). These parameters are determined by oxygen saturation and haemoglobin concentration. $C(a\text{-}v)O_2$ is thought to remain fairly constant throughout exercise, and hence changes in O_2 pulse is primarily secondary to increases in stroke volume. A normal O_2 pulse curve has a hyperbolic profile in response to increasing work rates [210], and a flattened O_2 pulse curve therefore reflects reduced stroke volume.

1.8.1.7 VE/VCO₂ Slope

VE/VCO_2 slope is a marker of gas exchange efficiency. Efficient gas exchange is dependent on ideal ventilation and perfusion matching. Maldistribution of pulmonary blood flow, secondary to right to left shunting, ventricular dysfunction, and pulmonary hypertension, causing V/Q mismatch will affect the VE/VCO_2 slope [215]. During aerobic stages of exercise, minute ventilation (VE) increases linearly with VCO_2, with VE/VCO_2 slope being a marker of ventilatory equivalence of carbon dioxide. VE remains relatively constant until metabolic acidosis develops. This causes a compensatory increase in VE out of proportion of the increasing VCO_2 causing an inflection in VE/VCO_2 slope. The normal increase in VE/VCO2 slope reflects the onset of metabolic acidosis and compensatory hyperventilation [211]. A steeper slope is suggestive of a ventilation / perfusion mismatch secondary to pulmonary or cardiac pathologies, or can be secondary to hyperventilation [216].

1.8.2 Submaximal Parameters

Maximal exercise testing is often difficult to obtain in many patients, particularly in those with a Fontan circulation [217]. However, a submaximal test can affect the interpretation of the CPET results. Therefore, several submaximal exercise parameters have been examined, and American Thoracic Society and the American College of Chest Physicians have recommended the need for submaximal exercise reference values. A multi-centre study (FUEL trial) conducted across North America and the Republic of Korea analysing the effects of Udenafil in 400 Fontan patients demonstrated improvements in submaximal parameters at ventilatory anaerobic threshold but not in peak parameters, highlighting the potential utility of submaximal parameters [218].

1.8.2.1 Oxygen Uptake at Anaerobic Threshold

Anaerobic threshold (AT) is used as an estimate of exercise capacity. It is the point during exercise when the body switches from aerobic to anaerobic mechanisms, when oxygen supply is unable to meet metabolic demand. It is reflected by accumulation of lactic acid and therefore the gold standard for identifying AT is through invasive lactic acid measurements. However, respiratory parameters, namely VO_2, can also accurately determine the beginning of metabolic acidosis. There is a minimal time delay between the start of bicarbonate buffering and its expression in CO_2 expiration and VE response [210], allowing timely detection in ventilation parameters. The highest VO_2 obtained without an increase in lactate is AT, and usually occurs at 50-60% of VO_2 max. AT can be determined and confirmed through a combination the V-slope method (point at which VCO_2 accelerates in relation to VO_2, in the absence of hyperventilation) and ventilatory equivalent method (increase in VE/VCO_2 without an increase in VE/VO_2) [210]. VO_2 at AT is normally reported as a percentage of predicted peak VO_2.

1.8.2.2 Oxygen Uptake Efficiency Slope

Ventilatory equivalent for oxygen (VE/VO_2) represents a patient's ventilatory response to aerobic exercise [219]. A steeper slope is indicative of less efficient ventilation. Oxygen uptake efficiency slope (OUES) also represents the relationship between VO_2 and VE but is expressed as the slope of VO_2 versus log VE. Due to the linear relationship between the two parameters throughout exercise testing, measurement at submaximal exercise is similar to when measured at maximal exercise [210]. A steeper slope suggests that VO_2 is more efficient relative to VE.

1.8.2.3 Exercise Oscillatory Ventilation

Patients with CHF may demonstrate instability of ventilation. Since the early 1800s Cheyne, and later Stokes, described a severe form of disordered breathing in a patient with CHF, which later became known as Cheyne-Stokes breathing [220,221]. Exercise oscillatory ventilation (EOV) is characterised by regular fluctuations in tidal volume in a crescendo-decrescendo pattern without apnoea (figure 1.7) [222]. EOV occurs during submaximal testing and can be identified through standard exercise testing.

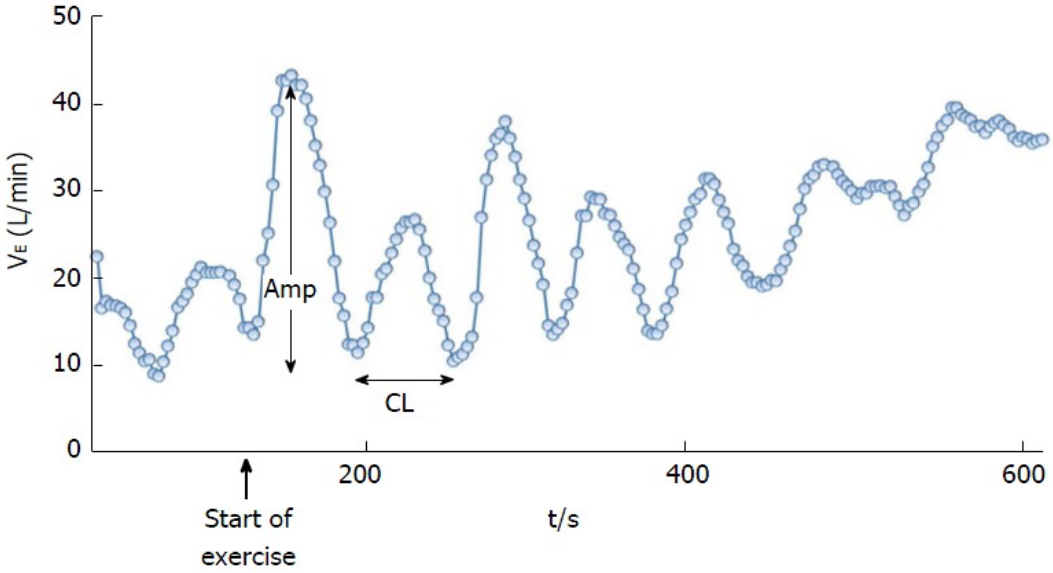


Figure 1.7. Oscillatory ventilation during exercise

CL: cycle length; Amp: amplitude of oscillation; VE: ventilation efficiency. Reproduced from Dhakal et al. [222]

Dhakal and Lewis explored the mechanisms behind EOV [222]. They summarised these changes as circulatory delay, increased chemoreceptor sensitivity to both arterial partial pressure of CO_2 and O_2, and baroreflex impairment. EOV has been identified in symptomatic CHF patients with reduced and preserved LV-EF [223-225], and may be useful in diagnosis, prognostication and surveillance of these patients [222,226]. Additionally, studies have shown reversal of EOV after stabilisation of ventilation and aerobic training [226,227].

1.9 Impaired Exercise Capacity

1.9.1 Exercise Impairment in Congenital Heart Disease

Patients with CHD can have altered haemodynamic adaptations, affecting the normal cardiorespiratory interaction, including abnormal pulmonary vasculature, sinus node dysfunction, ventricular dysfunction, residual shunts and associated pulmonary and skeletal muscle disorders [228]. These factors can adversely affect exercise capacity in this cohort. Historically, stringent exercise restrictions have been in place for many patients with CHD, predominantly due to the perceived potential risk of sudden death and aortic dissection [229,230]. However, the risk of obesity and acquired heart disease in these patients is also significant, and over time the importance of a healthy lifestyle and promotion of regular physical activity has been highlighted [231].

The benefits of physical activity and exercise training are now well-documented [232,233], and guidelines around managing CHD now include exercise recommendations, with the majority of CHD patients being encouraged to participate in some level of competitive sport [233-235]. Broadly, there are two types of exercise – dynamic sports requiring changes in muscle length without significant force, for example running; and static sports using large intramuscular force with minimal change in muscle length, for example weight lifting [215]. Miliaresis et al. describes that dynamic exercises rely on an increase in cardiac output, as it uses a large muscle mass which require an increase on oxygen consumption [215]. The increases in cardiac output are achieved through changes in stroke volume and heart rate. Anaerobic static exercises, however, results in an increase in blood pressure, with little change in oxygen consumption.

Patients with CHD on average have at least moderate exercise impairment, which is comparable to those with CHF [11,236]. In a meta-analysis of 25 studies, involving a total of 770 children with CHD, maximal VO_2 was significantly lower than in healthy controls, with a difference of 9 ml/kg/min [237]. Maximal heart rate was also lower, with an inverse relationship to maximal VO_2, suggestive of chronotropic incompetence. A recent systematic review revealed an elevated VE/VCO_2 slope; and significantly reduced peak VO_2, maximum heart rate, maximal workload, and oxygen pulse in children and adolescence with CHD compared to their healthy peers [238]. Even patients with simple CHD lesions, such as a surgically repaired ventricular septal defect, have reduced exercise capacity [11,239]. Inuzuka et al. found a mean peak VO_2 of 66% percent predicted across the whole cohort of adult CHD patients and demonstrated a decline in percent predicted peak VO_2 across the spectrum of CHD, with the lowest VO_2 in patients with Eisenmenger physiology followed by complex CHD [236]. These abnormal findings are highlighted by the finding of Kempney et al., who published age, gender, and diagnosis specific reference values for VO_2 and VCO_2 in patients with various CHD [240]. They confirmed that all patients had significantly reduced peak VO_2 compared to healthy controls.

Reduced exercise capacity is strongly associated with poor prognosis, increased risk of hospitalisation and early mortality in patients with CHD [9,11,51]. Peak VO_2, change in heart rate with exercise and an elevated VE/VCO_2 slope have all been predictive of increased

mortality in CHD patients. In a study of 1,375 adults with CHD, Inuzuka found peak VO_2, heart rate reserve and VE/VCO_2 slope to be predictors of midterm survival [236]. During a median follow-up of 5.8 years, 8.5% of patients died, with the main causes being heart failure, sudden cardiac death, perioperative mortality, and infection. Percentage of predicted peak VO_2 was an independent predictor of 5-year mortality [236]. In the noncyanotic group, VE/VCO_2 slope was related to increased mortality, but not in the cyanotic cohort.

Chronotropic incompetence, indicated by a blunted increase in heart rate (<80%) with exercise and a low heart rate reserve, is common following cardiac surgery for CHD [241-243]. It is also associated with poor prognosis in CHD patients [244], with Inuzuka et al. demonstrating heart rate reserve as a predictor of outcome, even in patients treated with negative chronotropic agents. These authors proposed that chronotropic incompetence is related to increased risk of arrhythmic events and sudden death [236].

1.9.2 Exercise Impairment in Fontan Patients

Although the Fontan circulation improves oxygen saturation in patients with a FSV and significantly improves mortality compared to the natural history, **patients with a Fontan circulation have been found to have reduced exercise tolerance** when compared to their healthy peers. Initial studies following the introduction of the Fontan procedure demonstrated an improvement in exercise tolerance in FSV patients after Fontan completion. For example, Zellers et al. found an increase in maximal oxygen uptake of 19% from 22 ml/kg/min to 27 ml/kg/min [245] and Driscoll et al. showed a similar improvement of 16% from 20.5 ml/kg/min to 24.3 ml/kg/min [246]. However, despite the initial improvement in exercise tolerance, we now know that patients with a FSV continue to have reduced exercise capacity compared to their healthy peers even following the Fontan completion [11,84,239,246-251]. Normal haemodynamic response to exercise is secondary to adaptation of the cardiac pump (ventricles), ventilatory pump (lungs and respiratory muscles) and peripheral pump (skeletal muscles) to increase oxygen demand of working muscles. In Fontan patients, all these mechanisms can be impaired.

Many Fontan patients are less active than their peers, have limited involvement in any regular physical activities, and do not meet the recommendations for physical activity in the European and American guidelines [252]. Even though many report normal physical activity on

questionnaires, the correlation between the self-reported activities and objective measures on CPET is weak [253]. The ability of Fontan patients to exercise may be affected by altered haemodynamics, previous surgeries, arrhythmias, underlying respiratory pathologies, skeletal deformities, muscular weakness, and associated co-morbidities including syndromic diagnoses associated with gross motor disability [254]. Exercise tolerance has been shown to be reduced by ~30-40%, in comparison to healthy controls, with progressive decline over time [242,245,248,249,255-258].

CPET can aid in differentiating the primary underlying aetiology of exercise intolerance – cardiac or respiratory impairment and is useful in guiding treatment and prognostication [215,259].

1.9.2.1 Peak Oxygen Consumption

Peak VO_2 is markedly reduced in Fontan patients, with less than 30% achieving a normal peak VO_2 on exercise testing [84]. Ohuchi et al. found a percent predicted peak VO_2 of less than 55% was associated with increased unplanned hospitalisation and mortality [260]. Reports of maximal VO_2 achieved has a wide range from approximately 40 to 80 percent-predicted, likely related to the wide spectrum of cohort characteristics including underlying cardiac anatomy, era of surgery and follow-up time. Anderson et al. found mean percent predicted peak VO_2 to be 65% ± 16% of normal [84]. Callegari et al. found similar values of 67 +/- 17.6% predicted in their total cohort of 214 patients, with a difference in mean peak VO_2 between those <20 years old and the older subjects [197]. Greutmann et al. found lower peak VO_2 in the Fontan subgroup compared to other CHD patients (51±12% compared to 70±15%) [261]. Brassard et al. (2006) reported values of VO_2 max ranging from 15-29 ml/kg/min in Fontan patients, equating to 43-78% of normal [248]. Fredriksen et al. found a much lower VO_2 at an average of 35.7% of predicted [179]. In 2008, Anderson et al. and Paridon et al. reported a cross-sectional analysis of outcomes, including exercise data, in a large cohort of Fontan patients as part of the Pediatric Heart Network [84,242]. In 411 Fontan patients, only 28% achieved a normal peak VO_2, with an average peak VO_2 of 26.3ml/kg/min (65% predicted) [199]. VO_2 at both peak exercise and anaerobic threshold were reduced compared to predicted values for age and gender.

Deterioration in VO_2 with time from surgery has been demonstrated. For example, Giardini et al. showed a deterioration of 2.5% of predicted peak VO_2 per year [262]. Fernandes et al. and Egbe et al. demonstrated a slower decline of 1.25% and 1.7% per year, respectively [257,263]. More recently Goldberg et al. and Illinger et al. have found a much slower decline in peak VO_2 of 0.8% and 0.4% per year, respectively [258,264]. This may be suggestive of improvement in medical and surgical management of Fontan patients over time, however variation in patient cohort, follow-up periods and normative values may also need to be considered. Illinger et al. identified the highest peak VO_2 in males to occur between 6-10 years of age, with a decline until the thirties. They compared this to a normal peak at 14-16 years of age in healthy males, highlighting the importance of Fontan-specific normative data [264].

Although all measures of exercise performance were reduced in the Fontan cohort examined by Goldberg et al., VO_2 at AT was better preserved than at peak exercise [258]. When divided into tertiles, a better percent predicted VO_2 at AT was associated with the same parameters as a higher percent predicted peak VO_2, namely younger age, lower body mass index (BMI) and female gender. This suggests that **submaximal parameters may also be useful in the assessment of exercise capacity in the Fontan cohort**.

Fredriksen et al. found that both age at Fontan completion and age at testing, negatively correlated with peak VO_2, with age at Fontan being the single most important predictor on multivariate analyses [179]. Goldberg et al. found younger age at testing to be associated with the highest tertile of peak VO_2 [258]. Illinger et al. identified female gender and high BMI as predictors of deterioration in percent predicted VO_2 [264]. This is in keeping with Lambert et al. and Goldberg et al. who both reported a negative association between BMI and oxygen consumption [258,265]. Lambert et al. also found increased oxygen consumption with higher height z-scores. The association of gender with peak VO_2 and its decline is variable, with Goldberg et al. finding poorer exercise capacity in males than females [258]. Correlation of ventricular morphology with exercise capacity has been inconsistent. Giardini et al. found a slower decline in exercise capacity in those with a systemic left ventricle morphology and a TCPC type Fontan [262]. Although Goldberg et al. noted a single left ventricle morphology was more common in the highest exercise performing tertile [258,266], others have demonstrated no impact of ventricular morphology on peak VO_2 [264].

1.9.3 Causes for Impaired Exercise Capacity in Fontan Patients

As previously discussed, the altered haemodynamics of the Fontan circulation leads to chronically elevated central venous pressures and preload insufficiency (see section 1.6 – Altered Cardiopulmonary Interaction in Fontan Patients). This results in an impaired ability to increase cardiac output and limit perfusion of oxygenated blood to respiratory and skeletal muscle. The presence of systemic desaturation and restrictive lung disease further impairs exercise capacity.

1.9.3.1 Impaired Cardiac Output

In patients with a Fontan circulation, cardiac output is reduced to 60-70% of normal [246,255,267-270]. Driscoll et al. measured cardiac output at rest and during exercise in children and adults with a Fontan circulation using acetylene-helium rebreathing technique [246]. They found at rest cardiac output was lower than the normative mean in all cases, with 11 out of 25 being below the lower limits of normal. Cardiac output increased with exercise in all subjects but remained abnormally low. Cortes et al. however found resting cardiac index at rest to be comparable to the control group, but also demonstrated impaired augmentation of cardiac output with exercise [270]. Stromvall-Larsson et al. demonstrated the same in 20 non-fenestrated Fontan patients [271]. Through dye-dilution technique, cardiac output was measured at rest and after 4 and 6 minutes of each workload. They found all but one patient had a peak cardiac output below a standard deviation of -2, with a rise in cardiac output from 4 L/min at rest to 8L/min at peak exercise [271].

Several factors can potentially limit cardiac output in the Fontan circulation. Primarily, the lack of a subpulmonary ventricle results in preload insufficiency [272,273] with consequent adverse ventricular remodelling [274,275] and impaired diastolic ventricular filling [84,88]. Other factors that may impact cardiac output in the Fontan circulation include systolic dysfunction [47,274], increased systemic afterload [276] and abnormal ventriculo-arterial coupling [277].

Augmentation of cardiac output is highly dependent on preload reserve [268]. Gledhill et al. demonstrated that in athletes with a normal circulation, ventricular filling contributes more to stroke volume than myocardial contractility or heart rate [278]. However, in the Fontan circulation, absence of a subpulmonary pump results in limitation of pulmonary artery forward flow and thereby pulmonary venous return and ventricular filling. This preload insufficiency

is exaggerated with ventricular dilation and/or hypertrophy [279]. Additionally, augmentation of cardiac output is further impaired secondary to reduced pulmonary venous return from elevated systemic venous resistance, and vasoconstriction of peripheral arterioles in response to low cardiac output through the baroreflex control [267].

Further increases in heart rate may have a blunted ability to increase cardiac output in the Fontan circulation [280]. Indeed, the so-called chronotropic incompetence seen in patients with a Fontan circulation may be an adaptive response to maintain ventricular filling in the setting of preload insufficiency [268]. Patients with a Fontan circulation have a reduced stroke volume at rest in comparison to the normal population, as well as an impaired ability to increase heart rate in response to exercise (chronotropic incompetence) [249,281,282]. Additionally, as the pulmonary blood supply is sourced directly from systemic venous return and bypasses a subpulmonary ventricle, the ability to augment pulmonary blood flow response in response to exercise is also limited. This consequently also causes reduced left ventricular filling, reduced stroke volume and ultimately reduced cardiac output [248]. Mean peak heart rate achieved in Paridon's cross-sectional study was 154bpm (74% predicted) [199].

O_2 pulse index (VO_2 divided by heart rate; an indicator of stroke volume) was also reduced in Fontan subjects. It has been proposed that this may be due to abnormal autonomic heart rate control or sinus node dysfunction [199,245,246]. As cardiac output is a product of heart rate and stroke volume, a reduced maximal heart rate can result in a blunted increase in cardiac output in response to exercise. However, Illinger et al. demonstrated that although oxygen pulse is significantly lower in the Fontan cohort compared to their healthy peers, there was no impairment in the ability to augment oxygen pulse from rest to peak exercise [264]. This is in keeping with La Gerche et al. suggesting ventricular function is not the limiting factor of oxygen delivery in a Fontan circulation [283]. Fredriksen et al. also showed no association between peak VO_2 and maximal heart rate, again indicating that chronotropic incompetence is not being the main limiting factor of exercise capacity [179].

1.9.3.2 Reduced Preload

The presence of chronically reduced preload in the Fontan circulation impairs maximal aerobic capacity [267]. Senzaki et al. demonstrated limited preload reserve through the use of low dose dobutamine [273,276]. In Fontan patients, they found that cardiac index was dependent on

ventricular preload, as measured by end-diastolic area, and not by contractility or ventricular afterload. They proposed that inadequate ventricular preload was the cause of reduced cardiac output augmentation with beta-adrenergic stimulation. Robbers-Visser et al. results were also suggestive of this. Dobutamine normal increases stroke volume, however Robbers-Visser et al. described no change stroke volume based on CMR volumetric assessments in response to dobutamine, with an adequate increase in heart rate, ejection fraction and cardiac index [280]. These findings demonstrate the inadequacy of ventricular preload, a contributor to cardiac output during exercise, in the Fontan circulation.

1.9.3.3 Elevated Afterload

In up to moderate levels of exercise, blood pressure in Fontan patients is relatively maintained, with increases in both systolic and diastolic pressures [179,245]. At peak exercise, however, systolic blood pressure is lower than healthy controls [251]. During exercise, reduction in systemic vascular resistance is blunted compared to normal [251]. This is thought to occur through increased sympathetic vasomotor tone and represent a compensation for limited cardiac output [283,284].

1.9.3.4 Impaired Contractility

Due to the altered haemodynamics from the anatomical changes of a FSV, the systemic ventricle is initially volume overloaded, dilated, and hypertrophied. Following the Fontan operation, there is a sudden reduction in volume and the ventricle is under-filled. However, in majority of patients systolic function is generally preserved [84,280]. In a cohort of 546 children, Anderson et al. found 73% had normal ejection fraction [84]. Robbers-Visser et al. assessed ventricular response to dobutamine using cardiac magnetic resonance (CMR) imaging [280]. In 32 teenagers and young adults, they demonstrated an adequate increase in heart rate, ejection fraction and cardiac index. These studies demonstrate that in majority of Fontan patients impaired contractility is not a significant cause of impaired exercise capacity.

1.9.3.5 Pulmonary Vascular Resistance

Numerous studies have shown the importance of PVR in the Fontan circulation and is now thought to be the major determinant of cardiac output [268,285]. As the systemic and pulmonary circuits in the Fontan circulation are connected without a pump in between, stroke volume is dependent on pulmonary venous return (preload), which in turn is dependent on

PVR. Gewillig and Goldberg described changes in cardiac output with alterations in ventricular systolic function and PVR [286]. Mild increases in PVR secondary to many factors including patency of the Fontan circuit, branch pulmonary arteries, pulmonary capillary bed and pulmonary veins and respiratory function, caused significant reduction in cardiac output. This is highlighted by the findings of improvement in exercise capacity with the use of pulmonary vasodilators [287-290]. In 2011 Goldberg et al. performed a double-blind randomised controlled crossover trial on the effects of sildenafil (a potent selective vasodilator) on exercise performance [289]. Twenty-eight children and young adults with a Fontan circulation were randomised to either placebo or sildenafil for 6 weeks. After a 6-week washout period, subjects were crossed over for another 6 weeks. They found improvements in respiratory rate and minute ventilation, and a reduction in VE/CO_2 slope at peak exercise following sildenafil treatment. Although there was no change in peak VO_2, there was a trend towards improvement at AT. A larger prospective randomised trial was reported by Hebert et al. assessing the effects of Bosentan (an endothelin-1 receptor blocker) on PVR in Fontan patients [287]. Sixty-nine adolescents and adults with a Fontan circulation were randomised to 14-weeks of treatment with Bosentan. They found improvement in peak oxygen consumption following treatment with Bosentan, with no serious adverse effect.

Pulmonary vasculature development is abnormal in patients with complex congenital heart. Antenatal hemodynamic factors can alter pulmonary artery size and arborization, and pulmonary venous and lymphatic anatomy. The connection between the cardiac and pulmonary circulations is evident embryologically with lung endoderm protruding into the mesoderm as the heart tube elongates and folds [291]. By 20-weeks gestation, pre-acinar pulmonary arteries have already formed, and any mal-development of cardiac structure has already occurred. Therefore, hemodynamic changes within the circulation, such as a restrictive atrial septum in HLHS, can adversely affect pulmonary artery development. Lack of pulsatile pulmonary flow in the Fontan circulation has been shown to be associated with endothelial dysfunction and abnormal vascular development [166,187]. This has also been shown on histological specimens, with Levy et al. finding thick-walled distal intra-acinar pulmonary arteries on lung biopsies at the time of Fontan procedure, in patients with poor outcomes from the Fontan procedure [292]. Whether abnormalities of pulmonary vascular development can impact alveolar development remains to be determined.

Augmentation of cardiac output is usually achieved by increases in heart rate, preload and/or myocardial contractility and reduced afterload. With exercise, for example, biventricular stroke volume increases in the setting of adequate preload reserve [268]. In the Fontan circulation ventricular function does not predict exercise performance, suggesting it is not the main limitation of exercise capacity [293]. The subpulmonary ventricle has now been shown to also play a significant role during exercise in the normal circulation [283,294]. La Gerche and Gewillig elegantly discussed the effects of increased RV afterload, reducing LV preload and consequently cardiac output; and further proposed that without a subpulmonary pumping chamber in the Fontan circulation, PVR becomes the limiting factor of cardiac output limitation [283].

1.9.3.6 Impaired Respiratory Function

As previously discussed, Fontan patients are known to have impairment in respiratory function, particularly reduced lung volumes and restrictive lung physiology (see section 1.7.2 – Lung Function Abnormalities in Patients with a Fontan Circulation). Factors that may alter lung function in patients with a Fontan circulation include previous lateral thoracotomy, bypass inflammation, diaphragmatic palsy and, as discussed below, inspiratory muscle weakness. As previously discussed, pulmonary blood flow is likely to be highly dependent upon efficient respiratory function.

In Fontan subjects, reduced FVC is associated with low VO_2 and reduced exercise capacity and is a predictor of survival in adults with CHD [10,12]. In adults with CHD, presence of restrictive lung disease is a strong predictor of exercise capacity [12]. It is now recognized that a significant proportion of patients with a Fontan circulation have ventilatory rather than circulatory limitations to exercise capacity [164,197,200].

Guenette et al. reported higher activity-related dyspnoea in their Fontan cohort compared to controls [200]. In keeping with previous studies, Guenette et al. also found significantly reduced FEV_1, FVC, maximal voluntary ventilation, and DLCO, with a restrictive ventilatory pattern [200]. They examined the cardiopulmonary response to exercise in Fontan subjects, noting significantly reduced VE compared to controls, secondary to reduced peak tidal volume. Fontan subjects adopted a more rapid breathing pattern at any given exercise intensity. To further characterize ventilatory limitation to exercise, these authors performed inspiratory

flow-volume loops during exercise. This demonstrated a higher end inspiratory lung volume, indicative of reduced inspiratory reserve volume. There was no evidence of dynamic lung hyperinflation. VE/VCO_2 slope during exercise was significantly elevated. Their findings suggest that the restrictive pattern of lung function in Fontan subjects contributes to an abnormal ventilatory response to exercise. Previous studies have also shown elevated ventilatory equivalence of oxygen (VE/VO_2) at both rest and exercise in functionally single ventricle patients [295] Like other studies [197,198], Turquetto et al. also found a strong correlation between peak VO_2 and lung function parameters (FEV_1, FVC, TLC and DLCO) [198].

Impairments in pulmonary function can impact exercise capacity, with Opotowsky et al. demonstrating that a low FVC was predictive of low peak VO_2, and a stronger determinant than ventricular morphology or dysfunction [164]. They also showed that Fontan subjects with an elevated VE/VCO_2 slope were more likely to have a low breathing reserve (BR), suggestive of ventilatory limitation of exercise. In comparing those with ventilatory limitation of exercise (defined as BR<20%) and those with presumed cardiac limitation (BR>20%), low FVC, high VE/VCO_2 slope and high body mass index independently predicted ventilatory limitation. Callegari et al. demonstrated a positive association between FEV1 and peak VO_2 percent predicted on exercise testing [197].

1.9.3.7 Skeletal and Respiratory Muscle Weakness

Normally, lower limb skeletal muscles are important during standing and upright exercise. The contraction of these skeletal muscle assists in systemic venous return [296,297]. In 1960 Wang et al. demonstrated the influence of the skeletal muscle pump on cardiac output, through measurement of cardiac output, heart rate and stroke volume in 4 healthy men [297]. There was a fall in cardiac output by 41% and a subsequent rise in heart rate with standing. In the Fontan circulation, there is a greater dependence on respiratory and skeletal muscles to aid systemic venous return to the lungs, due to the absence of a subpulmonary pump [57,163,298,299]. In a cross-sectional study on exercise in Fontan patients, Paridon et al. found that 57% of Fontan patients did not achieve maximal aerobic capacity (defined as RER ≥1.1) and potentially did not reach cardiovascular exhaustion. Other limiting factors include respiratory or musculoskeletal fatigue, or lack of motivation [199]. More recently, reduced skeletal muscle mass is now being recognised in the Fontan cohort [300].

Adults with acquired CHF commonly have associated generalized myopathy, involving both respiratory and skeletal muscles, affecting exercise capacity [301-305]. Middlekauff reviewed skeletal myopathy in adults with CHF summarising abnormal musculature in these patients with reduced muscle bulk, replacement of normal oxidative type I fibres to glycolytic type IIb fibres, reduced capillary and mitochondrial density, and abnormal metabolism [306]. All these features correlate with lower peak oxygen consumption in these patients [307,308]. The importance of adequate perfusion to contracting skeletal muscle in determining exercise tolerance in CHF patients has been determined [303]. These studies precipitated an analysis of respiratory muscle strength in adults with repaired CHD, due to the overlapping physiology with those with heart failure [309,310].

Like those with acquired heart failure, respiratory muscle weakness has been reported in patients with CHD, affecting both inspiratory and expiratory muscles. Greutmann et al. studied a group of 41 adults with different forms of CHD (repaired Tetralogy of Fallot, systemic right ventricles and Fontan circulations) finding reduced respiratory muscle strength comparable to adults with advanced heart failure [261]. Inspiratory muscles were affected more than expiratory muscles, with maximal inspiratory pressure (MIP) significantly reduced in the congenital heart disease group, measuring 75±26 cmH_2O (77±27%) compared to 102±32 cmH_2O in the control group. They went on to show that MIP and maximum expiratory pressures (MEP) correlated with peak VO_2, demonstrating an association between reduced respiratory muscle function and reduced exercise capacity. Additionally, subjects with globally reduced respiratory muscle strength (both MIP and MEP) had lower maximal voluntary minute ventilation, which at peak exercise was also associated with peak VO_2. In their discussion, they proposed that the reduced muscle strength may result in an overestimation of the degree of lung impairment due to the respiratory muscle contribution to spirometry measures. Upper limb skeletal muscles were also shown to be weak, with reduced handgrip strength.

Similarly, Turquetto et al. examined respiratory muscle strength in 27 Fontan patients [198] by using two non-invasive modalities, MIP and sniff nasal inspiratory pressure (SNIP), They also found reduced muscle strength, measuring MIPs of 76±23 cmH_2O (63±16% predicted) in males and 81±33 cmH_2O (71±32% predicted) in females. SNIP was measured at 99±24 cmH_2O (72±31%) in males and 84±13 cmH_2O (82+/-12%) in females. Furthermore, they found an

association between SNIP and peak VO_2. This respiratory muscle weakness may contribute to reduced lung volumes, as seen in Fontan patients.

Skeletal muscle weakness has also been documented in Fontan patients. Cordina et al. was one of the first to assess skeletal muscle mass in the Fontan cohort [300]. In 16 relatively active (New York Heart Association (NYHA) class I or II) adults with a Fontan circulation, they found significantly reduced skeletal mass, based on dual X-ray absorptiometry, compared to age and sex matched reference values. Twenty-five percent had muscle mass in the sarcopenic range. Lean mass positively correlated with peak VO_2 and oxygen pulse [300]. Avitabile et al. found lower leg lean mass Z-scores in 50 Fontan patients compared to healthy controls. This was associated with a worse peak VO_2 on exercise testing, and there was a strong correlation between lower leg lean mass and change in cardiac index and indexed systemic flow on CMR imaging (following supine exercise) [311,312]. Turquetto et al. further explored skeletal muscle weakness in the Fontan patients, demonstrating abnormal skeletal muscle strength with reduced thigh muscle volume and blunted blood flow to skeletal muscle. They found that the low blood flow was associated with a reduced cross-sectional area of the thigh, which is turn correlated with abnormal peak VO_2 on exercise testing [253]. Compared to healthy controls, concentrations of norepinephrine and muscle sympathetic nerve activity were increased, as found in previous studies [313]. Multivariate analyses showed a negative correlation between muscle sympathetic nerve activity, and FVC and stroke volume. The authors proposed that this was an adaptive mechanism to the increased systemic vascular resistance, resulting in reduced skeletal muscle flow [253]. Shafer et al. performed a study to assess the relative contribution of the skeletal and respiratory muscle pumps to stroke volume in Fontan patients [163]. The largest increase in stroke volume in both the Fontan and control cohorts was during zero-resistance cycling, with an increase in cardiac index by 30% in the Fontan patients. With the addition of respiratory muscle involvement through isocapnic hyperpnoea, there was no significant change in cardiac index to either group. They concluded that peripheral skeletal muscles contribute more to stroke volume than respiratory muscles, however both are important in maintaining cardiac output, with a drop in stroke volume with expiratory load [163]. These studies highlight the importance of lower limb skeletal muscles during exercise in improving systemic venous return and cardiac output. The lack of regular and intensive physical exercise in many of these patients contributes to the reduced muscle mass.

Several mechanisms have been proposed for the aetiology of respiratory and skeletal muscle weakness [314]. Increased levels of tumour necrosis factor and interleukin-6 with enhanced sympathetic activation, pathways associated with skeletal muscle atrophy and altered metabolism, have been shown in adults with CHD [303,309]. As previously discussed, many patients with CHD and/or a Fontan circulation have a more sedentary lifestyle and may have exercise restrictions in place by their parents or treating physician. This leads to general deconditioning with a reduction in skeletal and respiratory muscle strength. In their study, Greutmann et al. found patients with CHD had reduced levels of activity compared to healthy controls [261]. Despite Fontan patients have lower exercise capacity than other forms of CHD, respiratory muscle strength was similar across all patients with CHD. This is in keeping with a detraining effect. Turquetto et al. showed respiratory muscle weakness to be associated with poor functional capacity [315].

Brassard et al. demonstrated abnormal skeletal muscle function in Fontan patients, with a higher ergoreflex contribution to diastolic blood pressure compared to healthy controls [316]. Their results were suggestive of impaired skeletal muscle endurance and metabolism. Inai et al. also found reduced skeletal muscle perfusion and endothelial dysfunction, which correlated with peak VO_2, and demonstrated attenuated oxygen re-saturation post exercise [317]. These findings are suggestive of the important role respiratory and peripheral skeletal muscles play in determining exercise capacity.

1.9.3.8 Desaturation During Exercise

Although Fontan completion improves the arterial saturation of patients by reducing right to left shunting, these patients still have lower baseline saturations at rest than healthy controls, with a further decrease at maximal exercise [248,249]. For example, in a cross-sectional study of Fontan patients, Paridon et al. found testing O_2 saturations were mildly reduced, with a mean of 94%, with further desaturation to 91% at peak exercise [199]. Baseline desaturation is not uncommon secondary to intracardiac or intrapulmonary right to left shunts. This may be in the form of a fenestration, veno-atrial collaterals, or pulmonary arterio-venous malformations. Further desaturation with exercise may be due to increases in vena caval pressure and congestion, increasing right to left shunting. Stromvall-Larsson et al. performed simultaneous arterial blood gas analyses during exercise testing on Fontan patients, to further elicit the mechanism behind impaired pulmonary gas exchange [271]. They found right to left shunting

as the major cause of desaturation with high alveolar-arterial oxygen partial pressure difference. Other causes of impaired gas exchange include hypoventilation, ventilation-perfusion mismatch, impaired gas diffusion, or low cardiac output.

Exercise intolerance in Fontan patients is related to both impaired cardiopulmonary function with inadequate preload reserve and an impaired ability to increase cardiac output, and respiratory and skeletal muscle weakness. Treatment regimens improving all these symptoms will be of most benefit.

1.10 Skeletal Muscle Training

Aerobic exercise training and more comprehensive cardiac rehabilitation programs have been shown to improve exercise capacity in adults with acquired CHF [318,319]. Additional benefits identified have been reduced HF-related hospitalisations and obesity; improvement in lipid profile and health-related quality of life; and improvement in overall cardiac mortality and morbidity [320,321]. There is also evidence for benefit in CHD patients, with improvement in peak VO_2 and oxygen pulse [322,323]. Van Dissel et al. performed a prospective randomised controlled trial in 40 adults with moderate or severe CHD and limited function (NYHA class II or III) on exercising training [323]. Their exercise program consisted of 3 exercise sessions per week for 6 months, of any sport of choice. They found good adherence to the program, with significant improvement in peak VO_2.

Due to the confirmatory findings of reduced respiratory and skeletal muscle strength in Fontan patients (see section 1.9.3.7 – Skeletal and Respiratory Muscle Weakness), research has been undertaken to assess the role of exercise training in an effort to improve long-term morbidity. Cordina et al. demonstrated a case of pulsatile pulmonary flow being generated through lower limb exercise in a 30-year-old Fontan patient [324]. This finding is consistent with previous evidence demonstrating that the skeletal muscle pump aids in venous return, and the importance of maintain skeletal musculature strength in this cohort. Training programs have focused on aerobic training, resistance training and more general rehabilitation programs. Longmuir et al. evaluated exercise programs in 61 children with a Fontan circulation, using a 12-month home-based program [325]. They found that the introduction of the exercise program improved physical activity, gross motor skills and physical fitness. Numerous small studies

have been undertaken to evaluate the safety and efficacy of exercise training programs on Fontan patients. In 2015, Sutherland et al. performed a literature review on this topic [326]. They included a total of 23 articles, which involved a total of 201 Fontan subjects. Importantly there were no adverse effects identified following exercise training. Majority of studies demonstrated improvement in peak oxygen consumption, oxygen pulse, daily activity levels and quality of life. Improvement in muscle strength was equivocal [326].

More recent work have explored changes in central venous pressure (CVP) and exercise in Fontan subjects, and have shown increases in pressure with exercise. Asagai et al. measured CVP using cardiac catheterisation at rest and during hand ergometer exercise in 34 patients (median age 19.3 years) with a Fontan [327]. They found an increased in CVP in approximately 40% of their cohort by a mean of 8mmHg, with those in the high CVP group during exercise were younger, but had a longer duration from Fontan completion, than those in the low CVP group. This was also associated with changes in variables associated with a failing Fontan physiology. Colman et al. measured peripheral venous pressure (as a surrogate for CVP) in 46 adults (mean age 26.9 years) during rest and exercise on a cycle ergometer [328]. They found a similar increase (mean of 10 mmHg) in peripheral venous pressure from rest to peak exercise, with an association higher peak peripheral venous pressure and adverse outcome. These results are suggestive that measurement of CVP during exercise may help risk-stratify patients and identify those requiring close follow-up and further intervention. However, further studies are needed to understand if the increases in CVP during exercise poses a risk to our Fontan population.

1.10.1 Resistance Training

Due to the effects of gravity, when exercising, peripheral skeletal muscles need to pump against gravity to maintain diastolic filling. In the Fontan circulation, pulmonary blood flow is dependent on IVC return. Lower limb venous compliance correlated with calf surface area and muscle mass [329]. Cordina et al. was one of the first studies to examine isolated resistance training in the Fontan cohort [299]. They demonstrated that after 20 weeks of high-intensity resistance training, peak oxygen consumption, muscle strength and muscle mass increased in 6 Fontan patients. They went on to show regression in these parameters following de-training [299].

1.10.2 Aerobic Training

Minamisawa et al. examined the effects of a home-based aerobic training program for 2-3 months in 11 children and young adults (aged 11-25 years) [330]. After exercise training, there was an improvement in maximal workload and peak oxygen consumption, although these remained lower than in healthy age-matched controls. They noted a trend towards a lower heart rate; however, it did not reach statistical significance. Opocher et al. examined a younger cohort (aged 7-12 years) and implemented an 8-month aerobic training program twice a week in 10 Fontan patients [331]. They also noted improvement in peak oxygen consumption, as well as oxygen pulse and a reduction in heart rate, following aerobic exercise training. Turquetto et al. undertook a randomised controlled trial on 42 Fontan patients aged 12 to 30 years over 4 months [315]. In their final cohort, 10 underwent aerobic training consisting of three supervised 60-minute training sessions of running, light resistance training and stretching; 10 inspiratory muscle training (IMT) (see section 1.12 – Inspiratory Muscle Training) and 12 continued their usual physical activities with no involvement in exercise training programs. Four months of aerobic training and IMT improved peak VO_2, with a more significant effect from aerobic training. Pulmonary function, however, only improved in the IMT group with increase in MIP, FVC, FEV_1 and minute ventilation. Similarly, Jacobsen et al. noted improvement in peak oxygen consumption following 12 weeks of a moderate-high intensity training program [332]. The specifics of the exercise program were not detailed in their paper, however consisted of dynamic and static exercises. They found progressive improvement in peak oxygen consumption from baseline to 6-weeks and, to 12-weeks follow-up. Additionally, they noted improved parent-report health-related quality of life following the training program [332].

Hedlund et al. examined the effects of a 12-week endurance training program in 30 children with a Fontan circulation [333]. They found improvement in vital capacity and submaximal exercise, based on improved 6-minute walk test, however peak oxygen consumption was not significantly different after training or at a year follow-up. They also found improved quality of life, as reported by the Fontan patients and their parents; highlighting the importance of some form of exercise in this population [333].

1.10.3 Mixed Aerobic and Resistance Training

In one of the earlier studies assessing the effect of cardiac rehabilitation programs on patients with CHD, Rhodes et al. assessed 19 patients with CHD, of which 11 had a Fontan circulation

[322]. Through supervised 12 weeks of 1-hour sessions twice a week, they performed stretches, aerobic and resistance training exercises. Like other studies look at aerobic training alone, they found improvement in peak oxygen consumption and minute ventilation in all Fontan patients. Additionally, submaximal parameters (VO_2, oxygen pulse and work rate at AT) also improved. There was no improvement in ventilatory efficiency as measured by VE/VCO_2 slope [322].

In a small cohort of 17 adolescent Fontan patients, Sutherland et al. examined the effects of two 1-hour exercise training sessions per week over 8 weeks [334]. Six had a hospital-based training program, and the other 11 underwent a home-based program. Both consisted of stretches, aerobic exercise, and resistance training. With the combination of both exercise training, they found an increase in oxygen consumption at peak exercise and AT. Patients who had a hospital-based training program also had improved peak workload. Additionally, like Hedlund et al., they found improvement in self- and parent-reported quality of life scores post training program [334].

Wittekind et al. also demonstrated an improvement in peak VO_2 following exercise training in 10 paediatric Fontan patients with either HLHS or tricuspid atresia [335]. They also demonstrated improvement in submaximal parameters of lower heart rate and higher oxygen pulse throughout exercise. They went on to discuss that a higher oxygen pulse may be reflective of improved stroke volume or oxygen extraction efficiency. To further explore this, they measured stroke volume on echocardiography following exercise training and found no difference [335]. This is in keeping with previous findings of the impaired ability for Fontan patients to augment stroke volume likely secondary to impaired ventricular preload (see section 1.9.3 – Causes for Impaired Exercise Capacity in Fontan Patients). Wittekind et al. also examined changes in VE/VCO_2 slope (marker of ventilatory efficiency) in this cohort, and unlike Rhodes et al. demonstrated a significant decrease in VE/VCO_2 slope, with no change in baseline oxygen saturations. They proposed that this improvement may have been secondary to improvement in ventilation-perfusion matching.

Although studies have been with small numbers, the safety and efficacy of exercise training programs in Fontan patients has been established. Many studies have looked at aerobic training alone, or in combination with resistance training. These programs have been in both supervised hospital settings and at home. Studies which did not demonstrate improvement in exercise

parameters tended to have a shorter exercise program, suggesting the need for a longer duration of training. Although the above studies have varied in exercise programs, most have demonstrated improvement in both submaximal and maximal parameters, including improvement in oxygen pulse, suggestive of improved stroke volume or more efficient oxygen extraction. Additionally, the finding of improved self- or proxy-reported quality of life is significant.

1.11 Positive Pressure Ventilation in the Fontan Circulation

Pulmonary blood flow and PVR are the major determinants of cardiac output in the Fontan circuit and are significantly affected by respiration. In a normal circulation, PVR is the main determinant of pulmonary afterload and is affected by lung volumes [336]. Reduction in intrathoracic pressure with inspiration aids antegrade pulmonary blood flow and consequently cardiac output.

Positive pressure ventilation (PPV) is frequently used in the management of respiratory failure in post-operative cardiac patients. However, it has been shown to reduce cardiac output likely secondary to increases in PVR and reduced systemic venous return. The haemodynamics of a Fontan circulation are dependent on low PVR and sufficient venous return, and hence the adverse effect of PPV is augmented in the Fontan circulation [165]. PPV can be used to increase pressures during inspiration and expiration, resulting in increased intrathoracic pressures. Applying pressure during expiration prevents intrathoracic pressures from returning to normal, thereby limiting systemic venous return and cardiac output [337-339]. Cournand et al. showed reduction in cardiac output was inversely proportional to the pressure delivered [146]. This reduction in cardiac output is exaggerated in the Fontan circuit, also reducing antegrade flow from the SVC into the branch pulmonary arteries. These findings highlight the importance of early extubation and/or minimising positive pressure post-operatively after Fontan completion [158,340]. Ventricular-ventricular interaction is also present in Fontan patients with Fogel et al. demonstrating marked differences in the wall motion of systemic right ventricles depending on presence of a left ventricle, through a magnetic resonance tagging technique [341]. They concluded that this ventricular-ventricular interaction plays a significant role in the mechanisms of the systemic ventricle in the Fontan circulation. However, the role of

respiratory dependent septal shift in “biventricular” Fontan patients (i.e. those with two ventricles present) is unknown.

Since the 19th century negative pressure ventilation (NPV) has been used in paralysed patients with respiratory insufficiency. It has been shown to improve both pulmonary blood flow and cardiac output, particularly in the Fontan circulation, and is associated with improved systemic venous return [157,342-345]. NPV applies sub-atmospheric pressures to the thorax during inspiration, causing the thorax to expand thereby reducing alveolar pressures, lowering PVR and augmenting systemic venous return [346].

Shekerdemian et al. converted patients in intensive care after Fontan completion from PPV to NPV and demonstrated acute improvement in pulmonary antegrade flow [344]. This was associated with improved mixed venous saturations and increasing cardiac output, without changes in heart rate, by over 50% during both the acute and late post-operative periods [344]. Charla et al. also examined the role of ten minutes of NPV and biphasic ventilation (BPV) in the ambulatory Fontan population [347]. Using CMR, they also found baseline low pulmonary blood flow compared to controls. With both NPV and BPV, there was a significant improvement in both pulmonary blood flow and cardiac output compared to controls. This was most likely secondary to changes in intrathoracic pressures, as previously demonstrated with normal inspiration [157]. They saw a greater improvement with BPV, which was postulated as being due to BPV supporting both the inspiratory phase as well as maintaining intra-abdominal pressures during expiration thereby minimizing retrograde flow [347]. This was supported by their demonstration of increased IVC and hepatic venous flows. Additionally, subjects tolerated short term external ventilation well and their willingness to continue external ventilation correlated with the improvement in pulmonary blood flow [347]. These studies examining NPV in Fontan patients have assessed acute hemodynamic response only. Long term safety, tolerability, and efficacy of NPV remains an interesting area for future research.

1.12 Inspiratory Muscle Training

1.12.1 Testing of Respiratory Muscle Strength

Assessment of respiratory muscle strength is utilised in both research and clinic practice settings. Several methods have been developed and are used to aid diagnosis and response to

treatment, particularly in patients with respiratory or neuromuscular disorders. The European Respiratory Society have relatively recently performed an extensive review of respiratory muscle testing in 2019 [348]. There are three main methods of measuring respiratory muscle strength: i) maximal static inspiratory and expiratory pressure; ii) maximal sniff nasal inspiratory pressure; and iii) peak cough flow.

1.12.1.1 Maximal Static Inspiratory and Expiratory Pressure

Different devices can be used to measure maximal static inspiratory and expiratory pressures. It allows a simple assessment of general respiratory strength. Many devices are small and handheld, while others are laboratory-based larger devices [349]. Maximal inspiratory pressure (MIP) is the maximal pressure generated when breathing in against an occluded airway [348,350,351]. It is usually measured after maximal forced expiration, at residual volume, and reflects inspiratory muscle strength, namely diaphragmatic muscle strength [261]. Maximal expiratory pressure (MEP) is measured at total lung capacity and reflects abdominal and expiratory muscle strength. Both MIP and MEP testing require a degree of co-ordination and co-operation from the subject. Like baseline spirometry, the manoeuvre is repeated to obtain three measurements with less than 10% variation [348]. Numerous studies have assessed normal values of MIP in healthy individuals and in adults with CHF [351-353]. Average MIP is higher in males than females and decreases with age [350].

1.12.1.2 Maximal Sniff Nasal Inspiratory Pressure

Maximal sniff nasal inspiratory pressure (SNIP) test is performed with a small probe placed in the nostril. This is useful in patients who are unable to obtain a tight seal around the mouthpiece for MIP testing, and therefore may be more practical in younger ages [348]. SNIP measures the pressure generated during maximal inhalation through one nostril. Therefore, it is less reliable in the presence of significant nasal obstruction.

1.12.1.3 Peak Cough Flow

Peak cough flow is predominantly used in patients with neuromuscular disorders and assesses the effectiveness of expiratory muscles and mucous clearance [348]. Measurements are obtained during a maximal cough after complete inhalation [354].

1.12.2 Inspiratory Muscle Training

Like skeletal muscles, respiratory muscles can be trained with regular pressure loading. Respiratory muscle weakness affects exercise capacity by predisposing them to fatigue, with an increased perception of dyspnoea [355]. During maximal exercise, 14-16% of cardiac output supplies the respiratory muscles [356]. It has been speculated that strengthening respiratory muscles can augment skeletal blood flow by reducing blood diverted to the respiratory muscles. IMT has been studied in a number of conditions including CHF [353,357,358]. It has been shown to improve exercise capacity through strengthening of the inspiratory muscles and attenuating the exaggerated peripheral vasoconstriction in exercising limbs [359].

Inspiratory muscle training (IMT) has been tested in patients with chronic obstructive pulmonary disease (COPD), asthma, respiratory muscle disorders, spinal cord injuries and CHF; to improve exercise capacity through strengthening of the inspiratory muscles. Inspiratory muscle weakness is generally defined as a MIP of $\leq 60 cmH_2O$ [360]. Like skeletal muscles, respiratory muscles can be trained with regular pressure loading. Weakness of inspiratory and expiratory muscles affects exercise capacity by predisposing them to fatigue, and an increased perception of dyspnoea [355]. During maximal exercise, 14-16% of cardiac output supplies the respiratory muscles [356,361]. IMT is a simple and safe intervention that can be used to improve respiratory muscle strength.

It has been proposed that respiratory muscle weakness causes reduced exercise capacity, a symptom found commonly in patients with COPD, CHF and those with a FSV. To study this hypothesis, Demspey et al. looked at the respiratory muscle metaboreflex. They found that by unloading the work of respiratory muscles with assisted ventilation, work of breathing and cardiac output were increased and there was an increase in peripheral blood flow to quadricep muscles [362]. With a reduction in work of breathing by 50-60%, they found a decrease in limb fatigue and vice versa, with an increase in limb fatigue during resistive loading causing an increase in work of breathing by 80%. Hence, they concluded that work of breathing plays a role in determining exercise duration until limb fatigue, and ultimately exercise ability. They postulated that inspiratory muscle (diaphragm and accessory muscles) fatigue during exercise from hyperventilation causes build-up of metabolites, which activates type IV phrenic afferent nerves. This causes sympathetic vasoconstriction reducing blood supply to peripheral muscles including active skeletal muscles [362,363]. Through the increase in respiratory effort and

reduced oxygen supply, there is increased activation of the respiratory muscle metaboreflex causing further reduction in peripheral muscle perfusion. Therefore, improving work of breathing through strengthening of inspiratory muscles may result in improved exercise capacity.

The concept of IMT was initially addressed in the late 1970s, with Leith and Bradley looking at the concept of respiratory muscle strength and endurance training in healthy subjects [364]. They found that like skeletal muscles, respiratory muscles could be trained, improving respiratory muscle strength and endurance. In their study, Leith and Bradley allocated healthy participants to three training groups – strength training (performing maximum static inspiratory and expiratory manoeuvres), endurance training (hyperventilation until exhaustion) and a control group (no respiratory muscle training). The participants in the strength training group had increased respiratory muscle strength (increased MIP) after training and the endurance group had increased endurance (time to exhaustion). In sedentary subjects, the respiratory system was found to limit exercise tolerance, with Boutellier et al. demonstrating an increase in cycle endurance by 50% in subjects after respiratory training [365]. Respiratory effort has also been shown to affect exercise tolerance. Harms et al. observed improved exercise tolerance with reduced work of breathing after respiratory muscle training [356]. They hypothesised that the mechanism may be due to respiratory muscle fatigue from increased work of breathing, and/or the altered blood flow distribution between the respiratory and skeletal muscles. These findings are suggestive that improved respiratory muscle strength through respiratory muscle training may improve overall exercise endurance.

There have been numerous studies looking at the effectiveness of respiratory (inspiratory and expiratory) muscle training (RMT) using different forms of training including flow resistance or targeted threshold training. These studies have looked at running, cycling and rowing as the method of exercise. Results and conclusions from these studies have been variable, with some studies showing improvement in maximal inspiratory and/or expiratory pressures, pulmonary function parameters such as FEV_1 and FVC, and/or perception of dyspnoea. However, studies performed have been limited by sample size and design, with few studies being conducted as a well-designed randomised controlled trial. McConnell and Romer published a review article discussing the findings from studies looking at RMT in healthy participants [355]. They concluded that RMT improved exercise performance during moderate intensity exercise, and

that initial studies looking at the effects of RMT on exercise performance demonstrated no improvement due to insufficient sample sizes. Each study demonstrated some improvement in the RMT group compared to the control groups, but these values were not of statistical significance.

Rochester and Arora published a review article in 1983 concluding that IMT improves respiratory muscle strength and endurance in COPD, cystic fibrosis and spinal cord injuries, but did not necessarily result in improvement in physical performance. In 1985 Sharp reviewed articles on IMT and found evidence of improvement in inspiratory muscle strength in healthy subjects post IMT training [366]. Subsequent studies analysing IMT in healthy individuals have found that IMT at low resistance training (≤40% baseline MIP) improves MIP by 15-20% but does not improve other markers of respiratory function such as diaphragmatic strength and exercise performance [367]. However, with higher resistance training (80% baseline MIP), contracted diaphragm thickness, lung volumes and work capacity did improve [368]. Downey et al. examined the effects of IMT in hypoxic and normoxic exercise conditions [369]. They also found increases in MIP (by 25%) and diaphragm thickness, with increased arterial saturations by 4% in the hypoxic group (breathing in 14% fraction of inspired oxygen). As rowing requires significant aerobic power and high minute ventilation, Volianitis et al. looked at the effects of IMT in female competitive rowers [370]. After 11 weeks of IMT at a training pressure of 50% of peak inspiratory pressure, MIP increased by 45.3±29.7% and performance in the 6 minute and 5000m tests improved compared to baseline measurements.

The mechanism for RMT-induced improved exercise performance still requires further evaluation. Cardiovascular adaptations were studied by Markov et al. looking at changes in stroke volume in patients with improved exercise endurance post RMT [371]. They found that despite finding an increase in cycling endurance, stroke volume and heart rate remained unchanged after RMT. This implies that the changes in exercise endurance from RMT are not due to changes in cardiovascular physiology. They concluded that RMT may affect exercise endurance through direct strength training of the respiratory muscles. McConnell et al. discussed possible mechanisms for RMT-induced improved exercise performance [355]. As respiratory muscles are a form of specialised skeletal muscle [372], RMT may have a direct effect on inspiratory and expiratory muscle strength as shown by increases in maximal velocity of shortening, maximal force production and endurance after RMT [364,373,374]. To further

demonstrate direct effects on respiratory muscles, Ramirez-Sarmiento et al. (2002) looked at structural changes of respiratory muscles after IMT through examining muscle biopsies pre- and post-IMT [375]. They found that along with increases in inspiratory muscle strength and endurance, there was hyperplasia of type I and II fibres in external intercostal muscles. RMT has also been shown to delay respiratory muscle fatigue, particularly of the diaphragmatic muscles [376,377]. This may contribute to reducing the sensation of breathlessness. Harms et al. found that at maximal exercise, blood flow to the lower limbs was affected by work of breathing [361]. They extended their study further looking at the effects of work of breathing on cardiac output. This demonstrated that at maximal exercise, a higher percentage of cardiac output was directed to the respiratory muscles and along with the effects of vasoconstriction, there was diminished blood flow to the lower limb muscles [361]. Therefore, reducing work of breathing through RMT, may improve blood flow to the lower limbs at peak exercise.

1.12.3 Inspiratory Muscle Training Devices

A wide range of IMT devices are available worldwide, with small hand-held devices being commercially available. There are three main types of IMT devices available: non-targeted resistive trainers, targeted resistive or threshold trainers and hyperventilation trainers (figure 1.8) [378,379]. All devices contain a one-way valve, providing an inspiratory load on breathing in when the valve closes, and no load during expiration as the valve opens.

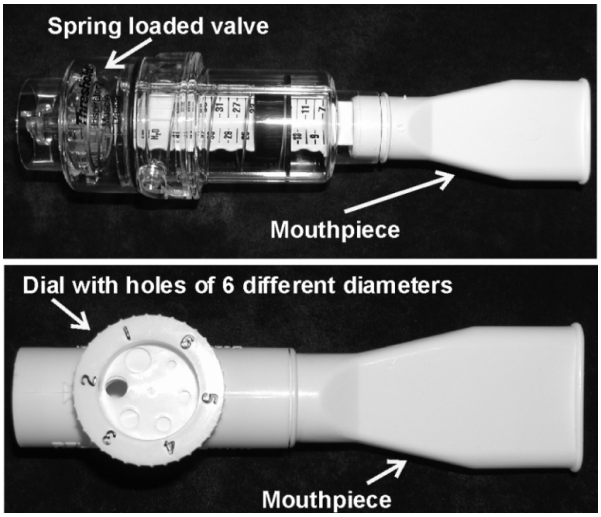


Figure 1.8. Inspiratory muscle therapy devices

Top – Threshold trainer; Bottom – Resistive trainer. Reproduced from Mueller et al. [380]

The inspiratory load of non-targeted trainers can be altered by selecting holes of various diameters for the patient to inhale through – the smaller the hole, the greater the inspiratory resistance. However, by slowing the rate of inspiration, the inspiratory load can be reduced and hence a breathing rate needs to be set. Resistive trainers have been shown to improve total lung capacity and inspiratory muscle strength by 55% [364].

Targeted threshold trainers provide a constant set inspiratory load, with the resistance being unaltered by small changes in respiration. The threshold trainer contains a spring-loaded valve that is adjustable according to the patient's baseline maximal inspiratory pressure. Targeted resistive trainers provide a target inspiratory pressure through visual feedback, such as a spirometer. The patient determines their inspiratory pressure, with an aim to achieve the target flow. Suzuki et al. showed an improvement in both inspiratory and expiratory muscle strength after pressure threshold training, increasing MIP by 30-40% and MEP by 16% [374]. Pressure threshold training has also been shown to improve performance, with Volianitis et al. showing improved rowing performance in 6 minute and 5000m tests [370]. Turner et al. demonstrated reduced oxygen requirement during voluntary hyperpnoea after threshold training [381].

The hyperventilation trainers are used mainly in research, allowing inspiratory muscle training at high respiratory rates. The participant is required to breathe at high rates for up to 30 minutes. The apparatus is attached to a rebreather bag supplied with oxygen, to maintain a constant CO_2 level and prevent hypocapnia. Studies have shown improvement in vital capacity, maximum sustained ventilatory capacity, maximum voluntary ventilation, and endurance with hyperventilation training [365,382].

The targeted IMT devices are the best device for inspiratory muscle training and have been used in most studies looking at the effects of IMT in patients with reduced inspiratory muscle strength. The non-targeted devices do not ensure a constant training inspiratory pressure and the hyperventilation trainers are not practical in a home setting. Hsiao et al. looked at the two types of targeted devices in a home-based training program [383]. Both devices demonstrated improvement in inspiratory endurance time with no difference between the two devices. They found that the resistive trainers gave patients positive visual feedback but patient's compliance to achieve target inspiratory pressures was difficult to accurately assess by the clinician. The threshold device on the other hand allowed consistent training intensity. However, the patient

must be able to generate enough pressure during inspiration to open the valve, highlighting the need for adequate training and supervision.

1.12.4 Indications for Inspiratory Muscle Training

1.12.4.1 Chronic Obstructive Pulmonary Disease

COPD is a common lung condition in adults affecting the function of bronchi, bronchioles, and alveoli. They commonly develop inspiratory muscle weakness secondary to hyperinflation from air trapping, hypoxaemia and chronic increased work of breathing; presenting with symptoms of dyspnoea and reduced exercise tolerance [384]. Initial studies published on IMT in COPD demonstrated improvements in inspiratory muscle strength but did not consistently show significant clinical improvements including exercise capacity [385,386]. The use of IMT in COPD has now been studied extensively with positive results of increased inspiratory muscle strength, improved exercise tolerance and reducing the sensation of dyspnoea [357,387]. Geddes et al. published a systematic review in 2006 analysing the effect of IMT in adults with COPD.

1.12.4.2 Neuromuscular Disorders

Neuromuscular disorders affect both skeletal and respiratory muscles, with respiratory failure being a common complication. As well as affecting inspiratory and expiratory muscles, bulbar muscles (muscles that control speech and swallowing) can also be affected, impairing cough and airway protection and increasing the risk of aspiration and pneumonia. [388] Both respiratory and skeletal muscle weakness affect patients' exercise capacity and activities of daily living. A systematic review was performed by Reyes et al. looking at RMT, inspiratory and expiratory muscle training, in neurodegenerative disorders. They concluded that there is some evidence that RMT improves respiratory function in patients with Parkinson disease and multiple sclerosis [358]. Of their initial 24 studies, only 6 were included in their systematic review, all looking at patients with Parkinson disease or multiple sclerosis. They found that studies looking at expiratory muscle training showed improvements in mean maximal expiratory pressures with a mean increase of 25-50%, and improvement in cough function. IMT was also shown to improve inspiratory muscle strength, with improvements in mean MIP by 26-44%. Fry et al. also showed improvement in expiratory function after IMT, with

increases in FEV_1 and FVC after training [389]. There was also evidence for IMT reducing the patient's sensation of dyspnoea in Parkinson disease and MS [358].

1.12.4.3 Heart Failure

Two major clinical symptoms of CHF, dyspnoea and reduced exercise capacity, are thought to be due to a combination of alterations in the cardiovascular system as well as the neuroendocrine, immune and musculoskeletal systems [390]. Poor respiratory muscle strength correlates with the degree of symptoms experienced by patients with CHF [391]. Initial research examined ventricular dysfunction as a causative factor for reduced exercise capacity in adults with CHF, however little evidence was found to support this hypothesis [392,393]. Adults with CHF have mild ventilation and perfusion mismatch, adversely affecting gas exchange. This is associated with increased work of breathing, particular during exercise. With increased work of breathing and blunted cardiac output response during exercise in adults with CHF, Olson et al. proposed that reducing respiratory work (with the use of BiPAP) improves skeletal blood flow through reducing steal to diaphragmatic muscles [143].

Skeletal muscle abnormalities are thought to play a significant role in the development of these symptoms, with Coats et al. describing the muscle hypothesis [394]. They proposed that deterioration in left ventricular function causes abnormalities in muscular metabolism and subsequent muscle wasting and weakness, consistent with CHF patients having generalised muscle atrophy. This in turn causes sympathetic stimulation with vasoconstriction and further ventricular dysfunction through an increase in afterload. Right ventricular dysfunction also plays a role in impaired exercise capacity, limiting pulmonary blood flow particularly during exercise. Coats et al. also proposed that inadequate inspiration secondary to respiratory muscle weakness may also affect ventilation, demonstrated by reduced tidal volume, minute ventilation and VE/VCO_2 slope in CHF patients [394].

As exercise causes increased blood flow to respiratory muscles, diverting blood flow away from skeletal muscles, Borghi-Silva et al. proposed that by assisting the respiratory muscles through positive ventilation, blood flow could be redistributed to peripheral skeletal muscles improving exercise capacity [395]. They demonstrated improvement in submaximal exercise capacity in patients with CHF with assisted positive pressure ventilation compared to controls, with associated improvement in skeletal muscle oxygenation through the use of near infrared

spectroscopy. This suggests that by unloading the work of respiratory muscles during exercise (though positive ventilation or muscle strength training), perfusion and oxygenation to exercising muscles can be improved, resulting in improved exercise capacity.

Other suggested mechanisms for poor exercise tolerance in CHF include poor lung perfusion, reduced skeletal muscle blood flow, impaired oxidative metabolism, sympathetic overdrive and cytokine activation [396,397]. Structural changes have been found in skeletal and respiratory muscles in patients with CHF [308,375]. Adults with CHF have been shown to have reduced respiratory muscle strength, with the degree inspiratory muscle weakness correlating with the degree of dyspnoea experienced by patients during daily activities [391,398]. Patients with respiratory muscle weakness have a more significant and abrupt reduction in skeletal muscle blood flow at rest and during exercise [359]. Reduced exercise capacity, measured by peak VO_2, is an independent predictor of rehospitalisation and mortality [399]. MIP is also a predictor of mortality and correlates with the severity of CHF [400].

Several mechanisms for improvement in exercise capacity post IMT have been proposed. As histological changes in diaphragmatic biopsies have been found in adults with CHF, it is likely that respiratory muscle training may reverse some of these changes and improve their strength and function [301,353,375]. Improvement in exercise capacity through alterations in aerobic and anaerobic metabolism has also been suggested, delaying fatigue of respiratory muscles [401].

The use of IMT in adults with CHF has been studied since the 1990s. There is evidence for IMT improving inspiratory muscle strength, lung function, exercise capacity and sensation of dyspnoea [353]. From a literature search using Medline and Embase, 32 trials were identified looking at the benefits of IMT in adults with CHF with 24 of the studies being conducted as randomised controlled trials. The majority of studies utilised targeted threshold trainers, set at inspiratory pressures of 20-60% of measured MIP [402,403]. The duration of training ranged from a single training session to a 12-month home based program [404,405]. The most commonly used IMT program was 30% of measured MIP for 30 minutes daily for 5-7 days over a 6-12 week period. These studies demonstrated an improvement in MIP, MEP, peak VO_2, oxygen uptake at anaerobic threshold, 6-minute walk test and/or perceived dyspnoea; suggestive of respiratory muscle weakness being a causative factor in reduced exercise capacity

in this population. Meyer et al. found inspiratory muscle strength to be an independent predictor of survival [406].

Montemezzo et al. published a meta-analysis on inspiratory muscle training in chronic heart failure patients with inspiratory muscle weakness [353]. They found that the baseline MIP ranged from 46.5 to 82.8cmH_2O in the CHF group, compared to a range of 50.7 to 84.2cmH_2O in the control groups. Methods of conducting training sessions varied between studies, ranging from patients using the device as frequently as twice a day to using the device 3 sessions a week. Training sessions were 10 to 30 minutes long, or in 3 studies until patient was fatigued, with most studies having supervised sessions. The total duration of the program ranged from 4 to 12 weeks. Their meta-analysis showed a mean improvement in MIP of 22.6cmH_2O (CI 3.7-41.54, p=0.02) post IMT in comparison to the control group. There was also an increase in sustained maximal inspiratory pressure and peak oxygen consumption. Improvement in MIP and minute ventilation was greater in people with inspiratory muscle weakness (MIP ≤60cmH_2O) [353]. Their review of articles also demonstrated an improvement in patients' 6-minute walk test, a representative test of activities of daily living. A systematic review from Cahalin et al. found similar findings to Montemezzo, with improvements in peripheral muscle strength, ventilatory parameters, cardiac performance and quality of life [407]. Patients with inspiratory muscle weakness (MIP <70% of predicted) had a greater improvement in parameters than those without inspiratory muscle weakness. From the studies reviewed, the Threshold IMT devices were the most common and effective IMT device, consistently demonstrating positive outcomes. Their review included three studies that analysed IMT combined with other exercise modalities including anaerobic exercise and resistance training, demonstrating additional improvements in MIP, peak VO_2, power and quality of life [403,407,408]. This data is suggestive that additional benefits can be obtained in CHF with the use of IMT combined with another form of exercise training. Inspiratory muscle weakness with MIP<70% of predicted, shortness of breath at rest or on exertion, and pulmonary hypertension are clinical features for IMT in CHF [407]. Contraindications include worsening heart failure or respiratory muscle strength after commencing IMT, desaturation with IMT, persistent thoracic or abdominal muscle discomfort and severely elevated left ventricular end-diastolic pressure and volume [407]. These contraindications were listed by Cahalin et al. following reports of patients being unable to adhere to their IMT program due to their symptoms. No

long-term follow-up studies have been performed in patients with CHF looking at maintaining inspiratory muscle strength and improved ventilation and exercise capacity.

Chiappa et al. reported approximately 30% of their CHF patients secondary to left ventricular systolic dysfunction having inspiratory muscle weakness defined as <70% of predicted MIP [359]. They found that a 4-week IMT program at 30% of measured MIP for 30 minutes daily resulted in a significant improvement in MIP by 72% and diaphragm thickness. In keeping with the previously described respiratory muscle metaborelex, they also found a slight reduction in calf blood flow during respiratory muscle loading with a threshold device. Patients with CHF had an exaggerated response with premature and more pronounced reduction in calf flow with the same respiratory load, with improvement in limb blood flow after IMT [359].

IMT has also been evaluated in conjunction with aerobic training. Aerobic training has been well established as a useful tool in rehabilitation programs for adults with CHF, maintaining activities of daily living and improving patients' quality of life [396,409]. However due to findings of respiratory muscle weakness, studies have been undertaken to combine IMT with aerobic training to maximise potential improvements in exercise capacity in this group of patients. Adamopoulos et al. performed a randomised control trial finding additional improvements in maximal inspiratory pressure, quality of life and dyspnoea sensation [410]. However, there were no additional increases in exercise parameters in the combined IMT and aerobic training group compared to the aerobic training and unloaded IMT (at 10% of measured MIP). Both groups demonstrated an improvement in MIP, with no significant additional benefit from the IMT cohort. This is suggestive that even at minimal pressures (10% of measured MIP), IMT can improve inspiratory muscle strength. Adamopoulos et al. proposed that their study did not show additional improvements in exercise parameters, such as peak VO_2, due to the small changes in diaphragmatic muscle strength from aerobic training [410].

In a small cohort, Winkelmann et al. studied the effects of the addition of IMT to aerobic exercise training in adults with CHF. In 24 patients, they found improvement in exercise capacity and respiratory muscle strength, as measured by MIP, VO_2 peak, OUES, ventilatory efficiency and ventilatory oscillation.

Studies conducted in patients with CHF have shown IMT to be beneficial, with improvements in respiratory muscle strength, quality of life, sensation of dyspnoea, pulmonary function, and exercise parameters. Studies have shown baseline MIP in adults with CHF ranging from 50-80cmH_2O and mean increase in MIP by 17-30cmH_2O post IMT. The addition of IMT to aerobic training further improves respiratory muscle strength and quality of life. The most proposed mechanism is that by increasing respiratory muscle strength and improving ventilation, work of breathing is reduced, and hence steal of blood flow from peripheral muscles is also limited.

1.12.4.4 Congenital Heart Disease

Few studies have been conducted utilising IMT in children, with only one study involving children with CHD where they examined the effects of IMT in 2 ventilated children with CHD. Smith et al. described two cases of IMT in infants with CHD, to assist in weaning ventilation [411]. One of their patients had truncus arteriosus, patent ductus arteriosus, ventricular septal defect and interrupted aortic arch with DiGeorge syndrome. Two months after insertion of an RV-PA valved conduit and repair of the ventricular septal defect and interrupted arch, the child represented in respiratory distress and required re-intubation. The child was noted to have pulmonary hypertension, LV dysfunction, PA stenosis and a residual ventricular septal defect. Their second patient was an infant with a tetralogy-type pulmonary atresia, ventricular septal defect and aortopulmonary collaterals. After complete repair with an RV-PA conduit the child developed cardiorespiratory failure and required extracorporeal membranous oxygenation. Both infants had difficulties in weaning ventilation. A form of IMT was introduced through the ventilator, with improvement in their MIP, respiratory rate and tidal volumes. IMT has been shown to be of some benefit in adults to aid in ventilation weaning through increasing inspiratory muscle strength [412]. The cases reported by Smith et al. suggest that **IMT may also be effective in improving inspiratory muscle strength in infants with CHD**. Therefore, further evaluation of IMT is required in children and in children with CHD.

1.13 Summary

The altered haemodynamics in a Fontan circulation leads to chronic elevation of central venous pressure and restricted ventricular preload. The presence of pulmonary vascular dysfunction, valvar regurgitation, and ventricular dysfunction all further contribute to abnormal cardiac filling and increase systemic venous pressure.

Exercise impairment is well established in the Fontan cohort, and initial studies were focused on alterations within the cardiovascular system. However, the aetiology of exercise impairment is multifactorial, and other mechanisms such as lung function and skeletal muscle strength clearly play a role. Fontan patients have been shown to have reduced lung volumes with restrictive lung disease, and abnormal lung gas transfer, which has been associated with parameters of exercise testing and mortality. Additionally, they have reduced skeletal and respiratory muscle strength, further limiting exercise capacity.

This thesis will provide more detailed assessment of respiratory function and exercise parameters in Fontan patients and assess the altered cardiopulmonary interaction. An in-depth understanding of the cardiopulmonary interaction in the Fontan circulation is required to tailor medical and surgical management to improve the morbidity of these patients long-term.

CHAPTER TWO: GENERAL METHODOLOGY

Parts of this chapter is based on the following publications:

Laohachai K, Winlaw D, Selvadurai H, Gnanappa GK, d'Udekem Y, Celermajer D, Ayer J. Inspiratory muscle training is associated with improved inspiratory muscle strength, resting cardiac output, and the ventilatory efficiency of exercise in patients with a Fontan circulation. JAHA. 2017; 21: 6(8).

Laohachai K, Badal T, Thamrin C, Robinson PD, Kennedy B, Rice K, Selvadurai H, Weintraub R, Cordina R, d'Udekem Y, Ayer J. Older age at Fontan completion is associated with reduced lung volumes and increased lung reactance. Int J Cardiol. 2022 Oct 1;364:38-43

2.1 The Australia and New Zealand Fontan Registry

Parts of the data included in this thesis (Chapters 3 and 5) were acquired as part of the Australia and New Zealand Fontan Registry (ANZFR) Functional Outcomes after Fontan study. Due to the variability in management of patients with a Fontan circulation, the Australia and New Zealand Fontan Registry was set-up to collate data on patients with a Fontan circuit across Australia and New Zealand. The ANZFR Functional Outcomes after Fontan study was conducted across Sydney, Melbourne, and Auckland, aiming to capture all patients with a Fontan circulation across Australia and New Zealand. Inclusion criteria for this study included age ≥13 years and ≥5 years since Fontan completion. Exclusion criteria were those with severe heart failure, a history of significant exercise-induced arrhythmia, severe systemic outflow tract obstruction or severe systemic hypertension at rest, or severe cognitive impairment/ intellectual disability. Clinical data for Fontan participants were retrospectively collected from the ANZFR REDCap database. These patients underwent transthoracic echocardiogram, spirometry, cardiopulmonary exercise testing and exercise cardiac MRI, as described below.

2.2 International Physical Activity Questionnaire (IPAQ)

Physical activity levels were assessed (Chapter 4) through a self-administered International Physical Activity Questionnaire (IPAQ) short form (see section 8.1 – Appendix 1). The IPAQ estimates the amount of time spent doing three specific levels of activity: walking, moderate-intensity, and vigorous intensity, based on the patient's activities over the last 7 days. A metabolic equivalents value per week is then calculated by multiplying the duration and frequency of each activity by the known metabolic equivalent for each activity. Based on the calculated weekly metabolic equivalents value, participants are identified as undertaking low, moderate, or high levels of activities. Craig et al. demonstrated that both the short and long forms of the IPAQ were comparable to other established self-reports [413].

2.3 Echocardiography

Transthoracic echocardiography images were obtained without sedation on all Fontan participants by on-site paediatric sonographers or trained medical practitioners, using local

ultrasound equipment. Procedure was explained to the parents and/or child prior to the study. Participants were connected to ECG and respiratory monitoring throughout the study. Standard echocardiography guidelines were followed [414], and image quality was optimised prior to acquisition. Two-dimensional still and cine images were acquired to determine the following characteristics: a) underlying cardiac anatomy; b) ventricular morphology classified into RV-dominant, LV-dominant or balanced ventricles; c) type of Fontan circulation; d) ventricular function (qualitatively assessed); e) severity of atrioventricular valve regurgitation; and f) presence of fenestration. Findings were recorded and collated for each study. This data was utilised in Chapters 3, 4 and 5.

2.4 Lung Function Testing

2.4.1 Spirometry

Spirometry is considered the gold standard of lung function testing. It measures the maximal amount of air one can inspire and expire, depicting volume or flow as a function of time [415]. Forced vital capacity (FVC) is the full volume of air with forceful expiration from full inspiration and forced expiratory volume in 1 second (FEV_1) is the amount of volume expired within the first second (figure 2.1). Spirometry was performed using CareFusion SentrySuite software and obtained via standard spirometry techniques according to American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines [415]. Measurements were obtained with the participant sitting upright and a nose peg in place. After explanation and demonstration of the manoeuvre to the patient, FVC and FEV_1 were obtained. Results were reviewed after each manoeuvre to ensure acceptable results. The test was repeated until 3 acceptable tests met repeatability criteria, with the values varying by less than 150 mls, or it was accepted that an adequate test would not be obtainable. The largest FVC and FEV_1 were reported and the FEV_1/FVC ratio was calculated.

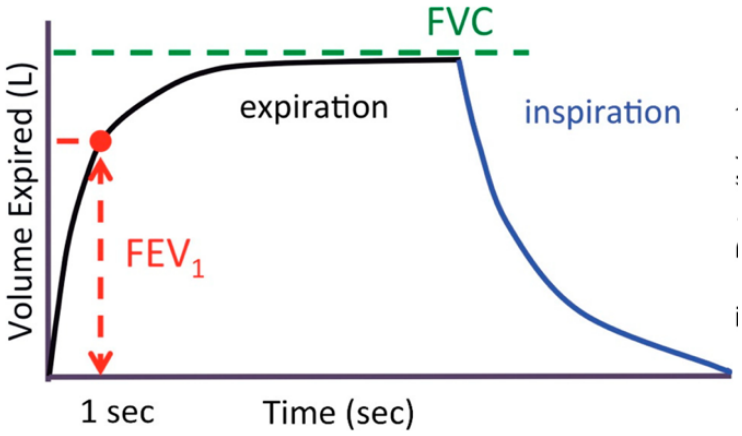


Figure 2.1. Graphical representation of spirometry parameters

FVC – forced vital capacity, FEV1 – forced expiratory volume in 1 second, L – litres, TLC – total lung capacity, RV – residual volume. Reproduced from Almazioum et al. [416]

Results were assessed for validity according to the ATS/ERS guideline [415] and any technically poor results were excluded. Presence of an obstructive or restrictive pattern was determined from the results obtained. Z-scores and percent predicted values were calculated using published reference values, corrected for age, height, gender, and ethnicity [417,418]. Spirometry was utilised in Chapters 3, 4 and 5.

2.4.2 Body plethysmography

Body plethysmography primarily measures functional residual capacity and specific airway resistance [419]. With deep inspiration and expiration, it can also determine total lung capacity and residual volume, measurements which cannot be obtained with spirometry alone (figure 2.2).

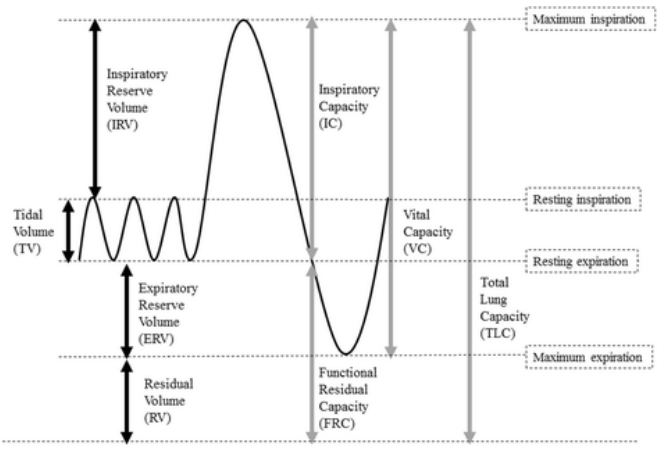


Figure 2.2. Standard lung volumes on spirometry

Reproduced from Lutfi [420]

This testing was performed by trained respiratory technicians using standard techniques (figure 2.3) [211]. Testing was performed with the patient seated and a nose peg in place. Initial measurements were acquired with normal respiration, followed by panting; producing pressure-volume loops. These data were used in Chapter 3.

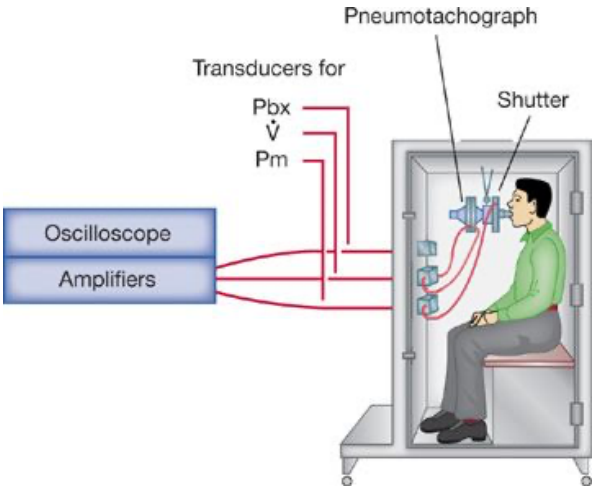


Figure 2.3. Body plethysmography.
Reproduced from Grippi and Tino, 2015 [6]

2.4.3 Oscillometry

Oscillometry is a simple, non-effort dependent lung function test performed during tidal breathing, making it more suitable for younger children than standard spirometry. This technique provides measures of respiratory mechanics by superimposing pressure oscillations onto the airway opening during normal breathing into a mouthpiece and examining the resultant flow and its relationship with the oscillating pressure. Oscillometry was performed according to ERS technical standards [421]. Thirty-second recordings were made in triplicate, and each breath within a recording was visually checked for artefacts such as leaks, glottal closures, swallows and coughs, and excluded if present.

We examined two oscillometry measures of interest: resistance (R5) and reactance (X5) at 5 Hz. R5 is a measure of airway calibre, which in comparison to spirometry is more sensitive to changes in the small airways. X5 predominantly reflects the elastic properties or stiffness of the respiratory system and is sensitive to changes such as small airway closure or reduced communicating lung volume. R5 and X5 were expressed as Z-scores based on previously published reference values [422]. Oscillometric data were used in Chapter 3.

2.4.4 Multiple Breath Washout

Multiple breath nitrogen washout (MBNW) assesses heterogeneity (or unevenness) in the distribution of ventilation within the lungs, by studying the progressive changes in end-tidal resident nitrogen values over successive breaths as the patient breathes in 100% oxygen. MBNW was performed according to ERS/ATS consensus statement recommendations using a tidal breathing protocol [423]. Technically acceptable trials were performed in triplicate and the mean values reported for FRC and a global measure of ventilation heterogeneity termed lung clearance index (LCI). These data were used in Chapter 3.

2.4.5 Diffusion Capacity of the Lungs for Carbon monoxide and Double Diffusion

Diffusion capacity of carbon monoxide (DLCO) is a measure of lung gas transfer from alveolar gas to haemoglobin within the pulmonary capillaries. It is affected by diffusion across the alveolar-capillary membrane, haemoglobin levels and capillary blood volume. DLCO was measured by the single-breath technique according to ATS standards [424]. Z-scores were calculated based on Global Lung Initiative reference values [425].

The double diffusion test was used to help identify the mechanisms behind impaired DLCO [426] (figure 2.4). By using two gases (carbon monoxide (CO) and nitric oxide (NO)) with different relative contributions to gas diffusion resistance from the alveolar/capillary membrane versus red cell uptake, it is possible to not only calculate the diffusion capacity for both gases (DLCO and DLNO), but also partition DLCO into alveolar-capillary membrane diffusing capacity for carbon monoxide (DmCO) versus pulmonary blood capillary volume (VC). NO has a higher affinity to haemoglobin, making it less affected by blood volume and a better assessment of the alveolar-capillary membrane function. Thus, the DLNO/DLCO ratio can help determine the aetiology of the impaired diffusing capacity. Furthermore, by measuring the rate of uptake of carbon monoxide from alveolar gas (KCO) it is possible to determine the contribution of alveolar volume (VA) to DLCO. DLCO values were corrected for the subject's haemoglobin value.

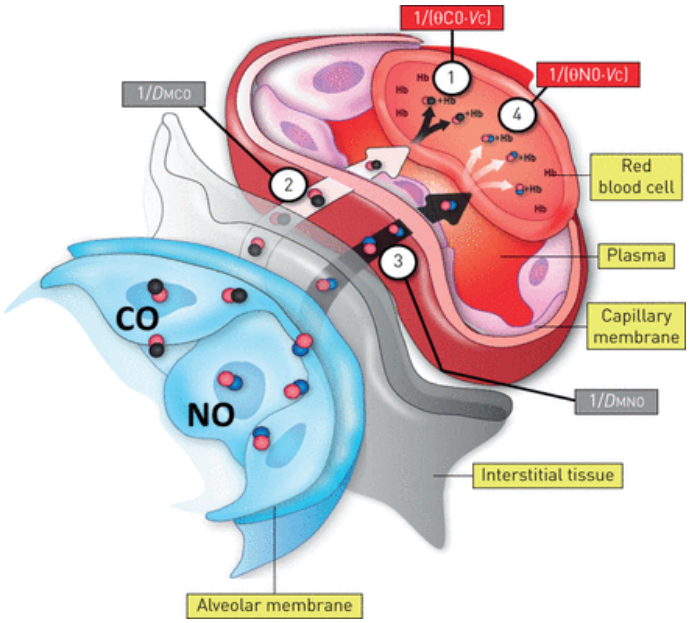


Figure 2.4. Uptake pathways of inhaled nitric oxide and carbon monoxide
Reproduced from Zavorsky et al. [427]

2.5 Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing (CPET) provides an in-depth assessment of cardiac and respiratory physiological response to exercise. Results of exercise testing that form parts of this thesis (Chapters 3-6) were performed on an upright cycle ergometer (Ergoline Ergoselect 200) (figure 2.5). Gas calibration was undertaken on a daily basis and volume calibration was performed prior to each testing. A ramp protocol was utilised, starting at 20W, and increasing by 10W every minute if the participant was less than 50kg, and by 15W every minute if the participant was greater than 50 kg; with an aim of exercising between 8-12 minutes. Baseline heart rate, blood pressure and oxygen saturations were performed prior to starting the test.

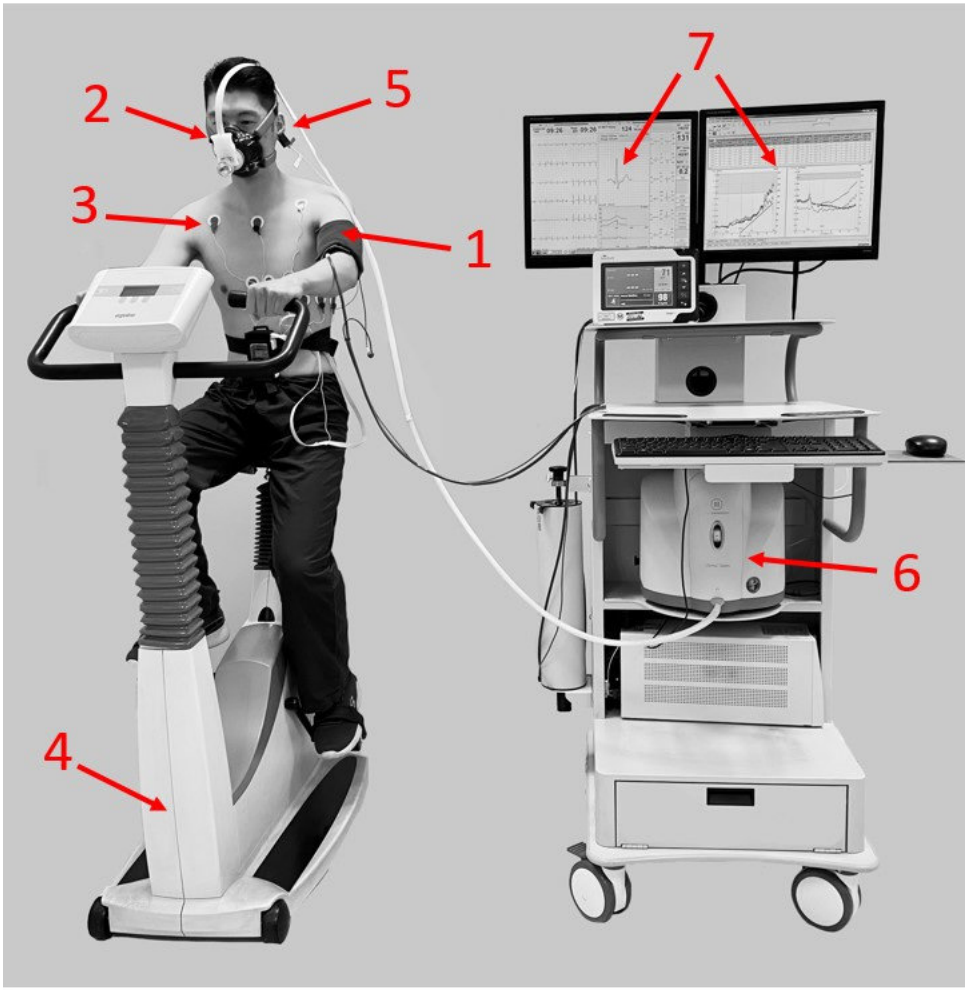


Figure 2.5. Cardiopulmonary exercise testing on a cycle ergometer

1 – blood pressure monitor; 2 – mask, volume sensor and gas analyser tubing; 3 -12-lead ECG; 4 – cycle ergometer; 5 – pulse oximeter; 6 – gas analyser; 7 – display of breath-by-breath data and exercise ECG. Reproduced from Pritchard et al. [428]

Participants were given 5 minutes to familiarise themselves with the exercise bike prior to commencing the study. The participants were instructed to maintain a cycle speed of 50 to 60 rpm throughout the testing, with electronic arrows on the cycle's display screen indicating if they needed to increase or decrease their speed. A blood pressure recording was performed every 3 minutes during exercise and at 1 and 5 minutes in recovery, and ECG and oxygen saturation monitoring were continuous. The ECG tracing was monitored to ensure no significant arrhythmias. Oxygen and carbon dioxide levels were monitored throughout the testing and until 5 minutes into recovery. Patients were encouraged to continue exercising to maximal exertion. A Borg score was noted from each participant during and at the completion of their CPET. Reasons for stopping exercise were recorded.

The data were then stored and analysed using CareFusion SentrySuite and CardioSoft ECG software. Using the standard 9-panel plot (figure 2.6), the following parameters (see section 1.8 – Determinants of CPET parameters in health) were recorded or calculated, and analysed: 1) at maximal exercise: exercise duration, maximal workload, peak heart rate, peak VO_2, RER, VE/VCO_2 slope, oxygen pulse, tidal volume, heart rate and breathing reserve; and 2) at calculated anaerobic threshold: workload, VO_2, VE/VCO_2 slope, oxygen pulse, oxygen uptake efficiency slope. Measurements were averaged over 30 seconds, and the highest averaged measurement was identified as the peak value.

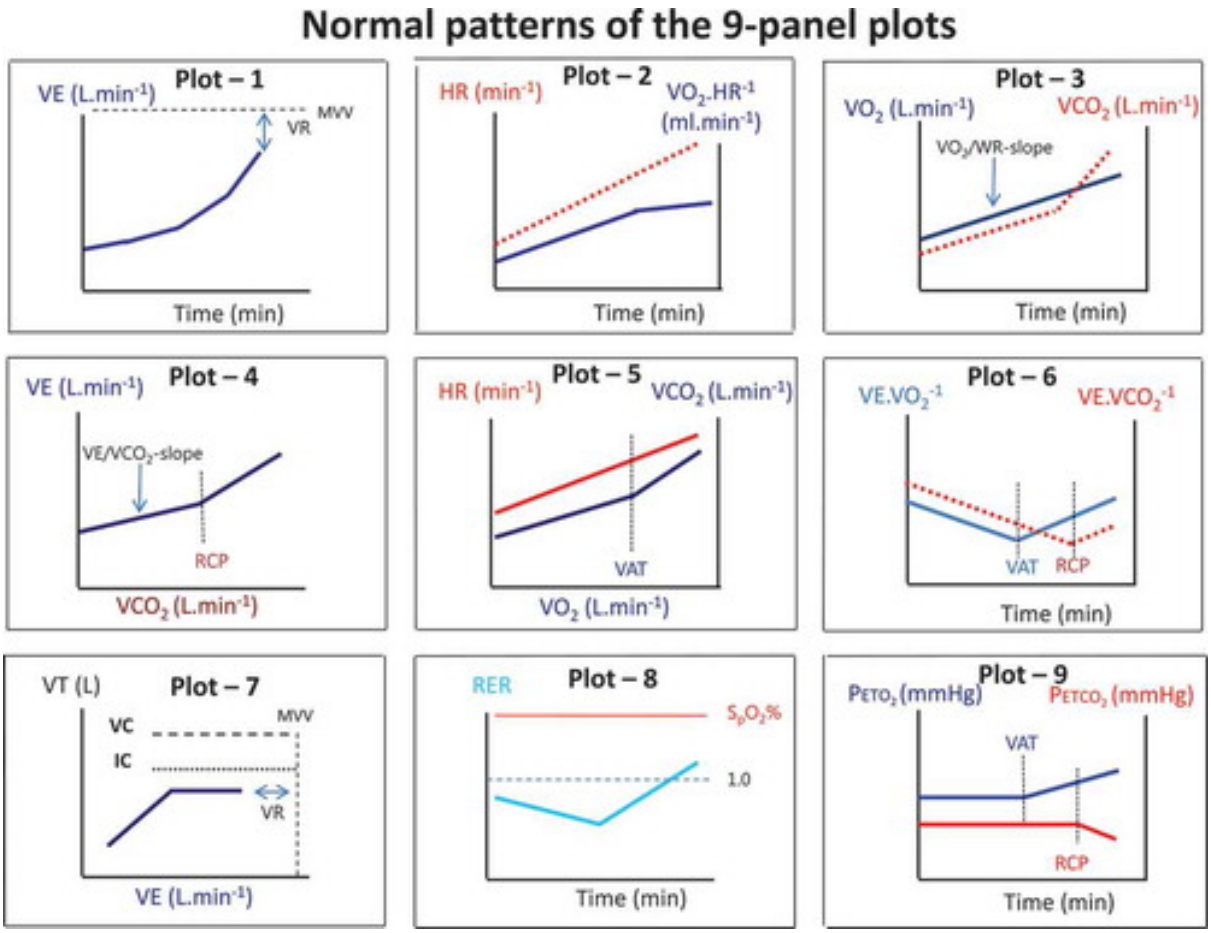


Figure 2.6. 9-panel plot demonstrating normal response to exercise

Reproduced from Takken et al. [219]

Predicted values were calculated based on established normative data [210,429-431]. For subjects under 18 years, Blanchard equations were used for predicted workload. These equations were based on corrected body mass in those with a BMI >85th percentile.

2.6 Exercise Cardiac Magnetic Resonance Imaging

For our study examining the effects of inspiratory muscle training (IMT), a non-contrast cardiac magnetic resonance imaging (CMR) was performed in a Phillips 1.5T magnetic resonance imaging (MRI) scanner. Participants and their parents completed an MRI safety questionnaire prior to commencing the study. The procedure was explained to them in detail, including the exercise protocol. Participants were attached to ECG, peripheral pulse unit and respiratory monitoring. Participants were given 5 minutes to familiarise themselves with the MRI-compatible ergometer prior to starting the scanning. After scout images were acquired to determine patient position, baseline images were acquired to assess underlying anatomy. These images were performed in real time with free breathing, obtaining an axial stack, 2- and 4-chamber views, short axis stack, left ventricular outflow tract view and an LPA or RPA orthogonal view. Flows were obtained in the ascending aorta, descending aorta at the level of the diaphragm, SVC, extra-cardiac conduit, and LPA or RPA (figure 2.7). Branch PA flows were used as validation of other flows. The velocity encoding gradient was set to 200 cm/s for the ascending and descending aorta, and to 150 cm/s for the other vessels, unless aliasing was present.

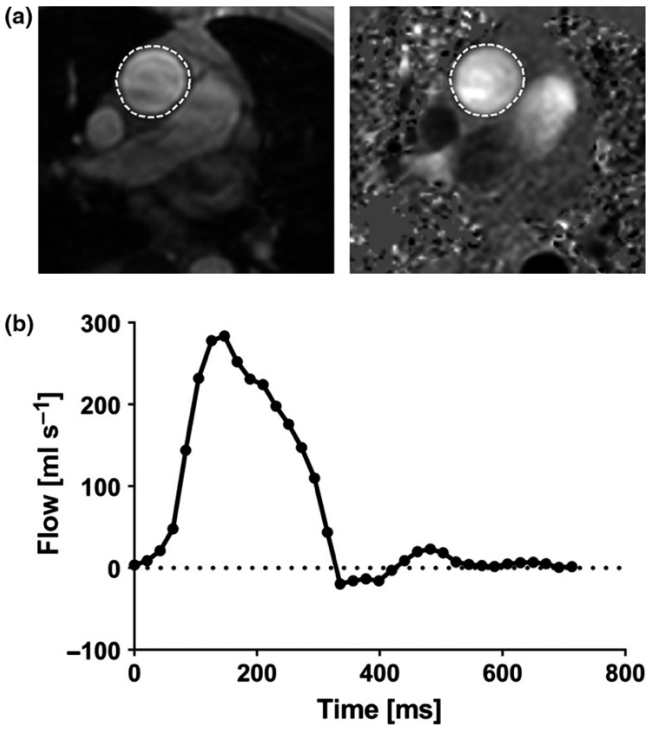


Figure 2.7. Phase contrast flow on cardiac magnetic resonance imaging
Reproduced from Bidhult et al. [432]

Using the Ergospect Ergometer (figure 2.8), participants exercised following a step protocol. Initial workload was set to 30W and increased by 20W every 3 minutes until exhaustion. Imaging was acquired from 50% of the participants' maximum workload on the upright cycle while exercising, and at every stage thereafter until fatigue. At each stage, a short axis stack was acquired for volume analysis and flow data was acquired in the following vessels: ascending aorta, descending aorta, inferior and superior vena cava, and left or right pulmonary artery. Participants were asked to indicate their level of exhaustion using the Borg scale, as a comparison to their effort on the upright cycle ergometer.

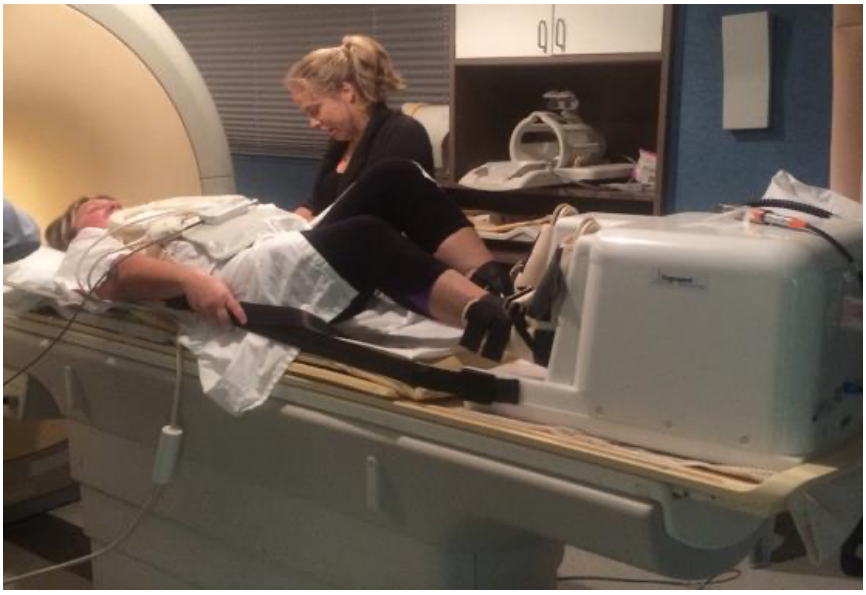


Figure 2.8. Ergospect Ergometer

Ventricular volumes were analysed by two experienced practitioners independently to ensure inter-observer consistency. The endocardial borders were traced manually in OsiriX Lite v7.5 on short-axis cine imaging measuring end diastolic volumes (EDV) when the ventricular chamber was the largest and end systolic volumes (ESV) when the ventricular chamber was the smallest. Papillary muscles and ventricular trabeculae were excluded from the ventricular volumes. EDV and ESV were calculated from these tracings (figure 2.9). The stroke volume (SV) was calculated as the ESV subtracted from the EDV and the ejection fraction (EF) was calculated as the SV / EDV x 100. These values were calculated for each participant at each stage pre and post IMT. These parameters were then analysed using paired t-tests between the baseline and peak workload for each patient pre and post IMT training, and between the peak workload pre and post IMT training.

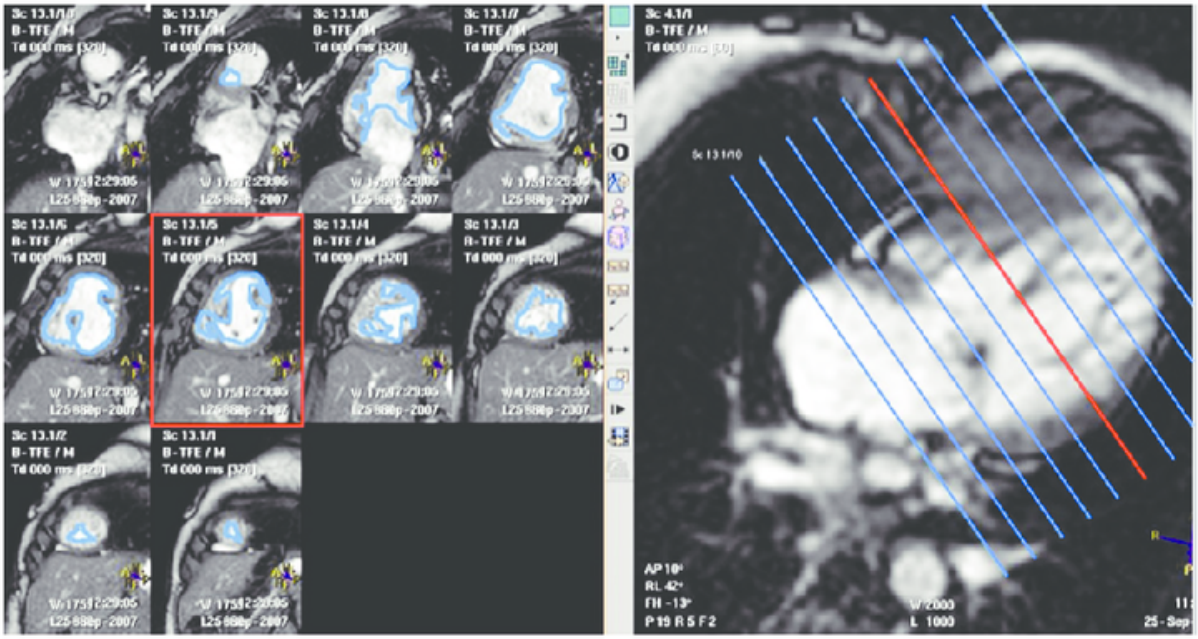


Figure 2.9. Ventricular segmentation

Reproduced from Bell et al. [433]

The flow data for each vessel at each workload was extracted by outlining the vessel of interest, producing a time versus flow curve over a period of 15 seconds. Using a trapezoidal method, the total area under the curve was calculated in GraphPad Prism v6.0, giving the total flow in each vessel over a 15 second period. The pulse wave acquired simultaneously allowed calculation of the participant's heart rate at the time, giving flow through the vessel in mls per beat.

Using the physiology log files, containing respiratory and pulse wave data, created by the MRI scanner during acquisition of the images, a code was written in Matlab to identify the respiratory phase. The code segmented the data in the physiology log files to identify when the scanner was active and acquiring flow data. The respiratory data was then filtered using a Butterworth filter. Respiratory states (inspiration and expiration) were classified by taking the first derivative of the filtered data. Respective flow values were then calculated by trapezoidal integration on the same time scale. This outputted the total flow in each vessel during inspiration and during expiration. Inspiratory to expiratory ratio (I:E) was calculated. The I:E was then compared between baseline and peak workload pre and post IMT training, and between peak workload pre and post IMT training.

2.7 Ethics

Ethics approval was obtained from each local institutional Human Research Ethics Committee where data was collected. All participants and/or their parents provided consent to take part in the studies.

2.8 Statistical Analyses

All data was collated in an Excel spreadsheet and statistical analyses were performed using a statistical software package, GraphPad Prism versions 6-9 and SPSS version 27. Data was tested for normality using D'Agostino-Pearson omnibus normality test, and a p-value of <0.05 was considered statistically significant. For descriptive representation, parametric data was presented as or mean+/-standard deviation, and non-parametric data as median (interquartile range). For analyses between groups or cohorts, data was compared using Chi-square tests or t-tests for parametric data, and Mann-Whitney U-tests for non-parametric data. One-way ANOVA was used for comparison between more than 2 groups. Correlations between continuous data were determined using Pearson or Spearman correlation as appropriate and multiple linear regression models were examined. Data was also viewed graphically to aid in interpretation. A p-value <0.05 was considered statistically significant.

2.9 Personal Involvement

I was personally involved in different parts of each study presented in this thesis. Parts of the studies were undertaken by others, of whom I have recognised in my acknowledgement at the beginning of this thesis. I am grateful for their contribution to these studies.

I was involved in the conceptualisation and methodology of the project undertaken in Chapter 3 (Lung function Abnormalities). Data was collected in different sites across Australia and New Zealand. I compiled the spirometry and CPET data to ensure consistency in measurements between sites, and validity. I interpreted and performed statistical analyses on the data and, in conjunction with my supervisor and respiratory colleagues, formulated the discussions around our results.

For Chapter 4 (Inspiratory Muscle Training), I was involved in the experimental design and methodology, patient recruitment, data collection and interpretation. I undertook patient selection and recruitment. With the assistance of a respiratory physiotherapist, I provided education and supervision of MIP measurements and IMT and performed follow-up phone calls to ensure compliance. I supervised and interpreted the CPETs and exercise CMR studies, and set-up the exercise CMR equipment in our institution. I analysed the echocardiograms performed. I undertook all analyses and was actively involved in the interpretation and discussion of the results.

For Chapter 5 (Oxygen Pulse Slope) I was involved in the supervision and interpretation of the CPET and exercise CMR data of cohort A. Cohort B was data collected across many sites. I collated the raw CPET data and performed analyses on the data to allow analyses of O_2 pulse slopes. I also collated the echocardiographic and cardiac MRI data and performed analyses on the data and was involved in the interpretation and discussion of the results.

Chapter 6 (Normative Values) data was extracted from a publicly available dataset, the Pediatric Heart Network. I reviewed the available data and analysed the questionnaires to assist with our study design. Due to the complexity of the statistics, a statistician was involved in deriving the normative curves and equations. Part of the external validation dataset was from the IMT study cohort. I performed the analyses, interpretation, and discussion of the clinical data.

CHAPTER THREE: LUNG FUNCTION ABNORMALITIES

This chapter is based on the following publication:

Laohachai K, Badal T, Thamrin C, Robinson PD, Kennedy B, Rice K, Selvadurai H, Weintraub R, Cordina R, d'Udekem Y, Ayer J. Older age at Fontan completion is associated with reduced lung volumes and increased lung reactance. Int J Cardiol. 2022 Oct 1;364:38-43

Abstract

Background: Fontan patients have abnormal lung function, in particular restrictive lung disease and low diffusing capacity of carbon monoxide (DLCO). We sought to further characterise these abnormalities with detailed pulmonary function testing and examine associations with clinical parameters.

Methods: 132 Fontan patients across Australia and New Zealand underwent spirometry. Measurement of diffusion capacity (DLCO) including its components (alveolar volume (VA) and rate of uptake of CO (KCO)) and oscillometry (reactance (X5) and resistance (R5)) were assessed in a subset of Fontan patients (n=44) and healthy controls (n=12). Double diffusion (to assess diffusing capacity of nitric oxide (DLNO), capillary blood volume (Vc), alveolar capillary membrane function (DmCO)) was performed in Fontan patients (n=18) and healthy controls (n=12).

Results: FEV_1 and FVC z-scores were low in Fontan subjects (mean -1.67±1.24 and -1.61±1.29 respectively) and correlated with exercise capacity. Compared to controls, z-scores for X5, DLCO, KCO, VA and DLNO were significantly lower in Fontan patients. R5, Vc and DmCO z-scores were preserved. X5 was associated with VA (r=0.41,p=0.009) and DmCO (r=0.61,p=0.008). Older age at Fontan completion was associated with lower z-scores for FEV_1 (r=-0.46,p=0.002), FVC (r=-0.47,p=0.002), X5 (r=-0.32,p=0.033) and VA (r=-0.36,p=0.022).

Conclusion: Fontan patients have a reduced DLCO which is largely driven by low VA. Lung stiffness (X5) is increased which is associated with VA and DmCO. These parameters negatively correlate with older age of Fontan completion suggesting that earlier Fontan completion may have a beneficial effect on lung function.

3.1 Introduction

Children with single ventricle anatomy undergo staged surgical procedures in early life, resulting in a Fontan circulation. Significant developments in management have improved life expectancy, however, morbidity is still high, with reduced exercise capacity and quality of life. In the Fontan circulation, systemic venous return is passively redirected into the pulmonary vasculature, bypassing a subpulmonary ventricle. This leads to chronically elevated central venous pressure and restricted ventricular preload [71]. These limitations can be compounded by multiple factors including chronotropic incompetence, non-uniform pulmonary blood distribution, chest wall and spinal deformities, pleural adhesions, diaphragmatic palsies, and respiratory and skeletal muscle weakness.

In the Fontan circulation, ventilation has a significant influence on pulmonary blood flow and cardiac output, at both rest and exercise [163]. Restrictive lung disease (measured by spirometry) and impaired gas transfer (measured by the diffusing capacity of carbon monoxide (DLCO)) have been observed in patients with a Fontan circulation [12]. Reduced lung volumes in children with a Fontan circulation have been shown to correlate with reduced aerobic capacity [164].

The aim of our study was to further characterise abnormalities of lung function and gas transfer in Fontan patients by examining: i) respiratory system mechanics and ventilation distribution, especially in the peripheral airways; ii) causes of low DLCO by assessing the function of components of gas diffusion capacity - alveolar volume (VA), capillary blood volume (Vc) and the alveolar capillary membrane (DmCO); iii) the relationship between DLCO (and its components) and measures of small airway mechanics and, iv) associations between lung function and clinical parameters.

3.2 Methods

3.2.1 Recruitment

Subjects with a Fontan circulation were identified through the Australian and New Zealand Fontan Registry (ANZFR). Subjects recruited were part of the ANZFR Functional Outcomes after Fontan study conducted across Sydney, Melbourne, and Auckland. Inclusion criteria for

this study included age ≥13years and ≥5years since Fontan completion. Exclusion criteria were those with severe heart failure, a history of significant exercise-induced arrhythmia, severe systemic outflow tract obstruction or severe systemic hypertension at rest, or severe cognitive impairment/ intellectual disability. Clinical data for Fontan participants were retrospectively collected from the ANZFR REDCap database.

Participants in the full cohort (n=132) underwent spirometry, exercise testing and echocardiography across three cities – Sydney (The Children's Hospital at Westmead, CHW and The Royal Prince Alfred Hospital, RPAH), Melbourne (The Royal Children's Hospital) and Auckland (Starship Children's Hospital) (figure 3.1). Detailed pulmonary function testing (PFT) was offered only to participants in Sydney through CHW and RPAH (n=44). Detailed PFT was performed in the respiratory laboratories at CHW (≤16 years old) and Woolcock Institute of Medical Research, WIMR (>16 years old). Healthy controls were recruited through the WIMR and underwent detailed PFT (n=12).

3.2.2 Spirometry

Spirometry was performed using standard techniques according to ATS/ERS guidelines [415]. Z-scores for forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), and FEV_1/FVC ratio were calculated using published reference values, corrected for age, height, gender and ethnicity [417]. Results were assessed for validity according to the ATS/ERS guidelines [415] and any technically poor results were excluded.

3.2.3 Cardiopulmonary Exercise Testing

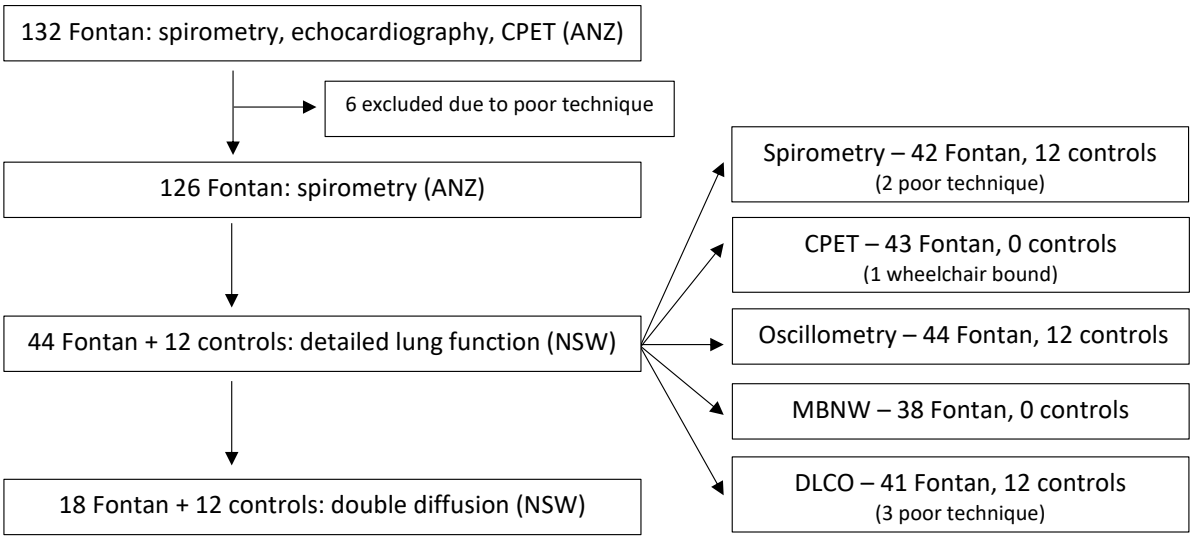
CPET was performed on an upright cycle ergometer (Ergoselect200, Ergoline) using a progressive ramp protocol, aiming to achieve peak oxygen consumption (VO_2) within 7-10 minutes. Baseline heart rate (HR), blood pressure and oxygen saturations were recorded from rest to recovery. Breath-by-breath VO_2, carbon dioxide consumption (VCO_2) and minute ventilation (VE) were measured (Sentry Suite, Carefusion). VE/VCO_2 slope was calculated from the beginning of exercise to the respiratory compensation point. Anaerobic threshold was calculated using a combination of the V-slope, ventilatory equivalents and respiratory exchange ratio methods. HR reserve, breathing reserve, and oxygen pulse were calculated. Oxygen uptake efficiency slope (OUES) was calculated and expressed as %predicted [429].

3.2.4 Echocardiography

Echocardiograms were performed as part of the ANZFR Functional Outcomes after Fontan study and the following were obtained: anatomy, ventricular dominance, atrioventricular (AV)-valve regurgitation (none-mild, moderate, more than moderate) and global systolic dysfunction as a qualitative measurement (less than mild: ejection fraction (EF) >55%, mild-moderate: EF=30-55%, more than moderate: EF<30% impairment).

3.2.5 Detailed Pulmonary Function Testing

A subgroup of participants (n=44, figure 3.1) underwent detailed pulmonary function testing (PFT) in the following order: oscillometry (Thorasys, TremoFlo®C-100, version1.0.44), multiple breath nitrogen washout (MBNW, Ecomedics ExhalyserD, Spiroware, version3.1.6; at CHW only), spirometry and DLCO. A further subset of patients (n=18, figure 3.1) were invited for double diffusion testing (Medisoft, HypAir+, Leeds, United Kingdom), performed only at WIMR due to equipment availability. Spirometry was repeated at this visit to ensure consistency to baseline.



CPET = cardiopulmonary exercise testing, ANZ = Australia and New Zealand, NSW = New South Wales, MBNW = multiple breath nitrogen washout, DLCO = diffusing capacity of carbon monoxide

Figure 3.1. Study Design

3.2.5.1 Oscillometry

Oscillometry is a simple, non-effort dependent lung function test performed during tidal breathing that provides measures of respiratory mechanics by superimposing pressure oscillations onto the airway opening during normal breathing into a mouthpiece and examining the resultant flow and its relationship with the oscillating pressure. Oscillometry was performed using published guidelines [421], with each recording visually checked for artefacts, and breaths were excluded if present.

We examined two oscillometry parameters: respiratory system resistance (R5) and reactance (X5) at 5Hz. R5 is a measure of airway calibre, which in comparison to spirometry is more sensitive to changes occurring within the peripheral airways. X5 predominantly reflects the elastic properties or stiffness of the respiratory system and is sensitive to changes such as small airway closure or reduced communicating lung volume. R5 and X5 were expressed as z-scores based on published reference values [422].

3.2.5.2 Multiple Breath Nitrogen Washout

Multiple Breath Nitrogen Washout (MBNW) is another tidal breathing technique which assesses heterogeneity (or unevenness) in the distribution of ventilation within the lungs. It examines the progressive washout of resident nitrogen values over successive breaths as the patient inhales 100% oxygen. MBNW was performed using a recommended tidal breathing protocol [423]. The mean values from ≥2 technically acceptable trials were obtained for functional residual capacity (FRC) and lung clearance index (LCI), a global measure of ventilation heterogeneity.

3.2.5.3 Diffusion Capacity of Carbon monoxide (DLCO) and Double Diffusion

DLCO is a measure of lung gas transfer from alveolar gas to haemoglobin within the pulmonary capillaries. It is affected by diffusion across the alveolar-capillary membrane, haemoglobin levels and capillary blood volume. DLCO was measured by a single-breath technique in duplicate at 4-minute intervals according to ATS standards [424]. DLCO parameters (z-scores for DLCO, VA, rate of uptake of CO (KCO)) were calculated based on Global Lung Initiative reference values [425]. Additional double diffusion parameters (diffusion capacity of nitric oxide (DLNO) and DmCO were calculated as z-scores, apart from DLNO/DLCO which was calculated as % predicted, using existing reference equations in the literature [426,434].

The double diffusion test was used to help identify the mechanisms behind impaired DLCO [426]. By using CO and nitric oxide (NO) simultaneously, it is possible to calculate the diffusion capacity for both gases (DLCO and DLNO), and partition DLCO into its components (DmCO and Vc). As NO has a higher affinity to haemoglobin than CO, it is less affected by blood volume and provides a better assessment of alveolar-capillary membrane function. Thus, the DLNO/DLCO ratio provides insight into the aetiology of the impaired diffusing capacity. Furthermore, by measuring KCO it is possible to determine the contribution of VA to DLCO.

4.1.1 3.2.6 Statistical Analyses

Statistical analyses were performed using GraphPad Prism 9 and SPSS (version 27). Non-parametric and parametric data are presented as median (range) or mean+/-standard deviation, respectively. Categorical and continuous data were compared between healthy and Fontan groups using Chi-square tests, t-tests, or Mann-Whitney U-tests, as appropriate. Correlations between double diffusion parameters and lung mechanics clinical factors, were determined using Pearson or Spearman correlation and multiple linear regression models. A p-value <0.05 was considered statistically significant.

4.1.2 3.2.7 Patient and Public Involvement

Patients and the public were not directly involved in the study design or analyses. This study was endorsed by the ANZFR steering committee, which include patients and their representatives.

3.3 Results

3.3.1 Study Populations

In the full cohort, 132 Fontan subjects underwent spirometry with six having technically unsatisfactory spirometry. Baseline characteristics of these subjects are shown in table 3.1. Forty-four Fontan subjects and 12 healthy controls underwent more detailed PFT (figure 3.1). The only significant difference in baseline characteristics between the full Fontan cohort and those who had detailed PFT was fenestration at the time of Fontan completion, which related to an institutional preference for no fenestration at CHW. CPET findings from the Fontan subgroup are shown in table 3.2.

In the healthy control group, 58% were females, age was 30.5±5.7years, height 169±10.5cm, weight 69.8±10.5kg and BMI 24.4±2.6kg/m^2. In the Fontan subjects (n=18) who returned for double diffusion testing at visit 2, demographic and clinical characteristics were not significantly different to the Fontan group who underwent detailed PFT (n=44). There was good agreement in the spirometry results between the two visits, indicating that subjects remained at their baseline when further tests were done.

Table 3.1. Anthropometrics and Clinical Characteristics of the Fontan subjects

	Groups	**Spirometry (n=126)**	**Subgroup (n=44)**	**p-value**
Demographics				
Gender (M:F)		65 (52%): 61 (48%)	25 (57%): 19 (43%)	0.55
Ethnicity	Caucasian	-	33 (75%)	
	Black	-	0 (0%)	
	North-east Asia	-	0 (0%)	
	South-east Asia	-	1 (3%)	
	Other	-	10 (23%)	
Current age (yrs)		20.5 (16.0-28.0)	20.1 (16.5-27.7)	0.71
Height (cm)		165.6 ± 9.9 (144-188)	166.2 ± 9.1 (145-188)	0.67
Weight (kg)		65.0 ± 15.4 (29.9-110.0)	65.3 ± 15.9 (30.2-106.0)	0.78
BMI (kg/m^2)		23.6 ± 4.9 (14.4-37.9)	23.6 ± 5.2 (14.4-34.9)	0.96
Clinical characteristics				
Ventricular morphology	Left	77 (61%)	25 (57%)	0.51
	Right	37 (29%)	12 (27%)	
	Biventricular	12 (10%)	7 (16%)	
Isomerism	No	111 (88%)	39 (89%)	0.34
	Left	5 (4%)	0 (0%)	
	Right	10 (8%)	5 (11%)	
Cardiac diagnosis	Tricuspid atresia	26 (21%)	10 (23%)	0.80
	DORV	24 (19%)	9 (20%)	
	DILV	22 (17%)	6 (14%)	
	HLHS	13 (10%)	2 (5%)	
	AV septal defect	11 (9%)	4 (9%)	
	Pulmonary atresia	14 (11%)	4 (9%)	
	Other	16 (13%)	9 (20%)	
Thoracotomy (Y:N)	*4 unknown*	-	24 (55%): 16 (36%)	
Type of Fontan	ECC	80 (63%)	30 (68%)	0.76
	Lateral tunnel	30 (24%)	8 (18%)	
	Atriopulmonary	15 (12%)	5 (11%)	
	Other	1 (<1%)	1 (2%)	
Fenestration (Y:N)		36 (29%): 89 (71%)	5 (11%): 38 (86%)	0.02
Age of Fontan (yrs)		6.1 ± 4.4 (1.5-27.5)	5.5 ± 2.7 (1.9-14.6)	0.42
Years since Fontan		17.0 ± 6.7 (6.6-32.8)	16.6 ± 6.9 (7.7-30.6)	0.73
NYHA (*1 in wheelchair)*	Class I	94 (75%)	33 (77%)	0.82
	Class II	26 (21%)	10 (23%)	
	Class III	1 (<1%)	0 (0%)	

Echocardiography				
AV-valve regurgitation	None-mild	84 (67%)	33 (75%)	0.32
	Moderate	33 (26%)	10 (23%)	
	Moderate-severe	1 (<1%)	1 (2%)	
Ventricular dysfunction	Less than mild	100 (79%)	39 (89%)	0.13
	Mild-moderate	6 (5%)	4 (9%)	
	Severe	1 (<1%)	1 (2%)	

BMI - body mass index, DORV - double outlet right ventricle, DILV - double inlet left ventricle, HLHS - hypoplastic left heart syndrome, AV - atrioventricular, ECC - extracardiac conduit, NYHA - New York Heart Association

Table 3.2. Cardiopulmonary exercise testing results in Fontan subgroup

Cardiopulmonary exercise test, resting data (n=43)		
Baseline HR (bpm)		89 ± 18 (60-137)
Baseline saturations (%)		94 ± 4 (83-100)
Baseline systolic BP (mmHg)		112 ± 12 (87-130)
Resting VO_2 (L/min)		0.3 ± 0.1 (0.1-0.5)
Cardiopulmonary exercise test, exercise data (n=43)		
Ramp Protocol	10W	4 (9%)
	15W	38 (88%)
	20W	1 (2%)
Max heart rate (%Pred)		79.3 ± 10.3 (58.3-98.4)
Max systolic blood pressure (mmHg)		111 ± 12 (87-130)
Lowest saturations (%)		90 ± 5 (78-98)
Max workload (%Pred)		53.8 ± 10.8 (32.6-82.8)
Peak VO_2 (ml/kg/min)		22.7 ± 5.7 (11.3 – 36.2)
Peak VO_2 (%Pred)*		53.5 ± 13.2 (31.9-93.7)
Max RER		1.2 (1.15-1.32)
VE/VCO_2 slope		29.5 ± 6.0 (20.7-40.6)
Heart rate reserve		41 ± 20 (3-84)
OUES (%Pred)		57.8 ± 14.9 (28.8-90.2)
VO_2 / work		9.03 (8.07-9.96)
O_2 pulse (%Pred)		73.02 (60.27-82.11)
Breathing reserve (%)		45 (36-53)

Data presented as mean ± SD (range); median (IQR).
VO_2 – oxygen consumption, RER – respiratory exchange ratio, VE – ventilation, VCO_2 – carbon dioxide production, OUES – oxygen uptake efficiency slope

3.3.2 Spirometry in Full Fontan Cohort (n=126)

In the full cohort, Fontan subjects had reduced spirometry with mean FEV_1 z-score of -1.67 ± 1.24 and FVC z-score of -1.61 ± 1.29. FEV_1/FVC z-score was normal (-0.24 ± 0.97), consistent with a restrictive pattern of lung function. FEV_1 and FVC z-scores were associated with %predicted peak VO_2 (FEV_1 r=0.28, p=0.005; FVC r=0.34, p<0.001), %predicted OUES (FEV_1 r=0.25, p=0.005; FVC r=0.28, p=0.002), and VO_2/workload (FEV_1 r=0.24, p=0.009; FVC r=0.26, p=0.004) on CPET. Age of bidirectional Glenn (BDG) correlated with age of

Fontan completion (r=0.65, p<0.001). Older age of Fontan had a weak but significant negative association with FEV_1 (r=-0.24, p=0.008) and FVC (r=-0.3, p<0.001) z-scores. Age of BDG also had a significant negative association with FVC z-score (r=-0.25, p=0.03).

On CPET, 27% had a blunted increase in tidal volume (<2x increase) with median (IQR) of 138.3% (98.5%). Breathing reserve, an indicator of ventilatory limitation, was inadequate in 12%, with a median (IQR) of 38% (21%).

3.3.3 Detailed Pulmonary Function

MBNW was normal in Fontan subjects, with an FRC of 2.1±0.58L and a median LCI z-score of 0.30(0.46-2.10), suggesting no evidence of ventilatory heterogeneity. Differences in lung function measures between Fontan subjects and healthy controls are shown in table 3.3. Within this subset, compared to our healthy controls, Fontan subjects had the same pattern of abnormality described above with lower mean FEV_1 and FVC z-scores (figure 3.2) and equivalent FEV_1/FVC z-scores, consistent with a restrictive lung function defect.

Table 3.3. Lung function parameters between healthy controls and Fontan subjects

Parameter	Healthy controls	Fontan subjects	p-value
Spirometry	*n=12*	*n=42*	
FEV_1 z-score	0.50 ± 0.98	-1.24 ± 1.23	<0.0001
FVC z-score	0.62 ± 0.95	-1.03 (2.50)	0.008
FEV_1/FVC z-score	-0.28 ± 0.73	-0.18 ± 1.03	0.77
Oscillometry	*n=12*	*n=44*	
R5 z-score	0.06 ± 0.68	0.62 ± 1.16	0.12
X5 z-score	0.12 ± 0.78	-1.70 ± 1.45	<0.0001
DLCO	*n=12*	*n=41*	
DLCO z-score	-0.30 ± 1.15	-3.15 ± 1.63	<0.0001
VA z-score	-0.21 (0.78)	-1.82 ± 1.13	0.001
KCO z-score	-0.05 ± 1.26	-1.68 ± 1.83	0.006
Double Diffusion	*n=12*	*n=18*	
DLNO z-score	-1.25 ± 0.46	-2.13 ± 1.39	0.04
DmCO z-score	-1.08 ± 0.48	-1.45 ± 1.61	0.38
Vc z-score	-1.05 ± 0.62	-1.67 ± 1.14	0.10
DLNO/DLCO %pred	95 (4.25)	85.43 ± 21.44	0.76

FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity, R5 – airway resistance at 5Hz, X5 – reactance at 5Hz, DLCO – diffusing capacity of carbon monoxide, VA – alveolar volume, KCO – rate of uptake of carbon monoxide, DLNO – diffusing capacity of nitric oxide, DmCO – membrane diffusing capacity for carbon monoxide, Vc – capillary volume

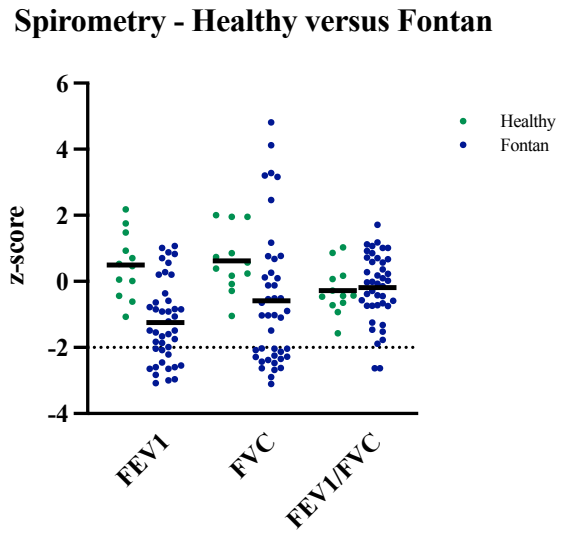


Figure 3.2. Spirometry in healthy controls and Fontan subjects

FEV1 – forced expiratory volume in 1 second, FVC – forced vital capacity

For oscillometry, X5 z-scores were significantly reduced ($p<0.001$) but R5 z-scores did not differ compared to healthy controls (figure 3.3), suggesting normal small airway resistance but increased respiratory reactance or stiffness.

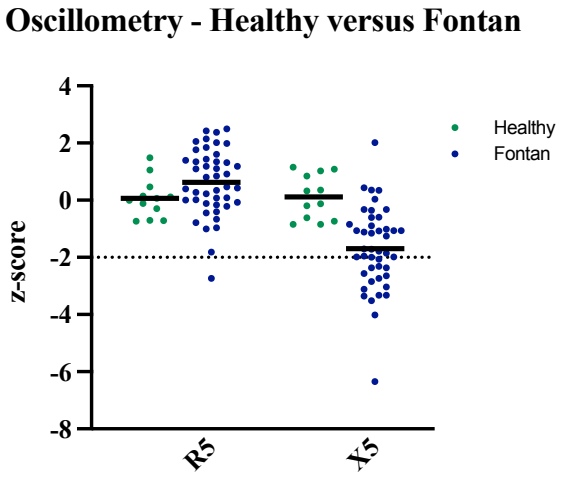


Figure 3.3. Oscillometry in healthy controls and Fontan subjects

R5 – airway resistance at 5Hz, X5- reactance at 5Hz

Consistent with existing literature, DLCO z-score was lower in Fontan subjects. Both VA ($p=0.001$) and KCO ($p=0.006$) were low compared to controls (table 3.3, figure 3.4). Forty-four percent (18/41) of Fontan subjects had a VA z-score <-2. From the double diffusion results, DLNO z-score was lower in Fontan subjects compared to controls with 44% (8/18)

having a DLNO z-score <-2. Thirty-nine percent (7/18) had DmCO z-score <-2, however mean values were not significantly different from the healthy controls. DmCO/VA z-scores were normal (z-score -0.85±1.63, p=0.74), suggestive of reduced alveolar capillary membrane function in a subset of patients. Vc z-scores were low-normal in Fontan patients with 8/18 (44%) having z-scores <-2, however this was not statistically different from the healthy controls. The DLNO/DLCO ratio was not statistically significant between Fontan patients and controls.

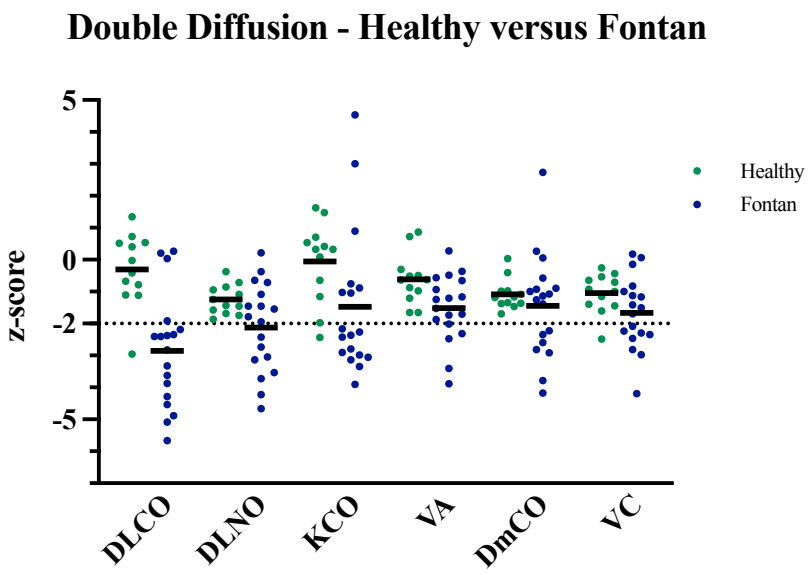


Figure 3.4. Double diffusion in healthy controls and Fontan subjects

DLCO – diffusing capacity of carbon monoxide, DLNO – diffusing capacity of nitric oxide, KCO – rate of uptake of carbon monoxide, VA – alveolar volume, DmCO – membrane diffusing capacity for carbon monoxide, Vc – capillary volume

3.3.4 Relationship Between Gas Diffusion Capacity Measures and Lung Mechanics

Both VA (r=0.41, p=0.009) and DmCO (r=0.61, p=0.008) z-scores but not Vc (r=-0.14, p=0.57) were significantly associated with X5 z-score (figure 3.5). When VA and DmCO were assessed together in multiple linear regression as predictors of X5, VA was a stronger predictor of X5 than DmCO (VA standardised β=0.96, p=0.009; DmCO standardised β=0.56, p=0.03); this two factor model explained 48% of the variance in X5 ($F(2,13)=5.98$, p=0.01) .

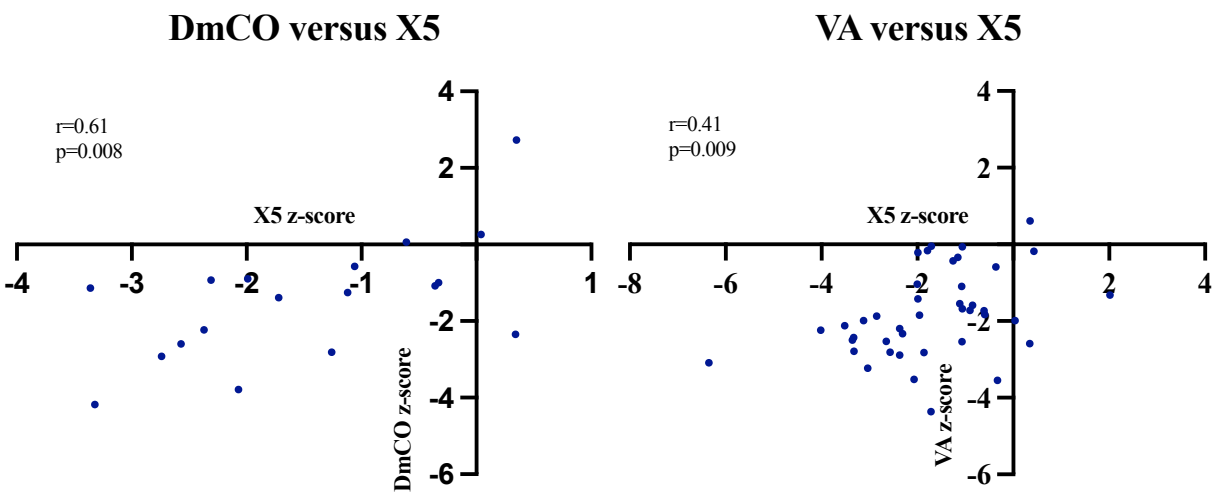


Figure 3.5. X5 (x) against VA and DmCO (y)

DmCO – membrane diffusing capacity for carbon monoxide, X5 – reactance at 5Hz, VA – alveolar volume

3.3.5 Relationship Between Lung Function and Clinical Parameters

We examined the associations between lung function, and clinical and exercise parameters in those who had detailed lung function measures (table 3.4). Older age at Fontan completion was associated with lower FEV_1 (r=-0.46, p=0.002), FVC (r=-0.47, p=0.002), X5 (r=-0.32, p=0.033) and VA (r=-0.36, p=0.022) z-scores. To explore this further, subjects in the full cohort were divided into tertiles by age of Fontan completion. Those in the upper tertile (Fontan completion >5.8years) had significantly lower FEV_1 and FVC z-scores compared to those in the lower two tertiles (F=5.11, p=0.007; and F=7.74, p<0.001; respectively) (figure 3.6).

Table 3.4. Association of lung function with clinical and exercise parameters in Fontan subgroup

	FEV_1 z-score		**FVC z-score**		**X5 z-score**		**VA z-score**		**DLCO z-score**		**DLNO z-score**	
	p	r / F	p	r / F	p	r / F	p	r / F	p	r / F	p	r / F
Clinical												
Morphology	0.78	(2,39) = 0.25	0.85	(2,39) = 0.17	0.97	(2,41) = 0.03	0.90	(2,38) = 0.11	0.98	(2,38) = 0.02	0.72	(2,15) = 0.33
Age of Fontan completion	<0.01	-0.46	<0.01	-0.47	0.03	-0.32	0.02	-0.36	0.53	-0.10	0.07	-0.43
Fontan Type	0.95	(3,38) = 0.11	0.89	(3,38) = 0.21	0.04	(3,40) = 2.96	0.70	(3,37) = 0.47	0.41	(3,37) = 0.98	0.08	(2,15) = 2.96
Echocardiography												
Ventricular function	0.13	(1,40) = 2.39	0.07	(1,40) = 3.36	0.06	(1,42) = 3.88	0.11	(1,39) = 2.75	0.19	(1,39) = 1.75	0.10	(1,16) = 3.16
AV-valve regurgitation	0.49	(1,40) = 0.50	0.05	(1,40) = 3.99	0.04	(1,42) = 4.58	0.88	(1,39) = 0.02	0.71	(1,39) = 0.14	0.25	(1,16) = 1.46
Cardiopulmonary exercise testing												
Max Workload	0.28	0.17	0.15	0.23	0.82	0.04	0.24	0.19	0.99	-0.003	0.77	-0.08
Peak VO_2 (%Pred)	0.56	0.09	0.17	0.22	0.5	0.1	0.25	0.18	0.75	0.05	0.66	0.11
VE/VCO_2	0.54	-0.1	0.73	-0.06	0.49	0.11	0.26	-0.18	0.51	0.11	0.51	0.16
O_2 Pulse	0.49	-0.11	0.7	-0.06	0.9	0.02	0.67	-0.07	0.4	0.14	0.13	0.6
Resting sats (%)	0.12	0.25	0.62	-0.08	0.001	0.48	0.90	0.02	0.08	0.28	0.93	-0.02
Lowest sats (%)	0.42	0.14	0.74	0.06	0.88	-0.03	0.77	-0.05	0.08	0.30	0.34	0.29
Breathing reserve (%)	0.09	0.27	0.42	0.13	0.06	0.29	0.59	0.09	0.97	-0.06	0.58	0.14
Heart rate reserve	<0.01	0.32	<0.01	0.33	0.25	-0.18	0.36	-0.15	0.74	0.05	0.08	0.42

FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity, X5 – reactance at 5Hz, VA – alveolar volume, DLCO – diffusing capacity of carbon monoxide, DLNO – diffusing capacity of nitric oxide, VO_2 – oxygen consumption, VE – ventilation, VCO_2 – carbon dioxide production

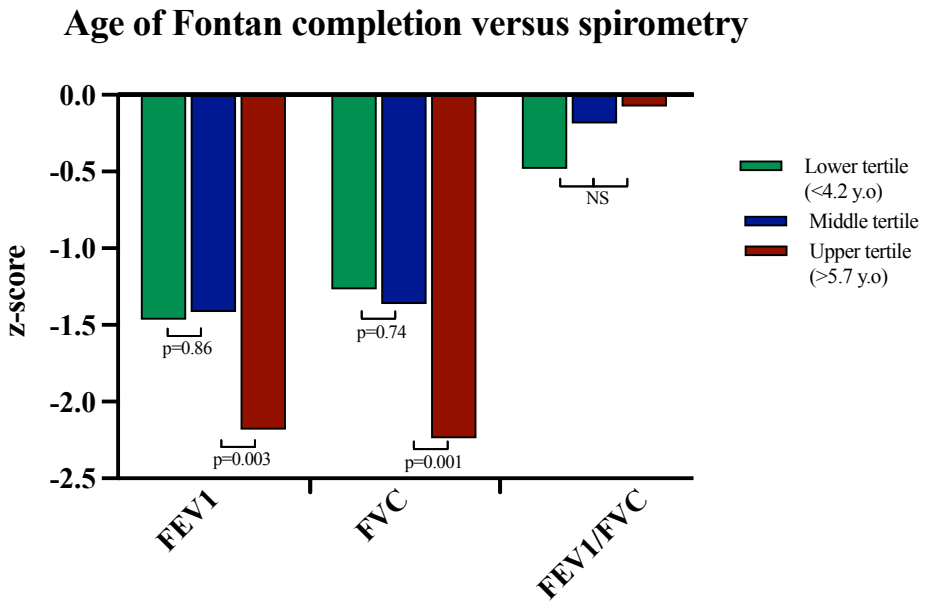


Figure 3.6. Age of Fontan completion versus spirometry

FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity

In the subjects who underwent detailed PFT, lung function was not associated with oxygen saturation, ventricular dominance, Fontan type, years since Fontan completion or previous thoracotomy. There was also no association between lung function and AV-valve regurgitation or ventricular dysfunction on echocardiography. Markers of exercise capacity on CPET did not correlate with detailed pulmonary function measures.

3.4 Discussion

Our results confirm and extend the findings of previous studies, demonstrating that Fontan patients have reduced FEV_1, FVC and DLCO [192,195]. We found evidence of ventilatory limitation in 12% of our cohort, which is slightly slower than previously reported [164,435]. In our full cohort, we have also confirmed that reduced lung volumes are associated with reduced measures of aerobic capacity, also in keeping with previous studies [164,192,436].

Our novel findings provide unique mechanistic insight into the lung impairments present in Fontan subjects. We found that: i) Fontan patients have abnormalities in respiratory reactance measured by X5; ii) low DLCO in Fontan patients is largely driven by low alveolar volume (VA) and in some patients by reduced alveolar capillary membrane properties, but not by reduced capillary blood volume; iii) the strongest association with X5 is VA, suggesting a link

between small airway mechanics, alveolar volume and thereby DLCO; and iv) younger age at Fontan completion may be associated with improvements in large and small airway function.

3.4.1 Reduced Gas Transfer in Fontan Patients

Reduced DLCO may be related to reduced capillary blood volume available for gas transfer, reduced alveolar volume and/or abnormal alveolar membrane conductance. For example, patients with heart failure have been found to have reduced DLCO, predominantly related to alveolar capillary membrane abnormalities [437]. DLCO is also reduced in patients with pulmonary arterial hypertension primarily due to reduced Vc, secondary to vascular remodelling, low cardiac output, and localised thrombosis in the pulmonary vascular bed [438]. Reduced DmCO has also been documented in pulmonary arterial hypertension, but this may be secondary to reduced Vc with proportional reduction of functional gas exchange area.

In patients with a Fontan circulation, in contrast to heart failure and pulmonary arterial hypertension, we have found that the predominant mechanism for reduced DLCO relates to reduced alveolar volume. Lung volume, alveolar surface area and number increases from 29-weeks gestation to at least 12-weeks postnatally, with a close association to body weight [175]. Alveolar growth continues through childhood and into adolescence, although at a reduced rate [176]. In single ventricle patients, many factors can potentially adversely impact alveolar development including abnormal pulmonary perfusion, mechanical ventilation, thoracotomies, kyphoscoliosis, pleural effusions, lymphatic dysfunction, and diaphragmatic palsy.

Lack of pulsatile pulmonary flow in the Fontan circulation is associated with endothelial dysfunction and abnormal vascular development [166,187]. In a study of lung biopsies from 18 Fontan patients, Levy et al. documented variable intimal proliferation and muscularisation of terminal bronchiole and alveolar duct arteries [292]. We speculate that such changes in intra-acinar arteries could distort acinar architecture and form the basis for our findings of reduced alveolar volume and reduced small airway elastic properties.

Although DmCO was lower in some Fontan subjects, our small subject numbers preclude further analysis of the factors impacting alveolar capillary membrane function. Idorn et al. found that low DLCO in Fontan patients (n=87) was related to abnormal Vc [195]. However, we showed no significant difference in mean Vc between the healthy and Fontan populations.

Vc z-scores were abnormal in 44% of our subjects, and statistical significance may have become apparent with a larger sample size. Additionally, in comparison to Idorn et al., our sample had a larger proportion of patients with an extracardiac conduit and dominance of the left ventricle.

3.4.2 Reduced Lung Volumes and Abnormal Lung Mechanics in Fontan Patients

Our results confirm a restrictive pattern of lung function in Fontan patients [12,195]. Potential causes include respiratory muscle weakness, diaphragmatic palsy, recurrent thoracotomy and sternotomies, and chest wall and spinal abnormalities. Oscillometry provides novel measures of lung mechanics not available with standard spirometry. As it is undertaken during passive tidal breathing, it is more suitable for assessing lung mechanics in very young children. Through oscillometry, we have shown abnormal reactance (X5) but normal airway resistance (R5) in Fontan patients. These findings are suggestive of either abnormal elastic properties of the lung, as occurs in pathologies such as pulmonary fibrosis and/or reduced lung communicating volume due to small airway closure, gas trapping, or reduced alveolar volume. Consistent with this, both DmCO and VA (lung tissue changes and reduced alveolar volume, respectively), but not Vc (pulmonary blood capillary abnormalities), were strong predictors of X5. However, only VA was significantly reduced in Fontan patients compared to health.

3.4.3 The Effect of Earlier Age of Cavopulmonary Connection on Lung Development

Importantly we have found that abnormal lung volumes, reduced VA and better X5 are associated with older age at both BDG anastomosis and Fontan completion. We speculate that stabilisation of pulmonary blood flow and unloading of the functional single ventricle improves development of the pulmonary vasculature and hence alveolar development. Previous studies have shown associations of earlier Fontan completion with improved aerobic capacity and improved mortality [66,179,260,439]. Graneggar et al. demonstrated that children with complex congenital heart disease had reduced somatic growth, which normalised after Fontan completion [440]. They, and others, found that earlier age of Fontan completion was associated with improved catch-up growth [441,442]. Aortopulmonary collaterals are common following BDG, associated with increased pulmonary blood flow (Qp) and regress after Fontan completion [443]. Grosse-Wortmann et al. found that older age at Fontan completion was associated with a higher Qp:Qs, consistent with increased aortopulmonary collateral flow [444]. It is possible that longer duration of aortopulmonary collateral flow associated with older

age of Fontan completion may adversely affect later lung function. Further studies are required to establish the mechanistic link between age at Fontan completion and lung function. However, our results are in keeping with previous studies favouring earlier Fontan completion.

3.5 Limitations

Our sample size was small and due to equipment availability only a subset of patients underwent double diffusion testing, limiting the ability to correlate our double diffusion findings and clinical variables. Like many studies involving exercise testing, selection bias may have inadvertently been introduced due to the more physically active Fontan subjects more likely agreeing to participate, as indicated by our high percentage in NYHA class I. Our small sample were relatively young and hence rates of complications were low, making it difficult to assess correlation of lung function with Fontan related outcomes such as plastic bronchitis, transplant and mortality. Our association between younger age of Fontan completion and better lung function may have been mediated by clinical factors not ascertainable in this retrospective study. Larger prospective studies examining changes in lung function throughout single ventricle palliation are required.

3.6 Conclusion

We have shown that Fontan patients have low lung volumes and abnormal lung function with abnormal DLCO and DLNO. This study provides us with a better understanding of the mechanism behind impaired DLCO in Fontan patients. Our findings are indicative of these abnormalities being largely driven by low alveolar volume (VA). Additionally, Fontan patients have increased respiratory reactance or stiffness (X5), which strongly correlates with VA. This suggests a link between respiratory mechanics, VA and thereby DLCO. Lung reactance, measured by oscillometry, is a non-effort dependent measure of lung function that may serve as a useful marker of changes in lung function in single ventricle patients, and can be performed in children of any age. Importantly, older age at Fontan completion was associated with both reduced lung and alveolar volumes, and increased lung reactance, suggesting that earlier Fontan completion may have a beneficial effect on lung function.

CHAPTER FOUR: INSPIRATORY MUSCLE TRAINING

This chapter is based on the following publication:

Laohachai K, Winlaw D, Selvadurai H, Gnanappa GK, d'Udekem Y, Celermajer D, Ayer J. Inspiratory muscle training is associated with improved inspiratory muscle strength, resting cardiac output, and the ventilatory efficiency of exercise in patients with a Fontan circulation. JAHA. 2017; 21: 6(8).

Abstract

Background: Patients with a Fontan circulation have reduced exercise capacity and respiratory muscle strength. Inspiratory muscle training (IMT) improves exercise capacity and quality of life in adults with heart failure. We assessed if 6 weeks of a home-based program of IMT improves inspiratory muscle strength and the ventilatory efficiency of exercise (VE/VCO_2 slope) in adolescents with a Fontan circulation.

Methods and Results: Twenty-three adolescents (aged 16 ± 2 years) with a Fontan circulation underwent 6 weeks of IMT for 30 minutes daily. Respiratory muscle strength (maximal inspiratory pressure, MIP, and expiratory pressure, MEP), lung function and exercise capacity (cardiopulmonary exercise testing) were assessed. Fourteen of 23 subjects also underwent exercise cardiac magnetic resonance imaging (exCMR) to examine the effects of IMT on cardiac output and systemic and pulmonary blood flow. Six weeks of IMT improved MIP by 36 ± 24cmH_2O (61% ± 46%) with no change in MEP. VE/VCO_2 slope improved after 6 weeks of IMT (from 34.2 ± 7.8 to 32.2 ± 5.6, p=0.04). In those who underwent exCMR, IMT increased resting cardiac output (from 4.2 ± 1.2 to 4.5 ± 1.0 L/min, p=0.03) and ejection fraction (from 50.1 ± 4.3 to 52.8 ± 6.1, p=0.03).

Conclusions: Six weeks of IMT is associated with improved inspiratory muscle strength, VE/VCO_2 slope and resting cardiac output in young Fontan patients. IMT may be a simple beneficial addition to the current management of Fontan patients, potentially reducing exercise intolerance and long-term morbidity and mortality.

4.2 Introduction

Since its original description in 1971, the "Fontan operation" has undergone a number of modifications and a widening of the indications for its use [72]. There is a growing population of children and adults with congenital heart disease who are living with a Fontan circulation. In the current era, medium term survival after Fontan completion is high. However, these patients still face significant morbidity and a higher mortality than the general population [72]. Treatments to improve outcomes for Fontan patients are limited.

The Fontan circulation, where systemic venous return bypasses a subpulmonary ventricle, has inherent limitations of chronic elevation of central venous pressure and restricted ventricular preload [71]. These inherent limitations may be compounded by chronotropic incompetence, non-uniform distribution of pulmonary blood flow, elevated pulmonary and systemic vascular resistance [71], and reduced skeletal muscle blood flow and strength [261]. The limitations of a Fontan circulation manifest during exercise, and various parameters of exercise performance have been associated with adverse outcomes in Fontan patients [263].

Respiratory muscle weakness has been demonstrated in adults with congenital heart disease including patients with a Fontan circulation [261]. Such weakness has previously been described in adults with heart failure[301] and inspiratory muscle training (IMT) in heart failure patients, for periods of between 4 and 12 weeks, results in improved inspiratory muscle strength, exercise capacity and quality of life [359,445,446]. Fatiguing inspiratory muscles activate phrenic afferents which produce sympathetic peripheral vasoconstriction (the so called, "inspiratory muscle metaboreflex" [362]) and, in adult heart failure patients, IMT attenuates the exaggerated peripheral vasoconstriction in resting and exercising limbs [359].

To our knowledge, the effects of IMT have not been previously reported in patients with a Fontan circulation. The aims of the current study in Fontan patients were therefore to test if IMT improved: i) inspiratory muscle strength and ii) objective measures of exercise capacity. In regards to the latter aim, we specifically tested the hypothesis that IMT improves the ventilatory efficiency of exercise, as measured by VE/VCO_2 slope. A high VE/VCO_2 slope may be determined by two factors potentially altered by IMT, namely physiologic dead space ventilation (Vd/Vt) and increased ventilatory drive mediated by a complex metabolic reflex by

which peripheral chemoreceptors in muscles register local metabolic by-products during exercise and initiate a neural reflex that drives hyperventilation [447].

4.3 Patients and Methods

Patients with a Fontan circulation were identified through databases of The Children's Hospital at Westmead, Sydney. Details of the underlying cardiac anatomy and previous surgery were obtained. In order to maintain a relatively homogenous population, only subjects with a non-fenestrated extracardiac conduit aged 12-20 years were included. Exclusion criteria were moderate to severe ventricular dysfunction or atrioventricular valve regurgitation, a history of exercise-induced syncope, recent arrhythmia or clinical instability, major musculoskeletal disease, or intellectual disability. Written informed consent was obtained from all participants and/or parents and the study was approved by the institutional ethics committee.

The study consisted of 3 visits over a 6-week period (figure 4.1). Anthropometric data were obtained at each visit. Subjects underwent the following at visits 1 (week 0, baseline) and 3 (week 6, study end): i) assessment of physical activity levels (self-administered standardised questionnaire; International Physical Activity Questionnaire, IPAQ short form, with physical activity rated as low, moderate or high [413]); ii) testing of inspiratory and expiratory muscle strength; iii) spirometry and iv) cardiopulmonary exercise testing (CPET). In an exploratory sub study, to evaluate the effects of IMT on resting and exercise pulmonary and systemic blood flow, an exercise cardiac magnetic resonance imaging (ex CMRI) study was performed at visits 1 and 3. At visit 1, subjects were instructed on how to use the IMT device and asked to train for 30 minutes daily. At visit 2 (week 3) subjects underwent repeat respiratory muscle and lung function testing. The load of the IMT device was adjusted for any change in MIP between visits 1 and 2. Between visits, phone contact was made with subjects and/or parents to assess compliance to IMT. Compliance to IMT was additionally assessed by twice daily subject diary entry. Baseline testing at visit 1 also included a transthoracic echocardiogram where AV valve regurgitation and ejection fraction of the dominant ventricle were measured.

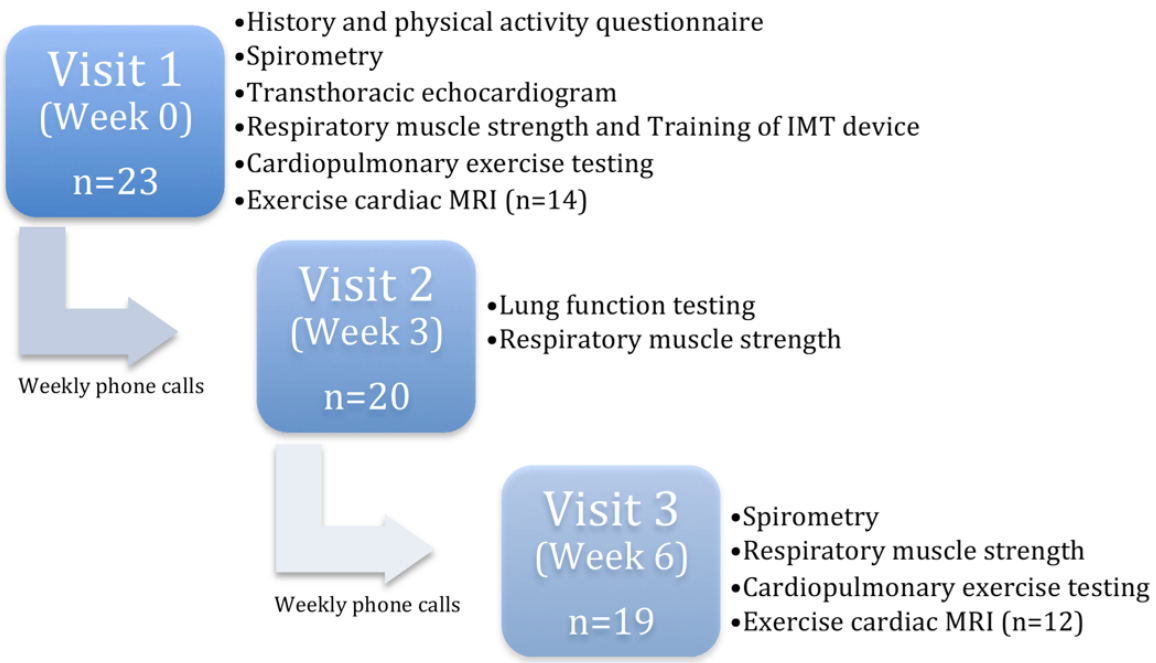


Figure 4.1. Study Design

Sixty-seven potential participants aged 12-20 years were identified from the institutional database. Twenty-three subjects agreed to participate and underwent baseline testing. Four subjects dropped out of the study after visit 1 due to commitments unrelated to the study, with 19 completing 6 weeks of IMT. The first 14 consecutive subjects were recruited into the ex CMRI sub study, 2 of whom withdrew after visit 1.

4.3.1 Inspiratory Muscle Testing and Training

Inspiratory and expiratory muscle strength, measured as maximal inspiratory (MIP) and expiratory pressures (MEP) at the mouth were assessed using a MicroRPM (Respiratory Pressure Meter, Carefusion, Germany), as recommended in the 2002 ATS guidelines [351]. Briefly, maximal inspiratory and expiratory manoeuvres were supervised by an experienced respiratory physiotherapist and measurements were performed with a nose peg in place and the subjects sitting with their feet flat on the floor. Up to 10 measurements were performed for MIP and MEP, until three consistent results (within 5 cmH_2O) were obtained. The maximum value of these three readings was recorded. The device was calibrated against a fluid-filled manometer at the start of the study.

Initial instruction on IMT was provided by an experienced respiratory physiotherapist. The IMT was done using a Threshold Inspiratory Muscle Training device (Philips Threshold IMT, Best, The Netherlands). The inspiratory load on the device was set to 30% of the participant's measured MIP. Technique was checked twice, at visit 1 and then again at visit 2. All subjects kept a diary of their usage at home and the total time using the device was calculated and averaged to minutes per day.

4.3.2 Inspiratory Muscle Strength

Inspiratory and expiratory muscle strength, measured by maximal inspiratory (MIP) and expiratory pressures (MEP) were assessed using CareFusion MicroRPM (Respiratory Pressure Meter) (figure 4.2). Measurements were performed with a nose peg in place and the patients sitting with their feet flat on the floor. Participants were instructed on how to use the equipment and supervised during the measurements. Up to 10 measurements were performed for MIP and MEP, until 3 consistent results (within 5 cmH_2O) were obtained. The highest consistent result was recorded. These values were analysed for each participant comparing the values pre and post IMT training using paired T-tests.

Figure 4.2. Respiratory pressure meter.

4.3.3 Inspiratory Muscle Training

Inspiratory muscle training was provided under the guidance of a respiratory physiotherapist. Participants were demonstrated how to use and clean the Philips Threshold IMT device (figure 4.3). The IMT device was set to 30% of the participant's measured MIP. The participant demonstrated their ability to use the device at the time of training and again an hour later after completing their exercise MRI. All participants kept a diary of their daily usage at home (see section 8.2 – Appendix 2) and the total time using the device was calculated and averaged to minutes per day. Participants were phoned weekly to encourage compliance and answer any questions of concerns they may have regarding the IMT device and training.

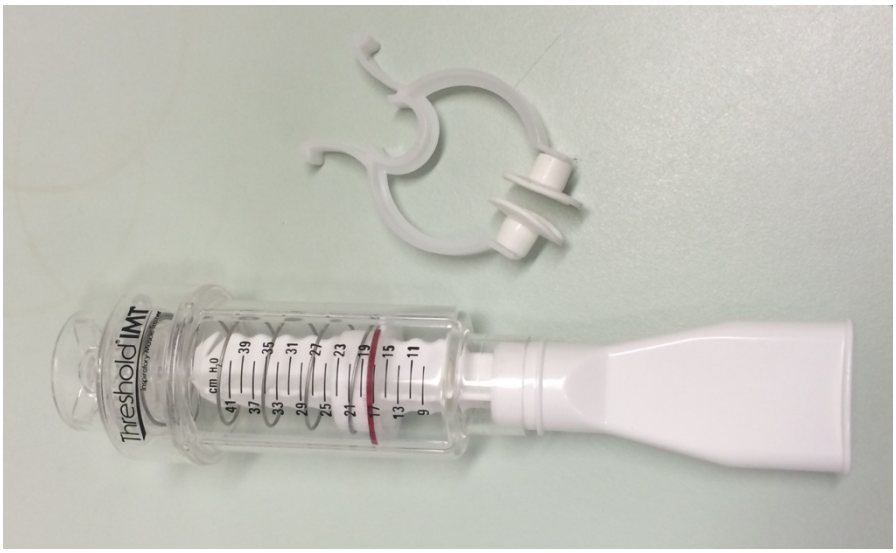


Figure 4.3. Philips Threshold IMT device

Patients and their parents were shown how to use the inspiratory muscle trainer (IMT), which was set at 30% of their maximum inspiratory pressure (MIP) based on inspiratory muscle strength testing. Participant's technique was re-tested at the end of their visit for verification of correct technique. Participants were provided with instructions on how to clean the device, and a diary to record their daily usage. They were asked to use the device for 30 minutes per day in 2-3 divided sessions.

Patients returned for a second visit at week 3 of the study to perform repeat inspiratory and expiratory muscle strength, adjustment of their IMT pressure to reflect their new MIP, repeat spirometry and body plethysmography. At this visit, the participant's IMT diary was reviewed, and encouragement was provided to continue using the device for 15 minutes twice a day.

The third visit consisted of follow-up investigations, to assess any changes from the IMT. This visit was similar to the initial visit and consisted of assessment of inspiratory and expiratory muscle strength, spirometry, cardiopulmonary exercise testing on an upright cycle ergometer and an exercise MRI study. Patients were tested at the same time of the day on both occasions in a thermo-regulated environment.

In between visits, patients were contacted via phone or email weekly to review any difficulties and to encourage compliance. Details of their compliance were collected on their IMT diary at the end of the study (see section 8.2 – Appendix 2).

4.3.4 Cardiopulmonary Exercise Testing

CPET was performed on an upright cycle ergometer (Ergoselect 200, Ergoline, Germany) using a ramp protocol. The initial workload started at 20 Watts (W), increasing by 10 W every minute for participants < 50 kg and by 15 W every minute if >50 kg, until exhaustion [448]. Baseline heart rate (HR), blood pressure and oxygen saturations were recorded prior to test commencement and during exercise. Breath-by-breath oxygen consumption (VO_2), carbon dioxide production (VCO_2) and minute ventilation (VE) were measured (SentrySuite, Carefusion, Germany). VE/VCO_2 slope was calculated using the linear relationship between VE and VCO_2 between 25-75% of maximum VCO_2 [449]. Anaerobic threshold was calculated using the V-slope method, and by confirming the value with the ventilatory equivalent method and respiratory exchange ratio (RER) [450]. O_2 pulse, a surrogate for stroke volume, was calculated by dividing peak VO_2 by peak HR. Vd/Vt was calculated non-invasively using Jones' equation, calculating arterial CO_2 from end-tidal CO_2 [451]. Maximal voluntary ventilation (MVV) was estimated using FEV_1 x 35 and breathing reserve was then calculated as a percentage using the formula (1- peak VE/MVV) x 100 [211]. The Borg score of perceived exertion was recorded at test completion.

4.3.5 Exercise Cardiac Magnetic Resonance Imaging

Ex CMRI was performed (1.5T, Phillips Intera, Best, The Netherlands) at visits 1 and 3 in a subset of subjects (n=14 visit 1, n=12 visit 2). Vectorcardiography (VCG), peripheral pulse (PPU) and respiratory belt monitoring were applied, and the data recorded in the scan physiology log file. Resting and exercise non-gated real time phase contrast flow (200 consecutive dynamics, dynamic scan time 75 milliseconds, corresponding to approximately 15

seconds of flow data) was measured in the ascending aorta, descending aorta at the level of the diaphragm, superior vena cava (SVC), extra-cardiac conduit, and left or right pulmonary artery (LPA or RPA, depending upon the presence of a left sided or right sided bidirectional Glenn shunt). Retrospective-gated (VCG or PPU) balanced steady-state free-precession cine imaging was performed (axial stack, 2- and 4-chamber, left ventricular outflow tract and two LPA or RPA orthogonal views at rest and a short axis stack at rest and during each stage of exercise). Typical imaging parameters were field of view 350 × 350 mm (approximately); reconstruction matrix 240 x 240; scan percentage 94%; flip angle 60°; sensitivity encoding (SENSE) factor 2; repetition time 2.5 milliseconds; echo time 1.3 milliseconds; reconstructed voxel size, 1.46 × 1.46 × 10 mm; temporal resolution 34 milliseconds.

Exercise was undertaken using an MRI compatible stepper (Ergospect, Innsbruck, Austria). The initial workload was set to 30 W and increased by 20 W every 3 minutes until exhaustion. Flow and cine imaging was acquired from 50% of the participants' maximum workload based upon the prior CPET testing, and at every stage thereafter until fatigue.

Ventricular volumes were determined by manual contouring of the endocardial border of each short axis slice, at end-diastole (EDV) and end-systole (ESV). Papillary muscles and ventricular trabeculae were excluded from the blood pool. Stroke volume (SV) was calculated as EDV-ESV and ejection fraction (EF) as SV/EDV.

The flow data for each vessel at each workload was extracted by contouring the vessel of interest in each dynamic, producing a flow versus time curve over the approximately 15 second time period. Using a trapezoidal method, the total area under the flow-time curve was calculated (GraphPad Prism 6 v6.0, USA), giving the total flow in each vessel over the sample period. Simultaneously acquired VCG and PPU data allowed calculation of the participant's HR, giving vessel flow in ml/ beat. Vessel blood flow during inspiration (I) and expiration I was determined using simultaneously recorded respiratory data. An in-house code was written (Matlab, MathWorks, USA) to apply a Butterworth filter to the respiratory data and the I and E states were classified by taking the first derivative of the filtered data. Respective flow values were then calculated by trapezoidal integration on the same time scale. This outputted the total flow in each vessel during inspiration and expiration. The proportion of blood flow during inspiration (I:I+E) was calculated. The I:I+E was then compared between baseline and peak

workload pre and post IMT training, and between peak workload pre and post IMT training. Pulmonary blood flow was calculated as SVC + extracardiac conduit flow. Cardiac output was calculated as aortic flow x HR. Aorto-pulmonary collateral (APC) flow was calculated as ascending aortic flow – (SVC + conduit flow), as previously described [444]. APC flow indexed to aortic flow (Indexed APC flow, %) was also calculated.

4.3.6 Power and Statistical Analysis

For the primary study questions, the following sample size calculations were made:

i) 6 weeks of IMT improves MIP: we determined that a sample size of 20 would have an 80% power at α=0.05 to detect a change of 16 cmH_2O (20% difference); assuming a (mean ± standard deviation) MIP of 80 ± 25 cm H_20 and a within-subject correlation of 0.6, based upon previously published literature [452]. We only allowed for a conservative estimate of MIP improvement (20%) even though previous literature in adults with heart failure demonstrated substantially greater increases in MIP after 6 weeks of IMT [446,453,454].

ii) 6 weeks of IMT improves VE/VCO_2 slope: we determined that a sample size of 14 would have an 80% power at α=0.05 to detect a change of 2, assuming VE/VCO_2 slope SD of 3 in adolescents with a Fontan circulation [288]. In a recent systematic review, IMT in heart failure was associated with a mean reduction in VE/VCO_2 of 2.28 (95% CI 1.3 to 3.25) [455].

Statistical analyses were performed using SPSS Version 24. Data are presented as median (range) or mean ± standard deviation, as appropriate. Pre- and post-IMT data were compared using paired t-tests for continuous variables. Correlation or linear regression was used to analyse associations with MIP change. Additionally, subjects were categorised into two groups (upper tertile and lower two tertiles) based upon MIP change and compared by Chi-square tests for categorical variables and independent sample t-tests for continuous data. A p value of <0.05 was considered to be statistically significant. Mean change was calculated as post-IMT values minus pre-IMT values, with a 95% confidence interval.

4.4 Results

Baseline demographics are shown in table 4.1. The morphological diagnoses of subjects were representative of the Fontan population of Australia and New Zealand [72]. Thirty per cent of the subjects had exposure to cigarette smoke at home or at work, but all subjects were themselves non-smokers. The majority of subjects self-reported their usual activity levels as moderate or high.

Table 4.1. Baseline Characteristics of Participants at Visit 1

Parameter	Value	
Total number of patients	23	
Age (years)	16 ± 2 (12-20)	
Gender (M:F)	12(52%): 11(48%)	
Height (cm)	164.9 ± 8.3	
Weight (kg)	61.4 ± 15.0	
BMI (kg/m^2)	22.5 ± 4.4	
Dominant ventricle	LV	13(57%)
	RV	8(35%)
	Balanced	2(9%)
Smoke exposure (Y:N)	7(30%): 16(70%)	
Age at Fontan (years)	5 ± 2 (3-9)	
Lateral thoracotomy (Y:N)	12(52%): 11(48%)	
Diagnosis	Pulmonary atresia	4(17%)
	Tricuspid atresia	5(22%)
	DILV	2(9%)
	HLHS	3(13%)
	Other	9 (40%)
Baseline Saturation (%)	93 ± 4 (83-97)	
IPAQ	Low	3(13%)
	Moderate	10(43%)
	High	10(43%)
% Predicted FEV_1	89 ± 11	
% Predicted FVC	86 ± 11	

BMI – body mass index, LV – left ventricle, RV – right ventricle, DILV – double inlet left ventricle, HLHS – hypoplastic left heart syndrome, IPAQ – international physical activity questionnaire, FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity

4.4.1 Respiratory Muscle Strength

Males had a greater baseline MIP than females (75 ± 28 versus 62 ± 11 cmH_2O, p=0.02). Inspiratory muscle training improved MIP by 61 ± 46%, p<0.01 (table 4.2). As expected, there was no significant change in MEP. The prescribed training time was 30 minutes daily, and based on self-completed diaries, subjects trained for an average of 24 ± 5.7 minutes per day. Figure 4.4, demonstrating individual participant MIP, highlights variability in the magnitude

and tempo of change. However, the grouped data (figure 4.4), highlights that the largest magnitude change occurred between visits 1 and 2 (mean increase of 26 cmH_2O) with a plateau between visits 2 and 3 (mean increase of 9 cmH_2O).

Table 4.2. Effects of inspiratory muscle training on respiratory muscle strength

Parameter	Pre-IMT	Post-IMT	p-value
MIP (cmH_2O)	69 ± 22 (M 74 ± 38; F 62 ± 11)	103 ± 32	<0.001
MEP (cmH_2O)	67 ± 23	73 ± 33	0.10
Change in MIP (cmH_2O)	36 ± 24		
%Change in MIP	61 ± 46		

MIP – maximal inspiratory pressure, MEP – maximal expiratory pressure, M – male, F – female

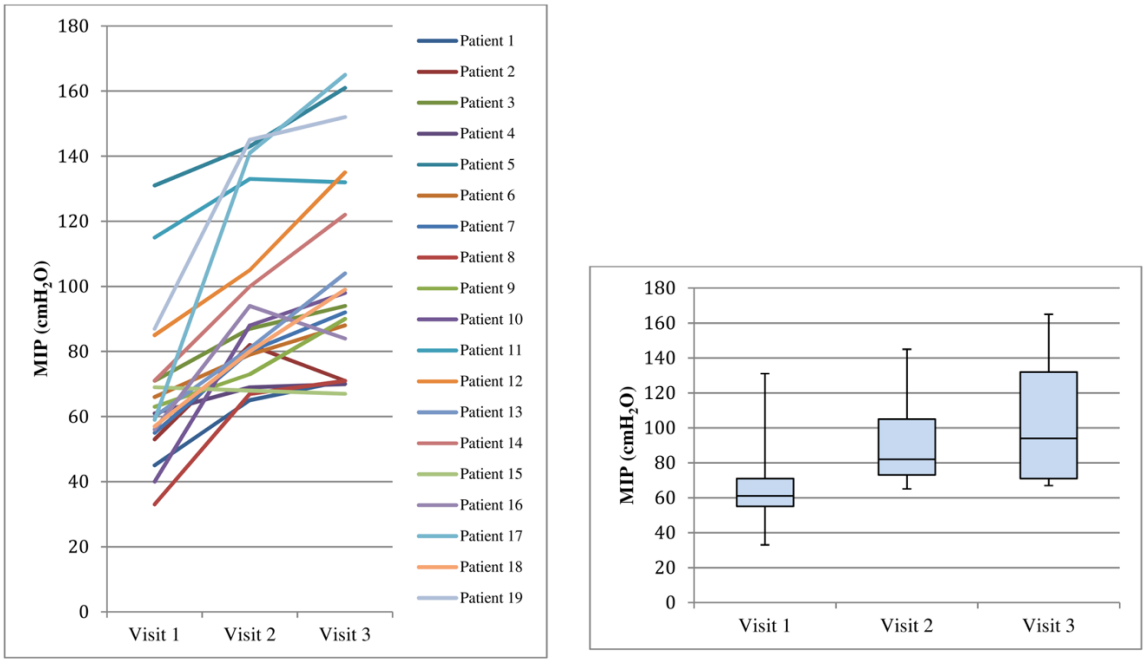


Figure 4.4. Change in mean inspiratory pressure

Left – Individual change in MIP; Right – Grouped change in MIP; MIP = maximal inspiratory pressure

Males tended to have a greater change in MIP than females (45.7 ± 29 versus 25.8 ± 10 cmH_2O, p=0.07). Change in MIP was positively correlated with baseline peak VO_2 (R=0.474, p=0.04). Change in MIP was not related to the subjects' baseline MIP, age, self-reported compliance based on IMT usage in minutes/day, baseline oxygen saturations, predominant ventricle, ejection fraction as measured on echocardiogram, previous thoracotomy, or self-reported physical activity. There were no adverse effects reported from IMT.

4.4.2 Exercise Capacity

All participants achieved maximal exertion on CPET both before and after IMT, as evidenced by an RER ≥1.1 and Borg score ≥15. Consistent with other studies in young Fontan subjects, pre IMT peak workload was 120 ± 28 W and peak VO_2 was 26.8 ± 6.8 ml/min/kg. IMT improved the ventilatory efficiency of exercise as evidenced by reduced VE/VCO_2 slope (table 4.3, 34.2 ± 7.8 versus 32.2 ± 5.6, p=0.04). IMT did not alter other exercise parameters including peak VO_2, O_2 pulse and Borg Score.

Table 4.3. Effects of inspiratory muscle training on CPET parameters

Parameter	Pre-IMT	Post-IMT	p-value	Change *
Workload (Watts)	120 ± 28	118 ± 28	1.00	-0.1 (-5.2 to 5.0)
Peak HR (beats/min)	174 ± 15	171 ± 19	0.19	-4.1 (-10.4 to 2.3)
Peak systolic BP (mmHg)	133 ± 23	134 ± 15	0.66	4.2 (-3.5 to 11.9)
Peak diastolic BP (mmHg)	68 ± 8	70 ± 9	0.91	3.7 (-2.3 to 9.7)
Lowest saturation (%)	87 ± 7	86 ± 6	0.37	-1.6 (-5.2 to 2.1)
RER	1.3 ± 0.1	1.3 ±0.2	0.32	0.04 (-0.05 to 0.13)
Peak VO_2 (ml/kg/min)	26.8 ± 6.8	26.0 ± 7.2	0.05	-6.04 (-7.66 to 4.43)
O_2 Pulse (mls/beat)	9.2 ± 2.2	8.7 ± 2.0	0.32	-0.26 (-0.79 to 0.28)
VE/VCO_2 slope	34.2 ± 7.8	32.2 ± 5.6	0.04	-2.24 (-4.63 to -0.18)
Peak VT (L)	1.6 ± 0.5	1.6 ± 0.6	0.67	-0.03 (-0.17 to 0.11)
Peak VE (L/min)	64.2 ± 13.5	62.2 ± 18.3	0.56	-1.42 (-6.41 to 3.56)
Peak VCO_2 (L/min)	1.7 ± 0.5	1.6 ± 0.5	0.27	-0.05 (-0.16 to 0.05)
Breathing Reserve (%)	33.0 ± 9.6	34.5 ± 9.8	0.54	0.01 (-0.04 to 0.06)
Borg Score	17 ± 2	18 ± 1	0.51	0.3 (-0.7 to 1.3)

** Calculated as post IMT values minus pre IMT values, expressed as mean change (95% confidence interval)*
IMT – inspiratory muscle training, CPET – cardiopulmonary exercise testing, HR – heart rate, BP – blood pressure, RER – respiratory exchange ratio, VO_2 – oxygen consumption, VCO_2 – carbon dioxide production

Subjects in the upper tertile of MIP change, as compared to those in the lower two tertiles, had lower baseline VE/VCO_2 slope (table 4.4, 27.3 ± 3.4 versus 37.9 ± 7.2, p=0.02) but equivalent baseline MIP, peak workload and peak VO_2. Those in the lower two tertiles of MIP change tended to have a larger improvement in VE/VCO_2 slope (table 4.5, -3.6 ± 5.0 versus 0.1 ± 2.4, p=0.16), suggesting that even small changes in MIP may improve VE/VCO_2 in those with the lowest baseline ventilatory efficiency of exercise.

Table 4.4. Comparison of baseline characteristics in tertiles of MIP change

Baseline (Pre-IMT) Parameter	Upper Tertile of MIP Change	Lower Tertiles of MIP Change	p-value
MIP (cmH_2O)	67 ± 18	67 ± 27	0.64
Compliance (mins)	22.9 ± 7.1	24.5 ± 5.1	0.64
Baseline O_2 saturation (%)	93 ± 3	92 ± 4	0.43
Lowest O_2 saturation (%)	86 ± 7	88 ± 7	0.87
Workload (Watts)	129 ± 29	113 ± 24	0.52
Peak VO_2 (ml/min/kg)	33.2 ± 4.7	24.8 ± 5.7	0.69
VE/VCO_2 slope	27.3 ± 3.4	37.9 ± 7.2	0.02

IMT – inspiratory muscle training, MIP – maximal inspiratory pressure, VO_2 – oxygen consumption, VCO_2 – carbon dioxide production

Table 4.5. Comparison of Change in CPET Parameters between Upper and Lower Tertiles of MIP Change

Parameter	Upper Tertile of MIP Change	Lower Tertiles of MIP Change	p-value
Change in workload (Watts)	1.2 ± 8.2	-0.7 ± 11.8	0.71
Change in peak VO_2 (L/min)	-0.5 ± -1.7	-1.9 ± 3.6	0.26
Change in VT (L)	139.0 ± 156.5	-106.3 ± 305.9	0.50
Change in VE/VCO_2	0.1 ± 2.4	-3.6 ± 5.0	0.16

CPET – cardiopulmonary exercise testing, MIP – maximal inspiratory pressure, VO_2 – oxygen consumption, VT – tidal volume, VCO_2 – carbon dioxide production

4.4.3 Lung Function

Only 1 subject had spirometry consistent with restrictive lung disease. All other subjects had normal baseline lung function (table 4.6). There were no statistically significant changes in lung function after IMT, particularly there was no change in Vd/Vt.

Table 4.6. Effects of IMT on Lung Function

Parameter	Pre IMT	Post IMT	p-value	Change *
FEV_1	2.9 ± 0.7	2.7 ± 0.7	0.48	-0.04 (-0.02 to 0.10)
FVC	3.3 ± 0.8	3.3 ± 0.8	0.10	0.07 (-0.02 to 0.16)
FEV_1/FVC	87.8 ± 5.6	83.3 ± 7.5	0.06	-3.30 (-7.32 to 0.73)
IC (rest)	2.3 ± 0.5	2.3 ± 0.7	0.31	-0.10 (-0.30 to 0.10)
Vd/Vt	0.2 ± 0.04	0.2 ± 0.10	0.98	-0.02 (-0.03 to 0.01)

** Calculated as post IMT values minus pre IMT values, expressed as mean change (95% confidence interval)*
IMT – inspiratory muscle training, FEV_1 – forced expiratory volume in 1 second, FVC – forced vital capacity, IC – inspiratory capacity, Vd/Vt - physiologic dead space ventilation

4.4.4 Exercise Cardiac Magnetic Resonance Imaging

In subjects who underwent ex CMRI, neither the resting or peak heart rate, or aortic or pulmonary blood flow changed significantly after IMT (table 4.7). However, resting cardiac output improved following IMT (4.2 ± 1.2 versus 4.5 ± 1.0 L/min, p=0.03). Cardiac output at peak exercise remained unchanged. The relative proportion of aortic flow during inspiration (I:I+E), both at rest and peak exercise, did not change significantly after IMT (table 4.7). The relative proportion of resting pulmonary blood flow during inspiration also remained unchanged but there was a trend towards an increase of the inspiratory proportion of pulmonary blood flow at peak exercise after IMT (0.47 to 0.54, p=0.06). Both SV and EF increased significantly from rest to peak exercise, before and after IMT (table 4.8). Although IMT did not change either rest or peak exercise SV, it did result in increased resting EF (table 4.7). APC flow at rest and peak exercise did not change significantly after IMT.

Table 4.7. Effects of IMT on CMRI parameters

Parameter	Pre IMT	Post IMT	p-value	Change *
Resting heart rate (bpm)	73.3 ± 15.1	75.7 ± 14.4	0.41	2.33 (-3.66 to 8.33)
Peak heart rate (bpm)	121.0 ± 20.0	118.8 ± 19.6	0.53	-2.25 (-9.88 to 5.38)
Resting aortic flow (mls/beat)	58.5 ± 19.2	60.0 ± 14.3	0.64	1.45 (-5.20 to 8.11)
Peak aortic flow (mls/beat)	55.3 ± 14.6	59.3 ± 11.5	0.17	4.03 (-2.06 to 10.11)
Resting cardiac output (L/min)	4.2 ± 1.2	4.5 ± 1.0	0.03	0.29 (0.03 to 0.54)
Peak cardiac output (L/min)	6.6 ± 1.5	7.0 ± 1.4	0.16	0.36 (-0.22 to 0.95)
Resting pulmonary flow (mls/beat)	52.2 ± 11.2	55.1 ± 12.2	0.37	2.82 (-3.82 to 9.45)
Peak pulmonary flow (mls/beat)	55.4 ± 11.6	54.8 ± 6.5	0.81	-0.52 (-5.29 to 4.24)
Resting stroke volume (ml)	60.6 ± 7.6	63.7 ± 10.4	0.10	3.08 (-0.65 to 6.81)
Peak stroke volume (ml)	70.5 ± 11.5	68.2 ± 9.0	0.26	-2.33 (-6.70 to 2.03)
Resting ejection fraction (%)	50.1 ± 4.3	52.8 ± 6.1	0.03	2.67 (0.41 to 4.92)
Peak ejection fraction (%)	57.6 ± 6.0	55.3 ± 5.1	0.14	-2.25 (-5.40 to 0.90)
Resting APC flow	9.4 ± 9.4	6.9 ± 6.0	0.24	-2.45 (-6.79 to 1.89)
Indexed resting APC (%)	13.8 ± 11.9	10.9 ± 8.8	0.32	-2.90 (-9.02 to 3.23)
Peak APC flow	4.2 ± 5.8	6.0 ± 6.1	0.25	1.80 (-1.47 to 5.07)
Indexed peak APC (%)	6.3 ± 7.7	9.0 ± 8.6	0.23	2.71 (-1.97 to 7.40)
I:E Ratio[#]				
Resting aortic flow (%)	48.6 ± 6.5	48.4 ± 7.6	0.94	-0.18 (-4.76 to 4.41)
Peak exercise aortic flow (%)	54.5 ± 7.3	55.1 ± 4.8	0.81	0.99 (-4.81 to 6.78)
Resting pulmonary flow (%)	55.1 ± 10.2	56.1 ± 10.2	0.84	1.07 (-10.1 to 12.2)
Peak exercise pulmonary flow (%)	45.4 ± 8.7	50.1 ± 9.0	0.11	6.98 (-0.40 to 14.36)

***** Calculated as post IMT values minus pre IMT values, expressed as mean change (95% confidence interval)
[#] *Percentage of Inspiration and Expiration of Aortic and Pulmonary Blood Flow at Rest and Peak Exercise*
IMT – inspiratory muscle training, CMRI - cardiac magnetic resonance imaging, bpm – beats per minute, APC - aorto-pulmonary collateral, I – inspiratory, E – expiratory

Table 4.8. Change in Stroke Volume and Ejection Fraction Between Rest and Peak Exercise

Parameter	Rest	Peak Exercise	p-value
Pre IMT stroke volume (mls)	64.0 ± 12.7	72.4 ± 12.6	0.003
Pre IMT ejection fraction (%)	50.1 ± 3.9	56.7 ± 6.1	0.001
Post IMT stroke volume (mls)	63.7 ± 10.4	68.2 ± 9.0	0.03
Post IMT ejection fraction (%)	52.8 ± 6.1	55.3 ± 2.1	0.02

IMT – inspiratory muscle training

4.5 Discussion

To our knowledge, this study is the first report of the effects of IMT in subjects with a Fontan circulation. The two principal findings are that a 6-week home-based program of IMT increases inspiratory muscle strength by an average of 61% and is associated with an improvement in the ventilatory efficiency of exercise, as evidenced by reduced VE/VCO_2 slope, in young Fontan patients. We have also found an increase in resting cardiac output and EF after IMT.

A number of lines of evidence suggested a rationale for IMT in Fontan patients. Firstly, adults with congenital heart disease, including those with a Fontan circulation, have been shown to have reduced respiratory muscle strength compared to controls [261]. Secondly, pulmonary blood flow in the Fontan circulation may be significantly influenced by breathing and the respiratory pump [159,161]. Lastly, Fontan physiology overlaps with that of adults with heart failure, a patient group in whom IMT has been shown to have benefit [193,455].

4.5.1 Improvement in MIP

We have demonstrated a magnitude of improvement in MIP which is similar to that shown in studies of IMT in adults with heart failure [453,456-458]. The majority of these IMT trials in adult heart failure have utilised targeted threshold trainers, set at inspiratory loads of between 20-60% of measured MIP with training durations of between 1 session and 12 months [353,407]. We based our program of IMT upon the most frequently reported protocols of 6-12 weeks of training for 30 minutes per day, 5-7 days/week and with inspiratory loads of 30% of measured MIP. Most [408,445,459] but not all [446] studies of IMT in adults with heart failure, either alone or in combination with aerobic training, have shown improvements in measures of exercise capacity, functional status and quality of life.

4.5.2 Improvement in VE/VCO_2

We have also demonstrated a magnitude of improvement in VE/VCO_2 which is similar to that shown in studies of IMT in adults with heart failure [455]. Potentially, IMT could influence one or more of the major mechanisms contributing to elevated VE/VCO_2, namely Vd/Vt, early lactic acidosis and impaired breathing control related to activation of peripheral or central chemoreceptors. Although we cannot exclude some effect of IMT on ventilation-perfusion mismatch, we did not demonstrate a change in Vd/Vt or tidal volume (VT) to suggest an effect of IMT on dead space ventilation. Although blood lactate during exercise was not measured in the current study, we were unable to demonstrate significant changes after IMT in O_2 pulse or O_2 saturation on CPET or peak exercise cardiac output on ex CMRI, pointing against an effect mediated by reduced lactic acid production. Studies in both healthy controls and adults with heart failure have suggested an effect of IMT on reducing activation of peripheral chemoreceptors by inspiratory muscle fatigue ("inspiratory muscle metaboreflex"). For example, Witt et al., in a study of 16 healthy subjects who undertook 5 weeks of either IMT (n=8) or sham IMT (n=8), demonstrated a blunted increase in HR and mean blood pressure induced by eucapnic resistive breathing after IMT [401]. Chiappa et al., in study of 18 patients with heart failure and inspiratory muscle weakness, demonstrated improved resting calf blood flow and exercise forearm blood flow after a 4-week program of IMT [359]. Although yet to be proven, our data points to a similar mechanism in patients with a Fontan circulation whereby IMT may attenuate vasoconstriction to exercising peripheral muscles induced by fatiguing inspiratory muscle.

In adults with heart failure, VE/VCO_2 slope is independently associated with severe cardiovascular events including death with perhaps a better ability to predict risk than peak VO_2 [460]. In adults with all forms of congenital heart disease, including those with a Fontan circulation, VE/VCO_2 slope is higher than in normal control subjects. In examining the association between exercise parameters and mortality, in a large population of adults with congenital heart disease, Dimopoulos et al. demonstrated that the strongest independent association in non-cyanotic patients was with VE/VCO_2 (HR 1.076 per unit, 95% CI 1.038 to 1.115, $p<0.05$) [390]. Therefore, improvements in ventilatory efficiency of the order of magnitude that we have documented after just 6 weeks of IMT, if sustained over longer durations, hold the promise of translating into improved morbidity and mortality for Fontan patients.

4.5.3 Improvement in Resting Cardiac Output and Ejection Fraction

We have documented increased resting cardiac output and ejection fraction after IMT, in a sub-study of subjects who had ex CMRI. EF is a load dependent measure of cardiac function and may be influenced by preload, afterload, and contractility. Resting cardiac output in the Fontan circulation has been thought to be importantly dependent upon ventricular preload and thus on low resistance to pulmonary blood flow [249]. We were unable to detect a change in either resting pulmonary blood flow or APC flow after IMT, suggesting a mechanism other than an effect on ventricular preload. The effects of IMT on afterload, ventricular-vascular coupling and myocardial contractility, all which have been reported as abnormal in patients with a Fontan circulation [88,273], may be of interest in future studies. Therefore, IMT holds the promise of arresting the progressive decline in resting cardiac output brought about by the inherent limitations of the Fontan circulation.

4.5.4 Future Directions

In order to limit underlying patient heterogeneity, we included only young subjects with a non-fenestrated extracardiac conduit. We demonstrated a positive association between MIP change and baseline peak VO_2 and greatest improvements in MIP in those with the lowest baseline VE/VCO_2. These data suggest that improvements in MIP may be greatest in those with higher baseline exercise capacity. However, we also demonstrated a trend for the largest improvements in VE/VCO_2 slope in those in the lowest two tertiles of MIP change, suggesting that IMT may be most beneficial in those with the highest baseline VE/VCO_2 slope. Taken together, these findings imply that the greatest benefit of IMT may occur in Fontan subjects that are the least fit and that in this group, even small changes in MIP may be of value. Conversely, although fitter Fontan patients may get a greater improvement in MIP the benefit they get in terms of lowering VE/VCO_2 appears to be smaller. As both peak VO_2 and VE/VCO_2 slope show a gradual decline with age in patients with congenital heart disease [288,461] and as VE/VCO_2 may be higher in non-extracardiac conduit types of Fontan circulation, we speculate that IMT could have an even greater benefit in older adults with either a lateral tunnel or AP type Fontan circulation.

We did not demonstrate any improvement in exercise duration, workload or peak VO_2 related to IMT. Peak VO_2 is influenced by a number of potential factors that limit the ability of the circulation to increase cardiac output during exercise, including preload inadequacy, excessive

afterload, systolic and diastolic dysfunction and chronotropic incompetence. Although studies in adults with heart failure have demonstrated improvements in peak VO_2 related to IMT, it is possible that our study was either under powered to detect such a change and/or that the interaction between IMT and cardiac output during exercise is substantially different in Fontan and heart failure patients. The effects of combining IMT with aerobic and/or resistance training on peak VO_2 and other exercise parameters will be a focus of future trials.

Several potential explanations are possible for why we did not demonstrate any improvement in lung function or pulmonary blood flow after IMT. Firstly, baseline lung function may have been better preserved in our cohort than that reported in other Fontan patients. For example, in a study of 52 adult AP Fontan patients (age 26.5 (18-45) years, age at Fontan 17.5 (5-36) years), Fredriksen et al. reported a % predicted forced vital capacity (FVC) of 77% with much lower baseline exercise capacity (VO_2 max 15.9 ml/kg/min) than in our study [179]. Opotowsky et al., in a study of 260 young Fontan patients (age 13.2 ± 3.0 years, 57 extracardiac conduit (ECC), 152 lateral tunnel (LT) and 43 atrio-pulmonary connection (AP)) reported a % predicted FVC of 79 ± 14.8% with FVC correlating with peak VO_2 [164]. Ohuchi et al., in a study of 101 younger Fontan patients (age 13.6 ± 4.6 years, 29 AP type and 72 LT or ECC) found lung function closer to that in our cohort (% predicted FVC 80 ± 20%) [193]. Similarly in a study of 33 Norwegian children with a Fontan circulation (mean age 12.6 years), Matthews et al. reported a % predicted FVC of 86 ± 17% and % predicted FEV_1 of 94 ± 19% [192]. In our study of younger patients with an ECC Fontan, higher baseline lung function and self-reported levels of physical activity may have limited the scope for IMT to produce further improvements in lung function and pulmonary blood flow. Secondly, trials of IMT in adults with heart failure have not demonstrated improvement in either FEV_1 or FVC, despite significant improvements in inspiratory muscle strength and exercise capacity [445,459], suggesting that in heart failure the beneficial effects of IMT may be independent of changes in lung function. Finally, although there was not even a trend toward improvement, we acknowledge that we did not power our study to detect a change in either lung function or pulmonary blood flow.

4.5.5 Potential Study Limitations

We utilised an IMT program that was home-based and feasible for adolescents and young adults to undertake. Although the training was not fully supervised by the investigators,

subjects demonstrated their use of the IMT device at the end of each study visit and parents were encouraged to supervise their child's training. Compliance was checked by diary entry and also weekly phone contact with subjects. All our subjects were young and had a non-fenestrated extracardiac Fontan, and hence our results cannot necessarily be extrapolated to the general Fontan population where a larger clinical trial may be required. The duration of IMT in our study was based upon adult heart failure trials which demonstrate the greatest magnitude of MIP improvement over the first 6 weeks with a plateauing thereafter. We are therefore unable to draw firm conclusions about the longer-term effects of IMT or the effects of detraining after ceasing IMT.

We acknowledge that volumetrics may be affected by the respiratory state (e.g. end-expiration), however our volumetric analyses of real-time exercise MRI data did not account for changes in respiration. Future studies could incorporate the use of respiratory data in volumetric analyses to further assess changes in cardiac output and ejection fraction. Our study was powered to meet our primary outcomes of change in MIP and VE/VCO_2 slope. Therefore the study may have been insufficiently powered to detect differences after IMT in other exercise parameters. In order to limit the likelihood of type 2 statistical error, we did not undertake adjustment for multiple comparisons in the results from the exploratory ex-CMRI component of our study. Although we have demonstrated interesting findings in relation to the effects of IMT on resting cardiac output and ejection fraction, we acknowledge that our results may be subject to type 1 statistical error and require confirmation in other trials of IMT in patients with a Fontan circulation.

In this short-term study, we were also not able to examine the effects of IMT on potentially clinically meaningful end points, such as Fontan failure and mortality. Although maximal exertion was achieved in all CPET studies (RER $\geq$1.1, Borg score > 15), the ventilatory compensation point (VCP) was not clearly identifiable in all subjects in the plots of VE versus VCO_2. We therefore chose to determine VE/VCO_2 slope as occurring between 25-75% of maximum VCO_2, as previously described [225,449], rather than across the whole test. In those subjects who did have a clear VCP, we performed a correlation between the VE/VCO_2 slope calculated by the two methods demonstrating that the two values were highly correlated ($R=0.894$, $p<0.01$).

4.6 Conclusion

Six weeks of inspiratory muscle training is associated with improved inspiratory muscle strength, the ventilatory efficiency of exercise and resting cardiac output in young patients with a Fontan circulation. Although the mechanisms for these improvements and the effects of IMT combined with other forms of exercise prescription remain to be determined, our data suggest that IMT may be a simple beneficial addition to the current long-term management of Fontan patients, potentially improving exercise capacity and late morbidity and mortality.

CHAPTER FIVE: OXYGEN PULSE SLOPE

This chapter is based on the following manuscript (submitted for publication):

Laohachai K, Cordina R, d'Udekem Y, Rice K, Weintraub R, Ayer J. O2 pulse slope correlates with stroke volume during exercise in patients with a Fontan circulation. Submitted for publication.

Abstract

Background: Peak oxygen pulse (O_2 pulse = oxygen consumption / heart rate) is calculated by the product of stroke volume (SV) and oxygen extraction. It has been shown to be reduced in patients with a Fontan circulation. However, in the Fontan population it may be a poor marker of SV. We propose that the slope of the O_2 pulse curve may be more reflective of SV during exercise.

Methods: We analysed cardiopulmonary exercise test data in twenty-two subjects with a Fontan circulation (cohort A) and examined the association between peak SV during exercise (aortic flow measured on exercise cardiac magnetic resonance imaging), and O_2 pulse parameters (absolute O_2 pulse and O_2 pulse slopes up to anaerobic threshold (AT) and peak exercise). In a separate Fontan cohort (cohort B, n=131), associations between clinical characteristics and O_2 pulse kinetics were examined.

Results: In cohort A, peak aortic flow was moderately and significantly associated with $O_2pulseslope^{PEAK}$ (r=0.47, p=0.02). However, neither absolute O_2pulse^{AT} nor O_2pulse^{PEAK} were significantly associated with peak aortic flow. In cohort B, $O_2pulseslope^{PEAK}$ and $O_2pulseslope^{AT}$ were not significantly associated with clinical parameters, apart from a weak association with forced vital capacity.

Conclusion: The slope of the O_2 pulse curve to peak exercise may be more reflective of peak stroke volume in the Fontan population than a single peak O_2 pulse value.

5.1 Introduction

Cardiopulmonary exercise testing (CPET) is an important tool in assessing the haemodynamic response to exercise in patients with a Fontan circulation, and certain CPET parameters are emerging as useful in the prognostic assessment of these patients [51,257]. However, maximal exercise is not met in a substantial proportion of Fontan patients [199] and hence there is significant interest in sub-maximal exercise parameters, their determinants and association with clinically relevant outcomes [217,462].

One maximal parameter, peak oxygen (O_2) pulse, has been shown to be reduced in patients with a Fontan circulation [199,463]. O_2 pulse represents the relationship between oxygen consumption (VO_2) and heart rate (HR) (O_2 pulse = VO_2/HR) and is determined by the product of stroke volume (SV) and oxygen extraction (arteriovenous oxygen difference (a-vO_2)) [216].

In healthy populations the change in a-vO_2 between rest and peak exercise is predictable, such that peak O_2 pulse has been used as a non-invasive surrogate measure of SV [464,465]. However, as SV plateaus from mid-range exercise onward [214], O_2 pulse from mid exercise is mainly determined by a-vO_2. As skeletal muscle abnormalities are highly prevalent and variable in patients with a Fontan circulation [300,316], with the potential of impaired oxygen extraction by exercising muscles [335], peak O_2 pulse may be a poor marker for SV in this cohort [466].

O_2 pulse can be evaluated across the whole of exercise and O_2 pulse kinetics, including the slope of the O_2 pulse curve, have been examined in patients with a Fontan circulation [467]. Fontan patients have been shown to have an altered lower O_2 pulse slope compared to healthy controls, with different slope patterns. Given that O_2 pulse slope takes into account both submaximal and maximal responses to exercise, we postulated that it, rather than peak O_2 pulse, may be more strongly associated with SV.

The relationship between SV during exercise and O_2 pulse slope in Fontan patients has not been determined. Thus, the primary aim of this study in patients with a Fontan circulation was to examine the association between SV (assessed as ascending aortic flow measured by exercise cardiac magnetic resonance) obtained during exercise, and peak O_2 pulse and O_2 pulse

slope during CPET. As a secondary aim we explored associations between clinical characteristics and both peak O_2 pulse and O_2 pulse slope in a larger cohort of patients with a Fontan circulation.

5.2 Methods

5.2.1 Recruitment

Two cohorts of Fontan participants were used in this study as follows (figure 5.1):

i) In the first cohort (cohort A – 22 subjects with 40 CPETs and 26 exercise cardiac magnetic resonance imaging (exCMR) analysed) we examined the association between absolute O_2 pulse, O_2 pulse slope and SV during exercise. These participants were part of a study analysing the effects of inspiratory muscle training, as previously described [468]. Cohort A were participants identified through the cardiology database at The Children's Hospital at Westmead (CHW) in Sydney, Australia. Only participants with a non-fenestrated extracardiac conduit aged 12-20 years were included.

ii) In the second cohort (cohort B – 131 subjects with 131 CPETs and transthoracic echocardiograms (TTE)) we examined the association between clinical characteristics and O_2 pulse parameters. These participants with a Fontan circulation were identified through the Australian and New Zealand Fontan Registry (ANZFR) and were part of the ANZFR Functional Outcomes after Fontan study conducted across Sydney, Melbourne, and Auckland. Inclusion criteria for this study were age ≥13years and ≥5years since Fontan completion.

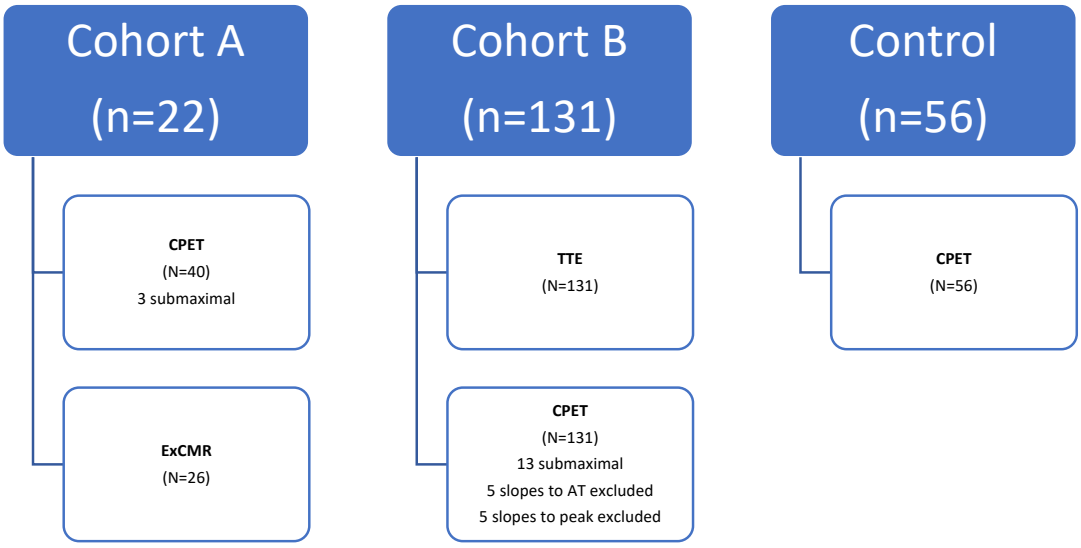


Figure 5.1. Study participants

n – number of subjects, CPET – cardiopulmonary exercise test, N – number of tests, ExCMR – exercise cardiac magnetic resonance imaging, TTE – transthoracic echocardiogram, AT – anaerobic threshold

Exclusion criteria for both cohorts were those with severe heart failure, a history of significant exercise-induced arrhythmia, severe systemic outflow tract obstruction or severe systemic hypertension at rest, or significant cognitive impairment or intellectual disability. Clinical data for Fontan participants were retrospectively collected from the local cardiology database at CHW (cohort A) and the ANZFR REDCap database (cohort B, hosted by the Murdoch Children's Research Institute).

Cohort A subjects underwent CPET at CHW. A subset of these patients underwent exCMR, also performed at CHW [468]. Cohort B subjects underwent CPET and TTE at one of four sites – Sydney (CHW and The Royal Prince Alfred Hospital, RPAH), Melbourne (The Royal Children's Hospital) and Auckland (Starship Children's Hospital).

To compare O_2 pulse parameters between Fontan subjects and healthy controls, data from healthy controls were obtained through the exercise labs at CHW, RPAH, and the Women's and Children's Hospital in Adelaide, Australia (n=56). These subjects had no underlying cardiac, respiratory, or neurological conditions and were undergoing exercise testing for non-specific symptoms.

This study had received institutional ethics committee approvals.

5.2.2 Cardiopulmonary exercise testing (CPET)

CPET was performed in all participants, in a standardised fashion on an upright cycle ergometer using a progressive ramp protocol, aiming to achieve peak oxygen consumption (VO_2) within 7-10 minutes. Cohort A underwent CPET at baseline and at 6 weeks after inspiratory muscle training (n=40). Heart rate, blood pressure and oxygen saturations were recorded from rest to recovery. Breath-by-breath VO_2, carbon dioxide consumption (VCO_2) and minute ventilation (VE) were measured. Anaerobic threshold (AT) was calculated using a combination of the V-slope, ventilatory equivalents and respiratory exchange ratio methods.

O_2 pulse was calculated throughout testing, from the start of exercise until the beginning of recovery. Absolute O_2 pulse was determined at AT (O_2pulse^{AT}) and at peak exercise (O_2pulse^{PEAK}). O_2 pulse against time curves were reviewed and the curves were determined to have an overall linear pattern, without clear inflection points at consistent time points. Due to this pattern, lines of best fit for O_2 pulse against exercise time up to AT ($O_2pulseslope^{AT}$) and peak ($O_2pulseslope^{PEAK}$) were determined by regression (GraphPad Prism 9), and the slope of these regression lines were taken as the O_2 pulse slope (figure 5.2 as representative). Lines of best fit were visually checked by two independent observers (KL, JA) and poorly fitting lines were excluded by agreement (5 slopes from cohort A and 5 slopes from cohort B were thus excluded). No slopes from the control cohort were excluded. For analysis, indexing of O_2 pulse metrics to body surface area was not undertaken given that indexing of aortic flow was also not undertaken.

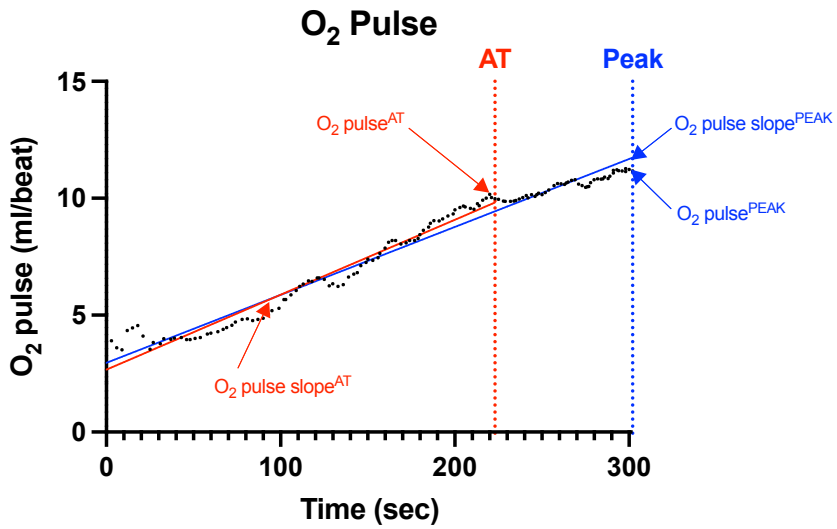


Figure 5.2. O_2 pulse parameters

AT – anaerobic threshold

5.2.3 Spirometry

Spirometry was performed in all participants using standardised techniques [415]. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured, and percent predicted FVC and FEV_1 were determined [418].

5.2.4 Exercise Cardiac Magnetic Resonance Imaging

Exercise cardiac magnetic resonance imaging (ExCMR) was performed (1.5T, Phillips Intera, Best, The Netherlands) at baseline (n=14) and then 6 weeks after inspiratory muscle training (n=12) [468]. Exercise in the magnetic resonance imaging (MRI) scanner was undertaken using an MRI compatible stepper (Ergospect, Innsbruck, Austria). The initial workload was set to 30W and increased by 20W every 3 minutes until exhaustion. Flow and cine imaging was acquired from 50% of the participants' maximum workload based upon the prior CPET, and at every stage thereafter until fatigue. Resting and exercise non-gated real time phase contrast flow (200 consecutive dynamics, dynamic scan time 75 milliseconds, corresponding to approximately 15 seconds of flow data) was measured. Ascending aortic flow was measured at rest (Aortic $flow^{REST}$) and at the final stage prior to exhaustion (Aortic $flow^{PEAK}$), as a measure of SV. Typical scan parameters for balanced steady-state free-precession cine imaging have been described previously [468]. Ventricular volumes were determined by manual contouring of the endocardial border of each short axis slice, at end-diastole (EDV) and end-systole (ESV). Ejection fraction was calculated (EF = (EDV-ESV)/EDV x 100%). To study the association between SV and O_2 pulse metrics, all ExCMR studies and their corresponding CPET (n=26) were analysed.

5.2.5 Echocardiography

Participants in cohort B had transthoracic echocardiograms performed and the following were obtained: anatomy, ventricular dominance, atrioventricular (AV)-valve regurgitation (none-trivial, or mild or more) and global systolic dysfunction as a qualitative measurement (normal or impaired).

5.2.6 Statistical analyses

Statistical analyses were performed using GraphPad Prism 9. Non-parametric and parametric data are presented as median (range) or mean+/-standard deviation, respectively. Categorical and continuous data were compared between healthy and Fontan groups using Chi-square tests,

t-tests or Mann-Whitney U-tests, as appropriate. Correlations between clinical Fontan and O_2 pulse parameters, were assessed using Pearson or Spearman correlation and multiple linear regression models. A p-value <0.05 was considered statistically significant.

5.3 Results

The baseline characteristics of cohort A and cohort B are shown in table 5.1. Cohort B included a wider age-range, with an older mean age than cohort A (23.0 versus 15.8 years). Due to the inclusion criteria of cohort A, more subjects in cohort B were fenestrated at the time of Fontan completion and had non-extracardiac conduits. Other baseline characteristics were similar between the two Fontan groups. Characteristics of the control subjects were: thirty-three (59%) males, age 29.5+/-12.0 years, height 169.8+/-11.8cm, weight 77.5+/-22.2kg and body mass index 22.7+/-6.0.

Table 5.1. Anthropometrics and Clinical Characteristics of Fontan subjects (cohorts A and B)

	Cohort A Fontan (n=22)	**Cohort B Fontan** (n=131)	**p-value**
Age (years)	15.8 ± 2.3	23.0 ± 7.9	<0.01
Gender (Male: Female)	11 (50%) : 11 (50%)	67 (51%) :64 (49%)	>0.9
Height (cm)	164.3 ± 8.1	165 ± 10.0	0.56
Weight (kg)	60.6 ± 14.8	64.9 ± 15.7	0.23
Body mass index	22.0 ± 4.5	23.5 ± 4.9	0.27
Dominant ventricle (Right: Other)	14 (64%) : 8 (32%)	92 (70%) : 39 (30%)	0.62
Age of Fontan (years)	5.2 ± 2.0	6.0 ± 4.3	0.40
Type of Fontan (Extracardiac conduit: Other)	22 (100%) : 0 (0%)	82 (63%) : 49 (37%)	<0.01
Fenestration (No: Yes)	22 (100%) : 0 (0%)	89 (64%) : 41 (36%) *1 unknown*	<0.01

There was no significant difference in O_2pulse^{PEAK}, O_2pulse^{AT}, $O_2pulseslope^{PEAK}$ or $O_2pulseslope^{AT}$, between cohort A and B (figure 5.3). Both Fontan cohorts had significantly lower peak VO_2, O_2pulse^{PEAK}, O_2pulse^{AT} and $O_2pulseslope^{PEAK}$ compared to healthy controls. There was no difference in $O_2pulseslope^{AT}$ between cohort B and healthy controls (table 5.2, figure 5.3).

Table 5.2. O_2 pulse parameters

Parameter	Healthy Controls	Cohort A Fontan	Cohort B Fontan
Peak VO_2 (ml/kg/min)	36.0 ± 10.3	25.1 ± 7.1*	20.9 ± 7.1*
O_2 $pulse^{PEAK}$ (ml/beat)	13.2 ± 4.1	9.8 ± 2.2*	9.3 ± 2.9*
O_2 $pulse^{AT}$ (ml/beat)	10.9 ± 3.5	8.4 ± 1.8*	8.7 ± 2.8*
O_2 pulse $slope^{PEAK}$	0.01 ± 0.005	0.008 ± 0.003*	0.009 ± 0.005*
O_2 pulse $slope^{AT}$	0.01 ± 0.006	0.01 ± 0.004*	0.01 ± 0.0005**

** Compared to healthy controls, p<0.01*
*** Compared to healthy controls, not statistically significant (p>0.05)*

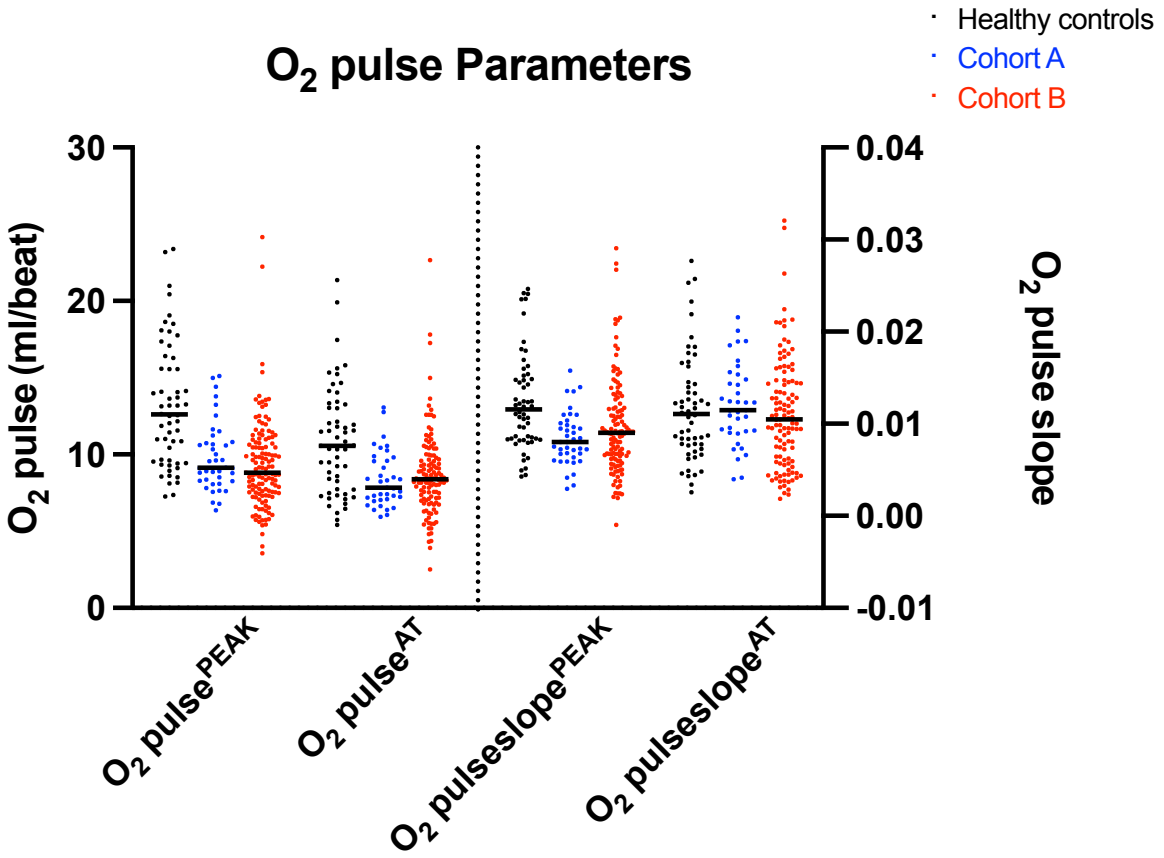


Figure 5.3. O_2 pulse parameters

In cohort A, aortic $flow^{PEAK}$ was moderately and significantly associated with $O_2pulseslope^{PEAK}$ (r=0.47, p=0.02) but was not significantly associated with $O_2pulseslope^{AT}$ (r=0.37, p=0.09). Neither O_2pulse^{PEAK} nor O_2pulse^{AT} correlated with aortic $flow^{PEAK}$ (r=-0.05, p=0.82; r=0.20, p=0.33 respectively). There was a weak negative association between ejection fraction (EF) measured at peak exercise on exCMR and O_2 pulse at peak and rest (r=-0.40, p=0.03; r=-0.53, p<0.01, respectively). There were no significant associations between EF and other O_2 pulse parameters.

In cohort B, neither resting nor peak oxygen saturation were correlated with any of the O_2 pulse parameters. $O_2pulseslope^{PEAK}$ correlated with O_2pulse^{PEAK} (r=0.56, p<0.01) and $O_2pulseslope^{AT}$ with O_2pulse^{AT} (p<0.01, r=0.44). O_2pulse^{PEAK} and O_2pulse^{AT} were negatively associated with age of Fontan completion (r=-0.19, p=0.03; r=-0.20, p=0.03, respectively). $O_2pulseslope^{AT}$ was associated with severity of AV-valve regurgitation (U=847, p=0.03) with a lower slope in those with at least mild regurgitation. However, AV-valve regurgitation was not associated with $O_2pulseslope^{PEAK}$. $O_2pulseslope^{PEAK}$ and $O_2pulseslope^{AT}$ were not significantly associated with other clinical variables including ventricular morphology or function, Fontan type, age of Fontan completion, presence of fenestration or pacemaker, presence of permanent pacemaker, or use of HR-limiting medications (table 5.3). Baseline FVC %predicted but not FEV_1 was weakly associated with O_2pulse^{PEAK} $O_2pulseslope^{PEAK}$ (r=0.2, p=0.03) and $O_2pulseslope^{AT}$ (r=0.21, p=0.03).

Table 5.3. Association of clinical variables with O_2 pulse parameters (cohort B)

Variable	**O_2 pulse ^{PEAK}**	**O_2 pulse ^{AT}**	**O_2 pulse slope $_{PEAK}$**	**O_2 pulse slope ^{AT}**
Ventricular dominance (Right vs left or biventricular)	U=1428, p=0.07	U=1227, p=0.15	U=1376, p=0.12	U=1177, p=0.24
Ventricular function (Normal vs impaired)	U=1645, p=0.79	U=1299, p=0.85	U=1387, p=0.33	U=1117, p=0.61
Fontan type (Extracardiac vs other)	U=1644, p=0.08	U=1504, p=0.34	U=1783, p=0.78	U=1450, p=0.64
Age at Fontan completion	r=-0.19, p=0.03	r=-0.20, p=0.03	r=-0.11, p=0.24	r=-0.07, p=0.48
Fenestration	U=1774, p=0.80	U=1197, p=0.12	U=1593, p=0.75	U=1232, p=0.75
AV-valve regurgitation (None-trivial vs >mild)	U=1334, p=0.10	U=1248, p=0.78	U=1513, p=0.97	U=847, p=0.03
Presence of permanent pacemaker	U=586, p=0.60	U=444, p=0.51	U=458, p=0.31	U=405, p=0.29
Heart-rate limiting medications	U=1236, p=0.61	U=1123, p=0.98	U=1102, p=0.79	U=986, p=0.92
FEV_1 %predicted	r=0.16, p=0.08	r=0.11, p=0.26	r=0.13, p=0.16	r=0.16, p=0.1
FVC %predicted	r=0.19, p=0.03	r=0.12, p=0.21	r=0.20, p=0.03	r=0.21, p=0.03

AV – atrioventricular, FEV1 – forced expiratory volume at 1 sec, FVC – forced vital capacity

5.4 Discussion

We have demonstrated a positive association between O_2 pulse slope to peak exercise and SV, in patients with a Fontan circulation. We have also confirmed the lack of association between

peak O_2 pulse and SV, as previously demonstrated by other authors [466]. Our data suggest that O_2 pulse slope, which incorporates O_2 pulse kinetics across exercise, may be a better surrogate marker than absolute peak O_2 pulse of maximal SV during exercise in Fontan patients.

5.4.1 Stroke Volume and a-vO_2 Difference

O_2 pulse is determined by SV and oxygen extraction (a-vO_2 difference). In untrained healthy subjects, SV increases during exercise but then plateaus at a sub-maximal load [469]. The a-vO_2 difference however increases as a linear function during exercise in healthy subjects, and is the main determinant of O_2 pulse from mid-exercise [214]. SV and a-vO_2 difference responses across exercise in patients with a Fontan circulation appear to be more heterogeneous.

SV has been shown to increase throughout exercise in some Fontan patients but plateau or decrease in others at maximum exertion [246,269,290,466,470]. This variable SV response may relate to differences in underlying patient characteristics but also to differences in the way SV is measured across different studies. Although submaximal and maximal HR is lower in Fontan patients than healthy controls [463,470,471], the slope of the response of HR against workload or VO_2 appears to be higher or equivalent [463,470]. The exercise chronotropic response of patients with a Fontan circulation may thus be adaptive to preload insufficiency in order to maintain ventricular filling and cardiac output. In keeping with these data, we did not find a difference in the O_2 pulse slope up to anaerobic threshold between Fontan patients and controls.

In a small study of 10 Fontan subjects before and after a single dose of sildenafil, Van Bruaene et al. showed decreasing SV (measured by ExCMR) and increasing a-vO_2 difference (measured from direct arterial and venous blood sampling) from rest to peak exercise [290]. In a study of 15 children with a Fontan circulation, Larsson et al. showed increasing a-vO_2 difference across exercise but with considerable variability in the difference at peak exercise (median 16.8, range 10.5-21.5 ml/100 ml) [269]. Rosenthal et al. demonstrated higher a-vO_2 difference in 43 children with a Fontan circulation compared to healthy controls at rest and across all stages of exercise [472].

The impact of chronic cyanosis on a-vO_2 has been poorly studied. It is possible that cyanosis, related to the either the presence of a fenestration or systemic venous collateral, results in a reduced ability to augment oxygen extraction at peak exercise or impaired microcirculatory function. We found no association between O_2 pulse parameters and either the presence of a fenestration or resting or peak oxygen saturation. This is in keeping with a study by Loomba et al. showing similar exercise arterial-venous saturation difference (utilising regional near infrared spectroscopy) in fenestrated versus non-fenestrated Fontan patients [473] and a study by Strieder et al. showing similar tissue oxygenation during exercise in cyanotic versus repaired acyanotic CHD [474].

5.4.2 Association Between O_2 Pulse Slope and Clinical Variables

In our exploratory study, we found no association between clinical variables and O_2pulseslope^{PEAK} or O_2pulseslope^{AT}, apart from a weakly positive association with baseline FVC %predicted. There was no association with other spirometry parameters.

Fontan subjects have been shown to have abnormal spirometry, with an association between FVC and peak VO_2 [164,475]. Its association with O_2 pulse slope is beyond the scope of this study, however may reflect a ventilation driven inability to augment pulmonary blood flow, and therefore SV.

5.4.3 O_2 pulse and Long-term Outcomes

Neither O_2 pulse nor its change over serial testing have been shown to predict long term adverse Fontan outcomes [476]. We speculate that, in part, this relates to its poor correlation with stroke volume in Fontan patients. It would be of interest to investigate the association between O_2 pulse slope and its change and long-term Fontan outcomes.

5.4.4 Implications and Study Limitations

In a study of 411 Fontan subjects (age 12.4 ± 3.2 years, 166 achieving a maximal exercise test) Paridon et al. found, as expected, that percent predicted peak O_2 pulse was strongly associated with percent predicted peak VO_2 and moderately with percent predicted VO_2 at AT [199]. Based upon the strength of these associations, the authors inferred that SV limitation was solely responsible for the variation in aerobic performance. However, our data suggests caution in using peak O_2 pulse as a surrogate marker for SV in Fontan patients. We speculate that this

may relate to a greater variability in the a-vO_2 difference at peak compared with sub-maximal exercise.

As this was a preliminary study, our study cohorts were small, and we did not have exCMR data available for cohort B. However, even based on our small cohort A, we were still able to demonstrate a moderately significant association between O_2 pulse slope to peak exercise and peak stroke volume.

Our exCMR protocol was performed using recumbent exercise, likely producing sub-maximal exercise testing; compared to our upright cycle ergometer CPET. The use of serial gas measurements, and CMR-augmented CPET would have allowed simultaneous assessment of gas exchange, and a-vO_2 during exercise.

Our healthy controls were retrospectively obtained from subjects previously undergoing CPET and therefore we did not have a prospectively matched cohort. However, given the older age of the control subjects, we are more likely to have underestimated the significance of the difference between our Fontan and control cohorts.

Given our relatively small sample size and short-term follow-up we were unable to examine whether O_2 pulse slope predicted adverse long term Fontan outcomes. In our exploratory analysis of cohort B, adjustments for multiple comparisons were not performed, increasing the risk of type I error. Further larger studies would be of interest to analyse the association between O_2 pulse parameters and clinical variables.

5.5 Conclusion

Peak O_2 pulse slope may be more reflective of peak stroke volume in the Fontan population than a single peak O_2 pulse value, taking into account changes in a-vO_2 during exercise. The O_2 pulse slope may be a useful submaximal marker of stroke volume.

CHAPTER SIX: NORMATIVE VALUES

This chapter is based on the following manuscript (pending submission):

Laohachai K, Webb A, Ayer J. Deriving Fontan-specific normative exercise data from well-functioning patients. Pending submission.

Abstract

Background: Exercise capacity is reduced in patients with a Fontan circulation compared to their healthy peers secondary to altered haemodynamics of the Fontan circulation. Cardiopulmonary exercise testing is routinely used in Fontan patients to assess their cardiopulmonary response to exercise and help guide management decisions, however their results are routinely compared to normative data derived from healthy individuals.

Method: Using data available from the Pediatric Heart Network, we performed a retrospective study to derive Fontan-specific normative values in a subgroup of well-functioning adolescent patients.

Results: We generated Fontan-specific regression equations in adolescent male and females for normative values of peak oxygen consumption (VO_2), maximal workload and VO_2 at anaerobic threshold.

Conclusion: We propose that Fontan-specific normative values is a more useful comparison than healthy normative data due to the known circulatory limitations of the Fontan circulation and will assist in management decision and prognostication.

6.1 Introduction

In the absence of a subpulmonary pump, the Fontan circulation is characterised by preload insufficiency. The resistance of blood flow through the pulmonary vascular bed necessitates a chronically elevated central venous pressure. These inherent limitations mean that the majority of patients with a Fontan circulation have reduced exercise capacity compared to their healthy peers [199,268]. Cardiopulmonary exercise testing (CPET) is now routinely used in Fontan patients to assess their cardiopulmonary response to exercise, and to help guide management decisions [476]. Serial testing allows prognostication, with changes in percent predicted peak oxygen consumption (peak VO_2) being a predictor of 5-year risk of adverse cardiac events [257].

As exercise parameters are altered by patient factors such as age, gender, height and weight, interpretation of results are dependent on comparison to normative data [430]. Current normative data used to interpret CPET results in patients with a Fontan circulation have largely been derived from healthy subjects with a normal circulation [51,210]. Given the inherent limitations of the Fontan circulation, a potentially more clinically meaningful comparison of CPET data is to that derived from other Fontan patients. Fontan specific normative data have been derived but are from a spectrum of Fontan patients, irrespective of their functional class [477].

We propose that a more relevant normative dataset is that derived from well-functioning asymptomatic Fontan patients (equivalent to New York Heart Association (NYHA) class 1). We therefore designed a study to derive normative data from adolescents with a Fontan circulation who are asymptomatic during ordinary physical activity. We then utilised CPET results from a separate group of adolescents with a Fontan circulation to test the derived normative data and to characterise patients with low exercise capacity.

6.2 Methods

6.2.1 Pediatric Heart Network Test Dataset

The Pediatric Heart Network (PHN) collated data on the general health status, cardiac function, and exercise capacity of 546 children and adolescents with a Fontan circulation, aged 6 to 18

years [84]. We utilised the public use dataset from the PHN Fontan cross-sectional study where of the 546 patients, 411 had complete data on CPET. The CPET protocol for this study has previously been described [199]. Briefly, subjects had performed a maximal ramp exercise test on a cycle ergometer, starting with 3 minutes in an unloaded state then increasing at an individual slope based on a predicted maximal work rate to be achieved after 10-12 minutes of cycling. Oxygen consumption, carbon dioxide production and minute ventilation were measured, and continuous electrocardiographic measurements and oxygen saturations were performed. Anaerobic threshold (AT) was determined using the V-slope method when it could be accurately determined. We extracted data on three CPET variables: peak VO_2, maximal workload, and VO_2 at AT, as described in detail below.

Using the PHN data obtained from the Congenital Heart Adolescent and Teenager (CHAT) Questionnaire, we classified patients into well-functioning and lower-functioning groups. The CHAT Questionnaire asked adolescents (aged 10-19 years) with a Fontan circulation how often their heart condition stops them from undertaking particular physical activities (figure 6.1). Those who answered never or rarely (score <2 per category) for vigorous (a), moderate (b), and light (c) activity were classified as having a NYHA class of 1 and formed the test dataset for derivation of normative CPET values [478].

Section D: PHYSICAL ACTIVITIES

The following questions ask about how your heart condition affects your ability to do physical things/activities.

D1. How often does your heart condition stop you from doing any of the following?

	NEVER	RARELY	SOMETIMES	OFTEN	ALWAYS
a. Vigorous activity like running/gym/lifting heavy objects	0	1	2	3	4
b. Moderate activity like dancing/shopping and carrying	0	1	2	3	4
c. Light activity like walking	0	1	2	3	4
d. Washing/bathing yourself	0	1	2	3	4
e. Going to places you would like to visit	0	1	2	3	4
f. Taking part in hobbies you would like to do	0	1	2	3	4

Figure 6.1. Congenital Heart Adolescent and Teenager Questionnaire

6.2.2 Statistical Analysis and Derivation of Normative Data

The means and standard deviations of exercise parameters were computed for the whole sample, the higher performing (NYHA1) group and the lower performing (NYHA>1) group. Significant differences between the two groups were investigated using an independent samples t-test for continuous variables and chi-square tests for categorical variables. Additionally, differences in the trends in the exercise parameters across age for the whole sample compared to the higher performing (NYHA1) cohort were investigated graphically.

In view of the known differences in normative values between gender and age [430], among the higher performing NYHA1 group, the means and standard deviations of the CPET variables were computed for each two year age strata (10-11 years, 12-13 years, 14-15 years, 16-17 years and 18-19 years) for the group as a whole and then separately for males and females. Differences in the means across age strata were investigated using independent samples t-tests adjusted for multiplicity, to control the overall type I error level for each comparison to 0.05.

Two methods for establishing normative values for this cohort were investigated. In the first method ("Regression Method"), we fitted a linear regression model with the exercise parameter as the outcome and an interaction between age (as a continuous variable) and gender as the predictor. Polynomial terms for age were considered to account for possible non-linearity in the trend. These regression models were then used to obtain the predicted percentiles of interest (2.5th, 10th, 25th, 50th, 75th, 90th, 97.5th) across age for each gender. In the second method ("Observed Method'), we obtained the percentiles of interest directly from the observed data for each age strata within each gender. Percentile lines across age groups were then represented graphically by fitting a smoothed curve. All statistical analysis was performed in R v4.0.1 and a p-vale of <0.05 was considered as statistically significant.

6.2.3 Validation Data Sets

Validation of the normative values obtained was explored using an external validation dataset. The external dataset were adolescents (aged 12-17 years) with a Fontan circulation, who had participated in one of two studies: i) the Australian and New Zealand Functional Outcomes after Fontan study conducted across Sydney, Melbourne and Auckland, as previously described [475] (n=37), or ii) an inspiratory muscle training study, as previously described [468] (n=16).

All subjects in the external validation datasets undertook CPET on a cycle ergometer with a protocol very similar to that undertaken in the PHN study. Performance of the normative values against the validation dataset were examined by Q–Q plots.

6.3 Results

6.3.1 Derivation of the Test Dataset

From the PHN study, a total of 291 patients had CPET data and completed the CHAT questionnaire. One-hundred and thirty-seven were categorised into NYHA1 and 154 into NYHA>1 based upon their responses in the CHAT questionnaire. Comparison of the baseline characteristics of NYHA1 and NYHA>1 are presented in table 6.1. Age at testing was similar between the two groups. In comparison to NYHA>1 patients, NYHA 1 had a lower body mass index and were less likely to be fenestrated. All other baseline characteristics were similar. Comparison of the CPET data of NYHA1, NYHA>1 and the whole PHN group are presented in table 6.2. The NYHA1 group had a significantly higher mean peak VO_2 ($p < 0.01$), peak tidal volume ($p = 0.03$) and higher VO_2 and oxygen pulse at AT ($p < 0.01$ and $p=0.03$ respectively). Maximum work rate was higher in the NYHA1 group but of borderline significance ($p=0.07$). Based upon this we chose to focus on normative data for peak VO_2, maximum work rate and VO_2 at AT. The NYHA1 group had a different and higher trajectory across age groups compared with the whole PHN group for these three variables (figure 6.2).

Table 6.1. Demographics

Clinical characteristics		**NYHA1 (n=137)**	**NYHA>1 (n=154)**	**p-value**
Gender (M:F)		89 (65%): 48 (35%)	83 (54%): 71 (46%)	0.06
Height (cm)		152.0 (13.1)	153.7 (13.8)	0.5
Weight (kg)		45.1 (14.6)	49.2 (16.5)	0.03
Body mass index		19.0 (3.6)	20.3 (4.2)	<0.01
Age of testing (years)		13.5 (2.5)	14.0 (2.7)	0.12
Dominant ventricle	Left	87 (64%)	83 (54%)	0.1
	Right	42 (31%)	62 (40%)	
	Biventricular	8 (6%)	9 (6%)	
Age of Fontan (years)		3.4 (1.9)	3.9 (2.7)	0.06
Type of Fontan	LT	83 (61%)	93 (60%)	0.09
	ECC	16 (12%)	29 (19%)	
	Other	38 (28%)	32 (21%)	
Fenestration (Y:N)		75 (55%): 62 (45%)	103 (67%): 51 (33%)	0.04
Complications (Y:N)	Thrombus	6 (4%): 131 (96%)	5 (3%): 149 (97%)	0.8
	Stroke	3 (2%): 134 (98%)	11 (7%): 143 (93%)	0.06
	PLE	1 (1%): 136 (99%)	6 (4%): 148 (96%)	0.1
	Arrhythmia	21 (15%): 116 (85%)	41 (27%): 113 (73%)	0.2

Data presented as mean (SD). NYHA – New York Heart Association, LT – lateral tunnel, ECC – extracardiac conduit, PLE – protein-losing enteropathy

Table 6.2. Descriptive statistics of exercise parameters for the two cohorts

Exercise Parameter	**Whole PHN group**	**NYHA 1 group**	**NYHA >1 group**	**p-value**
Peak VO_2	25.49 (8.58)	27.75 (6.95)	23.09 (8.20)	<0.01
Maximum workload	77.28 (37.96)	92.07 (37.55)	84.09 (36.40)	*0.07*
Maximum oxygen pulse	7.79 (3.95)	8.03 (3.09)	7.50 (4.33)	0.24
Peak VE	54.28 (22.27)	56.26 (21.86)	52.52 (21.78)	0.15
Peak tidal volume	0.95 (1.55)	1.13 (0.47)	0.80 (1.85)	0.03
VE/VCO_2 slope	35.81 (21.42)	37.83 (17.84)	34.02 (19.71)	0.09
VO_2 at AT	14.54 (11.16)	16.81 (9.30)	12.51 (9.53)	<0.01
Oxygen pulse at AT	5.19 (5.45)	5.89 (4.46)	4.57 (5.46)	0.03

Data presented as mean (SD). VO2 – oxygen consumption, VE – minute ventilation, VCO2 – carbon dioxide production, AT – anaerobic threshold

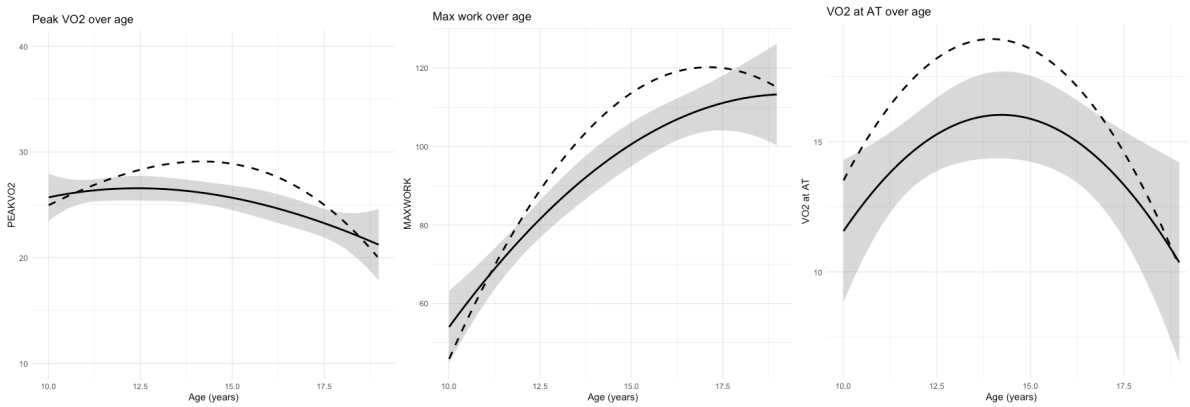


Figure 6.2. Exercise parameters across age

Solid line indicates trend for whole sample, with grey shaded area indicating the 95% CI of this trend. Dotted line indicates the trend for the NYHA1 cohort.

Within the test dataset NYHA1 group, changes in CPET parameters by age and sex are shown in table 6.3. Sex appeared to have an important influence on peak VO_2, maximum work rate and VO_2 at AT. Based upon these values separate normative values were derived for males and females.

Table 6.3. Exercise parameters for gender and age group in the NYHA1 cohort

	n	**Peak VO_2**	**Max work**	**VO_2 at AT**
Whole cohort	135			
10-11 years	46	27.06 (7.11)	65.54 (21.18)	15.69 (11.44)
12-13 years	39	29.42 (6.28)	87.44 (21.64)*	19.04 (7.47)
14-15 years	24	27.71 (5.57)	119.88 (37.65)*	17.23 (6.09)
16-17 years	17	28.45 (8.48)	128.29 (33.22)*	17.47 (10.39)
18-19 years	9	21.99 (7.52)	106.89 (58.31)*	9.93 (7.69)
Males	88	29.75 (6.42)	97.13 (40.03)	18.54 (9.39)
10-11 years	28	28.75 (7.09)	66.21 (19.91)	16.34 (12.17)
12-13 years	31	29.98 (6.39)	87.84 (22.34)*	19.76 (7.24)
14-15 years	16	29.61 (3.19)	130.44 (32.11)*	19.66 (4.24)
16-17 years	10	32.40 (8.66)	141.20 (34.87)*	21.36 (10.96)
18-19 years	3	28.87 (6.35)	157.00 (70.55)*	10.13 (10.96)
Females	47	23.83 (6.36)	82.94 (31.59)	13.45 (8.46)
10-11 years	18	24.43 (6.47)	64.50 (23.57)	14.68 (10.45)
12-13 years	8	27.25 (5.73)	85.88 (19.99)	16.22 (8.19)
14-15 years	8	23.91 (7.43)	98.75 (41.04)*	11.99 (6.01)
16-17 years	7	22.81 (4.01)	109.86 (21.10)*	11.90 (6.81)
18-19 years	6	18.55 (5.64)	81.83 (34.48)	9.83 (6.83)

** Age group mean is significantly different from reference level (10-11 years)*
Data presented as mean (SD). VO_2 – oxygen consumption, AT – anaerobic threshold

6.3.2 Derivation of Normative Values

Due to the small numbers in the 18-19 year cohort, these patients were excluded from the following analyses. Curves for each sex (females at the top, males at the bottom) showing normative data for maximal workload, peak VO_2, and VO_2 at AT are shown in figures 6.3 and 6.4 respectively). Curves from both derivation methods are shown (Regression on the left, Observed on the right).

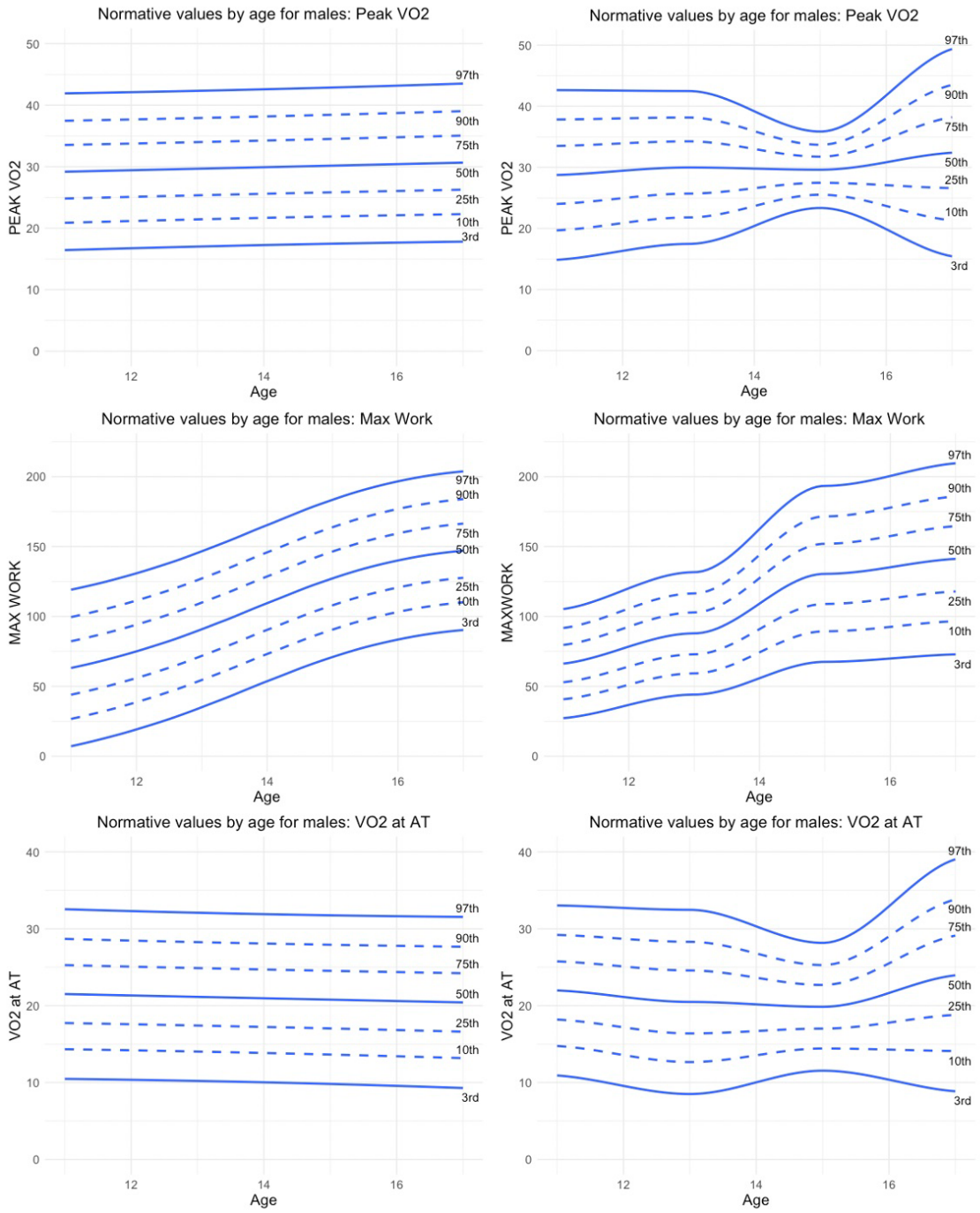


Figure 6.3. Normative values for males

VO_2 – oxygen consumption, AT – anaerobic threshold

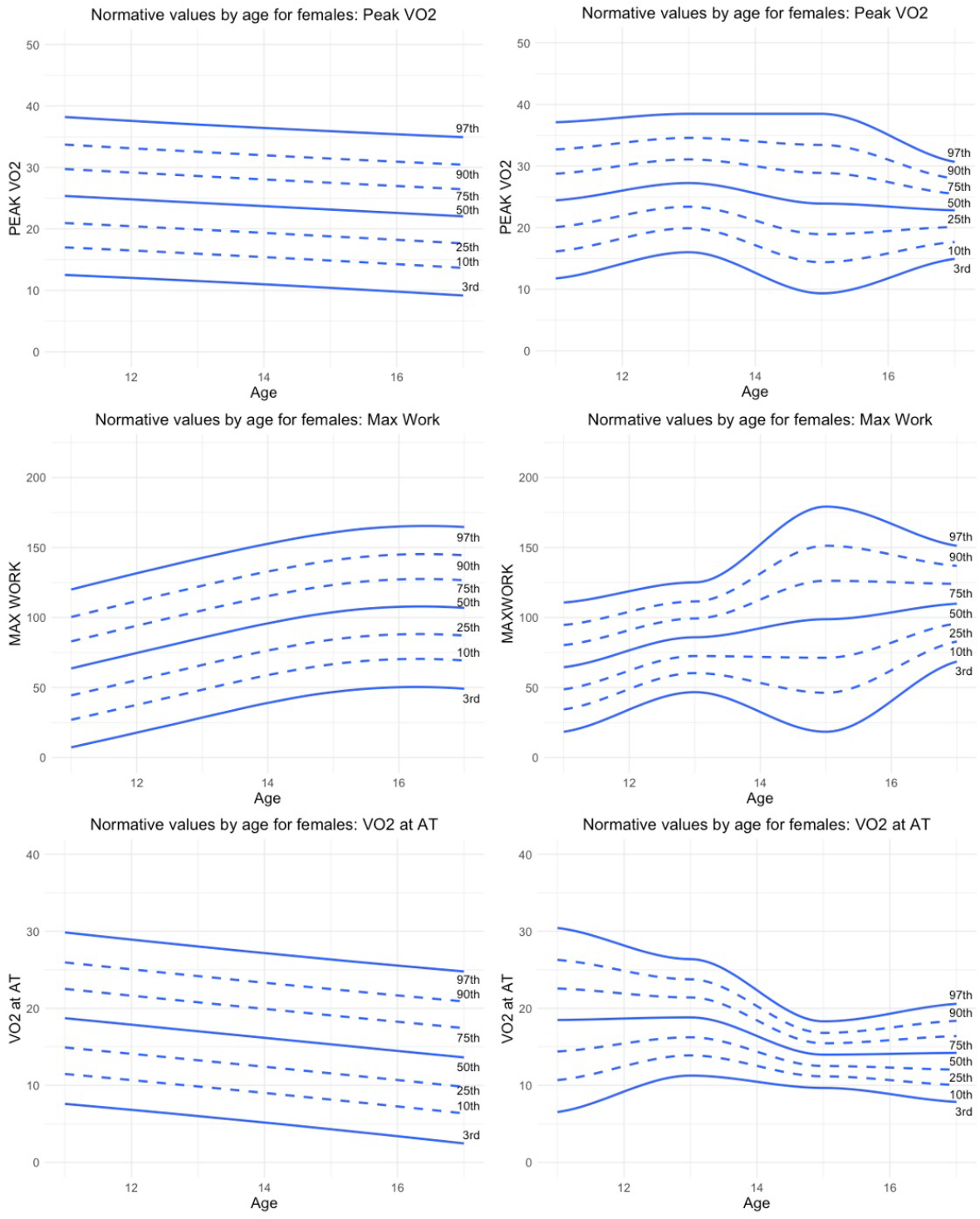


Figure 6.4. Normative values for females

VO_2 – oxygen consumption, AT – anaerobic threshold

6.3.3 Derivation of the External Validation Dataset

Comparing the external validation cohort to the NYHA1 group, the validation cohort were older (mean age 14.8 versus 13.5 years, $p<0.01$) and had higher mean height (162.5 versus 152 cm, $p<0.01$), weight (58.7 versus 45.1 kg, $p<0.01$) and body mass index (22.0 versus 19.0 kg/m^2, $p<0.01$). Additionally, the validation cohort had an older average age of Fontan completion (4.8 versus 3.4 years, $p<0.01$). Due to the selection criteria of the previous studies, all patients in the validation cohort had extracardiac conduits and a smaller proportion were fenestrated 55% versus 26%, $p<0.01$). The external CPET values for subjects in the validation cohort are plotted on the corresponding normative curves (maximal workload, peak VO_2, and VO_2 at AT for males and females are shown in figures 6.5 and 6.6 respectively). The Q-Q plots for the Regression method (left panel) and the Observed Method (right panel) are shown in figure 6.7 and 6.8. From these Q-Q plots it appeared that the distribution of data from the validation and test cohorts were best approximated by the Regression method.

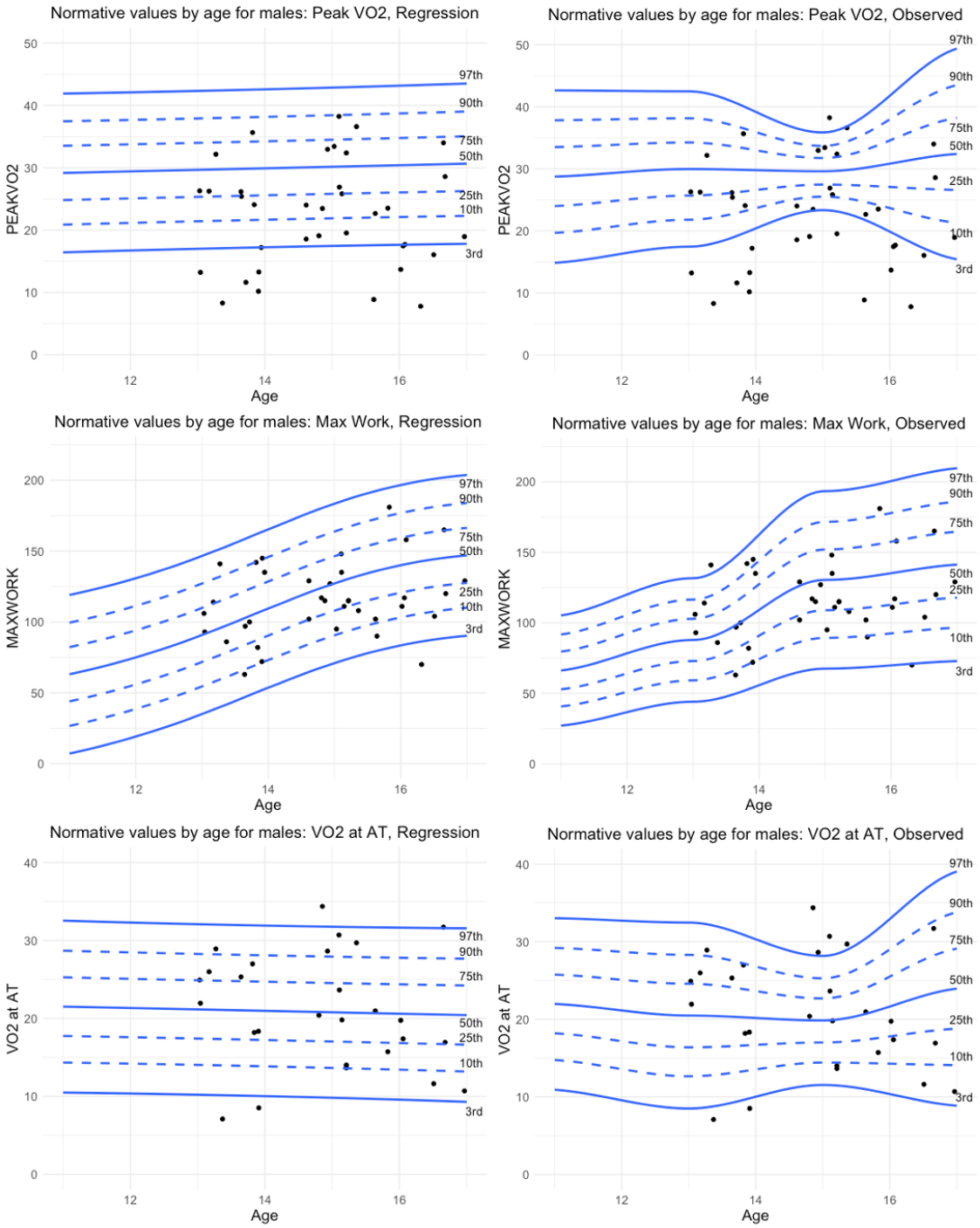


Figure 6.5. External validation for males

VO_2 – oxygen consumption, AT – anaerobic threshold

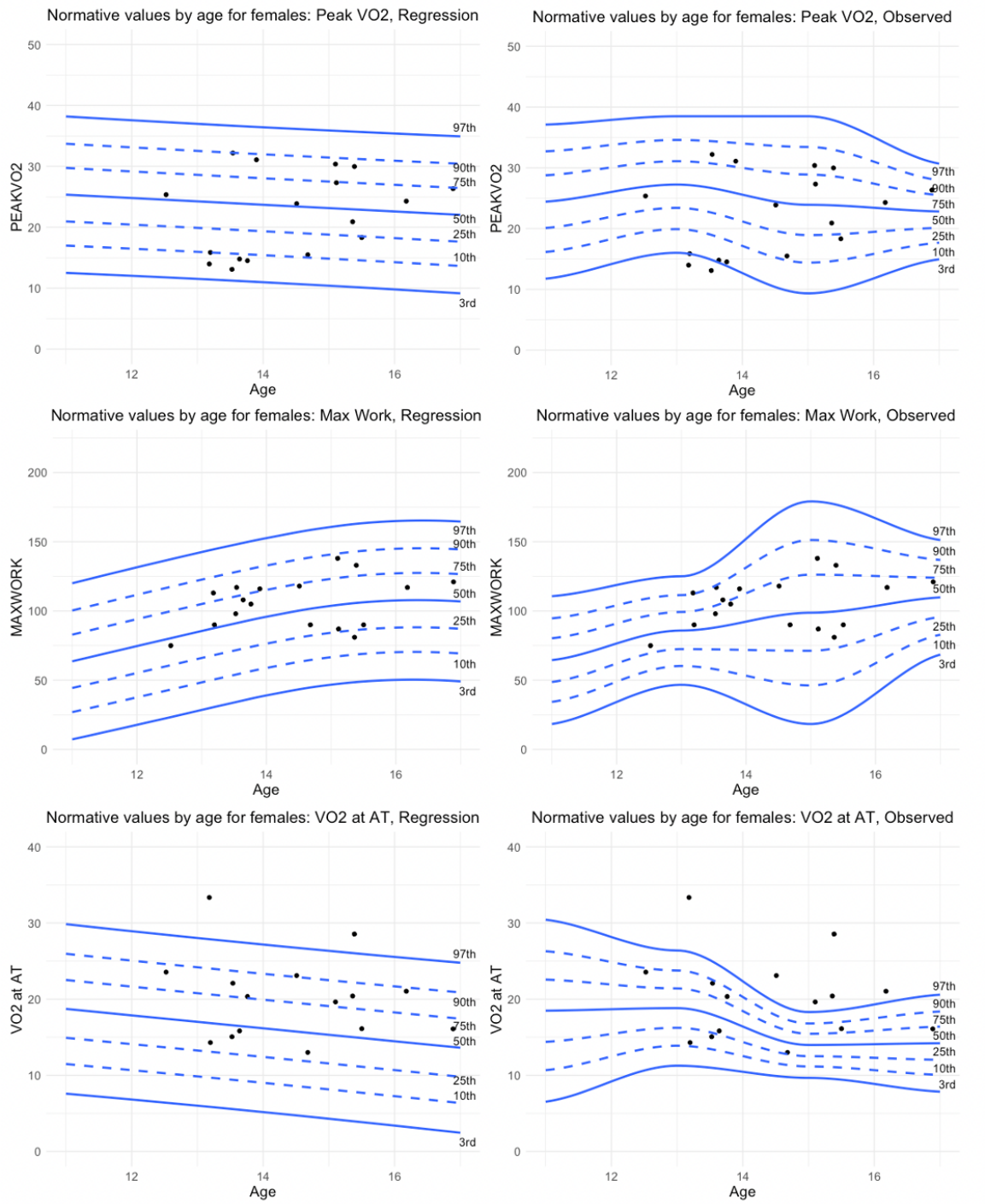


Figure 6.6. External validation for females

VO_2 – oxygen consumption, AT – anaerobic threshold

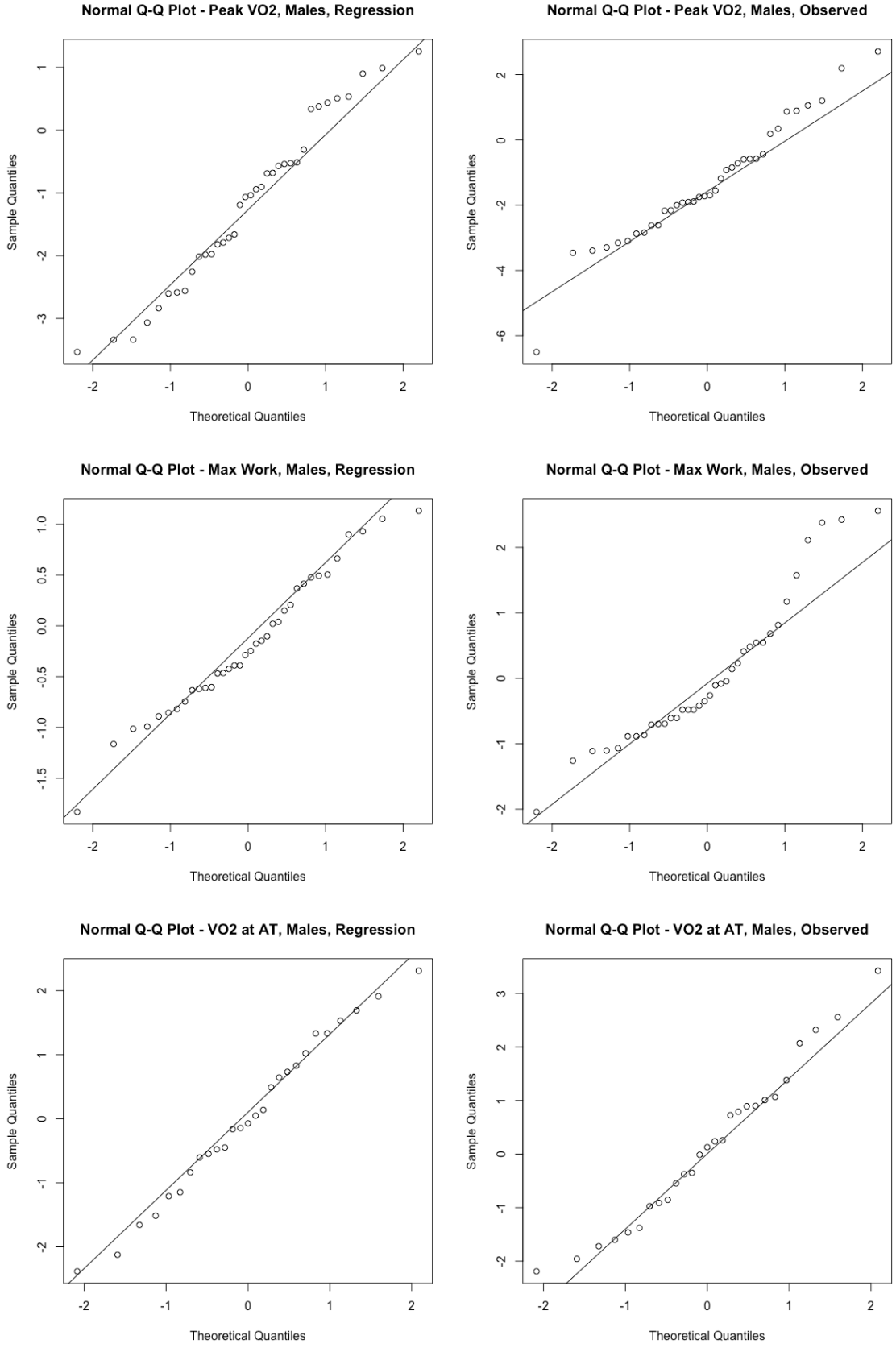


Figure 6.7. Q-Q plots for males

VO_2 – oxygen consumption, AT – anaerobic threshold

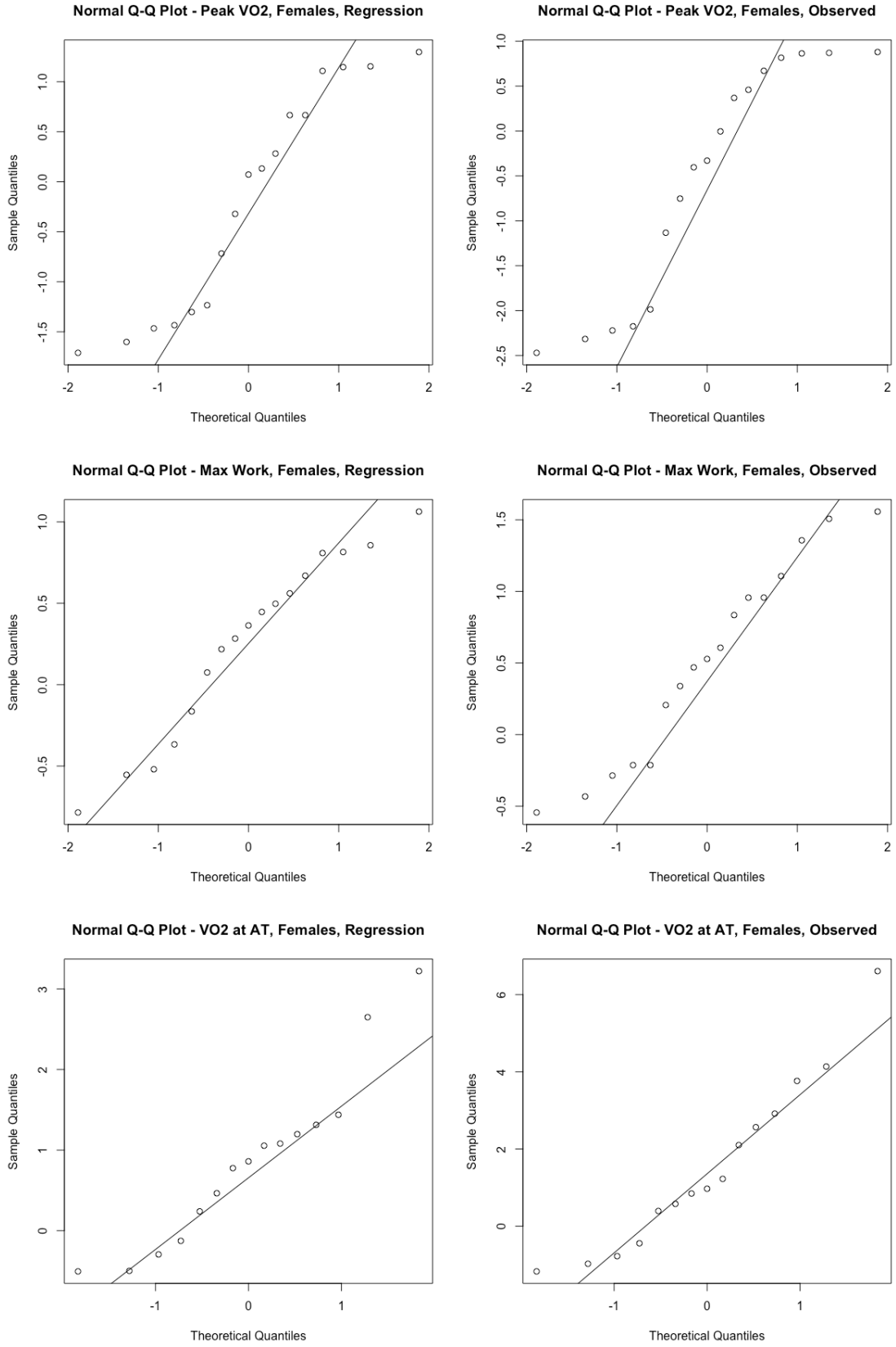


Figure 6.8. Q-Q plot for females

VO_2 – oxygen consumption, AT – anaerobic threshold

From these Q-Q plots it appeared that the distribution of data from the validation and test cohorts were best approximated by the Regression method. From the regression method, the equations describing the normative data were as follows (VO2 – oxygen consumption, AT – anaerobic threshold):

$$Z_{\text{Peak VO2,Male}} = \frac{\text{PeakVO2} - (26.45 + 0.25 \times \text{Age})}{6.42}$$

$$Z_{\text{Peak VO2,Female}} = \frac{\text{PeakVO2} - (31.43 - 0.55 \times \text{Age})}{6.36}$$

$$Z_{\text{MaxWork,Male}} = \frac{\text{MaxWork} - (97.83 + 330.48 \times \text{Age} - 60.48 \times \text{Age}^2 - 89.33 \times \text{Age}^3)}{40.03}$$

$$Z_{\text{MaxWork,Female}} = \frac{\text{MaxWork} - (84.14 + 166.36 \times \text{Age} - 92.59 \times \text{Age}^2 - 40.30 \times \text{Age}^3)}{31.59}$$

$$Z_{\text{VO2atAT,Male}} = \frac{\text{VO2 at AT} - (23.51 - 0.18 \times \text{Age})}{5.87}$$

$$Z_{\text{VO2atAT,Female}} = \frac{\text{VO2 at AT} - (28.07 - 0.85 \times \text{Age})}{5.12}$$

6.3.4 Comparison of Subjects in the Validation Dataset

We performed analyses of clinical characteristics of the validation cohort comparing those who fell in the bottom 25th percentile of maximum workload with those who were above the 25th percentile (table 6.4). The lower performing group had a higher body mass index (20.3 versus 23.2, p=0.02) and had a higher proportion with fenestration (47.6% versus 13.0%, p=0.02). The other baseline characteristics were similar between the groups.

Table 6.4. Clinical characteristics of lower and upper 25th centiles

Clinical characteristic	Lower 25th centile (n=20)	Upper 25th centile (n=23)	p-value
Age (years)	14.7 ± 1.1	14.8 ± 1.2	0.86
Height (cm)	161.5 ± 9.3	163.8 ± 9.3	0.38
Weight (kg)	61.7 ± 19.0	54.7 ± 10.2	0.12
Body mass index	23.2 ± 5.4	20.3 ± 2.8	0.02*
Age of Fontan	4.8 ± 1.4	4.9 ± 1.1	0.88
Ventricular dominance (L:R:B)	16:12:2	12:8:3	0.15
Fenestration (Y:N)	11 :9	3:20	<0.05*

L – left, R – right, B – biventricular

6.4 Discussion

In this study, we have utilised data from the PHN public use dataset to generate normative values for CPET parameters in adolescents with a Fontan circulation. We have utilised the same dataset as Seckler et al. but limited the data to only well-functioning Fontan patients (equivalent to NYHA 1) [477]. We have been to establish that the CPET parameters were different in the NYHA 1 compared to the whole group. We have also been able to establish the different patterns related to age in females versus males. Finally, we propose that modelling normative values by a regression method may be more appropriate than modelling percentiles of interest directly from the observed data.

Patients with a Fontan circulation have reduced exercise capacity with less than 30% of patients achieving a normal VO_2 on exercise testing [84]. Maximal peak VO_2 achieved is ~65% [84,197,199]. Absence of a subpulmonary pump in the Fontan circulation results in inherent circulatory limitations including chronically elevated systemic venous pressure and pulmonary resistance, and reduced ventricular preload, resulting in reduced stroke volume [267]. Other factors that can further limit augmentation of stroke volume with exercise are systolic and diastolic ventricular dysfunction and increased arterial stiffness [479]. Given these inherent limitations, it may be more useful to compare Fontan patients against normative data for healthy, well-functioning Fontan patients. This would allow both clinicians and patients to gauge cardiorespiratory performance in relation to other well patients with a similar circulatory physiology.

6.4.1 Study Limitations

Our classification of a well-functioning Fontan patient was limited to only three simple self-answered questions based on the CHAT questionnaire. Although we were not able to validate this with other parameters, we have shown that those that we classed as NYHA 1 had lower weight and body mass index that the NYHA>1 group, in keeping with a higher physical functional class.

We validated our normative data with only a small test set of 53 patients. Although we found good agreement of the distribution with the normative data, further validation is required against other Fontan cohorts.

Scaling against body composition (e.g., fat-free mass and lean mass) may be better than measures of body mass for expression of peak oxygen consumption and other exercise parameters [480]. However, this data was not available on our cohort of patients and may be useful in establishing future Fontan-specific nomograms.

6.5 Conclusion

We have derived normative data for well-functioning adolescents with a Fontan circulation. We propose that this is a more useful comparison than healthy normative data due to the known circulatory limitations of the Fontan circulation and will assist in management decision and prognostication.

CHAPTER SEVEN: CONCLUSION AND FUTURE DIRECTION

Fontan patients have altered haemodynamics with chronically elevated central venous pressures and reduced preload. With absence of a subpulmonary pumping chamber, patients with a Fontan circulation are more reliant on adaptation of the respiratory and skeletal muscle function during exercise. They have abnormal lung function with reduced lung volumes and restrictive lung physiology. Their exercise capacity is reduced compared to healthy peers.

In this thesis we aimed to: i) explore the mechanisms for and determinants of altered lung function, 2) investigate a specific intervention targeting respiratory function, namely inspiratory muscle training and, iii) explore new measures of exercise performance. This thesis provides a more in-depth understanding of respiratory function, haemodynamic responses to exercise, and altered cardiopulmonary interactions, in patients with a Fontan circulation. We also present novel measures of exercise performance in these patients. This knowledge will provide insight into improving the long-term outlook of Fontan patients.

7.1 Inspiratory Muscle Training

We have shown that six weeks of inspiratory muscle training is associated with improved inspiratory muscle strength, improved ventilatory efficiency of exercise and increased resting cardiac output in young patients with a Fontan circulation. Since the publication of our results three other studies have examined the impact of IMT in patients with a Fontan circulation, with one study comparing IMT to aerobic training [315,481,482].

Wu et al. recruited 11 adults with a Fontan circulation and examined the effects of 12 weeks of IMT [482]. Median age was 28.8 years, and median age of Fontan completion was 7.8 years. Seven had a lateral tunnel Fontan, 3 with an AP connection and 1 with an ECC. The IMT device was set to 40% of measured MIP and their training program consisted of 30 minutes per day (in 1 or 2 sessions), 5 days a week for 12 weeks. The training sessions were self-recorded, and subjects were contacted weekly for review. Resistance was increased by 2 cmH_2O every 2 weeks, up to a maximum of 41 cmH_2O. At baseline, they found average MIP to be within the normal range (98.8% of predicted), with only 2 patients having a MIP of less than 70%. They found no correlation between baseline MIP and peak VO_2. Following IMT there was an improvement in maximal work rate and a trend towards improvement in peak VO_2 and VE/VO_2 slope, however neither of these were statistically significant. They found no change in MIP or

peak tidal volume. Wu et al. reported that only 8 out of the 11 subjects were compliant with the IMT program, with the 8 adhering to at least 2 consecutive weeks of at least 120 minutes per week [482]. This is low compared to their prescribed program of 150 minutes per week for 12 weeks, and the lack of improvement in MIP is suggestive of ineffective training. The sample size was also small, further limiting the ability of the training program to show a change in measures of exercise performance.

Fritz et al. also examined IMT in adults with a Fontan circulation [481]. Their larger cohort of 42 patients were randomised into an intervention or control arm. The intervention cohort underwent telephone-supervised daily IMT for 10-30 repetitions a day for 6-months, an individually adjusted load. They found improvement in resting oxygen saturations, indicative of improvement in ventilation/perfusion matching; but there no improvement in lung function or exercise capacity [481].

More recently, Turquetto et al. performed a randomized controlled trial looking at combined IMT and aerobic training [315]. Fontan subjects were randomized to either personalised aerobic training or IMT; and a non-exercise group was used as a control. Their IMT regime consisted of 3 sets of 30 repetitions at 60% of individual MIP for four months, with adjustment in load throughout the duration of the trial. Aerobic training consisted of 60-minute supervised, individually prescribed exercise training (treadmill, light resistance and stretching) 3 times a week for the 4 months. They found an improvement in peak VO_2 with both these training regimes, but a higher improvement in the aerobic training group [315].

In addition to these studies on IMT, Ait Ali et al. undertook an assessment of controlled breathing (respiratory training) in Fontan patients, training both the inspiratory and expiratory muscles [483]. They used a method which forms the basis of a yoga practice, involving conscious diaphragmatic contraction and relaxation. This involved weekly 2-hour sessions for three months of respiratory training. Diaphragmatic respiration increased intrathoracic negative pressure, optimizing systemic venous return. They demonstrated improvement in peak VO_2 and endurance time.

To limit underlying patient heterogeneity, our study only included young subjects with a non-fenestrated extracardiac conduit. We demonstrated a positive association between MIP change

and baseline peak VO_2 and greatest improvements in MIP in those with the lowest baseline VE/VCO_2 slope, suggesting improvements in MIP may be greatest in those with higher baseline exercise capacity. However, we also demonstrated a trend for the largest improvements in VE/VCO_2 slope in those in the lowest two tertiles of MIP change, suggesting that IMT may be most beneficial in those with the highest baseline VE/VCO_2 slope. Taken together, these findings imply that the greatest benefit of IMT may occur in Fontan subjects that are the least fit and that in this group, even small changes in MIP may be of value.

As both peak VO_2 and VE/VCO_2 slope show a gradual decline with age in patients with congenital heart disease [288,461] and as VE/VCO_2 may be higher in non-extracardiac conduit types of Fontan circulation, we speculate that IMT could have an even greater benefit in older adults with either a lateral tunnel or AP type Fontan circulation. This is supported by Wu et al. demonstrating improvements in workload after IMT in an older cohort with a high proportion with non-extracardiac conduits.

These studies are suggestive that with the correct training regime, including adjustments of the load according to the patient's individual MIP, both IMT and respiratory training have the potential to improve exercise capacity. However, larger studies need to be undertaken to determine the potential benefits, particularly in the setting of other physical activity programs. IMT is a simple and safe intervention that can be used to improve respiratory muscle strength.

Studies assessing the benefits of IMT in Fontan patients are still limited with small study sizes. It would be beneficial for future studies to specifically examine the effects of aerobic and resistance exercise training on inspiratory muscle strength. Furthermore, the effects of combining IMT with aerobic and/or resistance training on peak VO_2 and other exercise parameters should be the focus of future trials.

Lambert et al. have shown elevated muscle sympathetic nerve activity and reduced endothelial function in adults with a Fontan circulation [313]. Chiappa et al. have shown that IMT improves blood flow to resting and exercising muscle in adults with acquired heart failure [359]. Assessing the effect of IMT on muscle sympathetic nerve activity, endothelial function and skeletal muscle blood flow would be of interest.

7.2 Lung Function

We have shown that Fontan patients have low lung volumes and abnormal lung function with abnormal DLCO and DLNO. Our findings suggest that these abnormalities are largely driven by low alveolar volume. On oscillometry, we found increased reactance (lung stiffness as measured by X5) in our cohort after Fontan completion strongly correlates with alveolar volume.

To explore the changes in lung stiffness that occur along the single ventricle palliation pathway, it would be of interest to undertake oscillometry during the interstage period. Oscillometry requires less patient cooperation than other forms of lung function assessment and can be performed in infants and young children. The impact of changes in pulmonary blood flow on lung stiffness brought about initial palliation, bidirectional Glenn anastomosis and Fontan completion would be of interest.

Larger studies examining reactance in patients in a Fontan circulation may along determination of the association of lung stiffness with other clinical factors, exercise capacity and long term outcomes.

7.3 O_2 Pulse Slope

We have demonstrated that oxygen pulse slope may be a useful submaximal marker of stroke volume in Fontan patients. The oxygen pulse slope up to peak exercise may be more reflective of peak stroke volume in the Fontan population than a single peak O_2 pulse value, taking into account changes in arteriovenous oxygen differences during exercise. Examining the association between oxygen pulse slope and stroke volume, as measured by exercise CMR, in other cohorts will help validate our findings.

MRI assisted CPET or invasive measures of arterial and venous oxygen content along with exercise MRI would allow simultaneous measure of stroke volume, the arterio-venous oxygen difference and oxygen pulse.

To date, peak O2 pulse has not been found to be associated with long term Fontan outcomes. Whether the O2 pulse slope performs better as a marker of risk should be studied in other large Fontan cohorts.

7.4 Normative Data

We have derived normative data from well-functioning adolescents with a Fontan circulation. We propose that this is more clinically useful normative data in comparison to utilising normative data derived from healthy adolescents. However, we acknowledge that our approach needs to be validated using other test cohorts. Establishing a multi-institutional Fontan exercise registry would allow pooling of data for further research to be undertaken and facilitate standardisation of testing protocols and normative data.

7.5 Summary

This thesis characterises the impaired lung function and abnormal exercise capacity in patients with a Fontan circulation. While there are inherent limitations of the Fontan circulation, a better understanding of the mechanisms behind the dysfunction will provide insight into improving management and morbidity for these patients. In this thesis, we have identified factors that can potentially be modified and improve outcome, such as inspiratory muscle weakness and age of Fontan completion. We have demonstrated a therapy (inspiratory muscle training) that may improve exercise capacity and propose a new measure of submaximal exercise (oxygen pulse slope). Lastly, as cardiopulmonary exercise testing is becoming part of routine surveillance of Fontan patients, we have derived normative data for well-functioning Fontan patients to allow more meaningful percent predicted calculations. Further research involving larger cohorts are required to improve exercise capacity and the long-term outcomes of all patients with a Fontan circulation.

These studies demonstrate that the aetiology of exercise intolerance in the Fontan cohort is multi-factorial, and that single measures are not reflective of significant alterations in haemodynamics. A comprehensive assessment including echocardiography, lung function testing, cardiopulmonary exercise testing and cardiac magnetic resonance imaging; is required

in all Fontan patients at multiple time points to accurately assess and monitor their functional status. Future studies are required to further understand the mechanisms of the physiological changes and optimise potential treatment.

CHAPTER EIGHT: APPENDICES

8.1 Appendix 1. International Physical Activity Questionnaire

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information
More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

Reproduced from Craig et al. [413]

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

8.2 Appendix 2. Patient Inspiratory Muscle Training Diary

INSPIRATORY MUSCLE TRAINING DIARY

Dr J Ayer, A/Professor G Sholler, Professor D Winlaw, **The Heart Centre for Children, The Children's Hospital at Westmead, Ph: 02 9845 2345**

A/Professor H Selvadurai, **The Department of Respiratory Medicine, The Children's Hospital at Westmead, Ph: 02 98453395**

Dr Neil Caplin, **The Department of Medical Imaging, The Children's Hospital at Westmead, Ph: 02 9845 2931**

DATE	TIME IMT STARTED	DURATION OF IMT	COMMENTS	SIGNATURE

Version 2, SEPTEMBER 6TH 2014

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