Exercise Pathophysiology and Exercise Therapy in Pulmonary Arterial Hypertension

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Summary

Pulmonary hypertension (PH) is an incurable disorder of the pulmonary circulation, characterised by progressive vascular remodelling, vasoconstriction and increased right ventricular afterload. The direct consequence of this is impaired cardiac output, initially in response to exercise and in later stages of the disease at rest. Progressive exercise limitation is the cardinal clinical feature and ultimately premature death from right heart failure ensues.

Available disease targeted treatments slow progression, however, PAH remains incurable with a high symptom burden and prognosis remains poor. Lung transplantation provides the only hope of cure. A small proportion of patients are eligible and fit enough for transplantation, notwithstanding the availability of suitable donor lungs and associated post-transplant morbidity in those who survive. Current drug therapy is expensive and is limited to three classes of pulmonary arterial vasodilator. The most effective form of treatment, epoprostenol, is invasive, requiring central venous access and significantly impacts on quality of life despite the associated improvements in exercise capacity.

The abnormalities in the right ventricle and pulmonary circulation are well established in PH. More recent evidence has highlighted multi-system abnormalities in PH patients, with muscle dysfunction from a clinical to cellular level, systemic inflammation and insulin resistance. It is unclear whether these changes are a result of the atrophying effects of low cardiac output, or a systemic process associated with PH but independent from cardiac function.

Historically exercise had been avoided in PAH, however recent evidence suggests it provides significant benefit in terms of exercise capacity and quality of life. Exercise is an attractive therapeutic option for many reasons. With established infrastructure, it is cost effective, sustainable and provides wide-ranging benefits out with the pulmonary circulation, such as reduced risk of diabetes, cardiovascular disease and improved mental health. The exercise programmes studied in PAH have been wide ranging in terms of the approach used and have shown variable degrees of success. The most successful programmes have employed fairly intensive exercise regimens and many countries do not have the

resource or infrastructure to adopt this approach. The role of exercise therapy in PAH and the mechanisms by which it exerts a beneficial effect remain poorly understood. It is also unclear what the best outcome measure is to determine the efficacy of exercise-based interventions in PAH. The feasibility of intensive exercise therapy in countries where residential exercise programmes are not robustly established remains untested.

The aims of this thesis are

- To establish the population demand, feasibility and safety of PH specific exercise therapy in a UK pre-capillary PH population.

- To explore potential physiological and biological mechanisms behind the improvements seen in exercise capacity in order to gain a greater understanding of the disease process.

- Identify key components of the exercise programme to allow recommendations for effective PAH exercise prescriptions moving forwards.

To address the aims of the thesis, three studies were performed. The first two studies were precursors to the main study discussed in chapters 5 to 9:

1. “Assessing the demand for exercise therapy in a Scottish PAH population” (Chapter 2):

2. “The ventilatory, gas exchange and haemodynamic response to upright and supine exercise”, (Chapter 3),

3. “The effect of adding exercise training to optimal drug therapy in Pulmonary Arterial Hypertension”.

Chapter 1 provides an introduction to exercise physiology in PAH and describes the current state of knowledge regarding exercise therapy in PAH

Chapter 2 describes the current standard of care for patients with PAH in Scotland and the demand for exercise therapy in the Scottish PAH population. From this

data, it is clear that there is significant enthusiasm for exercise rehabilitation in PAH. Potential barriers to its uptake or implementation were explored and these included employment, carers commitments and proximity to the exercise venue.

In Chapter 3, the ventilatory, gas exchange and haemodyanamic responses to upright and supine exercise are discussed in order to determine the degree to which exercise capacity is reduced in the supine position and the physiological changes that accompany this. This information was used to help design the protocol used for supine endurance exercise with invasive haemodynamic measurements, discussed in Chapter 7. From this data, it is clear that exercise capacity is significantly reduced in the supine position, this appeared to be due to changes in ventilatory efficiency and was associated with significant alterations in the stroke volume – heart rate response to exercise.

Chapter 4 outlines the study protocol and exercise intervention for the main study in this thesis "The effect of adding exercise training to optimal drug therapy in Pulmonary Arterial Hypertension".

Chapter 5 describes the overall efficacy of exercise therapy in PAH in relation to the primary outcome measures of the study "The effect of adding exercise training to optimal drug therapy in Pulmonary Arterial Hypertension"; specifically, 6 minute walk distance (6MWD), quality of life and right ventricular ejection fraction. Analysis of potential "responders" and "non-responders" and factors associated with poorer prognosis are discussed in an exploratory post-hoc analysis.

Chapters 6 to 9 adopt a systems-based approach to describe the physiological and pathobiological changes that are present in the studied PAH cohort and the factors that change with exercise therapy. Each chapter discusses the specific relationship with responders and non-responders to exercise therapy in more detail. The specific methods used to assess each system are discussed in the individual chapters and the overall protocol for the exercise therapy is discussed in Chapter 2.

Chapter 6 discusses the impact of exercise therapy on lung function, looking at cardiopulmonary exercise testing (CPET), pulmonary function tests and mouth

pressures. The sensitivity of different exercise outcome measures are also explored including cycling endurance time, 6MWD, and incremental CPET.

Chapter 7 investigates invasive haemodynamic responses to exercise therapy using both resting and exercise right heart catheterisation. A steady state exercise protocol is used to assess serial measurements of haemodyamics and oxygen extraction throughout exercise.

In Chapter 8, a non-invasive approach to assessing cardiac status before, during and after exercise therapy is investigated using cardiac magnetic resonance imaging and N-terminal pro brain natriuretic peptide (NTproBNP). These changes are correlated with invasive haemodynamic markers.

Chapter 9 discusses the impact of exercise therapy on muscle function, metabolism and levels of systemic inflammation. Serum and muscle biomarkers are explored to provide pilot data on potential mechanisms for PAH myopathy and how it may be reversed.

Collectively these chapters demonstrate improved exercise capacity and quality of life in response to exercise therapy in PAH. This is associated with improved ventilatory efficiency and cardiovascular function, with changes being linked to prognosis. Potential mechanisms behind these improvements are explored and include the reversal of deconditioning, lung recruitment, improved vascular endothelial health, reduced atrial stretch and reduced hyperventilation. Chapter 10 outlines the major findings and conclusions of this research and future research avenues to be explored.

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Declaration

The work presented in this thesis was undertaken during my post as a Clinical Research Fellow at the Scottish Pulmonary Vascular Unit at the Golden Jubilee National Hospital, Glasgow and the Institute of Cardiovascular and Medical Sciences at the University of Glasgow. I was supervised by Dr Martin Johnson and Dr David Welsh.

The work reported in this thesis was undertaken by me, with the assistance of a number of colleagues. Joanna Ford, Golden Jubilee National Hospital, provided physiotherapy input and assisted with scheduling of testing and exercise sessions. Dr Michael McGettrick (MMc), Scottish Pulmonary Vascular Unit, reported Cardiac MRI scans. Dr Karine Pinel (KP) and Josephine Cooney (JC) in Dr Christian Delles's lab at the University of Glasgow, provided assistance with mirco-RNA experiments, quantitative PCR and cytokine assays. Dr David Ritchie (DR) and Dr Mark McCleary (MM), Musculoskeletal Radiologists at Gartnavel General Hospital and Glasgow Royal Infirmary, assisted with learning of the muscle biopsy technique. All statistical analyses were performed by me.

Work relating to this thesis has been published or submitted to peer-reviewed journals and presented at international conferences.

The writing of this thesis constitutes my own work, written solely by me. Signed

Alison MacKenzie, March 2020

Publications and abstracts relating to thesis

Publications

Medical therapies for the treatment of pulmonary arterial hypertension: How do we choose? ***MacKenzie AM,*** Peacock AJ. Curr Hypertens Rep. 2015 Jul;17(7):56.

Demand for exercise training in patients with pulmonary arterial hypertension in Scotland. ***MacKenzie AM,*** Johnson MK. Eur Respir J. 2015 Nov;46(5):1513-5.

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Abstracts

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Efficacy and feasibility of pulmonary hypertension specific exercise rehabilitation in a UK setting, British Thoracic Society, December 2018

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Adherence to exercise training in PAH and impact of concurrent health problems. European Respiratory Society. September 2017

Role of heart rate recovery in detecting response to treatment in PAH. European Respiratory Society 2016.

Patient perspectives on exercise training in pulmonary arterial hypertension. European Respiratory Society. August 2015

The haemodynamic, ventilatory and gas exchange response to upright and supine exercise. Scottish Sports Medicine Symposium. May 2015.

Definitions and abbreviations

AE	Adverse event
ACSM	American College of Sports Medicine
AT	Anaerobic threshold
ATS	American Thoracic Society
AUC	Area under the curve
BMI	Body mass index
BMPR2	Bone Morphogenic protein receptor type 2
BP	Blood pressure
CHD-PAH	Congenital heart disease associated PAH
CI	Cardiac index
CMR	Cardiac magnetic resonance imaging
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise test
CRP	C-reactive protein
CTD-PAH	Connective tissue disease associated PAH
CTEPH	Chronic thromboembolic pulmonary hypertension
ddCt	Delta delta threshold cycle
EOS	End of study
ECG	Electrocardiogram
eRHC	Exercise right heart catheterisation
ERS	European Respiratory Society
FEV1	Forced expiratory volume in one second
FPG	Fasting plasma glucose
FPI	Fasting plasma insulin
FVC	Forced vital capacity
GJNH	Golden Jubilee National Hospital
HDL	High density lipoprotein
HOMA	Homeostasis Model Assessment
HPAH	Heritable pulmonary arterial hypertension
HR	Heart Rate
HR/VO_2 slope	Slope of heart rate / oxygen uptake
IPAH	Idiopathic Pulmonary Arterial Hypertension

IR	Insulin resistance
KCO	Transfer coefficient of the lung for carbon monoxide
LDL	Low density lipoprotein
LVEDV	Left ventricular end diastolic volume
LV	Left ventricle
MAPK	Mitogen activated kinase
mGPS	Modified Glasgow prognostic score
MEP	Maximal expiratory mouth pressure
MIP	Maximal inspiratory mouth pressure
miR	Micro-RNA
mPAP	Mean pulmonary artery pressure
MRI	Magnetic resonance imaging
MVC	Maximum voluntary contraction
N	Newtons
NLR	Neutrophil to lymphocyte ratio
NTproBNP	N-terminal pro-Brain Natriuretic Peptide
PAEC	Pulmonary artery endothelial cell
PAH	Pulmonary arterial hypertension
PA-aO_2	Alveolar – arterial gradient
$Pa0_2$	Partial pressure of Oxygen
$PaCO_2$	Partial pressure of Carbon Dioxide
PAP	Pulmonary artery pressure
PAWP	Pulmonary artery wedge pressure
Pca	Pulmonary artery compliance
$PETO_2$	End tidal partial pressure of oxygen
$PETCO_2$	End tidal partial pressure of carbon dioxide
PFT	Pulmonary Function Test
PH	Pulmonary hypertension
PLR	Platelet to lymphocyte ratio
POPH	Portopulmonary hypertension
PVR	Pulmonary vascular resistance
Q	Flow
RAP	Right atrial pressure
REC	Research ethics committee
RER	Respiratory exchange ratio

RHC	Right heart catheter
RNA	Ribonucleic acid
RR	Respiratory rate
RT-PCR	Reverse transcription polymerase chain reaction
RV	Right ventricle
RVEDV	Right ventricular end diastolic volume
RVEF	Right ventricular ejection fraction
RVESV	Right ventricular end systolic volume
RVOPF	Right ventricular oscillatory power fraction
SAE	Serious adverse event
SaO_2	Arterial blood oxygen saturation
SD	Standard deviation
SPAP	Systolic pulmonary artery pressure
SpO_2	Peripheral oxygen saturations
SPVU	Scottish Pulmonary Vascular Unit
SVO_2	Mixed venous oxygen saturations
SV	Stroke volume
TCA	Tricyclic acid
TLC	Total lung capacity
TPR	Total pulmonary resistance
TLCO	Transfer factor of the lung for carbon monoxide
QOL	Quality of life
VCO_2	Carbon dioxide output
VA	Alveolar volume
V_D	Dead Space
V_D/V_T	Dead Space to Tidal Volume Ratio
VE/VCO_2 slope	Slope of minute ventilation / carbon dioxide output
V_E	Minute ventilation
V_E/VCO_2	Ventilatory equivalent for carbon dioxide
V_E/VO_2	Ventilatory equivalent for oxygen
VLDL	Very low density lipoprotein
VO_2	Oxygen uptake
VO_2/HR	Oxygen Pulse
VO_2/WR slope	Slope of oxygen uptake / work rate
V/Q	Ventilation to Perfusion Ratio

WHO FC	World Health Organisation Functional Class
WR	Work rate
WU	Wood Units
6MWD	Six minute walk distance
6MWT	Six minute walk test
ρ	Spearman correlation coefficient
Δ	Delta (change)
%B	Beta cell sensitivity
%S	Insulin sensitivity

Chapter 1 Introduction

1.1 Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is an incurable disorder of the pulmonary circulation that results in breathlessness, reduced exercise capacity, syncope, right heart failure and ultimately death.

Proliferation of vascular endothelial and smooth muscle cells lead to thickening of the tunica intima and tunica media in the pulmonary arteries. Accumulation of proteins such as collagen, formation of plexiform lesions, and occlusion of small pulmonary arterioles occur. This results in a severe and progressive remodelling of the pulmonary arterial tree. The mechanical consequences of this are an increase in pulmonary vascular resistance (PVR), an increase in vascular stiffness and elevation of pulmonary artery pressure (PAP) at rest and on exercise. The cumulative effect of these changes is an increase in right ventricular (RV) afterload, this eventually leads to RV failure, the principal cause of death in PAH.

Typical symptoms of PAH are exercise intolerance, fatigue and dyspnoea. As RV failure ensues, pre-syncope, chest pain and ankle swelling develop. Clinical features are often subtle in the early stages. As the condition progresses, a tricuspid murmur will be heard, an RV heave may be present and a split second heart sound. Later clinical signs are of resting tachycardia, hypoxaemia and eventually features of right heart failure, with elevated JVP and peripheral oedema.

1.1.1 Clinical classification

Pulmonary Hypertension (PH) is defined by a mean pulmonary artery pressure (mPAP) greater than 25mmHg. Pulmonary Arterial Hypertension (PAH) is characterised by an mPAP ≥ 25 mmHg, Pulmonary Artery Wedge Pressure (PAWP) ≤ 15 mmHg and a Pulmonary Vascular Resistance greater than 3 Wood Units (WU)[1, 2]. Pre-capillary PH encompasses Group 1 and Group 4 PH (Table 1.1)

Table 1.1 Clinical classification of Pulmonary Hypertension

1. Pulmonary Arterial Hypertension	
1.1	Idiopathic
1.2	Heritable
	1.2.1 BMPR2 mutation
	1.2.2 Other mutations
1.3	Drug or toxin induced
1.4	Associated with
	1.4.1 Connective Tissue Disease
	1.4.2 Human immunodeficiency virus (HIV) infection
	1.4.3 Portal Hypertension
	1.4.4. Congenital Heart Disease
	1.4.5 Schistosomiasis
1' Pulmonary Veno-occlusive disease and / or pulmonary capillary haemangiomatosis	
1'.1	Idiopathic
1'.2	Heritable
1'.3	Drug, toxin and radiation induced
1'.4	Associated with
	1'.4.1 Connective tissue disease
	1'.4.2 HIV infection
2.	Pulmonary Hypertension due to left heart disease
3.	Pulmonary Hypertension due to lung disease and / or hypoxia
4.	Chronic Thromboembolic Pulmonary Hypertension and other pulmonary Artery Obstructions
	4.1 Chronic Thromboembolic Pulmonary Hypertension
	4.2 Other Pulmonary Arterial Obstruction
	4.2.1 Angiosarcoma
	4.2.2 Other intravascular tumours
	4.2.3 Arteritis
	4.2.4 Congenital pulmonary arterial stenosis
	4.2.5 Parasites (hydatidosis)
5	Pulmonary Hypertension with unclear and / or multifactorial mechanisms

Adapted from ERS guidelines on the diagnosis and treatment of pulmonary hypertension [3]

1.1.2 **Treatment of PAH**

Treatment of PAH can be disease targeted or supportive. Supportive treatment consists of:

- Diuretics
- Oxygen

- Maintaining sinus rhythm (cardioversion or with drug therapy)

- Vaccination (influenza and pneumococcal)

There are three classes of disease targeted therapy in PAH and all are pulmonary arterial vasodilators[4]:

- Endothelin Pathway (Endothelin Receptor Antagonists)

- Nitric Oxide Pathway (Phosphodiesterase Type 5 Inhibitors and Soluble Guanylate Cyclase Stimulators)

- Prostacyclin Pathway (IP prostacyclin-receptor agonists)

Therapy is prescribed in accordance with international guidelines, based on a clinical assessment of severity [5]. Two significant changes have occurred in the last 20 years, which have resulted in improved survival or requirement for lung transplantation. Intravenous Epoprostenol was introduced in 1996 and was the first and to date only therapy to demonstrate robust survival benefit [6]. Secondly, goal orientated therapy has been adopted, with more patients being on combination therapy at an earlier stage in their disease [7]. Despite these advances, Pulmonary Hypertension remains incurable with significant associated morbidity.

A number of novel drug targets and therapies have been investigated over the past ten years, to address other pathological mechanisms that drive vascular remodelling and systemic manifestations of PAH[8].

- The BMPR2 pathway (Tacrolimus) [3]

- Inhibition of platelet derived growth factor using the tyrosine kinase inhibitor Imatinib [9]

- Insulin resistance: Metformin and Ranolizine [10]

- Inflammation and immune modulation (Rituximab[11], Tocalizumab[12])

- Oxidative stress (Apoptosis signal-regulating kinase 1 inhibitor)[13]
- Serotonin antagonists (Terguride) [14]
- Pulmonary artery denervation [2]

None of these pathways or targets have yielded clinically beneficial effects with tolerable or acceptable safety profiles to date. There remains an un-met need for additional therapies in order to improve survival and morbidity in PAH. For this reason, there has been increasing interest in exercise therapy as a potential adjunct to disease targeted therapy. Impressive improvements in exercise capacity, both maximal and submaximal, and quality of life (QOL) have been demonstrated in several studies[15] however there are a number of unknown factors that prevent its widespread adoption. These factors are discussed in further detail below.

1.2 **Exercise physiology**

Exercise requires the production of force by muscles, which requires the breakdown of adenosine triphosphate (ATP). This occurs through the transfer of oxygen from the atmosphere to muscle mitochondria to allow ATP production through cellular respiration, with the simultaneous removal of the waste product carbon dioxide. Internal cellular respiration needs to be coupled to external gas exchange to meet the metabolic requirements of the working muscle, this occurs through the integrated responses of the cardiovascular and respiratory systems[16]. The physiological response to exercise is dependent on the intensity, duration and frequency of the exercise as well as environmental conditions[17]

As exercise progresses, oxygen uptake (VO_2) and carbon dioxide output (VCO_2) increase. Oxygen consumption can be described by the Fick equation:

$$VO_2 = CO \times (CaO_2 - CvO_2)$$

VO_2 = Oxygen Consumption, CO = cardiac output, CaO_2 = arterial oxygen content, CvO_2 = venous oxygen content.

This equation can be expanded to interrogate the individual contributors to a given VO_2

$$VO_2 = (SV \times HR) \times (HB \times (SaO_2 - SvO_2))$$

SV = stroke volume, HR = heart rate, Hb = haemoglobin concentration, SaO_2 = arterial oxygen saturation, SvO_2 = venous oxygen saturation.

The Fick equation encompasses the requirements for efficient coupling of internal and external respiration[18]:

1. Appropriate intracellular structure, energy substrate and enzyme concentration

2. Effective heart pump function to circulate oxygenated blood

3. A network of blood vessels capable of distributing blood flow to match local tissue gas exchange requirements

4. Normal Haemoglobin concentration

5. Normal lung mechanics

6. Ventilatory control mechanisms capable of regulating arterial blood gas tensions and hydrogen ion concentrations.

The normal exercise response can be broadly divided into the cardiovascular response, ventilatory response and the muscle and metabolic response.

1.2.1 **Muscle and metabolic response**

Skeletal muscle consists of two basic fibre types, type I and type II, classified on the basis of their contractile and biochemical properties[16]. Type I fibres are slow twich fibres, with higher levels of myoglobin and mitochondria, therefore containing a greater concentration of oxidative enzymes. Type I fibres are predominantly involved in longer duration, aerobic exercise. Type II muscle fibres are termed fast twitch muscle fibres and have a higher glycolytic potential than type I fibres, they are involved in higher intensity, shorter duration activity. Type II fibres are further subdivided into type IIa (fast oxidative) and type IIx (fast glycolytic), type IIa have higher myoglobin and mitochondrial concentration compared to type IIx fibres. Type IIA fibres therefore have a higher resistance to fatigue than IIX fibres.

Four biochemical energy supply systems exist in skeletal muscle

1. Breakdown of inherent Adenosine Triphosphate (ATP)

2. Breakdown of phosphocreatine (AT-CP system)

3. Generation of ATP by anaerobic glycolysis

4. Generation of ATP by oxidative phosphorylation

At rest, less than 20% of the body's resting energy expenditure is attributed to skeletal muscle metabolism. Almost all the changes that occur during exercise are related to increased energy metabolism, largely within the skeletal muscle. During intense exercise, energy expenditure is up to 15-25 times greater than at rest. The immediate energy source for muscles during exercise is adenosine triphosphate. The inherent intramuscular ATP concentration is small and only sufficient to power a short duration of muscle contraction, for example, the concentration of ATP in a human quadriceps muscle is typically 8mM [19], while the average rate of ATP turnover during moderate intensity knee extension is 24 mM min $^{-1}$[20]. The rate of ATP breakdown ranges from 70 to 140 mM min^{-1} during isometric contractions of various intensity to as much as 400 mM min^{-1} during intense, dynamic activity. Metabolic pathways must therefore be activated during exercise to maintain ATP

synthesis to meet the demands of the exercising muscle. Buffering of ATP by phosphocreatine extends the duration of activity possible, however sustained activity requires continual regeneration of phosphocreatine. This is achieved through oxidative processes when there is sufficient tissue oxygen content (Krebs cycle and electron transport chain) or anaerobic glycolysis during intense activity, when oxygen content is insufficient[21]. Aerobic metabolism is far more efficient than anaerobic glycolysis at producing ATP, as demonstrated in Figure 1.1

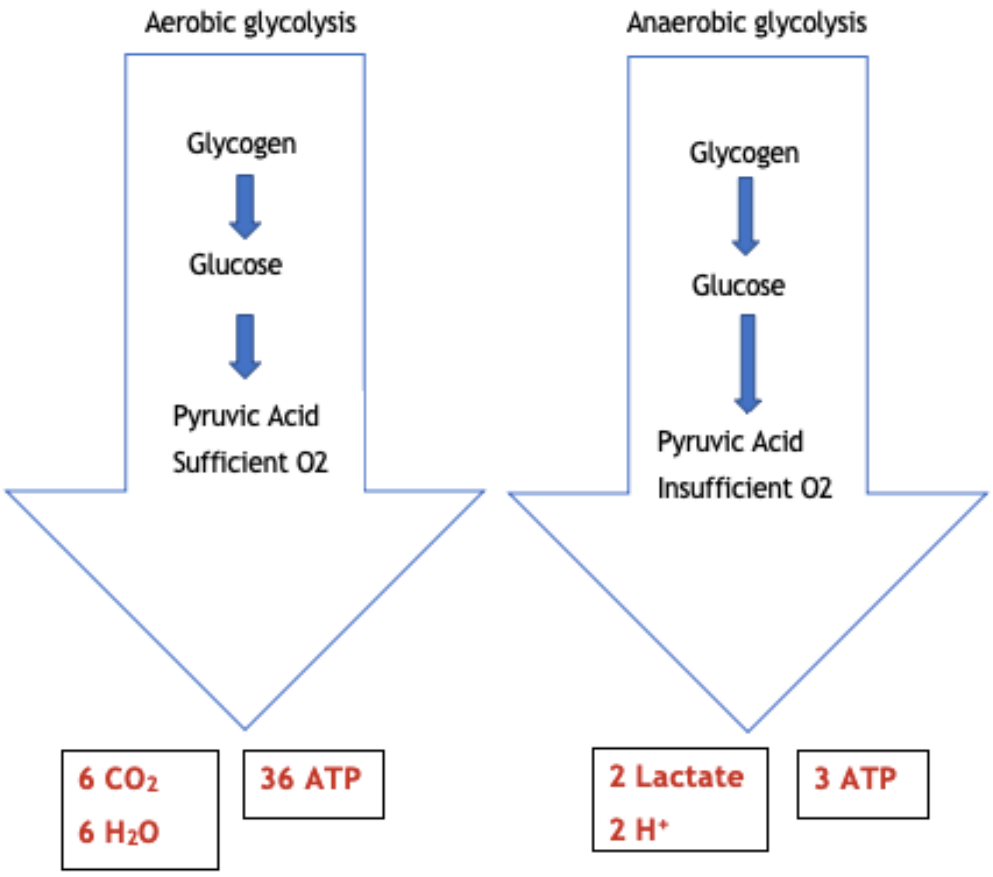


Figure 1.1 Aerobic and anaerobic metabolism energy generation

Different intensities of exercise result in different metabolic responses (**Error! Reference source not found.**). The contribution of anaerobic sources to exercise energy metabolism is inversely related to the exercise duration; energy to perform short duration, high-intensity exercise primarily comes from anaerobic sources, whereas the energy during prolonged exercise comes predominantly from aerobic metabolism.

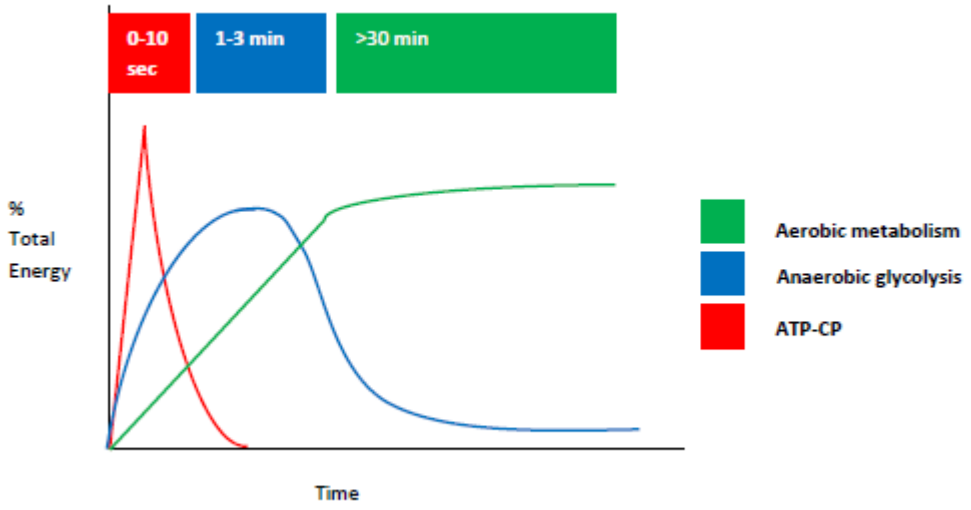


Figure 1.2: Relative contribution of metabolic systems through different exercise durations

In transition from rest to light exercise, steady state oxygen output (VO_2) and heart rate (HR) are reached within 1-4 minutes in untrained adults, this time may be longer in disease states and shorter in highly trained individuals. The time required to reach a steady state increases at higher work rates. Because VO_2 does not increase instantaneously to steady state, anaerobic energy sources contribute to meet the energy demand at the beginning of exercise. Once steady state is achieved, aerobic metabolism then takes over. In normal environmental conditions, steady state VO_2 can usually be maintained for 10-60 minutes of submaximal continuous exercise [22].

In progressive incremental exercise, oxygen uptake increases in a linear fashion with work rate until peak VO_2 is reached; in the early stages of incremental exercise, ATP comes predominantly from aerobic metabolism, during later stages of exercise, there is increasing reliance on anaerobic metabolism and blood levels of lactate increase. The point at which the rate of lactate production surpasses the rate of lactate clearance is known as the anaerobic threshold (AT)[22].

Short term, high intensity exercise relies on the AT-CP system at durations under 5 seconds (e.g. weight lifting) and a combination of AT-CP and anaerobic glycolysis at durations between 5 to 60 seconds.

In recovery from exercise, oxygen consumption remains elevated above resting levels. Metabolism is higher after high intensity exercise than light or moderate intensity and likewise is more prolonged with longer duration exercise than shorter.

Exercise testing, including measurement of the metabolic response to exercise is covered in Chapter 6.

1.2.2 **Cardiovascular response**

The cardiovascular system responds to exercise by increasing blood flow to meet the requirement of skeletal muscle metabolic activity. Increasing oxygen demand reflects the energy requirement of the working muscles and increased blood flow also serves to allow removal of the metabolic products carbon dioxide (CO_2) and lactate in order to maintain acid-base homeostasis [23] These changes are relative to the workload faced by the active muscles.

Cardiac output increases through increases in Stroke Volume (SV) and Heart Rate (HR). HR increases initially due to a reduction in vagal tone, followed by increase in sympathetic tone. In health, HR rises in a linear fashion with WR and VO_2 . SV increases almost immediately due to increased venous return from contracting muscles, decreased intrathoracic pressure due to increased tidal volume and increased cardiac inotropy. It then plateaus at around 50% of maximum WR; further increases in CO thereafter are through increasing HR. At peak exercise, SV may decrease due to reduced diastolic filling time. The pulmonary vascular bed dilates to accommodate the increased RV SV, without significant increase in afterload.

In addition to increased CO, blood flow is diverted to exercising muscles from the skin, splanchnic, renal and hepatic circulations. Cerebral blood flow is maintained. Local blood flow distribution is determined by the autonomic nervous system and local humoral control, with endothelial nitric oxide resulting in skeletal muscle vasodilation.

There is a linear rise in systolic blood pressure (BP) with increasing WR. BP is directly related to cardiac output and peripheral vascular resistance and therefore

can provide non-invasive information about cardiac performance during exercise. Mean pulmonary artery pressure (mPAP) rises during exercise due to increased CO and increased left atrial pressure (LAP). The mPAP-CO relationship during exercise is used as a surrogate for total pulmonary vascular resistance (TPR). An abnormal exercise response is felt to be present when TPR is greater than 3 WU and mPAP is ≥ 30mmHg [24]

1.2.3 **Ventilatory response**

At rest, minute ventilation (VE) is approximately 6L/min in a healthy man. Ventilation must increase appropriately to maintain arterial oxygen tension and eliminate the H^+ associated with CO_2 and lactate. The rate of increase is linear to VO_2 until AT is reached, after which VE increases more rapidly than VO_2 . At low work rates, increasing ventilation is achieved primarily through increases in tidal volume, beyond AT, respiratory rate increases to a greater extent. At maximal exercise, VE increases 15 to 25 fold from resting values.

The ventilatory system is not normally the limiting factor to peak aerobic exercise, however in some healthy individuals exercise induced arterial hypoxaemia (EIAH) can occur. EIAH can occur in untrained individuals[25] but is more prominent highly trained athletes at high exercise intensities (>80% VO_2 max), particularly if a large proportion of muscle mass is engaged in the exercise process[26].

1.3 **Factors affecting exercise performance**

The limit to peak aerobic exercise capacity in most healthy untrained adults has traditionally been attributed to the cardiovascular system. Exercise limitation is more complex than this however, and a number of factors must be taken into account including technical, physiological, social and psychological factors[27].

The traditional and widely held model of “peripheral fatigue” or “cardiovascular” exercise limitation was first proposed by Archibald Hill in 1923. This model theorises that an inadequate supply of oxygen to the heart causes myocardial ischaemia, this limits cardiac output and means skeletal muscle blood flow is inadequate to match the high skeletal muscle oxygen demands during maximal exercise[28, 29]. A resulting skeletal muscle anaerobiosis then causes lactic acid to

accumulate, which impairs skeletal muscle relaxation, ultimately terminating exercise.

A central governor model has been proposed more recently as an alternative mechanism of exercise limitation, by Noakes et al[30]. The model proposes that it is the central nervous system maintains homeostasis in all bodily systems during exercise, by integrating both psychological inputs (e.g, motivation, previous experience, self belief, expectations) and physiological inputs (e.g. hydration status) to then "feed forward" to regulate the number of motor units recruited by the exercising muscles, thus adjusting metabolic demands. The central nervous system simultaneously receives physiological "feedback" from all organs, in order to maintain homeostasis and modify exercise behaviour. Thus it is the central nervous system that limits exercise performance in this model.

1.4 **Exercise limitation in PAH**

Exercise intolerance is a hallmark symptom of pulmonary hypertension. Traditionally, exercise limitation in PAH has been attributed predominantly to impaired right ventricular (RV) function and an inability of the heart to increase stroke volume (SV) in response to exercise [31]. Disease targeted therapy improves SV by reducing pulmonary vascular resistance (PVR) and therefore afterload[32], with combination drug therapy being superior in this regard[33]. Despite advances in medical therapy, most patients remain symptomatic on optimal drug treatment, with the vast majority of patients being in WHO functional class III in the 2019 UK PH National Audit [34]. This lack of improvement in exercise tolerance suggests additional mechanisms other than poor SV are responsible for exercise limitation. In addition to a high symptom burden, survival remains poor, with 40% survival at 5 years for group 1 patients[35]. It is therefore desirable to explore the persistent exercise intolerance in PAH to identify possible pathways that may be amenable to therapeutic intervention and this will be explored throughout the thesis in a systems based approach.

Examining the processes that underpin efficient exercise reveals possible mechanisms for persistent exercise intolerance in PAH. Pathological changes in PAH that may be potentially linked to exercise intolerance in PAH are summarised

in Table 1.2. Chapters 5 to 9 go on to describe the changes in muscle function, respiratory physiology and cardiovascular function and exercise capacity in PAH in a system based approach and explore the role of exercise therapy in modulating these pathological changes.

Robust data do not presently exist to support the relative contributions of the problems presented in Table 1.2 to exercise intolerance in PAH, nor have studies to date explored psychological and behavioural contributions despite the high prevalence of psychological and psychiatric comorbidity in PAH[36].

Table 1.2: Potential mechanisms of exercise limitation in PAH

Exercise Requirement	Pathology impairing exercise performance in PAH
Appropriate intracellular structure, energy substrate and enzyme concentration	Reduction in skeletal muscle oxidative enzyme activity. Reduced mitochondrial function [37]
Effective heart pump function to circulate oxygenated blood	Impaired stroke volume response to exercise and chronotropic incompetence[38].
A network of blood vessels capable of distributing blood flow to match local tissue gas exchange requirements	Microvessel rarefaction at a muscle level[39]. Pulmonary arterial vasculopathy and vascular bed obliteration, resulting in V/Q mismatch[40].
Normal Haemoglobin concentration	High prevalence of iron deficiency and anaemia[41]
Normal lung mechanics	Respiratory muscle weakness[37]
Ventilatory control mechanisms capable of regulating arterial blood gas tensions and hydrogen ion concentrations	High prevalence of hyperventilation. Altered baroreceptor sensitivity[42]

1.5 **Exercise prescription**

Exercise prescription ranges from optimising performance in athletes to disease prevention and treatment of chronic illnesses. Prior to an exercise prescription being made, an individual must have an assessment of cardiovascular and musculoskeletal fitness along with an assessment of comorbidities. It is important that exercise prescription is tailored to the individual and the benefits of exercise are balanced with risks, including injury and aggravation of any underlying medical condition[22].

Exercise prescription is based on 5 principles: type, duration, frequency, intensity, and volume. Type refers to mode of exercise training, with the main forms being aerobic (i.e. endurance training), resistance (i.e. strength training), flexibility, and balance. The length and number of exercise sessions performed within a given time frame, are described by duration and frequency, respectively.

Generic prescription recommendations for the initiation of exercise programmes have been made by the American College of Sports Medicine (ACSM) [22]. The exercises used in the study “The effect of adding exercise therapy to optimal treatment in pulmonary hypertension” used aerobic and resistance exercises, therefore these will be discussed in more detail below.

Table 1.3 Adapted from ACSM recommendations for exercise prescription

Type	Frequency	Intensity	Time/Duration	Examples
Aerobic (Endurance)	5 days/week	Moderate	30 minutes (for 150 minutes per week)	Vigorous walking, jogging, swimming, hiking, cycling
Resistance (Strength)	2-3 days/week	60-70% of 1 rep max (novice), 40-50% of 1 rep max (sedentary person, older adult)	8-12 repetitions, 2-4 sets with 2-3 minutes rest in between	Free weights, bodyweight exercise
Flexibility	2-3 days/week	Until feeling of tightness.	Hold 10-30 seconds, 2-4 times to accumulate 60 seconds per stretch	Ballistic, static, dynamic, proprioceptive neuromuscular facilitation
Balance	2-3 days/week	Has not been determined.	20-30 minute	Tai Chi

1.5.1 **Aerobic exercise prescription**

Intensity is the level of effort being exerted by the participant and can be measured in a variety of ways[22]. Common measures of aerobic intensity include:

- The Borg Rating of Perceived Exertion scale
- Target heart rate: % Maximum heart rate, Karvonen Formula or Heart Rate Reserve (HRR)
- Metabolic Equivalents (METS)
- Percentage of peak VO_2

Examples of these measures in quantifying exercise intensity are summarised in Table 1.4

Table 1.4: Examples of measurement of aerobic intensity using different scales

Measurement	Low Intensity	Moderate Intensity	Vigorous Intensity
Modified Borg (0-10)	< 5	5-6	≥ 7
HR max	50- 63%	64- 76%	77-93%
% peak VO_2	20-39%	40-59%	60-84%

The dosage of an aerobic exercise programme is a function of the frequency (F), intensity (I) and duration (time) (T) of the exercise performed in combination with the type (T) of activity, the FITT principle[43]. The level of intensity and frequency required to generate improvements in an individuals fitness is dependent to a large extent on their baseline level of fitness, for example Swain et al found that subjects with a relatively low VO_2 under 40 $mL.Kg^{-1}.min^{-1}$ were successful at increasing peak VO_2 after completing an exercise programme at low intensity (30% peak VO_2 or less) compared to subjects with a VO_2 of 40-50 $mL.Kg^{-1}.min^{-1}$, who demonstrated no improvement in peak VO_2 at this intensity [44].

In terms of optimising benefits from an aerobic exercise programme, it has been demonstrated that interval training (repetitions of short periods of vigorous intensity (e.g. 2 minutes) interspersed with recovery or low intensity (e.g. 5 minutes)) results in greater improvements in peak aerobic exercise capacity, in comparison with continuous training at low, moderate or vigorous intensity in both health and chronic medical conditions and also appears to be safe[45, 46]. The ACSM therefore recommend the incorporation of interval training into exercise programmes.

1.5.2 **Resistance exercise prescription**

A resistance programme can be adjusted depending on the goal of the programme, with heavier load and lower repetitions for increasing strength (< 8 repetitions)

and higher repetitions with lighter load (> 15 repetitions) if endurance is the aim[22].

1.5.3 Progression of exercise prescription

Progression involves some or all of increasing the frequency, intensity, and duration of an exercise programme. Advancement of an exercise program should be incremental to encourage participant adherence and avoid injury. Overall volume should be monitored for adverse effects and decreased if necessary.

Aerobic exercise is progressed by initially increasing the duration of each session by 5-10 minutes every 1 to 2 weeks over the first 4 to 6 weeks, then increasing the frequency and intensity as tolerated over subsequent months[43].

Resistance exercise is progressed by increasing repetitions before increasing load. Once the maximum repetitions for a target range have been achieved, load can be increased by approximately 5% so that no more than the lower limit of repetitions can be performed.

1.6 Exercise therapy in disease states

Wide-ranging beneficial effects of exercise and exercise therapy exist in a variety of chronic illnesses and these are outlined below in sections 1.6.1 and 1.6.2. Left heart disease and chronic pulmonary diseases such as Chronic Obstructive Pulmonary Disease (COPD), share many common features with PAH including exercise intolerance, fatigue and dyspnoea. Additionally, pathophysiological abnormalities such as myopathy, autonomic dysfunction and inflammation are common in both conditions. Exercise therapy has been more widely investigated in these diseases, with established clinical guidelines regarding its role[47, 48]. It is therefore of interest to explore the beneficial effects and mechanisms of these benefits in chronic cardiorespiratory conditions. There are clear differences however, between the optimum mode of training and the mechanisms by which it exerts its beneficial effects between different disease groups, therefore highlighting the need for dedicated research for individual disease entities.

1.6.1 **Exercise therapy in left heart disease**

In medically stable patients with left ventricular failure, exercise therapy has been shown to be safe and efficacious across a wide range of aetiologies, age groups, ethnic groups and disease severities [49]. Exercise capacity and health related quality of life improve, hospital admissions are reduced [50] improvements are also seen in serum markers of inflammation [49] and autonomic function[51]

Improvements in exercise capacity are related to the exercise intervention, with different results seen in resistance versus aerobic exercise and more marked improvements in high versus moderate intensity exercise. In recent years, in addition to continuous exercise training, high-intensity interval training and pyramid training have effectively been established for coronary artery disease (CAD) patients. [52].

1.6.2 **Exercise therapy in chronic respiratory conditions**

Pulmonary Rehabilitation is established as a key component in the management of many chronic respiratory conditions and more recently as a pre-operative adjunct to surgery in lung cancer [53], with improved longer term post-operative exercise capacity and function. The main body of literature relates to COPD, with clear evidence of benefit in exercise capacity, quality of life and symptoms [54], positive effects exist in other conditions such as interstitial lung disease, but with relatively a smaller body of evidence compared to COPD[55]. As is seen in PAH, there is clear evidence of cachexia, inflammation and skeletal muscle dysfunction in COPD, with potential epigenetic mediated improvements with exercise therapy[56]

Unlike left heart disease, increasing exercise intensity during interval training does not appear to provide additional benefit, compared with moderate intensity exercise in COPD[57]. Despite equivalent improvements in symptoms, resistance training appears to have a less potent effect on skeletal muscle oxidative function than aerobic training. Limited data suggest that neither resistance or aerobic training result in significant improvements in vascular function or capillarisation [58].

1.6.3 **Exercise therapy in PAH**

Exercise therapy in PAH has a smaller body of evidence in comparison to other chronic cardiorespiratory conditions. Historically, exercise in PAH had been viewed as hazardous due to potential harm from increased RV afterload [59] and it was recommended that physical activity was minimised, however no evidence existed to support this recommendation.

Over the past decade, it has been demonstrated that exercise training in PAH can improve exercise capacity and quality of life (QOL). Exercise training has been shown to result in more significant improvements in exercise capacity and QOL than the majority of pharmacological therapies[60], with reassuring safety and health economics [61]. The level of evidence is now sufficient that the 2015 ESC/ERS guidelines on the management of pulmonary hypertension, recommended supervised exercise training for stable PH patients (class II, level of evidence B) [5]. The evidence supporting this recommendation is discussed below in section 1.6.3.1

1.6.3.1 **Mode of training: exercise type, duration, setting and outcome measures**

Within the current literature, a variety of approaches to delivering exercise therapy have been adopted, with exercise training being initiated either in an inpatient hospital setting (11 studies) or as an outpatient based programme (14) with 12 published outpatient hospital programmes or less commonly, entirely home based approaches (2). These studies are summarised in Table 1.5, including outcome measures, specific PH subgroups studied, duration of exercise, exercise type, monitoring of study participants and trial design. The majority of randomised control trials (RCTs) have been conducted at a single centre in Heidelberg, Germany, therefore this protocol is referred to in Table 1.5 as the “Heidelberg model”. This specific exercise protocol is described in further detail in section ‘ Personal experience: training in exercise rehabilitation1.6.3.3’.

Most studies have utilised the 6MWD as a primary outcome measure, along with a measure of QOL, most commonly SF-36. Few studies have utilised endurance tests as outcome measures despite the majority of exercise programmes employing

aerobic and low intensity exercises. In COPD, endurance tests such as the continuous work rate ergometer test and the endurance shuttle walk test are more responsive to interventions (both pharmacological and exercise based) than incremental tests and the 6MWT[62]. There is less evidence for endurance testing in PAH, however it is possible that the same effect may be seen. There is a suggestion that this may be the case in the study published by De Man [63], where quadriceps endurance improved significantly after an outpatient exercise programme but no improvements were seen in peak VO_2 or the 6MWD. Only one study has included invasive haemodynamic measurements on exercise[64], this was a step wise incremental protocol that was uniform amongst all participants, despite widely ranging exercise capacity, therefore limiting the value of the data obtained. Given the critical role of the RV and pulmonary circulation on prognosis, exercise intolerance and symptoms in PAH, it would be highly desirable to investigate this area further. The ability to develop an appropriate exercise testing protocol during right heart catheterisation is limited by the fact that this test is generally performed in the supine position and it is unknown to what extent exercise capacity is affected by this posture and what physiological changes accompany this, this is discussed further in **Error! Reference source not found.**. Further research is therefore required to establish these differences in order to formulate an optimal protocol for exercise right heart catheterisation.

PAH and CTEPH are the most represented PH subgroups within the current literature, the majority of studies have included more than one sub-group, in order to have sufficient power, reflecting the relatively low incidence of IPAH.

Table 1.5 Evidence for exercise therapy in Pulmonary Hypertension

Study Design, Population, Author, Year	Length of programme	Mode and frequency of training	Monitoring	Outcome measures (bold = statistically significant)
Inpatient				
RCT IPAH and CTEPH Mereles, 2006 [60]	15 weeks Weeks 1–3 inpatient Weeks 4–15 at home	Heidelberg model	Subjective physical exertion HR <120 beats·min^{-1} S_{aO2} >85% (if lower, supplemental oxygen supplied)	**6MWD** **QOL (SF-36)** **WHO FC**
Prospective cohort Group 1 PH Grunig, 2011[65]		Heidelberg model		**6MWD** **QOL (SF-36)** **WHO FC**
Prosepective cohort CHD APAH Becker-Grunig 2013 [66]		Heidelberg model		**6MWD** **QOL (SF-36)** WHO FC
RCT Group 1 and CTEPH Ehlken, 2014 [67]		Heidelberg model		**QOL (SF-36)**
RCT Group 1 and CTEPH Ley, 2013 [68]	3 weeks inpatient	Heidelberg model		**6MWD**

Study Design, Population, Author, Year	Length of programme	Mode and frequency of training	Monitoring	Outcome measures (bold = statistically significant)
Prospective cohort CTD-PAH Grunig, 2012 [69]		Heidelberg model		**6MWD** **QOL (SF-36)** WHO FC
Prospective Cohort Group 1 and CTEPH Grunig, 2012 [71]		Heidelberg model		**6MWD** **QOL (SF-36)** **WHO FC**
Prospective cohort CTEPH Nagel, 2012 [72]		Heidelberg model		**6MWD** **QOL (SF-36)** WHO FC
RCT Group 1 and CTEPH Elkhen, 2016 [73]		Heidelberg model	Supplemental oxygen if S_{aO2} <90%. Described in Mereles et al[70]	**6MWD** **QOL (SF-36)** Peak VO_2
Prospective cohort Group 1 Kabitz, 2014 [74]	15 weeks Weeks 1–3 inpatient Weeks 4–15 at home	Heidelberg model	HR ≤130 beats·min^{-1} Supplemental oxygen if S_{aO2} <90%. Mereles et al[60]	**6MWD** **Respiratory muscle strength**
Non-randomised controlled trial.	12 weeks Week 1 in hospital	Walking (30–60 min) Bicycle ergometer Resistance	Borg scale 12–13 (scale 6/20) HR 40–60% of HR	**Peak VO_2** PHQ-9 **SF-36**

Study Design, Population, Author, Year	Length of programme	Mode and frequency of training	Monitoring	Outcome measures (bold = statistically significant)
Fukui, 2016 [75]	Weeks 2–12 at home	training (low weights) Frequency: week 1 daily, weeks 2–12 outpatient session twice per week	reserve S_{aO2} ≥90%	**Quadriceps strength**
Outpatient				
Case reports, Group 1, Shoemaker, 2009 [76]	6 weeks	Bicycle ergometer constant WR (45 min) 3 x per week	Subjective exertion <4/10, HR ≤80% age-predicted maximum, blood pressure ≤180 mmHg, S_{aO2} >91%	6MWD
Prospective cohort, De Man , 2009[63]	12 weeks	Progressive cycle ergometer interval training and quadriceps strength and endurance training three times per week	S_{aO2}>85% HR <120 beats·min^{-1}	6MWD **Quadriceps endurance time Muscle strength**
Non-randomised controlled trial, Group 1 and CTEPH, Martinez-Quintana 2010[77]	12 weeks	24 minutes Bicycle ergometer interval training and 10 minutes of upper and lower limb resistance training twice weekly	Borg scale, HR	SF-36 6MWD **WHO-FC**

Study Design, Population, Author, Year	Length of programme	Mode and frequency of training	Monitoring	Outcome measures (bold = statistically significant)
Case series, IPAH, Mainguy[78]	12 weeks	10-15 min continuous bicycle ergometer, 15 minutes brisk walking and upper / lower limb body weight resistance exercises 3 times per week	Borg scale <6/10 Resting allowed Intensity reduced if S_{aO2} <85%	**6MWD**
Non randomised controlled trial, Group 1 and CTEPH, Fox 2011[79]	12 weeks		Subjective exertion, rest permitted, HR, S_{aO2} "monitored" S_{aO2} >90% (if lower, supplemental oxygen supplied)	**6MWD** **Peak VO_2**
RCT, Group 1, Chan 2013[80]	10 weeks	Treadmill interval training and low weight dumbbell weight training twice weekly	Subjective exertion, S_{aO2}, HR "monitored" (no values given)	**SF-36** **CAMPHOR** **6MWD** Peak VO_2
RCT, Group 1, Weinstein 2013 [81]	10 weeks	Continuous treadmill walking at least twice weekly	Subjective exertion, S_{aO2}, HR "monitored" (no values given)	**6MWD**
Retrospective cohort,	>8 weeks	"60 minutes Aerobic training" on either treadmill,	Borg scale	

Study Design, Population, Author, Year	Length of programme	Mode and frequency of training	Monitoring	Outcome measures (bold = statistically significant)
aetiology not reported, Raskin 2014 [82]		crosstrainer or bicycle 2-3 times weekly		**6MWD**
RCT, Group 1 and CTEPH, Gonzalez-Saiz, 2017 [83]	8 weeks	Bicycle ergometer interval training 5 x week, inspiratory muscle training 6 x week, large muscle group resistance training 3 x week	S_{aO2} >80% BP reduction <20 mmHg BP systolic <220 mmHg, diastolic <110 mmHg No ECG abnormalities	**SF-36** 6MWD
Retrospective cohort, Group 1, Talwar 2017[84]	12 weeks	Continuous treadmill walking 30-60 min, 3 x week	Only safety equipment specified (blood pressure monitor, ECG, pulse oximetry, supplemental oxygen)	**Treadmill speed**
Case series, Group 1, Busotti 2017 [85]	4 weeks	Continuous bicycle ergometer 30 min, low weight resistance training and respiratory muscle training 5 x week	HR <70% of max at CPET Borg scale <5 S_{aO2} >90%	**HADS** **6MWD**
Home				

Study Design, Population, Author, Year	Length of programme	Mode and frequency of training	Monitoring	Outcome measures (bold = statistically significant)
Prospective cohort, CTEPH, Inagaki 2014, [86]	12 weeks	>20 min continuous walking, respiratory muscle training and resistance training 1 x week	Subjective exertion	**SGRQ** **6MWD**
Prospective cohort, Group 1, Ihle 2014 [87]	40 weeks	Bicycle ergometer, resistance leg exercises and respiratory training	Subjective exertion: Borg scale <7/10 HR increase <30 beats·min^{-1} S_{aO2} >85%	6MWD SF-36
BP: blood pressure; ECG: electrocardiogram; HADS: Hospital anxiety and depression score; HR: heart rate; S_{aO2}: oxygen saturation; CPET: cardiopulmonary exercise testing; RCT randomised control trial; SGRQ: St George's Respiratory Questionnaire. *Content adapted from the ERJ 2019 clinical statement on exercise training in Pulmonary Hypertension.* [88]				

1.6.3.2 **Adverse events**

There is no direct evidence from human studies that exercise in PH is harmful, however safety is a logical concern. Physiologically, exercise results in increased pulmonary artery pressures and therefore could potentially precipitate right heart failure in PH patients[89]. Additionally some patients may be at risk of exercise-induced hypoxaemia and malignant arrhythmia[90], such as those with congenital heart disease. There have been no human studies published of exercise training in patients with clinically unstable PH, however in an animal model, exercise training significantly reduced the survival of rats that received higher monocrotaline dosage representing a progressive form of PH compared to those with stable PH, implying that exercise is potentially harmful in unstable PH[70]. Careful patient selection and close monitoring are therefore very important.

The recently published European Respiratory Journal guidelines on exercise training in PAH [91]comprehensively reviewed adverse events in a pooled analysis of 674 patients participating in exercise rehabilitation studies, with 511 in an inpatient setting and 138 in an outpatient setting. Events were classified as related to exercise or unrelated to exercise. No cases of death were reported. The most common adverse event related to exercise was reported as desaturation in 16 of the 674 participants (2.4%), followed by dizziness (1.2%), arrhythmia (0.4%), hypotension (0.1%), syncope (0.1%) and fatigue (0.1%). Clinical worsening of symptoms and heart failure related to exercise therapy were not reported in any of the studies included in the pooled analysis.

Of adverse events not directly related to exercise, the most frequent adverse event (3.4%) was respiratory tract infection, which led to antibiotic treatment and short discontinuation of the training. Other reported events included syncope (0.3%), Pre-syncope (0.7%), mild haemoptysis (0.1%), other infections (0.2%).

1.6.3.3 Personal experience: training in exercise rehabilitation and development of study protocol

To gain further experience in the rehabilitation of patients with pulmonary hypertension, I spent two weeks in Heidelberg, Germany, under the supervision of Professor Ekkehard Grünig. This placement involved attending two sites, the Thorax Clinic in Heidelberg and a rehabilitation centre, Rehaklinik Heidelberg-Königstuhl, where there is an established rehabilitation programme for patients with chronic cardiorespiratory conditions, including PH.

At the Thorax clinic, patients were referred from across Germany for consideration of suitability to take part in the pulmonary hypertension specific exercise rehabilitation programme at the Rehaklinik in Königstuhl. The programme was run by Professor Grünig, with a team of specialist physicians and physiotherapists. I attended the Thoraxclinic and shadowed the PH physicians during their assessment and investigation of patients referred. A typical patient journey involved a clinical assessment by PH physician, this included WHO functional class determination, current medication, symptoms and physical examination. Along with this assessment, some or all of the following investigations were performed if the patient was deemed suitable to participate: CMR, RHC, exercise echo using a 25

watt stepwise protocol and 6MWT. Once clinical stability was confirmed by means of the above assessments, patients were enrolled in the exercise programme and given a date to attend for this in the forthcoming months.

At the Rehaklinik in Königstuhl, I shadowed a group of patients for ten days in total to observe typical components of rehab programme. The exercise programme observed consisted of a three week residential programme consisting of four daily sessions that were a combination of bicycle ergometer, walking or treadmill, education sessions, progressive muscle relaxation sessions, respiratory muscle training and resistance exercises. The initial exercise prescription was completed by a PH physician and monitored and titrated by specialist physiotherapists. Sessions took place in small groups of 8-10. Each of the following were performed five times per week for the first three weeks of a fifteen week programme

- Bicycle ergometer sessions: intervals of 1 min higher intensity (~ above anaerobic threshold) and 30 seconds lower intensity (~ below anaerobic threshold) for 10–25 min depending on the fitness of the participant.

- Walking with “positive mental imagery” (60 min) (described below)

- Resistance exercise; low-weight dumbbell training or body weight upper and lower limb strength training (30 min)

- Respiratory muscle training (30 min)

- Progressive muscle relaxation – 1-2 times per week, (30 min)

I conducted informal interviews with specialist physiotherapists in order to learn about individual components of the exercise programme and how parameters were set, including training zones, safety parameters and how patients were monitored. A comprehensive physical assessment had been performed at the Thorax clinic prior to the patient starting the programme and all aerobic sessions were monitored either using a pulse oximeter (walking) or telemetry with continuous monitoring (bicycle ergometer). These assessments were used to inform the target power for bicycle ergometer sessions and guide the duration and intensity of

walking session, the latter being judged subjectively by experienced physiotherapists.

When the three week residential rehabilitation programme was completed, patients performed a 6MWT and were provided with an exercise programme for home, this consisted of around 1-1.5 hours of cumulative exercise daily, including the components above. A physiotherapist called the patients every 1-2 weeks to determine if the programme needed altered. Patients then returned for a final 6MWT and assessment of quality of life, using the SF-36 questionnaire, to determine the effectiveness of the programme.

Mental imagery was incorporated into the walking programme in Heidelberg. Mental images are defined are cognitive constructions of hypothetical events or reconstructions of real events. Different types of mental imagery can promote goal-directed behaviours; it has been shown in a healthy adult population that regular exercisers frequently use imagery and that by employing imagery exercisers can learn exercise tasks (technique imagery) and set appearance-related goals (appearance imagery)[92]. Duncan et al demonstrated that targeted imagery interventions could be used to increase self-efficacy for exercise among female exercise initiates enrolled in a 12-week cardiovascular exercise programme[93]. Examples of mental imagery used in the Heidelberg were of successfully completing frequently encountered physically challenging situations such as an incline in the participants area of residence, or a flight of stairs. The participant was instructed to visualise themselves having successfully completed the hill or the flight of stairs before the session began and a representative terrain at the rehabilitation facility was then used to practice walking.

In terms of developing a protocol for the study “The effect of adding exercise training to optimal therapy in Pulmonary Arterial Hypertension”, the two main influences for this were the training I received in Heidelberg, along with the current evidence demonstrating the “Heidelberg model” appeared to be the most consistent and result in the most significant improvements in exercise capacity and quality of life. This led to a modified version of the Heidelberg exercise protocol being used, this protocol is described in detail in Chapter 4.

1.6.3.4 **Current barriers to implementing exercise therapy in PAH**

Despite being widely recognised as a safe, efficacious and cost-effective therapy [91], exercise therapy is not part of standard care in the UK and many other European countries. There are several unanswered questions that pose a barrier to its widespread implementation; these fall into three main domains:

1. Relationship with drug therapy

With robust risk stratification and goal-orientated therapy now being the standard of care, the majority of patients with PAH should be on combination therapy at an early stage [94]. Current evidence supporting exercise therapy for PAH is based on studies pre-dating this treatment strategy and consequently the majority of patients are on monotherapy [61]. No study has exclusively assessed the effect of exercise training in addition to optimal PAH therapy.

2. Optimal mode of delivery

The optimal mode of delivering exercise therapy has not been definitively established. This remains a key research question that is essential to answer in order to deliver an effective treatment. Current evidence demonstrates that the "Heidelberg model" adopted by Mereles et al [61], is more likely to be efficacious in improving exercise capacity compared with outpatient rehabilitation [63]. In order to best ascertain what components of the programme are essential and what components can be excluded or refined, a clearly structured programme that is highly likely to be efficacious first needs to be adopted and the physiological and biological changes associated with improvement tested. It can then be refined to exclude the less useful components and enhance the general applicability by reducing the time required at hospital for the patient and the resource and time burden for the NHS.

3. Potential mechanisms underlying improvements in exercise capacity and quality of life with exercise therapy

Limited data exist to explain the beneficial effects of exercise training in PAH. There are a number of pathophysiological and pathobiological processes in PAH

that may impair the exercise response. These factors have not been studied in relation to the effect of exercise training. In order to best prescribe a PAH specific training programme, it is essential that the underlying mechanisms of improved exercise capacity are fully understood; this will dictate the content, duration and intensity of exercise. It is likely that it affects some or all of the factors listed below, Chapters 5-9 will discuss these factors in detail, they are summarised briefly below.

Respiratory muscle function

Inspiratory and expiratory muscle strength are reduced in IPAH, independently of haemodynamic severity, leading to a reduced ventilatory capacity[95]. Specific respiratory muscle training has been shown to be an important component in exercise training programmes.[96].

Cardiac function

In animal models, exercise training reduced RV hypertrophy and pulmonary artery remodelling, suggesting a direct effect on the pulmonary vasculature and myocardium[97, 98]. Exercise training in patients with stable PH on treatment improved cardiac index and reduced mPAP[64]. In rats with stable monocrotaline induced PAH, exercise trained rats had increased capillary density in cardiomyocytes and improved exercise endurance compared with sedentary matched controls.[99]

Autonomic function

A higher resting heart rate (HR), reduced heart rate recovery (HRR), reduced HR variability (HRV)[100] and evidence of altered baroreceptor sensitivity (BRS) support autonomic dysfunction in PAH. These findings are independent of haemodynamic severity but correlate with peak oxygen uptake (VO_2)[38]

Systemic inflammation and metabolism

Inflammatory cytokines may contribute to proteolysis and damage contractile proteins involved in skeletal muscle function. Cytokines such as interleukin (IL)-6,

IL-8, IP-10 and monokine induced interferon-γ (MIG) are elevated in the serum of IPAH patients. In chronic thromboemolic pulmonary hypertension (CTEPH), IP-10 negatively correlates with cardiac index and 6MWD [101]. In left ventricular failure, cytokines such as TNF-alpha reduce with exercise training and correlate with improved exercise capacity [102].

Epigenetics

Systemic angiogenic defects contribute to skeletal muscle microcirculation rarefaction and exercise intolerance, independently of haemodynamic severity. Reduction in the expression of pro-angiogenic miR-126 in the skeletal muscle of humans with PAH correlates with capillary density and peak VO_2 and is significantly reduced compared with healthy controls. In a PAH rat model, miR126 down regulation reduce capillary density and this correlates with exercise capacity.[103] In health, change in expression of miRs such as miR-20a correlate with changes in VO_2 following exercise training[104].

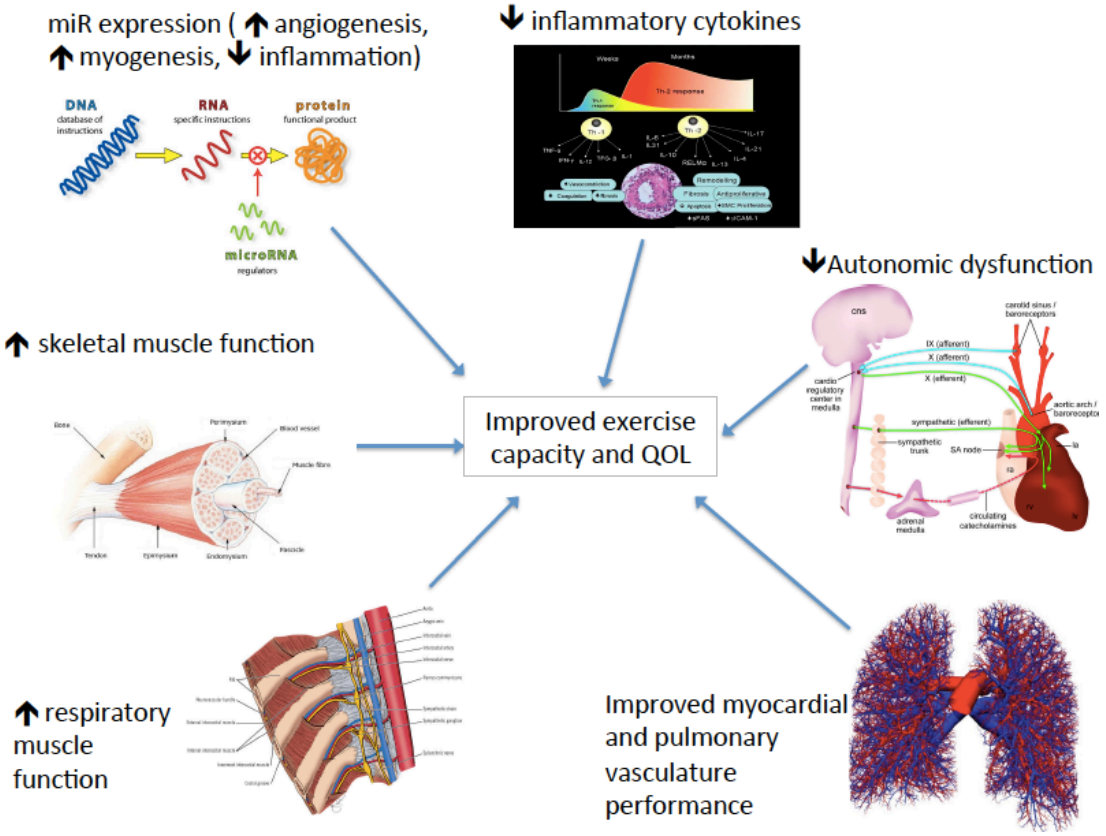


Figure 1.3 Potential mechanisms for improved exercise capacity in PAH

1.7 **Thesis Aims**

1. Establish the demand for exercise therapy in the Scottish PAH patient population and potential barriers to its uptake

2. Determine the degree to which cycle ergometry exercise capacity is reduced in the supine position in order to determine an appropriate protocol to use during exercise right heart catheterisation.

3. Determine whether exercise therapy can enhance exercise capacity and quality of life when undertaken in addition to optimal therapy in PAH in a UK setting.

4. Generate pilot data on the mechanisms of exercise limitation and the factors that improve with training, assessing:

    I. Exercise capacity

    II. Cardiac function

    III. Gas exchange, lung function and respiratory muscle function

    IV. Clinical markers of muscle function and serum and muscle markers of muscle function

    V. Profile of systemic inflammation and metabolism

1.8 **Original hypotheses of thesis**

Sufficient demand exists amongst the PAH population in Scotland to pursue research into establishing a PAH-specific exercise rehabilitation programme.

Supervised exercise training in patients with stable PAH on optimal therapy in a Scottish health care system is safe, feasible and improves exercise capacity, quality of life and right ventricular (RV) ejection fraction.

The improvement in exercise capacity occurs for reasons beyond improvements in cardiac output and RV function

Exercise training affects the control mechanisms for skeletal muscle function. Improvements are due to changes in the inflammatory cytokine profile and in expression of micro-RNA associated with angiogenesis, myogenesis and inflammation.

Endurance testing is a more sensitive tool for detecting change following exercise therapy in PAH than incremental exercise testing.

Chapter 2 Assessing the demand for exercise therapy in a Scottish PAH population

2.1 Introduction

Over the past 10 years, the body of evidence supporting exercise training as an effective treatment for PAH has grown significantly, such that the 2015 consensus statement on the management of PAH upgraded its recommendation to class I, level A. The consensus recommends that exercise training of PAH patients should be undertaken by centres experienced in both the management of PAH and rehabilitation of compromised patients[105]. Despite this, dedicated exercise training programmes do not exist in the UK and many other European countries. In these countries, exercise training is delivered to a small, unselected population of PH patients in an ad hoc manner, by services specialised for chronic obstructive pulmonary disease or left heart failure.

Several factors may hinder the widespread adoption of specialised exercise training for patients with PAH. Firstly, the infrastructure and expertise that exists in German rehabilitation clinics, where the much of this research has been conducted, does not exist in many other centres, raising the question of whether these results can be replicated elsewhere. Secondly, the successful approach described by Mereles et al [60] involved an initial intense inpatient phase followed by a monitored outpatient period, using multi-modality, PAH specific rehabilitation. Such an approach is demanding both on patient time and hospital resources. The optimal structure for a rehabilitation programme has not been established and other investigators have evaluated lower intensity exercise programmes, with less successful outcomes. De Man et al utilised existing outpatient cardiac rehabilitation facilities and observed improved muscle endurance but failed to show an improvement in six minute walk distance [63] suggesting that there are advantages to the initial intensive residential approach. Finally, no long-term data exist to support a prognostic benefit.

The Scottish Pulmonary Vascular Unit (SPVU) in Glasgow serves a population of 5.3 million and is the national referral centre for PAH in Scotland, with a prevalent population of 47 per million[106]. Scotland is a geographically challenging country

to deliver intensive health care in certain sub-populations, due to low population density and large distances between tertiary centres and remote areas. To establish a training programme for Scottish PAH patients, it must first be determined that there is demand amongst the patient population for such a treatment and established potential logistical difficulties.

2.2 **Aims**

The aims of this study were

1. Establish the demand for a dedicated PAH specific exercise rehabilitation programme in the Scottish population in order to determine the viability of establishing a PAH specific rehabilitation programme.

2. Explore potential barriers to the uptake of exercise rehabilitation

2.3 **Methods**

The West of Scotland Research Ethics Committee assessed the research proposal and deemed that formal ethical review was not required.

At SPVU, demographic and clinical data is prospectively maintained on the InfoFlex® database, Heretfordshire, UK by a clinical data manager. The Infoflex database was used to generate a list of suitable survey participants in September 2014, based on the following criteria:

- WHO functional class I-III

- 6MWD ≥ 150m

- PAH diagnosed by right heart catheterization (mean pulmonary artery pressure ≥ 25mmHg*, pulmonary capillary wedge pressure ≤15mmHg and PVR > 3 WU) (*prior to the 2019 proposed new haemodynamic definition of PAH)

Patient interest and willingness to participate in a PAH-specific exercise rehabilitation programme of 2 weeks residential rehabilitation at the Golden Jubilee National Hospital, followed by 12 weeks outpatient, remotely monitored rehabilitation mirroring that of Mereles et al[60] was assessed. The programme described consisted of aerobic and resistance exercises, respiratory muscle training and education. The full survey can be found in Appendix 1: Patient survey letter.

Demographics and routinely measured clinical and prognostic variables were collected from the database

- RHC haemodynamics at diagnosis
- Most recent quality of life score (EmPHasis-10)
- Most recent 6MWD
- NTproBNP
- PAH subtype
- WHO functional class
- Use of oxygen therapy.

2.3.1 **Statistics**

Data are presented as mean (SD) for normally distributed data. NT-proBNP data were not normally distributed therefore a log transformation was performed to produce normal data. Paired analyses were performed using an unpaired t-test. Comparisons of unpaired categorical variables were performed with a chi-squared test. IBM® SPSS® Statistics software was used to perform all statistical analyses.

2.4 **Results**

224 patients met the inclusion criteria. These patients were contacted by mail with a copy of the survey.

Forty three percent of patients (96/224) responded to the survey. 62.5% (60/96) were interested in all components of the rehabilitation programme (residential and outpatient), a further 11.5% (11/96) were interested in outpatient rehabilitation only.

Those interested in the rehabilitation programme tended to be younger, with better functional class and were less likely to be on oxygen therapy. There were no significant differences between groups in baseline haemodynamics, quality of life or NTproBNP (Table 2.1). Some participants did not identify themselves, therefore it was not possible to phenotype them; 6 of the "not interested in rehabilitation" responders and 2 of the "interested in rehabilitation responders.

Table 2.1 Characteristics of survey respondents

	"Not interested" responders (N 30)	**"Interested" responders (N 58)**	**P value**
Haemodynamic			
mPAP (mmHg)	45.3 ±12.47	44.7 ± 14.40	0.640
SVO_2 (%)	64.1 ± 8.9	64.7 ± 8.63	0.970
Thermodilution CO (L/min)	4.5 ± 1.33	4.7 ± 1.58	0.640
Functional			
CAMPHOR total	26.0 ± 19.4	31.6 ± 18.2	0.070
QOL	7.2 ± 6.7	10.0 ± 7.9	0.060
Activities	10.8 ± 6.4	10.6 ± 6.1	0.500
Symptoms	9.3 ± 7.1	11.2 ± 6.8	0.150
EmPHasis-10	22.07 ± 15.2	25.5 ± 11.6	0.180
6 minute walk distance (metres)	348 ± 129	381 ± 111	0.250
Age	68.1 ± 13.1	56.42 ± 13.32	**0.001**
Log NT-pro BNP	2.62 ± 0.67	2.57 ± 0.60	0.707
Male	53.3% (16/30)	45% (27/60)	0.439
Disease category			
IPAH	23% (7/30)	34.5% (20/58)	0.234
CTD-PAH	30% (9/30)	12.1% (7/58)	
CHD-PAH	6.7% (2/30)	12.1% (7/58)	
POPH	6.7% (2/30)	6.9% (4/58)	
CTEPH	30% (9/30)	24.1% (14/58)	
HIV-PAH	0%	1.7% (1/58)	
PVOD	0%	3.4% (2/58)	
Group 5	0%	5.2% (3/58)	
WHO FC			
Group I	0% (0/30)	12.2% (7/57)	**0.047**
Group II	70% (21/30)	47.4% (27/57)	
Group III	30% (9/30)	40.4% (23/57)	
Oxygen therapy	5/32 (15.6%)	3/61 (4.9%)	**<0.005**

Additionally, patients provided free text responses. Many reported enthusiasm towards the proposal and felt it would improve their confidence, overall health and quality of life. The following reasons were cited in patients who felt at least one aspect of the programme would be unsuitable, Figure 2.1:

- Too far from home, 19.4% (7/36)
- Work or carer commitment 27.8% (10/36)
- Too old or too many comorbidities 27.8% (10/36)
- Too big a time commitment 5.6% (2/36)
- Active enough 8.3% (3/36)
- Equipment in their house would be unacceptable 2.8% (1/36)
- No reason given 8.3% (3/36)

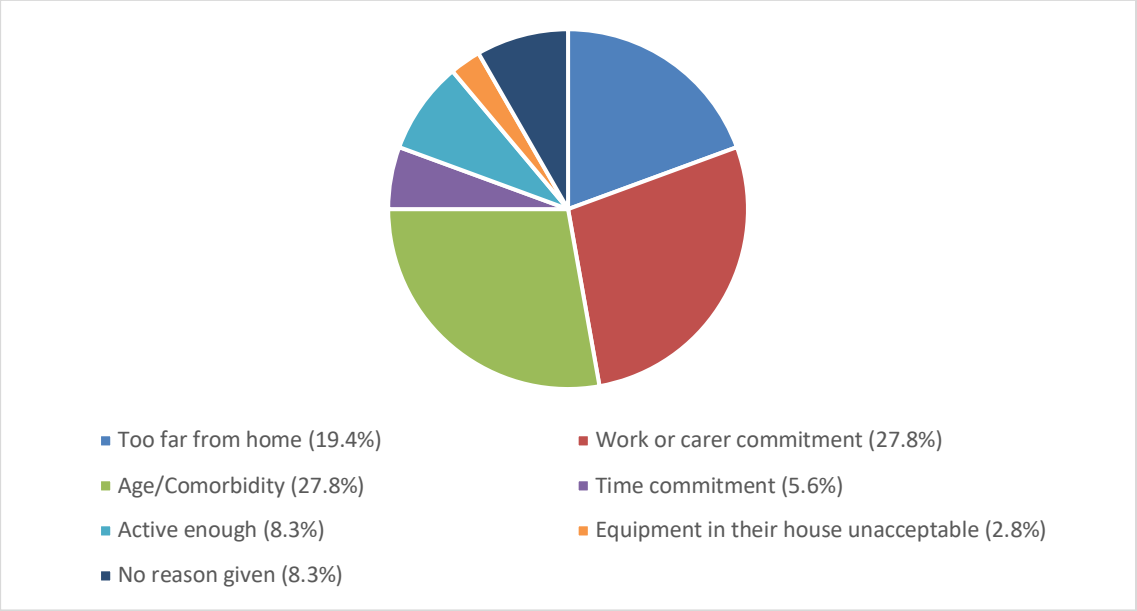


Figure 2.1 Reasons given for not wishing to participate in PAH-specific exercise rehabilitation

2.5 **Discussion**

The population included in this study had typical features of pulmonary hypertension, with reduced exercise capacity, poor quality of life and cardiorespiratory failure. All those included were on optimal disease targeted therapy, further underlining the need for additional treatment options for PH patients.

Amongst the population who responded, there was significant interest in a dedicated PAH exercise programme, particularly amongst patients with IPAH. Exercise rehabilitation in PAH has been shown to improve quality of life and exercise capacity across a range of disease sub-types and functional classes. Despite this, those who did not show interest in participating, had poorer functional class and QOL. From assessment of free text responses, this may be due to age or other comorbidities. Additionally, the intensive time commitment required in the initial residential phase of the exercise programme appeared to discourage those with jobs or carers commitments.

A concerted effort must be made to ensure patients with a higher burden of disease are reassured regarding the safety of exercise therapy in PAH and efforts are made from a logistical and rehabilitation perspective to make such an intervention more acceptable. Barriers need to be understood and overcome in order to deliver exercise therapy to those who are geographically isolated or committed to work or carer responsibilities in order to ensure an equitable service and deliver effective therapy to those who need it most. Further research is needed to establish the optimal timing, structure, content and duration of PAH-specific rehabilitation.

2.6 **Conclusions**

Sufficient demand exists in the PAH patient population in Scotland to develop a PAH-specific rehabilitation programme. Employment, social and geographic factors contribute significantly to the proportion of patients who do not wish to consider this treatment option.

Chapter 3 The ventilatory, gas exchange and haemodynamic response to upright and supine exercise

3.1 Introduction

Standard right heart catheterisation is performed in the supine position. Exercise can be performed during right heart catheterisation with the use of a supine cycle ergometer. Exercise haemodynamics provide valuable insights into the response of the pulmonary circulation and heart to exercise both at an isolated time point and in response to treatment. Indications for performing exercise right heart catheterisation in PH are:

1. To assess for "exercise induced" pulmonary arterial hypertension in at risk populations with normal resting pulmonary artery pressures: [107-109]
    a. Connective Tissue Disease
    b. Chronic Thromboembolic Disease
    c. Family History or suggestive symptoms / investigations
2. To assess for occult left heart disease by measuring the pulmonary artery wedge pressure before and during exercise
3. As a research tool to assess the impact of an intervention on the pulmonary circulation

As discussed in Chapter 1, evaluating the response of the pulmonary circulation to exercise before and after an exercise rehabilitation programme by means of exercise RHC may be pivotal in understanding the mechanisms by which exercise capacity may improve symptoms and exercise capacity in PAH. Unlike upright standard incremental exercise testing, standardised protocols do not exist for exercising in the supine position; there is a paucity of data on the degree to which exercise capacity is altered in supine versus erect exercise and the changes in physiology that accompany the two different postures. In order to formulate

suitable supine exercise testing protocols and interpret results, it is important to understand the normal physiological responses to supine exercise and the magnitude to which exercise capacity differs from the upright position.

Although RHC is a standard and necessary test in PH, in studies of healthy individuals who do not otherwise require RHC, a non-invasive approach to assessing the exercise response is desirable, both to reduce the risk to research participant and to increase the ease to which the test can be serially performed.

Cardiopulmonary exercise testing provides information not only on the degree of exercise impairment but also on the relative contribution to that impairment from ventilation, gas exchange, and oxygen transport and delivery as discussed in Chapter 6.

Inert gas rebreathing (IGR) is an established technique for the non-invasive measurement of CO. It is based on the principle that when rebreathing in a closed circuit, the rate at which a specified non physiological gas dissolves in blood is proportional to the blood flow through the pulmonary capillaries. The pulmonary blood flow (PBF) is equal to the cardiac output in the absence of a significant intracardiac or intrapulmonary shunt, therefore CO and stroke volume can be calculated. IGR has been validated against the direct Fick method using acetylene and mass spectrometry in chronic heart failure[110] and using photoacoustic analysis of nitic oxide in pulmonary hypertension against invasive haemodynamics and CM [111].

IGR and CPET therefore provide reliable and accurate means of non-invasively assessing the exercise response and relevant physiological changes that occur between the upright and supine position.

3.1.1 **Effect of body posture on exercise capacity**

Current evidence is relatively sparse and conflicting in terms of the normal response to supine exercise in health and disease. Comparisons between studies are also difficult as a variety of incremental and endurance protocols have been used. Stepwise protocols have the advantages of achieving periods of steady state, to allow measurements of gas exchange to be performed and appear to provide

similar information in relation to gas exchange[112]. Incremental cycle exercise testing in the erect position compared with supine is associated with an increase in exercise capacity, both peak VO_2 and in total exercise time [113]. The factors contributing to this reduction in exercise capacity are discussed below.

3.1.1.1 **Ventilation and gas exchange**

When the body position is changed from upright to supine there is a reduction in FVC in healthy individuals, variation exists between subjects depending on age and sex. The supine position promotes airway closure in dependent lung regions, this change is more prominent in older subjects. Movement of the rib cage and abdomen also contribute to reduction in FVC.

At rest, In the supine position, perfusion (Q) is increased to dorsal dependent regions, however less is known about the distribution of ventilation (V), with limited data to suggest the distribution does not change significantly[114], therefore matching between ventilation and perfusion may be impaired in the supine position with increased dependent blood flow but reduced ventilation[115]. Attempts have been made to discriminate between PAH and CTEPH with resting breath-by-breath measurements of VO_2 and VCO_2 in the supine and erect position[116], however the effect of posture on gas exchange has not been studied in PAH during exercise.

In healthy individuals during stepwise erect and supine exercise, the VE/VCO_2 slope was not affected by posture, however minute ventilation was higher [117]. A small study of 9 patients with heart failure demonstrated no difference in perception of exertion (RPE) nor the ventilatory response to CO_2, but found that peak ventilation was higher in the erect position [118].

3.1.1.2 **Cardiovascular function**

At rest, stroke volume is increased in the supine position compared to erect. This is a consequence of increased venous return to the right heart from the elevated legs[119]. The increased SV is associated with a lower supine heart rate due to vagal activation and sympathetic withdrawal.

In coronary artery disease, exercising in the supine position versus upright, resulted in higher left ventricular end diastolic volumes and pressures, and higher stroke volume at rest and during sub-maximal exercise[120]. Earlier studies however, demonstrated reductions in supine heart rate during stepwise, interrupted exercise, but with no changes in ventilatory parameters or RPE and similar HR/VO_2 slopes [120]. Less conventional exercise protocols have been studied, including leg raises and static forearm exercise, this study suggested that SV reduced 5-8% during supine exercise, however did not compare this with erect responses [121].

3.1.1.3 **Muscle function**

The gravitational effect of exercising with elevated legs in the supine position decreases blood flow to the active muscles[122].

Near infrared spectroscopy (NIRS) non-invasively assess dynamic changes in skeletal muscle oxygenation. The NIRS signal provides continuous, non-invasive monitoring of the relative concentration changes in oxygenation ($[HbO_2]$), deoxy-hemoglobin ([HHb]) and total hemoglobin ([Hbtot]) concentrations[123]

In a small study of healthy individuals, using constant load cycling and NIRS, found that muscle oxygenation was reduced in the supine position, this may be due to increased oxygen extraction and did not alter the rate of muscle fatigue compared to the same load in the upright position [124]. More recent data using EMG demonstrated that fatigue was significantly greater during supine compared with upright high-intensity cycling and was accompanied by a reduced activation of the exercising muscles[125].

3.2 **Aims**

Determine the normal cardiovascular and ventilatory responses to erect and supine exercise during cardiopulmonary exercise testing (CPET)

Determine the extent to which exercising in the supine position reduces peak work rate in order to inform exercise protocol design in the supine position

3.3 **Methods**

This study was assessed by the West of Scotland Research Ethics Committee and did not require formal ethical approval.

Inclusion criteria:

1. No significant current or past medical health problems

2. No contraindications to exercise testing such as unexplained syncope

3. Able to provide informed consent

Thirteen healthy volunteers participated in the study. Subjected were recruited through written and verbal advertisement at Glasgow University Medical School and the Golden Jubilee National Hospital. Recruited took place between September 2014 and April 2015. The study and all exercise tests were performed in the SPVU at the Golden Jubilee National Hospital, Clydebank, Scotland.

Four exercise tests were performed in total (Figure 3.1). Two maximal incremental tests; one erect and one supine. These tests were used to determine the peak erect and peak supine work rate. Subjects then performed sub-maximal step-wise exercise with inert gas rebreathing (IGR) measurements of pulmonary blood flow at 20, 40, 60 and 80% of peak work rate. There was a minimum rest period of 24 hours between exercise tests to allow recovery.

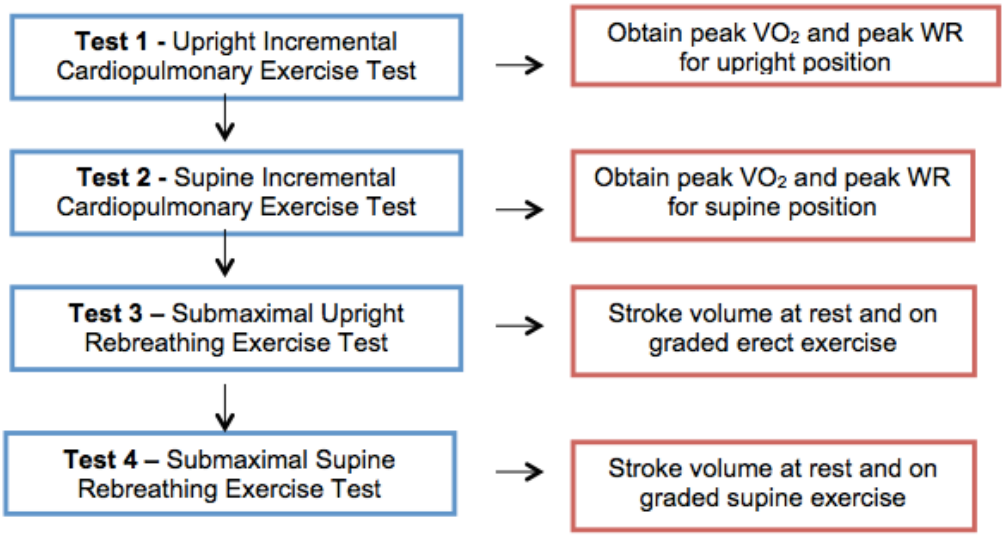


Figure 3.1: Protocol for exercise testing

3.3.1 **Incremental cardiopulmonary exercise testing**

Cardiopulmonary exercise testing was performed as described in Chapter 6 using the Lode™ electromagnetically braked upright ergometer and the Lode™ supine ergometer. Breath by breath measurements were performed using the lovemedical® software in upright and the Innocor®, Innovsion, Denmark in the supine position.

In the incremental test, the work rate varied from a ramp of 15 to 30 watts per minute and was determined by the study investigator (AM) based on the subjects' level of fitness.

Comparisons of resting, unloaded and peak variables were made between incremental tests. Isotime comparisons were also made, isotime being defined as the same time point in loaded exercise between two tests. The ramp rate was the same for individual participants between erect and supine tests, therefore isotime corresponded to isowork. Highest isotime comparisons were used in this study, meaning the highest time point was taken from the test of shortest duration for that individual, comparisons were then made using the equivalent time point in the test of greater duration (Figure 3.2)

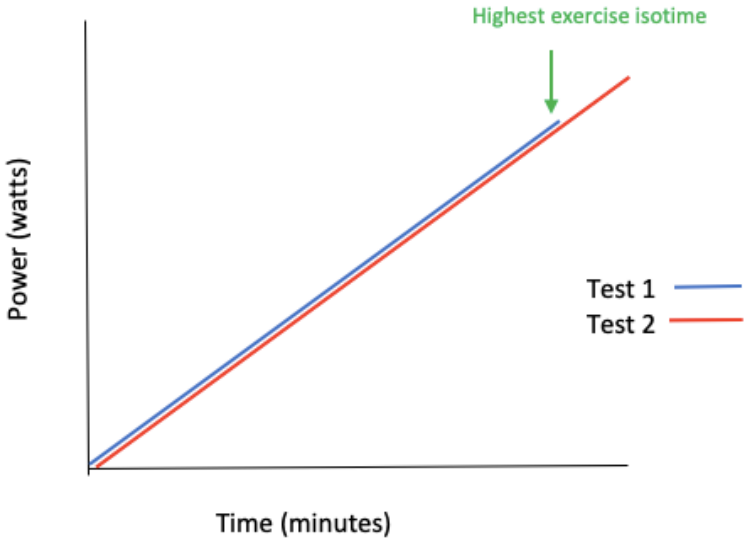


Figure 3.2: Illustration of isotime measurements on an incremental exercise test

3.3.2 Inert Gas Rebreathing with Innocor

Inert gas rebreathing (IGR) was performed using Innocor®, Innovision, Denmark (Figure 3.3). IGR was used to determine pulmonary blood flow and therefore right ventricular stroke volume at rest and during exercise.

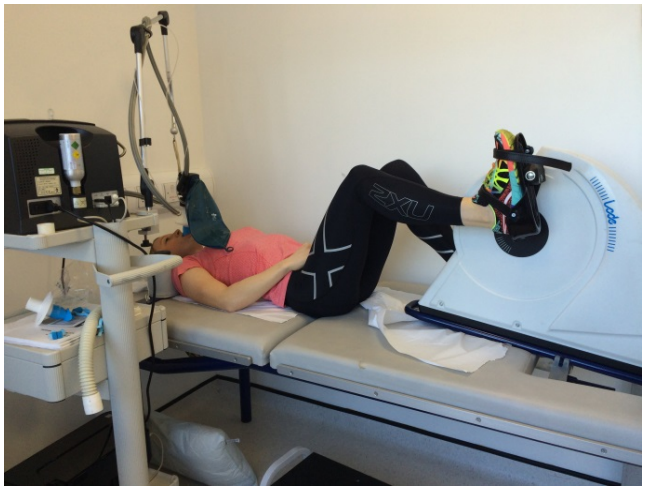


Figure 3.3: The LodeTM supine ergometer in use during a supine exercise test with inert gas rebreathing

Prior to each IGR measurement, a Douglas bag was pre-filled with an oxygen enriched mixture containing blood soluble 0.5% nitrous oxide (%) and blood insoluble 0.1% sulphur hexafluoride (SF6). This was obtained by mixing a bolus

from a gas bottle containing 94% oxygen, 5% N_2O and 1% SF6 with ambient air. Under normal resting conditions, an automated bolus fraction of 10% and air fraction of 90% was used to give a gas mixture of 28.3% oxygen, 0.5% N_2O and 0.1% SF6. The volume in the rebreathing bag was adjusted to accommodate for the increasing tidal volume and oxygen demand during exercise. The target volume was calculated automatically by the Innocor software and was the highest of the following volumes: 44% of predicted vital capacity, averaged tidal volume during the preceding five breaths and the volume required to meet the maximum carbon dioxide (default 6%) and minimum oxygen limits (default 13%) in the rebreathing bag. At higher work rates, the increased oxygen demand was met by either increasing the bolus fraction or rebreathing bag volume.

To perform the test, the subject sealed their mouth around a mouthpiece, a nose clip was used to create a sealed circuit from the Innocor machine to the lungs, with a gas delivery line and a gas sampling line. The patient was instructed to breathe at a rate of at least 20 breaths/min and empty the Douglas bag during each inspiration. Once gas concentrations were stabilised after 2 to 3 breaths, the re-breathing test was commenced by AM and the one-way respiratory valve was activated. At end expiration the subject inhaled the gas mixture and a photoacoustic infrared gas analysed measured the rate of disappearance of N_2O from the exhaled gas over four to five respirations. This did not take longer than 30 seconds, to avoid recirculation of N_2O. Alveolar N_2O concentration was normalised for changes in lung volume using SF6 concentration before the start of each PBF calculation.

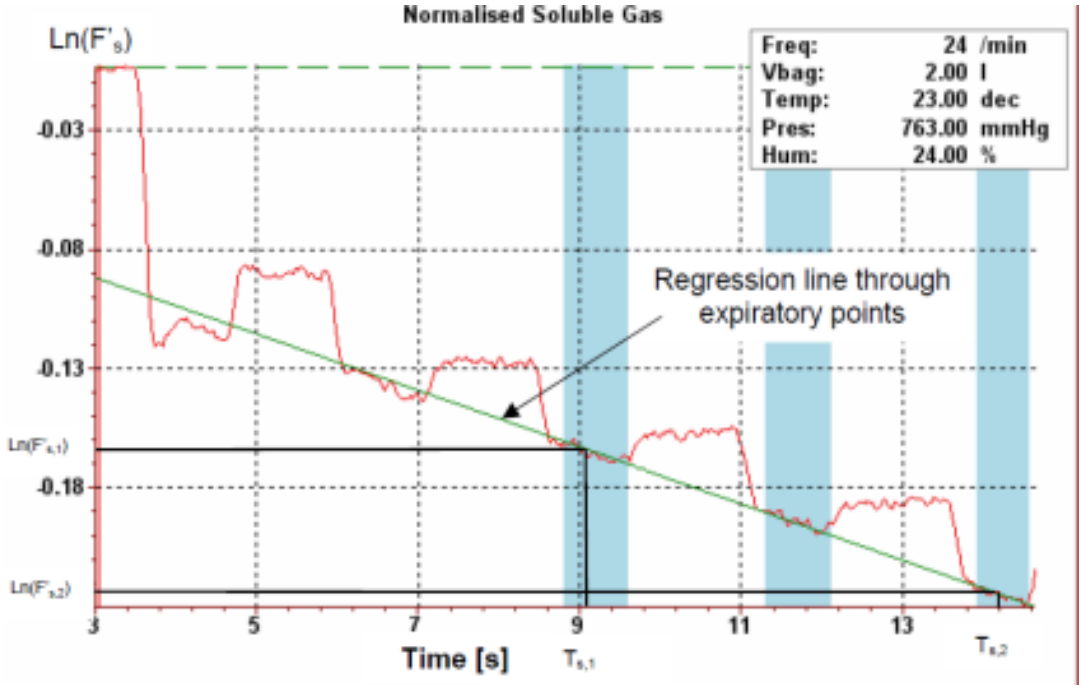


Figure 3.4: Normalised N_2O concentration against time

PBF was calculated automatically by the Innocor software using the following formula

$$PBF = \frac{-\beta \cdot V_L \cdot C_1 + C_2}{\alpha_b}$$

β = Slope of the regression line

V_L = Total systemic volume (automated calculation from initial and end SF6 concentration in relation to the volume of the rebreathing bag)

C_1 = Ambient pressure – 47mmHg / 760 mmHg

C2 = Constant for the absorption of N_2O into lung tissue (0.407 STPD x 0.6L)

α_b = Bunsen solubility coefficient for N_2O in blood (0.412 STPD)

Pulmonary blood flow is equivalent to cardiac output, therefore stoke volume could be calculated as:

$$SV = \frac{PBF}{HR}$$

3.3.2.1 **Resting IGR measurements**

At the first visit the subjects were shown how to perform an IGR manoeuvre and practice attempts with only air in the rebreathing circuit were performed until the patient could perform the test with ease. Before subsequent tests, a practice test was performed to confirm familiarity with the technique.

For supine tests, subjects lay at rest for 10 minutes to ensure the recorded measurements were a true reflection of the resting supine state. After the first IGR manoeuvre, 5 minutes was given between subsequent tests to ensure N_2O washout had occurred (<0.002%). The IGR manoeuvres were repeated in the supine position until 3 technically acceptable results were obtained.

3.3.2.2 **Exercise IGR measurements**

Stepwise exercise was performed in the supine and erect position. 3 minutes of rest was followed by 3 minutes of unloaded exercise then a stepwise work rate increasing by 20% of peak work rate every 3 minutes until peak exercise (Figure 3.5). At each increment in the exercise protocol, IGR measurements of PBF and HR were taken. If the N_2O remained greater than 0.002%, exercise continued at that step until full washout had occurred. This was rarely more than 30 seconds beyond the allocated 3 minutes.

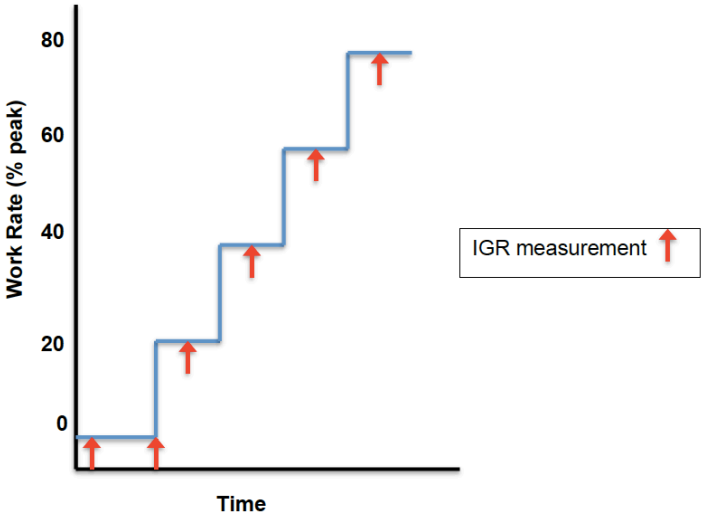


Figure 3.5: Inert gas rebreathing measurement protocol, each step was 3 minutes in duration, IGR measurements occurred at points marked by the arrow

3.4 **Statistics**

The primary outcome measure was the difference in peak oxygen consumption (VO_2) between the upright and supine position. Sample size calculation based on the study by Terkelsen et al [117]. With a power of 0.8 and two sided significance (α) of 0.05, this generated a sample size of 10. 13 subjects were recruited to account for the possibility of non-analysable data and dropouts.

Statistical analyses were performed using IBM ® SPSS ® Statistics, version 26. Comparisons of paired data were made using Wilcoxon signed-rank test given the small population size. No corrections were made for multiple comparisons. $P < 0.05$ was considered statistically significant.

3.5 **Results**

Useable data were collected from twelve of the thirteen participants, one subject was excluded due to an inability to reliably perform the IGR measurements.

7/11 (63.6%) subjects were male, the average age was 28 (22- 53) and was BMI 22.5 (19.6 – 29.7).

3.5.1 **Exercise capacity**

Peak work rate was higher in the erect position 249 watts (43) than the supine position 222 watts (37), p 0.003. VO_2 at anaerobic threshold was lower supine (1.614L/min) than erect (1.713L/min) (p 0.004) and total exercise duration was higher in erect 668 seconds (66) versus 602 seconds (80) in the supine position, p0.020. VO_2 and RER differences are displayed in Table 3.1

Table 3.1: Markers of exercise capacity in the supine versus upright position

VO_2 (L/min)	Erect	Supine	p
Rest	0.43 (0.16)	0.38 (0.04)	0.410
Unloaded	0.65 (0.10)	0.63 (0.13)	0.814
Peak	2.9 (0.6)	2.7 (0.4)	0.182
VO_2 ml/kg			
Unloaded	9.5 (1.6)	8.9 (1.6)	0.637
Peak	42.6 (11)	39.2 (7.3)	0.099
RER	Erect	Supine	p
Rest	0.85 (0.11)	0.85 (0.06)	0.937
Unloaded	0.84 (0.06)	0.83 (0.08)	0.937
Peak	1.21 (0.1)	1.25 (0.1)	0.185

3.5.2 **Cardiovascular response – CPET**

Heart rate was higher throughout all stages of exercise in the erect position than supine (Table 3.2)

Table 3.2: Cardiovascular response to exercise in erect versus supine position

Heart rate	Erect	Supine	p
Rest	80 (12)	66 (11)	**0.002**
Unloaded	90 (16)	78 (13)	**0.006**
Peak	170 (12)	159 (13)	**0.012**
VO_2/HR	Erect	Supine	p
Rest	5.4 (1.7)	6.1 (1.2)	0.158
Unloaded	7.2 (1.3)	7.9 (1.4)	0.182
Peak	17.5 (4.1)	17.3 (2.8)	0.754

3.5.3 **Cardiovascular response – IGR**

Stroke volume was higher in all stages of exercise in the supine position, reaching statistical significance in the later stages of exercise. Peak stroke volume was

reached at an earlier stage in exercise in erect cycle ergometry than supine (**Error! Reference source not found.**), (Table 3.3, Figure 3.6)

Table 3.3: Stroke volume response to erect and supine exercise

SV (mL) at different stages of peak work rate	Erect	Supine	p
Rest SV	72.6 (21.0)	92.2 (23.6)	0.116
Unloaded SV	87.6 (4.7)	101.2 (17.1)	0.144
20% SV	104.7 (14.0)	103.7 (12.0)	0.878
40% SV	103.2 (10.9)	103.6 10.9)	0.721
60% SV	97.9 (13.9)	110.6 (17.7)	0.050
80% SV	91.7 (9.1)	102.7 (13.2)	0.036
Peak SV	92.1 (12.6)	105 (15.2)	0.028
Mean Highest SV in test	111 (14.4)	119 (12.1)	0.345
% peak WR at highest SV	28 (18)	55 (14)	0.026

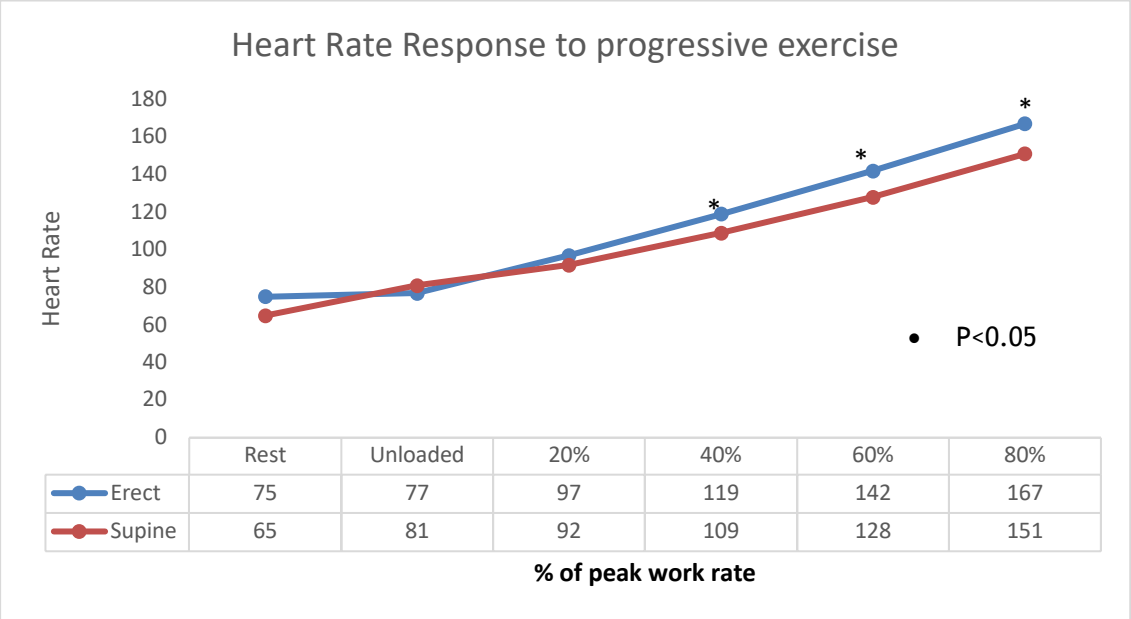


Figure 3.6: Heart rate response to supine and erect exercise

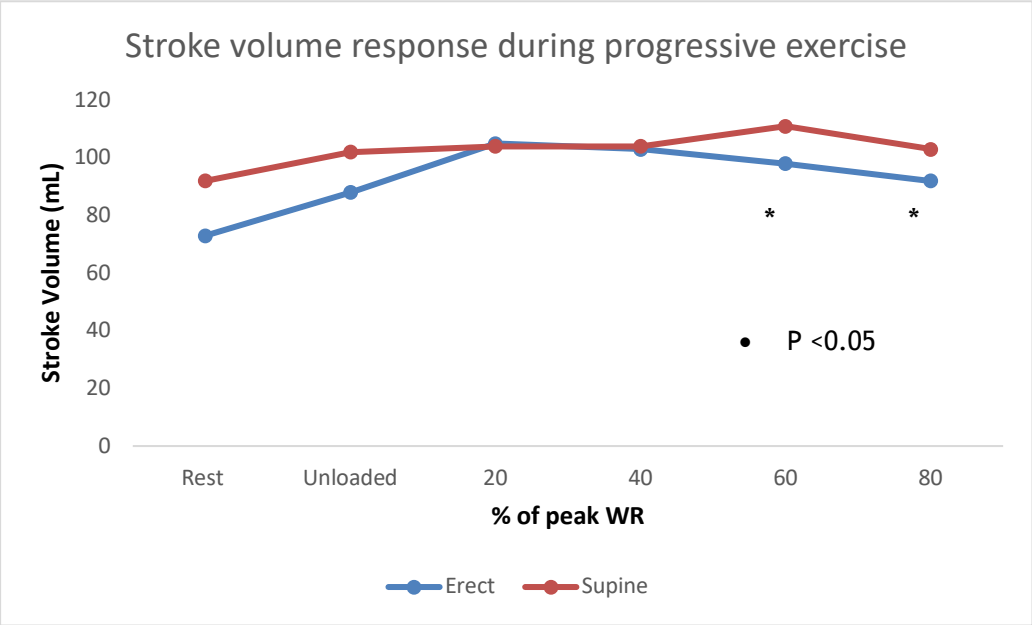


Figure 3.7: Stroke volume response to supine and erect exercise

No statistically significant difference were found in CO, a trend was seen in the early stages of exercise to higher CO in the supine position and in the later stages of exercise, to higher CO the erect position Table 3.4

Table 3.4: Cardiac output response to exercise in erect and supine position

Cardiac output (L) at progressive stages of exercise (% peak WR)	Erect	Supine	p
Rest CO	5.7 (1.5)	6.8 (1.5)	0.116
Unloaded CO	6.6 (1.6)	7.7 (1.1)	0.273
20% CO	10.0 (1.6)	9.5 (1.5)	0.674
40% CO	12.1 (1.2)	11.4 (1.7)	0.407
60% CO	13.9 (1.1)	14.2 (2.4)	0.575
80% CO	15.5 (1.1)	15.3 (2.2)	0.889
Peak CO	15.8 (1.7)	15.1 (2.3)	0.310

3.5.4 **Ventilatory response**

Tidal volume was higher in all stages of erect exercise and there was an associated lower respiratory rate. No changes were observed in minute ventilation (Table 3.5,Figure 3.8)

Table 3.5: Ventilatory responses to erect and supine exercise

RR	Erect	Supine	p
Rest	14 (5)	16 (5)	0.128
Unloaded	17 (5)	19 (4)	0.182
Peak	36 (5)	40 (6)	**0.023**
VE (l/min)	Erect	Supine	p
Rest	14.0 (3.5)	13.3 (2.7)	0.433
Unloaded	19.7 (3.3)	18.7 (3.3)	0.530
Peak	100.3 (20)	97.4 (13.5)	0.480
Vt (L)	Erect	Supine	p
Rest	1.1 (0.4)	0.9 (0.2)	0.083
Unloaded	1.3 (0.4)	1.1 (0.3)	**0.050**
Peak	2.85 (0.6)	2.51 (0.42)	**0.050**

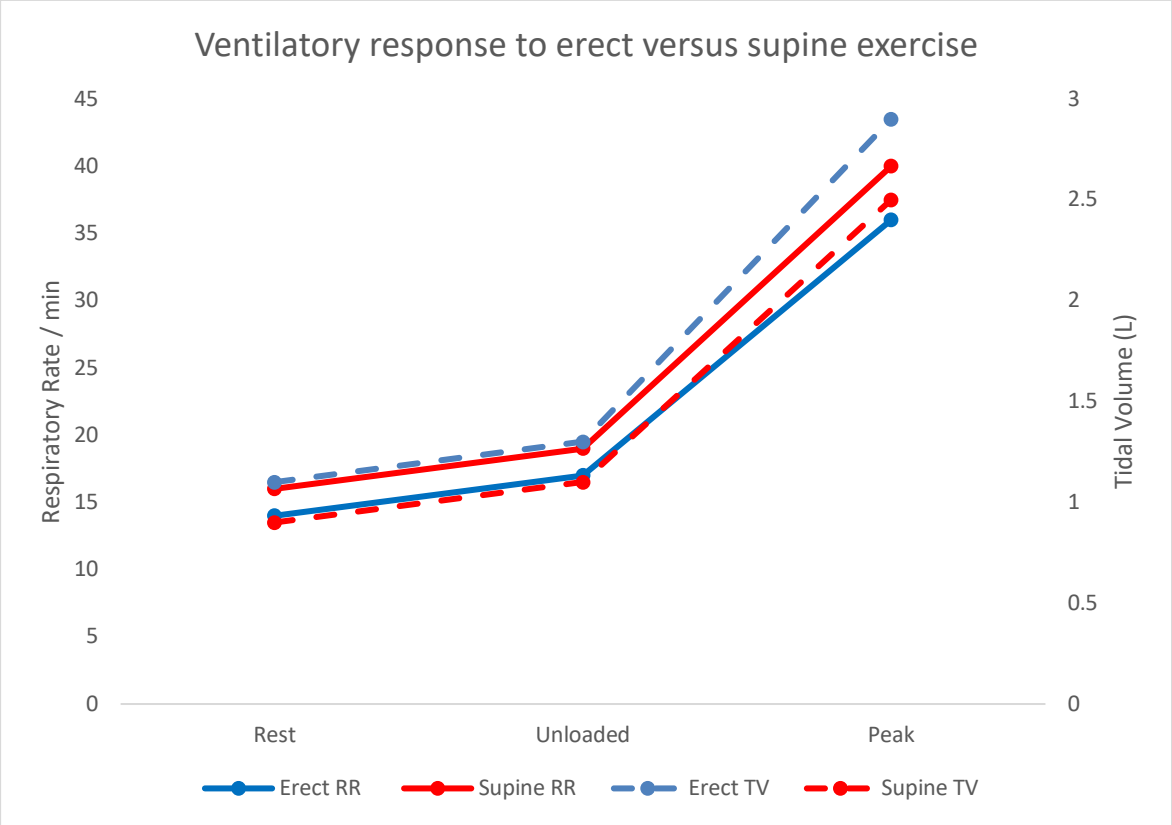


Figure 3.8: Tidal volume and respiratory rate changes between erect and supine exercise

3.5.5 **Gas exchange response**

No significant changes were noted in gas exchange variables (Table 3.6)

Table 3.6: Gas exchange variables supine and erect exercise

$PETCO_2$ (mmHg)	Erect	Supine	p
Rest	5.2 (0.6)	5.5 (0.7)	0.099
Unloaded	5.4 (0.7)	5.6 (0.6)	0.346
Peak	5.7 (0.8)	5.6 (0.7)	0.638
VE/VCO_2	Erect	Supine	p
Rest	35.1 (7.1)	33.7 (4.6)	0.368
Unloaded	32.8 (5.1)	31.0 (5.1)	0.209
Peak	27.6 (4.4)	27.4 (3.7)	0.929

3.5.6 **Isotime CPET analysis**

Highest exercise isotime was used to compare the exercise response between upright and supine incremental tests. Ventilation and gas exchange were more efficient, with a trend to higher VO_2, higher WR and lower HR in the erect cycling position (Table 3.7)

Table 3.7: Highest isotime CPET comparison between erect and supine ergometry

Highest Isotime	Erect	Supine	p
VO_2 (ml.kg.min)	41 (9.8)	39.8 (7.4)	0.317
Heart Rate (bpm)	162 (13)	162 (13)	0.878
Respiratory Rate	29.7 (6.2)	39.6 (6.2)	**0.004**
VE (L/min)	83.2 (10.7)	95.8 (12.9)	**0.010**
VCO_2 (L/min)	3.1 (1.0)	3.4 (0.4)	**0.041**
VO_2 (L/min)	2.8 (0.5)	2.7 (0.4)	0.594
RER	1.1 (0.3)	1.2 (0.1)	**0.007**
VO2/HR	17.7 (4.7)	17.4 (2.8)	0.722
PET O2 (mmHg)	15.3 (0.8)	16.2 (0.6)	**0.008**
PET CO_2 (mmHg)	6.2 (0.8)	5.7 (0.5)	**0.013**
$VeqCO_2$	25.4 (3.4)	26.6 (2.5)	0.230
$VeqVO_2$	28.7 (4.2)	32.9 (3.3)	**0.016**
Vt (L)	2.9 (0.5)	2.5 (0.4)	**0.007**

3.6 **Discussion**

This study demonstrated that exercise capacity is greater in the upright position. Work rate was seen to be 12% higher in the upright position than supine, the anaerobic threshold was reached at a later stage and exercise duration was 11% longer. These changes are in keeping with overall reduced efficiency of exercise in the supine position and reduced VO_2/WR relationship. Knowledge of the degree to which peak work rate is reduced in the supine position helps to determine appropriate work loads for supine exercise testing for PAH patients undergoing exercise right heart catheterisation. The factors contributing to this reduction in efficiency in the supine position are discussed below.

Efficiency of ventilation appeared to be contribute to this difference. Peak RR was lower and tidal volume higher in the erect exercise, suggesting more efficient ventilation. The improved ventilatory efficiency was also evident at highest exercise isotime, with lower VCO_2, $VeqCO_2$ and respiratory rate in the upright position and higher tidal volume. This may be due to reduced diaphragmatic excursion in the supine position due to the effects of the abdominal compartment. It is well established that vital capacity is reduced in the supine position[3],

Cardiovascular changes did not reach statistical significance, however there was a trend to higher stroke volume in the supine position, associated with lower heart rate. This may be due to increased venous return from the elevated lower limbs and lower vagal tone in the spuine position, further research, for example looking at regional muscle blood flow, would be required to verify this .

Changes in muscle function or muscle fatigue were not measured in this study and may contribute to the observed changes seen.

This data allows prediction of suitable work rates for supine incremental and steady state exercise testing in health, based on incremental upright CPET data. It is possible that an exaggerated difference may be seen in supine and upright exercise in PAH, due to the pre-existing myopathy, ventilatory and cardiovascular inefficacy.

3.7 **Conclusions**

Exercise capacity is reduced by approximately 12% in the supine position in young, healthy individuals.

The reduction in exercise capacity appears to be multifactorial, with reduced a ventilatory efficiency and a trend towards reduced cardiovascular efficiency. Muscle function and fatigue were not assessed in this study and could also be a contributing factor.

Chapter 4 Methods "The effect of adding exercise training to optimal therapy in pulmonary arterial hypertension"

4.1 Introduction

After establishing sufficient demand for exercise training in Scotland, the study "The effect of adding exercise training to optimal therapy in pulmonary arterial hypertension", was conducted. The protocol design was based on existing evidence on exercise training in PAH as discussed in Chapter 1. The most effective strategy to date appears to be that adopted by Grunig et al [61], involving 3 weeks of supervised residential exercise comprising personalised resistance and aerobic exercises – both endurance and interval training, followed by 12 weeks of remotely monitored exercise with home exercise equipment and specific goal setting. This strategy resulted in the greatest increase in QOL scores and 6MWD, therefore was the most likely to provide a translatable benefit in the UK, where no formal exercise rehabilitation exists for PAH and provide insights into the physiological changes that occur. In designing the study protocol, primary end points were chosen to reflect exercise capacity, cardiac function and quality of life. A number of exploratory secondary end points were employed to investigate the physiological and biological changes with exercise therapy.

4.2 Ethical Approval

This study was approved by the West of Scotland Research Ethics Committee on 4th September 2015 (REC Reference 15/WS/0197, IRAS project ID 181697). NHS National Waiting Times Board acted as the study sponsor.

Within the main study, two optional sub-studies existed. One to assess invasive haemodynamic responses to exercise therapy, requiring exercise right heart catheterisation. The second to assess muscle structure and function, requiring muscle biopsy before and after the exercise programme. Both sub-studies required separate written consent.

4.3 **Study Setting**

This research was conducted at the SPVU, Golden National Jubilee Hospital, Clydebank, Glasgow. The SPVU serves a population of 5.3 million and is the Scottish tertiary referral centre for PAH. The incident population of treated PH patients during the period this research was conducted rose from 498 in 2014 to 649 in 2018.

Exercise rehabilitation sessions were performed at the Golden Jubilee National Hospital (GJNH) Physiotherapy Gym. Muscle biopsies were performed between Gartnavel General Hospital and Glasgow Royal Infirmary Radiology departments. All other investigations were conducted at GJNH.

4.4 **Study Population**

Patients with PAH, confirmed by right heart catheterisation (RHC) in accordance with European guidelines, were recruited from the Scottish Pulmonary Vascular Unit (SPVU) outpatient clinic. Written information regarding the study was provided and prospective participants were asked to provide a response within 14 days.

Inclusion criteria:

1. World Health Organisation functional class (WHO-FC) II-III
2. Stable on optimal disease targeted PAH therapy for ≥ 3 months
3. 18 years of age or older
4. Able to provide written informed consent

Exclusion criteria:

1. Unable to provide informed consent
2. Significant peripheral vascular disease, neurological or musculoskeletal comorbidity precluding exercise

3. Exercise induced syncope, cardiac arrhythmia or chest pain

4. Pregnancy

5. Specific component exclusions: Cardiac MRI (CMR): Any contraindication to MRI

4.5 **Study design**

This was a prospective, single centre, controlled crossover study. It included two groups: a group receiving exercise therapy for 15 weeks (Group A) and a group receiving standard care for 15 weeks (Group B). Crossover to the exercise arm occurred with group B participants after 15 weeks of standard care. In group A, the study intervention was performed in two phases.

Phase 1: A 3-week residential exercise training programme, based at the Golden Jubilee Hospital, Clydebank

Phase 2: A 12-week ambulatory exercise training programme remotely monitored by a specialist physiotherapist.

In Group B, the study intervention was performed in 3 phases:

Phase 1: 15 weeks of standard medical care for PAH

Phase 2: A 3-week residential exercise training program, based at the Golden Jubilee Hospital, Clydebank

Phase 3: A 12-week ambulatory exercise training program remotely monitored by a specialist physiotherapist.

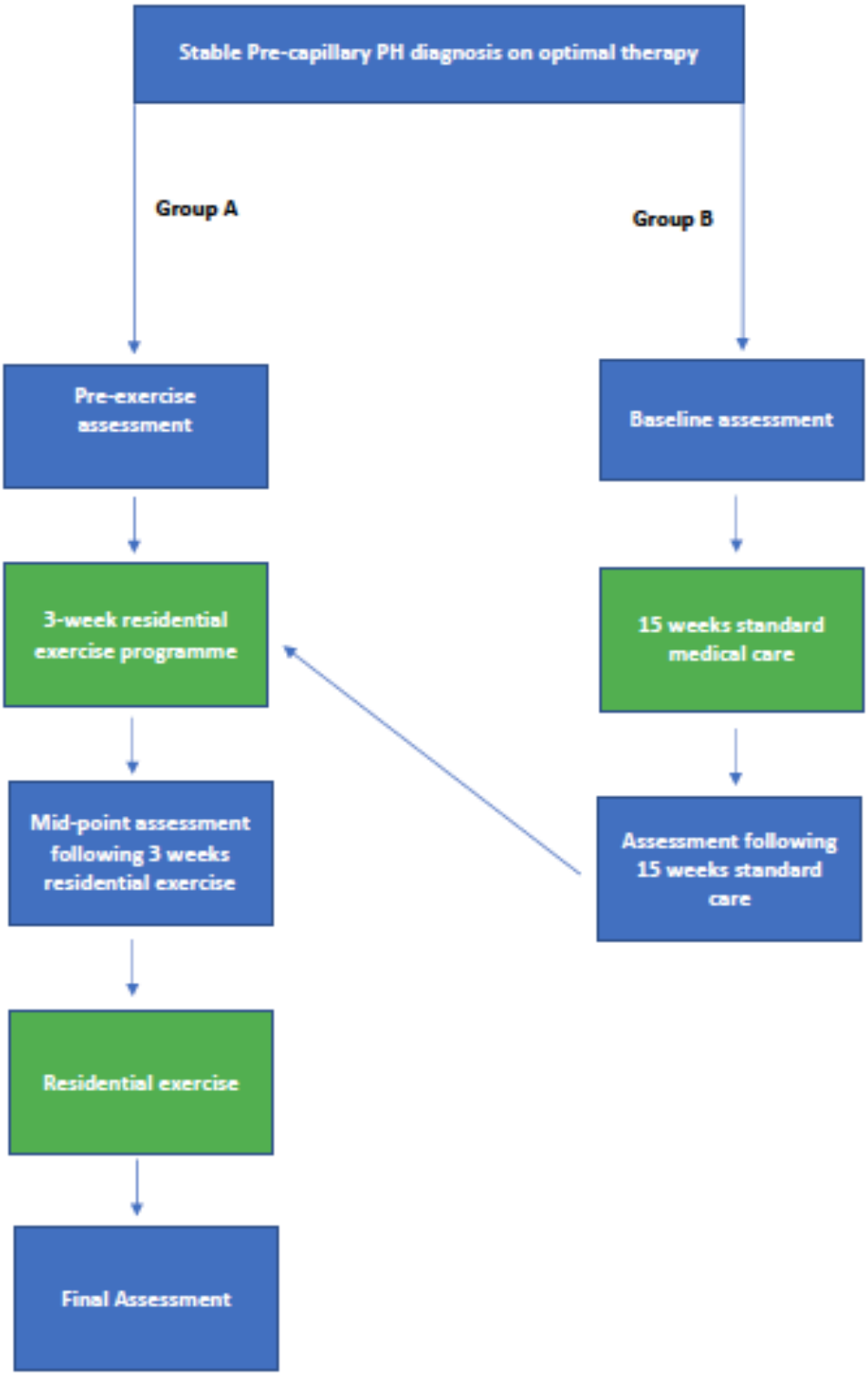


Figure 4.1 Flow chart of study protocol. Green indicates intervention, blue indicates assessment time point

4.5.1 Outcome measures

4.5.1.1 Primary outcome measures

To reflect the potential physiological and functional changes relevant to clinical improvement in PAH, three well validated primary end points were selected for the study.

1. Change in 6-minute walk distance (6MWD) from baseline to end of study

2. Change in quality of life (QOL) parameters (SF-36, CAMPHOR and EmPHasis-10) from baseline to end of study

3. Change in resting RV ejection fraction measured by Cardiac MRI (CMR) from baseline to end of study

6MWD: The 6-minute walk test (6MWT) is validated and commonly used outcome measure in PAH. It is a sub-maximal exercise test where the subjects walks as far as they can within the 6 minute time frame. The 6MWD was performed in line with the ATS standards for conducting the 6MWT [126].

SF-36™ version 2 by Optum® is an established patient reported quality of life scoring system. It reports on eight domains: Physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, and mental health. There are two summary scores, physical and mental. The SF scoring software system was used to generate scores and an appropriate software license was obtained. (Appendix 12: SF-36)

EmPHasis-10, by the Pulmonary Hypertension UK Society, is a PAH-specific health-related quality of life (HrQOL) score that consists of 10 items relating to breathlessness, fatigue, control and confidence (
Appendix 10: EmPHasis-10)

CAMPHOR (Cambridge Pulmonary Hypertension Outcome Review), Galen Research, is an earlier PH specific HrQOL tool, consisting of 65 items relating to symptoms, activities and quality of life (

Appendix 11: Camphor)

4.5.1.2 **Secondary outcome measures**

A variety of secondary outcome measures were collected to generate pilot mechanistic data. The rationale and methods of each testing modality are discussed in detail in chapters 5 to 9 and are briefly outlined below:

1. Exercise capacity:
    a. Standard incremental cardiopulmonary exercise test (CPET) (Chapter 6)
    b. Supine ergometer endurance time
    c. Cardiac and haemodynamic function
2. Cardiac function

- Cardiac MRI [127] (0)
- N-terminal pro brain natriuretic peptide (NTproBNP) concentration [128]
- Standard RHC measurements at rest and supine exercise performed on a cycle ergometer (Chapter 7)

3. Ventilatory function and gas exchange (Chapter 6)
    a. Full pulmonary function tests
    b. Mouth pressures
4. Peripheral muscle function (0)
    a. Volitional quadriceps and grip strength measured by a myometer.
5. Profile of systemic inflammation and metabolism
    a. Interleukin-6 cytokine assay

b. Specific micro-RNA primers reflecting angiogenesis, myogenesis and inflammation

c. C-peptide and fasting glucose concentration

4.5.1.3 Sample size calculations

The primary outcome measure was change in 6 minute walk distance (6MWD) at 15 weeks. Power calculation for 6MWD was performed based upon a between group difference at 15 weeks of 74m [61] with a standard deviation of 56m, power of 80% and confidence level of 0.05. This provided a sample size in each group of 11. Thirty participants were enrolled to cover for unexpected dropouts.

The table below summarises the power calculations for the other key outcome variables, including QOL and RVEF, the two additional primary outcome measures. The calculations are based on paired changes, treatment compared with control, with a power of 80% and confidence level of 0.05

Table 4.1 Sample size for change in secondary outcome measures

Variable	Change Expected in Treatment Group	SD of change	Sample size	Reference for expected change	Clinical relevance
SF36: Physical Functioning	17	14	22	[61]	Minimum clinically important difference (MCID) defined in a paediatric population of 10 [129]
Right Ventricular Ejection Fraction (RVEF) (%)	5	4.9	32	[127, 130]	No validated MCID in PAH, mean change of 4.6% over 1 year observed in a multicentre study on the effect of disease targeted therapy. This was

					associted with improved 6MWD.[131]
NTproBNP (ng/L)	-400	-175	8	[132]	No defined MCID exists for NTproBNP in PAH, maintenance in low category (< 300ng/L) associated with improved prognosis versus intermediate (300-1400 ng/L) or high (>1400 ng/L)[133]
Exercise RHC CI ($L/min/m^2$)	1.0	1.0	32	[134]	Change in exercise CI with pulmonary vasodilator therapy resulted in a mean of 0.4. Change in exercise CI following exercise therapy in PAH is also associated with improved 6MWD[135].
Peak VO_2 (L/min)	0.2	0.12	12	[134, 136]	A change of 0.2L/min has been seen following exercise therapy in PAH and associated with improved QOL [64]
Maximum inspiratory mouth pressure (MIP) (kPa)	3	2	14	[100, 137]	MCID does not exist for MIP, a lower limit of normal has been proposed as 9.95 kPa for men and 7.43 kPa for women. [138]
Quadriceps Strength (Nm)	12	3.5	4	[63, 139]	An MCID of 7.5nM has been defined following COPD rehabilitation [140]

4.5.2 **Allocation to groups**

Randomisation was planned to be performed with the use of pre-sealed envelopes. Due to the time commitments for subjects involved in the study (3 weeks residential stay for exercise therapy) and competing commitments (childcare, employment), it proved very difficult for many subjects to accept their randomised study allocation. Therefore, in order to maintain recruitment, these subjects were offered the most convenient study slot for them. Thereafter, subjects were allocated to study group based on subject availability and balanced clinical and demographic factors. This amendment was discussed with the approving research ethics committee.

4.5.3 **Assessments**

The contents of assessments are outlined in Table 4.2 and the timeline for these assessments are described below. All assessments with the exception of muscle biopsy, were performed within the designated assessment periods mentioned below. Muscle biopsies were performed at least one week prior to commencing the exercise programme to allow sufficient healing time and on completion of the exercise programme, biopsies were performed in the following week.

Group A had three assessments

1. A.1 Baseline

2. A.2 End of the residential exercise therapy (Week 3)

3. A.3: End of study (Week 15)

Group B had four assessments

1. B.1: Baseline

2. B.2: After 15 weeks of standard care, prior to commencing the exercise programme (Week 15)

3. B.3: At the end of 3 weeks residential exercise (Week 18)

4. B.4 End of study (Week 30)

Table 4.2: Outline of assessment visits and investigations performed

Assessment time point	Content of assessment	
Assessment B.1	6MWD CMR QOL NTproBNP CPET	
Assessment A.1 and B.2 Prior to exercise programme	6MWD CMR QOL NTproBNP CPET PFT Metabolic blood panel	Exercise RHC** Muscle Biopsy **
Assessment A.2, B.3 End of residential exercise programme	6MWD QOL NTproBNP CPET PFT	
Assessment A.3, B.4 End of study	6MWD CMR QOL NTproBNP CPET Metabolic blood panel	Exercise RHC** Muscle Biopsy **
Abbreviations: 6-minute walk distance (6MWD), Cardiac MRI (CMR), Quality of life (QOL), N-terminal pro brain natriuretic peptide (NTproBNP), Cardiopulmonary		

exercise test (CPET), Right heart catheterisation (RHC)

**** In subjects who consented to these sub-studies**

4.6 **Safety**

Exercise was supervised continuously by a specialist physiotherapist and doctor when required. All patients were confirmed as being in a clinically stable condition by a clinician with PAH expertise prior to commencing the rehabilitation programme. Oxygen saturations and heart rate were monitored intermittently during exercise using a Nonin® Go2 pulse oximeter™.

1. For all exercise sessions, supplemental oxygen was used if oxygen saturations were below 90%, using a static oxygen concentrator for stationary exercises and oxygen cylinders for dynamic exercises. The modified Borg scale (

Appendix 2: Modified Borg dyspnoea scale) was used to assess subjective levels of breathlessness and leg fatigue.

For safety reasons, exercise intensity was reduced or terminated according to the following criteria:

- Heart rate (HR) was consistently over 120 beats per minute.
- Modified Borg level was greater than 5.
- Physician or physiotherapist discretion
- Patient request

4.7 **Study intervention**

The exercise programme consisted of low dose resistance and aerobic training. Five main components were included in the programme

1. Walking
2. Stationary bicycle ergometer interval training
3. Upper and lower limb resistance training
4. Respiratory muscle training and breathing control techniques
5. Relaxation and education sessions

Prior to commencing the exercise programme, patients were provided with an exercise diary (
Appendix 3: Exercise Diary). They used this diary to log pre-defined goals they wished to achieve by the end of the exercise programme. The diary was also used to monitor compliance and provide reinforcement of the exercise techniques.

The daily programme (

Appendix 5: Sample residential) consisted of a combination of aerobic and resistance exercise, education or relaxation and respiratory muscle training. A total of 1.5 to 2 hours per day of exercise and education were performed over four sessions, with rest intervals between. At weekends, lower intensity, unsupervised exercise was prescribed, mirroring the outpatient phase. Ongoing exercise prescription and titration of exercise intensity was made by the medical and physiotherapy team on an individual patient basis. Exercise prescriptions were modified based on tolerability, progress and heart rate response.

4.7.1 **Walking and treadmill**

Sessions occurred on a PhysioMill® treadmill in the Golden Jubilee National Hospital (GJNH) Rehabilitation Department or in the grounds of the hospital. Sessions were 30-60 minutes in duration depending on the baseline functional level and the stage in the exercise programme (Figure 4.2).

To achieve steady state aerobic exercise, target heart rate range for walking sessions was 50-60% of peak heart rate and Borg was aimed for 3-4, therefore in a low intensity range, selected from a safety perspective to monitor initial tolerance and given the potential for exercise induced desaturation. The protocol for walking training and data collection can be found in
Appendix 6: Walking training.

In addition to providing aerobic cardiovascular training, walking sessions also focussed on 3 key areas:

1. **Controlling the level of exertion** (avoiding under or over exertion), patients were educated on the Borg scale , HR and SpO_2 to objectively assess this, for example being able to talk in short sentences. Once the subjects were familiar with the pulse oximeter and Borg scale, they were asked to modify exercise intensity based on these parameters, with the support of the physiotherapist (JF) or study doctor (AM)

2. **Psychological intervention**: a validated psychology method, positive mental imagery, reinforcing positive thinking towards achieving short term, realistic

goals relating to exercise and physical performance[93]. This is described in Chapter 1, Section “Personal experience: training in exercise rehabilitation and development of study protocol”

3. **Practical considerations**: Developing mechanisms for dealing with challenging environments or situations such as hills, stairs, uneven terrain.

Figure 4.2 Participants performing treadmill exercise with supplemental oxygen (left) and outdoor gait practice with supplemental oxygen (right)

4.7.2 **Bicycle ergometer**

Bicycle ergometer sessions occurred on an Ergoline Optibike ®, Bitz Germany, Ergometer in the GJNH rehabilitation gym.

An individualised interval programme was prescribed by the study doctor (AM) based on peak power (watts) from the study entry incremental CPET. To generate an interval programme that was predominantly aerobic, with short anaerobic spells, the following protocol was used:

1. 5 minutes warm up aerobic zone or unloaded cycling

2. 7.5 or 12 minutes of intervals at the following ratio depending on subject fitness

a. 1 minute at 40% of peak exercise capacity

b. 30 seconds at 60% of peak aerobic exercise capacity

3. 5 minutes cool down

Heart rate and SpO_2 monitoring were continuous throughout exercise. Borg was checked in the warmup phase, twice in the interval phase and in cool down. Exercise was modified by the study doctor on an individual patient basis depending on Borg and HR. Supplemental O_2 was provided if SpO_2 were consistently below 90%. Breathing control exercises (described below) were added to the ergometer sessions as the subject progressed (Figure 4.3). The full ergometer training protocol can be found in
Appendix 7: Bicycle ergometer protocol.

Figure 4.3: Bicycle ergometer training, with breathing control exercises (left)

4.7.3 **Respiratory muscle training**

Respiratory training involved 2 key components based on standard respiratory muscle training techniques[141]

1. Respiratory muscle strengthening

    a. Thoracic expansion techniques

b. Diaphragmatic breathing

2. Breathing control and control of hyperventilation

Passive and active exercises were performed whilst the subject was seated and a towel was used to provide proprioceptive feedback on thoracic expansion before and after the exercise session (Figure 4.4). The full protocol for respiratory muscle training can be found in
Appendix 8: Respiratory muscle training

Figure 4.4: Respiratory muscle training (top image) and using towel for thoracic expansion (lower image)

4.7.4 **Skeletal muscle training**

Body weight exercises or light weights were used and selected by the physiotherapist based on the subjects' initial assessment; the highest weight that could be lifted ten times easily was selected. Heavy weights were avoided due to the risk of increased pulmonary artery pressure and increased afterload.

The resistance programme was based on standard upper and lower limb rehabilitation methods typical of those used in pulmonary rehabilitation[142]. The full protocol for resistance training can be found in
Appendix 9: Skeletal muscle training. Each resistance training session consisted of the following sequence (Figure 4.5):

1. Upper and lower limb warm up stretches

2. Upper limb low weight repetitions (20 – two times sets of ten)

    a. Elbow flexion

    b. Shoulder extension

    c. Lateral raises

3. Lower limb body weight or low ankle weight repetitions (20- two times sets of ten)

    a. Knee extension

    b. Hip flexion

    c. Hip abduction

4. Cool down stretches

Figure 4.5: Low weight upper limb resistance exercises

4.7.5 **Outpatient phase**

After three weeks of residential, supervised exercise therapy, each participant was issued with an individually tailored training programme. This programme was based on their exercise parameters and progression during the 3-week residential exercise phase.

A cycle ergometer (domyos®, UK), dumbbell weights (domyos ® UK) and a pulse oximeter (Nonin ® UK) were provided for home. Participants were educated and independent in the use and interpretation of these devices prior to commencing the out-patient programme.

The daily home exercise programme mirrored the components of the residential phase, however, was less intensive and was designed to be incorporated into their daily routine. Typically, three sessions per day totalling 1.5 hours of exercise were

prescribed from a combination of: walking, cycle ergometry, resistance limb exercises, respiratory muscle training.

Participants were given the following advice on their safe exercise limits:

- Borg no greater than 6
- Heart rate no higher than 120 consistently
- SpO_2 no lower than 90%. Provided there were no contraindications to home oxygen, those with exertional hypoxaemia were supplied with an oxygen concentrator (Dolby vivasol ®). Relevant oxygen education was given by a respiratory nurse specialist (RT).
- Stop exercising if any of: chest pain, distressing breathlessness, lightheaded or dizzy.

Subjects returned an exercise log (
Appendix 4: Sample outpatient timetable) each week detailing the sessions completed, heart rate, Borg score and oxygen saturations. This was followed up with a weekly phone call by either the physiotherapist or study doctor. This information was used to assess safety and adverse events, determine compliance and whether the programme required adjustment.

Chapter 5 Efficacy of exercise training in patients on optimal therapy for Pulmonary Arterial Hypertension

5.1 Introduction

As discussed in chapter 1, treatment for pulmonary hypertension results in modestly improved symptoms and in the case of Epoprostenol, provides a survival benefit. Survival from PAH has improved significantly over the past 20 years since the advent of Epoprostenol and additional classes of disease targeted therapy[143]. This improvement is mirrored in the Scottish PAH cohort from 1997 to 2019 (Figure 5.1)

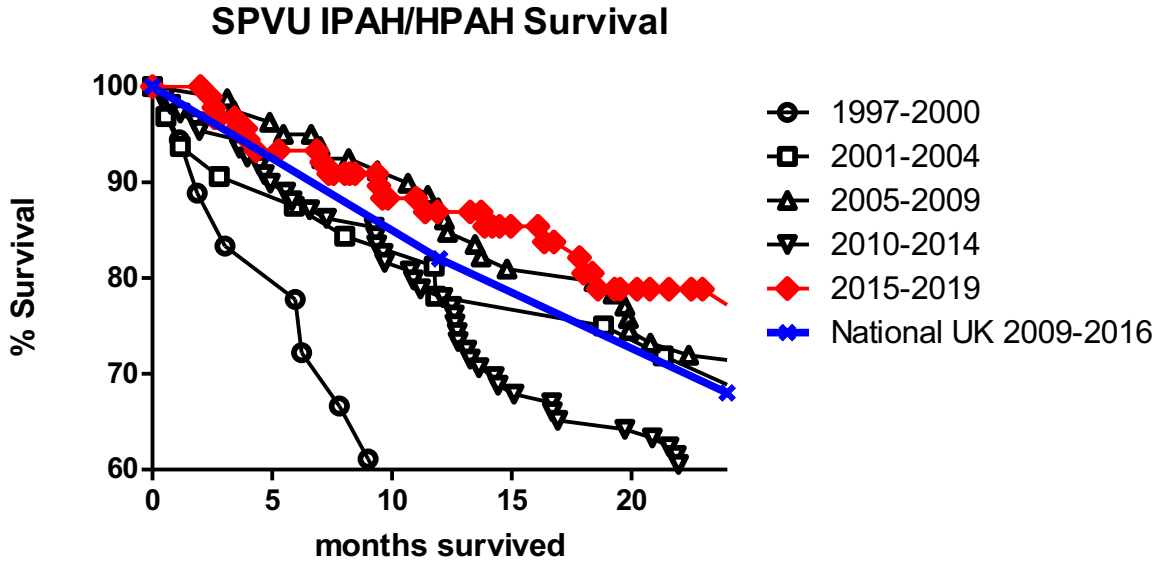


Figure 5.1: Kaplan Meier Curves for SPVU IPAH survival (1997-2019)

Despite this, Pulmonary hypertension remains a life limiting illness with around 55% of patients with IPAH being alive 5 years from diagnosis and under 50% with CTD-PAH (Figure 5.2) [34].

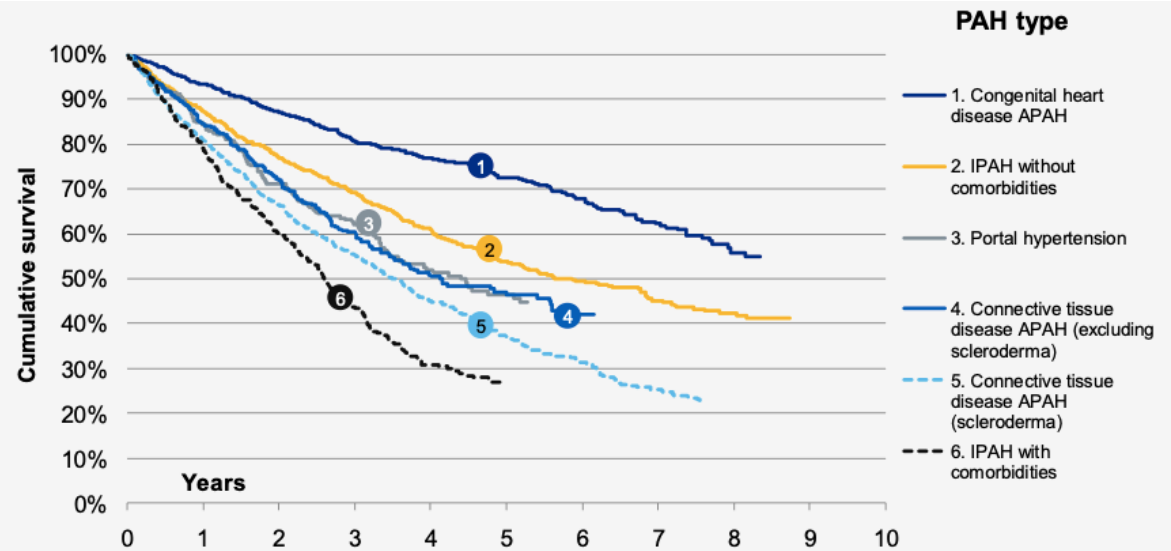


Figure 5.2: UK National Audit data 2009-2019 on PAH sub type survival

These patients have a significant symptom burden. Despite a small proportion of patients moving into more favourable functional classes, the majority remain in functional class III and have impaired quality of life despite optimal pharmacological therapy (Figure 5.3).

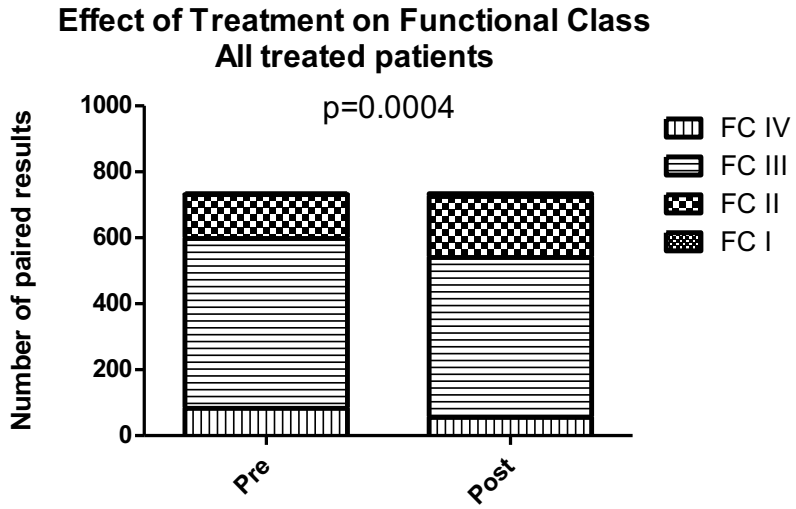


Figure 5.3 Effect of disease targeted therapy on WHO-Functional Class in SPVU patients 2015-2019[144]

Multi-system effects are evident in PAH, such as skeletal muscle atrophy and systemic inflammation, therefore pulmonary arterial vasodilators alone do not target all the manifestations of the disease. This has led to exercise therapy being explored as a potential to target these systemic effects and improve morbidity and mortality in PAH.

PAH specific rehabilitation has been shown to be safe and effective in single European and North American centres and as such has been recommended for stable patients with PAH in a statement recently published by the European Respiratory Society [91], however the availability of PAH specific exercise therapy out with a small number of countries remains low.

5.1.1 Determining efficacy

In determining the efficacy of exercise therapy, it must be ensured that appropriate outcome measures are used, the population selected are representative of the disease process being studied and the intervention is delivered in a way that will provide meaningful conclusions about its true effect. As discussed in Chapter 1, single centre studies from Germany have established that in patients with PAH on a variety of treatment regimens, exercise rehabilitation in a monitored inpatient setting prior to ongoing outpatient therapy is more efficacious than outpatient rehabilitation alone. It is unclear if this translates to different health care systems or populations on optimal therapy. The best outcome measures and timing of therapy have not yet been established.

5.1.1.1 Outcome measures

To encompass the physiological and functional hallmarks of PAH – exercise limitation, RV impairment and impaired quality of life, three primary end points were selected for this study.

1. Change in 6 minute walk distance

2. Change in RV ejection fraction

3. Change in quality of life: SF-36, CAMPHOR and EmPHasis-10

The 6MWT is a sub-maximal test used to asses exercise capacity. It is self-paced and requires a flat, straight 30m corridor. Borg dyspnoea scores are recorded before and after the test. The subject is asked to walk as far as possible in the 6-minute period, heart rate and oxygen saturations are recorded continuously. The total distance walked in 6 minutes is recorded.

The 6MWT has been the most commonly employed primary end point in PAH pharmacotherapy studies over the past 20 years and has been utilised as the primary end point in the majority rehabilitation studies. The relationship between Δ6MWD and short- and long-term outcomes, such as need for hospitalisation, lung transplantation or death, have failed to consistently demonstrate significant associations[145]. For this reason, there is a move towards composite end points in PAH therapy, including time to clinical worsening and hospitalisation. In trials of exercise therapy in PAH, change in 6MWD has been associated with improved QOL scores [61] and appears to be a robust and relevant primary outcome measure. The 6MWD minimum clinically important difference (MCID) is generally accepted at around 30m in PAH [146]. There a ceiling effect in fitter patients, therefore limiting its use as a sole end point.

PAH-specific QOL scoring systems (EmPHasis-10 and CAMPHOR) relate to treatment response, prognosis and exercise capacity [147]. Most importantly QOL scores provide an objective means of assessing the impact of the intervention on the individual in their daily life. For PAH specific QOL, there are no accepted minimum clinically important difference (MCID) values.

Right ventricular ejection fraction is representative of right ventricular function and the load faced by the RV secondary to increased pulmonary vascular resistance. It is a strong determinant of patient outcome in PAH and relates to the underlying disease process[148]

5.1.1.2 **Patient selection and timing of intervention**

There is a paucity of evidence in relation to the optimal timing of PAH specific exercise therapy and how it should be incorporated into the treatment algorithm. It is also not clear what specific sub populations are most likely to derive benefit or conversely be unsuitable; no studies have been performed to look at the responses in pure populations of PAH subgroups [91]. All studies have included subjects who are clinically stable and established on drug therapy; however, this has ranged from monotherapy to triple therapy.

Compliance and patient motivation

In PAH specific rehabilitation, compliance ranges from 58% to 100% and benefits are dose dependent [149]. Dedicated studies have not assessed factors that influence or improve compliance in PAH. These areas have been more closely studied in cardiac and pulmonary rehabilitation [150, 151]. Common factors associated with reduced compliance are:

- Environmental: work commitments, travel, disruption to the patient's usual routine, cost burden.

- Medical: current smokers, lower baseline functional status, higher BMI

- Patient and physician beliefs: too ill or not ill enough, beliefs around the role or safety of exercise, cultural reasons

Developing an Individualised therapy programme, psychological support, regular telephone or email support and involving a family or friend in the exercise routine have all been shown to enhance compliance[152, 153].

Patient motivation and education directly influence compliance. Validated strategies exist to enhance motivation: Long term, realistic goal setting should take place at the beginning of a programme; Goals should be contextualised and adjusted as needed; Specific psychological techniques such as mental imagery can also be employed with specialist training of staff [60].

5.2 **Aims**

The aims of this chapter are

1. To establish the efficacy of PAH specific exercise therapy in a UK pre-capillary population stable on optimal disease targeted therapy

2. To determine the feasibility of PAH specific exercise rehabilitation, specifically relating to safety and compliance.

5.3 **Methods**

Chapter 2 describes the study protocol including recruitment, exercise intervention and outlines primary and secondary outcome measures.

5.3.1 **Primary outcome measures**

1. Change in 6 minute walk distance from baseline to end of study

2. Change in SF-36, EmPHasis-10 and CAMPHOR scores from baseline to end of study

3. Change in MRI right ventricular ejection fraction from baseline to end of study

5.3.1.1 **6MWD**

6 minute walk tests were performed according to ATS guidelines[126] in a 30m corridor. Oxygen saturations and heart rate were continuously recorded during the test and for 3 minutes afterwards. The total distance walked in 6 minutes was recorded. If the subject was on oxygen, the flow rate was standardised for each test

5.3.1.2 **QOL**

After initial instruction on how to complete the surveys, SF-36, EmPHasis-10 and CAMPHOR scoring sheets were completed by subjects without assistance. Subjects were familiar with the PAH specific scoring systems through routine clinical care.

5.3.1.3 **RVEF**

Right ventricular ejection fraction was measured by MRI as per standard clinical practice and is described in detail in Chapter 8:

Non-invasive assessment of the cardiovascular response to exercise therapy in PAH.

5.3.2 **Secondary Outcome measures**

Chapter 2 outlines the secondary outcome measures studied and these are discussed in detail in chapters 6 to 9. WHO Functional Class (FC) is also discussed in this chapter alongside quality of life. The WHO FC score is a commonly employed clinical tool in the initial diagnosis and follow up of patients with PAH. It is used in conjunction with other prognostic parameters to aid treatment initiation and escalation decisions and objectively inform on clinical stability. WHO FC was collected at baseline, end of the residential phase of exercise and at the end of the study.

5.3.3 **Safety reporting**

Serious Adverse Events (SAE) were defined in accordance with the HRA guidance [154] as an occurrence that met one of the following criteria:

- Resulted in death
- Life-threatening
- Required hospitalisation or prolongation of existing hospitalisation
- Resulted in persistent or significant disability or incapacity
- Otherwise considered medically significant by the investigator.

A prospective log was maintained of all AE and SAEs for individual participants in the exercise training phase and recorded in a master log. “Related” events were deemed to have resulted from research procedures, “Unexpected” events were unrelated to the study intervention and not listed in the protocol as an expected occurrence. Events reportable to the ethics committee were defined as both serious and related to the study intervention. Unrelated SAEs were recorded in a master AE log that was submitted to the R&D department with the final study report.

5.3.4 **Monitoring of adherence**

Subjects discussed personal goals or motivating factors for participating in the exercise programme at enrolment, this was recorded in their exercise diary. During the residential phase, attendance at sessions and adherence with the exercise programme was recorded in the subjects' exercise diary by the physiotherapist. In the outpatient phase, exercise logs were returned by email or post on a weekly basis. This allowed monitoring of adherence, HR and SPO_2data to assess safety and completed intensity / distance to allow titration / adjustment of exercise programme. This was followed up with a weekly telephone call and recorded in a master log.

5.3.5 **National PAH population data**

In order to provide context to the size of changes seen with exercise therapy, data on the treatment effect seen with medical therapy from the 2019 SPVU annual audit are presented in conjunction with the primary outcome measures. These data are obtained from the prospectively maintained InfoFlex® database on an annual basis. All data relates to treatment changes seen between diagnosis and following 3 months of medical therapy.

5.3.6 **Statistical analysis**

Statistical analysis was performed using IBM SPSS software version 26. Continuous variables were tested for normality using D'Agostino and Pearson omnibus normality test. Normally distributed variables are shown as mean (SD) and non-normally distributed variables are shown as median (IQR).

Categorical variables are presented as number (%). Comparison between baseline and follow-up continuous variables were made by Wilcoxon signed rank test. Comparison between baseline and follow-up WHO FC was made by x^2test. Comparison between control and treatment variables were made by Mann-Whitney U test. All tests were non-parametric due to the small sample size.

5.4 **Results**

5.4.1 **Population characteristics**

73% were female with a median age of 53 (26-73). The majority of patients had IPAH (Table 5.1)

Table 5.1: PAH sub type

Diagnosis	
IPAH	66.7% (20/30)
CTD-PAH	20% (6/30)
POPH	3.3% (1/30)
CHD-PAH	3.3% (1/30)
Heritable	3.3% (1/30)
CTEPH	3.3% (1/30)

All patients were stable, on optimal therapy as defined by the European Respiratory Society Guidelines on Pulmonary Hypertension [155]. 17/30 (57%) were on dual oral therapy and 13/30 (43%) were on triple therapy. Baseline characteristics were typical of a pre-capillary PH population, with reduced exercise capacity, impaired gas exchange and reduced quality of life (Table 5.2).

Table 5.2: Baseline characteristics

	Mean / Median	SD / Range
Age (y)	50	34-74
Sex (M)	8/30 (27%)	
Time since Diagnosis (months)	100	101
BMI (kg/m2)	35	20-48
6MWD (m)	422	96
DLCO (ml/min/Kpa)	4.68	1.96
DLCO (%)	52.3	21.1
Peak VO_2 (L/min)	0.99	0.29
VO_2 (ml/kg/min)	12.5	3.6
Peak WR (watts)	75	26
WR (%)	63	27
VE/VCO_2 gradient	42	12
Log NTproBNP	2.3	0.6
EmPHasis-10	26	14.3

At enrolment, Group A and B comparisons revealed well matched groups (Table 5.3)

Table 5.3: Drug therapy and PAH sub type between groups

	Group A (n 16)	Group B (n 14)
Drug therapy		
Triple Therapy	56% (9/16)	29% (4/14)
Dual Oral Therapy	44% (7/16)	71% (10/14)
Diagnosis		
IPAH	69% (11/16)	64% (9/14)
CTD-PAH	19% (3/16)	22% (3/14)
POPH	6% (1/16)	0
CHD-PAH	6% (1/16)	0
Heritable	0%	7% (1/14)
CTEPH	0%	7% (1/14)

Table 5.4: Group A and B unpaired comparisons

	Group A	SD / IQR	Group B	SD / IQR	P
Age*	54	17	48	21	0.715
Sex (M)**	25% (4/16)		21% (3/14)		0.544
Time since Diagnosis *	76	83	30	114	0.457
BMI (kg/m^2)*	29	13	26	11	0.193
6MWD (m)	413	101	427	91	0.559
DLCO (ml/min/Kpa)	4.51	2.15	4.96	1.7	0.486
RVEF (%)	53.1	24.1	58.9	15.4	0.156
Peak VO_2 L/min	0.93	0.21	0.97	0.38	0.440
VO_2 ml/kg/min	11.9	24.4	13.3	2.8	0.025
Peak WR (watts)	71	21	84	34	0.294
VE/VCO_2 gradient	43	14	42	11	1.000
Log NTproBNP	2.4	0.47	2.3	0.43	0.371
EmPHasis-10	28	6	24	7	0.232

*** Median / IQR displayed,**
**** CHI squared test**

5.4.2 **Recruitment and retention**

The target of 30 patients were recruited to the study between January 2016 and March 2018 (Figure 5.4). The final patient, final visit occurred in July 2018.

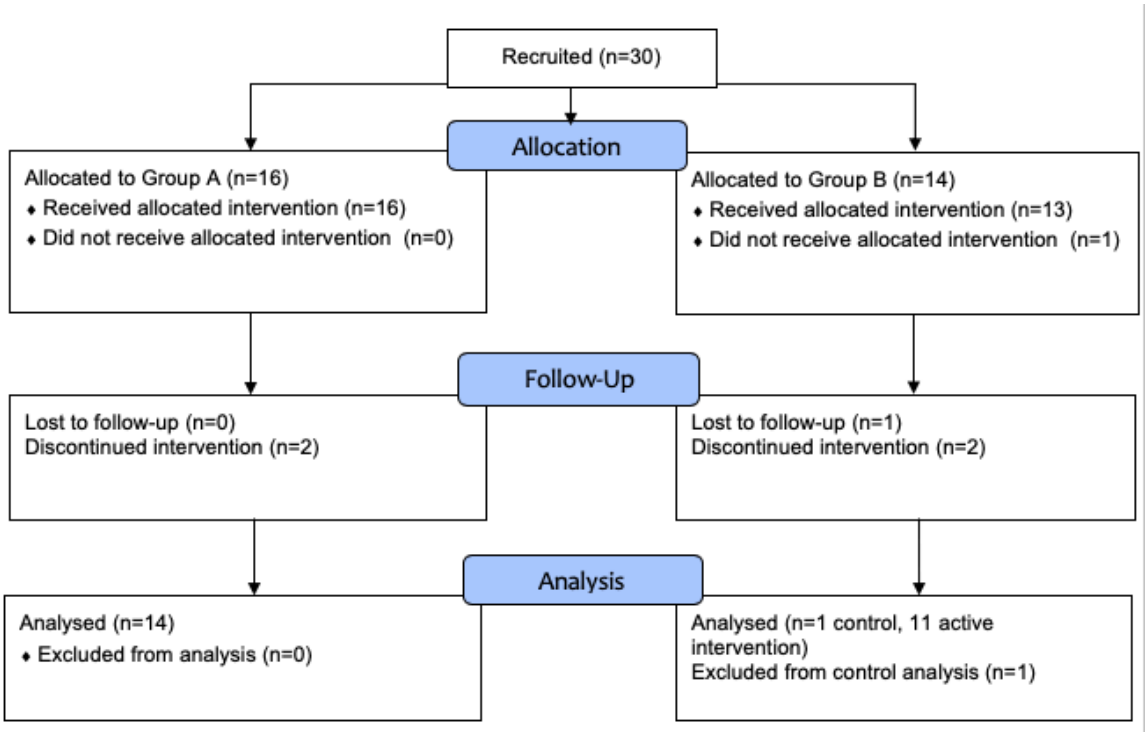


Figure 5.4: Study recruitment and retention flow diagram

5.4.2.1 **Subject withdrawal from study**

There were 5 dropouts from the study (16.7%), this is comparable to studies of PAH pharmacotherapy [156]. One subject from group B was withdrawn in the control phase because of worsening PH. Four subjects dropped out during the active intervention in the outpatient phase (two having started in Group A and two in Group B). The causes were recurrent chest infections, withdrawal due to worsening right heart failure (peripheral oedema) and renal function, lung transplantation (in a subject listed for this before entry into the study) and lost to follow up because of failure to attend for final assessment.

5.4.2.2 **Excluded from analysis**

One subject in Group A missed the week 3 assessment because of viral labyrinthitis. One subject in Group B was excluded as a control subject. He enrolled on a non-study structured exercise programme whilst in the control phase, resulting in a 100m improvement in 6MWD from 418 to 518m prior to starting the active intervention phase of the study. He has, however, been included in analyses of change during the active intervention.

5.4.3 **Primary Outcome measures**

5.4.3.1 **Six minute walk distance**

In the 28 patients who completed the initial 3 week residential phase of the exercise programme and the 6MWT, the 6MWD increased by 38m. Two subjects did not complete the week 3 6MWT due to intercurrent illness. Twenty five subjects completed the full exercise programme, 6MWD increased by 35m (Table 5.5)

n 28	Baseline	SD	Week 3	SD	P
6MWD (m)	429m	97	467m	108	0.0001
n 25	Baseline	SD	End of study	SD	P
6MWD (m)	443m	88	478m	100	0.002

Table 5.5: Change in 6MWD with exercise therapy

An unpaired comparison of change in 6MWD in all subjects who received exercise therapy (Group A & B) and in group A versus the control arm (Group B) demonstrated significant improvements in 6MWD with exercise therapy (Table 5.6, Figure 5.5)

	Exercise intervention	Control (B)	P
Delta 6MWD (A) (n 14)	32m (49)	5m (16)	0.045
Delta 6MWD (A+B) (n 25)	35m (47)	5m (16)	0.039

Table 5.6: Change in 6MWD control (B) versus exercise therapy

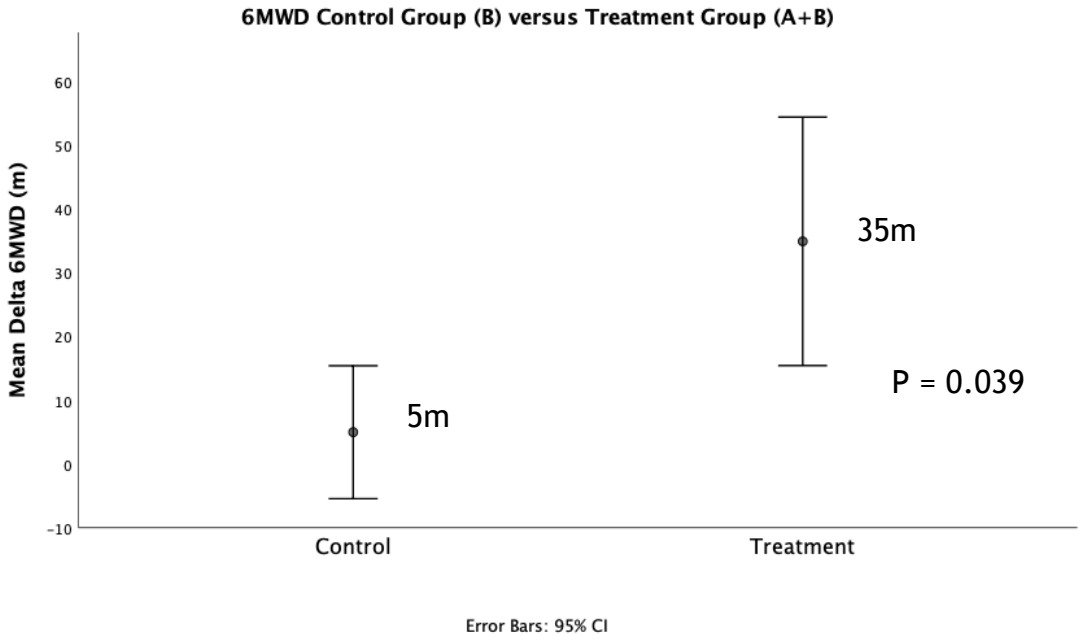


Figure 5.5 Change in 6MWD in control group (B) versus exercise therapy (A+B)

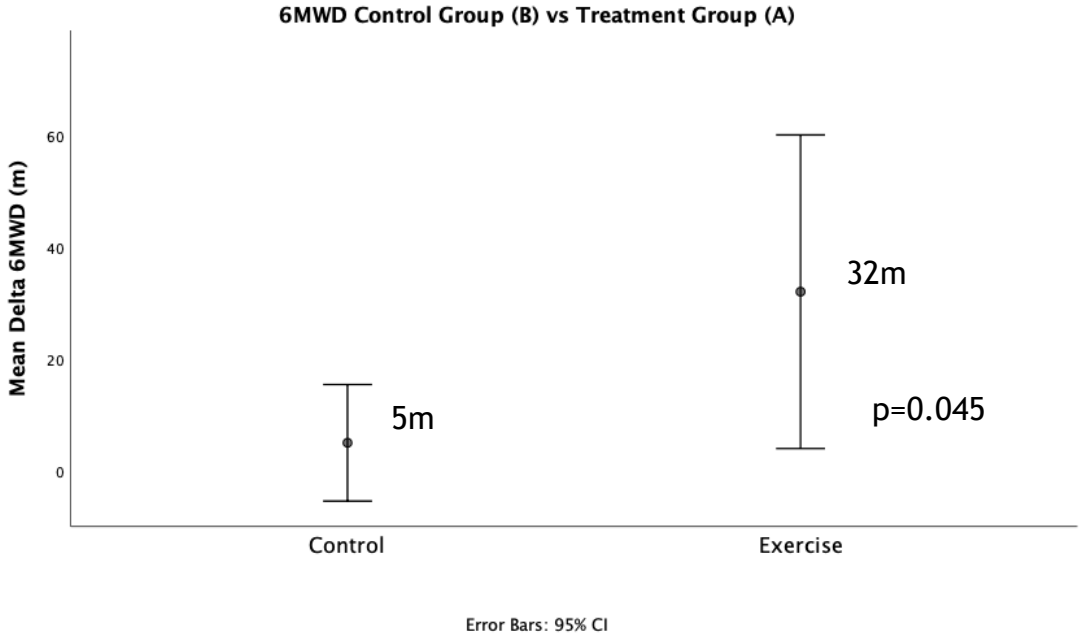


Figure 5.6 Change in 6MWD in control group (B) versus treatment group (A)

The majority of improvement in walk distance occurred in the initial residential phase (week 0 to week 3) and was maintained during the residential phase (Table 5.7)

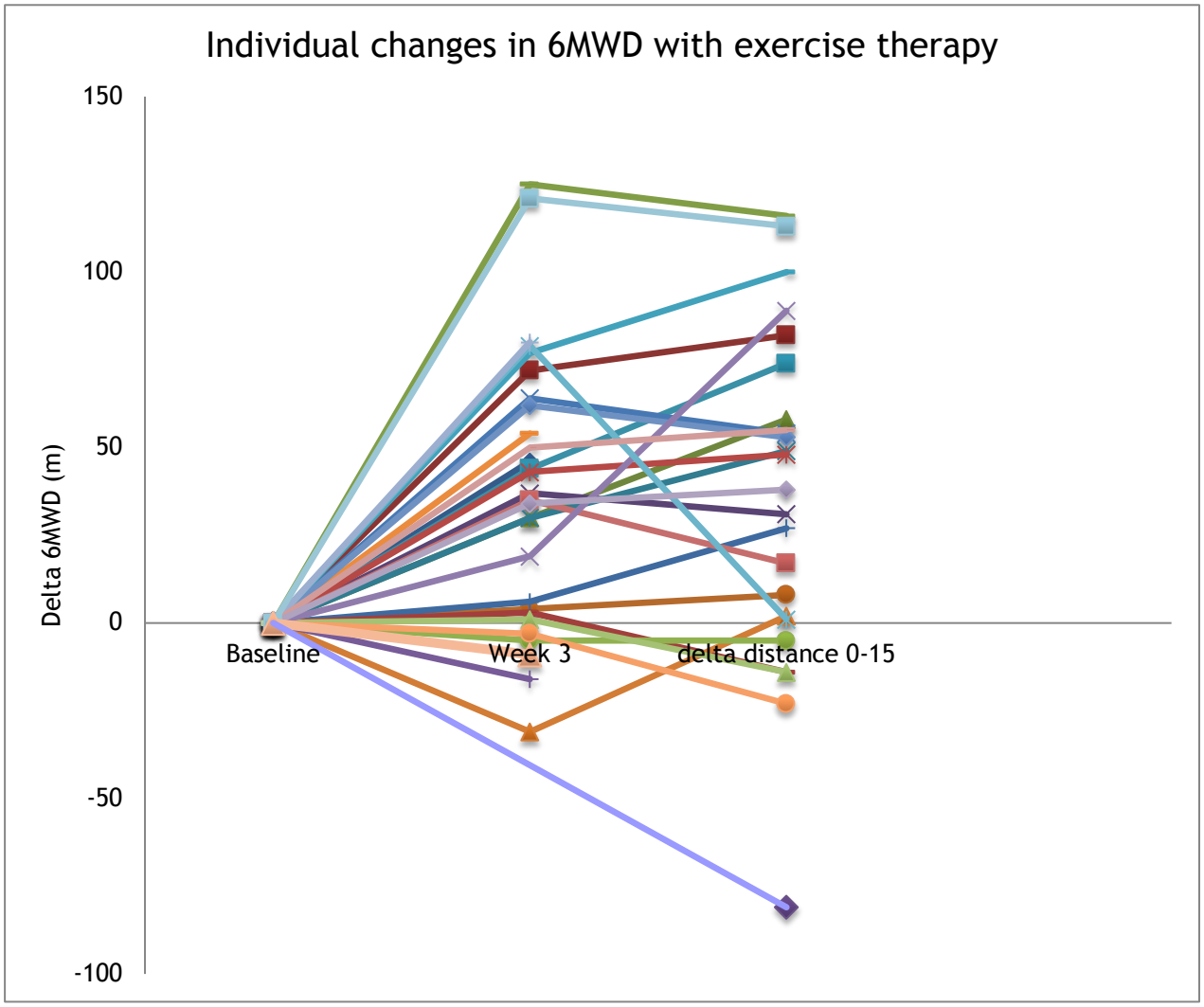


Figure 5.7: Individual change in 6MWD; baseline, end residential phase, end of study

The study was not designed to detect a difference in treatment response between PAH subgroups. Post hoc analysis was performed on the two largest subsets, IPAH and CTD-PAH (Table 5.7). Patients with IPAH had a higher pre-exercise 6MWD and appeared to have a more positive response to exercise therapy.

Table 5.7: Comparison of change in 6MWD between IPAH and CTD-PAH

	Baseline (SD)	End of Study (SD)	p
IPAH (17)	461 (82)	498 (73)	<0.005
CTD PAH (6)	337 (53)	349 (62)	0.204

In comparison, in the SPVU PAH population between 2015-2019, a treatment effect from initiation of medical therapy at diagnosis of 27m was seen in 6MWD (n 584)

(Figure 5.8), with the greatest effect being seen in IPAH and lowest effect in CTD-PAH (Table 5.8)

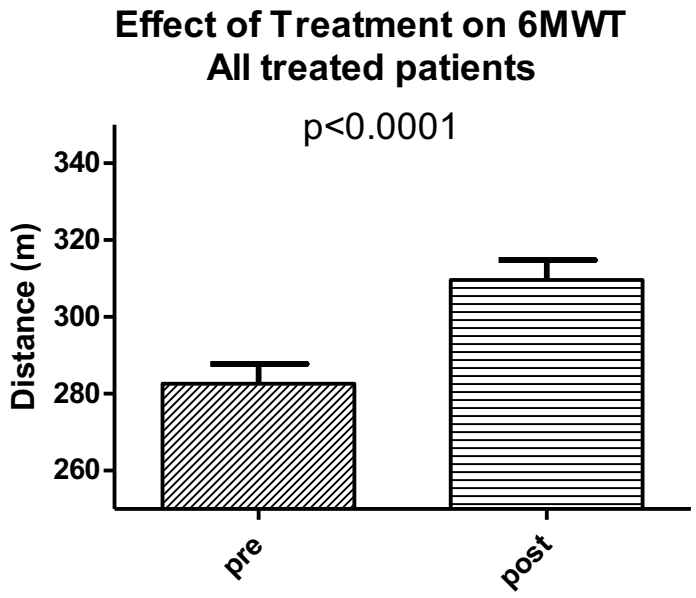


Figure 5.8 Mean change in 6MWD at 3 months following initiation of drug therapy in SPVU PAH population 2015-2019

Table 5.8: Effect of medical therapy on 6MWD in SPVU PAH sub-groups 2015-2019[144]

Diagnosis	Mean Improvement 6MWD (m)	N	p
IPAH	34	195	<0.0001
CTD-PAH	20	118	0.003
Portopulmonary	45	40	0.002
CTEPH (medical treatment)	20	121	0.0006

5.4.3.2 **Quality of life**

Pulmonary hypertension specific QOL.

A validated pulmonary hypertension specific QOL scoring system EmPHasis-10 was used in addition to a generic QOL scoring system (SF-36). QOL improved from a mean baseline score of 25 (14) to 19 (15) at week 3, p 0.013, and this improvement was maintained at the end of the study with a mean score of 18 (15), p 0.01. The improvement in QOL followed a similar pattern of initial significant improvement then maintenance, to changes seen with 6MWD (Figure 5.9,

Table 5.9).

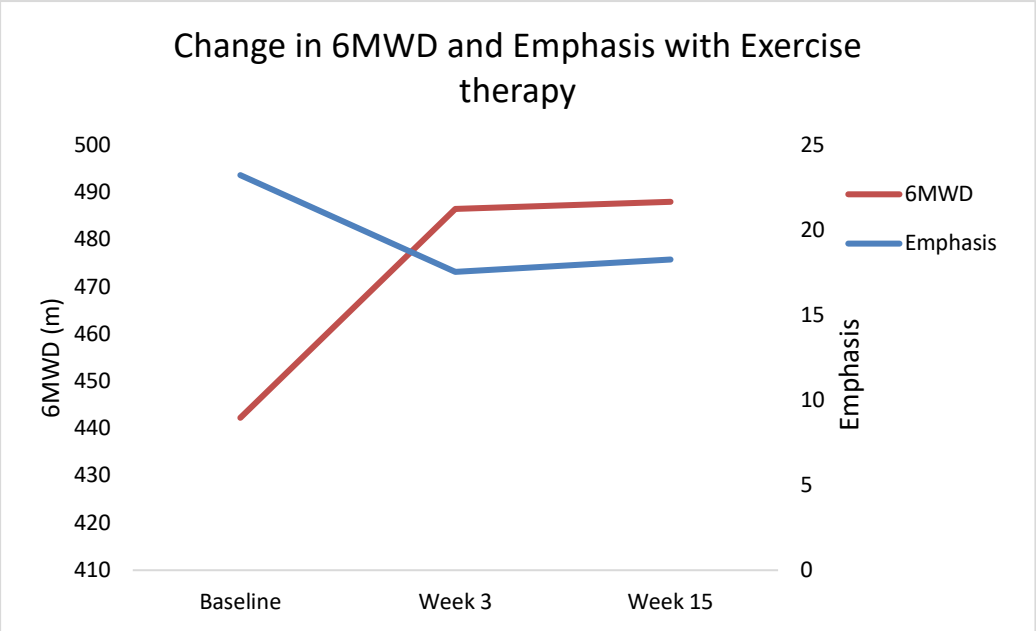


Figure 5.9 change in 6MWD and EmPHasis-10 with exercise therapy

n 26	Baseline	SD	Week 3	SD	P
EmPHasis-10	24.7	15	19.4	15	0.002
n 23	Baseline	SD	End of study	SD	P
EmPHasis-10	23.3	15	18.3	15	0.017

Table 5.9: Change in QOL with exercise therapy

This relationship was confirmed with a significant Spearman's correlation between baseline 6MWD and EmPHasis-10 score – 0.519, p 0.004 (Figure 5.10) and between change in ΔQOL and Δ6MWD at the end of study, Spearman's rho -0.519, p 0.004

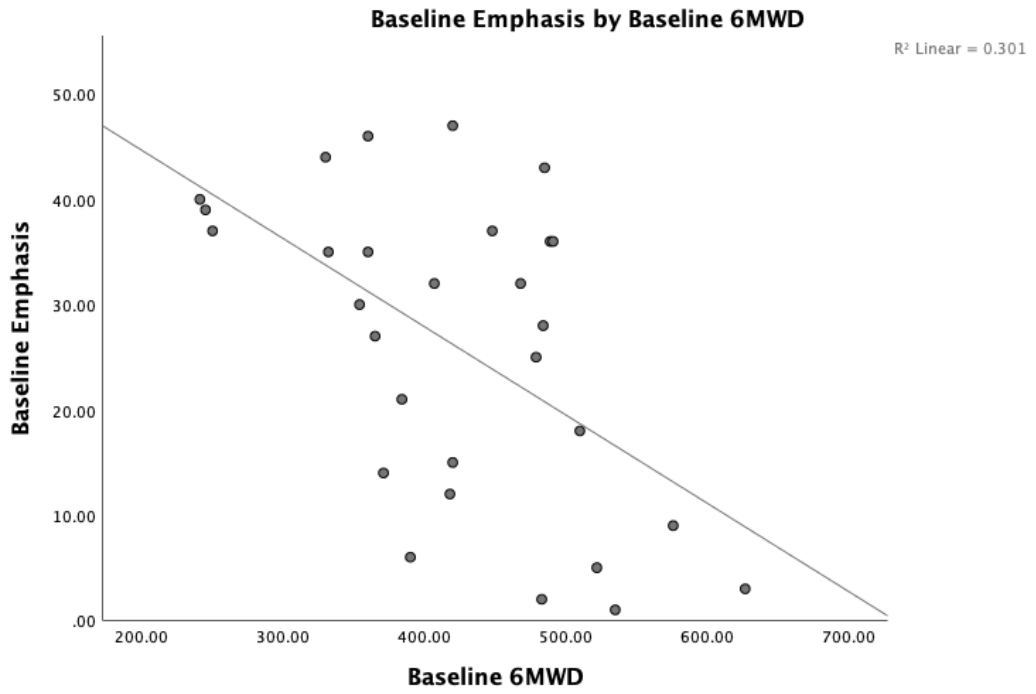


Figure 5.10: Scatter plot of baseline EmPHasis-10 and walk distance

EmPHasis-10 scored improved in those receiving exercise therapy in comparison with the control arm (Table 5.10)

	Exercise intervention	(n 10) Control (B)	P
EmPHasis-10 (A) (n 12)	-6.7 (14)	2.1 (4)	0.07
EmPHasis-10 (A+B) (n 22)	-5.2 (11)	2.1 (4)	0.02

Table 5.10: Change in QOL in exercise group versus control (B)

Changes seen with drug therapy at diagnosis in the SPVU PAH population from 2015-2019 demonstrated a trend to improved EmPHasis-10 score by a mean of 1.3 points across all subtypes of PAH (figure 13) with improved performance in IPAH patients (

Table 5.11), notably lower than the improvement seen with exercise therapy.

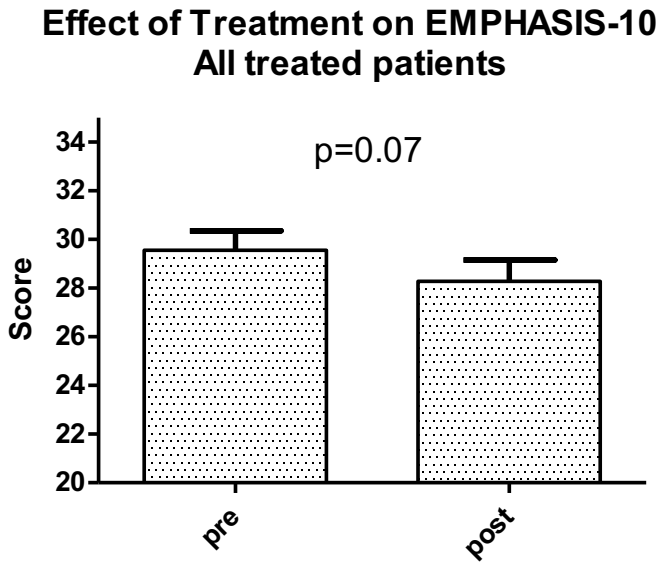


Figure 5.11: Effect of drug therapy on QOL in SPVU population 2015-2019 [144]

Table 5.11: Effect of treatment on QOL in SPVU PAH subtypes 2015-2019 [144]

	Mean Improvement in EmPHasis-10	Number of paired results	p-value
IPAH	2.9	64	0.047
CTD PAH	0.2	57	NS
Portopulmonary	0.3	13	NS
CTEPH (medical treatment)	1.3	53	NS

Generic QOL score: SF-36

SF-36 was measured at baseline, the end of residential exercise (ER) phase and at the end of the study (EOS). Complete surveys were available for 26 subjects from baseline to the end of the residential phase (Table 5.12) and for 23 patients from baseline to the end of the study (

Table 5.13)

Table 5.12 SF36 scores baseline to end of residential therapy (n=26)

SF-36 Component	Baseline	ER	p
Physical function	42 (24)	51 (26)	0.060
Role limitation due to physical problems	33 (40)	53 (44)	0.010
Pain	68 (28)	68 (25)	0.980
General health perception	36 (19)	44 (21)	**0.020**
Energy/vitality	45 (26)	59 (24)	**0.004**
Social functioning	61 (32)	71 (33)	0.009
Role limitation due to emotional problems	59 (46)	79 (33)	**0.020**
Mental health	63 (21)	73 (20)	**0.002**
Physical component summary	44 (21)	54 (23)	**0.001**
Mental component summary	53 (24)	65 (21)	**<0.0001**

Table 5.13: SF36 scores baseline to end of study (n=24)

SF-36 Component	Baseline	EOS	p
Physical function	44 (24)	56 (26)	**0.010**
Role limitation due to physical problems	35 (40)	56 (44)	**0.006**
Pain	70 (29)	67 (28)	0.590
General health perception	36 (20)	43 (25)	0.110
Energy/vitality	49 (25)	56 (24)	**0.010**
Social functioning	66 (31)	76 (24)	**0.080**
Role limitation due to emotional problems	65 (44)	71 (40)	0.470
Mental health	68 (20)	67 (20)	0.230
Physical component summary	46 (22)	55 (24)	**0.005**
Mental component summary	57 (24)	62 (23)	0.280

Changes in SF-36 score between baseline, end of the residential exercise phase and the end of the study in those completing the exercise programme are show in in (Table 5.14, Figure 5.12).

Table 5.14: Change in SF36 in subjects completing the exercise programme (n=22)

Assessment time point	Baseline	End residential	p	EOS	p
Physical function	45 (24)	54 (26)	0.080	57 (23)	**0.02**
Role limitation due to physical problems	36 (41)	56 (44)	**0.007**	57 (43)	**0.01**
Pain	71 (28)	70 (26)	0.810	67 (27)	0.590
General health perception	38 (19)	47 (21)	**0.020**	44 (24)	0.180
Energy/vitality	49 (26)	62 (25)	**0.002**	55 (24)	**0.02**
Social functioning	65 (32)	77 (32)	**0.005**	76 (24)	0.07
Role limitation due to emotional problems	62 (45)	79 (35)	0.060	70 (41)	0.380
Mental health	67 (21)	76 (20)	0.010	65 (20)	0.210
PCS	47 (21)	57 (24)	**0.001**	55 (23)	**0.009**
MCS	56 (25)	68 (21)	**0.0005**	62 (23)	0.130
PCS – physical component score, MCS - mental component score					

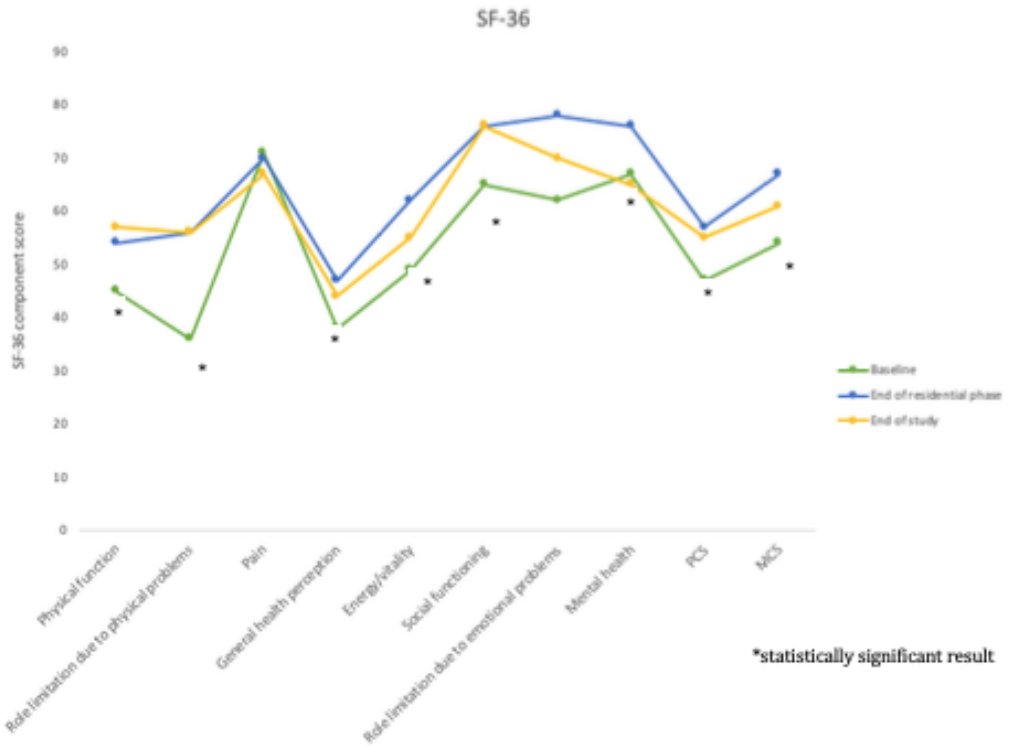


Figure 5.12: Change in SF-36 component scores with exercise therapy

When comparing the effect of the exercise intervention to the control arm, two analyses were performed. The first (Table 5.15) was an unpaired comparison of the control arm (Group B) vs the immediate exercise therapy arm (Group A). Of the SF-36 components, only "Mental Health" improved significantly.

Table 5.15: SF-36 control (Group B) versus Immediate Exercise therapy (A)

	Mean change treatment (A) (n 13)	Mean change control (B) (n 9)	P
Physical function	14 (25)	0.56 (19)	0.180
Role limitation due to physical problems	23 (31)	2.8 (34)	0.370
Pain	-5.6 (12)	0.0 (0.0)	0.230
General health perception	7.7 (18)	1.3 (8.4)	0.570
Energy/vitality	3.5 (11)	0.0 (5.0)	0.540
Social functioning	7.7 (27)	2.8 (16)	0.890
Role limitation due to emotional problems	2.6 (48)	15 (29)	0.390
Mental health	-3.4 (17)	3.1 (5.6)	**0.047**
PCS	8.4 (15)	1.0 (9.8)	0.460
MCS	0.64 (22)	4.6 (6.5)	0.300

In an unpaired comparison of all patients who received the exercise intervention (Group A and group B patients following the control phase) to the control arm, no variables showed a convincingly significant improvement (Table 5.16).

Table 5.16: SF-36 control (B) versus all subjects post exercise therapy (A+B)

	Mean change treatment (A + B) (n 24)	Mean change control (B) (n 9)	P
Physical function	13 (23)	0.56 (19)	0.15
Role limitation due to physical problems	21 (30)	2.8 (34)	0.35
Pain	-4.0 (12)	0.0 (0.0)	0.29
General health perception	7.3 (19)	1.3 (8.4)	0.49
Energy/vitality	6.5 (10)	0.0 (5.0)	0.07
Social functioning	9.9 (25)	2.8 (16)	0.54
Role limitation due to emotional problems	5.6 (41)	15 (29)	0.41
Mental health	-1.8 (14)	3.1 (5.6)	0.11
PCS	8.8 (13)	1.0 (9.8)	0.27

MCS	3.7 (18)	4.6 (6.5)	0.74

WHO FC

WHO FC was measured at baseline, the end of the residential phase and at the end of the study. No significant difference was detected pre and post exercise therapy in the 26 patients who completed the programme and final assessment, x^2 1.527, p 0.676 (Table 5.17, Figure 5.13).

Table 5.17: Contingency table comparing WHO FC between baseline and End of Study

* FC categories whose column proportions do not differ significantly from each other at the .05 level.		Functional Class			
		I	II	III	IV
Baseline	Count	1*	11*	14*	0*
	Expected Count	1.0	12.0	12.5	.5
EOS	Count	1*	13*	11*	1*
	Expected Count	1.0	12.0	12.5	.5

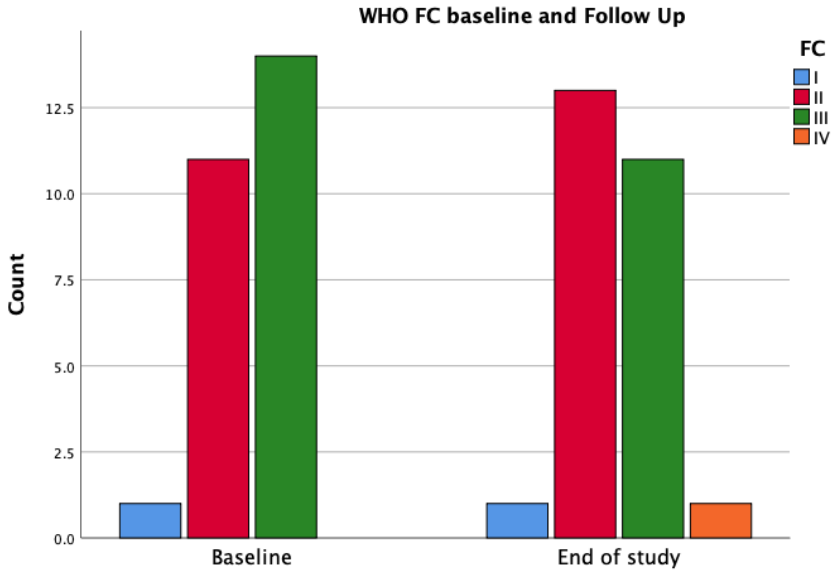


Figure 5.13: Distribution of WHO FC from baseline to EOS

5.4.3.3 Right ventricular ejection fraction

There was no significant change pre and post exercise therapy in right ventricular ejection fraction (RVEF); At baseline RVEF was 44.6% (13.3) and at the end of study was 47.5% (13.2), p 0.15

In those who received the active intervention (n 26) mean RVEF change was 2.93% (8.7) at the end of study versus -1.1% (11.2) in the control group, p 0.51

The response of cardiac MRI variables to exercise therapy is discussed in Chapter 8.

5.4.4 Adverse events

Adverse events were monitored during the active intervention by direct communication (in person or by phone) and through patient diaries.

5.4.4.1 Serious adverse events

No deaths occurred during the study and no study related serious adverse events (SAE) occurred. One death occurred within 30 days of a subject exiting the study (Subject A1). This subject had withdrawn from the study due to recurrent lower respiratory tract infection and comorbidities requiring hospitalisation. The cause of death (fall, rib fractures and pneumonia) was reported to the REC and deemed unrelated to the study intervention. All other subjects resumed the study intervention after the SAE resolved

Table 5.18 Serious Adverse Events

SAE	Frequency
Pneumonia or chest infection requiring hospitalisation	4
Viral illness requiring hospitalisation (labyrinthitis, non-specific)	2
Ventricular pauses requiring pacemaker	1
Cholecystitis	1
Syncope – noncompliance with PAH medication and fluid deplete	1
Rib fractures after fall (unrelated to exercise) and chest infection	1

5.4.4.2 Adverse events

The total number of adverse events recorded was 55, ranging from 0 to 5 per subject, with a median of 2 (Table 5.19, Figure 5.14). The miscellaneous adverse events observed or reported were urinary tract infection, paroxysmal second-

degree heart block, rectal bleeding, chronic cough, thrush, renal impairment, chest pain due to hypoxaemia, anxiety, headache and thrombocytopaenia

Table 5.19: Adverse events

	Distinct episodes	% of AEs
Musculoskeletal problems	13	24
Chest Infection	10	18
Viral illness	9	16
Dental problems	3	5
Peripheral oedema	3	5
Anaemia	3	5
Ear Infection	2	4
Fatigue	2	4
Miscellaneous (frequency 1)	10	18

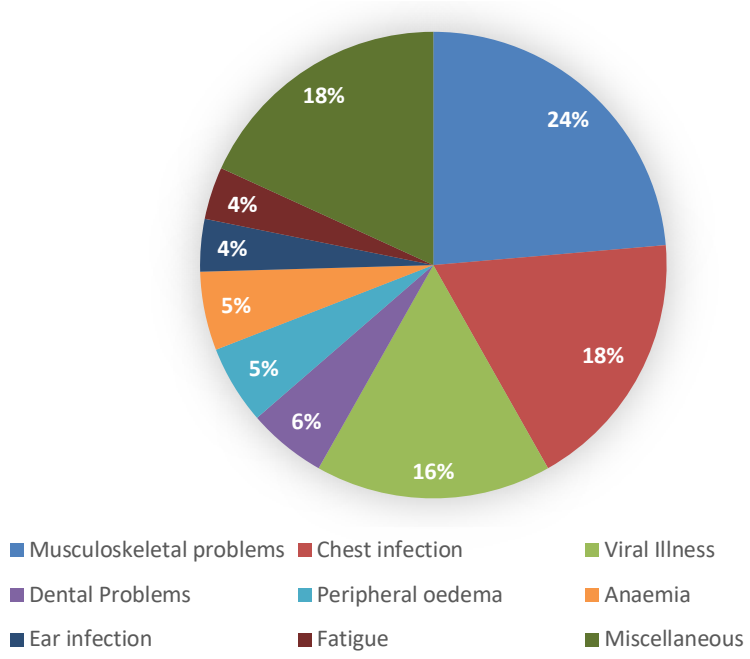


Figure 5.14: Adverse events

5.4.5 **Adherence**

Complete data for the residential phase was available for 24 subjects and 21 in the outpatient phase. The majority of the data omission was due to lack of completion of exercise logs. The subjects who did not return exercise logs on a weekly basis reported adherence with the study intervention during the weekly phone call to assess progress.

Six subjects (29%) reported 100% compliance with the study intervention and also reported additional exercise sessions on top of those provided in the daily schedule.

Adherence to the study intervention is reported in Table 5.20

Table 5.20: Adherence to exercise therapy

	Median N scheduled sessions (range)	Median N performed sessions (range)	Adherence (% completed sessions)
Residential n 24	40 (36-41)	39 (36-41)	98%
Outpatient n 21	228 (107-380)	206 (45-273)	82%
Total n 21	268 (148-421)	256 (81-314)	86%

Adherence was higher in all phases of the exercise programme in subjects who had an improved 6MWD following the study intervention, statistical significance was reached in comparisons of the residential phase only (Table 5.21)

Table 5.21: Improvement based on delta 6MWD and relationship with adherence

	Responders	n	Non responders	n	p
Residential	99.3% (±2)	14	96.8% (±5)	10	**0.026**
Outpatient	87.9% (±12)	13	73.1% (±28)	8	0.238
Overall	91.8% (±11)	13	76.2 (±25)	8	0.161

5.4.6 **Predicting response and prognosis**

A post hoc analysis was performed to explore factors predictive of a positive response to exercise therapy and transplant free survival.

5.4.6.1 **Responders and non-responders to exercise therapy**

A positive response to treatment was defined as an improvement in 6MWD of ≥ 30m. Twenty five subjects had complete pre and post intervention data, due to the drop outs discussed in section 5.4.2. 25 subjects were therefore analysed for both baseline predictors and pre and post exercise analysis.

15 subjects improved 6MWD greater than 30m (Responders), 10 showed no clinically significant change or deteriorated 6MWD (non-responders). Subjects who improved had lower baseline NTproBNP and were less likely to have CTD-PAH (Table 5.22)

Table 5.22: Baseline characteristics of responders versus non responders

Baseline	Responders n 15	Non responders n 10	p
Age (y)	53.5 (10.6)	52.6 (14.9)	0.814
Sex (M)	6/15 (40 %)	2/10 (20%)	0.739
Drug therapy			
Triple Therapy	7/15 (%)	5/10 (50%)	0.758
Dual Oral Therapy	9/15 (%)	5/10 (50%)	
Diagnosis			
IPAH / Heritable	12/15 (80%)	5/10 (50%)	0.317
CTD-PAH	2/15 (13%)	4/10 (40%)	
POPH	1/15 (7%)	0	
CTEPH	0	1/10 (10%)	
Time since Diagnosis (months)	97.5 (128)	81.2 (63.9)	0.877
BMI (kg/m^2)	29.3 (6)	30.0 (7.6)	0.715
6MWD (M)	439 (82)	438 (97)	0.657
EmPHasis-10	24.6 (14)	23.3 (15)	0.677
TLCO (ml/min/Kpa)	5.3 (1.8)	4.1 (2.2)*	0.156
Peak CPET WR (watts)	85 (21)	70 (15)	0.033
Peak VO_2 (L/min)	1.06 (0.2)	0.89 (0.1)	0.049
VE/VCO_2 gradient	38.4 (6.7)	49.4 (14.7)	0.035
Alive / transplant free at censoring	14/15 (93%)	8/10 (80%)	0.129
Log NT pro BNP	2.07	2.59	**0.019**

***8 subjects analysed for deteriorated TLCO, **9 subjects analysed for deteriorated NTproBNP Mann Whitney U test used for unpaired comparisons depending on normality. Anova for multiple comparisons**

In subjects who improved with exercise therapy, improvements were also seen in peak aerobic exercise capacity on CPET, ventilatory capacity at peak exercise, NTproBNP and quality of life (Table 5.23).

Table 5.23: Comparison of changes in key parameters with exercise therapy in those who improved vs deteriorated

	Responders (n 15)	Non responders (n10)	p
6MWD (m)	71.8 (29)	-10.3 (28.4)	**0.001**
Peak WR (W)	14.71* (15.7)	0.6 (9.9)	**0.011**
Peak (VO_2 ml/min/kg)	1.74* (2.0)	-0.1 (1.4)	**0.015**
End exercise O_2 pulse (% predicted)	5.86* (9.8)	-1 (7.1)	0.065
Minute Ventilation (L/min)	6.43* (10.5)	-0.4 (6.2)	**0.04**
Tidal Volume (L)	0.24* (0.18)	-0.17 (0.8)	**0.015**
Log NTproBNP	0.11 (0.22)	-0.38*** (0.9)	**0.034**
VE/VCO_2 at AT (mmHg)	-4.2* (4.1)	0.68*** (5.1)	0.051
Ve/VCO_2 gradient	-3.1* (4.8)	-5.69*** (10.1)	0.313
EmPHasis-10	-8 (12)	0.75** (2.1)	**0.018**
N analysed *14, **8, ***9			

5.4.6.2 **Transplant free survival**

At the point of censoring (January 2019), 8 patients enrolled in the study had been transplanted (2) or died (6). There were no deaths during the study intervention. Twenty nine of the 30 subjects enrolled in the study were analysed. One subject was not analysed (B11) due to dropping out in the control phase, with no baseline data. This subject died after withdrawal from the study, therefore the analysed population had 22 alive, 5 deaths and 2 transplanted at censoring.

Subjects who remained alive had a higher baseline 6MWD, higher TLCO, lower NTproBNP and higher peak aerobic exercise capacity (Table 5.24)

Table 5.24: Baseline demographics of alive versus dead/transplant at censoring

Baseline	Alive at censoring (22)	Transplant or dead at censoring (7)	p
Age	52.2 (11.4)	53.7 (11.6)	0.755
Sex (M)	6/22 (27%)	2/7 (29%)	0.197
Drug therapy			
Triple Therapy	10/23 (43.4%)	3/7 (42.8%)	0.966
Dual Oral Therapy	13/23 (56.6%)	4/7 (57.2%)	
Diagnosis			
IPAH	17/23 (69.6%)	4/7 (57%)	0.317
CTD-PAH	4/23 (17.4%)	2/7 (28.6%)	

POPH	1/23 (4%)	0	
CHD-PAH	0	1/7 (14.3%)	
CTEPH	1/23 (4%)	0	
Time since Diagnosis	91 (112)	99 (55)	0.852
BMI	29.5 (9)	31.5 (7.1)	0.544
6MWD (M)	452 (77)	327 (87)	**0.004**
EmPHasis-10	24 (15)	31 (12)	0.283
CAMPHOR total	33 (18)	35 (15)	0.885
RVEF (%)	45 (12)	42 (15)	0.629

Due to the small size of the population, there were few statistically significant differences in variables pre and post exercise therapy in those alive without transplant at censoring (Table 5.25)

Table 5.25: Comparison of changes in variable in those alive versus dead or transplanted

	Alive	n	Dead / Transplant	n	p
6MWD (m)	45 (45)	21	9 (70)	4	0.200
EmPHasis-10	-5.5 (2)	21	0.5 (11)	2	0.470
Peak WR (%)	8 (10)	21	-9 (14)	3	0.026
Peak VO_2 (%)	6.6 (11)	21	-3 (0.6)	3	0.154
Log NTproBNP	-0.7 (1.4)	21	0.1 (0.2)	2	0.373
VE/VCO_2 gradient	-4.6 (6.2)	21	1 (15.8)	3	0.743

5.5 **Discussion**

This is the first UK based study of exercise therapy in PAH and the first study to assess patients exclusively on optimal therapy in accordance with ERS guidelines.

6MWD improved significantly, in conjunction with QOL following a 15 week PAH specific exercise programme. In those who had no improvement in 6MWD, QOL remained unchanged. The increase in 6MWD of 38m seen in this study is above the MCID for 6MWD but appears low in comparison with earlier studies[61], where mean changes of up to 60m were seen. This may be explained by the fact the latter population were on a combination of monotherapy and dual therapy, in comparison to optimal therapy with either dual or triple therapy in this study. A significant proportion of subjects were several years post diagnosis and the timing

between diagnosis and initiation of exercise therapy may also have bearing on the observed difference.

Despite the clear evidence that exercise is beneficial in PAH [60], the optimal outcome measures for assessing long-term efficacy have yet to be defined. There is a lack of data assessing the impact of exercise training on disease progression and survival. Recent studies of drug therapy in PAH have used composite end points and time to clinical worsening [8] it has also been proposed that time to clinical improvement may be a more meaningful outcome. In this study, baseline 6MWD correlated with QOL as did changes in 6MWD and QOL. These changes were associated with more favourable baseline prognostic biomarkers, therefore the 6MWD seems a robust primary outcome measure in determining the short term outcomes from studies of exercise intervention. Further research is required to identify appropriate biomarkers and patient centred outcomes that relate to transplant free survival and long term outcomes.

Adherence is strongly associated with the success of any exercise training programme. In this study, subjects who improved with the exercise programme had a higher adherence rate than those who deteriorated or had unchanged 6MWD at the end of study visit. Adherence was high in the initial residential phase where there was intensive physiotherapy and medical input and lower in the outpatient phase. The residential phase allowed safe, structured and accelerated acquisition of the exercise techniques and also produced rapid benefit, giving the subject positive feedback and encouraging ongoing and future compliance. The dropout rate for the study was 16%, similar to published clinical trials evaluating PAH therapy.

Given the intensive nature of the residential phase, and the lack of rehabilitation infrastructure in the UK, it is possible that an outpatient based rehabilitation programme would be more feasible from a resource perspective but have poorer compliance. It would therefore seem logical to target factors known to be associated with poor compliance such as mental health, social isolation and high BMI, with specific interventions such as psychology and dietetics to optimise compliance and yield from the therapy.

Training interruptions due to intercurrent illness were relatively common, predominantly due to respiratory tract infections and musculoskeletal problems. Due to the nature of the PAH disease process and associated comorbidities, training interruptions are likely inevitable and frequent. It is not known whether exercise and its associated anti-inflammatory effects have a negative effect on the immune system and result in higher rates of infection. Due to the absence of study related SAE or AEs, exercise therapy appears to be safe in a monitored and structured environment.

During the study period, one patient with advanced IPAH on triple therapy underwent successful lung transplantation, having been listed prior to enrolment in the study. This subject demonstrated a 54m improvement in 6MWD from baseline and had significantly improved QOL scores. Exercise therapy therefore shows promise not only as an adjunctive therapy to pharmacological management, but also may have a role in "pre-habilitation" of patients for major surgery such as lung transplantation or pulmonary endarterectomy.

A post-hoc analysis was performed to explore potential factors that may predict individuals more or less likely to respond well to exercise therapy and to see if this was associated with transplant free survival. The study was appropriately powered to detect a change in the primary outcome measures of 6MWD and QOL. It was not designed to predict survival or analyse sub-groups based on gender, disease category or survival. These data are therefore exploratory. Interesting signals as to sub-groups that are less likely to improve are present and discussed below, however a larger study with pre-defined and appropriately powered sub-groups would be needed to robustly address these questions and make definitive recommendations.

Following the post-hoc analysis, there appeared to be a distinct population of patients who did not improve with exercise therapy and two subjects deteriorated during the study period due to progressive PAH. Those who did not improve had lower baseline exercise capacity and markers of poorer prognosis. There did not appear to be a significant difference in the time from diagnosis, however given this data, the yield from exercise training may be higher when patients are at an earlier stage in their disease process with less deconditioning. A potential strategy

could be to embark on exercise therapy as soon as patients have been stabilised on initial medical therapy around 3 months after diagnosis.

The CTD-PAH population warrants specific study. A large proportion of the "non-responders" had CTD-PAH. It is unclear whether this group are less responsive to exercise therapy or the lack of improvement reflects disease severity and inevitable poor trajectory associated with CTD-PAH. It is possible that despite the "lack of improvement" seen in the PAH group, exercise therapy may slow disease progression and it is possible that this population require different outcome measures. A dedicated, controlled study assessing exercise therapy in CTD patients is required to address these factors.

5.6 **Conclusions**

Exercise therapy in a UK PAH population is efficacious as evidenced by significant improvements in quality of life and exercise capacity in an established PAH population on optimal drug therapy. With appropriate monitoring and expertise, exercise rehabilitation for PAH patients is feasible and safe outwith an established rehabilitation infrastructure, with an acceptable adherence rate.

Baseline prognostic markers indicative of increased disease severity, such as reduced TLCO, low peak VO_2 and high NTproBNP were associated poorer transplant free survival and a lack of improvement in exercise capacity. Further work is required to identify the optimal timing of rehabilitation in relation to initiation of drug therapy and the disease process.

Chapter 6 Assessing the impact of exercise therapy on gas exchange, ventilation and exercise capacity in PAH

6.1 Introduction

The most common presenting symptom in PAH is dyspnoea; it correlates closely with quality of life and self-reported dyspnoea scores have been shown to correlate with prognosis[23]. In PAH, dyspnoea is multifactorial and the pathophysiological abnormalities contributing to this symptom must be understood in order to develop therapies to improve it.

Efficient exercise requires the simultaneous, coordinated activation of the cardiovascular and ventilatory systems to meet the metabolic needs of the working muscles. The requirements for efficient exercise are discussed in chapter 1 and cardiovascular responses to exercise are discussed in chapters 7 and 8.

This chapter will explore how exercise training influences ventilation and gas exchange in PAH. It will also assess the role of different forms of exercise testing in determining the efficacy of exercise training in PAH.

6.1.1 Pulmonary Function in PAH

6.1.1.1 Overview of abnormalities in Lung function in PAH

The most prognostically relevant abnormality measured in PFTs in PAH is in gas exchange, however a number of other abnormalities in physiology are common. These changes are summarised in

Table 6.1 and discussed further below.

Table 6.1: Lung function abnormalities in PAH

Spirometry	Normal or Airflow obstruction[157]
Lung Volumes	Normal or reduced TLC[158]
Mouth Pressures	Normal or reduced [159]
Gas Transfer	Reduced [160]
Arterial Blood Gas	Normal or reduced $PaCO_2$[161] Normal or reduced PaO_2

6.1.1.2 **Principles of gas exchange and measurement**

The Roughton and Forster model describes gas transfer (total diffusion resistance, $mmol^{-1}$.min. kPa) as two resistances in series: the diffusion of the gas across the alveolar-capillary membrane (1/Dm), the transfer into the plasma and across the red blood cell membrane, and the chemical reaction of the gas with haemoglobin (Hb) ($1/\theta V_c$)[162]. The following equation summarises these concepts:

$$1/DL = 1/Dm + 1/\theta V_c$$

DL: total lung diffusing capacity; D_m alveolar-capillary membrane diffusing capacity; V_c volume of blood in the pulmonary capillaries; and θ is the rate of reaction of the gas with the red cell.

The transfer factor of the lung for carbon monoxide (TLCO) measures the partial pressure difference between inspired and expired carbon monoxide. TLCO provides information about the ability of the lungs to transfer gas from the alveolar space to the red blood cells in the pulmonary vessels. It is the product two measurements during breath holding at full inflation: KCO (the rate of uptake of carbon monoxide from alveolar gas), and the alveolar volume (VA). KCO is linearly related to the alveolar uptake efficiency for carbon monoxide and directly reflects the quality of alveolar–capillary gas uptake[163]. VA is the alveolar volume available for gas exchange.

$$TLCO = KCO \times VA$$

In normal subjects, the VA should be within 10% of the TLC. The VA/TLC ratio does not depend on age or body size, it decreases in maldistribution of ventilation and in intrapulmonary airflow obstruction. The same TLCO may occur with different combinations of KCO and VA, each representing different pathologies.

Decreased VA occurs in

1. Reduced alveolar expansion

2. Alveolar damage or loss

3. Maldistribution of inspired gases with airflow obstruction

Decreased KCO occurs with

1. Alveolar-capillary damage

2. Microvascular pathology

3. Anaemia.

Increased KCO occurs with:

1. Failure to expand normal lungs to predicted full inflation (extra-pulmonary restriction)

2. Increased capillary volume and flow; global (e.g. left to right shunt) or local flow and volume diversion (e.g. pneumonectomy)

6.1.1.3 **Gas exchange in PAH**

Untreated, three quarters of IPAH patients have TLCO of <80% predicted. This abnormality has been consistently observed across studies, including when adjusted for smoking status. Low TLCO in PAH is independently associated with poorer survival, particularly at values under 45% [164].

Both pulmonary membrane diffusion capacity and the pulmonary capillary blood volume are reduced in PAH. Reductions in pulmonary membrane diffusion capacity may be secondary to thickening of the alveolar capillary membrane due to endothelial cell proliferation. Reduction in pulmonary capillary blood volume may be the result of increased pulmonary vascular resistance, reduced cardiac output and local thrombosis [160].

Confounding factors may affect the TLCO in PAH. In an observational study of 166 IPAH patients, those with TLCO under 45% had higher BMI, older age, higher tobacco exposure and lower FEV1/FVC ratio[165].

From a physiological perspective, concern exists that reducing pulmonary arterial tone with vasodilators potentially results in a deterioration in V/Q matching[166], and therefore may worsen hypoxaemia; this effect has been seen with the vasodilator nitric oxide in critically ill patients with lung disease. More recent observational studies have suggested that improvements in gas exchange can occur with PAH therapy, with no deterioration in oxygenation[167]. Further prospective research is required to robustly establish the effect of different classes of vasodilators on gas exchange in PAH.

6.1.1.4 **Effect of exercise on gas exchange**

Acutely during exercise, TLCO and KCO rise, with a constant VA. This is due to recruitment of alveolar vessels and distension of the pulmonary capillary bed due to the rise in PAP associated with exercise. This results in increased capillary volume and membrane diffusion capacity.

No studies of exercise therapy in PAH have specifically looked at the effect of exercise training on changes in TLCO over time. A study comparing gas exchange in athletes to sedentary individuals, demonstrated that exercise diffusion capacity in athletes was higher due to increased membrane diffusing capacity rather than capillary blood volume. In the same study, resting alveolar capillary blood volume correlated with peak VO_2[168]. In COPD, a study of 137 patients undergoing pulmonary rehabilitation found that TLCO improved regardless of disease severity or ventilatory inhomogeneity. The mechanisms proposed for this varied depending on the degree of ventilatory homogeneity, defined as VA/TLC. Those with VA/TLC < 0.8 improved TLCO by increased alveolar volume, whereas those with VA/TLC >0.8 improved TLCO through improved KCO.

6.1.1.5 **Respiratory Muscle Weakness**

Histological changes associated with striated muscle in PAH are discussed in Chapter 9. Data regarding respiratory muscle function in PAH are from small, single centre studies. One study of 27 IPAH patients found reduction in maximal inspiratory (MIP) and expiratory pressures (MEP), together with an increased mouth occlusion pressure within first 0.1 s of inspiration (P0.1) at rest, suggesting inadequate muscle effort with regards to central drive[95]. Volitional and non-

volitional diaphragm strength is also reduced compared to age and sex matched controls[159].

Very little evidence exists on respiratory muscle function during exercise in PAH. A small study of ten IPAH and HPAH patients found that around half had dynamic hyperinflation, this was associated with increased levels of dyspnoea, but no differences were found in oesophageal measurements of inspiratory muscle strength. The authors proposed this was due to altered respiratory muscle mechanics rather than muscle weakness [169].

Hyperventilation is common in PAH and further exacerbates the imbalance in inspiratory muscle demand and the impaired capacity to generate force due to muscle weakness. This is likely to contribute to reduced exercise capacity, dyspnoea and quality of life in PAH [170]

Respiratory muscle training is an established component of pulmonary rehabilitation programmes. Inspiratory muscle training both in combination with aerobic exercise and in isolation, has been shown to improve dyspnoea scores and exercise capacity in IPAH, however the numbers studied have been small [171].

6.1.1.6 **Hyperventilation**

Hyperventilation occurs at rest, during exercise and has been observed during sleep in PAH. Low PaCO2 ($PaCO_2$ <4.25kPa) in the arterial blood of PAH patients is a strong, independent marker of poor prognosis, and relates to cardiac output and exercise capacity[172]. The degree of ventilatory inefficiency is related to survival, particularly the VE/VCO_2 slope (discussed below in 6.1.2.3) [173]. Intuitively, increased dead space secondary to V/Q mismatch results in an increased respiratory rate. End tidal carbon dioxide ($PETCO_2$) is consistently low in PAH, meaning dead space increases alone do not explain hyperventilation. Studies in IPAH and CTEPH have suggested increased chemosensitivity to both hypoxia and hypercapnia[174] through CPET and blood gas analysis, however no studies have yet assessed neural drive to further characterise this.

6.1.1.7 **Airflow obstruction**

Airflow obstruction has been observed in up to one third of IPAH patients compared to matched healthy controls, being defined as a forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) ratio (FEV1/FVC) of less than 0.7[175]. Mid-expiratory flow measurements are more commonly reduced in PAH; these measurements relate to peripheral airway obstruction and small airways disease. Parenchymal lung changes do not occur in IPAH, however it is postulated that hypertrophied blood vessels have a direct effect on small airways and can result in loss of distensibility. In keeping with this, some studies have observed that up to one quarter of patients have reduced total lung capacity (TLC)[158]

6.1.2 **Exercise testing in PAH**

Exercise tests are reliable, responsive to rehabilitation and pharmacological intervention and provide information on the mechanisms of exercise intolerance. Their incorporation into clinical trial design and use as end points in PAH has been recommended by European guidelines and the European Medicines Agency[62]. The most commonly utilised exercise tests in PAH are the 6MWT and Cardiopulmonary Exercise Test (CPET). Other tests such as the shuttle walk test and endurance tests have been used but have a smaller evidence base. The 6MWT is discussed in chapter 5, endurance testing and CPET are discussed below.

6.1.2.1 **Subjective exercise scoring systems**

The modified Borg dyspnoea score is a validated numerical score rated 0 to 10, used to measure patient reported dyspnoea during exercise testing. An equivalent scoring system exists for leg fatigue. In healthy individuals, leg fatigue is the predominant symptom at end exercise[176]. The Borg dyspnoea score is incorporated to various forms of exercise testing such as 6MWT and CPET.

6.1.2.2 **Cardiopulmonary Exercise Testing – General Principles**

The standard incremental CPET allows evaluation of the physiological responses to submaximal and peak exercise responses to produce an output known as the nine-panel plot (9PP) (Figure 6.1). The 9PP provides integrated data on exercise

capacity, effort, oxygen delivery, ventilation and gas exchange. The ATS provide robust guidelines on the required equipment and methods for performing an incremental cardiopulmonary exercise test[177].

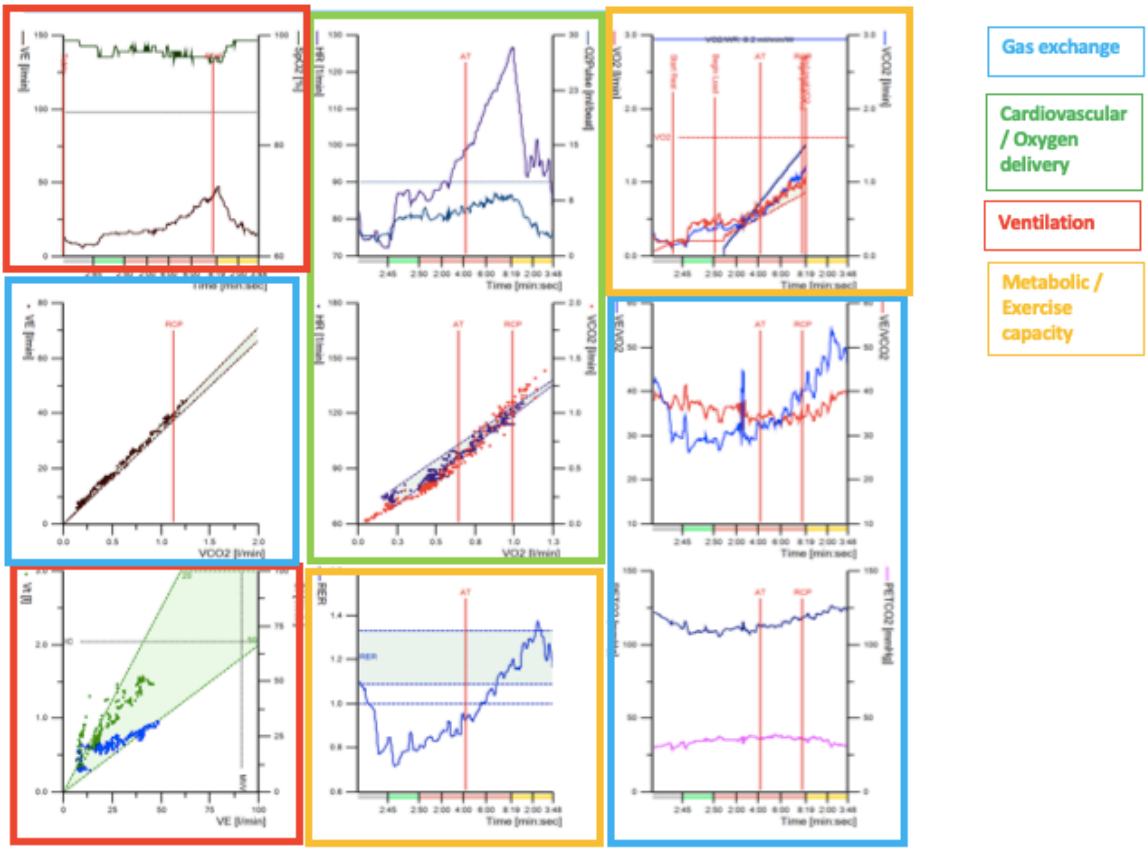


Figure 6.1: example of a 9-panel plot

Relevant parameters regarding CPET interpretation are discussed below[18]

1. Exercise capacity

End exercise work rate (WR): Maximum number of Watts produced at end exercise

Oxygen uptake (VO_2): measured from expired gas, the oxygen uptake each minute.

Respiratory Exchange Ratio (RER): The ratio of VCO_2 to VO_2 (VCO_2/VO_2), at rest should be equivalent the respiratory quotient (RQ) (VCO_2/VO_2 at tissue level). Affected by RQ and hyperventilation.

Anaerobic threshold (AT): An estimate of the onset of metabolic acidosis. Measured using the V-slope method, where there is an upwards deflection in the VCO_2-VO_2 relationship[178]

Ventilatory compensation point (VCP): when the VE/VCO_2 ratio increases in conjunction with the $PETCO_2$ falling. Correlates well with critical power and may not be present in sub-maximal exercise tests

2. Gas exchange

Alveolar-arterial oxygen difference (A-a gradient): Difference between alveolar and arterial pressure of O_2. Under 10mmHg is normal in young adults with values rising around 1mmHg for each decade of life. Raised values indicate gas exchange abnormalities

Dead space fraction (Vd/Vt): Physiological dead space; the fraction of inspired air that does not exchange gas with capillary blood

End tidal partial pressure of CO_2 ($PETCO_2$): partial pressure of CO_2 in exhaled gas, surrogate marker for alveolar [CO_2]

Ventilatory equivalents for CO_2 (VE/VCO_2): the number of litres of ventilation per litre of CO_2. Marker of ventilatory efficiency.

VE/VCO_2 slope: The relationship between minute ventilation and CO_2 output, a marker of ventilatory efficiency

3. Oxygen delivery (Cardiovascular Function):

Oxygen pulse: The volume of O_2 consumed per heartbeat. VO_2/HR, units mLO_2/beat

HR/VO_2 slope: Increases in this relationship imply lack of cardiac efficiency, reductions imply chronotropic incompetence.

VO_2/WR slope: In health the ΔVO_2/ ΔWR slope typically increases at 10ml/min/watt until peak exercise. Reduction in the gradient of VO_2/WR implies cardiovascular inefficiency.

4. Ventilation

*Minute ventilation (VE): T*he volume of air exhaled in one minute

Tidal volume (Vt): Volume of a single breath

6.1.2.3 **Cardiopulmonary exercise testing in PAH**

Cardiopulmonary exercise testing (CPET) is a powerful tool for diagnosis, assessment of treatment response and risk stratification in PAH. Exercise intolerance is the hallmark of PAH; CPET recreates an environment in which patients experience limiting symptoms and the physiological changes that occur with these symptoms can be captured. It allows assessment of disease severity by measuring known prognostic markers such as the VE/VCO_2 gradient. It is non-invasive and reproducible in expert hands[179] and several large case series have reported on the high level of safety, with no deaths and an adverse event rate of 0.2% or less [136, 180].

In PAH, peak aerobic exercise capacity is typically reduced, with impaired oxygen delivery and ventilatory inefficiency due to gas exchange abnormalities and hyperventilation. This produces a characteristic appearance on the 9PP (Figure 6.2).

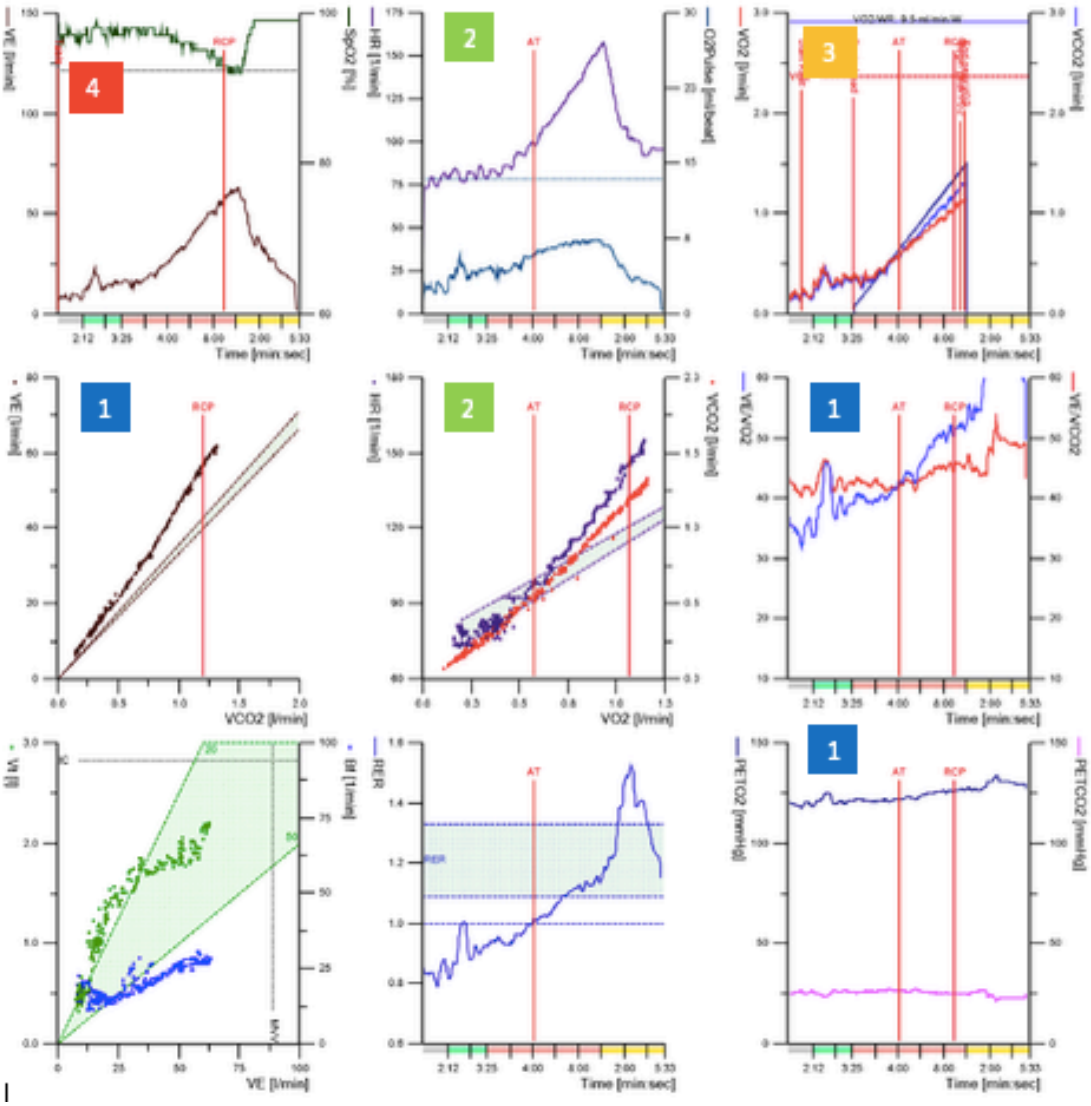


Figure 6.2: Typical 9PP in PAH

1 Gas exchange abnormalities: High Ve/VCO_2 gradient, low $PETCO_2$ at baseline and throughout exercise, high ventilatory equivalents for O_2 and CO_2.

2 Oxygen delivery abnormalities: High HR/VO_2 gradient, low peak O_2 pulse

3 Exercise capacity: Low peak VO_2

4 End exercise desaturation. Otherwise normal ventilation.

Exercise limitation and ventilatory inefficiency are strongly linked to survival; VO_2 < 15ml/min/kg and VE/VCO_2 slope > 36 are associated with poorer prognosis[181]. Peak HR has also consistently been shown to hold prognostic value.

CPET parameters have not been commonly employed end points of PAH clinical trials, despite their prognostic value. Early experience with CPET parameters as end points may have hindered future adoption in clinical trials; studies of Sitaxentan and Beraprost failed to demonstrate significant improvement in gas exchange or VO_2 despite improvements in 6MWD, however it was possible that there were methodological limitations to these studies. Subsequent smaller non-randomised studies of sildenafil and IV prostacyclin have shown improvements in peak VO_2 with associated mortality benefit[179].

In studies of exercise therapy, a meta-analysis of 469 patients with PAH and CTEPH reported a significant improvement in peak VO_2 of 1.84 ml/kg/min, however significant heterogeneity existed between included studies[182]. The majority of the studies were non-randomised, there were a combination of incremental and step-wise CPET protocols and a large proportion of patients were on monotherapy.

No minimum clinically important difference in CPET parameters have been established in PAH, however treatment goals exist and advocate aiming for a peak VO_2 of > 15ml/Kg/min and $VeqCO_2$ of < 45L.min.L.min [183]

6.1.3 Endurance tests

6.1.3.1 Concept of steady state exercise

Steady state exercise refers to aerobic exercise where there is equilibrium between energy supply and utilisation. At exercise levels below critical power, VO_2 increases exponentially until a steady state level Is reached [184](Figure 6.3). Under these circumstances, there is neither accumulation of lactate in muscle or blood, nor changes in muscle phosphocreatine concentration, and therefore VO_2

represents vast majority of metabolic energy liberation[185]

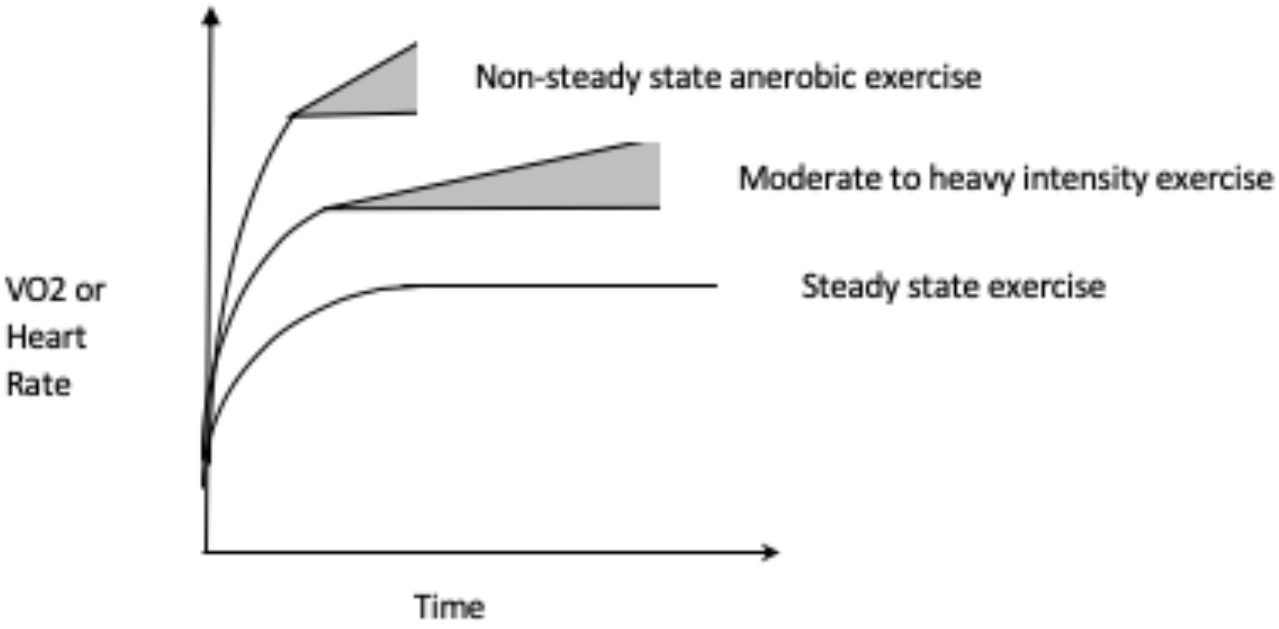


Figure 6.3: Oxygen kinetics in steady state exercise and exercise beyond the critical power

Steady state VO_2 can usually be maintained for 10-60 minutes of submaximal exercise. A limit to endurance is imposed by the availability of substrates sustaining aerobic metabolism.

An exercise testing protocol that achieves steady state has the advantages of being able to measure endurance time and make serial physiological measurements. The percentage of peak work rate corresponding to steady state will vary depending on individual levels of fitness; healthy and trained individuals will have a higher VO_2 at AT, therefore the percentage of peak work rate at which they remain in steady state will be greater than an untrained or diseased individual. VO_2 at AT is typically 50-60% of peak VO_2[184].

6.1.3.2 **Cardiac output – VO_2 relationship**

Cardiac output increases with VO_2 in a linear fashion; the increase in cardiac output is smaller than VO_2 for any given increase in power due to a positive y-axis

intercept (Figure 6.4) [185]

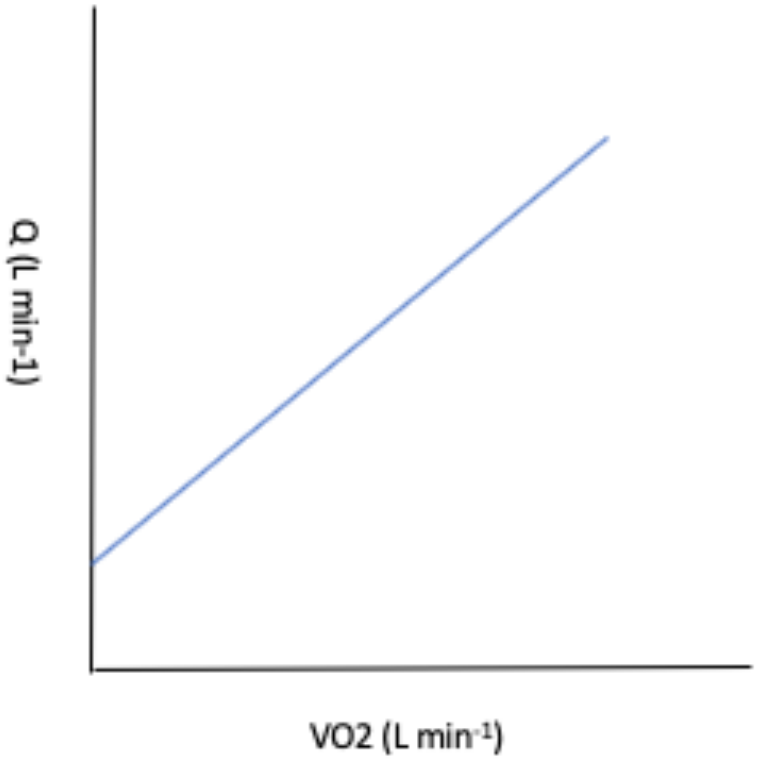


Figure 6.4: Cardiac Output (Q) and VO_2 relationship in steady state exercise

The slope of the line is equal to the reciprocal of the arterial-venous oxygen difference[186]. This relationship is evident in the Fick principle for cardiac output: CO is calculated as oxygen consumption divided by the arteriovenous oxygen concentration difference.

$$VO_2 = (CO \times Ca) - (CO \times Cv)$$

Therefore

$$CO = \frac{VO_2}{Ca\text{-}Cv}$$

6.1.3.3 **Endurance testing in PAH**

Selecting the most appropriate outcome measure to detect meaningful clinical change is essential in determining efficacy of exercise interventions. The exercise

test must be commensurate with the training modality. The majority of exercise rehabilitation programmes across chronic cardiac and respiratory conditions employ sub-maximal, aerobic exercise. Endurance tests are generally considered to be more sensitive at detecting improvement in functional capacity after exercise rehabilitation than 6MWT or maximal exercise tests [187]. In COPD, endurance testing has been recognised as a sensitive method for assessing treatment response. The endurance shuttle walk test is the most commonly employed testing modality, the cycling endurance test (CET) is another validated endurance test and in COPD has an MCID of 33% following pulmonary rehabilitation [188]

Endurance tests are not commonly employed end-points in PAH and have not been extensively investigated. A small study of 21 PAH patients suggested that endurance tests in PAH produced near maximal physiological changes and argued that changes in exercise capacity were related to modality (walking versus cycling) rather than incremental versus endurance testing protocols [189]. A possible limitation of this study is that the critical power may have been lower than anticipated, due to low VO_2 at AT in PAH and the "steady state" exercise may have been actually been anaerobic exercise.

6.2 **Aims**

The aims of this chapter are to determine the impact of exercise training in PAH on:

1. Respiratory physiology at rest and on exercise and explore potential mechanisms for these changes

2. Exercise capacity, utilising both endurance, sub-maximal and maximal exercise tests in order to determine the most suitable outcome measure(s) for assessing response to the exercise programme.

6.3 **Methods**

Exercise tests were performed at baseline, at the end of the residential phase of exercise and at the end of the study. Pulmonary function tests were performed at baseline and after the residential exercise phase was completed.

6.3.1 **Cardiopulmonary exercise testing**

Spirometry was performed before the test to allow reference values to be obtained for ventilatory measurements. Subjects wore a tight fitting mask, connected to the spirometer (

Figure 6.5). This mask was then used for the subsequent CPET.

Figure 6.5: Spirometry being performed prior to CPET

CPETs were conducted in accordance with ATS guidelines[190] using an electromagnetically braked cycle ergometer (Ergoselect 200, Ergoline GmbH, Bitz,

Germany). CPETs were carried out by a trained respiratory physiologist with medical supervision (AM). The face mask was connected to a metabolic cart for breath by breath measurement of VO_2 and VCO_2 (Medisoft, Sorinnes, Belgium; lovemedical, Manchester, United Kingdom). Oxygen saturations were measured by finger or ear probe and a continuous 12 lead electrocardiograph (ECG) was performed for cardiac monitoring. Systemic blood pressure was measured noninvasively by either an automated electronic or a manual sphygmomanometer at rest, during exercise and recovery (Figure 6.6). Borg scores for breathlessness and leg fatigue were obtained. Capillary blood gases were performed before and immediately after exercise

Figure 6.6: Incremental cardiopulmonary exercise test equipment set up

The ramp rate was determined by the physiologist and supervising doctor (AM) depending on the individual level of fitness, with the aim of achieving 8 to 12 minutes of loaded cycling. Saddle height was adjusted for optimal cycling mechanics. A two minute period of resting measurements were taken, followed by 3 minutes of unloaded cycling. A pre-determined progressive ramp rate then

commenced until symptom limited cessation of exercise. Verbal encouragement was given to the subject in accordance with guidelines.

Borg breathlessness score was performed before, during and after the test to assess breathlessness and leg fatigue, to determine subjective effort and the predominant limiting symptom. Capillary blood gases were taken before exercise and as close to peak exercise as possible.

CPET analysis and reporting was performed by AM using Blue Cherry® analysis software (Geratherm, Bad Kissingen, Germany). AT and RCP were manually determined.

6.3.1.1 **Isotime CPET measurements**

Isotime comparisons of CPET variables were performed before and after the exercise programme. Ramp rate was kept the same between tests for individual subjects, so that isotime corresponded to isowork. Isotime (and therefore isowork) was defined as the time elapsed from the beginning of the ramp to the lowest of the two peak work rates between tests.

6.3.1.2 **Steady state endurance testing**

Steady state endurance testing was performed at the time of exercise right heart catheterisation. The supine endurance exercise protocol is described in Chapter 7: Changes in invasively measured haemodynamics in response to exercise therapy in PAH.

6.3.2 **Statistics**

Normally distributed variables are shown as mean (SD) and non-normally distributed variables are shown as median (IQR). Categorical variables are presented as number (%). Due to the small sample size, non-parametric tests were used throughout. Comparison between baseline and follow-up measurements were made by Wilcoxon signed rank test. Comparisons of non-paired data were made using the Mann-Whitney test. Correlation between two variables was determined by Spearman correlation coefficient.

6.4 **Results**

6.4.1 **CPET**

Baseline characteristics are displayed in

Table 6.2. The findings were typical of a PAH population, with reduced peak aerobic exercise capacity, impaired oxygen delivery and gas transfer.

Using Jones VO_2 % predicted[191], 9 subjects had severely reduced exercise capacity (VO_2 < 50%), 6 had moderately reduced (51-70%), 4 had mildly reduced (<80%) and 9 had normal exercise capacity (>80%). From a prognostic perspective, six subjects had VE/VCO_2 gradient greater than 45% and 19 had VO_2 ml/kg/min < 15.

Table 6.2: Baseline CPET characteristics

N 28	Mean	Range
Peak WR (%)	65	22-109
Peak VO_2 L/min (%)	61	23-98
Peak VO_2 ml/kg/min	13	7-20
Peak O_2 pulse (%)	77	36-136
VO_2 / WR slope (ml/min/W)	8	3-11
VE (%)	68	48-98
TV (L)	1.5	0.7-2.5
RR (/min)	36	24-50
VeqCO_2	41	29-65
VE/VCO_2 gradient	41	28-73
PET CO_2 (mmHg)	30	17-45
% signifies percentage predicted		

6.4.1.1 **Change with exercise therapy**

24 patients had full CPET data from baseline to end of study based on previously discussed drop-outs (4) and equipment malfunction (1). Change in CPET parameters from baseline to end of residential phase are outlined in

Table 6.3. Significant improvements are seen in exercise capacity, oxygen delivery, gas exchange and improved tidal volumes.

Table 6.3: Change in CPET parameters from baseline to end of residential phase

N 27	Baseline (SD)	Week 3 (SD)	p
Peak WR (watts)	78.9 (27.6)	88.3 (34.3)	<0.001
WR (%)	65.5 (25.1)	73.3 (29.6)	<0.001
Peak VO_2 (L/min)	1.0 (0.3)	1.1 (0.4)	0.003
VO_2 (%)	61.1 (21.2)	69.2 (24.9)	0.002
Peak VO_2 ml/kg/min	12.8 (3.5)	14.4 (4.6)	0.001
VO_2 at AT (L/min)	0.69 (0.16)	0.78 (0.18)	0.001
VO_2 at AT (%)	44.0 (15.6)	49.3 (16.9)	0.003
Peak HR (BPM)	135 (22)	140 (22)	0.002
HRR	33 (20)	28 (21)	0.002
E.E O_2 Pulse (ml)	7.3 (1.7)	8.1 (2.0)	0.002
% O_2 Pulse	77.6 (24.7)	84.5 (28.1)	0.005
VO_2/WR (ml/min/W)	8.2 (1.5)	8.6 (1.4)	0.475
VE (L/min)	54.0 (17.4)	62.1 (21.2)	<0.001
VE (%)	67.7 (19.4)	77.6 (19.4)	0.006
BR (L/min)	26.7 (14.7)	18.7 (17.0)	0.028
TV (L)	1.5 (0.5)	1.7 (0.5)	<0.001
RR (/min)	36.7 (7.8)	36.3 (9.3)	0.903
Peak O_2 sats (%)	91.1 (7.9)	90.6 (7.2)	0.371
Veq CO_2 AT	41.5 (8.1)	39.7 (8.9)	0.006
Veq O_2 AT	40.4 (9.0)	38.1 (8.1)	0.036
VE/VCO_2 gradient	40.6 (10.1)	36.9 (9.9)	<0.001
$PETCO_2$ (mmHg)	29.9 (5.9)	29.9 (8.7)	0.976
Vd/Vt end exercise	0.36 (0.22)	0.14 (0.37)	0.293
PA-a O_2 gradient rest	63.7 (13.8)	48.2 (24.9)	0.043

Change in CPET parameters from baseline to the end of the study are described in

Table 6.4. A similar pattern is seen to the changes observed from the start of the study to end of the residential phase.

Table 6.4: Change in CPET parameters from baseline to end of study

N 24	Baseline (SD)	End of study (SD)	p
Peak WR (watts)	80.1 (29.0)	89.0 (37.9)	0.007
WR (%)	66.9 (25)	73.3 (30.3)	0.008
Peak VO_2 (L/min)	0.99 (0.3)	1.07 (0.35)	0.022
VO_2 (%)	61.4 (21.9)	66.8 (22.4)	0.037
Peak VO_2 ml/kg/min	12.7 (3.7)	13.7 (4.3)	0.020
VO_2 at AT (L/min)	0.68 (0.2)	0.76 (0.24)	0.030
VO_2 at AT (%)	44.1 (16.1)	47.8 (16.7)	0.077
Peak HR (BPM)	137 (21)	140 (23))	0.063
HRR (/min)	30.5 (19)	27.1 (20.2)	0.034

E.E O_2 Pulse (ml)	7.2 (2)	7.7 (2.1)	0.032
% O_2 Pulse	76.5 (25.7)	79.5 (25.9)	0.157
VO_2/WR (ml/min/W)	7.89 (2)	8.02 (1.4)	0.702
VE (L/min)	56.3 (17.6)	59.9 (19.3)	0.048
VE (%)	69.2 (12.5)	75.3 (15.5)	0.054
BR (L/min)	25.9 (14.8)	19.1 (15.2)	0.116
TV (L)	1.55 (0.5)	1.73 (0.5)	<0.005
RR (/min)	37 (7)	35 (9)	0.160
Peak O_2 sats (%)	90 (9)	89 (8.6)	0.072
Veq CO_2 AT	42.7 (8.2)	40.5 (9.6)	0.026
Veq O_2 AT	41.7 (9.1)	39.5 (10.0)	0.044
VE/VCO_2 gradient	43.1 (11.9)	38.1 (10.7)	0.004
$PETCO_2$ (mmHg)	29.1 (6.2)	29.5 (9.10)	0.047

Change in CPET parameters in the group receiving 15 weeks standard care (Group B) were compared to all subjects who completed the exercise training programme (Table 6.5). Exercise capacity, peak HR, minute ventilation and tidal volume were all significantly improved in the exercise therapy group compared with control, the VE/VCO_2 gradient just missed statistical significance, with a trend towards reduction.

Table 6.5: Standard care group versus exercise therapy group CPET changes

	Delta Ex. therapy n 24	SD	Delta Standard Care n 11	SD	P
Peak WR (watts)	8.8	15.1	-3.5	5.6	0.005
WR (%)	6.1	11.7	-3.9	5.5	0.002
Peak VO_2 (L/min)	0.08	1.43	0.00	0.12	0.224
VO_2 (%)	5.4	10.8	0.91	9.6	0.252
Peak VO_2 (ml/kg/min)	0.97	1.9	0.18	1.9	0.252
VO_2 at AT	0.08	0.14	0.032	0.1	0.409
VO_2 at AT (%)	3.6	9.0	2.1	6.9	0.451
Peak HR (BPM)	3.2	6.7	-4.5	5.9	0.007
HRR (/min)	-3.4	6.6	4.81	5	0.001
E.E O_2 Pulse (ml)	0.5	0.9	0.2	0.7	0.430
VO_2/WR (ml/min/W)	-0.21	2.3	0.02	1.1	0.958
VE (L/min)	3.6	9.4	-4.9	6.9	0.004
VE (%)	6.2	15.4	-9.5	14.1	0.004
BR (L/min)	-6.9	15.6	7.8	12.8	0.040
TV (L)	0.17	0.19	-0.01	0.3	0.009
RR (/min)	-1.6	4.7	-3.18	5.9	0.687
Peak O_2 sats (%)	-1.3	3.8	-0.36	3.6	0.283

Veq CO_2 AT	-2.2	4.9	-0.26	4.3	0.299
Veq O_2 AT	-2.2	5.3	-0.15	5.3	0.517
VE/VCO_2 gradient	-5	7.9	-0.2	5.9	0.061
$PETCO_2$ (mmHg)	0.4	4.8	0.9	2.9	0.740

6.4.1.2 **Isotime CPET analysis**

Isotime analysis was performed for baseline to week 3 (end of residential phase exercise) and baseline to end of study CPET as described in 6.3.1.1. Isotime improvements were noted in O_2 pulse, TV and gas exchange in both analyses (Table 6.6 and Table 6.7). There was an improvement in isotime VO_2 between baseline and week 3.

Table 6.6: Baseline to week 3 CPET highest isotime comparisons

n 27	Baseline	SD	Week 3	SD	P
Weight	80.7	18.1	80.3	16.9	0.572
VO_2 (L/min) Jones	0.99	0.26	1.1	0.23	0.012
VO_2 (L/min) Jones (%)	59	18	63	19	0.004
VO_2 ml/min/kg	12.7	3.5	13.5	3.8	0.010
RER	1.15	0.11	1.11	0.09	0.058
HR (BPM)	134	22	132	20	0.215
HR % predicted max	85	13	83	12	0.210
O_2 pulse (ml)	7.4	1.5	8	1.7	0.004
O_2 pulse (% predicted)	72.1	16.7	78	19.3	0.003
VE (L/min)	53.4	17.3	51.1	16.7	0.177
VE % predicted	68.6	20.8	66.8	24.3	0.393
TV (L)	1.51	0.45	1.68	0.42	0.001
RR (/min)	36.3	7.9	30.7	7.8	<0.005
O_2 saturation (%)	90.6	7.7	91.3	7.3	0.197
VE/VCO_2	43.9	11.8	41.2	11	<0.005
VE/VO_2	49.6	13.2	46.7	14.3	0.011
$PetCO_2$ (mmHg)	28.9	6.8	30	7.4	0.018

Table 6.7: Baseline to end of study CPET highest isotime comparisons

N 24	Baseline	SD	EOS	SD	P
VO_2 (L/min) Jones	0.98	0.29	1.0	0.3	0.507
VO_2 (L/min) Jones (%)	59.2	19.3	60.2	19.2	0.579
VO_2 ml/min/kg	12.5	3.7	12.9	3.9	0.299
RER	1.15	0.09	1.14	0.09	0.604
HR (BPM)	136	22	133	22	0.256
HR % predicted max	86.6	13	85.2	12	0.349

O_2 pulse (mL/beat)	7.29	1.68	7.65	2.11	0.087
O_2 pulse (% predicted)	70.9	17.9	74.1	20.1	0.128
VE (L/min)	54.6	18.3	51.3	15.8	0.140
VE % predicted	71.5	20.5	67.9	20.7	0.200
TV (L)	1.54	0.46	1.67	0.49	0.033
RR (/min)	36.3	8.1	31.2	7.6	0.001
O_2 saturation (%)	90.3	8.2	89.9	8	0.536
VE/VCO_2	45.5	12.6	43.3	12.9	0.046
VE/VO_2	51.2	14.1	48.2	13.8	0.012
$PetCO_2$ (mmHg)	27.9	7.1	29.3	7.7	0.008

6.4.2 **Endurance time and assessment of exercise capacity**

Four markers of exercise capacity were compared, two maximal (peak WR, peak VO_2), one sub-maximal (6MWD) and one endurance (cycling endurance time). All parameters improved significantly from baseline to the end of the study. Cycling endurance improved by 52% (19% greater than the MCID). 6MWD improved by 40m, 7m beyond the MCID. Proportionate changes are displayed in Figure 6.7 and

Table 6.8

Table 6.8: Exercise capacity % changes with exercise therapy

Group mean	Baseline	EOS	% change	p
Peak WR (W)	80 (29)	89 (38)	11.3%	<0.001
6MWD (m)	447 (86)	487 (90)	9.0%	<0.001
Peak VO_2 (L/min)	0.99 (0.3)	1.07 (0.35)	8.1%	0.022
Loaded cycling endurance time (sec)	669 (287)	1018 (437)	52%	0.004

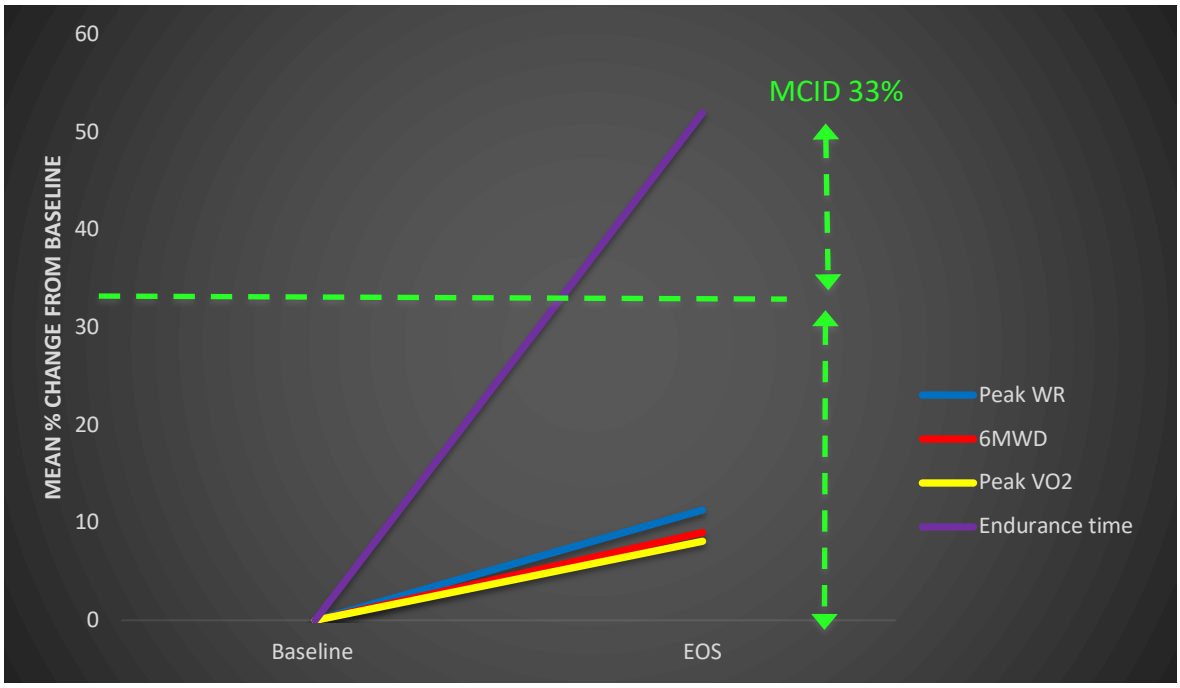


Figure 6.7: Endurance time versus conventional markers of exercise capacity

6.4.3 **Pulmonary Function**

Baseline characteristics are displayed in

Table 6.9 and are typical of a PAH population, with reduced gas transfer. TLCO was under 45% in 29% (8/28) of subjects, 32% (9/28) had evidence of airflow obstruction, air trapping (RV/TLC > 120%) was present in 18% (5/28), 14% (4/28) had ventilatory inhomogeneity (VA/TLC < 0.8).

Table 6.9: Baseline characteristics PFTs

N 28	Mean	Range
FEV1 (L)	2.28	1.11-3.33
FEV1 (%)	83.7	47-119
FVC (L)	3.2	1.38-4.39
FVC (%)	97.2	63-124
FEV1/FVC (%)	71.2	53-85
TLC (L)	5.11	2.85-7.90
RV/TLC (%)	37.37	19-61
MEP 1s (%)	67.39	18-166
MIP 1s (%)	64	17-101
KCO (Si)	1.09	0.29-1.83
KCO%	67.52	18-103
TLCO (ml/min/mmHg)	4.72	1.31-8.19
TLCO %	55.5	18-90
VA (L)	4.28	2.54-6.2
VA/TLC	0.84	0.69-0.95

Total lung capacity and gas exchange improved with exercise therapy .

Table 6.10: Effects of 3 weeks exercise therapy on pulmonary function

N 28	Baseline	SD	Week 3	SD	p
FEV1 (L)	2.28	0.55	2.254	0.586	0.451
FEV1%	83.7	19.5	82.89	20.02	0.647
FVC (L)	3.2	0.70	3.214	0.688	0.657
FVC%	97.2	17.3	93.45	25.32	0.989
FEV1/FVC	71.2	8.11	70.33	7.442	0.133
TLC (L)	5.11	1.11	5.263	1.196	0.042
RV/TLC	37.37	7.64	37.67	7.544	0.604
KCO (Si)	1.09	0.421	1.144	0.488	0.114
KCO%	67.52	22.97	71.11	26.93	0.034
TLCO (ml/min/mmHg)	4.72	1.98	4.92	2.10	0.105
TLCO %	55.5	21.1	57.5	22.0	0.190
VA (L)	4.28	1.31	4.40	1.1	0.785
VA/TLC	0.84	0.18	0.85	0.08	0.362

Expiratory mouth pressures improved following exercise therapy Table 6.11

Table 6.11: Change in mouth pressures with 3 weeks exercise therapy

N 27	Baseline	SD	Week 3	SD	p
MEP (1 sec)	65.9	28.5	72.2	21.9	0.150
MEP (%)	65.6	34.0	72.9	27.0	0.031
MIP (1 sec)	60.0	23.0	63.6	18.7	0.493
MIP (%)	63.1	23.3	65.1	25.2	0.486
MEP (peak)	105.6	38.7	105.8	29.7	0.656
MIP (Peak)	67.4	21.5	74.5	25.9	0.060
Maximum expiratory mouth pressure (MEP), Maximum inspiratory mouth pressure (MIP)					

6.4.4 Association between CPET and PFTs with prognostic and clinically relevant variables

CPET and PFT variables associated with exercise capacity, gas exchange and ventilation were correlated with clinically and prognostically relevant variables and are discussed further below.

6.4.4.1 **Exercise capacity**

Baseline markers of exercise capacity correlated with IL-6, PA compliance and gas exchange (Table 6.12). The cytokine IL-6 is discussed further in Chapter 9.

Table 6.12: Baseline exercise capacity correlations

Baseline	Baseline	ρ	p	N
WR (W)	IL6 (pg/L)	-0.641	<0.001	26
VO_2 (L/min)	Rca (ml/mmHg)	0.527	0.014	21
Peak VO_2 (%)	$VeqCO_2$	-0.565	0.006	28
Peak VO_2 (L/min)	Rca (ml/mmHg)	0.527	0.014	21
Peak WR (W)	TLCO (%)	0.746	<0.005	27
Peak WR (W)	IL6 (pg/L)	-0.641	<0.001	26

Change peak aerobic exercise capacity correlated with quality of life, peak exercise oxygen pulse (stroke volume surrogate) and peak minute ventilation (Table 6.13)

Table 6.13: Delta exercise capacity correlations

Delta	Delta	ρ	p	N
Peak WR (W)	EmPHasis-10	-0.425	0.048	22
Peak WR (W)	Peak O_2 pulse	0.603	0.002	24
Peak WR (W)	VE	0.647	0.001	24

6.4.4.2 **Gas exchange correlations**

Gas exchange at baseline was assessed through a number of parameters. A similar pattern was evident between gas exchange variables, of a correlation with exercise capacity, mixed venous O_2 saturations, pulmonary artery compliance and stroke volume (Table 6.14).

Table 6.14: Baseline gas exchange correlations

Baseline	Baseline	ρ	p	N
VE/VCO_2 at AT	% WR	-0.587	0.001	28
VE/VCO_2 at AT	% VO_2	-0.565	0.002	28
VE/VCO_2 at AT	SvO_2 %	-0.549	0.012	20
VE/VCO_2 at AT	SV mL (RHC)	-0.527	0.010	21
VE/VCO_2 at AT	Rca (ml/mmHg)	-0.544	0.011	21

VE/VCO_2 at AT	O_2 pulse %	-0.643	0.000	28
VE/VCO_2 gradient	Rca (ml/mmHg)	-0.684	0.001	21
VE/VCO_2 gradient	SV (RHC)	-0.545	0.011	21
VE/VCO_2 gradient	PVR (WU)	0.621	0.003	21
PET CO_2 at AT	PVR (WU)	-0.625	0.002	21
PET CO_2 at AT	Rca (ml/mmHg)	0.583	0.006	21
$PETCO_2$ at AT	O_2 pulse (mL/beat)	0.623	0.000	28
TLCO(ml/min/mmHg)	WR (W)	0.726	<0.001	26
TLCO(ml/min/mmHg)	VO_2 L/min	0.703	<0.001	26
TLCO(ml/min/mmHg)	VE (L)	0.528	0.006	26
TLCO(ml/min/mmHg)	RVOPF	-0.509	0.022	21
KCO (si)	VO_2 at AT	0.584	0.002	26
KCO (si)	VO_2 L/min	0.587	0.002	26
KCO (si)	% WR	0.562	0.003	26

Change in gas exchange with exercise therapy correlated with exercise capacity, stroke volume, minute ventilation and invasively measures PA pressures (

Table 6.15, Figure 6.8, Figure 6.9)

Table 6.15: Delta gas exchange variables

Delta	Delta	ρ	p	N
VE/VCO_2 at AT	6MWD (m)	-0.507	0.014	23
VE/VCO_2 at AT	SV (mL) (MRI)	-0.521	0.011	23
PET CO_2 at AT	mPAP (mmgHg)	-0.529	0.043	15
TLCO%	VE (L)	-0.655	0.006	22
TLCO %	RV SV (mL)	0.535	0.007	24
VE/VCO_2 gradient	RAP (mmHg)	-0.535	0.040	15

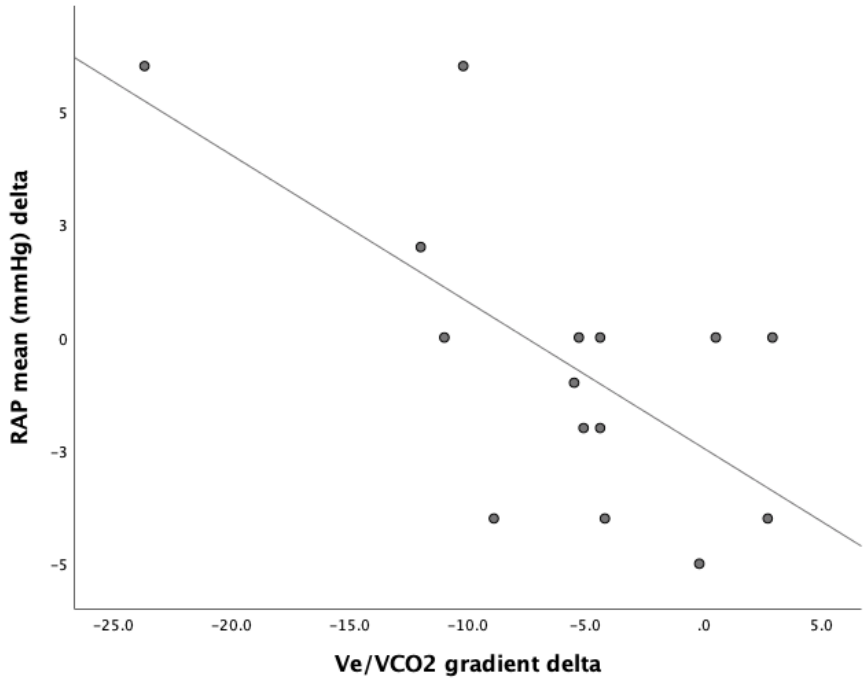


Figure 6.8: Change in VE/VCO_2 gradient and relationship with change in Right Atrial Pressure

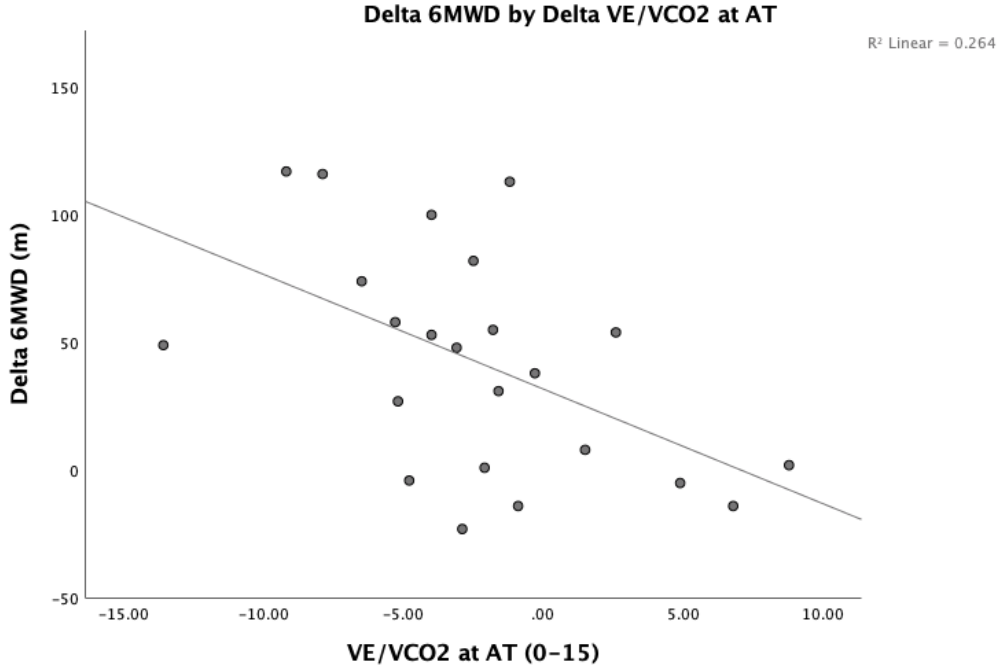


Figure 6.9: Change in Ventilatory Equivalent for CO_2 and change in 6MWD

6.4.4.3 Ventilation correlations

At baseline, tidal volume ad minute ventilation correlated with IL-6, quality of life scores and exercise capacity (

Table 6.16)

Table 6.16: Ventilation correlations at baseline

Baseline	Baseline	ρ	p	N
TV (L) (CPET)	6MWD (m)	0.493	0.008	28
TV (L) (CPET)	IL6 (pg/L)	-0.559	0.003	26
VE (L)	6MWD (m)	0.622	0.000	28
VE (L)	IL6 (pg/L)	-0.516	0.007	26
VE (L)	EmPHasis-10	-0.529	0.004	28

Change in ventilation with exercise therapy was associated with changes in stroke volume, cardiovascular efficiency and gas exchange (

Table 6.17)

Table 6.17: Delta ventilation correlations

Delta	Delta	ρ	p	N
VE (L)	VO_2 %	0.625	0.001	24
VE (L)	WR (W)	0.647	0.001	24
VE (L)	TLCO%	-0.566	0.006	22
VE%	RVOPF	-0.587	0.021	15
TV (L)(CPET)	SVI (RHC)	0.746	0.001	15
TV (L)(CPET)	SV (mL)	0.589	0.021	15
TV (L)(CPET)	CO (L) (RHC)	0.567	0.028	15
TV (L)(CPET)	O_2 Pulse (mL/beat)	0.484	0.017	24

6.4.5 **Relationship with prognosis and treatment response**

6.4.5.1 **Treatment response**

Comparison between baseline factors in those that improved, versus those who demonstrated no change or deteriorated (NC /D) are displayed in Table 6.18 and Table 6.19

Table 6.18: Improved 6MWD versus deteriorated or no change

Baseline	Improved (15)	SD	NC / D (10)	SD	p
Peak WR (W)	85	21	70	36	0.031
Peak VO_2 (L/min)	1.1	0.2	0.9	0.1	0.048
Peak VO_2 (mL/kg/min)	13.5	3.4	11.5	3.7	0.144
VO_2 at AT (L/min)	0.7	0.1	0.6	0.2	0.115
End ex HR %	83	9	79	13	0.495

HRR	28	15	36	23	0.428
Peak O_2 pulse (mL/beat)	7.6	1.4	6.6	2.3	0.196
VO_2/WR	8.4	1.9	7.3	1.8	0.071
VE (L)	55	11	58	25	0.978
TV (L)	1.6	0.4	1.5	0.5	0.727
RR	36	8	39	8	0.311
Ve/VCO_2 at AT	41	6	45	11	0.397
VE/VCO_2 gradient	38	7	49	15	0.036
PET CO_2 at AT	30	4	28	8	0.177

Table 6.19: Change in CPET parameters in improved group versus no change / deteriorated group

	Improved mean change (n14)	SD	Deteriorated mean change (N 9)	SD	p
Peak WR (W)	14.7	15.7	2.7	7.5	**0.024**
Peak WR % pred	10.5	10.3	2.8	7.7	0.053
Peak VO_2 (L/min) Jones	0.13	9.6	0.01	0.1	0.080
Peak VO_2 (L/min) Jones (% pred)	7.3	9.6	4.1	12.4	**0.013**
Peak VO_2 ml/min/kg	1.7	2.0	0.0	1.4	**0.023**
VO_2 at AT	0.11	0.16	0.03	0.08	0.159
VO_2 at AT (% pred)	4.2	10.9	2.8	6.2	0.692
End exercise HR	3.4	6.2	3.6	7.6	0.956
End exercise HR (% pred)	2.0	3.8	2.3	4.7	0.860
HRR	-3.6	6.2	-2.9	7.6	0.918
End exercise O_2 pulse (mL/beat)	0.8	1.0	0.3	0.9	0.251
End exercise O_2 pulse (% pred)	5.9	9.7	-0.2	7.1	0.100
VO_2/WR	0.16	1.9	-1.0	2.8	0.282
VE (L)	6.4	10.5	-0.6	4.1	0.104
VE % predicted	8.0	17.4	5.5	11.6	0.682
Breathing reserve	-7.9	17.0	-7.0	14.0	0.902
TV (L)	0.2	0.2	-0.2	0.8	0.179
RR	-1	4.2	-2	5.9	0.269
O_2 Sat rest (%)	-0.14	1.3	0.2	4.1	0.812
O_2 Sat peak (%)	-0.9	2.2	-1.2	5	0.841
VE/VCO_2 at AT	-4.2	4.1	0.7	5.4	**0.020**
VE/VO_2 at AT	-4.0	4.1	0.0	4.4	**0.042**
Ve/VCO_2 gradient	-3.1	4.1	-3.2	7.5	0.394
PetCO_2 at AT (mmHg)	1.8	1.9	1.0	1.7	0.677

6.4.5.2 **Prognosis**

Those who were dead, transplanted or had dropped out of the study at censoring, had lower baseline gas exchange and exercise capacity (

Table 6.20)

Table 6.20: Prognostically relevant CPET and PFT variables at baseline

Baseline	Alive at censoring (23)	Transplant or dead at censoring or drop out (7)	p
TLCO %	60.2 (19.8)	40.3 (18.9)	0.040
Peak WR (W)	83 (7)	52 (26)	0.001
Peak VO_2 (L/min)	1.0 (0.2)	0.77 (0.2)	0.015
Peak VO_2 ml/kg/min	13.3 (2)	9.5 (4)	0.012
A-a gradient	49 (22)	73 (16)	0.024
VE/VCO_2	39 (7)	52 (19)	0.059

From baseline to the end of study assessment, those who remained alive had improved gas exchange and exercise capacity, compared to those who were transplanted or had died (Table 6.21)

Table 6.21: Change in gas exchange variables in relation to transplant free survival

Delta	Alive at censoring (23)	Transplant or dead at censoring (7)	p
TLCO %	8.0 (11.6)	0.3 (5.6)	0.029
KCO **(si)**	0.17 (0.23)	0.02 (0.11)	0.042
Peak WR (W)	11 (14)	-7 (10)	0.045
Peak VO_2 ml/kg/min	1.2 (1.9)	-0.7 (10.9)	0.035

6.5 **Discussion**

Participants in this study displayed typical features of PAH, with reduced exercise capacity, impaired gas exchange and impaired of oxygen delivery on CPET. Despite being on optimal drug therapy, 68% of participants had peak VO_2 under 15 ml/kg/min and 21% had TLCO under 45%.

Wide physiological variation existed in this cohort, ranging from severe impairments of gas exchange, airflow obstruction and air trapping, to subjects with preserved gas exchange and supranormal lung volumes. This may be explained by the heterogeneity of the population both in age, BMI, PAH subtype and comorbidity. The second largest group behind IPAH was CTD-PAH, 6 subjects (20%) had associated lung disease, 2 subjects (13%) were smokers . It also highlights the need to pursue further research of a highly phenotyped population, with more specific inclusion criteria or pre-defined subgroup analyses.

Exercise capacity

Exercise therapy resulted in significant improvements in peak aerobic exercise capacity and endurance exercise capacity.

All markers of exercise capacity (Peak WR, Peak VO_2, cycling endurance time and 6MWD) improved and all were highly statistically significant. Assessing the varying degrees of sensitivity in exercise-based outcome measures may help inform future trial design in PH exercise rehabilitation studies. Comparing the performance of all parameters of exercise capacity in this study, the largest proportionate change was seen in loaded endurance time and this modality reflected the predominantly aerobic nature of the exercise programme. Additionally, it allowed serial measurements of haemodynamics and oxygen extraction (discussed in Chapter 7). The highest degrees of sensitivity from a statistical perspective were seen with peak WR and 6MWD. The 6MWT remains an inexpensive and reproducible test to perform, without the requirement for specific expertise. Incorporating both sub-maximal or maximal tests with an endurance testing provided valuable information on treatment response and the mechanisms of improvement in this study.

CPET

The improvement in exercise capacity was accompanied by a higher VO_2 at AT, higher peak oxygen pulse, greater peak minute ventilation secondary to greater tidal volumes and more efficient gas exchange. The effect was more pronounced within the first three weeks of exercise training but maintained at the end of the study.

In comparison to subjects receiving standard care, those who completed the exercise programme had significantly improved exercise capacity and ventilatory capacity. There was a signal towards improved gas exchange in the standard care versus treatment analysis, however this narrowly missed statistical significance.

Both at week three and the end of the study, isotime CPET measurements of gas exchange, oxygen pulse and tidal volumes were significantly higher and respiratory rate was significantly lower. Similar to the peak CPET comparisons, the improvement was maintained at the end of the study, but more pronounced within the first three weeks of exercise. Of note, VO_2 at isotime was higher at the end of the study, this may be due to improvements in the VO_2/WR relationship.

Lung function

Nearly half of participants had severely impaired gas exchange. Abnormalities were not restricted to gas exchange however, one third demonstrated airflow obstruction, 18% had air trapping and 14% ventilatory inhomogeneity. Despite this, the mean FEV1/FVC ratio was low normal as was VA/TLC

Following three weeks of exercise training, total lung capacity, gas exchange and expiratory mouth pressures improved. No significant change was seen in spirometry, RV/TLC or VA/TLC.

Clinical and prognostic relevance of observed changes

At baseline, a higher peak aerobic exercise capacity was associated with lower Il-6, greater pulmonary artery compliance and more efficient gas exchange (CPET). Improvement in peak WR correlated with improvements quality of life, oxygen pulse and minute ventilation.

The majority of gas exchange variables at baseline, both CPET and PFT, were associated with a number of relevant clinical variables. More efficient gas exchange was associated with greater exercise capacity, higher stroke volume (RHC and oxygen pulse), lower PVR and higher pulmonary artery compliance.

Following exercise therapy, improvements in gas exchange were associated with higher minute ventilation, improved stroke volume, reduced right atrial pressure, lower mPAP and increased 6MWD.

At baseline lower minute ventilation and tidal volumes were associated with lower 6MWD and poorer quality of life. Improvements in VE and TV were associated with improved exercise capacity, more efficient gas exchange, higher stroke volume (CMR , RHC and oxygen pulse) and cardiovascular efficiency (RVOPF).

In the post hoc analysis, those who improved with exercise therapy appeared to have higher baseline peak WR and VO_2 in addition to significantly lower VE/VCO_2 gradient. Peak aerobic exercise capacity (VO_2 and WR) correlated with 6MWD. In those who improved, minute ventilation and tidal volumes increased significantly compared to those who derived no benefit.

Gas exchange and exercise capacity at baseline appeared to be lower in those who were transplanted or dead at censoring. The group of subjects who improved, had improvements in gas exchange, whereas no change was noted in those transplanted or dead at censoring.

Mechanisms

This study verified the prognostic roles of exercise capacity and gas exchange in PAH, seen in the associations of these parameters with known prognostic markers such as PVR and WHO FC[5]. Improvements in exercise capacity were tracked by changes in gas exchange; a crucial relationship exists between the two, both at an isolated time point and in relation to the response to therapy. Those who did not demonstrate improvements in gas exchange were more likely to undergo lung transplantation or die and were less likely to improve functionally with exercise therapy. This relationship may exist because gas exchange measurements encompass many aspects of the pathophysiology of PAH including vascular remodelling, microthrombosis with damage to the capillary basement membrane, anaemia and low alveolar capillary blood volume. When inspecting the elements of physiology contributing to gas exchange, four potential reasons are evident to explain the mechanism of improvement

1) *Improvements in stroke volume* accompanied the gas exchange improvements occurred in this cohort. This could be explained by a larger alveolar capillary volume secondary to the higher cardiac output observed during exercise. The changes seen in TLCO were due to improved KCO rather than VA, this would be in keeping with improved alveolar capillary blood volume.

2) *Tidal volume improved* and correlated with gas exchange and *peak expiratory mouth pressured improved*. This may be secondary to recruited lung through increased respiratory muscle strength therefore improving V/Q matching

3) *Minute ventilation increased due to increased tidal volume, with reduced respiratory rate* → reduced hyperventilation and improved work of breathing / ventilatory efficiency secondary to lung recruitment and improved respiratory muscle strength.

4) *Reduced atrial stretch and improved pulmonary artery compliance* → Reduction in RAP correlated with reduced VE/VCO_2 gradient following exercise therapy. Additionally, a strong and clear relationship existed between pulmonary artery compliance and gas exchange, with higher pulmonary artery compliance being associated with better gas exchange across a number of variables. In keeping with this, higher RVOPF (i.e. a less efficient pulmonary circulation), correlated significantly with lower TLCO. Chapter 7 discusses the possible reasons for improved pulmonary artery compliance with exercise therapy. In reviewing the literature, animal models have demonstrated a close relationship between the RV, RAP and the ventilatory response. Pressure-related stimulation of mechanoreceptors in the right atrium and right ventricle result in an aggravated sensation of dyspnoea that increases ventilation[192]. RAP also has a strong negative association with exercise capacity and is linked to the afterload faced by the right ventricle[193].

There was no change in haemoglobin with exercise therapy and it is unlikely in the short time frame of the study, that there was a change in structure to the capillary

basement membrane. Therefore, the key mechanisms in gas exchange improvements appear to be: Lung recruitment secondary to strengthened muscles, reduced hyperventilation, improved stroke volume and improved pulmonary artery compliance, the latter two factors leading to increased capillary blood volume.

Subjects with improved 6MWD, had higher exercise capacity and more favourable prognostic markers at baseline. Those who deteriorated or did not improve, clearly had markers of more severe PAH at baseline. This group of "non-responders" with severe PAH are more difficult to analyse, it is unclear whether exercise therapy will slow an inevitable progression in this group of patients with severe PAH or will not impact on the disease course. No evidence from this data suggests the exercise therapy or testing caused harm. There were not enough participants in this study to perform an analysis of exercise therapy versus standard care those who did not improve, this would be a useful consideration for future studies to help clarify the role of exercise therapy in severe PAH.

Future research avenues to explore the improvements seen with gas exchange could include lung perfusion imaging to determine flow mediated contributions, exercise cardiac MRI to assess dynamic right atrial volume and the potential contribution of RA stretch, studies of respiratory muscle strengthening in isolation, to determine the impact of improved inspiratory muscle strength and lung recruitment and assessment of autonomic regulation, particularly of the pulmonary circulation, before and after exercise therapy.

6.6 **Conclusions**

Exercise therapy resulted in significant improvements in exercise capacity. Both endurance and incremental exercise tests were sensitive means of detecting treatment response and provided valuable and different information regarding the exercise response in PAH.

A significant proportion of subjects on optimal drug treatment had severe reductions in aerobic exercise capacity and gas exchange. These subjects were less likely to show a favourable response to exercise therapy. Lung function

abnormalities were common and suggest more specific inclusion criteria may be of benefit in future studies of exercise therapy.

Gas exchange and lung volumes improve with exercise therapy. These changes are associated with a number of relevant prognostic markers. The improvements in gas exchange are likely multifactorial, with lung recruitment, improved alveolar capillary blood volume and potentially reduced atrial stretch.

Chapter 7 Changes in invasively measured haemodynamics in response to exercise therapy in PAH

7.1 Introduction

7.1.1 Normal anatomy and physiology of the pulmonary circulation

The healthy adult human pulmonary circulation is a high flow, low pressure circuit [194], the function being defined by the pressure-flow relationship. Flow and pressure in the pulmonary circulation are pulsatile. Low pressure prevents fluid moving out of the pulmonary vessels into the interstitial space and allows the right ventricle to operate at minimal energy cost. In contrast to the systemic circulation, the arteries in the pulmonary circulation are thin walled, highly compliant vessels [195]. The pulmonary circulation is characterised by an inflow pressure (pulmonary artery pressure) and an outflow pressure (left atrial pressure), the difference between which is the transpulmonary pressure gradient (TPG).

The right ventricle pumps deoxygenated blood into the pulmonary arteries (Figure 7.1). The pulmonary arteries divide into pulmonary arterioles and then to the capillary bed, which supplies the deoxygenated blood to the alveoli, where gas exchange occurs (Figure 7.2).

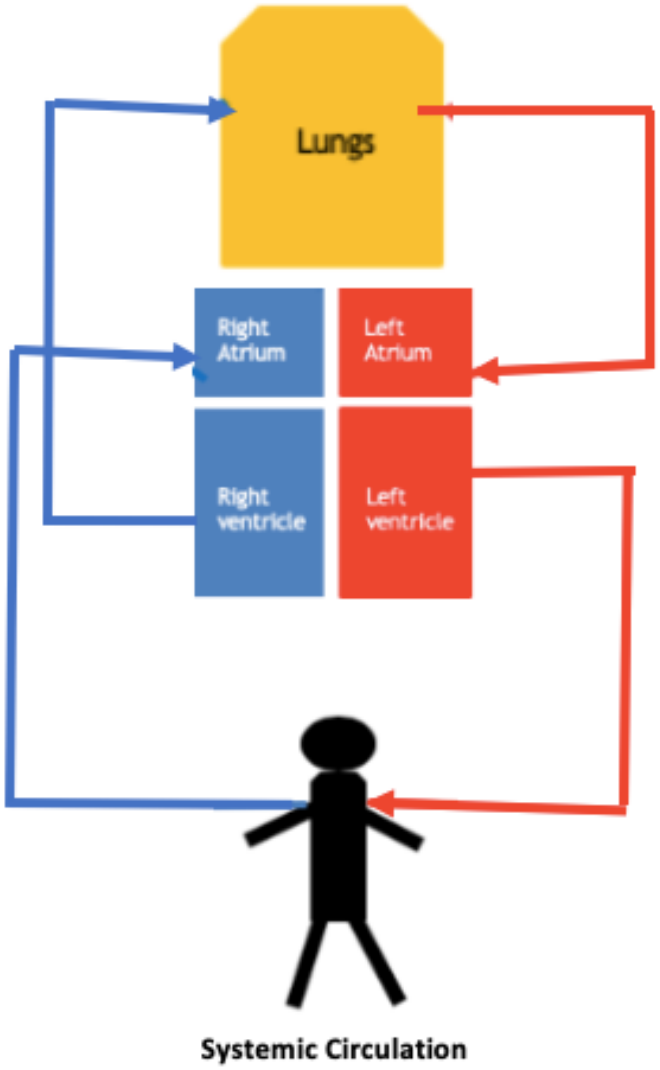


Figure 7.1 General anatomy of the human circulatory system – blue, deoxygenated blood; red, oxygenated blood.

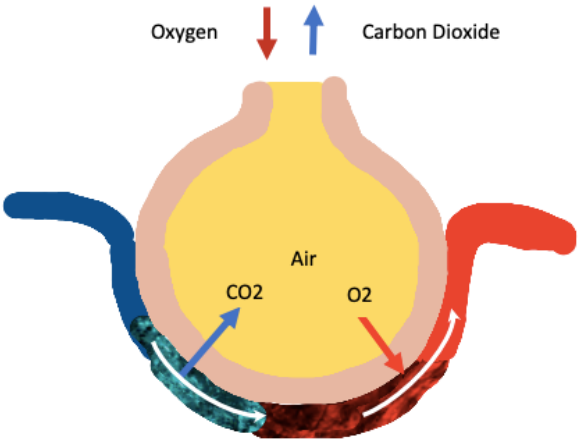


Figure 7.2: Alveolar gas exchange

Blood then drains into pulmonary venules, then veins, which transport blood to the left ventricle, allowing oxygenated blood to be delivered to the body via the systemic circulation.

7.1.1.1 Normal values and measurement of right heart and pulmonary artery pressures

Right heart catheterisation is the gold standard for assessment of the pulmonary circulation. A fluid filled, balloon tipped thermodilution catheter (Figure 7.3) with a pressure transducer is inserted into the systemic venous circulation via a central vein, allowing real time pressure wave monitoring. Vascular pressures are measured using a zero-levelled external manometer at the mid thoracic level (the hydrostatic indifferent point) [196] In the supine position, this is 5cm below the sternal angle [196].

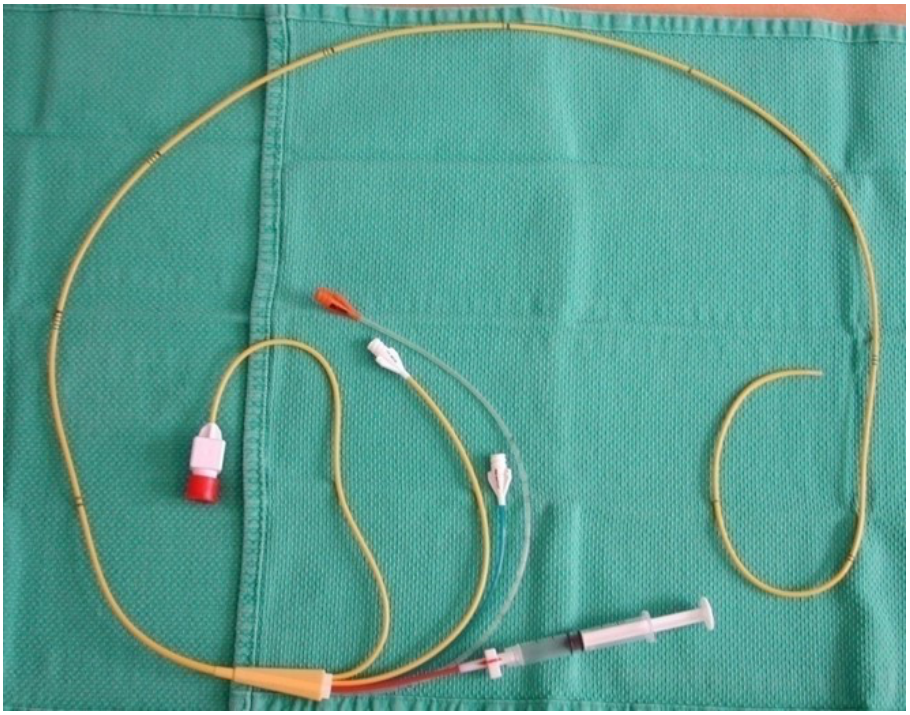


Figure 7.3: Swan Ganz thermodilution catheter

Once inserted into a central vein, the catheter is advanced into the right heart and pulmonary circulation under fluoroscopic guidance. With the balloon tip inflated, the catheter is then advanced to the most distal point of the pulmonary circulation that can be reached until the pulmonary artery is occluded. A wedged or occluded pulmonary artery pressure, is an acceptable estimate of left atrial pressure [197]. The pulmonary artery wedge pressure (PAWP) is obtained from the pulmonary pressure decay curve after a single arterial balloon occlusion. Measurements are performed at end expiration so as the lungs are at functional residual capacity (

Figure 7.4). At higher lung volumes, increased alveolar vessel resistance can cause an increase in PVR and at lower lung volumes, extra-alveolar vessel resistance rise, resulting in increased PVR. [198]

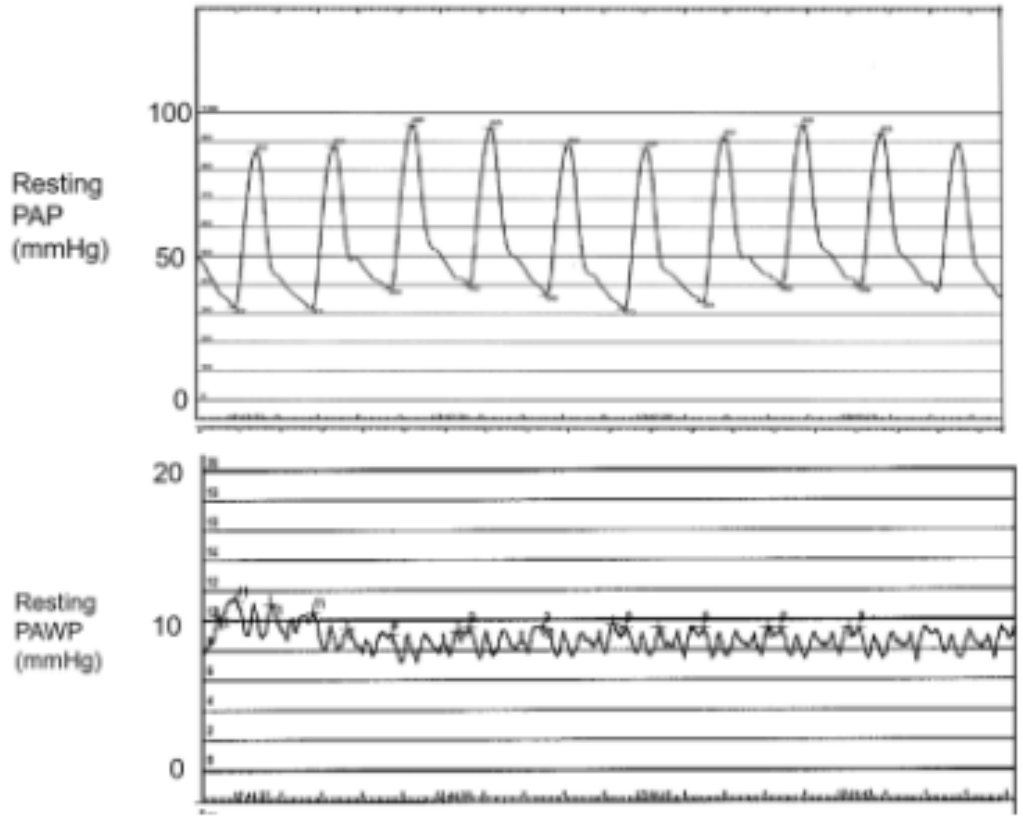


Figure 7.4: Pulmonary artery (top image)) and pulmonary arterial wedge (lower image) wave forms during right heart catheterisation

Table 7.1 covers the standard measurements made at right heart catheterisation and their normal range. Additional calculations that inform on pulmonary vascular function made at time of RHC using direct measurements. These calculations are displayed in Table 7.1 and are discussed in further detail below.

Table 7.1 Normal Pulmonary Artery and Right Heart Pressures [126]

	Measurement	Normal reference range
RAP (mmHg)	Direct	1-8
RVSP (mmHg)	Direct	15-30
RVEDP (mmHg)	Direct	2-8
Systolic PAP (mmHg) (SPAP)	Direct	13-26
Diastolic PAP (mmHg) (DPAP)	Direct	6-16
mPAP (mmHg)	2/3 DPAP + 1/3 SPAP	8-20
PAWP (mmHg)	Direct	5-12
CO (L/min)	Measured by thermodilution	4.5-8.5
CI	CO/Body Surface Area	2.5-4.0
PVR (WU)	mPAP-PAWP/CO	0.25-1.6
SvO_2 (%)	Direct – blood sampled from main pulmonary artery	60-80%
PA Compliance (ml/mmHg)	SV/(SPAP-DPAP)	3.8-12

7.1.1.2 **Pulmonary Vascular Resistance**

Resistance across the pulmonary vasculature consists of venous and arterial components. The site of most pulmonary vascular diseases is the resistive arterioles.

The Hagen-Poiseuille law is used to describe resistance in the pulmonary circulation. It is the physical law defining laminar flow of Newtonian fluid through non-distensible, straight, cylindrical tubes. It is governed by the following factors

- The viscosity of the fluid (n)
- The pressure gradient across the circuit (Δ P)
- The length (l) of the tubing
- radius (r) of the tubing

The law can be extrapolated to calculate the resistance in the pulmonary vasculature as the pressure gradient to flow (Q) ratio:

$$R = (l.n.8)/(\pi.r^4)$$

$$= \Delta P / Q$$

$$= mPAP - PAWP / CO$$

The fact that r in the equation is to the fourth power explains why resistance is exquisitely sensitive to small changes in calibre of these small vessels.

7.1.1.3 **Pulmonary artery compliance (capacitance)**

Pulmonary artery compliance (Pca) provides a measure of the ability of the pulmonary circulation to stretch in response to an applied pressure (cardiac output). It has the units $mmHg.mL^{-1}$ is calculated by the formula:

$$\frac{\text{Stroke Volume}}{\text{Pulmonary Artery Pulse Pressure}}$$

7.1.2 **Haemodynamic changes in PAH**

In PAH, as the vascular stiffness rises and luminal narrowing occurs, the PVR increases. This leads to increased PA pressure and RV afterload.[194]. Initially, cardiac output is maintained; heart rate rises and the right ventricle compensates for the increased afterload through right ventricular hypertrophy. As the afterload progressively increases, the right ventricle begins to fail, with both volume and pressure overload. This affects the left ventricle due to septal bowing and the interdependence of RV and LV function and reduced cardiac output to the systemic circulation (Figure 7.5).

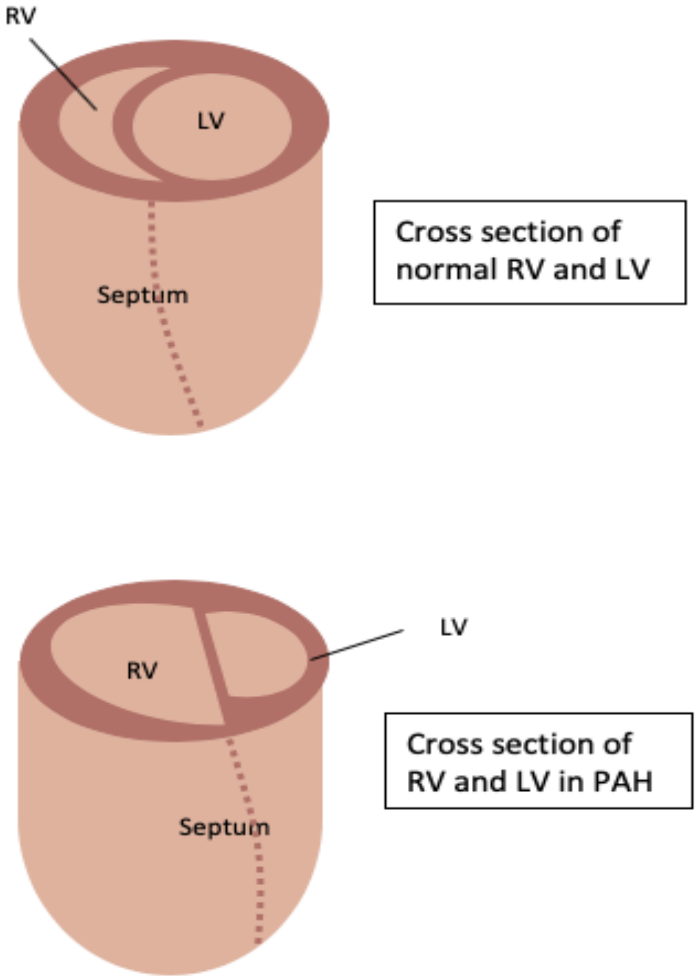


Figure 7.5: Change in cardiac dimensions in PAH

Changes that are seen in invasive measurements of the pulmonary circulation in PH are summarised in Table 7.2

Table 7.2: Haemodynamic changes in PAH

	Normal reference range	Pulmonary Arterial Hypertension
RAP (mmHg)	1-8	Normal or raised
RVSP (mmHg)	15-30	Raised
RVEDP (mmHg)	2-8	Normal or Raised
SPAP (mmHg)	13-26	Raised
DPAP (mmHg)	6-16	Raised
mPAP (mmHg)	8-20	Raised
PAWP (mmHg)	5-12	Normal or Low
CO (L/min)	4.5-8.5	Normal or Low
CI	2.5-4.0	Normal or Low

PVR (WU)	0.25-1.6	Raised
SvO_2 (%)	60-80%	Normal or Low
HR (bpm	40-100	Normal or High
Compliance (Pca) (ml/mmHg)	3.8-12	Reduced

Abbreviations: RAP: Right Atrial Pressure
RVSP: Right Ventricular Systolic Pressure
RVEDP: Right Ventricular End Diastolic Pressure
mPAP: Mean Pulmonary Artery Pressure
PAWP: Pulmonary Artery Wedge Pressure
CO: Cardiac Output
CI: Cardiac Index
PVR: Pulmonary vascular resistance
SvO_2: Mixed Venous Oxygen Saturations

There is a negative correlation between Pca and mortality in PAH [199]. Although PAH is classically viewed as a disease of the distal resistive vessels leading to increased PVR, it also results in a reduction in compliance, and both contribute to RV afterload. Right ventricular afterload is a key determinant of RV function, which is strongly related to patient survival in PAH.

7.1.2.1 **Right ventricular afterload**

Pulmonary Vascular Resistance has traditionally been used to characterise pulmonary haemodynamics, however it does not take into consideration pulsatile flow or arterial stiffness [200]. The proximal pulmonary arteries also play an important role in buffering pulsatile RV ejection and in RV-PA coupling. This is reflected in the finding that RV dysfunction is independently related to PA stiffness. There are two key components to right ventricular afterload [201]:

1. Pulmonary Vascular Resistance

2. Pulmonary Arterial Compliance

7.1.2.2 **Relationship between components of RV afterload**

In the pulmonary circulation, resistance and compliance are coupled in an inverse hyperbolic relationship, resulting in a constant product known as the RC time (Figure 7.6).

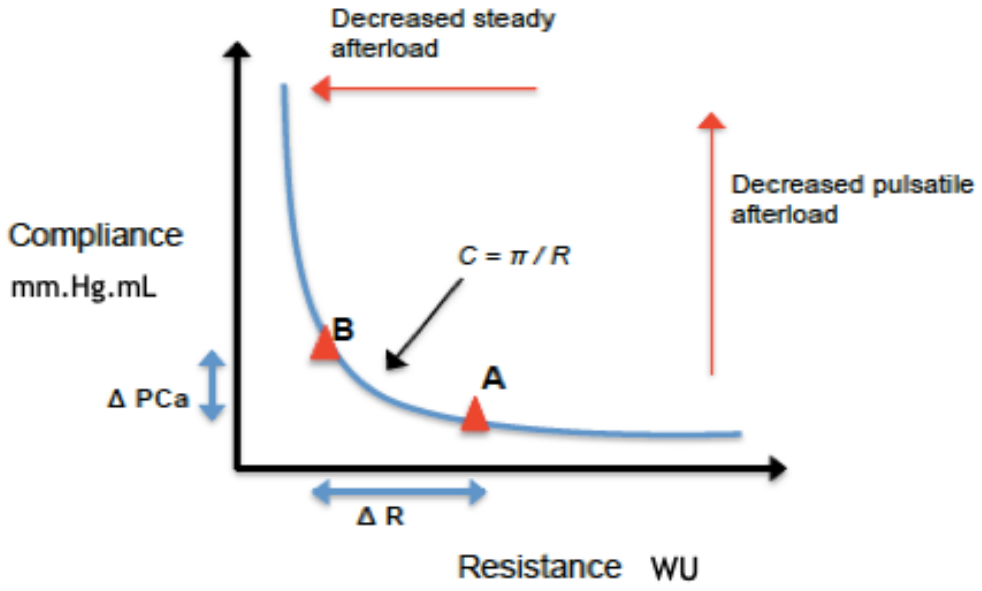


Figure 7.6: Relationship between compliance (mmHg.mL) and resistance (WU) in the pulmonary circulation

The Pulmonary Arterial time constant (RC time constant) represents the exponential pressure decay in the pulmonary artery during diastole. Units are in seconds. It is calculated as follows:

RC time constant = PVR x Pca.

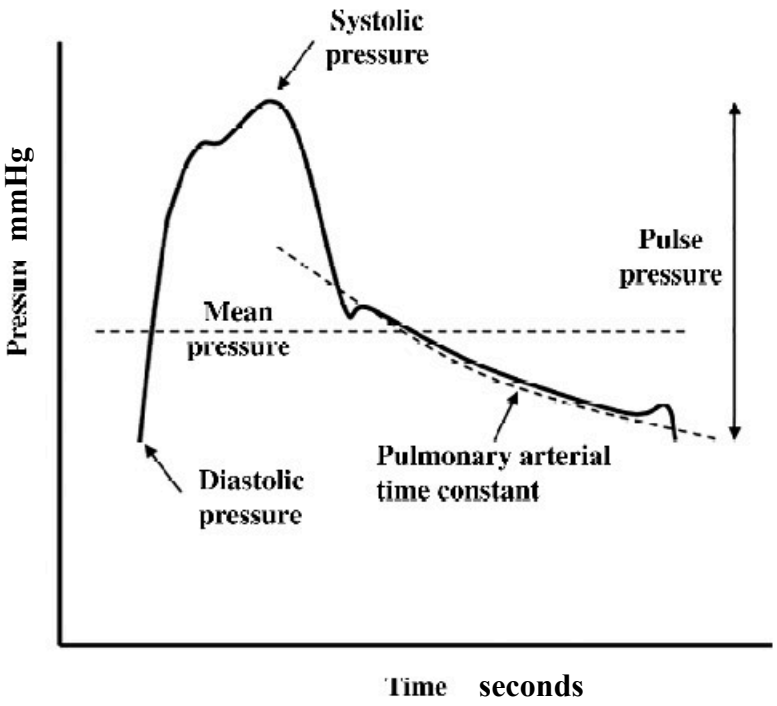


Figure 7.7: Pulmonary Artery Time Constant (Rc)

For any given resistance, a shorter time constant translates to faster diastolic pressure decay and a higher pulse pressure. It is independent of right ventricular function[199].

Unlike the systemic circulation, where RC time is highly variable, the RC time of the pulmonary circulation has traditionally been proposed to be constant in health and disease, including PAH of varying severity, pre and post treatment [202-204] (Figure 7.8).

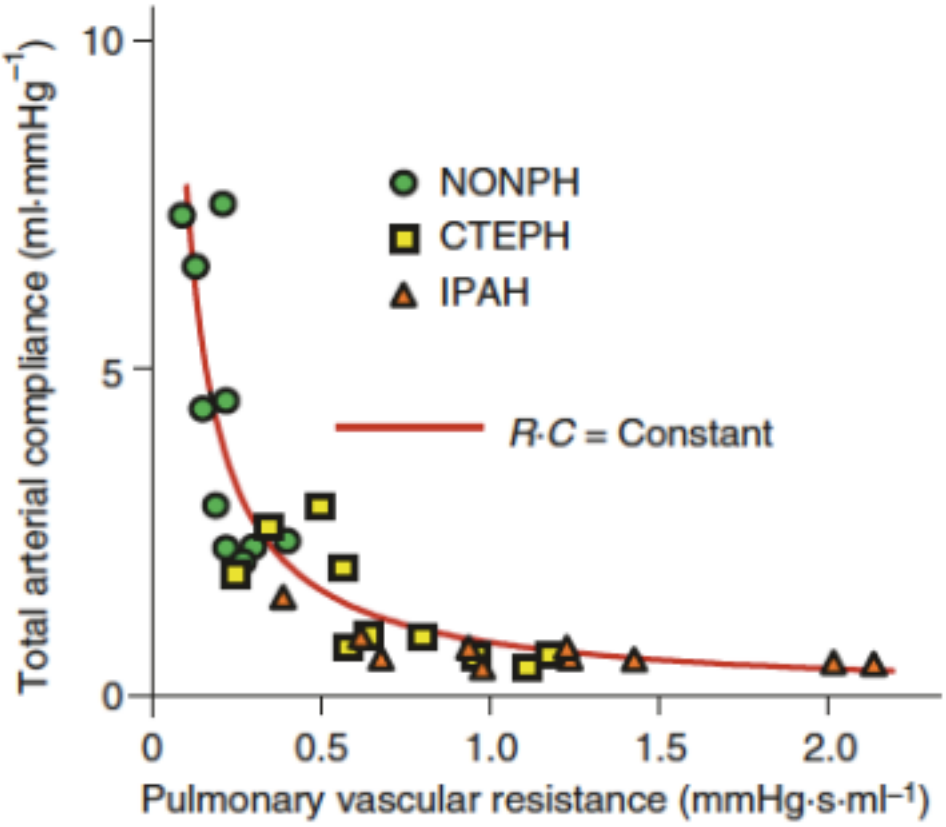


Figure 7.8: RC constant: The relationship between compliance and resistance in health and pulmonary vascular disease

This constancy implies that compliance cannot change independently from resistance and the two are tightly bound [205] . The proposed reasons for this fixed relationship [206] are:

1. The main pulmonary arteries account for only 15-20% of total pulmonary arterial compliance; the majority of vascular compliance is determined by vessels in the lung periphery; these vessels also account for the majority of pulmonary vascular resistance, meaning there is a tight anatomical correlation between the two.

2. Due to the anatomical and physiological properties of arterial vessels, elevations in PVR increase pressure within the vascular lumen leading to higher vessel wall tension and therefore increased vascular stiffness, as per the Law of Laplace.

Exceptions to this rule have been documented in a fairly large body of evidence, suggesting this relationship is not always fixed as has been previously thought. In studies of CTEPH [207], and left heart disease with high PAWP [208] RC time was reduced compared to normal; in a study of IPAH patients with cardiovascular risk factors versus those without cardiovascular risk factors, RC time was also significantly reduced[209]. Additionally, in an unselected population of pulmonary hypertension patients with a PAWP under 15mmHg, higher heart rate was associated with lower RC time independently of mPAP, PAWP and age[206]. More recently, in a large cohort of IPAH patients (717) versus those with no pulmonary hypertension (156), RC time was found to be significantly lower in the IPAH group and correlated with RV oscillatory power fraction [210].

Few studies have assessed dynamic changes in RC time in the pulmonary circulation on exercise. In healthy older exercising adults, reductions in RC time and compliance can occur during exercise, despite small reductions in pulmonary vascular resistance [211].

7.1.2.3 **Right Ventricular Oscillatory Power Fraction**

The right ventricular oscillatory power fraction (RVOPF) provides a measure of the efficiency of the pulmonary circulation. There are two components to the RVOF, reflecting the two key properties of the pulmonary arterial tree that determine afterload [212]:

Mean Power: energy per unit time expended to produce steady (non-pulsatile) net forward flow, calculated as the product of SPAP and mean flow (CO). The mean power is influenced by the calibre of small distal resistive arteries as reflected in the pulmonary vascular resistance

Oscillatory Power: The energy used in the production of the pulsatile component of flow and pressure. This estimates the contribution from elastic properties of the pulmonary arterial tree, which can be estimated by the total arterial compliance

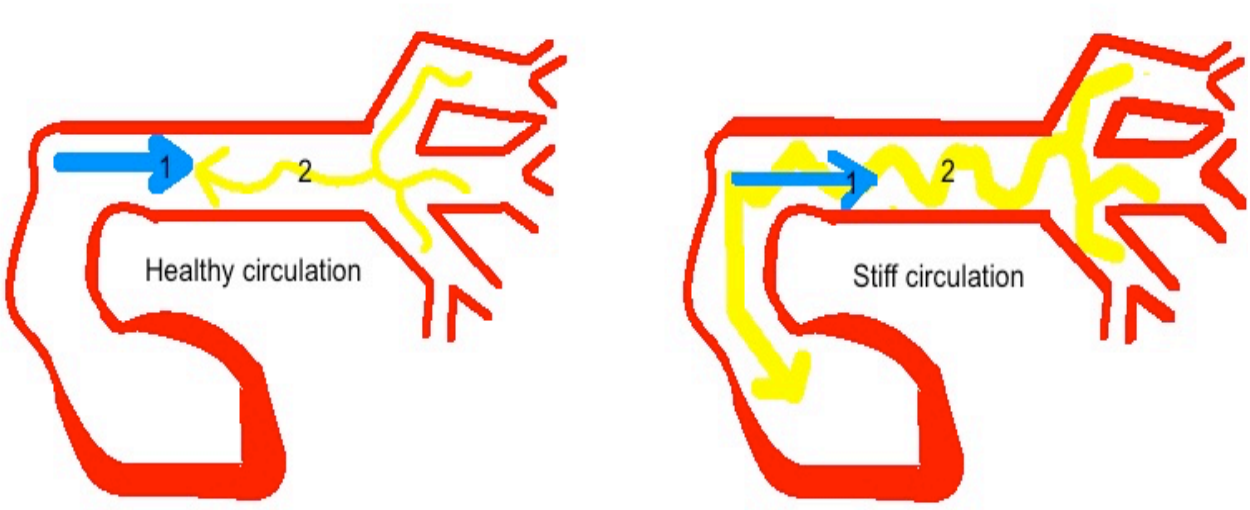


Figure 7.9: Oscillatory power fraction in healthy vessel (left) and stiff vessel (right) (Blue = mean power, yellow oscillatory power)

The sum of the mean power and oscillatory power is the total external hydraulic power generated by the ventricle.

Mean flow determines transport, therefore the mean power may be considered useful and oscillatory power wasted. The normal ratio is around 23-25% [213], with the lower the fraction, the more efficient the circulation. RVOPF can be calculated by the formula

$$1 - \frac{mPAP}{SPAP}$$

The formula is based on the following derivation [213].

Power (oscillatory) / (Power oscillatory + Power mean).

→ ((sPAP x CO) – (mPAP x CO))/ (SPAP x CO)

→ 1 – (mPAP / sPAP)

Similarly to RC time constant, it has been proposed that the RVOPF remains constant in health and disease[213], this constant fraction being explained by the

proportionality in mPAP and SPAP under all conditions [214]. In larger more recent studies, this concept has not held, with RVOPF being higher in patients with IPAH [210].

7.1.3 Haemodynamic responses to exercise

7.1.3.1 Physiology of exercise in the healthy human pulmonary circulation

The lungs receive the entire cardiac output with every stroke [202]. In young adults, the pulmonary circulation has an average resistance of 1mmHg.min.L-1 increasing to 2.5mmHg.min L-1 by the age of 60.

In health, mPAP and PAWP increase on exercise. mPAP rises approximately 0.5 – 3 mmHg per litre, per minute increase [215] with a smaller rise seen in PAWP [216]. The rise in mPAP is predominantly due to increases in Cardiac Output, which can be up to 6 fold at maximal exercise. This is to match the twenty fold increase in oxygen uptake and carbon dioxide output required to meet the convective transport needs of O_2 and CO_2 to and from the exercising muscle. In the elderly and in heart and lung disease, increasing filling pressures of the left ventricle and air trapping provide increasing contributions to the rising PAP during exercise.

7.1.3.2 Pulmonary Circulation and RV response to exercise in PAH

In PAH there is a steep rise in mPAP with a smaller increment in CO than is seen in health. The cardiac output response to exercise is blunted due to the failure of the RV to augment SV on exercise. This is due to impaired RV contractile reserve in the face of increasing RV afterload as exercise progresses. With increasing RV afterload, RV-PA uncoupling occurs, contributing to reduced SV .In severe PAH RV-PA uncoupling can be present at rest[217].

During exercise, the mPAP/CO gradient can be taken as a surrogate of TPR. The large increase in mPAP and relatively small increase in CO leads to a steeper mPAP/CO relationship with exercise.

Chronotropic incompetence also occurs, with a blunted heart rate response to exercise. In PAH, peak CO has been found to be related to both decreased peak SV and chronotropic incompetence.

7.1.4 **Haemodynamic responses to exercise therapy in PAH**

Limited data exist on the effects of exercise therapy on the pulmonary circulation in PAH. In the one controlled study to date assessing the impact of exercise therapy on invasive haemodynamics in PAH, a significant reduction in mPAP and PVR occurred, with improvement in CO at rest, however there was no significant change seen during exercise, on a standard 25 watt supine ergometer step wise protocol. No assessment was made of Pca, RC time or RVOPF[218].

7.2 **Aims**

The aims of this chapter are

1. Determine the impact of exercise training on resting pulmonary arterial haemodynamics in optimally treated pre-capillary PH patients

2. Assess the impact of exercise therapy on measures of RV-PA efficiency

    a. Right ventricular oscillatory power fraction

    b. Resistance-compliance time constant

3. Explore the change in invasively measured exercise haemodynamic before and after exercise therapy

7.3 **Methods**

7.3.1 **Study population**

The right heart catheterisation exploratory sub-study involved an opt in or opt out section in the consent form for the study "The effect of adding exercise training to optimal therapy in pulmonary arterial hypertension". Ethics, recruitment, consent and study intervention are described in the study protocol in Chapter 2. Separate written consent was performed for all right heart catheterisation procedures as is done in normal clinical practice. Local guidelines on anticoagulation were followed. Subjected who were anticoagulated were bridged with low molecular weight heparin until 24h prior to the RHC.

7.3.2 **Protocol**

Right heart catheterisation with exercise was performed before and after the study intervention (exercise therapy). There was a window of at least 24 hours between other exercise tests (6MWT and CPET) to allow sufficient time for recovery.

7.3.2.1 **Right heart catheterisation**

Resting right heart catheterisation

Resting right heart catheterisation was performed under full aseptic conditions, in the supine position via the left or right internal jugular vein. An 8Fr sheath was inserted under direct ultrasound visualisation, with fluoroscopic guidance. A balloon-tipped, flow-directed pulmonary arterial Swan-Ganz catheter was inserted through the introducer sheath and into the venous system. Under fluoroscopic screening, the catheter was passed into the right atrium, right ventricle and pulmonary artery. Standard pressure measurements were performed as discussed in section 7.1.1.1. Cardiac output was quantified by the thermodilution method [219] with the transducer zeroed at the mid thoracic point. Although the direct Fick method of cardiac output measurement is the gold standard, the thermodilution method has been accepted as a more practical alternative and has shown good agreement with the direct Fick method in patients with pulmonary hypertension, even in the context of low cardiac output or severe tricuspid regurgitation [110]. Cardiac output measurements were repeated until three readings were obtained with variability of ≤10% and the mean of these three readings was recorded as the CO. Measurements were captured and recorded using GE© digital healthcare software, UK. Mixed venous oxygen saturations (SvO_2) were measured by withdrawing 3ml of blood from the distal pulmonary arterial port of the catheter into a heparinised blood gas syringe (BD, Oxford, United Kingdom). This sample was immediately processed in a blood gas analyser (RAPIDLab 1265, Siemens Healthcare, Germany). To obtain an estimate of the relative oxygen extraction at peak exercise, the difference between the arterial and mixed venous saturations was expressed as a proportion of the arterial saturation. No systemic arterial blood sampling was undertaken as part of this study, therefore the peripheral arterial oxygen saturation as measured by pulse oximetry was used.

Following completion of resting measurements, subjects then went on to perform exercise (discussed below).

Exercise right heart catheterisation

As part of the study protocol described in Chapter 2, all subjects performed an initial standard increment cardiopulmonary exercise test. For the exercise RHC protocol, a constant work rate was used in order to achieve steady state exercise and allow serial measurements to be made. As discussed in Chapter 4, exercise capacity is significantly reduced in the supine compared to upright position during moderate to high intensity cycling and this is most marked during constant work rate exercise[113]. The supine constant work rate was calculated as 50% of the peak work rate achieved in the initial maximal upright cardiopulmonary exercise test.

An electromagnetically braked cycle ergometer (Corival Supine, Lode, Groningen, Netherlands) was placed on the cardiac catheterisation table and its position adjusted to allow comfortable supine cycling for each patient. The ergometer was secured to the table by bolts attaching it to the table's side rails to maintain its position during cycling. The patient's feet were then strapped to the pedals. This led to an elevation of the legs compared with the fully supine resting RHC.

Subjects were asked to start cycling, initially with zero resistance, at a cadence of 60 revolutions per minute for 3 minutes (unloaded cycling) as a warmup. The resistance was then increased to 50% of the peak WR achieved on erect incremental CPET. Subjects were asked to cycle until exhaustion. The following measurements were made: Total exercise time, Heart Rate, SpO_2, mPAP, Cardiac Output and SvO_2. It was not possible to reliably measure PAWP every two minutes due to lack of fluoroscopy during exercise and subject movement. Measurements were made at the following time points:

- At rest with the feet in pedals
- After 2min 30 of unloaded cycling

- After 3 min of loaded cycling

- Every 2 min thereafter until end exercise.

At the end of exercise, the pulmonary artery catheter was removed from the pulmonary arterial system under fluoroscopy screening. The introducer sheath was removed, firm pressure was applied and an occlusive dressing after bleeding had stopped. Subjects were observed for one hour after test completion with measurement of BP, HR and SpO_2 and review of puncture site.

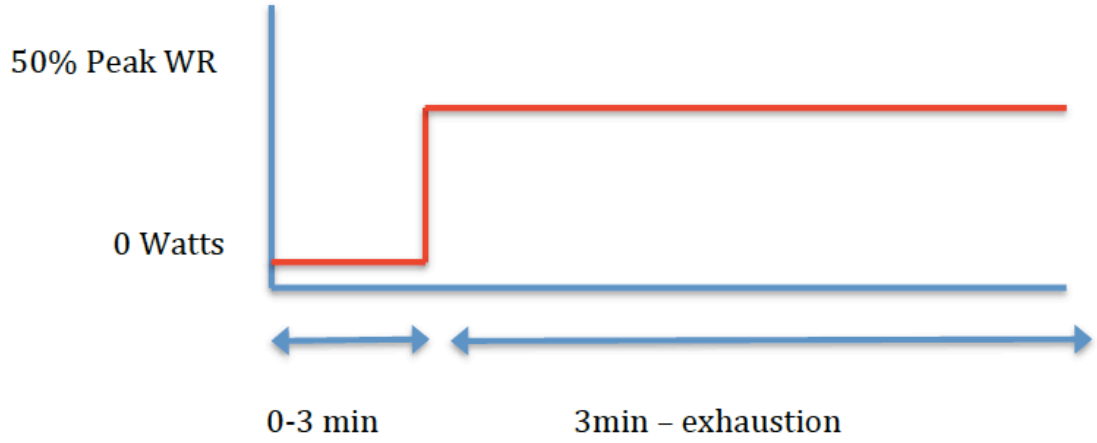


Figure 7.10: Exercise RHC protocol

As measurement of PAWP was not possible during exercise, the total PVR (TPR) was calculated as mPAP/CO. Despite not characterising the flow-resistive properties of the pulmonary circulation as accurately as PVR, TPR may be a more realistic estimate of right ventricular afterload, as the right ventricle is exposed to the mean pulmonary artery pressure and not to the difference between mPAP and PAWP.

7.3.3 **Statistics**

Normally presented data are presented as mean (SD), non-parametric data are presented as median and interquartile range (IQR). The Wilcoxon Sign rank test was used for paired data and Mann-Whitney U test for independent samples.

7.4 Results

7.4.1 Resting RHC

21 subjects had baseline right heart catheterisation performed. All were optimally treated; 48% (10/21) were on triple therapy and the remainder were on dual oral therapy.

The majority of the subjects were female; 62% (13/21). IPAH/HPAH was the most common diagnosis 61% (13/21). 5% (1/21) had CTD-PAH, 24% (5/21) CHD-PAH, 5% (1/21) POPH and 5% (1/21) residual CTEPH. The median age was 48 (34-74)

At rest 19% (4/21) had mPAP <25mmHg, these subjects all had PVR of 3.5 or less, indicating a good response to optimal drug therapy. 81% (17/21) had a normal CI (greater or equal to 2.5). 29 % (6/21) had an SvO_2 equal to or greater than 75% (Table 7.3)

Table 7.3: Resting RHC data at baseline

	Mean	SD	Range
BMI (kg/m2)	30.29	7.54	23-47
Sats (%)	93.24	4.72	83-100
HR (BPM)	77.05	12.55	56-107
SBP (mmHg)	119.38	17.85	97-177
DBP (mmHg)	71.57	9.92	51-87
RAP mean (mmHg)	4.95	3.68	0-11
RVP (mmHg)	61.76	18.89	30-85
SPAP (mmHg)	63.62	18.88	32-91
DPAP (mmHg)	23.33	9.91	6-39
MPAP (mmHg)	39.33	12.34	17-57
PAWP (mmHg)	7.48	4.68	1-17
CO (L/min)	6.22	1.62	3.3-9.4
CI (L/min/m^2)	3.22	0.78	1.8-4.4
PVR (WU)	5.50	2.70	2.3-13.2
PVRI (L.min^{-1}.m^2)	10.59	5.20	3.9-25.9
TPR (WU)	6.72	2.91	3.3-16
SV (mL)	81.95	21.55	33.3-120
SVI (ml/m^2)	41.68	10.78	17-61
SvO_2 (%)	69.89	8.05	49-82
Rca (ml/mmHg)	2.20	0.81	0.74-3.4

RVOPF	0.38	0.05	0.32-0.47
RC time (sec)	10.30	2.18	6.8-15.1

7.4.1.1 **RHC at rest pre and post exercise therapy**

Of the 21 subjects who had initial RHC, 16 completed end of study RHC. Reasons for the 5 subjects not completing follow-up RHC: 2 study dropouts, 1 subject transplanted, 1 subject declined repeat RHC, 1 subject had venous stenosis of left internal jugular vein and hickman line in the right internal jugular vein; advancement of the Swan-Ganz catheter into the right atrium was not feasible on the return visit.

Paired analysis in the 16 subjects who had pre and post exercise RHC revealed no significant differences in invasively measured haemodynamics at rest (Table 7.4)

Table 7.4: Baseline and End of Study Resting RHC data

	Baseline	SD	EOS	SD	p
HR (BPM)	74.2	10.6	76.1	12.2	0.269
SBP (mmHg)	119.6	19.5	107.1	11.9	**0.012**
DBP (mmHg)	72.1	8.5	70.2	7.9	0.311
RAP mean (mmHg)	4.9	3.8	4.4	4.3	0.639
RVP (mmHg)	54.4	17.6	53.2	19.2	0.522
SPAP (mmHg)	57.3	16.9	57.4	20.6	0.945
DPAP (mmHg)	20.8	9.3	21.5	8.8	0.551
MPAP (mmHg)	35.3	11.2	35.5	11.9	0.858
PAWP (mmHg)	6.6	4.6	7.19	4.7	0.318
CO (L/min)	6.3	1.5	6.7	2.2	0.284
CI ($L/min/m^2$)	3.3	0.7	3.6	1.1	0.211
PVR (WU)	4.8	2.2	4.7	2.5	0.701
PVRI ($L.min^{-1}.m^2$)	9.2	4.1	8.8	4.8	0.474
TPR (WU)	5.9	2.0	5.8	2.7	0.894
SV (mL)	84.1	16.7	87.7	22.9	0.547
SVI (ml/m^2)	42.8	7.9	46.3	12.1	0.264
SvO_2 (%)	69.8	6.3	70.1	7.1	0.879
Rca (ml/mmHg)	2.44	0.7	2.8	1.4	0.184
RVOPF	0.38	0.05	0.38	0.05	0.436
RC time (sec)	10.5	2.34	10.5	2.34	0.994

Those with paired resting RHC data who improved (11) versus those who had an unchanged or deteriorated 6MWD (5) were compared using a Mann-Whitney U test. There was a significant improvement in delta stroke volume, cardiac output and pulmonary artery compliance in those who had improved 6MWD versus those who did not (Table 7.5).

Table 7.5: Changes in haemodynamics in subjects with improved 6MWD vs those without

	Improved	SD	Deteriorated	SD	p
HR (BPM)	0.0	5.9	6.2	7.1	0.145
SBP (mmHg)	-10	19	-17	14	0.743
DBP (mmHg)	-1	4	-5	4	0.320
RAP mean (mmHg)	-1	3	0	4	0.661
RVP (mmHg)	-12	25	-13	31	0.583
SPAP (mmHg)	0.2	7.4	0.0	7.5	0.661
DPAP (mmHg)	0.8	5.0	0.5	5.1	0.913
MPAP (mmHg)	0.2	3.8	0.2	5.3	0.913
PAWP (mmHg)	0.4	2.2	1.2	3.0	0.583
CO (L/min)	1.0	1.7	-0.8	1.0	**0.038**
PVR (WU)	-0.4	1.0	0.6	1.1	0.115
TPR (WU)	-0.4	1.2	0.8	1.7	0.090
SV (mL)	12	21	-16	13	**0.005**
SvO_2 (%)	-0.6	6.3	2.1	2.8	0.221
Rca (ml/mmHg)	0.7	1.2	-0.3	0.5	**0.05**
RVOPF	-0.01	0.06	-0.01	0.04	1.00
RC time (sec)	0.35	2.9	-0.8	1.4	0.267

7.4.2 **Exercise RHC**

Comparison of standard haemodynamic variables at rest and throughout progressive stages of exercise are summarised in Table 7.6 pre and post exercise therapy. Lowest isotime was the earliest loaded measurement comparable at the same time on both tests, highest isotime was determined by the test of shortest duration; the time of last measurement before cessation of exercise in the shortest duration test was compared between both tests.

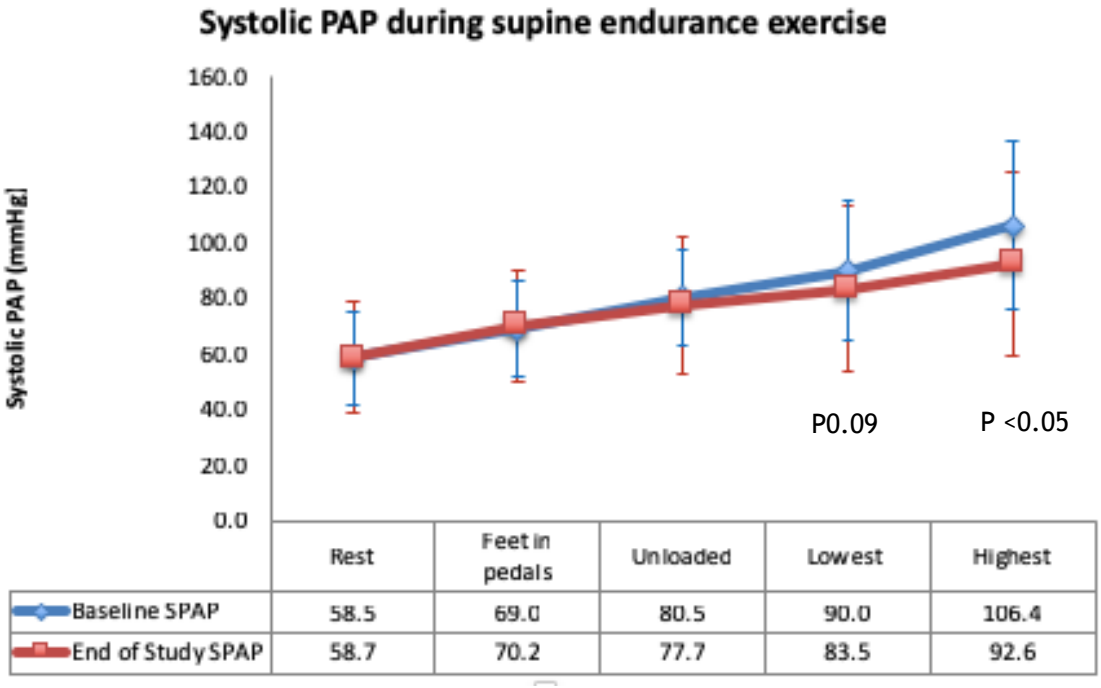
Table 7.6: Baseline and end of study exercise right heart catheterisation

Change haemodynamics following exercise therapy.			
	Baseline	End of Study	P
mPAP (mmHg)			
Rest	36 (11)	36 (12)	0.950

Unloaded	50 (9)	48 (13)	0.330
Lowest isotime	57 (12)	55 (16)	0.210
Highest isotime	66 (18)	60 (20)	**0.02**
End exercise	68 (18)	62 (20)	**0.04**
CO (L/min)			
Rest	6.3 (1.5)	6.7 (2.2)	0.280
Unloaded	8.9 (2.0)	8.7 (2.8)	0.630
Lowest isotime	10.7 (2.7))	10.9 (3.7)	0.730
Highest isotime	12.2 (3.7)	12.3 (4.2)	0.760
End exercise	12.2 (3.7)	12.5 (4.1)	0.630
CI			
Rest	3.3 (0.7)	3.6 (1.1)	0.210
Unloaded	4.6 (0.9)	4.5 (1.3)	0.690
Lowest isotime	5.5 (1.2)	5.6 (1.7)	0.680
Highest isotime	6.2 (1.7)	6.4 (2.0)	0.580
End exercise	6.4 (1.9)	6.7 (2.0)	0.530
SvO (%)			
Rest	69.8 (6.3)	70.1 (7.1)	0.880
Unloaded	58.0 (6.6)	58.4 (8.5)	0.780
Lowest isotime	45.8 (7.8)	45.8 (10.2)	0.990
Highest isotime	44.6 (7.6)	44.3 (8.0)	0.820
End exercise	42.9 (6.6)	42.2 (7.1)	0.650
HR (BPM)			
Rest	75 (11)	76 (12)	0.269
Unloaded	89 (10)	90 (11.0)	0.710
Lowest isotime	105 (14)	103 (15)	0.570
Highest isotime	127 (37)	119 (23)	**0.01**
End exercise	131 (27)	128 (25)	0.450
PCa (mmHg.mL)			
Rest	2.4 (0.7)	2.8 (1.4)	0.180
Unloaded	2.0 (0.7)	2.1 (1.0)	0.300
Lowest isotime	1.8 (0.7)	2.3 (1.4)	0.100
Highest isotime	1.5 (0.6)	2.4 (1.6)	**0.02**
End exercise	1.4 (0.5)	2.1 (1.2)	**0.03**
SV (mL)			
Rest	84 (17)	88 (23)	0.547
Unloaded	100 (19)	96 (26)	0.410
Lowest isotime	104 (27)	105 (31)	0.910
Highest isotime	96 (24)	104 (31)	0.110
End exercise	92 (22)	97 (24)	0.410
Pca; Pulmonary artery compliance. CI; Cardiac Index. SvO_2; Mixed venous oxygen saturation. HR; Heart Rate			

7.4.2.1 **Pulmonary artery pressure**

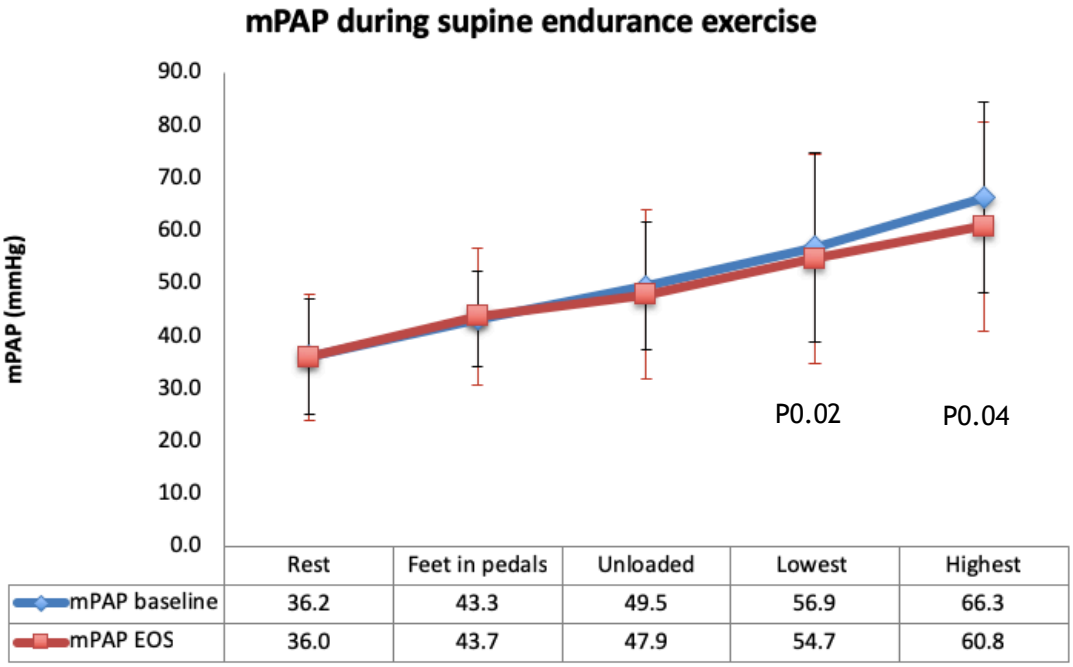
At rest, SPAP was unchanged pre and post exercise therapy. During progressive exercise, there was a significant reduction in SPAP at lowest and highest isotime point (Figure 7.11).



	Rest	Feet in pedals	Unloaded	Lowest	Highest
Baseline SPAP	58.5	69.0	80.5	90.0	106.4
End of Study SPAP	58.7	70.2	77.7	83.5	92.6

Figure 7.11: Pre and post exercise therapy changes in systolic PAP

mPAP displayed the same pattern in the pre and post exercise groups; At highest exercise isotime point and peak exercise, mPAP was significantly lower following exercise therapy (Figure 7.12).



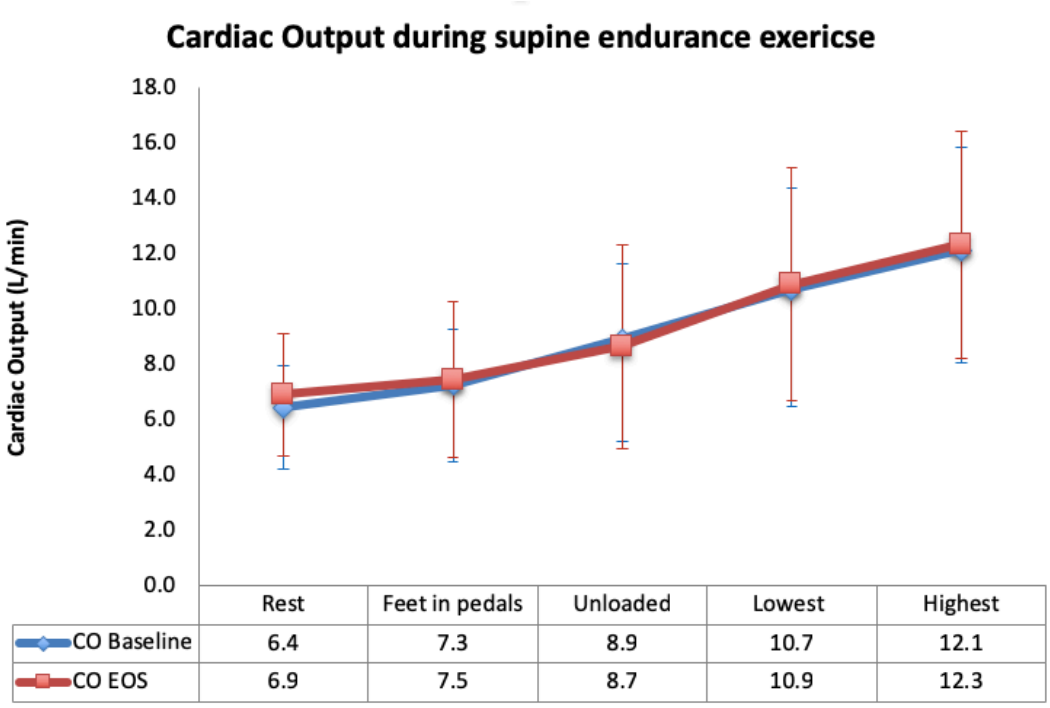
	Rest	Feet in pedals	Unloaded	Lowest	Highest
mPAP baseline	36.2	43.3	49.5	56.9	66.3
mPAP EOS	36.0	43.7	47.9	54.7	60.8

Figure 7.12: Pre and post exercise therapy changes in mPAP

There was no significant change in resting diastolic pulmonary artery pressures nor diastolic pulmonary artery pressures during exercise following the exercise programme.

7.4.2.2 Stroke volume, heart rate and cardiac output

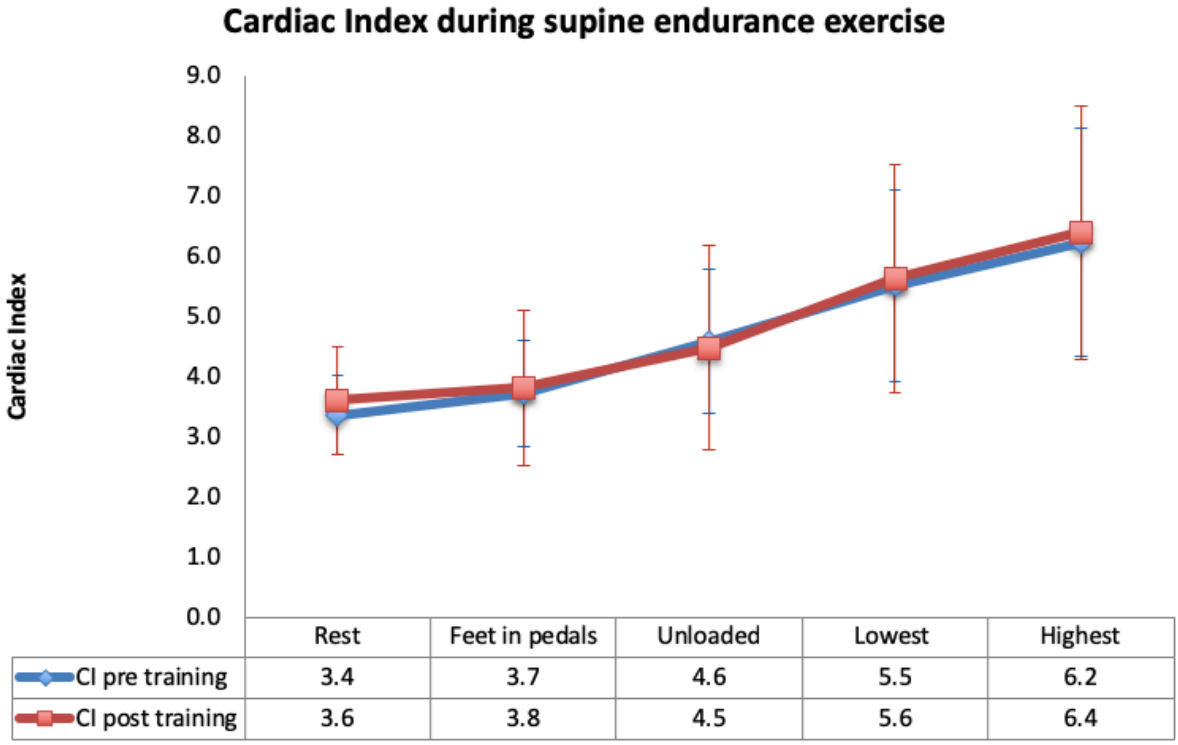
Change in cardiac output during exercise is show in Figure 7.13. No statistically significant difference was seen in CO changes during exercise before and after the exercise programme.



	Rest	Feet in pedals	Unloaded	Lowest	Highest
CO Baseline	6.4	7.3	8.9	10.7	12.1
CO EOS	6.9	7.5	8.7	10.9	12.3

Figure 7.13: Pre and post exercise therapy changes in CO

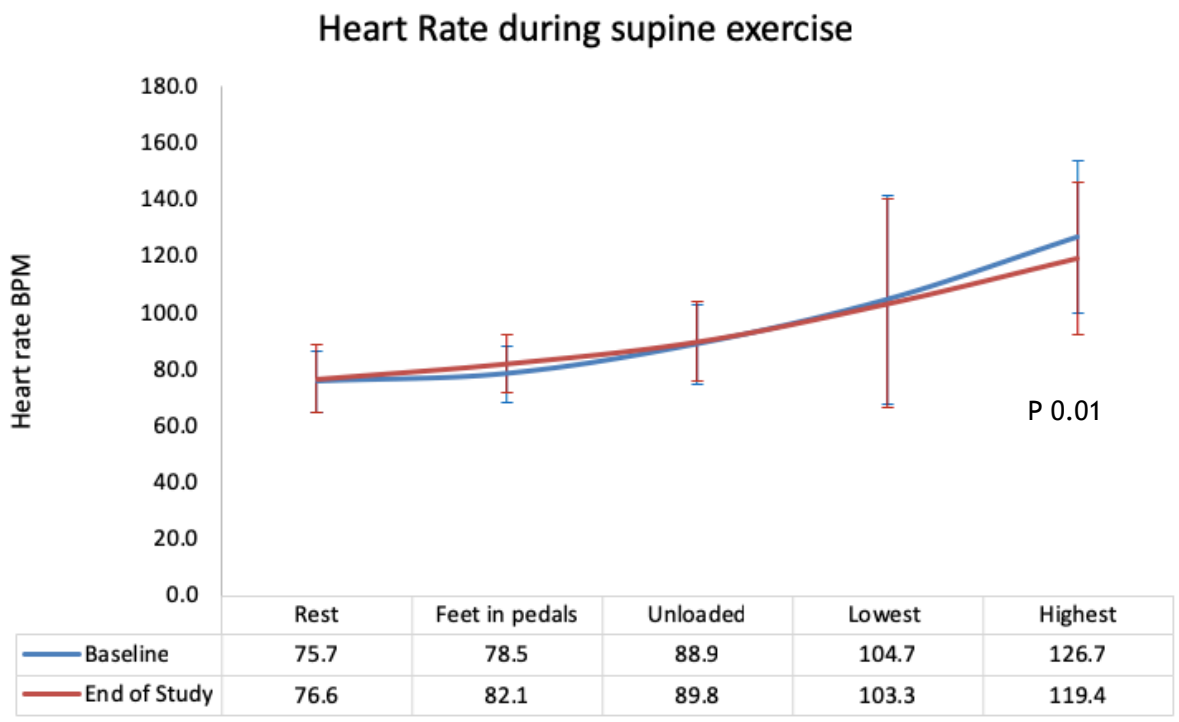
Cardiac index was higher in the post exercise therapy group at higher stages of exercise, however this did not reach statistical significance (Figure 7.14)



	Rest	Feet in pedals	Unloaded	Lowest	Highest
CI pre training	3.4	3.7	4.6	5.5	6.2
CI post training	3.6	3.8	4.5	5.6	6.4

Figure 7.14: Pre and post exercise therapy changes in Cardiac Index

Following completion of the exercise programme, heart rate was lower at highest isotime exercise than at baseline (Figure 7.15).



	Rest	Feet in pedals	Unloaded	Lowest	Highest
Baseline	75.7	78.5	88.9	104.7	126.7
End of Study	76.6	82.1	89.8	103.3	119.4

Figure 7.15: Pre and post exercise therapy changes in Heart Rate

SV improved at highest exercise isotime following exercise therapy, although missing statistical significance. SV can be seen to plateau and fall at an earlier stage in exercise in the pre exercise therapy group (Figure 7.16)

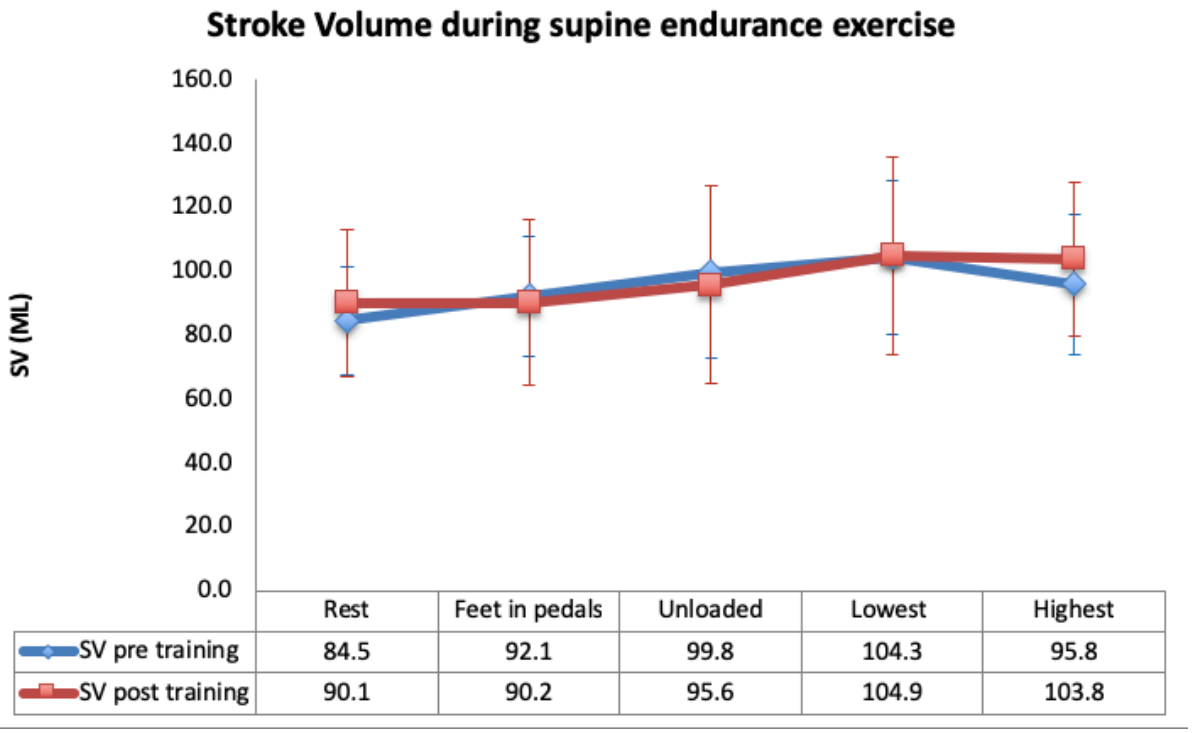


Figure 7.16: Pre and post exercise therapy changes in Stroke Volume

The changes in relative contributions of HR and SV to cardiac output pre and post exercise therapy can be seen in Figure 7.17, with improved SV and a lower HR during later stages of exercise in the post exercise therapy group. Indicative of a reduced reliance on chronotropic function to maintain CO.

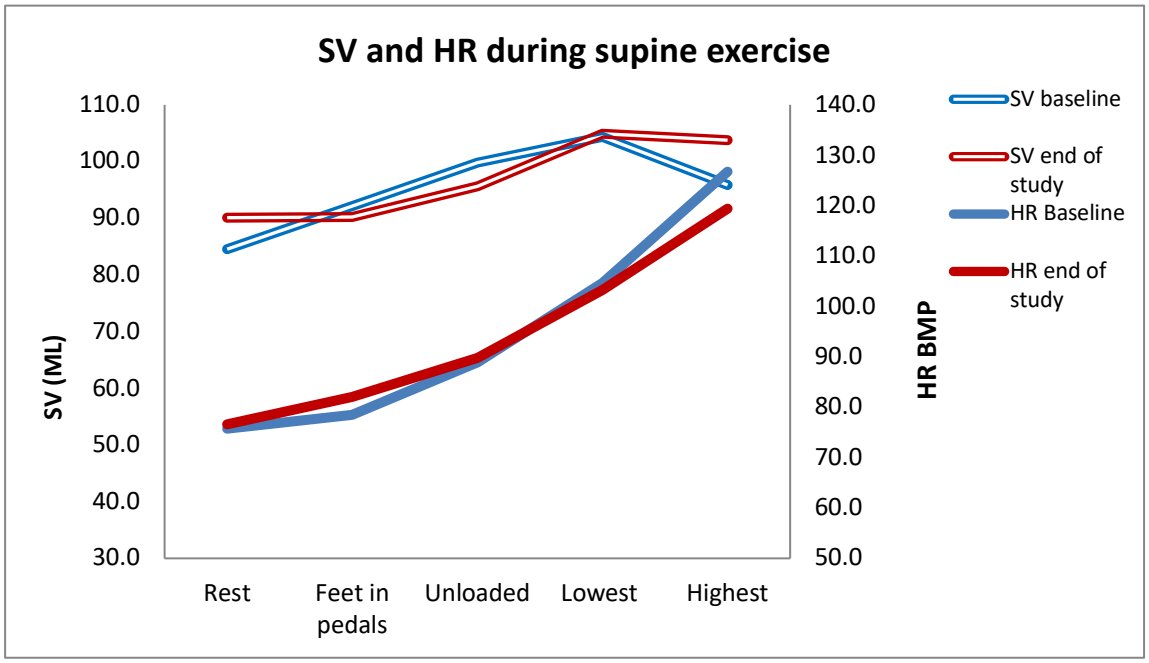


Figure 7.17: Relative changes in stroke volume and heart rate during exercise

7.4.2.3 **Pressure – Flow relationship**

The gradient between cardiac output and mean pulmonary artery pressure was taken as a surrogate of pulmonary vascular resistance. Total pulmonary resistance (mPAP/CO) provides an accurate reflection of the work faced by the right ventricle. The relationship between cardiac output and mPAP (TPR) was plotted at each exercise time point and the mean for the population was then plotted at baseline and the end of study (Figure 7.18,

Table 7.7). Linear trendlines were selected to best fit the progressive increase of mPAP with CO, with R^2 values close to 1 for both trendlines (equation shown in figure 7.18).

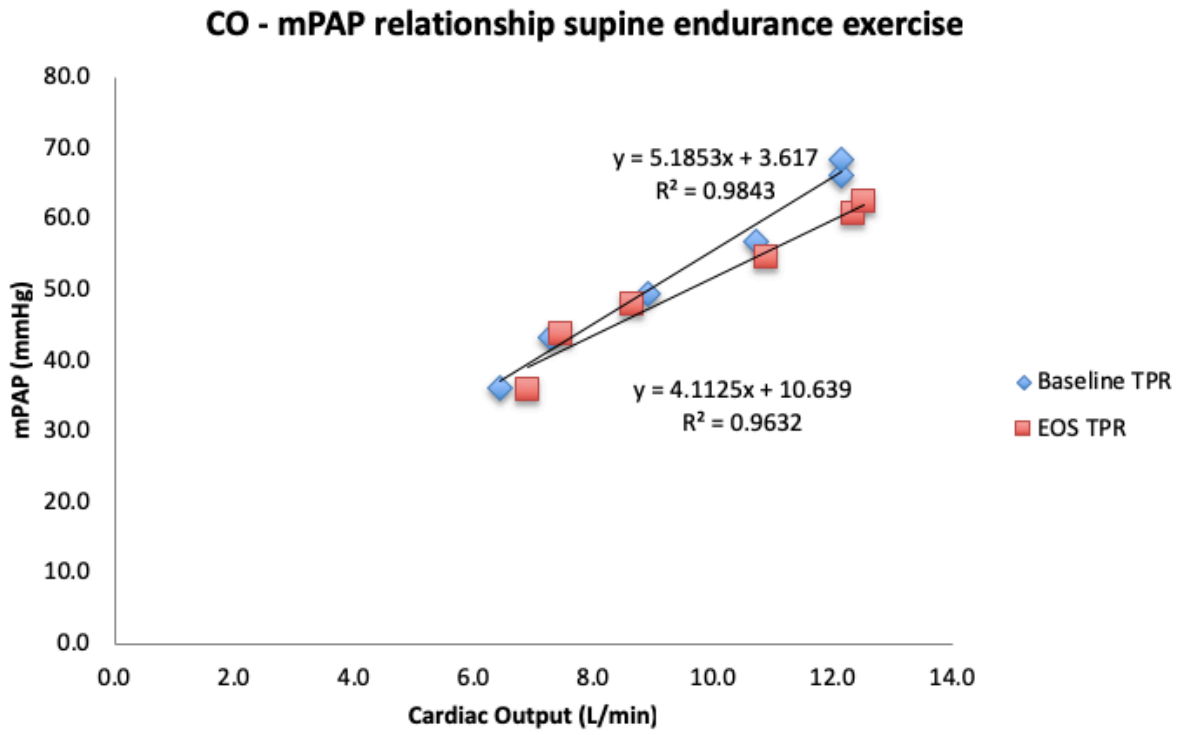


Figure 7.18: mPAP-CO gradient - change with exercise therapy

Table 7.7: Change in total pulmonary resistance with exercise therapy

	TPR baseline	SD	TPR EOS	SD	p
Rest	5.77	1.99	5.75	2.79	0.97
Feet in pedals	6.43	2.49	6.90	3.35	0.41
Unloaded	5.94	2.24	6.31	3.14	0.30
Lowest isotime	5.82	2.69	5.81	3.01	0.97

Highest isotime	6.27	3.80	5.77	3.39	0.18
Peak	6.56	4.02	5.89	3.49	0.11

For individual participants, the gradient was calculated for the mPAP-CO relationship throughout exercise at baseline and at the end of the study. Individual responses are shown in Figure 7.19 along with the population mean. The mean mPAP-CO gradient fell from 4.8 (2.2) to 3.8 (1.8), p 0.035.

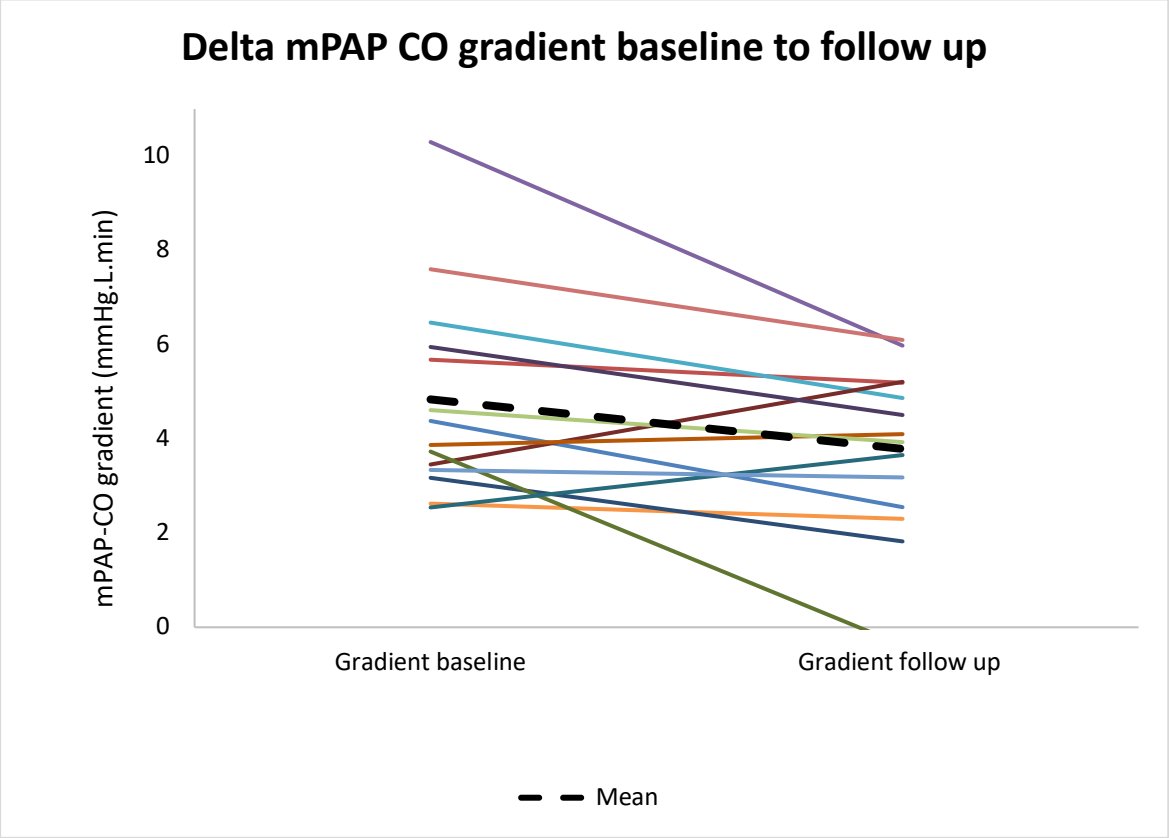


Figure 7.19: Individual changes in mPAP-CO gradient

7.4.2.4 **Pulmonary Artery Compliance**

Pulmonary artery compliance was measured at rest and during progressive exercise. Pulmonary artery compliance was significantly higher at highest isotime point and peak exercise (Figure 7.20).

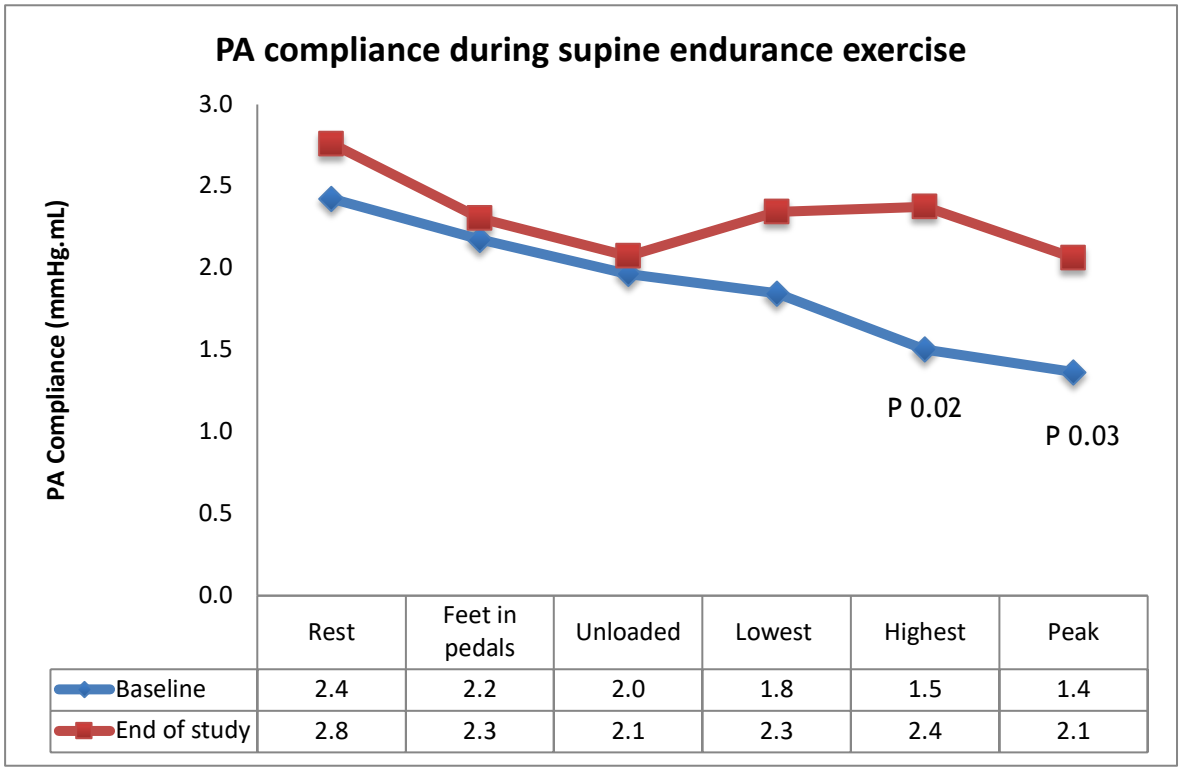


Figure 7.20: Changes in Pulmonary Artery Compliance

Pulmonary artery compliance is directly proportional to stroke volume, therefore the lower the PA compliance, the greater the increase in pressure for a given stroke volume and the more work required of the RV to maintain CO. This proportionate relationship can be seen in Figure 7.21 during progressive stages of exercise.

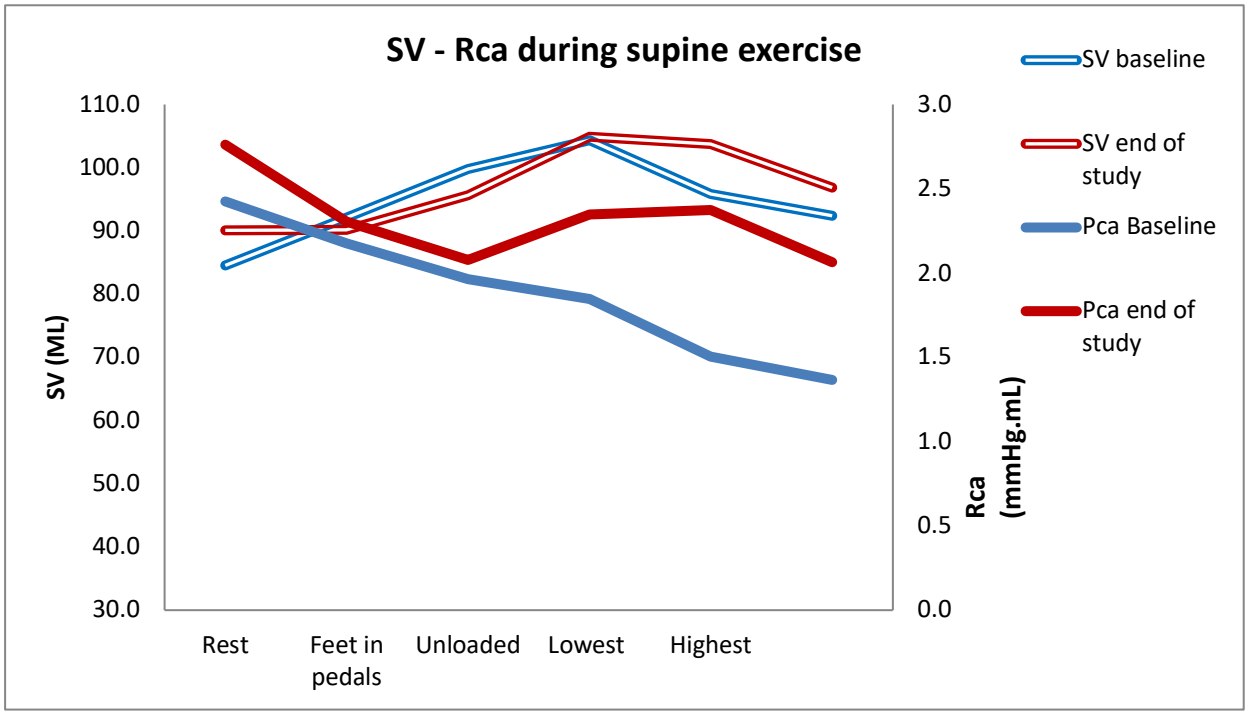


Figure 7.21: Stroke volume and PA Compliance relationship during progressive exercise

7.4.2.5 RC time constant

The time constant (in seconds) of a resistance compliance (RC) circuit is equal to the product of the circuit resistance and the circuit capacitance (compliance), called the RC time.

Resistance and compliance in the pulmonary circulation exhibit an inverse hyperbolic relationship, and therefore substantial declines in pulmonary artery compliance occur before increases in PVR or TPR. The further to the left of the inverse hyperbolic curve, the improved the relationship is i.e. a high compliance, low resistance circuit.

When measured following exercise therapy, the compliance - resistance relationship appeared to improve at rest, however not reaching statistical significance, p 0.609 (Figure 7.22). A power trendline was selected for both datasets, R^2 value is displayed in figure 7.22

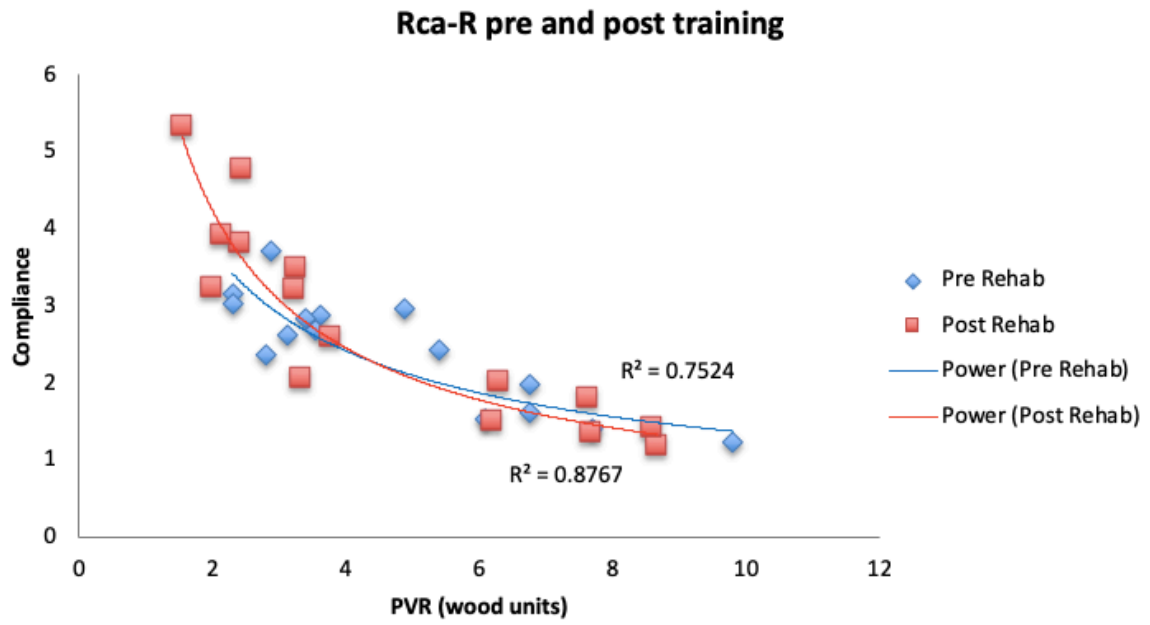


Figure 7.22: Change in compliance - resistance relationship at rest between baseline and end of study tests.

Combined curves of compliance and total pulmonary resistance are shown in Figure 7.23 and

Figure 7.24. During progressive exercise, the inverse hyperbolic relationship remained, however the curve shifted down and subjects are seen to move further to the right along the inverse hyperbolic curve, representing a deterioration in the efficiency of the pulmonary circulation during progressive exercise.

Following exercise therapy, subjects moved further to the left of the curve, with an improved compliance – resistance relationship. A Freidman repeated measures test was performed due to the non-parametric nature of the data to compare the different time points measured during exercise (Rest, unloaded, lowest isotime, highest isotime). This was performed at baseline and at the end of study (EOS), with the difference between exercise time points reaching statistical significance in both assessments.

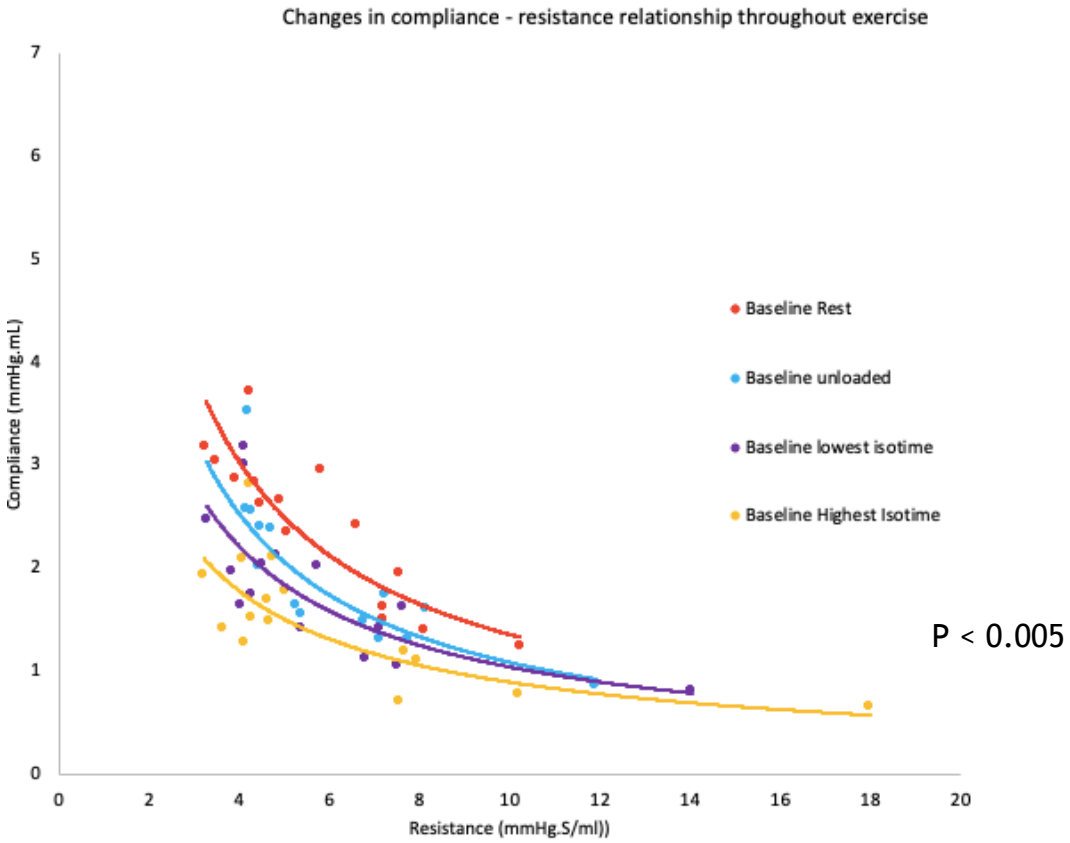


Figure 7.23: Baseline RHC. Compliance-Resistance curves during progressive stages of exercise

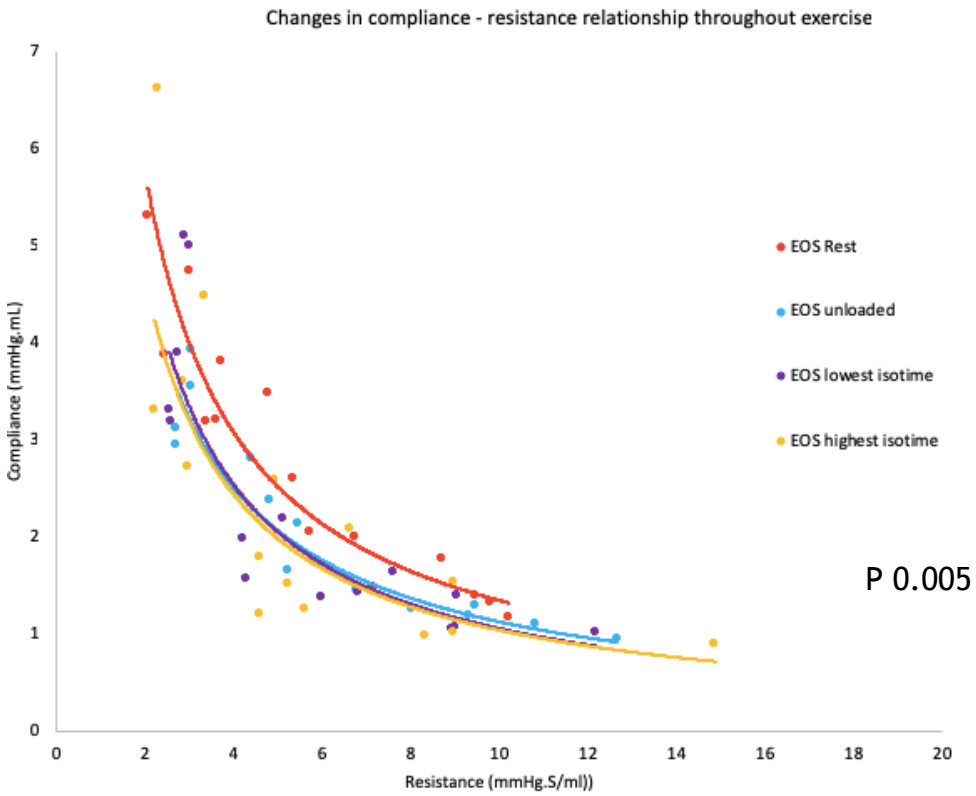


Figure 7.24: End of Study RHC. Compliance-Resistance curves during progressive stages of exercise

At highest isotime and peak exercise, there was a significant improvement in the resistance – compliance relationship (Figure **7.25**) with more subjects towards the left of the curve. This corresponded with a longer Rc time at highest isotime point and peak exercise (Figure 7.26)

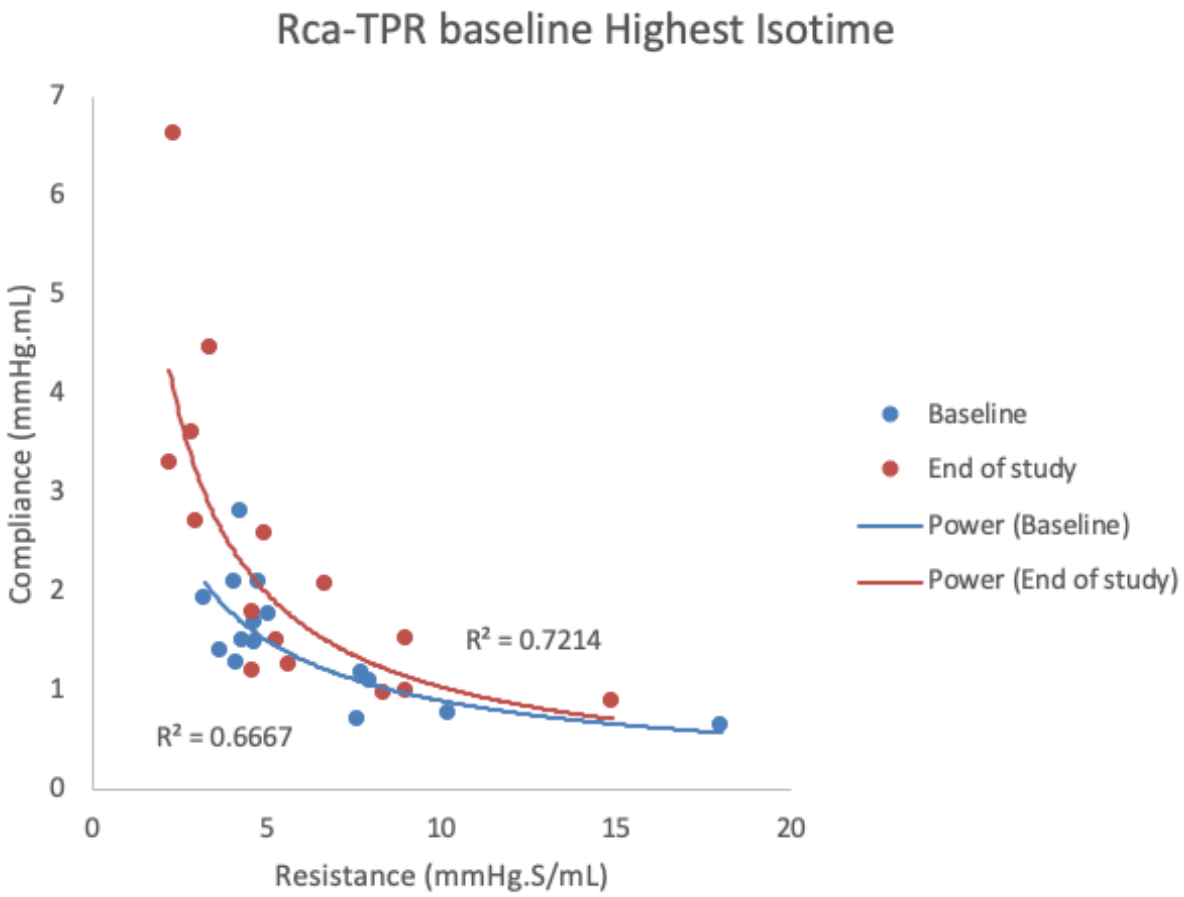


Figure 7.25 Comparison of resistance-compliance relationship at highest isotime point

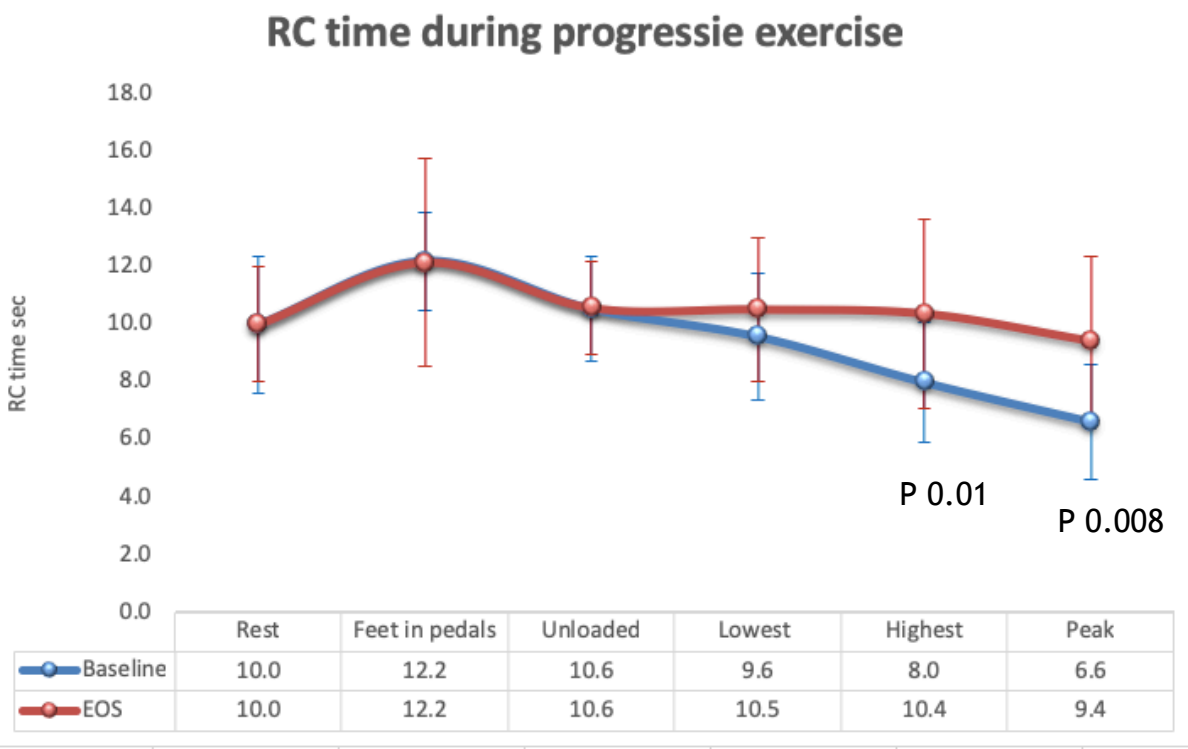


Figure 7.26: Progressive exercise change in RC time pre and post exercise therapy

7.4.2.6 Right Ventricular Oscillatory Power Fraction

At rest in the population studied, the mean baseline RVOPF was higher than in health at 38% (32-47%), with no subjects being within the normal range (20-25%). In later stages of exercise, the ratio of mean power to oscillatory power improved following exercise therapy in keeping with previously discussed invasively measured PA haemodynamic variables and improved efficiency of the pulmonary circulation (Figure 7.27).

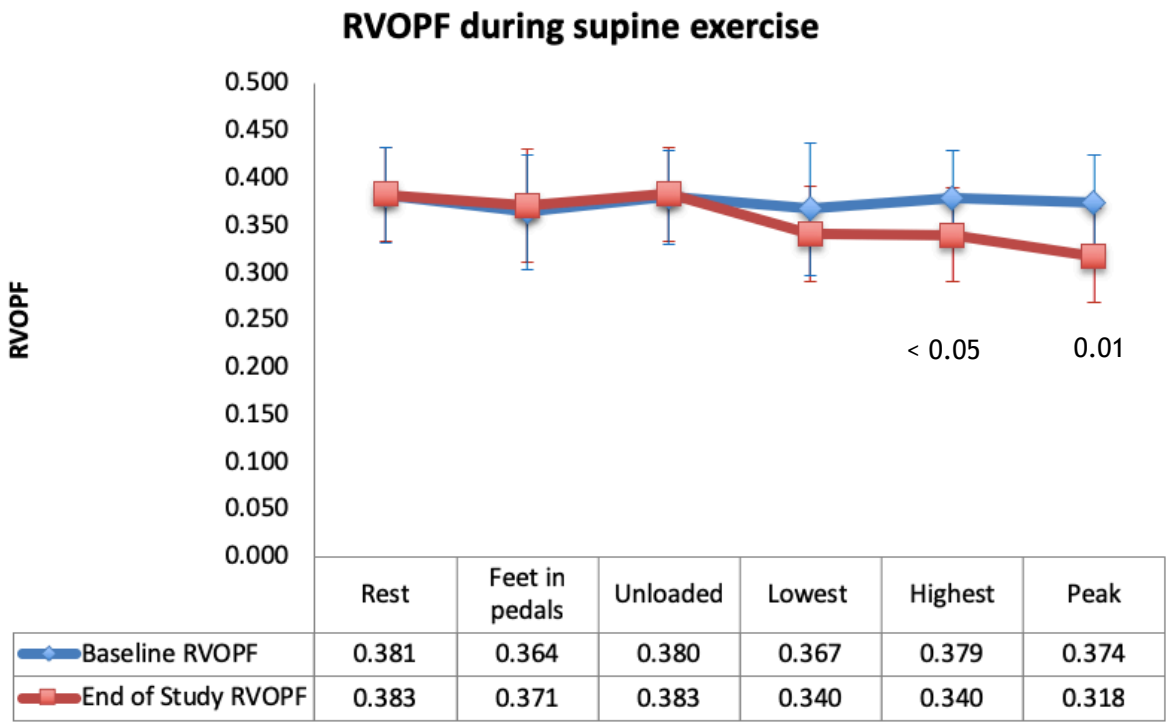


Figure 7.27: Pre and post exercise therapy right ventricular oscillatory power fraction during progressive exercise

7.4.2.7 Mixed venous oxygen saturation

As expected, during exercise, oxygen extraction increased and mixed venous oxygen saturation reduced. There was no significant difference in mixed venous oxygen saturation or oxygen extraction at any stage of exercise (Table 7.8,Figure 7.28). At baseline and at the end of exercise programme, O_2 extraction did not reach maximal expected values that would occur in health (0.8)[220].

Table 7.8: Oxygen extraction and mixed venous oxygen saturations during exercise RHC

Oxygen extraction					
	Baseline mean	SD	EOS mean	SD	p
Rest	0.24	0.05	0.24	0.05	0.84
Feet in pedals	0.25	0.06	0.27	0.05	0.21
Unloaded	0.36	0.06	0.35	0.07	0.74
Lowest isotime	0.50	0.08	0.49	0.11	0.74
Highest isotime	0.51	0.07	0.50	0.08	0.73
Peak	0.52	0.06	0.52	0.07	0.74
SvO (%)					
Rest	69.97	6.56	69.91	5.99	0.96
Feet in pedals	69.22	7.55	66.78	5.95	0.13
Unloaded	57.95	6.59	58.43	8.48	0.78
Lowest isotime	45.83	7.82	45.81	10.27	0.99

Highest isotime	44.58	7.58	44.30	7.99	0.82
Peak	42.89	6.62	42.18	7.11	0.65

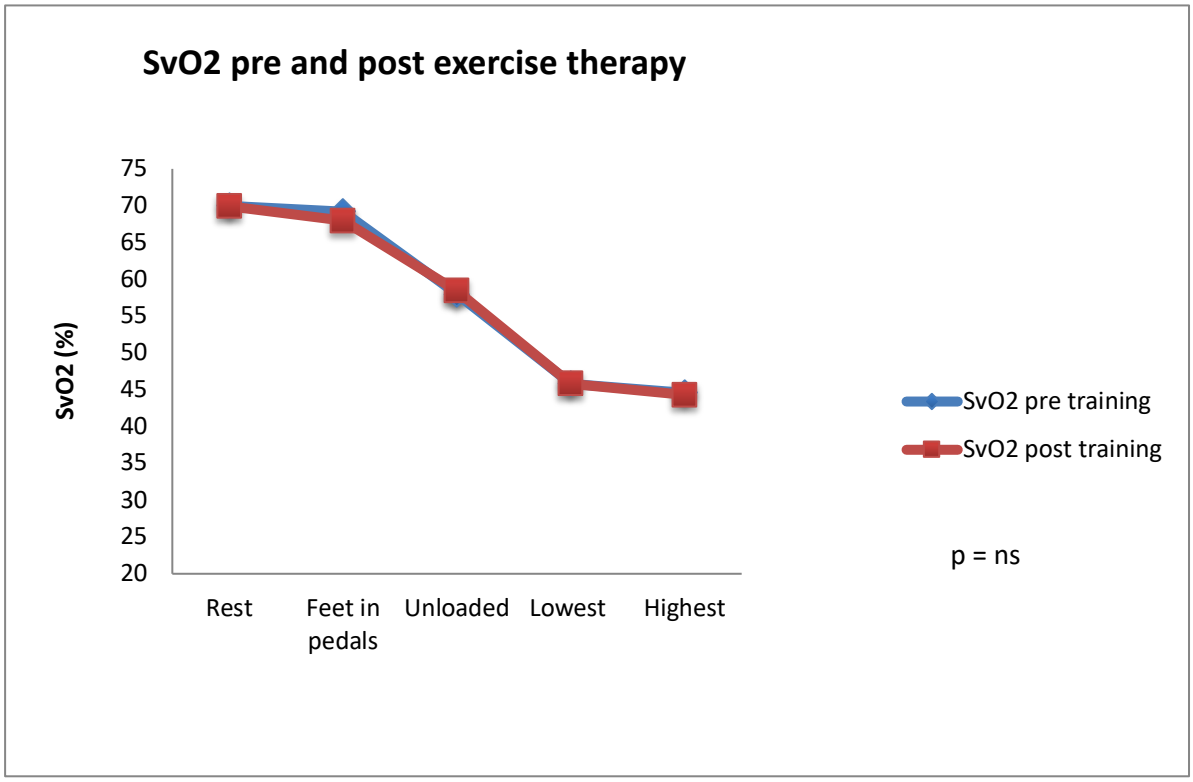


Figure 7.28: Pre and post exercise therapy SvO_2 during progressive exercise

7.4.2.8 **Endurance time**

Supine exercise time was measured at baseline and the end of the study. Endurance time improved significantly following exercise therapy from a baseline of 669 seconds (287) to 1018 seconds (437) at the end of study, p 0.004.

Comparison of endurance time to other markers of exercise capacity is discussed in Chapter 6.

7.5 **Discussion**

Haemodynamics in this cohort were typical of a pre-capillary PH population, with raised mPAP and reduced SvO_2. All patients were clinically stable at study entry on optimal drug therapy; this was reflected in the normal mean RAP, CO and CI. A subgroup of the studied population had near normal mPAP and PVR.

7.5.1 **Resting haemodynamics**

There were no significant changes in resting invasively measured haemodynamics pre and post exercise therapy, however a trend to improved PA compliance and stroke volume occurred. In those who had improved 6MWD, there was a significant improvement in stroke volume, cardiac output and PA compliance in comparison to the group who did not improve.

7.5.2 **Exercise haemodynamics**

Exercise therapy resulted in a similar pattern of improvement in all invasively measured exercise haemodynamics. Little or no change occurred at rest but as exercise progressed the majority of haemodynamic parameters improved following exercise therapy, with the most striking change occurring at peak exercise.

Following exercise therapy, significantly lower SPAP and mPAP were noted at later stages of exercise, along with lower heart rate and higher PA compliance. The mean mPAP-CO gradient was significantly lower, indicative of a reduced PVR during exercise. Although not reaching statistical significance, there was a trend to improved stroke volume and cardiac output. Pre- exercise therapy, there was an earlier and more pronounced fall in stroke volume during exercise, with a higher heart rate at equivalent stages of exercise, compared to SV and HR measurements post-exercise therapy.

7.5.3 **Cardiovascular efficiency**

The relationship between compliance and resistance deteriorated throughout progressive exercise. Exercise therapy resulted in improvements in this relationship, with a left and upwards shift of the inverse hyperbolic curve.

Contrary to literature on PA RC time, the RC time did not appear to be constant during dynamic measurements. As exercise progressed, the RC curve shifted downwards, meaning RC time was shorter for any given PVR. Exercise therapy resulted in improvements in RC time at later stages of progressive exercise.

At study entry, right ventricular oscillatory power fraction was elevated, in keeping with a high resistance, low compliance circulation. Following exercise therapy, the RVOPF improved in later stages of exercise, in keeping with a more efficient circulation and less wasted oscillatory power expenditure.

7.5.4 **Mechanisms for improvement**

Logically, increases in flow in the pulmonary circulation result in increases in PA pressure. Exercise therapy resulted in reductions in mPAP in the face of unchanged or improved cardiac output and lower heart rate. This maintenance or reduction in mPAP in the face of higher flow, is likely to be due to improvements seen in pulmonary artery compliance. These improvements are reflected in the assessment of the efficiency of the pulmonary circulation, with improved RC time and RVOPF, translating to reduced afterload and less wasted energy for the RV. The change in pulmonary artery compliance is probably the most striking of the changes seen following exercise therapy, along with improvements in endurance time – a clinical measure of the effects of the improved haemodynamics. It is unclear what is responsible for this improvement in PA compliance. Possibilities may include:

1. *Recruitment of capacitance vessels*; this may occur due to lung recruitment and / or improved flow secondary to improved cardiac output. Recruitment of capacitance vessels would fit with the variable RC time constant observed in this study. In the current literature, the constancy has been assumed to be due to the fact that the resistance in PAH is largely due to the smaller resistive vessels, and this is felt to be relatively fixed. Lung perfusion imaging may help to answer this question in future studies.

2. *Improved endothelial health* resulting in endogenous vasodilation. Exercise induced improvement in endothelial function and release of vasoactive mediators such as Nitric Oxide is a well-established phenomenon in the systemic circulation, both in health and disease states. This phenomenon would be difficult to study in the pulmonary circulation due to the lack of ability to biopsy the pulmonary artery. Consideration could be given as to whether changes in NO production and endothelial function in the systemic circulation would serve as a surrogate in future research[221]

3. *Autonomic function :* A number of studies have demonstrated evidence of autonomic nervous system disturbance in PAH. Wensel et al demonstrated reduced heart rate variability and blunted baroreceptor sensitivity associated with a reduction in peak VO_2[100]. Ciarka et al found that sympathetic nervous system activation in PAH was associated with lower 6MWD and NYHA FC[222]. In an animal model, pulmonary artery dilatation has also been shown to be induced by vagal stimulation[223]. More recently attempts to modulate the autonomic nervous system have been made with intravascular ultrasound pulmonary artery denervation; Rothman et al demonstrated improved mPAP and pulmonary artery compliance, associated with improvements in 6MWD[224]. Exercise training has been shown to improve autonomic regulation in a number of conditions including chronic heart failure[225], COPD[226] and coronary artery disease[227]. It is therefore possible that exercise improves autonomic regulation in PAH and reduces pulmonary arterial vasoconstriction, thus improving pulmonary arterial compliance. Further studies to look at the impact of exercise training on autonomic function in PAH would be of great interest.

No changes were seen in oxygen extraction or mixed venous oxygen saturation. One may have expected improvements in oxygen extraction with improved muscle blood flow and potentially improved skeletal muscle cellular function (Discussed in Chapter 9). Two possible explanations exist for this lack of change in oxygen extraction

1. Oxygen delivery (DO_2) proportionately rises with the improvements in oxygen uptake (VO_2), therefore the oxygen extraction ratio (O_2ER) remains the same

$$O2ER = \frac{VO_2}{DO_2}$$

   As discussed in chapter 6, peak VO_2 measured during CPET increases. CO also shows a trend to increase, in addition to improvements seen in O_2 pulse on CPET measurements. Therefore, it may be that delivery of oxygen

proportionately rises with oxygen consumption as the subjects' cardiovascular efficiency improves.

2. There may be additional pathophysiological factors that are unmodified with exercise therapy and contribution to impaired O_2 diffusion, local tissue O_2 delivery or O_2 extraction. Examining the Fick equation for diffusion of oxygen, this could be due to

    a. **O_2 gradient**. A smaller difference in O_2 gradient – i.e. patients with PAH are more prone to arterial hypoxaemia due to V/Q mismatch, therefore the concentration gradient is smaller.

    b. **Diffusion distance**. Increased diffusion distance in the alveolus; microvasculature disease with increased wall thickness.

    c. **Flow.** Although it appears that global blood flow improves there could be an unmodified problem with regional blood flow at a muscle level.

    d. **Oxygen extraction** at a local muscle level may be impaired (mitochondrial disease or cellular enzymatic dysfunction).

It Is clear from this data, that exercise therapy results in beneficial effects on the pulmonary circulation during exercise. The lack of change seen in resting figures supports the theory that the improvement in exercise capacity is multifactorial (as discussed in Chapter 6). The small population size means that this study may not be sufficiently powered to detect more subtle differences in invasive haemodynamics. Further research, with larger numbers of patients will help further characterise the nature of the improvements seen.

Assessment of resting haemodynamics only in this population could have led to inaccurate conclusions on the effects of exercise therapy on the pulmonary circulation. This should prompt further research into selecting the best outcome measures for PH trials and specifically for trials of exercise intervention.

7.6 **Conclusions**

Patients with pre-capillary pulmonary hypertension, treated with optimal drug therapy, can achieve satisfactory improvements in resting PA haemodynamics, as reflected in a sub-set of the population with normal mPAP and CO prior to embarking on exercise therapy. Exercise haemodynamics are crucial in the assessment of the pulmonary circulation and determining the response to an intervention; despite reassuring resting PA pressures, significantly abnormal exercise haemodynamic responses can occur, in keeping with impaired exercise capacity. These changes are responsive to exercise therapy.

In this study, exercise therapy resulted in reductions in mPAP during progressive exercise in the face of unchanged or improved cardiac output and lower heart rate. This is likely to be due to improvements in pulmonary artery compliance. These improvements are reflected in improved efficiency of the pulmonary circulation following exercise therapy, with improved RC time and RVOPF, translating to reduced afterload and less wasted energy for the right ventricle. The mechanisms underlying the improvement in pulmonary artery compliance requires further research.

Chapter 8 Non-invasive assessment of the cardiovascular response to exercise therapy in PAH

8.1 Introduction

Assessing the response of the right ventricle to exercise in PAH is essential, as it is RV function, rather than pulmonary artery pressure, that determines survival. To study the response of the right heart to exercise in PAH, the pathophysiology of RV failure in PAH must first be understood. Additionally, it is important to understand what modalities best assess right ventricular structure and function in relation the effect of exercise.

The gold standard for the diagnosis of PAH is right heart catheterisation[228]. This has disadvantages as a tool for follow up due to its invasive nature, requirement for a high level of operator expertise and lack of information on cardiac structure and volumes. Non-invasive, reproducible means of assessing cardiac status in PAH are desirable; CMR and NTproBNP are two of the most comprehensive and simple methods of doing so respectively. Their role as a means of assessing the response to exercise therapy in PAH has not been studied.

8.1.1 Pathophysiology of right ventricular failure in PAH

In PAH, RV failure is a late sign, after a long period of adaptation. Four stages occur in the adaptation and failure of the right ventricle [229]

1. Reduction in the calibre of blood vessel and increased resistance in the pulmonary vasculature, leading to increased right ventricular afterload

2. Right ventricular adaptation by increasing contractility (up to five fold) and RV hypertrophy

3. RV dilatation and increased heart rate to maintain cardiac output. Increased wall tension and oxygen consumption occur and leftward septal bowing occurs

4. “Uncoupling” occurs when the RV can no longer overcome the afterload resulting in a falling stroke volume and increasing RV end diastolic volume. In very late stages of the process, the mPAP can then fall as cardiac output falls

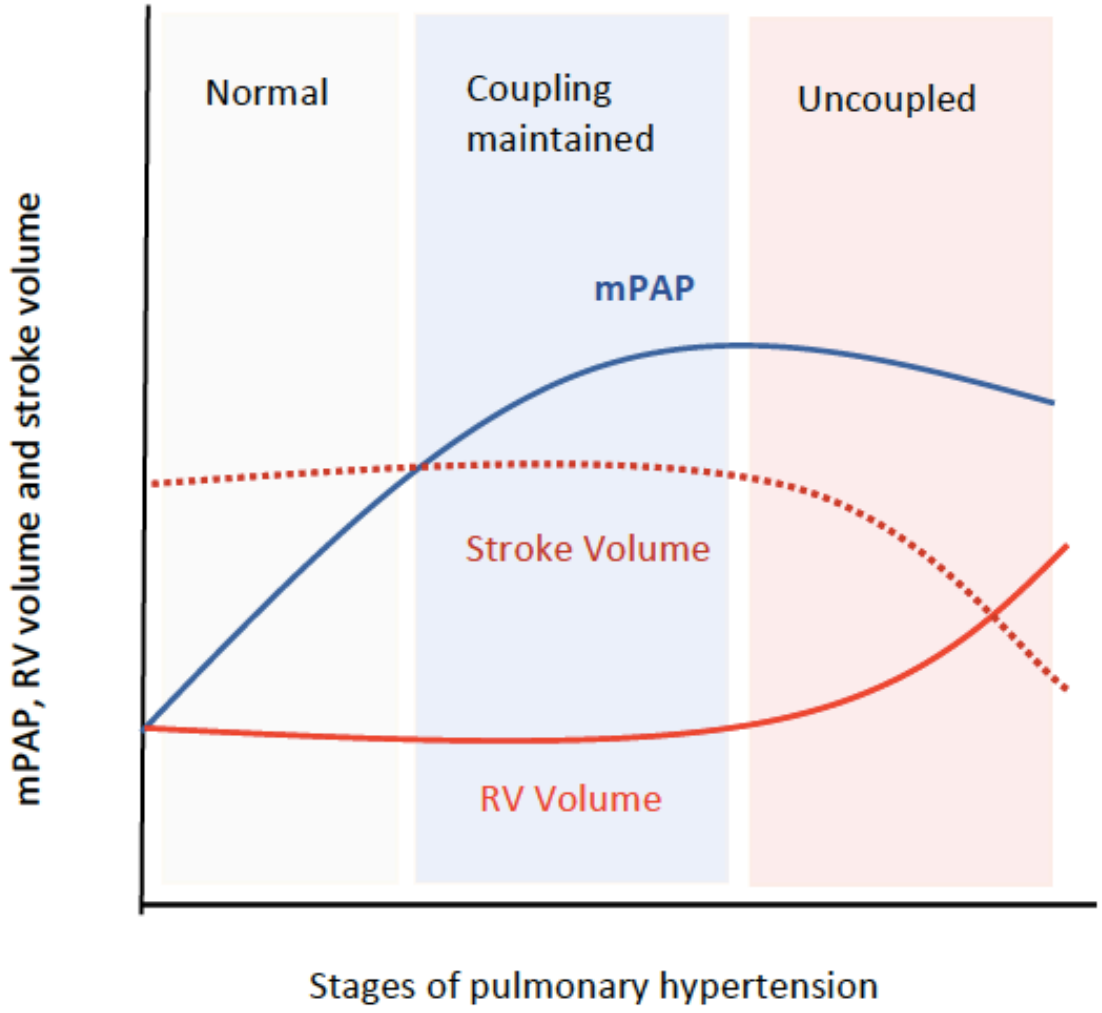


Figure 8.1 Coupling in PAH

In health and stable pulmonary arterial hypertension, RV-arterial coupling describes the matching of RV contractility to afterload[230]. Uncoupling only occurs in late stages of PAH, when the ventricle fails, or during exercise[231]. Cardiac output and stroke volume therefore provide prognostic information, however, are not sufficiently sensitive in identifying early signs of deterioration. Assessing earlier components of the RV adaptive pathway may facilitate earlier disease intervention and provide higher sensitivity for change in clinically stable patients.

The right ventricle is not the only important ventricle in PAH. Ventricular interdependency is a pathophysiological phenomenon and plays an important role in the pressure overloaded RV. Ventricular interdependency between the RV and LV in PAH can be due to

1. Parallel interaction: Leftward septal bowing, hampering filling of the LV. This is primarily due to a prolonged contraction time of the RV with respect to LV contraction time

2. Series interaction: decreased filling of the LV due to lowered RV SV (lower LV preload).

Chronic underfilling of the LV results in atrophy of the cardiac myocytes[232]. The importance of the LV in PH is underlined by the fact that stroke volume is closely related to LV end diastolic volume (LVEDV), but not to mPAP or RV end-diastolic volume[233]

8.1.2 **The role of cardiac magnetic resonance imaging in PAH**

Cardiac MRI is a non-invasive imaging modality that provides high resolution, three dimensional images, allowing assessment of cardiac structure, function and volumes[234]. Decreased RV ejection fraction is the strongest predictor of mortality in PAH meta-analyses [235], with RV end systolic volume (RV ESV) and LV End Systolic Volume (LV ESV) also providing useful prognostic information. Studies have also demonstrated that RV mass index of greater that 59gm-2 is associated with poorer survival, however this appears to have a weaker prognostic signal that end diastolic volumes and stroke volume.[236, 237]

Limited data exist on the use of CMR as a follow up tool to determine treatment response in PAH. From the limited data, low or falling RVEF appears to relate to treatment failure and poor prognosis and can occur in the face of falling PVR on treatment[238]. This highlights the pivotal role of the RV in determining survival and that the RV may not respond to treatment induced reductions in PVR. In patients with PAH and CTEPH, 1 year of treatment with PAH-specific therapy resulted in significant improvements in stroke volume which correlated with an increase in 6MWD and led to the calculation of a minimal clinically important

difference in SVI of 10 mL [237]. This MCID for SVI requires validation in larger cohorts.

8.1.2.1 **Cardiac MRI normal reference ranges**

The standard ventricular references ranges for structure and function have been extensively analysed in a number of studies[239], including 804 Caucasian males and females in the UK biobank study (Table 8.1) [240]

Table 8.1: CMR normal reference ranges

	Male	Female
Left ventricle		
LVEDV (ml	109-218	88-121
LVESV (ml)	39-97	31-68
LVSV (ml)	59-132	49-100
LV mass (g)	64-141	46-93
LVEF %	48-69	51-70
LV mass to volume ratio (g/ml)	0.42-0.84	0.39-0.71
Right ventricle		
RVEDV (mL)	124-248	85-168
RVESV (mL)	47-123	22-77
RVSV (mL)	62-131	48-99
RVEF %	45-65	47-68
RV mass (g)	25-57	21-49

8.1.3 **Changes in cardiac structure and function with exercise**

In health and athletes, changes in cardiac structure are specific to the type of exercise performed. Strength training (isometric exercise), involves short repetitive bursts of high-intensity muscle contraction and causes significant increases in systolic blood pressure with little effect on cardiac output. This affects mainly the left ventricle and aorta and leads to increase in left ventricular wall thickness without change in chamber size, resulting in concentric left ventricular hypertrophy (LVH). Endurance training (isotonic exercise) results in a high metabolic demand from exercising muscle, requiring a more persistent elevation in cardiac output; this functions as a volume load on all four cardiac chambers, with resultant biatrial and biventricular dilation. LV chamber size increases without increase in wall thickness, consistent with eccentric LV remodelling[241]. Exercise that has both high isotonic and isometric stresses result

in four chamber dilation with balanced increase in LV size and wall thickness consistent with eccentric LVH[242]

The majority of studies looking at changes in cardiac structure and function in athletes have underrepresented females. It appears that the male and female heart adapt differently to exercise therapy and gender appears to be an important modifier of the relationship between exercise and associated cardiac remodelling.[243]. Gender differences in the cardiac response to exercise in PAH is an unstudied, but relevant area, due to the "oestrogen paradox" in PAH, where a female preponderance exists, but with a less severe phenotype than men .

Evidence is sparse in relation to the effect of exercise therapy on the structure and function of the heart in PAH. In children and young adults with corrected tetralogy of Fallot or Fontan circulation, a 12 week aerobic exercise training programme resulted in improvements in peak aerobic exercise capacity, but no significant changes in cardiac structure or function[244]. In left ventricular systolic heart failure, endurance exercise can reverse LV remodelling with improvement of systolic and diastolic LV function and reduction of LV end-diastolic diameters. The reverse remodelling is a consequence of peripheral training effects and direct cardiac effects[245]

Exercise MRI is an emerging and potentially valuable tool to assess dynamic structural and functional cardiovascular changes in PAH, such as RV-PA uncoupling during exercise[246]. It has the advantage of recreating the environment in which the patient is most symptomatic (exertion) in order to delineate the changes from the resting state likely to be contributing to symptoms and identify features of RV dysfunction at an early stage in the disease process.

8.1.4 **The role of NTproBNP in PAH**

Brain Natriuretic Peptide (BNP) is secreted by ventricular cardiomyocytes in response to increased ventricular stretch, as a result of cardiac wall stress. N-terminal pro Brain Natriuretic Peptide (NTproBNP) is an inactive fragment of a 108 amino acid peptide. The 108 amino acid peptide is also metabolised to the active fragment BNP; BNP is less stable than NT-proBNP and therefore is a less convenient biomarker [247]. Serum levels of NT-proBNP correlate with mPAP, TPR and RV mass,

and inversely correlate with cardiac output and RV ejection fraction [128]. High plasma levels have a strong, independent association with mortality, however absolute values have not been shown to effectively predict treatment response.

8.2 Aims

The aim of this chapter is to determine the efficacy of non-invasive means of assessing the cardiac response to exercise therapy in PAH, specifically looking at changes in

1. Cardiac MRI: Structure and function

2. NTproBNP

3. How these parameters relate to invasively measured haemodynamics

8.3 Methods

As discussed in Chapter 2, General Methods, assessments were performed, pre and post exercise. Group B (control) subjects, had an additional assessment before and after 15 weeks of standard care, prior to entry to the exercise programme.

8.3.1 NT proBNP

N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured on venous blood, sampled by venepuncture and collected in vacuum tubes containing ethylenediaminetetraacetic acid (EDTA). NT-proBNP was sampled prior to the exercise therapy sessions or testing. Samples were processed in the Golden Jubilee Hospital Laboratory as per standard clinical practice.

8.3.2 Cardiac MRI

CMR imaging was performed as per standard clinical practice at the Golden Jubilee Hospital, in the supine position on a 1.5-T magnetic resonance imaging scanner (Sonata Magnetom, Siemens, Erlangen Germany) and images were analysed Circle Cardiovascular imaging, version 5.1 (Calgary, Canada).

CMR reporting was performed by a doctor (MMcG) trained in the analysis of cardiac MRI. The reporting Doctor was blinded to the subject number and assessment time point of the subject. All anonymised, blinded CMR scans were analysed twice and intra-observer agreement was calculated as discussed in 8.3.3.

RV and LV volumes and mass were determined by manual tracing endocardial and epicardial borders of end diastolic and end systolic images on the short axis stack obtained during breath-hold [248].

Stroke volumes (RVSV & LVSV) were calculated by EDV-ESV. Stroke volume and cardiac output were also measured using flow mapping using techniques previously described in publications from the SPVU [249]. Right ventricular SV was measured using MR phase-contrast flow quantification in an image plane positioned perpendicular to the main pulmonary artery. Left ventricular SV (aortic flow) was also measured, approximately 2–4 cm above the aortic valve and distal to the coronary arterial ostia.

Right and left ventricular mass (RVM and LVM) were determined as the product of myocardial volume for each ventricle and the quoted density of cardiac muscle (1.05 g/cm3). RV mass was determined as RV free wall mass, while the Interventricular Septum (IVS) considered part of the LV in accordance with accepted practice.

Ejection fraction (RVEF and LVEF) was determined as a percentage (%) using planimetry derived stroke volume measurement:

$$RVEF = (RVEDV-RVESV)/RVEDV \times 100$$

$$LVEF \text{ as } LVSV/LVEDV \times 100.$$

8.3.3 **Statistics**

Data was assessed for normality using the Shapiro-Wilk test due to the small sample size. For cardiac MRIs, scans were blinded and analysed twice by the same operator (MMcG), the mean being taken. Intra observer variability was determined using Cohen's Kappa. NTproBNP values were transformed to Log NTproBNP due to its non-normal distribution. Correlations were performed using Spearman's correlation coefficient. Multiple linear regression was performed to identify significant relationships between cardiac MRI variables and NTproBNP as dependant variables with clinically relevant parameters. Normally distributed data are presented as mean +/- SD, non-normally distributed are presented as median + IQR. Paired differences were analysed using Wilcoxon and unpaired using Mann Whitney.

8.4 **Results**

8.4.1 **NTproBNP**

In the full cohort of subjects who completed the exercise programme, NTproBNP did not change following exercise therapy. At baseline Log NTproBNP was 2.28 (0.54) and at the end of the study, LogNTproBNP was 2.25 (0.54), p 0.296, N 25.

There was no significant change in NTproBNP in the exercise therapy group versus control group: Delta Control Log NTproBNP -0.11 (0.20), N 12, Delta treatment -0.12 (0.41), N 15, p 0.965.

8.4.1.1 Relationship with treatment response and prognosis

Treatment response

In subjects with improved 6MWD, baseline NTproBNP was significantly lower (

Table 8.2). At the end of study, the NTproBNP remained lower in the improved group, however not reaching statistical significance. There was no rise in NTproBNP in the group that deteriorated.

Table 8.2: NTproBNP in subjects who improved versus deteriorated with exercise therapy

NT pro BNP	Mean improved (N 15)	Mean deteriorated (N 9)	p
Baseline	2.07 (0.4)	2.59 (0.6)	0.019
End of study	2.18 (0.4)	2.48 (0.7)	0.201

Transplant free survival

In those who had died or undergone lung transplantation at censoring, NTproBNP was significantly higher at baseline (N 7) and follow-up (N 3), compared to those who were alive (N 21) (Table 8.3)

Table 8.3: NTproBNP in subjects who were alive versus deceased or transplanted

NT pro BNP	Mean dead or transplant	Mean alive (N 21)	p
Baseline	2.95 (0.5)	2.10 (0.4)	<0.005
End of Study	2.97 (0.6)	2.18 (0.5)	0.014

Association with relevant clinical variables

At study entry, a number of relevant clinical and prognostic variables correlated with baseline NTproBNP (Table 8.4)

Table 8.4: Baseline NTproBNP significant correlations

Variable	Spearman's R	p	N
TLCO (ml/min/mmHg)	-0.538	0.005	26
VO_2/WR	-0.504	0.007	27
mPAP (mmHg)	0.577	0.008	20
PVR (w.u)	0.552	0.012	20
TPR	0.681	0.001	20

RAP (mmHg)	0.516	0.012	20

Multiple regression analysis was performed for variables with significant spearman's correlation coefficient. TLCO, mPAP, PVR and TPR remained significantly associated with baseline NTproBNP, whereas VO_2/WR and RAP did not (Table 8.5).

Table 8.5: Multiple regression analysis for NTproBNP at baseline

Variable	B	Std. Error	Beta	t	p
(Constant)	1.97	0.596		3.306	0.006
TLCO (ml/min/mmHg)	-0.153	0.036	-0.507	-4.252	0.001
VO_2/WR	-0.041	0.063	-0.089	-0.646	0.530
MPAP (mmHg)	0.018	0.008	0.38	2.252	0.044
PVR (WU)	-0.264	0.121	-1.233	-2.19	0.049
TPR	0.325	0.114	1.636	2.846	0.015
RAP mean (mmHg)	-0.009	0.026	-0.059	-0.354	0.730

TLCO and TPR showed the stronger correlations with NTproBNP (Figure 8.2, Figure 8.3)

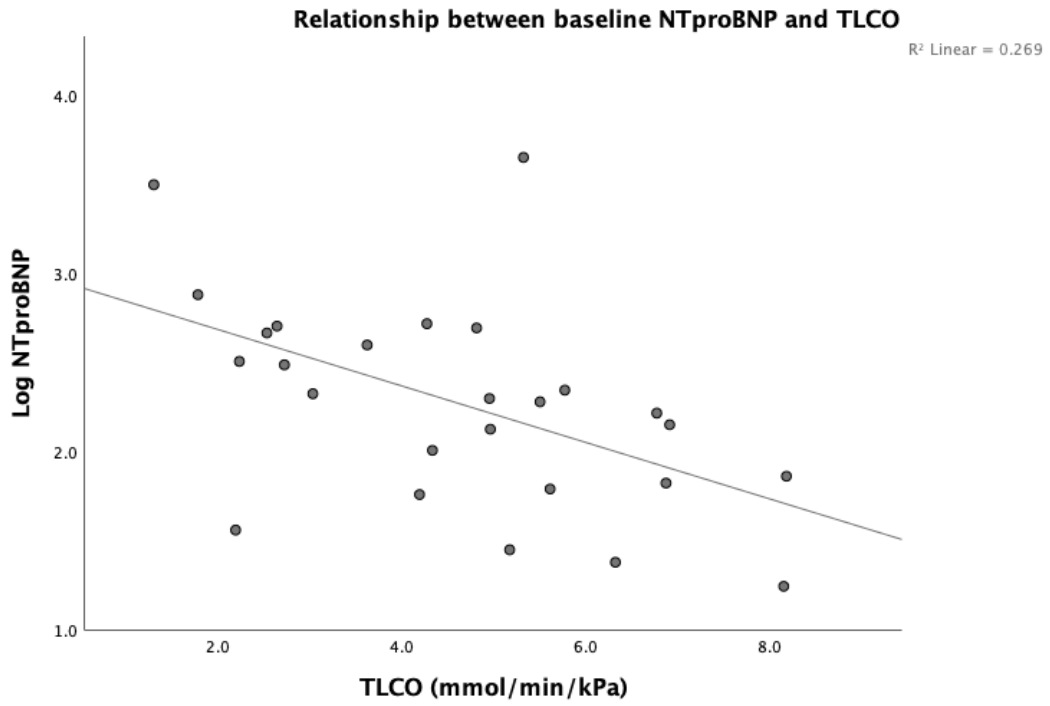


Figure 8.2: Correlation between NTproBNP and TLCO

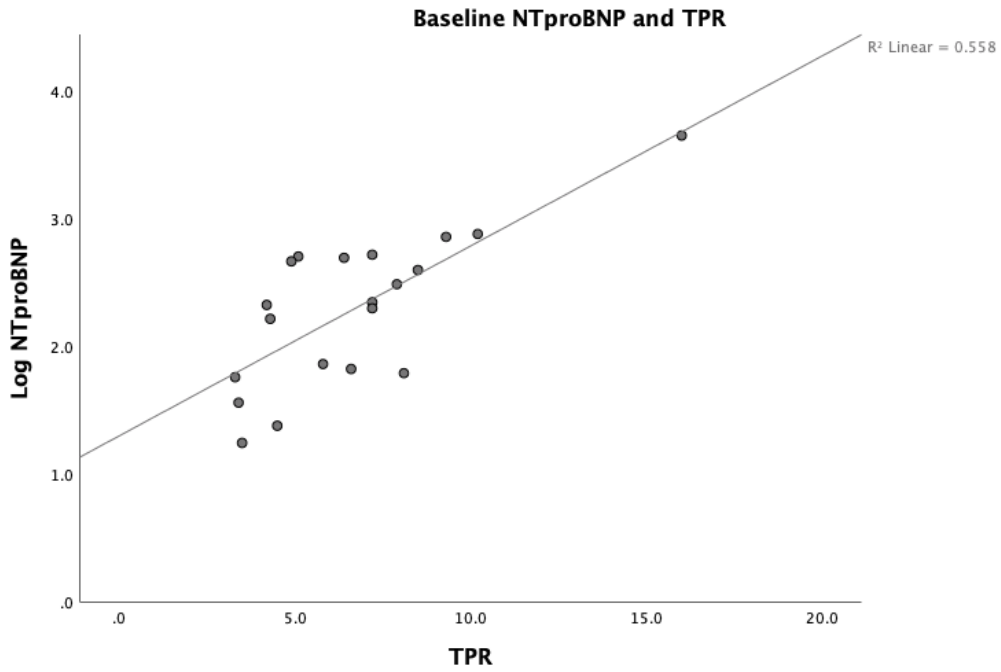


Figure 8.3: Correlation between NTproBNP and Total Pulmonary Resistance

End of study assessment

At the end of study assessment, NTproBNP correlated with biomarkers relating to exercise capacity, gas exchange and cardiovascular function. Multiple regression analysis was performed for the three domains.

Exercise capacity correlations are shown below and multiple regression analysis in

Table 8.6:

- 6MWD R^2 -0.564, p 0.005, n 23
- End exercise WR R^2 -0.606, p 0.004, N21
- Peak VO_2 ml/kg/min R^2 -0.662, p 0.001, N 21
- VO_2 at AT R^2 -0.483, p 0.026, N 21

Table 8.6: NT pro BNP linear regression with exercise capacity variables

	Beta	Std. Error	Adjusted Beta	t	p
(Constant)	3.324	0.587		5.662	<0.005
End exercise WR EOS (W)	0.002	0.006	0.109	0.285	0.779
Peak VO_2 ml/mi/kg EOS	-0.11	0.047	-0.905	-2.328	0.033
VO_2 at AT EOS	-0.042	0.646	-0.017	-0.065	0.949
EOS 6MWD (m)	0.001	0.002	0.153	0.566	0.579

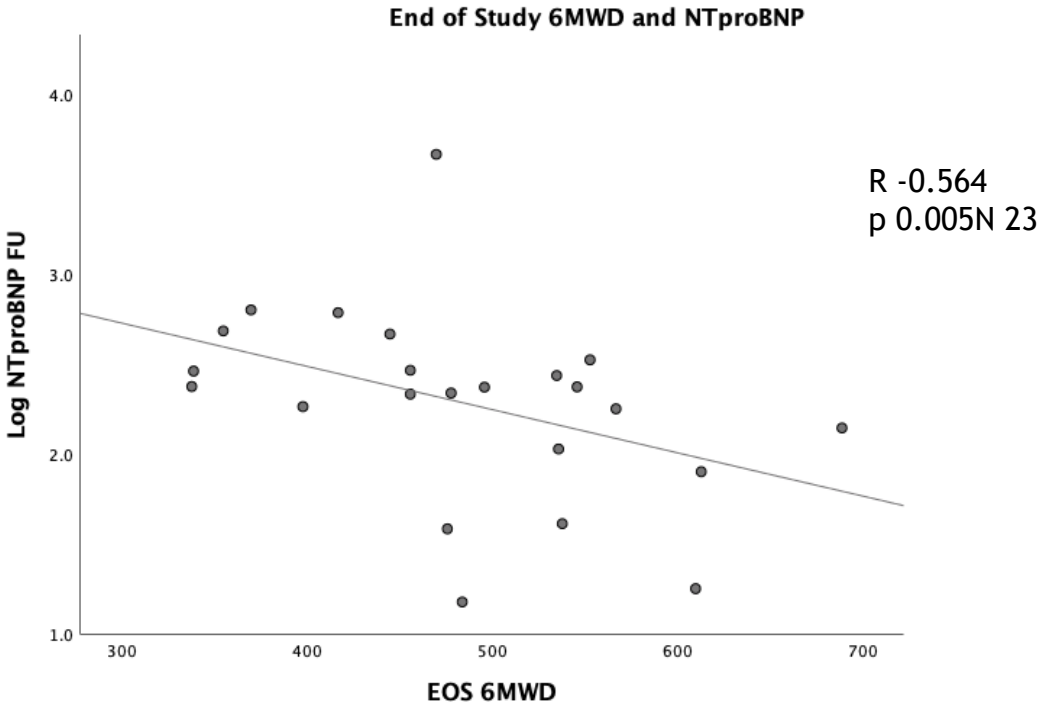


Figure 8.4: Correlation between end of study 6MWD and NTproBNP

Gas exchange (Table 8.8):

- O_2 saturations at rest R^2 -0.493, p 0.023, N 21
- Vd/Vt end exercise R^2 0.762, p 0.028, N 8

Table 8.7: NTproBNP and gas exchange linear regression

	Unstandardized beta	Std. Error	Standardized Beta	t	p
(Constant)	4.52	1.739		2.599	0.048
O_2 Saturation rest (%)	-0.023	0.018	-0.347	-1.275	0.258
Vd/Vt end exercise	0.254	0.11	0.628	2.307	0.069

Cardiovascular variables (Table 8):

- VO_2/WR R^2 -0.512, 0.018, N 21
- Right Atrial Pressure R^2 0.597, p 0.040, N 12

Table 8.8: NTproBNP and cardiovascular variables linear regression

	Unstandardized beta	Std. Error	Standardized Beta	t	p
(Constant)	3.105	2.037		1.524	0.171
RAP mean (mmHg)15	0.018	0.066	0.161	0.274	0.792
VO_2/WR 15	-0.114	0.211	-0.318	-0.539	0.606

Delta NTproBNP

Change in the primary outcome measures of 6MWD QOL and RVEF showed no significant correlation with change in NTproBNP, nor did change CMR variables or invasively measured haemodynamics. Significant variables are shown below (Table 8.9)

Table 8.9: Significant Spearman's correlation coefficients with change in NTproBNP

Variable	R	p	N
TLCO (ml/min/mmHg)	0.586	0.004	22
Delta VE (L)	0.482	0.02	23
RC time (sec)	0.48	0.044	18

Multiple regression model showed moderate correlation, with an adjusted R^2 of 0.444. TLCO remained the only significant variable (Table 8.10)

Table 8.10: Linear regression for delta NTproBNP with significant correlation coefficients

Delta NTproBNP	Unstandardized beta	Std. Error	Standardized Beta	t	p
(Constant)	-0.678	0.235		-2.887	0.014
TLCO (ml/min/mmHg)	0.065	0.021	0.6	3.05	0.010
RC time (sec)	0.035	0.021	0.334	1.666	0.122
VE delta (L)	0.009	0.011	0.166	0.83	0.423

8.4.2 **Cardiac MRI**

Cardiac output and stroke volume measured by flow map improved significantly in the entire population who completed the exercise programme (

Table 8.11)

Table 8.11: Change in Cardiac MRI variables pre and post exercise therapy (n 26)

	Mean / Median Baseline	SD / IQR	Mean / Median EOS	SD / IQR	p
LVEF %	62.5	10.4	64.7	11.4	0.140
LV EDV (mL)**	120	37.0	124	36.0	0.517
LV ESV (mL)	46.2	19.3	44.2	20.8	0.330
RV SV (mL)**	79.0	27	80.2	19.0	0.226
LV SV**	76.7	26.5	79.7	25.2	0.101
CO flow map (L/min)	5.9	1.7	6.3	1.73	0.040
LV Mass (g)	87.7	19.2	90.4	19.6	0.260
RV EF (%)	44.6	13.3	47.5	13.	0.100
RV EDV (mL)	180	56	179	62	0.450
RV ESV (mL)**	104.3	58.8	99.0	58.7	0.230
RV Mass (g)**	57.0	19.0	54.5	25.9	0.530
SV flow map (mL)	76.2	23.8	80.9	22.9	<0.005

Abbreviations and units: Left Ventricle (LV), Right ventricle (RV), Ejection fraction (EF, %), End systolic volume (ESV, mL), End diastolic volume (EDV, mL), Stroke volume (SV, mL), Mass (g)
** Non-parametric data; median / IQR values displayed

In both males and females, RV mass was greater than the normal range, in keeping with RV hypertrophy. In females, RV end systolic volume was greater than normal and in males, RV ejection fraction was lower than normal. In the female population, a significant improvement was seen in stroke volume.

Statistically significant differences between male and female CMR values were found in baseline LV mass: 83.9g (16.6) females, 103g (18.8) males, p 0.019; end of study RV mass, p 0.026; and end of study LV mass p 0.002; signified by (*) in Table 8.12. These values remained significantly different when indexed for BSA.

Table 8.12: Difference between males and females pre and post exercise therapy

	Mean Female baseline (18)	Mean Female EOS	p	Mean Male baseline (8)	Mean Male EOS	p
LVEF (%)	63.9 (9.2)	65.8 (12.1)	0.162	59.5 (12.8)	62.1 (9.9)	0.502
LVEDV (mL)	121 (39)	123 (34)	0.620	116 (29)	129 (42)	0.403
LVESV (mL)	44.0 (17.2)	41.9 (18.7)	0.227	51.3 (23.7)	49.5 (25.6)	0.754
RVSV (mL)	80.9 (26.8)	76.8 (17.4)	0.549	76.9 (26.5)	88.1 (21.5)	0.250
LVSV (mL)	77.4(27.8)	80.4 (24.5)	0.177	75.0 (26.3)	78.0 (28.5)	0.585
CO (L.min)	5.8 (1.8)	6.1 (1.8)	0.134	6.2 (1.7)	6.6 (1.9)	0.168
LV Mass (g)	83.9 (16.6)*	82.2 (14.6)*	0.609	103 (18.8)*	109 (16.7)*	0.330
RVEF (%)	45.7 (12.6)	50.2 (14.3)	0.112	***39.2 (12.7)***	***41.5 (8.8)***	0.543
RVEDV (mL)	181 (66)	169 (64)	0.616	197 (31)	200.7 (62.8)	0.500
RVESV (mL)	***104 (61)***	***91 (64)***	0.218	120 (35)	116 (42)	0.650
R Mass (g)	***57.9 (21.7)***	***53.9 (26.3)***	0.185	***69.3 (27.4)****	***70.6 (21.8)****	0.823
SV (mL)	74.9 (25.2)	80.0 (23.9)	0.002	79.0 (21.7)	82.9 (21.8)	0.174

Bold italics signifies values out-with the normal reference range
(*) signifies values that are significantly different between males and females.

8.4.2.1 **Exercise therapy versus standard care**

Change in cardiac MRI variables in all subjects (N 26) who completed the exercise programme, were compared to changes in control arm CMR following 15 of standard care (N 11) (Table 8.13)

Table 8.13: Control Group (B) Versus Exercise Therapy (A+B)

	Mean (26)	SD	Mean (11)	SD	p
LVEF (%)	2.1	7.1	-0.19	8.51	0.319
LVEDV (mL)	1.6	13.7	0.15	21.7	0.273
LVESV (mL)	-2.0	10.1	-0.92	12.98	0.642
RVSV (mL)	3.2	19.8	-0.15	38.90	0.485
LVSV (mL)	3.0	10.6	1.06	17.57	0.115
CO (L/min)(Flow Map)	0.4	0.0	-0.02	0.6	0.384
LV Mass (g)	2.7	11.7	2.6	18.59	0.883
RV EF (%)	2.9	8.6	-1.13	13.91	0.496
RV EDV (mL)	-1.5	19.6	-0.25	21.0	0.909
RV ESV (mL)	-5.7	17.3	-0.31	32.47	0.595
RV Mass (g)	-0.63	12.0	2.4	29.56	0.335
SV (Flow Map) (mL)	4.7	6.2	-0.30	11.19	0.058

Change in the individuals in Group A (N14, active intervention) versus Group B (N 11, standard care) are shown in

Table 8.14

Table 8.14: Group A (Exercise therapy) versus Group B (Control) changes in MRI variables

variable	Mean (14)	SD	Mean (11)	SD	p
LV EF (%)	3.65	8.42	-0.19	7.1	0.189
LV EDV (mL)	-5.1	8.20	0.15	21.7	0.784
LV ESV (mL)	-6.64	9.48	-0.92	12.98	0.106
RV SV (mL)	-0.39	21.70	-0.15	38.90	0.893
LV SV (mL)	0.46	10.48	1.06	17.57	0.784
CO (L)(Flow Map)	0.37	0.95	-0.1	0.60	0.139
CO (L)(short axis)	0.36	1.32	-0.02	1.35	0.443
LV Mass (g)	5.38	12.36	2.56	18.59	0.784
RV EF (%)	2.35	9.92	-1.13	13.91	0.547
RV EDV (mL)	-9.21	17.3	-0.25	21.0	0.274
RV ESV (mL)	-9.50	20.33	-0.31	32.47	0.274
RV Mass (g)	0.19	16.01	2.92	29.56	0.609

SV (mL)(Flow Map)	2.98	5.01	-0.30	11.19	0.317

8.4.2.2 **Responders and non-responders to exercise therapy.**

Responders to exercise therapy were defined, as previously discussed, as subjects who had a 30m or greater improvement in 6MWD. In this sub-group, change in left ventricular ejection fraction and cardiac output were significantly greater in the responder group (

Table 8.15, Figure 8.5, Figure 8.9).

Table 8.15: Delta CMR variables in responders versus non-responders to exercise therapy

	Responders Mean / Median	SD / IQD	Non responders mean / Median	SD / IQR	p
LVEF (%)	4.70	7.40	-1.52	5.74	0.04
LV EDV** (mL)	0.70	19.6	1.78	11.5	0.94
LV ESV (mL)	-4.15	11.98	0.33	6.73	0.30
RV SV(mL)	4.31	24.56	-4.61	23.11	0.29
LV SV(mL)	5.83	9.78	-2.22	11.00	0.07
CO (L)	0.67	1.17	-0.35	0.84	0.02
LV Mass (g)	3.22	14.47	1.85	8.22	0.79
RV EF (%)	3.67	9.35	0.77	7.57	0.42
RV EDV** (mL)	-7.5	40.3	-0.3	16.5	0.37
RV ESV (mL)	-6.88	19.97	-2.21	17.15	0.46
RV Mass (g)	2.79	13.35	-5.82	9.32	0.10
SV (mL)	5.33	7.62	3.64	4.46	0.53
** non-parametric data; Median, IQR displayed					

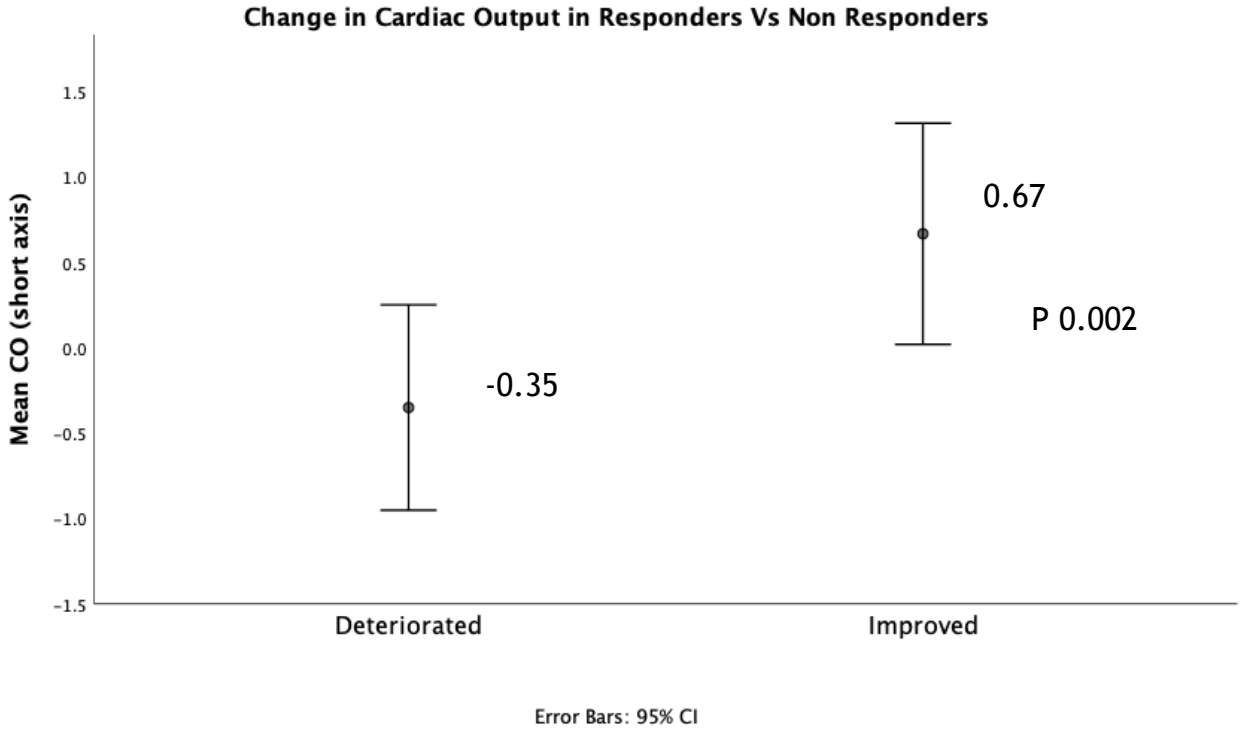


Figure 8.5: Change in cardiac output in responders versus non responders to exercise therapy

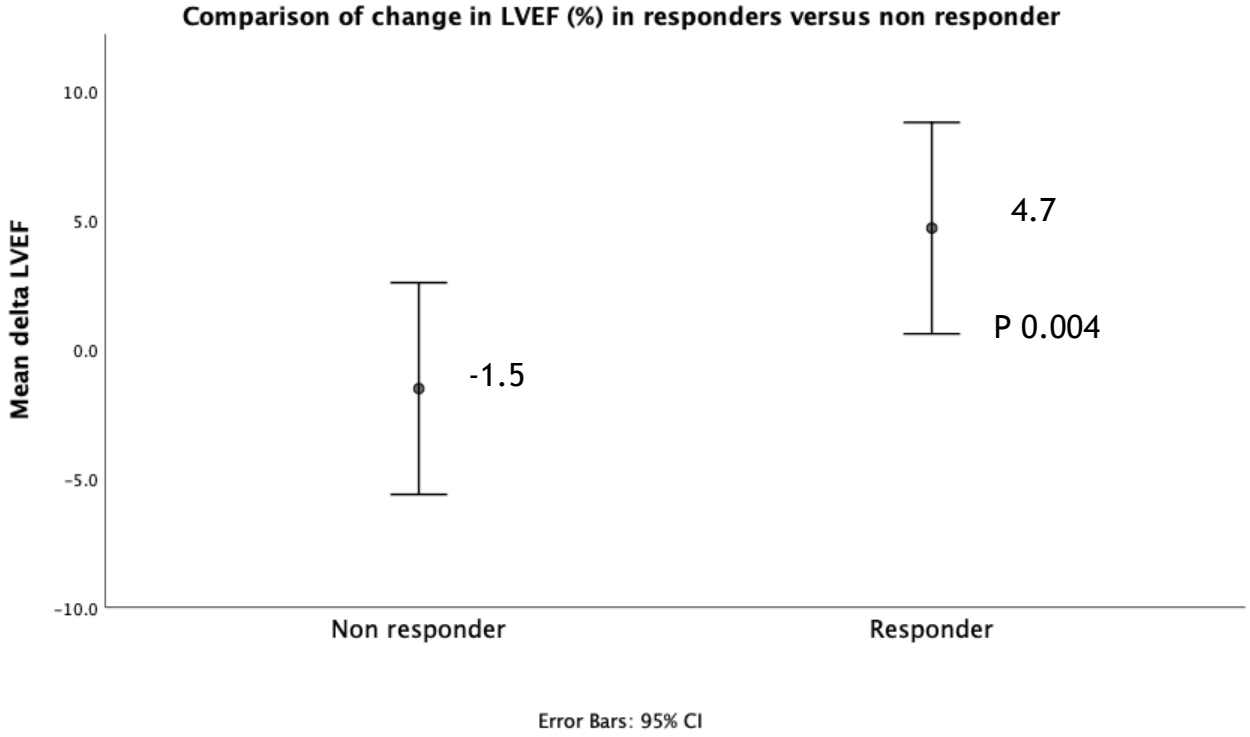


Figure 8.6: Change in LV ejection fraction with exercise therapy in responders and non-responders

Below are two examples of subjects participating in the exercise programme with CMR before and after, with one improvement and one with no change in 6MWD. The first subject (Figure 8.7) was a 33y old female on triple therapy for IPAH and had been diagnosed 1y and 8 months prior to embarking on the exercise programme. Her baseline 6MWD was 510m and end of study 6MWD was 610m. At baseline her LVEF was 65%, with an LV stroke volume of 75mls; at the end of study, LVEF was 67% with an LV stroke volume of 85mls and a 1.5L improvement in cardiac output. A clear improvement can be seen in RV morphology.

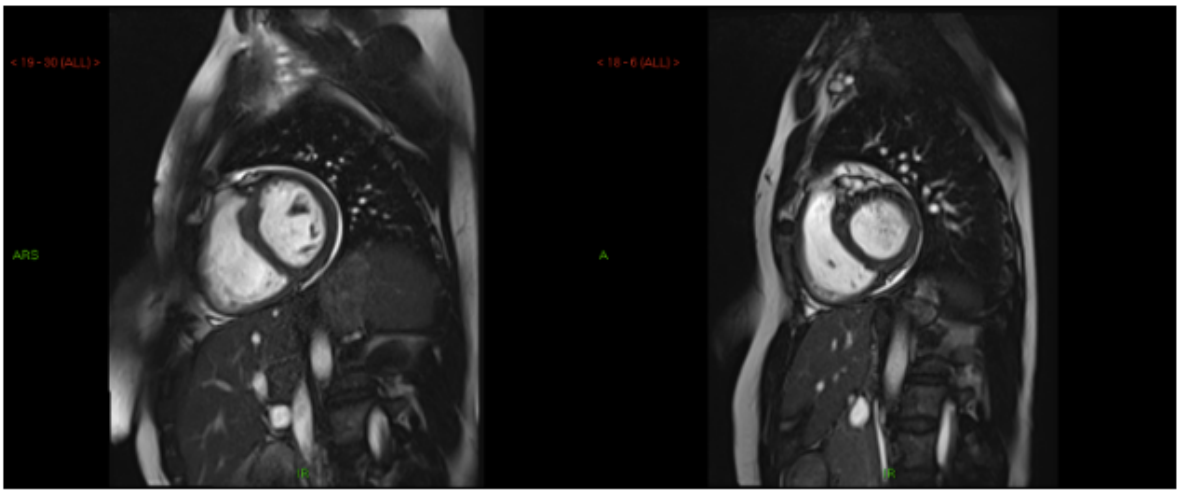
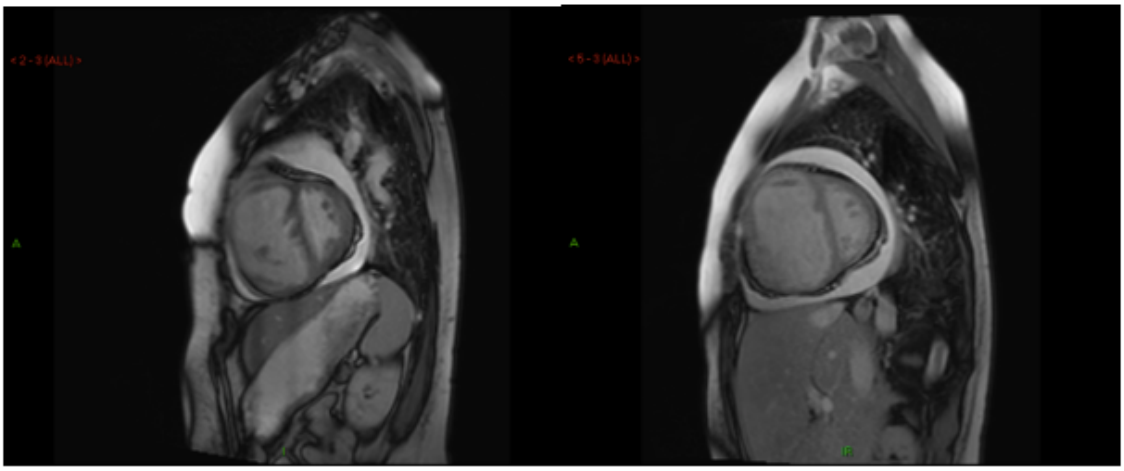


Figure 8.7:CMR before (left) and after exercise therapy (right) in a 33y old female subject with IPAH and improved exercise capacity

The second subject (Figure **8.8**) was a 38y old female, also on triple therapy for IPAH. She was diagnosed 12y and 6 months prior to the exercise programme and was undergoing work up for lung transplantation at time of enrolment. Her baseline 6MWD was 468m, at the end of study 6MWD was 470m. Despite no change in exercise capacity, this subject demonstrated a drop in LVEF from 41% to 33% at the end of the study, with an increase in RV end systolic volume from 312ml to 318ml and clear evidence of septal bowing and a "D-shaped" left ventricle. This subject was accepted for lung transplantation shortly after the exercise programme and went on to have a successful lung transplant in 2018.



Before After

Figure 8.8 **CMR before (left) and after exercise therapy (right) in 37y old female with IPAH and deterioration in 6MWD following exercise therapy.**

8.4.2.3 Transplant free survival

At censoring, 7 study participants had been transplanted (2) or had died (5). These subjects had poorer RV function both at baseline and the end of the study. They also had poorer LV function at the end of the exercise programme, compared to those who remained alive (

Table 8.16).

Table 8.16: Significant differences in CMR variables in those alive versus those dead or transplanted at censoring

	Dead or transplanted (7)	Alive (22)	p
LV EF EOS %	51 (15)	67 (9)	0.028
LV SV EOS mL	54 (25)	84 (23)	0.039
RV EF baseline %	32 (9)	48 (12)	0.008
RV EDV baseline mL	238 (71)	162 (51)	0.012
RV ESV baseline mL	169 (66)	89 (35)	0.001
RV ESV EOS mL	184 (92)	84 (36)	0.009
RV mass g	83 (33)	54 (18)	0.023
RV EF EOS %	30 (12)	51 (11)	0.012

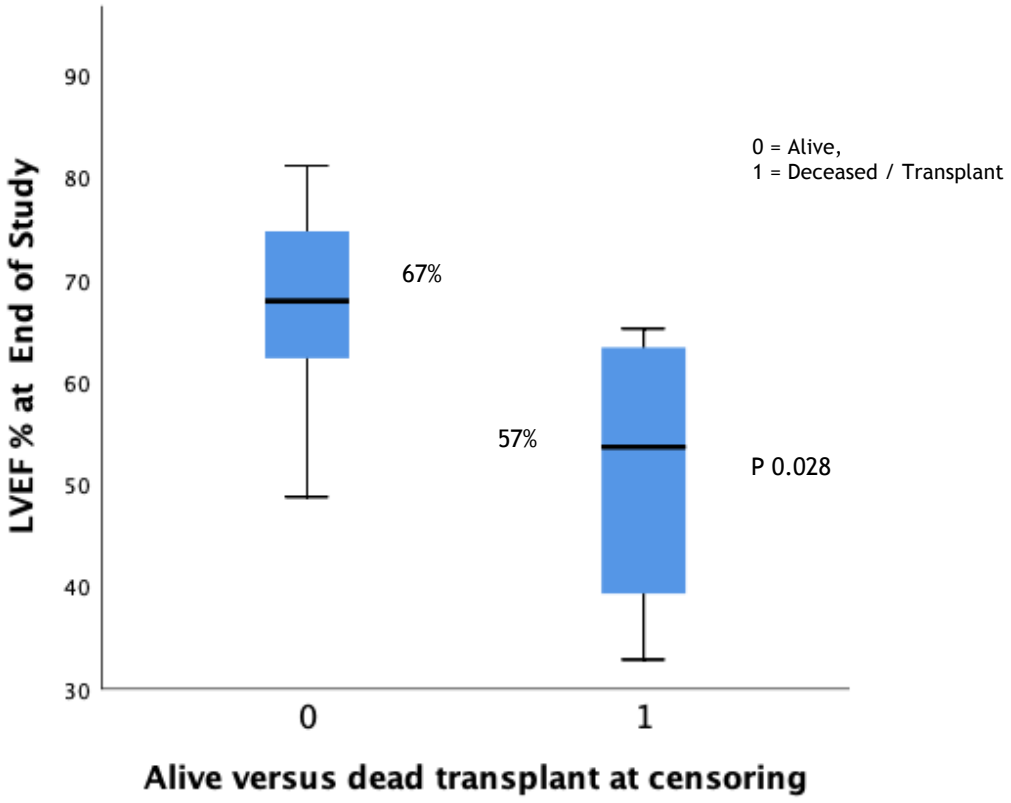


Figure 8.9: LV ejection fraction at the end of study in alive subjects versus transplanted or deceased

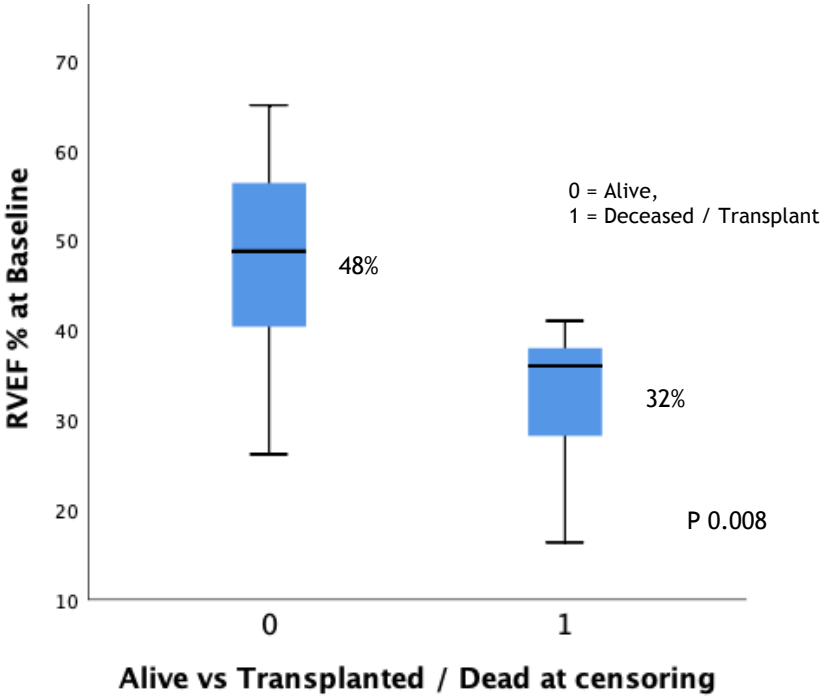


Figure 8.10: RV ejection fraction at study entry in alive subjects versus transplanted or deceased

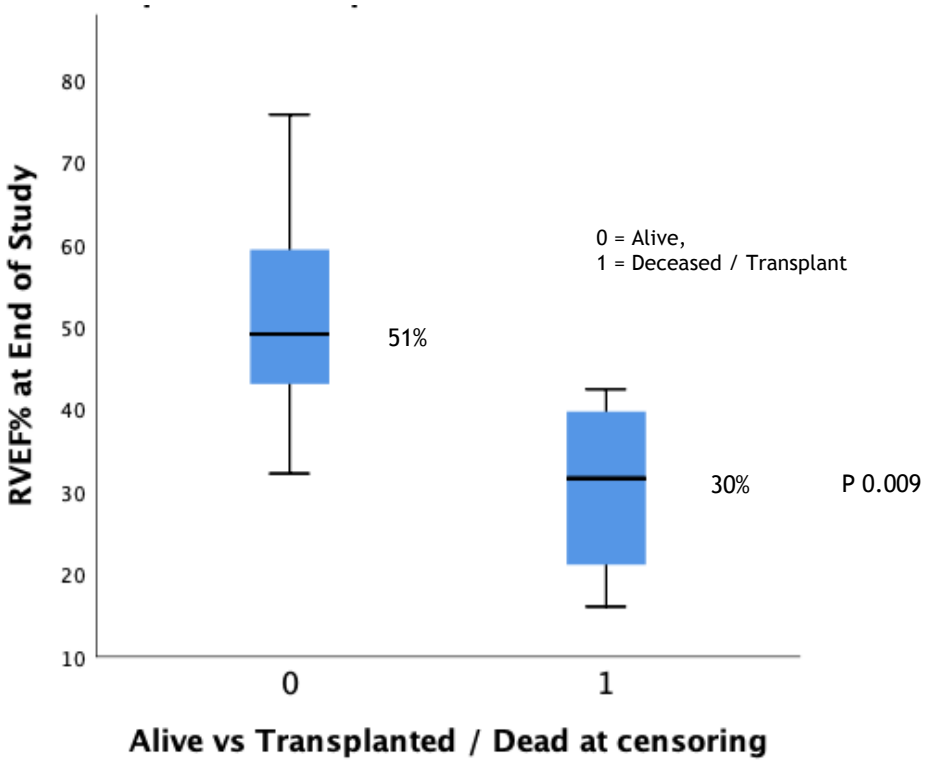


Figure 8.11: RV ejection fraction at the end of study in alive subjects versus transplanted

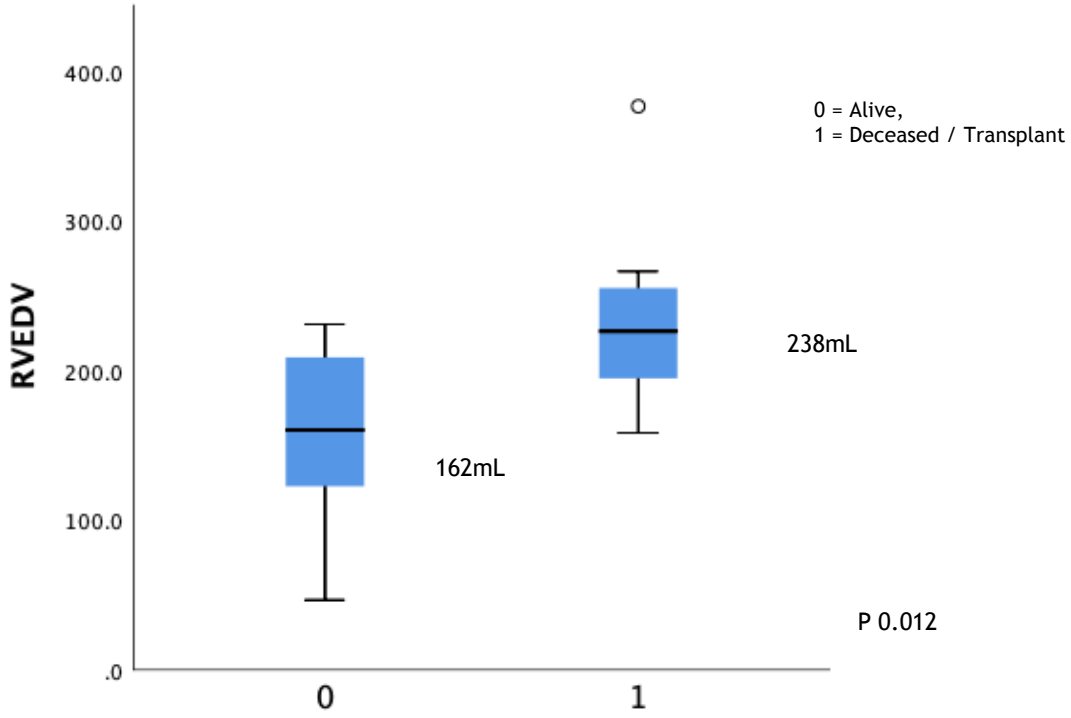


Figure 8.12: RV end diastolic volume at baseline in alive subjects versus transplanted

8.4.2.4 **Relationship between cardiac structure and function**

The relationships between Cardiac MRI measurements pre and post exercise in PAH and a number of invasive and non-invasive measures of cardiorespiratory function were explored. Specifically, cardiac function (flow and ejection fraction) and cardiac structure (mass and volumes).

Function

In keeping with ventricular interdependence and the impact of RV volume on LV function, a strong correlation was seen between RV and LV ejection fraction; R 0.727, p <0.005 and also between LV ejection fraction and RV end systolic volume; R -0.604, p 0.001.

CMR Measures of cardiac output showed good agreement with invasively measured CO by thermodilution during right heart catheterisation (Figure 8.13). CMR Stroke volume correlated with surrogates of SV on CPET and invasively measured SV and CO (Table 8.17).

Table 8.17: CMR CO and SV correlates

MRI variable	CO/SV Correlate	Spearman R	p	n
CMR CO flow map (L)	CO (RHC)	0.608	0.003	21
CMR CO flow map (L)	Delta O_2pulse (mL/beat)	0.573	0.003	24
CMR stroke volume (mL)	SV (mL) RHC	0.674	0.001	21

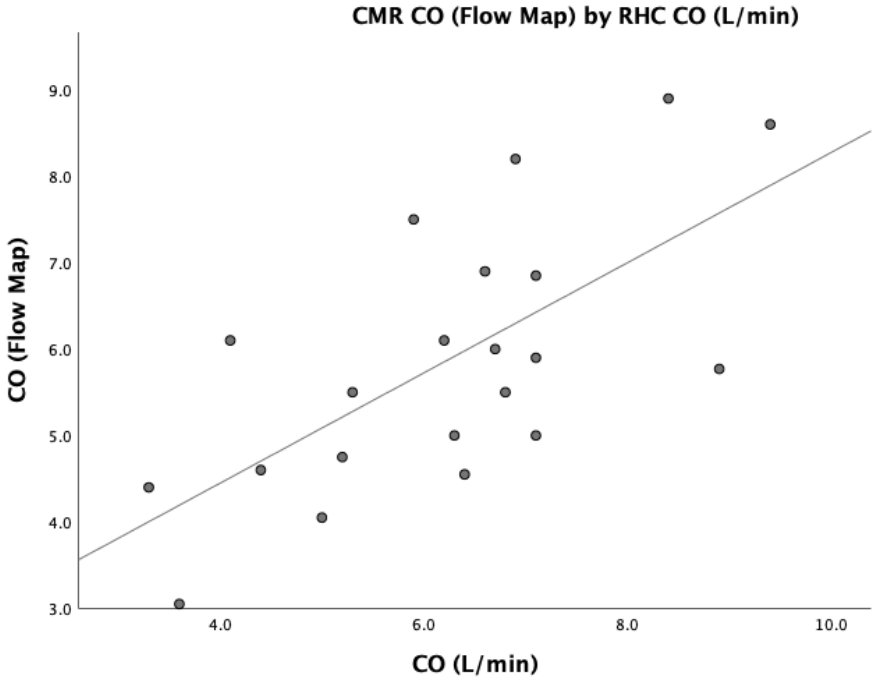


Figure 8.13: Correlation between Cardiac Output measured by RHC and CMR

Baseline CMR Stroke volume, measured by flow map also correlated strongly with peak aerobic exercise capacity and change in invasively measured pulmonary artery pressures following exercise therapy (Table 8.18).

Table 8.18: CMR stroke volume correlations

CPET variable	Spearman R	p	n
Peak VO_2 (L/min)	0.637	0.001	24
Delta SPAP (mmHg)	-0.639	0.008	16
Delta mPAP (mmHg)	-0.590	0.016	16

Change in SV on CMR correlated with gas exchange on CPET and change in SPAP measured during RHC (Table 8.19).

Table 8.19: Change in Stroke Volume correlations

Correlate	Spearman R	p	n
VE/VCO_2	-0.521	0.011	23
Delta SPAP (mmHg)	-0.639	0.008	16

Left ventricular stroke volume correlated with pulmonary artery compliance at baseline: R^2 0.619, p 0.003, N 21. (Figure 8.14)

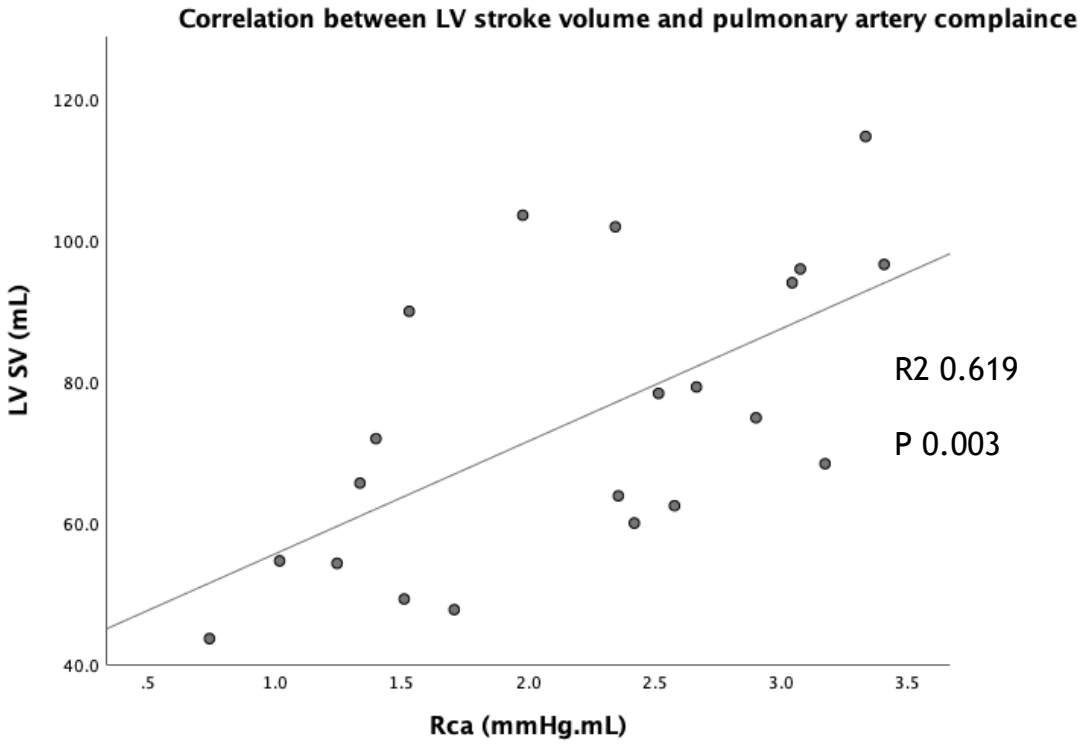


Figure 8.14: LV stroke volume and PA compliance correlation

Mass

LV mass demonstrated a moderate correlation with CO and SV as measured by RHC (Table 8.20, Figure 8.15)

Table 8.20: Correlations with LV mass

Correlate	Spearman R	p	n
CO (L) (RHC)	0.546	0.01	21
SV (mL) (RHC)	0.561	0.008	21

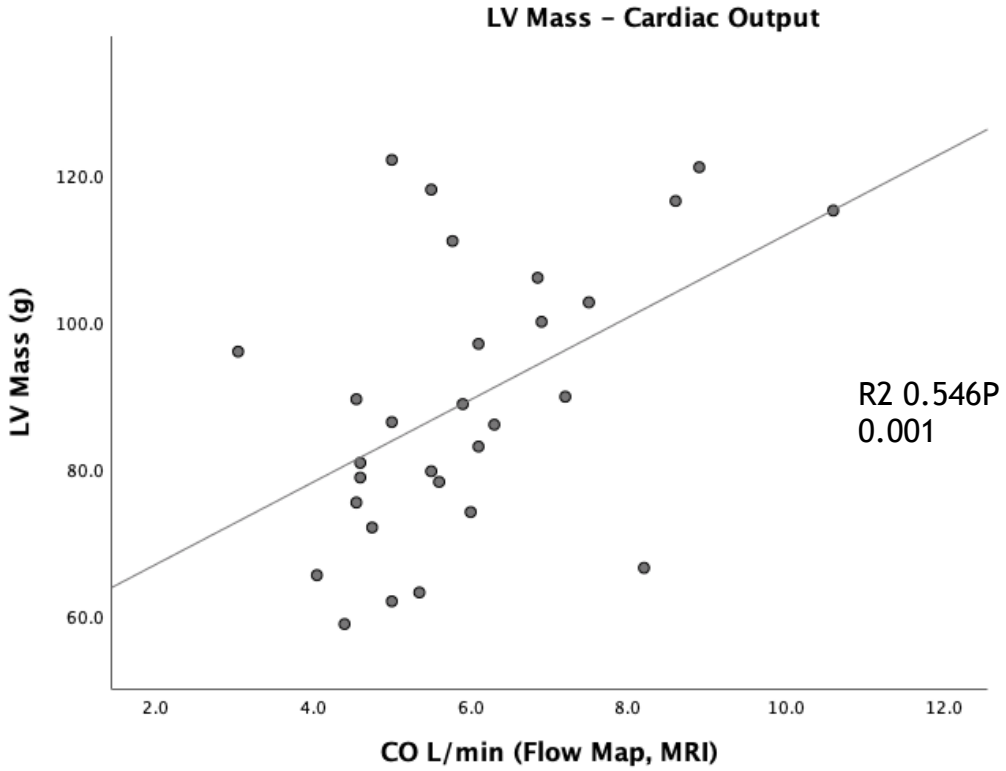


Figure 8.15: LV mass and cardiac output correlation

RV mass correlated with invasively measured pulmonary artery pressures at baseline and with change in RVOPF, Rca, SPAP and PAWP following exercise therapy (Table 8.21, Table 8.22).

Table 8.21: Correlation of RV mass with baseline invasive haemodynamic variables

Correlate	Spearman R	p	n
Baseline mPAP (mmHg)	0.627	0.002	21
Baseline SPAP (mmHg)	0.655	0.001	21

Table 8.22: Correlation of RV mass with change in invasively measured haemodynamics

Delta PAWP	0.602	0.014	16
Delta SPAP (mmHg)	0.547	0.028	16
Delta RVOPF (mmHg)	0.696	0.003	16
Delta Rca (ml/mmHg)	-0.559	0.024	16

There was no significant change in RV mass with exercise therapy and delta RV mass did not correlate with changes in invasively measured haemodynamic variables.

Volumes

Right ventricular end diastolic volume (RVEDV) correlated with CO, PVR and invasively measured PAPs (Table 8.23)

Table 8.23: RVEDV correlations with invasive haemodynamic measurements

	Spearman R	p	n
PVR (WU)	-0.504	0.02	21
CO RHC (L)	0.697	<0.005	21
SV RHC (mL)	0.642	0.002	21
mPAP (mmHg)	0.635	0.002	21
SPAP (mmHg)	0.65	0.001	21
DPAP (mmHg)	0.529	0.014	21
SPAP (mmHg)	0.65	0.001	21

Multiple regression analysis was performed for RVEDV. Adjusted R^2 for this model was 0.873, p 0.005. Cardiac Output and PVR remained significantly associated with RVEDV (Table 8.24).

Table 8.24: Multivariate analysis of RV end diastolic volume correlations

	B	Std. Error	Beta	t	p
(Constant)	-340.496	101.001		-3.371	0.005
PVR (WU)	55.915	12.305	2.118	4.544	0.000
CO (L/min)	64.698	20.051	1.466	3.227	0.006
SV (mL)	0.424	1.028	0.128	0.412	0.686
MPAP (mmHg)	4.874	7.767	0.843	0.628	0.540
SPAP (mmHg)	-4.624	3.253	-1.224	-1.421	0.177
DPAP (mmHg)	-5.058	4.806	-0.702	-1.053	0.310

RV end systolic volume also correlated with invasively measured pressures, however less strongly that RVEDV (Table 8.25).

Table 8.25: Correlation with RV end systolic volume

Variable	Spearman R	p	n
mPAP (mmHg)	0.57	0.007	21
SPAP (mmHg)	0.605	0.004	21
delta PAWP (mmHg)	0.62	0.010	16

LV end diastolic volume correlated strongly with both SV measured on CMR and RHC (Table 8.26,Figure 8.16). The relationship between stroke volume was stronger than that seen with RV volumes.

Table 8.26: Correlation between LV Volumes and Stroke Volume

Variable 1	Variable 2	Spearman R	p	n
MRI SV baseline (mL)	LVEDV Baseline	0.614	<0.005	29
RHC SV baseline (mL)	LVEDV Baseline	0.783	0.003	21
MRI SV EOS (mL)	LVEDV EOS	0.852	<0.005	26
RHC SV EOS (mL)	LVEDV EOS	0.572	0.020	16

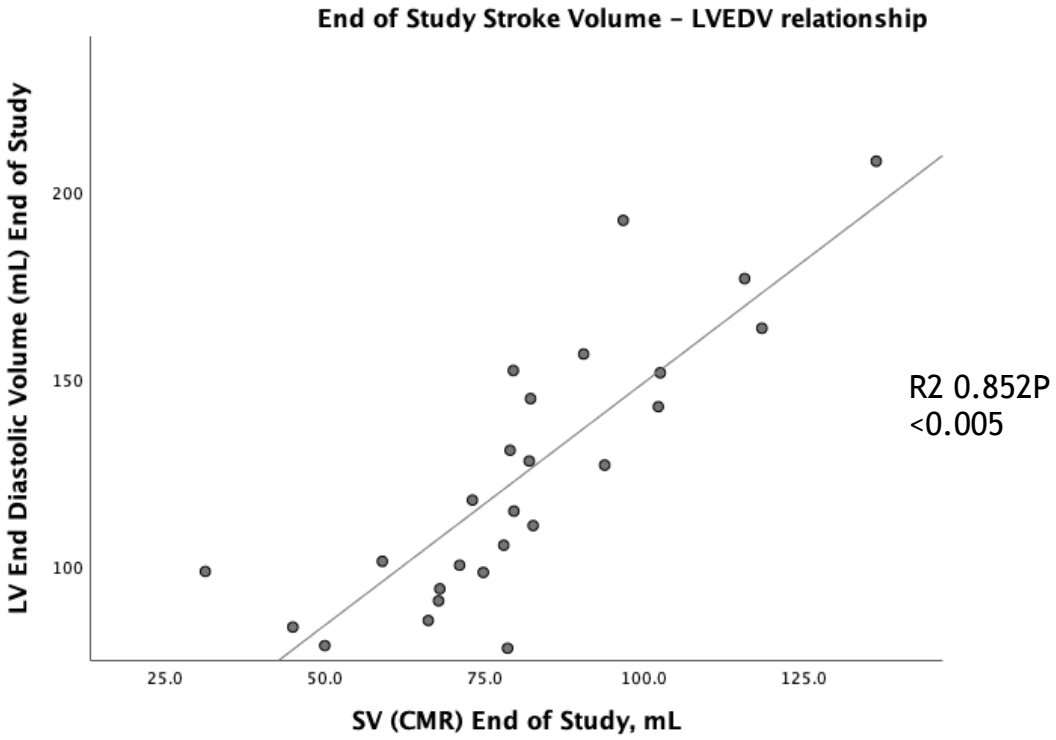


Figure 8.16: Stroke volume and LV end diastolic volume

8.5 **Discussion**

8.5.1 **NTproBNP**

No change in NTproBNP was noted with exercise therapy. NTproBNP was higher at baseline in those who did not improve with exercise therapy; there was no significant rise noted in this group. In smaller studies assessing treatment response in PAH, NTproBNP appears to show little or no change. Larger scale pharmacotherapy studies have demonstrated that changes can be detected following treatment [250, 251]; NTproBNP therefore appears to be a less sensitive biomarker for assessing treatment response or identifying a deteriorating patient who may require treatment escalation or cessation of the exercise programme.

Although NT pro BNP did not provide sensitive information about treatment response in isolation, the prognostic value of absolute NTproBNP was clear. NTproBNP was lower in those who were alive and untransplanted at censoring at baseline and follow up. Additionally, it correlated with a number of relevant prognostic variables at baseline and follow up relating to exercise capacity, cardiovascular function and gas exchange.

It is possible that the small sample size in this study limited the usefulness of the test.

8.5.2 CMR

8.5.2.1 Baseline characteristics

The population in this study had increased RV mass, however ventricular volumes and function were relatively preserved, despite impaired exercise tolerance. This is in keeping with previously published data, showing that RV ejection fraction can be maintained or even improve with drug therapy in the face of deteriorating haemodynamics and clinical condition[238].

Invasively measured Cardiac Output and stroke volume showed good agreement with CMR measures, highlighting the contribution that CMR can provide in the non-invasive assessment and following up cardiac function.

8.5.2.2 Changes in the right heart with exercise therapy

Function

With exercise therapy, cardiac output and stroke volume improved significantly in those who completed the exercise programme. CMR stroke volume correlated with exercise capacity (peak VO_2) and changes in pulmonary artery pressures, therefore demonstrating the use of non-invasively measured SV as a clinically relevant marker relating to invasive haemodynamics and disease severity. There was a trend towards improvement in RV ejection fraction, however this did not reach statistical significance.

Volumes

As previously published, ventricular volumes hold important prognostic value in PAH and can indicate deteriorating clinical status in the face of preserved RVEF[238]. Data from this study are in keeping with the published literature, with RVEDV correlating with CO, SV and invasively measured haemodynamics. RV end systolic volume also correlated strongly with invasively pressures. RVEDV and

RVESV were significantly higher in those that were transplanted or died in association with a lower RV ejection fraction.

Mass

RV mass correlated with pulmonary artery pressures and correlated with changes in PA compliance and right ventricular oscillatory power fraction. This is in keeping with the known pathophysiology of right heart failure in PAH; RV hypertrophy is an adaptive response to overcome increased RV afterload and the reduced efficiency of the RV-PA relationship, as reflected in the RVOPF. Exercise therapy did not appear to influence RV mass. It is possible that changes in RV mass may take longer to reverse than 15 weeks. Additionally, there may be counter effects of isometric exercise which would work to increase ventricular mass, whilst the presumed reduction in afterload would contribute to a reduction in ventricular mass.

8.5.2.3 Relationship between the Left Ventricle and the Right Ventricle-Pulmonary Circulation

The role of the left ventricle in pulmonary hypertension and its close relationship with the RV is important and clearly demonstrated in this study.

The impact of a volume overloaded RV on biventricular function was highlighted in the strong correlation between RV and LV function, as well as RV end systolic volume and left ventricular ejection fraction. There was a strong correlation between LV end diastolic volume and stroke volume both at baseline and at the end of the study.

LV stroke volume improved following exercise therapy and correlated with pulmonary artery compliance, therefore, the more compliant the pulmonary circulation, the higher the LV stroke volume. As discussed in Chapter 7, PA compliance seems to be a key marker of response to treatment and a more sensitive marker than invasive pressure or volume measurements. In subjects who died or were transplanted at censoring, LV ejection fraction and LV stroke volume were significantly lower.

LV mass showed moderate positive correlation with SV and CO (RHC); the latter observation would fit with the “deconditioned” and atrophic LV that occurs in more severe PAH due to reduced filling.

Interestingly, there seems to be scope to improve LV function without seeing significant improvements in RV function or RV volumes. It may be that the “deconditioned” LV is responsive in its own right to exercise training, as is seen in health and other medical conditions such as left ventricular systolic failure, where reverse remodelling can occur. It could also be that improvements in the LV are one of the earlier signs of positive change with exercise therapy.

In subjects with improved 6MWD, left ventricular ejection fraction and cardiac output were significantly higher than those who deteriorated or did not improve. In those who were alive and untransplanted at censoring, LV and RV function were significantly higher and ventricular volumes were significantly lower.

8.5.2.4 **Influence of gender**

Analysis of gender difference was performed in a post hoc analysis, therefore these data are exploratory as the study was not powered to detect a between group difference in response to exercise therapy between males and females. Gender differences appeared to exist both in baseline cardiac structure and function and in the response to exercise therapy in this study. In male subjects, RV ejection fraction was lower than normal. Female subjects appeared to have less severe haemodynamic impairment, however the only statistically significant differences between groups were in RV and LV mass, which may be expected given the naturally higher RV and LV mass in males. It is well established that PAH is more common in females[252], however a more severe clinical phenotype manifests in male patients; the so called oestrogen paradox[253]. Post hoc analysis showed trends to poorer indices of cardiac function in males. The exercise responses between male and female with PAH warrants further investigation.

8.5.2.5 **Future research and limitations**

Going forwards, research should focus on developing more sensitive methods of detecting treatment response and predicting RV failure. Given the changes seen in

Chapter 7, exercise CMR may capture earlier changes in dynamic RV function and may be more sensitive at detecting treatment response. This study may be limited by small sample size, therefore restricting evaluation of the role of CMR in follow up. Gender differences and associations with other clinical variables were post hoc and exploratory, future larger prospective studies would be useful to characterise the gender differences in cardiac structure and function PAH and response to exercise therapy.

8.6 **Conclusions**

PAH specific exercise therapy improves cardiac function, with improved cardiac output and stroke volume measured by MRI.

Cardiac MRI and NTproBNP provide useful and reliable non-invasive information regarding cardiac structural and functional changes with exercise therapy in PAH. Both modalities correlate with invasively measured haemodynamic variables and provide good surrogates for invasive measurements of cardiac function and disease severity. In isolation, NTproBNP is not sufficiently sensitive in detecting treatment response, however changes can be detected with Cardiac MRI.

Gender difference exist in cardiac structure, function and response to exercise therapy, with females displaying a less severe phenotype.

Chapter 9 Changes in muscle function, systemic inflammation and metabolism in response to exercise therapy in PAH.

9.1 Introduction

In PAH, improvements in exercise capacity occur with pulmonary arterial vasodilators, however a high degree of morbidity and exercise tolerance persist, despite optimal drug therapy. The pulmonary vasculature in PAH exhibits a cancer-like phenotype that promotes cell proliferation and resistance to apoptosis. Beyond the pulmonary circulation, it is well established that abnormalities in metabolic and muscle function exist and patients demonstrate a pro-inflammatory phenotype [254]. These changes have been associated with poorer RV function and survival[255] . This has led to an ongoing search for new therapies beyond the prostacyclin, nitric oxide and endothelin pathways

9.1.1 Metabolic function in PAH

The molecular origins of PAH promote remodelling of the pulmonary vasculature, characterised by hyperproliferation and increased cellular survival. Metabolic dysregulation has emerged as a candidate molecular driver of PAH pathogenesis [4]. Metabolic changes have been documented in the pulmonary arteries, right ventricle, skeletal muscle and serum of patients with PAH [256]. These changes are noted in the initial development of disease and early stages through to the end stages [5, 6].

9.1.1.1 BMPR2

The BMPR2 mutation is the most common genetic mutation in PAH, seen in up to 70% of heritable cases and 20% of idiopathic cases [257]. In metabolomic studies, it has been linked to downstream reprogramming in multiple independent metabolic pathways, with changes being more extensive in patients with higher disease severity [258]. In animal models and human PAECS, reduced BMPR2 expression resulted in pulmonary artery endothelial cell (PAEC) mitochondrial dysfunction,

promoting a pro-inflammatory or pro-apoptotic state and was associated with reduced microvessel density in pulmonary arteries[259]

9.1.1.2 **Insulin resistance**

Insulin resistance is a state in which a given concentration of insulin is associated with a sub-normal glucose response. The Homeostasis Model Assessment (HOMA) estimates steady state beta cell function (%B) and insulin sensitivity (%S), as percentages of a normal reference population, using fasting plasma glucose and fasting plasma insulin. As insulin sensitivity decreases, insulin production increases to compensate for decreasing beta cell function. The HOMA-IR model measures this relationship; as the HOMA-IR value increases, so does insulin resistance[260]. The original HOMA-IR relationship is described as below:

$$\text{HOMA1-IR} = \frac{\text{FPI x PFG}}{22.5}$$

$$\text{HOMA1-5B} = \frac{20 \text{ x FPI}}{\text{FPG} - 3.5}$$

FPI: Fasting plasma insulin, FPG: Fasting plasma glucose

An updated non-linear model was developed in 2004 to account for alterations in hepatic and peripheral glucose resistance, increases in the insulin secretion curve for plasma glucose concentrations greater than 10 mmol/L and the contribution of circulating proinsulin[261]. This model allows use of C-peptide in place of plasma insulin concentration. C-peptide is released from pancreatic beta-cells during insulin cleavage and has the reference range 0.26-1.03 nmol/L.

Metabolic profiling in advanced PAH lung tissue at time of transplant has demonstrated disrupted glycolysis in the cytoplasm, altered glucose metabolism through the TCA pathway in the mitochondria, and altered fatty acid metabolism through beta-oxidation in the smooth endoplasmic reticulum in addition to ß-oxidation in the mitochondria in the progression of severe PAH [262]. In animal models, insulin resistance (IR) rather than elevated lipid content is associated with

the presence of pulmonary hypertension in animal models. Insulin resistance has been documented in up to 45% of patients with IPAH compared with age, sex and BMI matched controls and has been associated with circulating markers of inflammation and vascular dysfunction. Insulin resistance has been associated with poorer 6 month event free survival (79% vs 58%) risk in IR versus insulin sensitive patients with PAH [263].

9.1.1.3 **Effects of exercise on metabolic function**

In health, exercise training enhances insulin sensitivity and induces increased expression of several key proteins involved in insulin-stimulated glucose metabolism, such as the glucose transporter GLUT4, Hexokinase-II, and glycogen synthase[264]. In a wide range of chronic health conditions, exercise therapy has been shown to improve metabolic function. In dialysis dependent chronic kidney disease, exercise training improved insulin sensitivity, associated with improvements in systemic blood pressure, without change in body weight[265]. Physical training improved hyperinsulinaemia and insulin resistance in patients with chronic left ventricular failure[266]. The effects of exercise have not been specifically studied in relation to metabolic function in PAH.

9.1.2 **Muscle Function**

9.1.2.1 **Skeletal muscle changes in PAH**

Skeletal muscle dysfunction in IPAH is well documented [267]. Clinically, leg fatigue and dyspnoea during exercise are potential indicators. Maximal volitional and non-volitional strength of both the quadriceps as well as the inspiratory muscles are reduced in PAH-patients and are closely correlated to exercise capacity[268]. Controversy exists as to whether PAH myopathy is due to the atrophying effects of low cardiac output, deconditioning or an independent systemic process (Figure 9.1).

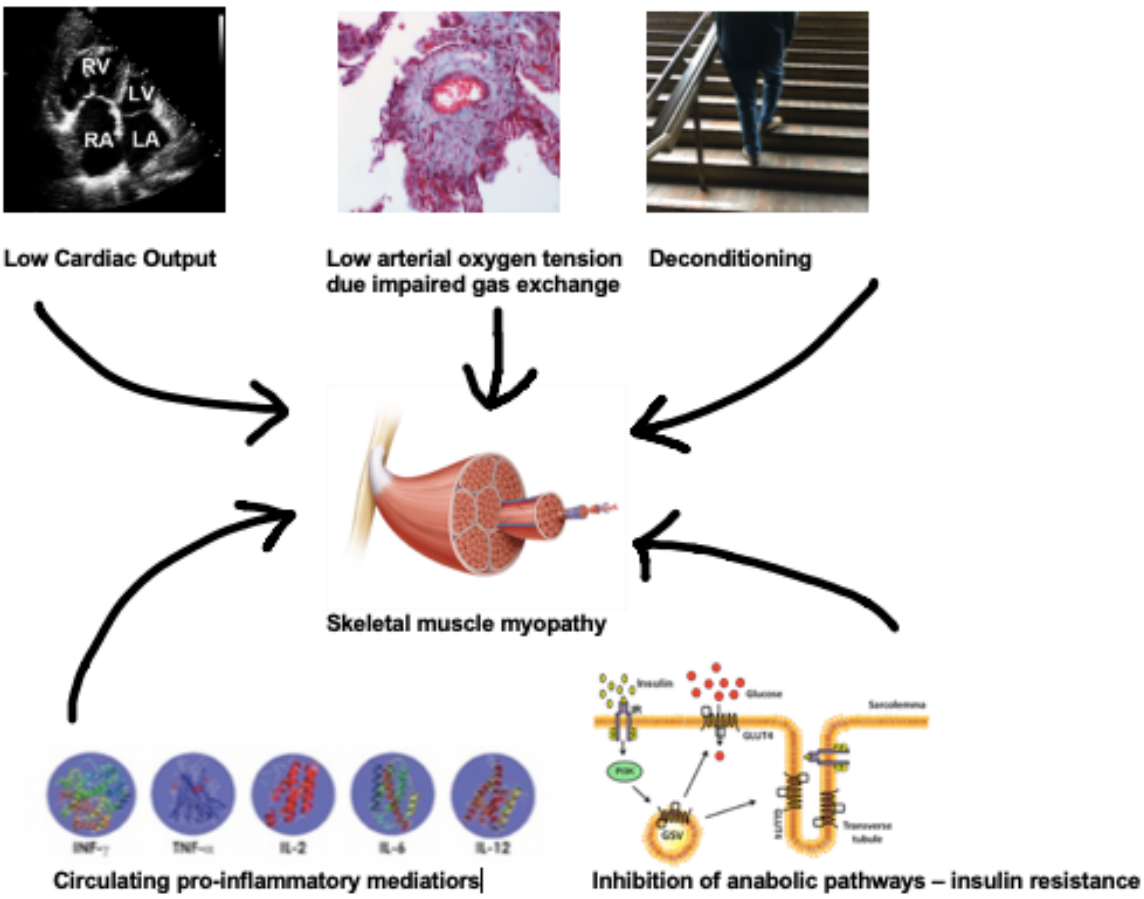


Figure 9.1: Potential mechanisms of skeletal muscle myopathy in PAH

At a physiological level, attempts have been made to study oxygen delivery and extraction balance in IPAH using near infrared spectroscopy at rest and on exercise. In PAH patients with higher PVR, the change in deoxygenated muscle haemoglobin on exercise was faster than the change in VO_2 (a so called "overshoot" phenomenon), suggesting a delivery – utilisation mismatch, with extraction being sufficient but delivery inadequate [269].

At a histological level, there is a shift from highly oxidative, fatigue resistant slow-twitch type I muscle fibres to fast twitch type II muscle fibres and reduction in peripheral skeletal muscle capillarisation occurs. Reduced skeletal muscle capillarisation correlates with markers of exercise capacity such as VO_2 and 6MWD [270].

At a cellular level, the Warburg effect describes the shift from oxidative phosphorylation to glycolysis in the presence of adequate oxygen tension[271, 272]. This phenomenon has been observed in the pulmonary circulation, RV and

skeletal muscle in PAH. There is a reduction in skeletal muscle oxidative enzyme capacity and reduced function and numbers of mitochondria, resulting in a higher potential for anaerobic metabolism compared with aerobic metabolism [267]. Importantly, these changes, correlate with exercise capacity and muscle strength, but are independent of the severity of pulmonary haemodynamics, suggesting a mechanism other than the atrophying effect of low cardiac output [273]. BMPR2 mutations, may again explain this phenomenon; in normoxia, reductions in BMPR2 are associated with increased ATP production, glycolysis, mitochondrial fission and a pro inflammatory, catabolic state[259].

9.1.2.2 Skeletal muscle changes in deconditioning and chronic health conditions

Deconditioning results in a number of structural and biochemical changes in skeletal muscle. Muscle mass decreases and type IIa fibres (fatigue resistant, high oxidative and glycolytic capacity) tend to convert to type IIb (low oxidative and high glycoloytic capacity; fatigue rapidly). There is a decrease in oxidative enzyme concentration, the number and density of mitochondria, and the number of capillaries. In other health conditions such as left ventricular failure, it has been demonstrated that in age, and VO_2 matched individuals, deconditioning did not account for changes in skeletal muscle oxidative capacity[274]. The contribution of deconditioning to myopathy in PAH has not been studied, however it is unlikely to be the sole driver for myopathy, given skeletal muscle changes are present in subjects with relatively preserved exercise tolerance.

9.1.2.3 Potential pathways and biomarkers in PAH myopathy

Micro-RNAs (miRs) are small, non-coding RNA molecules that are involved in post-transcriptional regulation of gene expression. Gene expression control by miRs is an important mechanism for maintenance of cellular homeostasis in response to different stimuli including exercise.

Specific pathways have been identified in the pathogenesis of myopathy in PAH. In skeletal muscle biopsies of patients with PAH, miR-126 expression was reduced by 60% compared with healthy controls and SPRED (a target of miR126) was upregulated [103], resulting in a downstream reduction in effectors of the VEGF

pathway. miR-126 expression correlated with capillary density and peak VO_2 in PAH, suggesting a role in angiogenesis in PAH. In this study, 3 patients had skeletal muscle biopsies before and after an exercise training programme with no significant difference in miR-126 expression. Systemic angiogenic defects may therefore contribute to skeletal muscle microcirculation rarefaction and exercise intolerance in PAH and appear to be unrelated to haemodynamic severity in this small study. A number of other miRs have been identified in relation to skeletal muscle function, blood vessel formation and inflammation in PAH, these are summarized in Table 9.1.

Table 9.1: Micro-RNA associations with muscle function and PAH

Function	Specific miR
Skeletal muscle specific miRs (myomirs) [275]	miR-1
	miR-133a/b
	miR-206
	miR-26(a)
	miR-29(a)
	miR-378
	miR-451
Non-specific miRs involved in skeletal muscle myogenesis [276]	miR-31
	miR-489
	miR-106b
	miR-25
	miR-29C
	miR-23(a/b)
Vascular Function and angiogenesis [277]	miR-17
	miR-221/222
	miR-126
	miR-92(a)
	miR-132
	miR-26(a)
Inflammation related / PAH cell signalling miRs [278-280]	miR-204
	miR-21
	miR-199a
	miR-145
	miR-155
	miR-124

9.1.2.4 Exercise training and skeletal muscle effects in chronic cardiorespiratory conditions

The effects of physical exercise on skeletal muscle are in many cases associated with changes in gene expression. The characteristics of exercise (intensity,

duration, frequency, modality, trained level) and age, play a central role in the regulation of gene expression. Physical exercise modulates expression of several miRs involved in protein synthesis, such as miR-26a, miR-29a, miR-378 and miR-451. In health, change in expression of miRs such as miR-20a correlate with changes in VO_2 following exercise training[104]. In health, miR-761 is related to skeletal muscle mitochondrial oxidative capacity and biogenesis via inhibition of p38 mitogen-activated protein kinase (MAPK) signalling pathway[281]; inhibition of this pathway is of relevance in PAH as expression of p38 MAPK is increased in animal models of the hypertrophied RV and in human pulmonary arteries[282]; inhibition of MAPK leads to diminished RV fibrosis and improved RV function in animal models[283].

The AKT pathway stimulates skeletal muscle growth via the pro anabolic peptide insulin-like growth-factor 1 (IGF-1)[284]. In left ventricular failure (LVF) and cancer cachexia, down regulation of the AKT pathway is linked to skeletal muscle atrophy. In LVF, this is predominantly due to a flow related decrease in blood and nutrient supply to the highly oxidative, fatigue-resistant type I muscle fibres, with exercise training exerting positive effects [285]. In a monocrotaline PAH rat model, the AKT pathway was unchanged in control versus PAH rats, whilst significant skeletal muscle loss and increased circulating inflammatory biomarkers (IL-1beta, CRP, myostatin) and local catabolic markers (MAFbx/atrogin-1 and protease) were noted [286]. The changes in PAH do not mirror the changes in left heart failure, therefore suggesting a different pathogenesis of myopathy and in keeping with changes not being solely flow related.

9.1.2.5 Exercise training and effects on PAH skeletal muscle

Studies with combined aerobic and resistance training in PAH have demonstrated improvements in quadriceps strength and peak aerobic exercise capacity [149]. Limited data exist on the effects of exercise and skeletal muscle at a histological level in PAH. In a small study of 5 IPAH patients, skeletal muscle biopsies pre and post exercise training resulted in a reduction in the proportion of type IIx fibres, this was associated with improved 6MWD and improved ventilatory efficiency on CPET [78].

Several studies have used less intensive exercise programmes. A Dutch study of 12 IPAH patients found that low intensity rehabilitation resulted in improved quadriceps strength and endurance time, with increased muscle capillarity and improved oxidative capacity, but importantly, no change in exercise capacity or prognostic variables [63]. Further studies utilising existing cardiac rehabilitation programmes achieved improvements in quadriceps strength and endurance, with increased capillarity density, improved succinate dehydrogenase activity and increased numbers of fast twitch fibres. The latter two changes being associated with quadriceps endurance [63], however no change was noted in exercise capacity.

From the existing evidence, it seems clear that exercise can induce positive effects in PAH myopathy, however the mechanisms in which to most effectively improve muscle function remain unclear.

9.1.3 **Inflammation**

Inflammation is an essential immune response and plays an important role in tissue repair. However, a persistence of inflammation beyond the initial repair, leading to chronically elevated levels of pro-inflammatory markers, exerts deleterious effects[287].

There is a pro-inflammatory phenotype in PAH. Increased circulating levels of monocyte chemo attractant protein-1, tumour necrosis factor- (TNF-α), interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70 and IL-64 in both PAH animal models and patients have been demonstrated [288]. Inflammatory infiltrates are also seen in plexiform lesions in pulmonary arteries. In animal models of PAH, over-expression of IL-6 leads to severe pulmonary hypertension in mice and under-expression provides a protective effect from experimental hypoxia driven PH [289].

Pro-inflammatory cytokines have detrimental effects on skeletal muscle through damage to contractile proteins and stimulation of proteolysis [290]. Furthermore, elevated levels of pro-inflammatory cytokines (interleukin-6, 8, 10, and 12p70) are associated with poorer survival in IPAH, irrespective of haemodynamics [291]. In a

study of chronic thromboemolic pulmonary hypertension (CTEPH) patients, IP-10 negatively correlated with cardiac index and 6mwd[101].

9.1.3.1 Composite serum marker of inflammation

The potential use of routinely measured, non-invasive serum measurements as biomarkers for systemic inflammation in PAH is appealing, particularly when assessing the response to therapy. Three scoring systems, discussed below, have been widely validated in prognostication of a wide range of cancers. Given the parallels between cancer and PAH pathobiology these scoring systems have potential to inform on levels of inflammation in PAH and have yet to be studied in this condition.

Modified Glasgow Prognostic Score

The modified Glasgow prognostic score (mGPS) is a simple scoring system combining serum CRP and Albumin. One point is allocated for a CRP greater than 10 mg/L, one point is allocated if the Albumin is under 35 g/L, with the possible scores ranging from 0 to 2. The mGPS was initially validated in colonic cancer, providing independent prognostic information. It has since been found to be associated with prognosis across a wide range of malignancies. It is also associated with muscle loss, poor performance status, increased comorbidity, increased pro-inflammatory and angiogenic cytokines[292].

Neutrophil to Lymphocyte Ratio

In several forms of cancer, neutrophilic inflammation has been associated with poorer prognosis. The Neutrophil to Lymphocyte Ratio (NLR) captures subclinical inflammation and is a marker of impaired cell-mediated immunity associated with systemic inflammation. The normal range is 1 to 3. [293]

Platelet to Lymphocyte Ratio

The platelet to lymphocyte ratio (PLR) has been extensively examined in cancer studies and out with oncology, in rheumatology and cardiovascular studies, PLR has been shown to correlate with the severity of inflammation and is helpful in

predicting infection and severe organ damage in SLE [294], the normal range is around 50-200[295].

9.1.3.2 Exercise therapy and inflammation

The effect of exercise on the pro-inflammatory cytokine profile has not yet been investigated in PAH. In health, the anti-inflammatory effects of exercise have been described for many years and are proposed form part of the protective mechanism against developing conditions such as type II diabetes[296]. Chronic inflammation underlies a variety of seemingly unrelated disease processes. In left ventricular failure, cytokines such as TNF-alpha reduce with exercise training and correlate with improved exercise capacity [102]. In type II diabetes, exercise training reduced high sensitivity CRP and pro-inflammatory cytokines[297]

9.2 Aims

The aims of this chapter are to explore the effect of exercise training in PAH on

1. Systemic inflammation: IL-6 and composite scores mGPS, NLR and PLR

2. Metabolism: Assessing insulin resistance through the HOMA2 score

3. Muscle function: Looking at clinical parameters of muscle function (myometry) and potential pathways - miRs involved in myogenesis (miR 21), angiogenesis (miR 126) and specific to PAH (miR 1).

9.3 Methods

30 PAH patients participating in an exercise rehabilitation study at the Scottish Pulmonary Vascular Unit were included. Those who had known diabetes (7) were excluded from metabolic function assessment. Fasting serum was taken before, during and after a 15 week aerobic and resistance PAH specific exercise programme, along with standard tests to assess PAH severity (described in Chapter 2). Serum and muscle biomarkers were correlated with standard clinical and prognostic variables and are described in more detail below.

9.3.1 **Metabolic function**

Insulin resistance was assessed by measuring fasting glucose, fasting C-peptide and calculating the HOMA2 score, insulin sensitivity (%S) and beta cell function (%B), as described in the introduction section: Metabolic function in PAH

Fasting whole blood samples were taken in a heparin plasma blood bottle for C-peptide and kept on ice until analysis. Fasting glucose samples were taken in a serum separator blood bottle. Serum glucose samples were analysed at the GJNH laboratory and C-peptide was analysed at the Queen Elizabeth University Hospital (QEUH) laboratory, as per standard clinical practice.

9.3.2 **Serum markers of inflammation**

9.3.2.1 **Serum IL-6**

Whole blood samples were taken in a serum separating blood tube and left for 30 min. Samples were then centrifuged at 3000 rpm for 10 minutes. Serum was pipetted into eppendorfs and frozen at -80C.

Samples were analysed in batch and thawed to room temperature. The Merck millipore SMC™ Human Interleukin-6 immunoassay kit was used to quantify plasma concentration of IL-6 according to manufacturer's instructions. The immunoassay was performed at the University of Glasgow ICAMS laboratory with the assistance of EB and JC.

Materials

Assay buffer, IL-6 coated beads, IK-6 standard diluent, IL-6 detection antibody, human IL-6 standard, Wash buffer.

Instrumentation

1. Jitterbug™ Microplate incubator / shaker

2. ALPS™ 50V microplate heat sealer (Thermo Fisher)

3. Centrifuge

4. 12-channel pipettes capable of transferring 20 µL - 250 µL

5. 8- or 12-channel pipette capable of transferring 15 µL

6. Rotisserie rotator

7. Microcentrifuge

8. MultiScreenHTS BV 96-Well Filter Plate (EMD Millipore)

9. 96-well V-bottom polypropylene plate, 500 µL (Axygen)

10. 384-well round bottom polypropylene plate, 120 µL (Thermo Fisher)

11. 0.2 µm syringe filter (EMD Millipore)

12. Universal plate cover (Thermo Fisher)

13. Sealing tape (Thermo Fisher)

14. Heat sealing plate foil (EMD Millipore)

15. 12-channel reagent reservoirs for preparing standards

16. 5 mL syringe

17. Microcentrifuge tubes

18. Container capable of holding 300 mL

19. 500 mL graduated cylinder

20. SMCxPRO™ 384–well Senso plate, sterile, flat bottom with lid

21. SMCxPRO™ Plate Holder (EMD Millipore)

22. Senso plate adhesive sealer (Thermo 276014) 23. SMCxPRO™ plate w/seals and holder (EMD Millipore PN 02-1003-00)

Process

1. All reagents and the standard curve were prepared according to the manufacturer's instructions

2. 75 μL of standard and samples and 100 μL of capture beads coated in antibody specific for IL-6 were pipetted to plate 1

3. The plate was covered and incubated for 2 hours at 25C on the microplate incubator/shaker to allow IL-6 present in the sample to bring to the capture antibody on the coated beads.

4. A post capture wash performed of plate 1 to wash away unbound molecules

5. The plate was removed from the magnet and 20μL of detection antibody was added to each well to recognize and bind to IL-6 captured on the beads

6. The place was covered and incubated for 1 hour at 25C on the microplate incubator/shaker.

7. The pre-transfer / post-detection was wash then performed on plate 1 to remove the unbound detection molecules

8. Manual plate transfer occurred to plate 2

9. Final aspiration was performed on plate 2

10. The magnet was removed and 10μL of Elution buffer was added to each well to dissociate the bound protein sandwich from the bead surface, releasing the labelled antibodies

11. Plate 2 was covered and incubated for 10 minutes at 25C on the microplate incubator/shaker

12. 10 μLL of buffer was added to each well in Plate 3

13. The contents of Plate 2 were transferred to Plate 3

14. Plate 3 was loaded onto the SMCxPRO™ system where the labelled molecules were detected and counted. The amount of flour-labelled detection antibodies counted was directly proportional to the amount of IL-6 present in the sample

9.3.2.2 **Composite markers of inflammation from whole blood**

Blood was drawn in ethylenediaminetetraacetic acid (EDTA) and serum separating blood tubes at baseline, the end of the residential phase of exercise, the end of the study, and in the control arm group before and after usual care. A standard panel of routinely measured laboratory tests were taken including: Full Blood Count (FBC), Urea and Electrolytes (U&E), Liver Function (LFT), Iron Studies, C-reactive Protein (CRP).

Samples were analysed in the GJNH laboratory in line with standard clinical practice and used to calculate

- mGPS: Score 1 for Albumin < 35 g/L, score 1 for CRP > 10

- PLR: Platelet to Lymphocyte Ratio

- NLR: Neutrophil to Lymphocyte Ratio

9.3.3 **Muscle Biopsy**

9.3.3.1 **Biopsy Technique**

A needle biopsy of the vastus lateralis was performed at baseline and at the end of the study. Biopsies were performed at Gartnavel General Hospital (GGH) and Glasgow Royal Infirmary (GRI) Radiology Departments. Ultrasound was used to identify the vastus lateralis muscle of the dominant leg, 3 cm proximal to the patella with the subject in the lateral decubitus position. The skin was prepared with chlorhexidine and anaesthetised with 2-5ml of 2% lidocaine. A 1cm skin incision was made. A size 14 Gauge, 9cm coaxial Achieve™ biopsy needle (Merit Medical, Jordan UT, USA) was used to take 2 to 3 biopsies to obtain roughly 200mg of skeletal muscle (Figure 9.2) with the assistance of, or by a musculoskeletal radiologist (MM or DR). Fat and hair were dissected off muscle samples prior to sample storage.

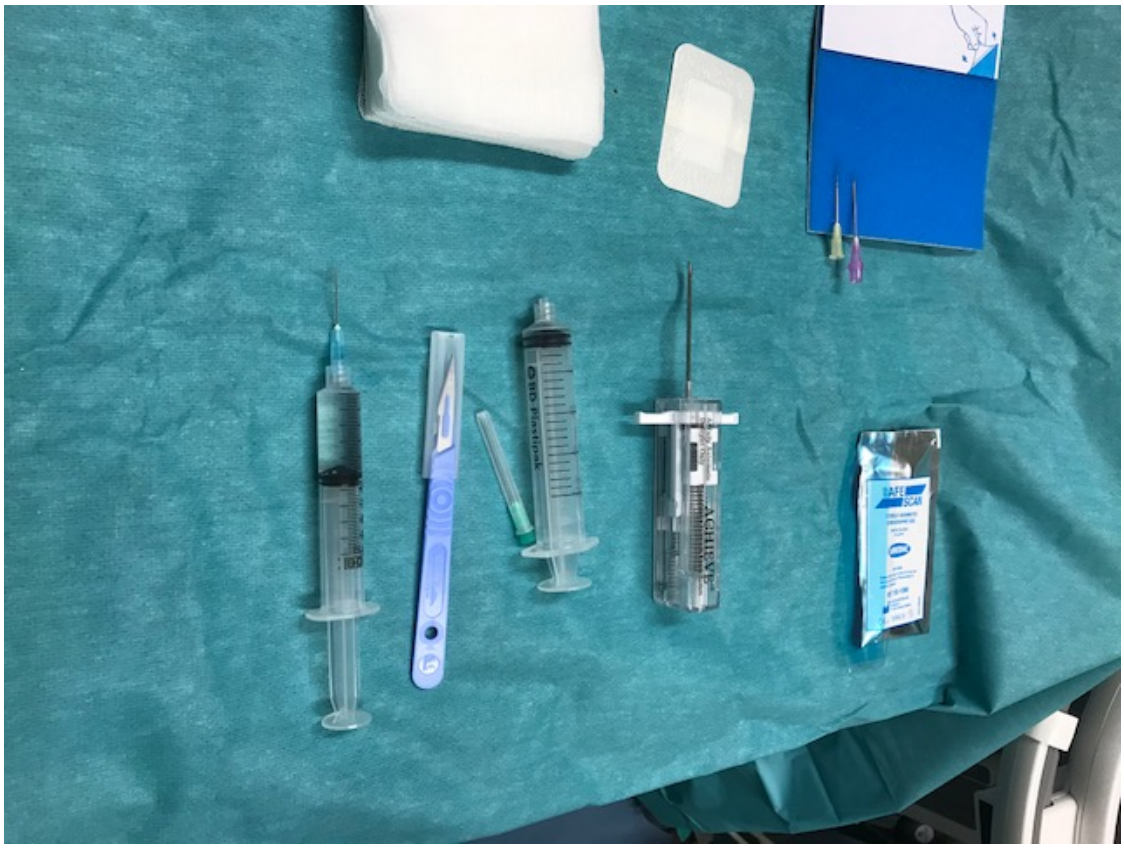


Figure 9.2 Muscle Biopsy Equipment

The muscle sample was transferred immediately to 5mLs RNA*later* aqueous solution (Thermofisher Scientific) for RNA stabilization and storage. Samples were transported to the SPVU laboratory at Glasgow University and placed on a rocker for 6 hours at 20 rpm in a cool room, they were then frozen at -20C for one week and thereafter stored at -80C. The samples stored in RNA later were used for assessment of miR expression.

9.3.4 **Micro RNA experiments**

9.3.4.1 **RNA extraction**

RNA extraction experiments were performed at the University of Glasgow ICAMS laboratory with the assistance of KP.

Apparatus

- Chloroform
- Ethanol (70% and 96–100%)
- Sterile, RNase-free pipet tips
- 2 ml microcentrifuge tubes
- 2 x Microcentrifuge for centrifugation at 4°C and at room temperature
- Disposable gloves
- Blunt-ended needle and syringe
- RNase-Free DNase Set (Qiagen, Switzerland)
- Qiagen TissueLyser II Machine, Figure 9.3

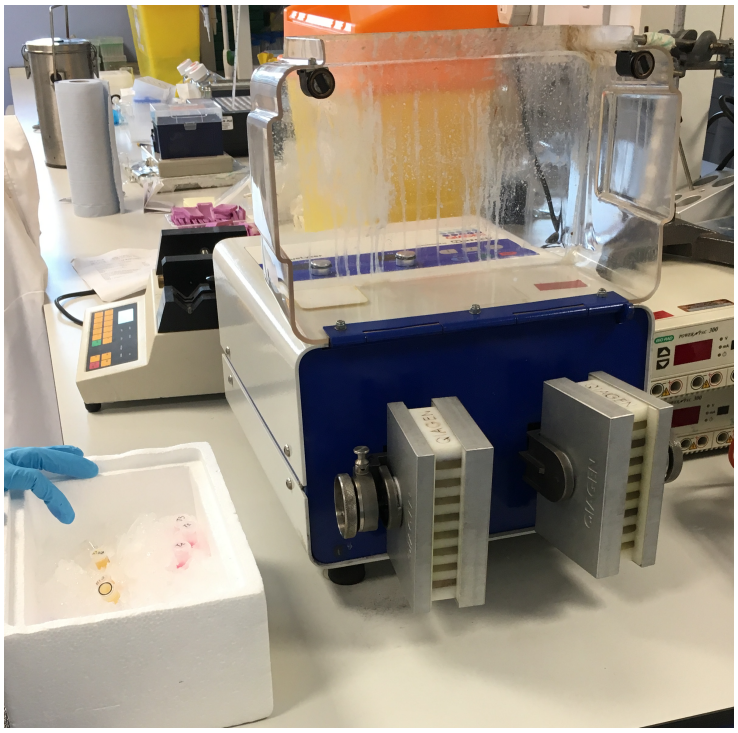


Figure 9.3: Tissue lyserQIAshredder disposable homogenizer tubes (Qiagen, Switzerland)

Muscle procedure

The experiment area was thoroughly cleaned with RNAse ZAP (Qiagen, Switzerland) to ensure an RNA free environment; sterile RNase-free water, collection tubes and pipet tips were used. Pre-prepared C. elegans miR-39 miRNA mimic (Qiagen, Switzerland) was used for the control.

1. 700 µ L of QIAzol Lysis Reagent was added to the skeletal muscle, tissue was disrupted and homoginized using 3mm stainless steel balls.

2. The homogenate was incubated at 15-25°C for 5 minutes

3. 140 µ L of chloroform was added and the sample vigorously shaken for 15 seconds

4. The sample was then incubated at room temperature for 2-3 minutes

5. Centrifuged for 15 minutes at 12,000g at 4°C

6. The upper aqueous phase was transferred to a new RNA free collection tube using a pipette.

7. 1.5 x the volume of the sample (~ 525 µL) of 100% ethanol and was mixed with the sample thoroughly by pipetting

8. 700 µL of the sample (including precipitate) into an RNA mini column in a 2mL collection tube. Centrifuge for 15 seconds at 10000G

9. Qiagen DNAse was used according to manufacturer's instructions, in order to remove genomic DNA (*KP)*

10. 700 µL of Buffer RWT was added to the RNeasy Mini Column and centrifuged for 15s at 10000G. Flow through was then discarded

11. 500 µL Buffer RPE was pipetted onto the RNeasy Mini column and centrifuged for 15s at 10000g and flow through was discarded

12. 500 µL Buffer RPE was added to the RNeasy Mini column and centrifuged for 2 min at 10000G

13. The RNeasy Mini column was placed in a new 2mL collection tube and centrifuged for 1 min at full speed to further dry the membrane

14. The RNeasy mini column was transferred to a new 1.5mL collection tube. 30 µL RNase-free water was pipetted directly onto the RNeasy Mini column membrane, then centrifuged for 1 min at 10000g to elute (Figure 9.4).

15. Purity and concentration were quantified using nanodrop technology. Between 200ng and 1000ng of RNA was obtained per sample

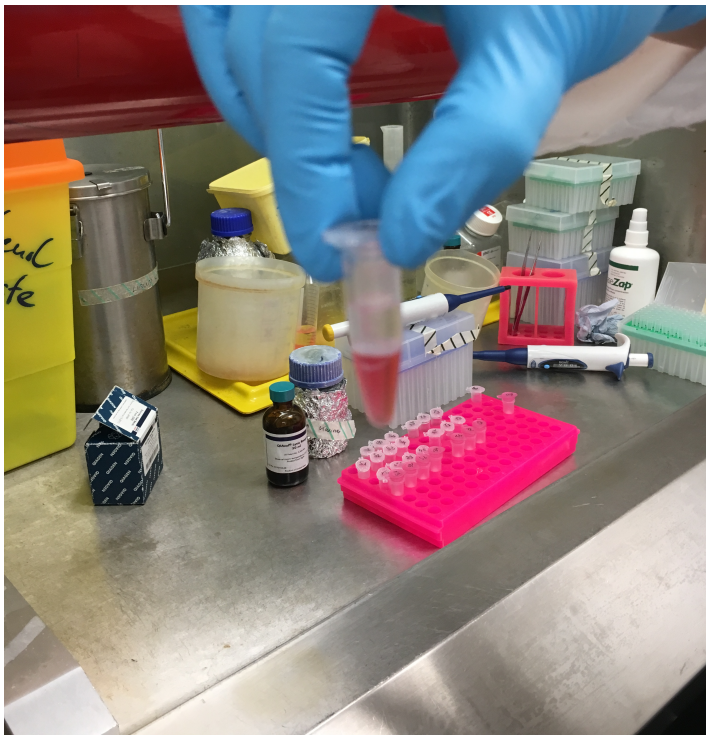


Figure 9.4: Final elute from muscle RNA extraction

Serum procedure

The miRNeasy Serum/Plasma kit (Qiagen, Switzerland), was used. The protocol was similar for muscle RNA extraction, the notable differences being in tissue homogenization and the DNAse step:

The procedure was as follows:

1. Serum samples were thawed in 37C water bath and immediately used

2. 500 μl of QIAzol Lysis Reagent was added to 100 μl of serum and kept at room temperature for 5 minutes

3. 3.5 μl miRNeasy control was added

5. 100 μl Chloroform was added to the tube and placed in the vortex for 15 seconds to allow phase separation; the tube was then kept at room temperature for 2-3 minutes.

7. The sample was centrifuged for 15 min at 12,000G (4°C). To allow separation into 3 phases: an upper, aqueous phase containing RNA; a white interphase; a lower, organic phase.

8. The upper aqueous phase was transferred to a new collection tube and 450 μl of 100% ethanol was added and mixed thoroughly

9. 700 μl of the sample was added to a RNeasy MinElute spin column in a 2 ml collection tube and centrifuged at 10,000 G for 15 s at room temperature. The flow-through was discarded. This step was then repeated for the remainder of the sample.

11. 700 μl of Buffer (supplied with the kit) was added to the RNeasy MinElute spin column and then centrifuged at 10,000 G for 15 seconds to wash the column, this process was repeated.

12. 500 μl of 80% ethanol (100% ethanol prepared with RNase free water) was added to the RNeasy MinElute spin column and then centrifuges 2 min at 10,000G to wash the spin column membrane. The flow through was discarded.

14. The spin column was placed in a new collection tube and centrifuged at full speed for 5 min to dry the membrane. The collection tube with the flow-through was discarded.

15. 14 μl RNase-free water was pipetted directly onto the center of the spin column membrane then centrifuge for 1 min at full speed to elute the RNA.

16. RNA quantification and purity were determined using nanodrop technology yielding a range of 580ng – 1270ng between serum samples

9.3.4.2 **miRNA PCR**

Qiagen miR RT kit was used to perform quantitative reverse transcription PCR on the extracted muscle and serum RNA, taqman probes were selected for miR1, miR 21 and miR 126. Housekeeping genes were used to normalize the mRNA levels of the genes of interest in serum and muscle samples before the comparison between

different samples by the real time PCR in triplicate. This experiment was performed by KP at the University of Glasgow, with assistance from AM.

miR-1, -21 and -126 were selected based on the following:

- miR-1 is involved in the development and physiology of muscle tissue, including cardiac muscle. High levels of circulating miR-1 have been used as a biomarker to identify cardiac ischaemia and miR-1 is down regulated in skeletal muscle following endurance exercise training programmes. In PAH, miR-1 has been associated with vascular remodelling[298].

- miR-21 is upregulated in the pulmonary vasculature of PAH patients and is also involved in control of skeletal muscle development[299]. Elevated levels of miR-21 have been found in a number of myopathies and also increased levels of circulating miR-21 have been found in a number of cancers, relating to inhibition of apoptosis. Long term inhibition of miR-21 has been associated with reduction in obesity in animal models and inhibition of tumour immune responses[300]. Endrunance training in healthy males was associated with downregulation of serum miR-12 after a 12 week endurance training programme.

- miR-126 has reduced expression in PAH skeletal muscle and is associated with impaired angiogenesis in PAH muscle and a number of other conditions such as retinopathy. It has pro-angiogenic and also pro-proliferative effects; it's role therefore remains controversial.

9.3.5 **Muscle Function**

Muscle function was assessed using a digital myometer and clinical analysis software (CAS) (MIE medical, Leeds, UK) (Figure 9.5). Hand grip and quadriceps strength and endurance were assessed at baseline, at the end of three weeks of exercise therapy and at the end of the study.

Figure 9.5: Myometer with pinch grip analyser

Muscle strength was determined by measuring the peak force (maximum voluntary contraction, Newtons (N)), the best of three attempts being taken.

For assessment of muscle endurance, the subject first performed 2 maximum contractions (MVC). The higher of the two contractions was used to perform the endurance test. The subject was asked to maintain 50% of their MVC for as long as possible (Figure 9.6) with real time audiovisual feedback via the CAS software. The total time, time within target range and total area under the curve were used as measures of Endurance (Figure 9.7).

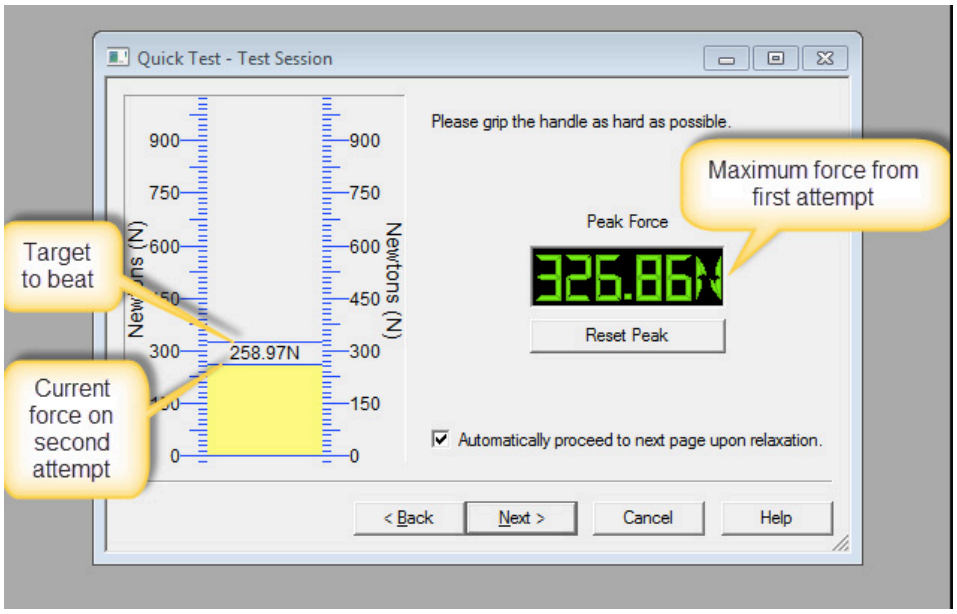


Figure 9.6: Digital display for endurance test

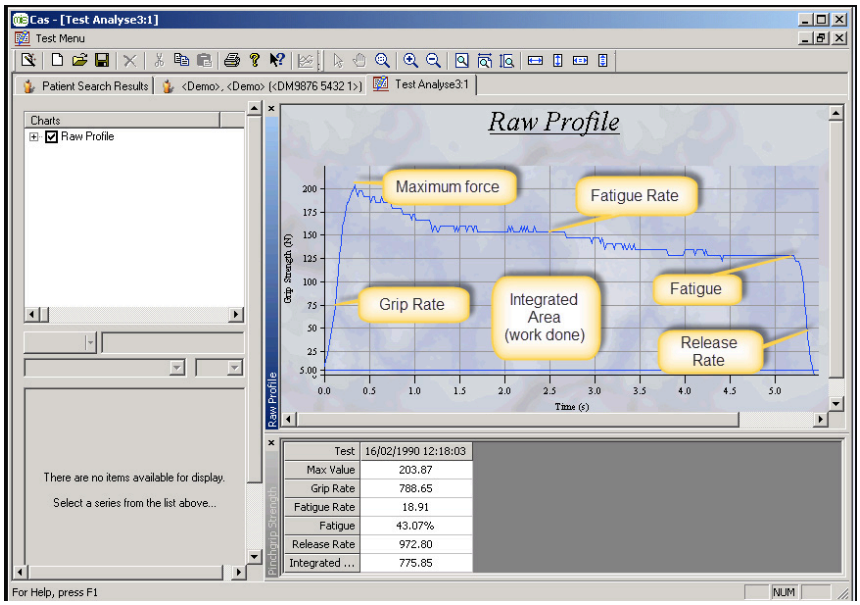


Figure 9.7: Result output example for endurance test

9.3.5.1 **Pinch grip testing**

Pinch grip testing was performed using an adjustable handle that connected to the myometer and measured force in newtons (N). The distance between two bars (cm) was measured at which a comfortable grip was produced. The test was performed in a seated position with the elbow resting on a table. The dominant hand was tested.

9.3.5.2 **Quadriceps testing**

Testing was performed with the subject seated with knees flexed at 90 degrees. The dynamometer shin strap was positioned to sit comfortably on the lower 1/3 of the tibia. MVC of Quadriceps was tested by knee flexion. A totally of 3 tests were performed on the dominant leg for strength and two for endurance.

9.4 **Statistics**

Normality was assessed using Shapiro Wilk. Normally distributed data are presented as mean (SD) and non-parametric data as median (IQR). All paired analyses were performed using the Wilcoxon test and non-paired data using Mann-Whitney due to the small sample sizes in this study. Spearman's correlation coefficient was used for correlation of variables. In IL-6 analysis, outliers were

identified using a box plot and analyses were performed with an without outliers present.

9.4.1 miR statistics

The ddcT (threshold cycle) method was used to calculate reverse transcription PCR (RT-PCR) results normalised to the housekeeper gene. For an individual subject ddC was calculated as follows:

$$ddC(t) = dCt(t) - dCt(c)$$

$$ddCt(c) = dCt(c) - dCt(c)$$

Where ***dCt*** was threshold cycle difference, ***dCt(c)*** was dCt of the control condition and ***dCt(t***) was dCt of the tested condition

For groups, the average dCT(c) and dCT(t) were used to calculate the ddCt.

The Relative Quotient (RQ) was then calculated as 2^{-ddCt}

Data were compared using the Wilcoxon test for paired data and Mann-Whitney for unpaired data. Correlations were performed using Spearman's.

9.5 Results

23 subjects were included without diabetes.

Population characteristics

- BMI 29.7 (19-41)
- Age 50.2y (34-74)
- Gender 22% Male (5/23)
- Pre-capillary PH population: 26% (6/23) CTD-PAH, 4% (1/23) Inoperable CTEPH,66% (15/23) IPAH, 4% (1/23) POPH

At baseline, a significant proportion of subjects had evidence of metabolic dysfunction and inflammation (Table 9.2, non-diabetic patients, N 23)

Table 9.2: Baseline profile of metabolism and inflammation

N 23	Mean	Median	Range	Normal Range	% of cohort Abnormal.
NLR	3.4	3.2	1.2-6.8	0.58-3.53	52% above
PLR	164	143	68-271	75-119	74% above
mGPS	0.13	0	0-1	≤1	14% above
CRP	5	4	1-16	≤ 10	14% above
Albumin (g/L)	43	43	34-50	35-55	3% below
Neutrophil (10^9/L)	4.1	3.8	2.1-9.2	2.5-7.5	6% above
Lymphocytes (10^9/L)	1.3	1.3	0.8-2.6	1.5-4.0	69% below
C-Peptide (ng/mL)	1.01	0.96	0.24-2.14	0.26-1.03	43% above
IL-6 (pg/mL)	3.52	2.38	0.7-11.6	≤6	23% above
Transferrin sat (%)	22	24	7-36	20-50	40% below
Serum iron	14	15	5-26	10-30	18% below

9.5.1 **Metabolic function**

10/23 (43%) had fasting C-peptide above the normal range. Median HOMA-IR score was 2.1 (range 0.5-4.65). (Table 9.3)

Table 9.3: Metabolic function pre and post exercise therapy

	Mean / Median Baseline	SD	Mean / Median EOS	SD	N	p
C-Peptide * (ng/mL)	0.94	0.81	0.92	0.86	15	0.938
%S*	46	40	53	61	15	0.820
%B*	133	92	157	125	15	0.570
HOMA-IR*	1.98	1.61	1.81	1.66	14	0.820
Glucose (mmol/L)	5.0	0.8	4.9	0.7	13	0.900
Total Chol (mmol/L)	4.1	1	4.3	0.9	13	0.670
Triglyceride (mmol/L)	1.2	0.4	1.4	0.6	13	0.326
HDL (mmol/L)	1.4	1	1.3	0.7	12	0.378

LDL (mmol/L)	2.1	0.7	2.2	0.7	12	0.726
VLDL (mmol/L)	0.6	0.2	0.6	0.3	12	0.157
Chol:HDL ratio*	3.9	1.9	3.6	2.1	12	0.187
***Median / IQR displayed**						

Exercise therapy versus standard care

In the exercise therapy group, there were no significant changes compared to the standard care group, Table 9.4

Table 9.4: Treatment versus control changes in metabolism

	Mean Exercise group change	SD	Mean standard care change	SD	p
Glucose (mmol/L)	-0.83	3.48	0.53	1.01	.226
Total Chol (mmol/L)	0.09	0.58	0.06	0.37	1.00
Triglycerides (mmol/L)	0.14	0.36	-0.05	0.05	.230
HDL (mmol/L)	-0.07	0.29	-0.04	0.08	.444
LDL (mmol/L)	0.01	0.36	0.30	0.73	.622
VLDL (mmol/L)	0.08	0.15	0.09	0.20	.687
cholesterol:HDL ratio	-0.24	1.11	0.39	0.79	.968

Those with elevated baseline C-peptide had poorer exercise capacity, higher symptom burden and higher levels of inflammation,

Table 9.5.

Table 9.5: Raised versus normal C-Peptide

	Increased C-peptide	SD	Normal C-peptide	SD	p
BMI	32	7	28	5	0.151
WHO FC	2.7	0.5	2.2	0.5	**0.044**
Peak CPET WR (W)	65	12	87	34	0.123
PEAK CPET VO_2 L/min	55	20	68	34	0.08

PEAK CPET HR	75	11	85	12	**0.021**
6MWD (m)	396	83	456	85	0.091
CRP (mg/L)	7	4.2	3	3.2	**0.023**
NLR	4.4	1.9	2.5	1.3	**0.016**
PLR	186	82	144	57	0.134
NTproBNP (pg/mL)	625	1293	273	285	0.449

9.5.2 **Serum markers of inflammation**

9.5.2.1 **Routinely measured biomarkers**

Neutrophil to lymphocyte ratio (NLR), Platelet to lymphocyte ratio (PLR) and modified Glasgow Prognostic Score (mGPS) were assessed at baseline, at the end of the residential exercise (week 3) and end of the exercise programme (EOS). Routinely measured variables including full blood count and iron studies were also measured.

27 subjects had complete baseline and week 3 data. Between baseline and week 3, the CRP reduced significantly. There were no other statistically significant variables, but there appeared to be a trend to reduced NLR and PLR (Table 9.6)

Table 9.6: Change in profile of inflammation from baseline to week 3 of exercise

N 27	Baseline	SD	Week 3	SD	mean delta	p
Hb (g/dl)	13.2	3.2	12	4.5	1.17	0.123
Hct (L/L)	0.39	0.09	0.36	0.13	0.03	0.123
MCV (fl)	84	16.7	78.7	27.2	5.32	0.203
WCC* (g/L)	6.2	2.2	5.1	2.6	0.54	0.275
Plt (10^9/L)	193	65	180	89	13.00	0.312
Neutrophil (10^9/L)	3.9	1.9	3.5	2.2	0.37	0.344
Lymphocyte* (10^9/L)	1.37	0.49	1.3	0.39	0.06	0.121
Creatinine (mmol/mL)	74	28	68	34	6.43	0.461
EGFR (mL/min)	56	12	51	21	5.07	0.092
Albumin* (g/L)	41.6	8.7	37.1	15.3	4.50	0.087
CRP (mg/L)	5.13	4.5	2.53	2.6	2.60	0.001
NLR	3.18	1.77	2.94	1.95	0.25	0.427
PLR	155	71	144	75	10.87	0.322

***Median / IQR displayed**

No significant differences were noted in the 24 subjects with complete baseline to end of study data (Table 9.7)

Table 9.7: Change in profile of inflammation from baseline to end of study

N 24	Mean / Median Baseline	SD	Mean / Median EOS	SD	p
Hb (g/dl)	13.7	2.1	13.6	2.2	0.303
Hct (L/L)	0.4	0.06	0.4	0.06	0.686
MCV (fl)	86.7	4.8	88.0	4.1	0.080
WCC* (g/L)	5.9	2.4	5.6	2.6	0.685
Plt (10^9/L)	205	56	199	57	0.157
Neutrophil (10^9/L)	4.1	1.9	4.1	1.9	0.861
Lymphocyte* (10^9/L)	1.2	0.6	1.2	0.5	0.647
Creatinine (mmol/mL)	81	23	80	19	0.475
EGFR (mL/min)	59	3.5	60	1.6	0.070
Albumin* (g/L)	42	2	43	6	0.834
CRP (mg/L)	5.3	3.9	4.5	4.4	0.569
Transferrin sat (%)	22	10	25	14	0.872
Serum Iron	14.6	7	16.3	8.3	0.773
Transferrin	2.8	0.4	2.8	0.5	0.300
NLR	3.3	1.7	3.1	1.3	0.424
PLR	160	50	152	44	0.362
***Median / IQR displayed**					

Standard care versus exercise therapy

There were no significant changes following standard care (control group) versus exercise therapy (ET) (Table 9.8)

Table 9.8: Control versus treatment change in routinely measured blood tests and serum markers of inflammation

	N	Mean ET group change	SD	N	Mean control change	SD	p
Hb (g/dl)	24	-0.10	1.30	11	0.03	0.99	.390
Hct (L/L)	24	0.00	0.03	11	0.00	0.03	.540
MCV (fl)	24	1.51	4.88	11	-2.82	5.99	.072
WCC* (g/L)	24	0.13	1.83	11	-0.19	0.83	.687

Plt (10^9/L)	24	-10.37	34.25	11	-11.10	34.40	.847
Neutrophil (10^9/L)	24	0.06	1.75	11	-0.24	0.79	.687
Lymphocyte* (10^9/L)	24	0.01	0.28	11	0.05	0.21	.958
Creatinine (mmol/mL)	24	-0.17	11.92	11	-1.27	10.77	.766
EGFR (mL/min)	24	0.79	2.62	11	0.09	1.14	.587
Albumin* (g/L)	24	0.04	3.32	11	-0.18	2.36	.687
CRP (mg/L)	24	-0.75	3.71	11	0.00	1.89	.820
NLR	23	-0.25	1.2	9	0.69	2.30	.273
PLR	23	-8.1	37.2	9	-6.3	58.9	.930

9.5.2.2 **Interleukin-6**

Normal Interleukin-6 (IL-6) serum concentration is under 6. IL-6 was non-parametrically distributed, therefore data are presented as Median (IQR). Table 9.9 demonstrates the scatter. Four subjects dropped out (as discussed in Chapter 5). 22 subjects had baseline to week 3 data and 21 subjects had baseline to end of study data. 41% (9/22) subjects had IL-6 concentrations greater than 6pg/mL

Table 9.9: IL-6 values at different time points in the exercise programme

	Mean	Median	IQR	Range	N
Baseline	7.5	3	6	0.7-49	27
End residential Phase	4	3.4	3.5	0.7-16	25
EOS	7.3	3.6	4	0.7-65	22
Control	3.4	2.9	2.6	1.7-6.3	10

9.5.2.3 **Change with exercise therapy**

IL-6 was measured at baseline and compared to serum IL-6 concentration at the end of residential (ER) phase exercise (Table 9.10) the end of the study (EOS) (Table 9.11). Group B subjects had IL-6 measurements performed pre and post 15 weeks of standard care (Table 9.12)

Table 9.10: Change in exercise therapy following 3 week residential phase exercise

Baseline Median	IQR	ER Median	IQR	P	N
2.7	4.1	3.6	3.1	0.848	22

Table 9.11: Change in IL-6 with 15 week exercise programme

Baseline Median	IQR	EOS Median	IQR	P	N
2.4	4.9	3.6	4.4	0.339	21

Table 9.12: IL-6 concentration with standard treatment (Group B)

Baseline	IQR	Entry to exercise programme	IQR	P	N
2.3	8.8	2.3	2.1	0.953	9

9.5.2.4 **IL-6 Outliers**

When analysing the data, 6 clear outliers were identified using a box plot (Figure 9.8,

Table 9.13)

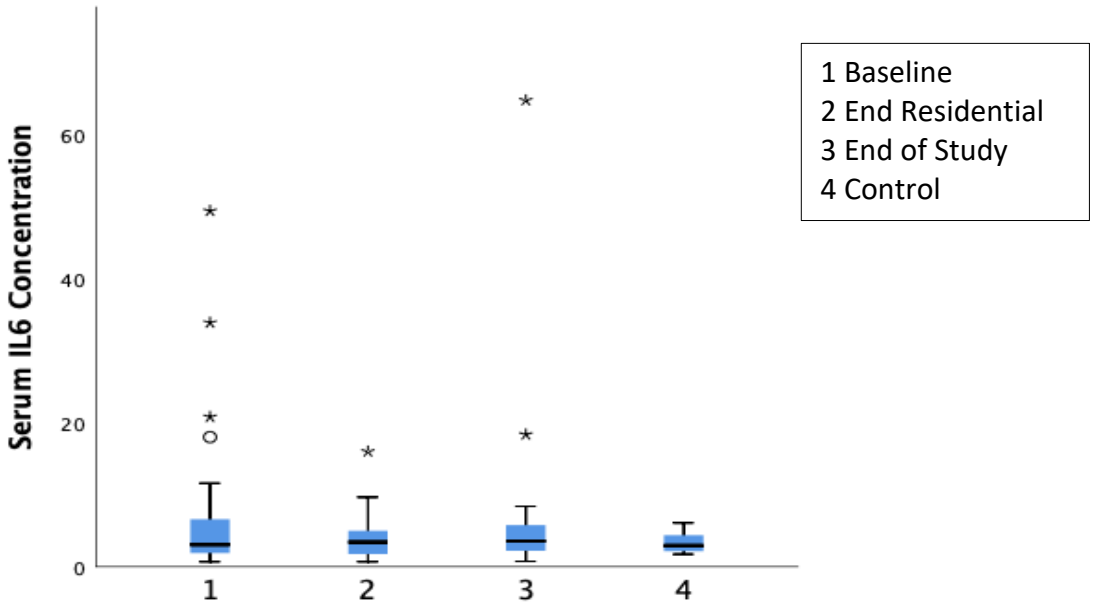


Figure 9.8: Serum IL-6 concentrations (pg/mL) at different time points during the study

Table 9.13: IL-6 concentration (pg/mL) outliers

Subject	Start Residential Phase (1)	End Residential Phase (2)	End of study (3)	Control (4)
A1	49.5	6.06	n/s	
A2	1.18	3.57	64.824	
A3	3.47	n/s	5.68	
A4	4.77	3.43	7.45	
A5	1.91	2.88	2.52	
A6	20.8	4.99	5.13	
A7	8.14	9.67	7.69	
A8	2.15	0.82	0.74	
A9	4.51	3.85	5.73	
A11	11.6	n/s	18.4	
A12	5.33	4.8	8.4	
A13	2.22	5.33	1.38	
A14	5.14	16.1	n/s	
A15	1.87	5.17	3.64	
A16	4.17	4.76	n/s	
B1	0.7	0.7	0.82	
B2	7.84	1.44	n/s	
B3	1.12	1.36	1.73	
B4	2.21	6.72	5.76	5.73
B5	n/s	2.91	2.43	n/s
B6	33.91	n/s	5.78	4.36
B7	2.44	2.75	2.7	4.00
B8	2.31	2.43	3.07	2.02
B9	1.47	1.29	n/s	2.2
B10	1.62	2.23	2.21	2.9
B11	n/s	n/s	n/s	6.1
B12	3.04	3.65	2.22	2.98
B13	18.0	1.75	3.5	2.15
B14	1.77	1.34	n/s	1.73

Outliers identified above, were analysed separately to look for any common factors to determine whether these results were a true reflection of serum IL-6 or error in the experimental process. Of the outliers, four were in the baseline samples.

These subjects had the following characteristics: One CHD-PAH, two CTD-PAH, one IPAH with autoimmune hepatitis. In relation to exercise capacity and metabolic function, they appeared to have a lower baseline exercise capacity and higher degree of metabolic dysfunction in comparison to the entire study population, none of these results were statistically significant due to the small numbers. (Table 9.14)

Table 9.14 Comparison of high IL6 concentration (pg/mL) baseline outliers to study population

	IL-6 Outliers			Study Population		
	Baseline	EOS	Delta	Baseline	EOS	Delta
Baseline 6MWD (m)	385	455	70	438	477	39
C-peptide (ng/mL)	1.8	1.36	-0.44	1.03	1.02	-0.01
HOMA-IR	3.85	2.83	-1.02	2.23	2.19	-0.04

9.5.2.5 IL-6 change with outliers out (Standard care v exercise therapy)

Paired analyses were performed with the outliers removed. There was no significant change in IL-6 following residential exercise phase (

Table 9.15), however a significant increase was seen following completion of the 15 week exercise programme (Table 9.16).

Table 9.15: Change in IL-6 following residential exercise (pg/mL)

Baseline Median	IQR	End residential Median	IQR	P	N
2.2	2.9	3.4	3.6	0.156	21

Table 9.16: Change in IL6 following 15 week exercise programme (pg/mL)

Baseline Median	IQR	End of study Median	IQR	P	N
2.3	2.8	3.1	4.6	0.031	17

Change in individual IL-6 concentration before and after exercise therapy can be found in Figure 9.9.

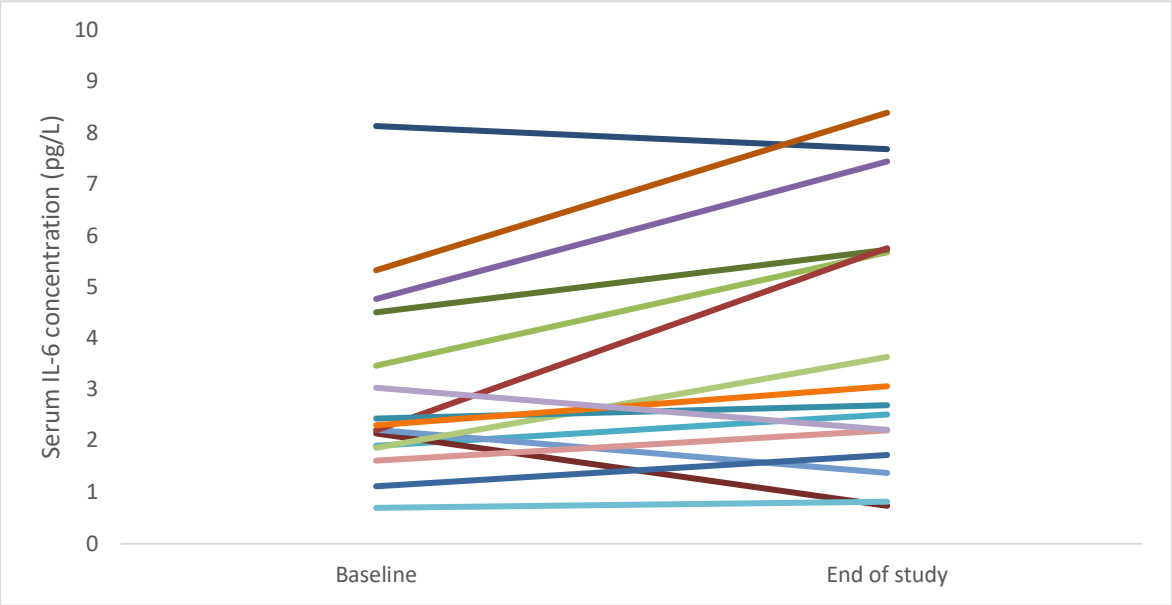


Figure 9.9: Individual changes in IL-6 concentration before and after exercise

In the control arm versus the treatment arm, there was a significant increase in serum IL-6 concentration with exercise therapy (Table 9.17, Figure 9.10)

Table 9.17: Control versus treatment change in IL-6 (pg/mL)

	Treatment Median (16)	IQR	Control Median (7)	IQR	N	p
Delta [IL-6 serum]	0.6	2.8	-0.3	1.4	23	0.027

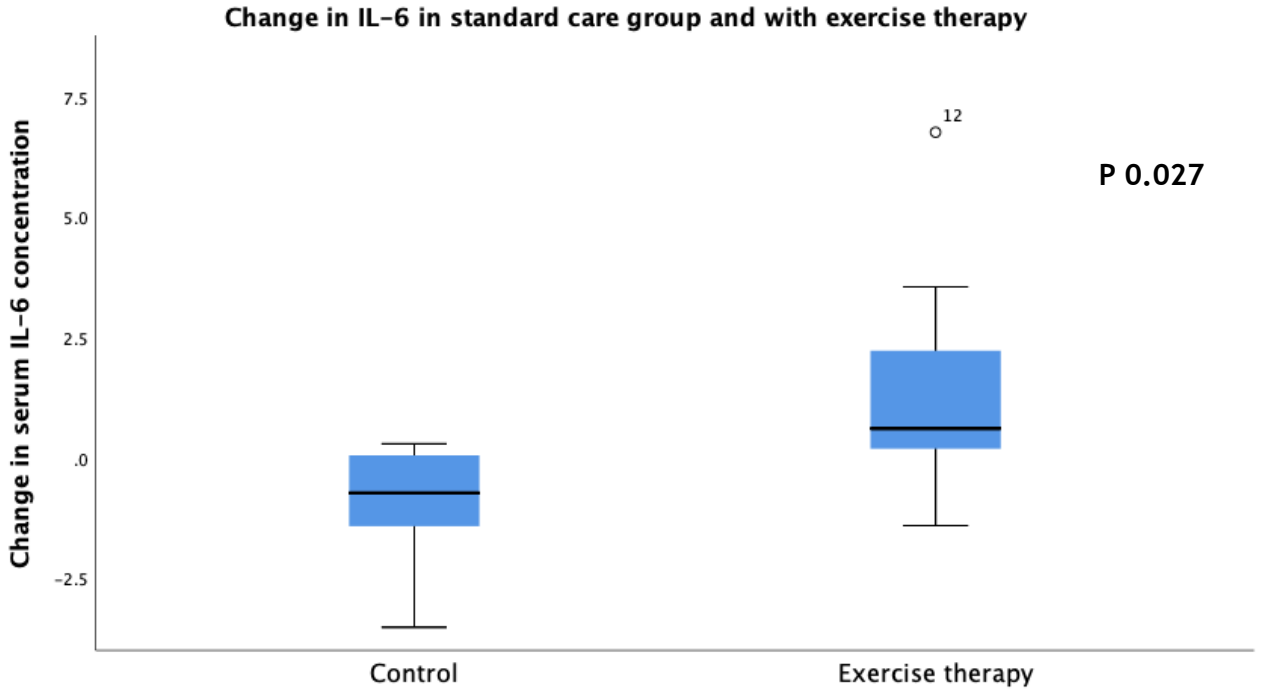


Figure 9.10**: Change in IL-6 standard care versus exercise therapy with outliers removed**

9.5.2.6 **Treatment response and prognosis**

Those who improved 6MWD (15 of 25) had a more favourable baseline metabolic and inflammatory profile, Table 9.18

Table 9.18: Differences in baseline metabolic function and inflammation in those with improved 6MWD versus no change or deteriorated (NC / D)

	Improved	SD	NC / D	SD	p
Hb (g/dL)	13.6	2.2	13.9	1.9	0.730
WCC (g/L)	5.4	1.5	7.2	2.4	**0.026**
Plt (10^9/L)	185	59.8	225	36.5	**0.042**
Neut (10^9/L)	3.6	1.2	5	2.5	0.063
Lymph (10^9/L)	1.3	0.5	1.5	0.4	0.204
Creat (mmol/mL)	81	24	66	26	0.131
eGFR (mL/min)	59	4	56	11	0.375
Albumin (g/L)	42.6	3.3	43.7	4.8	0.508
CRP (mg/L)	5.3	4.1	5.8	5.5	0.805
Transferrin sat (%)	26.2	18.9	25.9	13.8	0.969
Serum Iron	16.8	13.6	15.8	6.7	0.829
Transferrin	2.7	0.7	2.8	0.5	0.538

Glucose (mg/dL)	5.2	0.9	6.4	3.9	0.284
Total Chol (mmol/mL)	4	0.7	5.2	1.5	**0.014**
TG (mmol/mL)	1.2	0.42	1.8	0.85	**0.045**
HDL (mmol/mL)	1.2	0.3	1.7	1.2	0.157
LDL(mmol/mL)	2.2	0.7	2.7	1.3	0.237
VLDL(mmol/mL)	0.56	0.19	0.74	0.34	0.139
Cholesterol:HDL	3.4	0.89	3.7	1.6	0.543
mGPS	0.11	0.32	0.22	0.44	0.428
NLR	3.18	1.4	3.64	2.3	0.516
PLR	162	77	157	44	0.860
IL-6 (pg/mL)	6.4	8.9	5.6	6.5	0.675
C-Peptide (ng/mL)	1.08	0.62	1.00	0.59	**0.025**
HOMA2	1.69	1.54	1.72	1.04	0.961

Prognostic role

Those who were dead or transplanted (D/T) at censoring had higher baseline IL6 and higher baseline CRP than those who were alive (A). Although not reaching statistical significance, there was a trend to higher neutrophils, lower lymphocytes and higher HOMA2 score in those who had not survived or had been transplanted at censoring.

Table 9.19: Alive versus transplant / deceased at censoring

	Mean D/T (7)	SD	Mean A (22)	SD	P
Hb (g/dL)	14.7	2.0	13.4	2.1	0.217
Plt (10^9)	184	58	205	55	0.354
Neut (10^9)	5.0	1.7	3.7	1.7	0.070
Lymph (10^9)	1.2	0.5	1.4	0.5	0.304
Albumin (10^9)	43	5.1	43	3.3	0.980
CRP (mg/L)	7.9	5.4	4.5	4.0	0.055
Transferrin sat (%)	21	5.4	27	20	0.409
Serum iron	13	2.6	17	13	0.409
Glucose (mg/dL))	5.3	1.1	5.7	2.7	0.910
Total Chol (mmol/mL)	4.4	1.8	4.5	0.9	0.431
TG(mmol/mL)	1.5	1.1	1.5	0.5	0.392
HDL(mmol/mL)	1.3	0.4	1.4	0.8	0.940
LDL(mmol/mL)	2.3	1.6	2.4	0.6	0.319
VLDL(mmol/mL)	0.6	0.4	0.7	0.2	0.101
Cholesterol:HDL ratio	3.6	2.1	3.6	0.9	0.940
NLR	4.3	1.6	3.0	1.6	0.078

PLR	155	46	162	72	0.784
IL6 (pg/ml)	6.4	1.9	4.5	5.5	0.047
C-peptide (ng/mL)	1.0	0.8	0.9	0.4	0.971
HOMA2	1.9	2.0	1.5	1.2	0.798

Clinical relevance of IL-6

Baseline IL-6 correlated negatively with 6MWD (Figure 9.11), end exercise WR (Figure 9.12), VO_2 (CPET) and quadriceps muscle strength and positively with NTproBNP. No significant correlations with metabolic parameters HOMA-IR and C-peptide were noted (Table 9.20)

Table 9.20: Correlations between baseline IL-6 and relevant clinical and prognostic variables

	Spearman's coefficient	P	N
Baseline 6MWD (m)	-.534**	**0.004**	27
Age (y)	0.123	0.55	26
End exercise (WR)	-.641**	**<0.005**	26
Peak VO_2 (L/min) Jones	-.510**	**0.008**	26
Ve/VCO_2 gradient	-0.023	0.912	26
MPAP (mmHg)	0.156	0.523	19
PVR (WU)	0.008	0.974	19
Rca (mL/mm Hg)	-0.216	0.375	19
0_2 extraction (%)	0.214	0.379	19
RV SV (mL)	0.067	0.756	24
C-peptide (ng/mL)	0.345	0.136	20
Log NTproBNP	.569**	**0.002**	26
NLR	0.244	0.22	27
PLR	0.057	0.776	27
Quads MVC Baseline (N)	-.464*	**0.017**	26

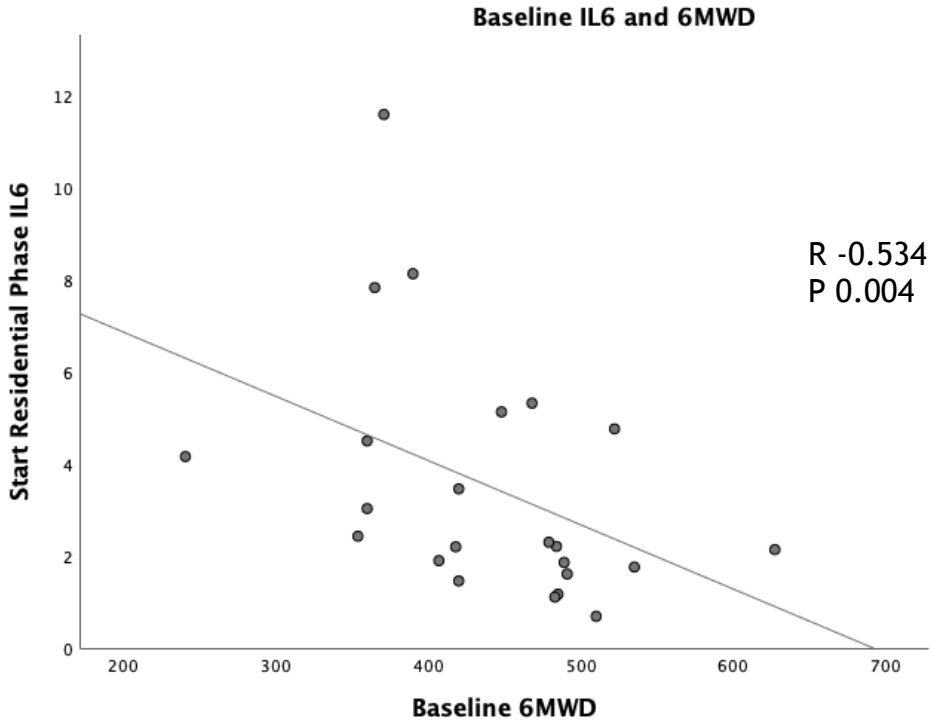


Figure 9.11 Correlation between baseline walk distance and serum IL6 concentration

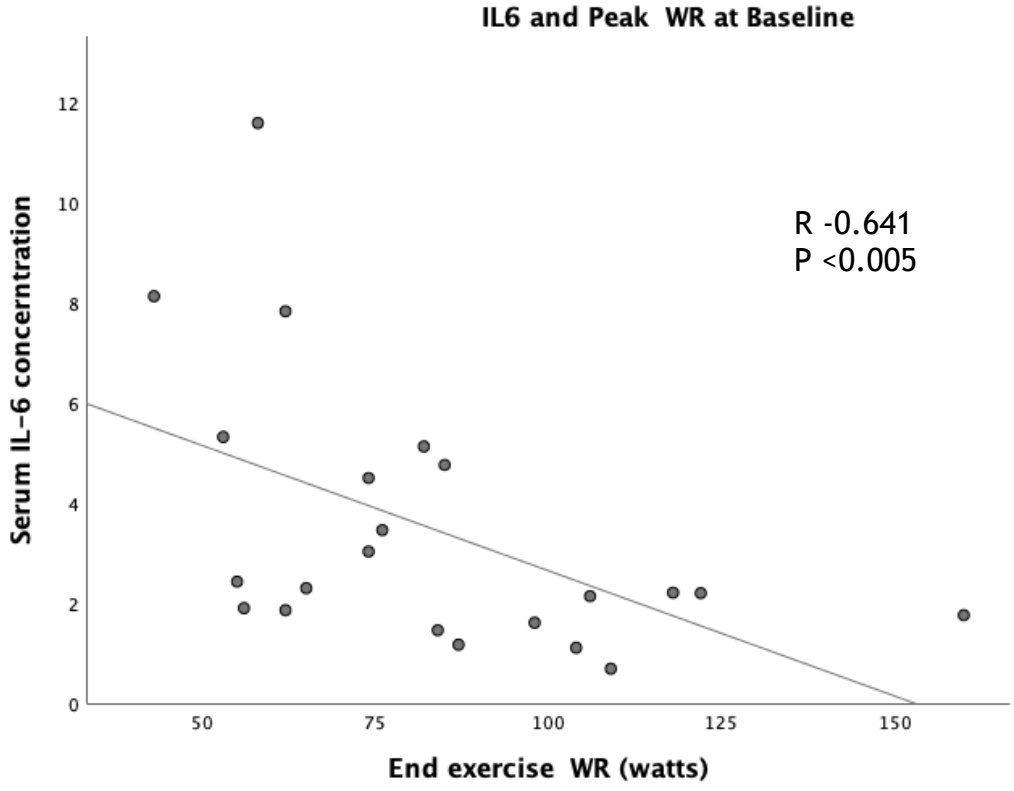


Figure 9.12 Correlation between baseline serum IL6 concentration and peak WR on CPET

9.5.3 **Muscle Function**

Muscle function was assessed at baseline, after 3 weeks of residential exercise and at the end of the study. Grip function and Quadriceps function were both assessed.

9.5.3.1 **Grip Strength and endurance**

Both grip maximum voluntary contraction force (strength) and grip endurance time improved significantly following three weeks of residential exercise therapy (Table 9.21). This improvement in endurance was maintained at the end of study visit. Grip strength remained higher than at baseline, but not reaching statistical significance (Table 9.22)

Table 9.21: Change in grip strength and endurance baseline to week 3

N 23	Mean Baseline	SD	Mean Wk 3	SD	p
MVC Grip (N)	197	60	226	63	0.002
AUC MVC Grip	1009	316	1224	350	0.001
Grip Endurance Time (sec)	102	63	143	103	0.053
Endurance time AUC	8701	4596	14210	11766	0.001

Table 9.22: Change in grip strength and endurance baseline to end of study

N 17	Mean Baseline	SD	Mean EOS	SD	p
MVC Grip (N)	194	59	223	56	0.196
AUC MVC Grip (N)	1012	342	1173	326	0.179
Grip Endurance Time (sec)	93	61	134	71	0.049
Endurance time AUC	8256	4436	11794	4860	0.044

9.5.3.2 **Quadriceps strength and endurance**

Quadriceps function improved significantly in the first 3 weeks of the exercise programme (Table 9.23) with quadriceps strength improvements being maintained at the end of study (Table 9.24)

Table 9.23: Quadriceps function baseline to week 3

N 23	Mean Baseline	SD	Mean week 3	SD	P
Quads diameter (cm)	50	10	51	10	0.42
MVC Quads (N)	256	115	355	176	**<0.005**

AUC MVC Quads	1332	639	1717	770	**0.015**
Quads Endurance Time (Sec)	157	139	215	175	**0.024**
Endurance time AUC	19705	18995	25751	20417	0.052

Table 9.24: Quadriceps function baseline to end of study

N 17	Mean Baseline	SD	Mean EOS	SD	P
Quads diameter (cm)	52	10	50	10	0.230
MVC Quads (N)	252	123	295	**120**	**0.049**
AUC MVC Quads	1396	724	1456	578	0.523
Quads Endurance Time (sec)	154	142	134	71	0.653
Endurance time AUC (sec)	20216	21035	31619	29635	0.088

No significant correlations were found with muscle endurance or strength with markers of exercise capacity.

9.5.4 **Micro-RNA**

9.5.4.1 **Serum miR**

20 subjects had pre and post exercise training complete serum miR values. There was a trend to down regulation of miR-1, -21 and -126, however statistical significance was not reached.

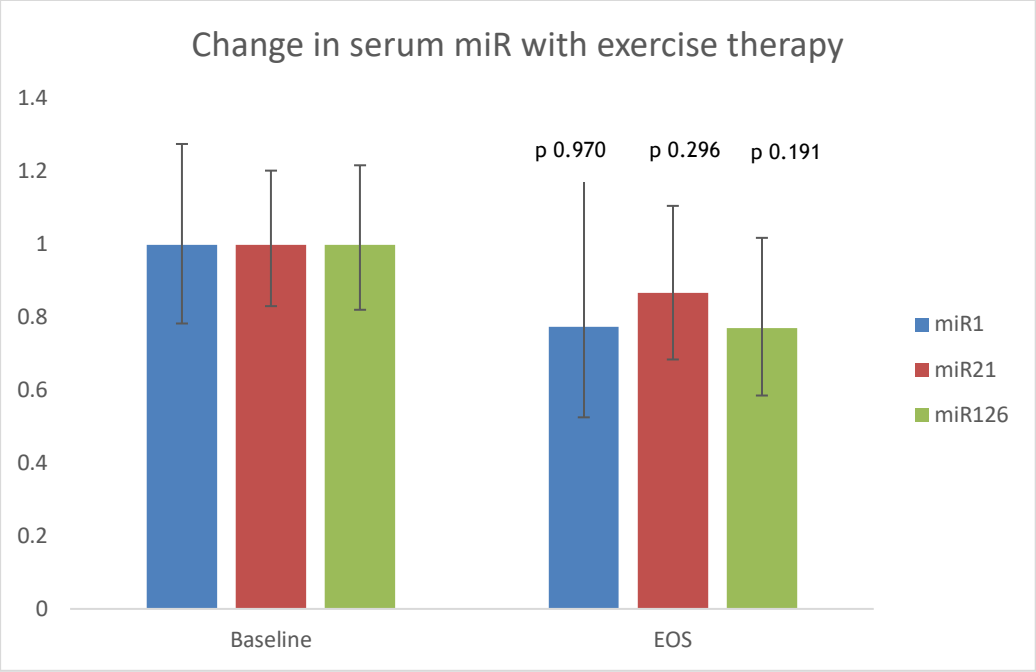


Figure 9.13: Change in serum miR expression with exercise therapy

With standard care, no significant differences were seen in serum miR-1, -21 and -126 (Figure 9.14).

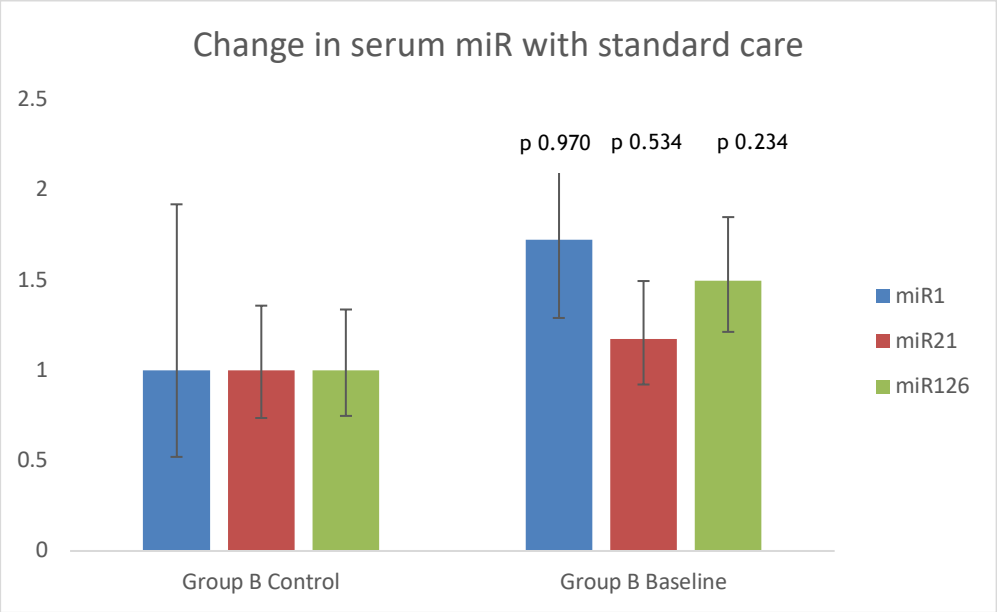


Figure 9.14: Change in serum miR with standard care

9.5.4.2 Muscle miR

19 subjects had baseline muscle biopsies and 10 had follow up biopsies. Of the 9 who did not have follow up biopsy, 4 were drop-outs from the study, 3 declined repeat biopsy and 2 subjects were unwell at the time of scheduled biopsy. Unpaired comparison of the whole population and paired comparisons in those who had pre and post exercise biopsies revealed very similar results, therefore paired data are presented in Figure 9.15, Figure 9.16 and Figure 9.17 below.

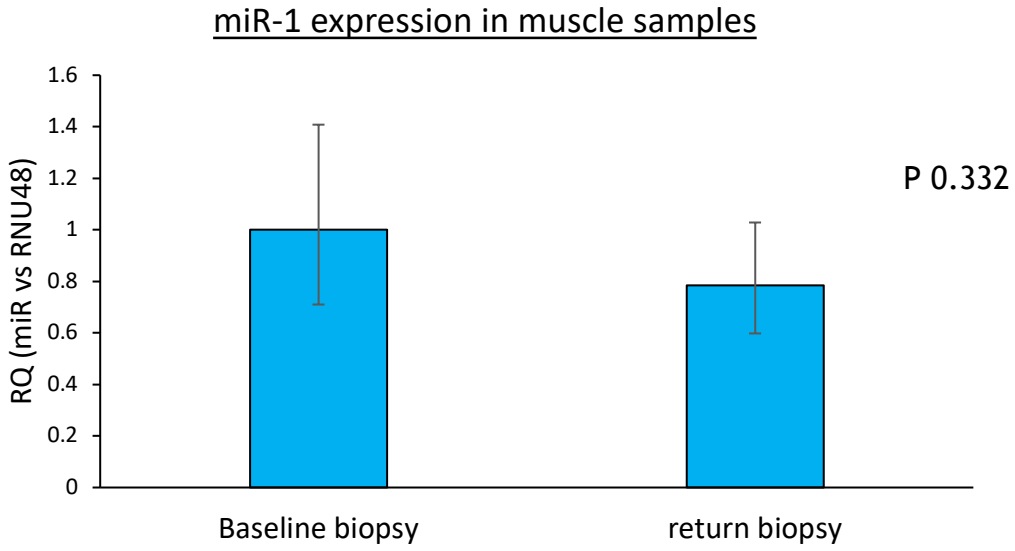


Figure 9.15: miR-1 expression pre and post exercise therapy

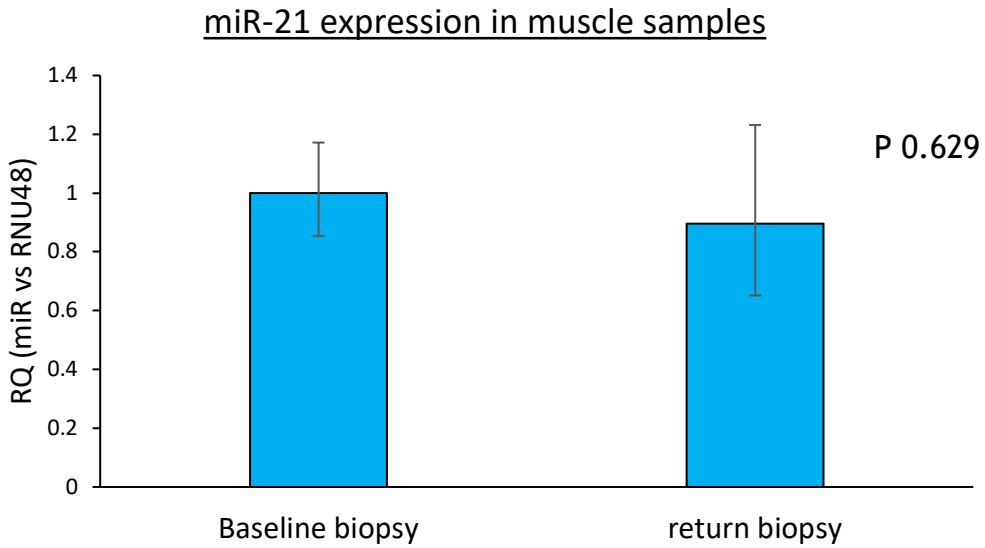


Figure 9.16: miR-21 pre and post exercise therapy

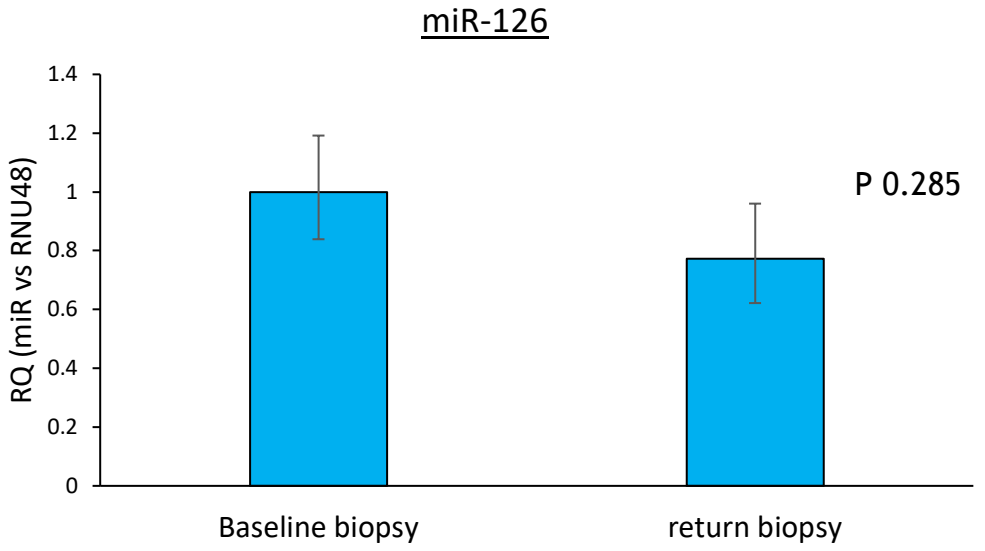


Figure 9.17: miR-126 skeletal muscle expression pre and post exercise therapy

Clinical improvement and transplant free survival

Muscle miR-126 expression increased significantly in those who deteriorated or did not improve with exercise therapy compared with those who did improve (Figure 9.18). A similar pattern was seen in those who were alive without transplant at censoring (Figure 9.19), however this did not reach statistical significance.

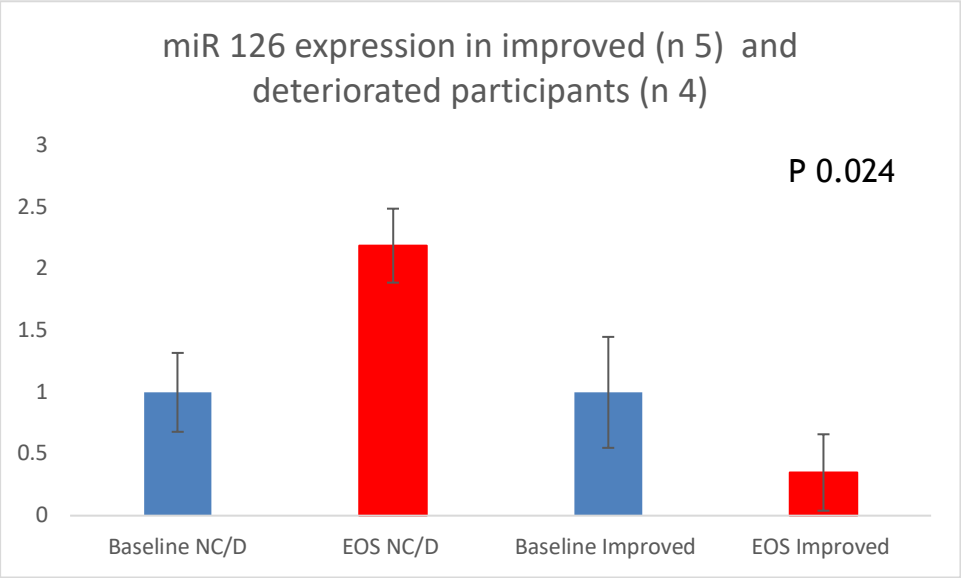


Figure 9.18: Differences in miR-126 skeletal muscle expression in improved versus deteriorated subjects

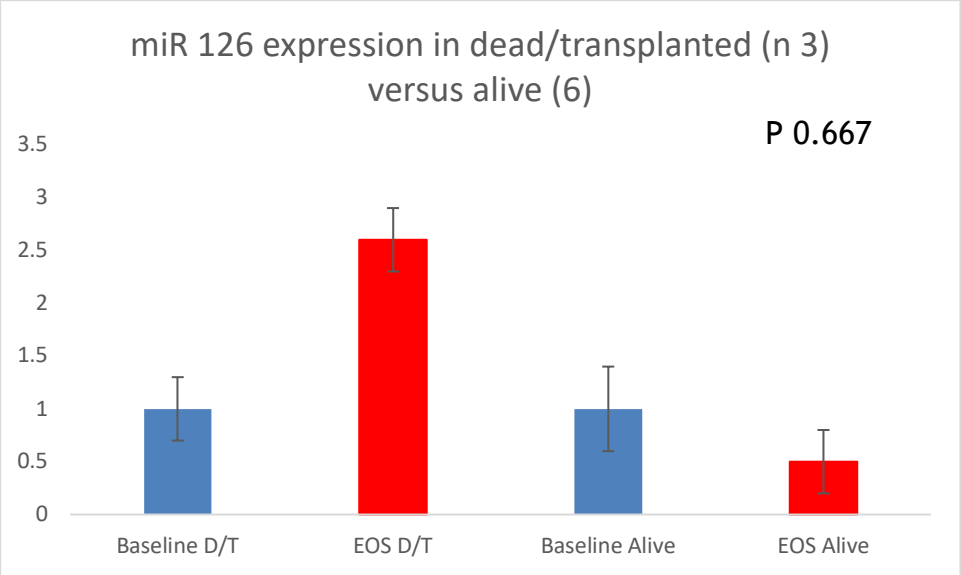


Figure 9.19: Difference in miR-126 muscle expression in relation to transplant free survival

There were no statistically significant differences in miR-1 and miR-21 in relation to transplant free survival or clinical improvement with exercise therapy.

Association with clinically relevant variables

Changes in miR-1, -21 and -126 expression correlated with prognostically relevant clinical variables such as 6MWD, mixed venous oxygen saturation and PVR (Table 9.25), however numbers were small.

Table 9.25: Clinical correlation with miR muscle expression

miR	Clinical variable	Spearman's R	p	N
miR 1	6MWD (m)	-0.750	0.020	9
miR 21	SvO_2 (%)	-0.857	0.007	9
miR 21	RV Mass (g)	0.703	0.035	9
miR 126	IL-6 (pg/L)	0.833	0.005	9
miR 126	PVR (WU)	0.833	0.010	9
miR 126	mPAP (mmHg)	0.714	0.047	8
miR 126	NLR	0.750	0.020	9
miR 126	Log NTproBNP	0.850	0.004	9
miR 126	KCO (%)	-0.929	0.001	8

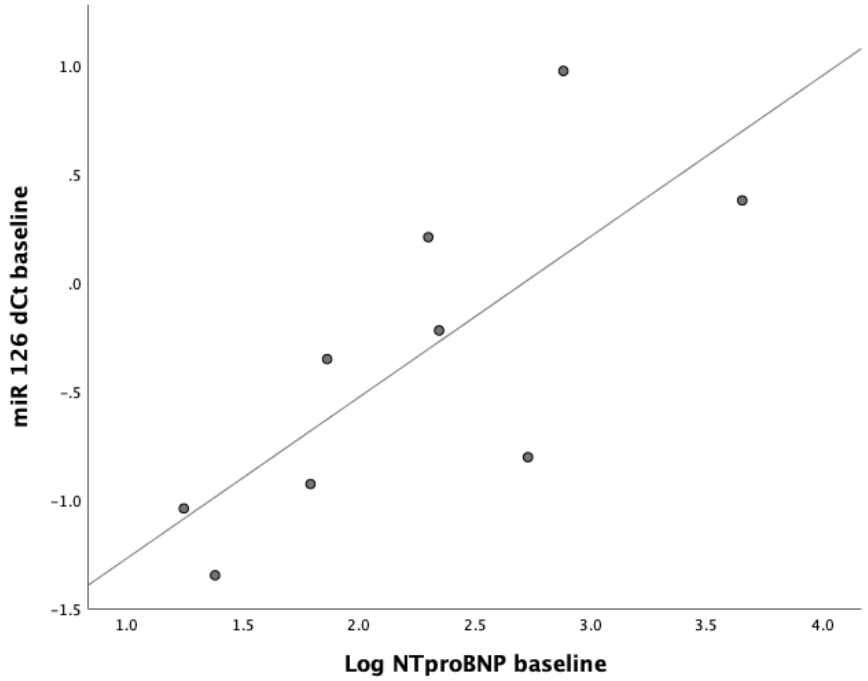


Figure 9.20: Log NTproBNP and miR126 muscle expression at baseline

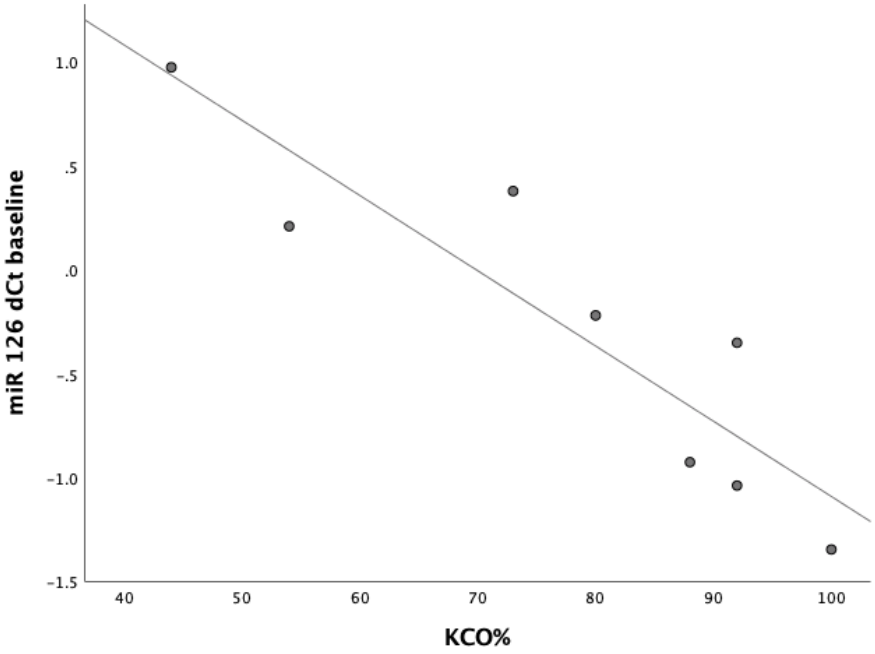


Figure 9.21: KCO (%) and muscle expression of miR-126

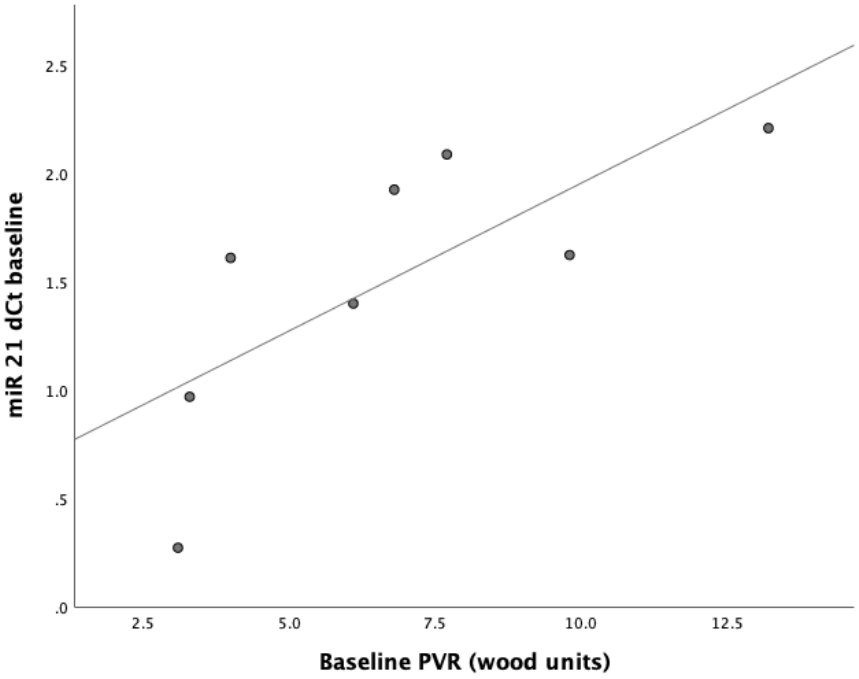


Figure 9.22: PVR (WU) and muscle expression of miR-21

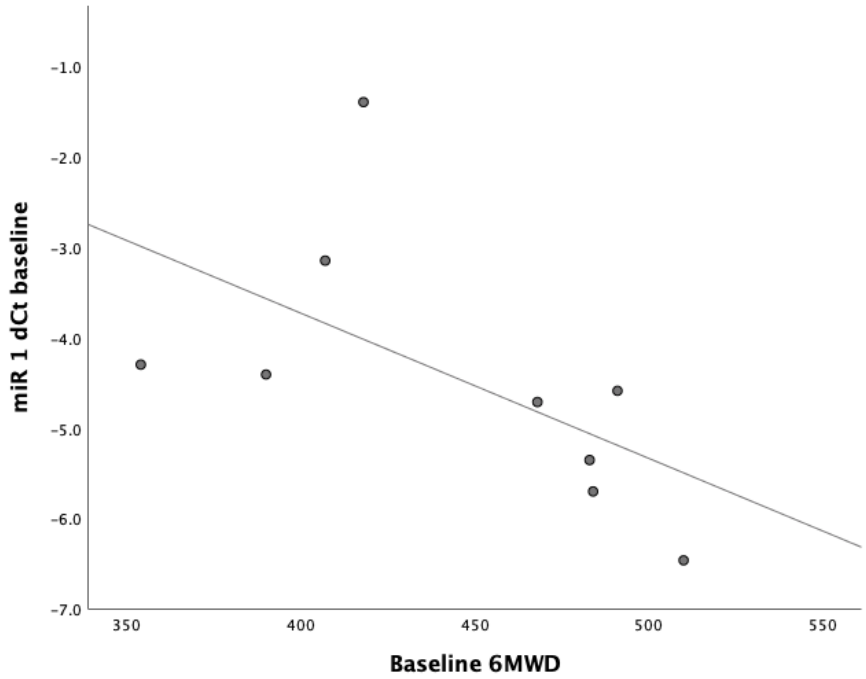


Figure 9.23: 6MWD and muscle expression of miR-1

Due to the small numbers, limited correlations were possible for tests of muscle function, however miR-126 at baseline and at the end of the study negatively correlated with grip endurance

Table 9.26: miR 126 and muscle function

miR expression	Muscle Function	R	p	N
miR 126 (Baseline)	Grip Endurance (sec)	-0.867	0.002	9
miR 126 (EOS)	Grip Endurance EOS (sec)	-0.821	0.023	7

9.6 **Discussion**

In keeping with published literature, this cohort of PAH patients displayed high rates of insulin resistance and systemic inflammation. As discussed in Chapter 7, a large proportion of subjects at baseline had normal cardiac output at rest and no change was seen in SvO_2 at rest or on exercise following exercise therapy, despite evidence of skeletal muscle myopathy and inflammation being present in baseline tests. These findings make flow driven causes of myopathy and inflammation less

likely and support the hypothesis of these changes being a reflection of the systemic nature of PAH as a pro-inflammatory condition.

9.6.1 **Effects of exercise therapy on metabolism**

There was a trend towards improved insulin sensitivity and beta-cell function, however these changes did not reach statistical significance. No changes were noted in serum lipid profile. The finding observed of improved insulin sensitivity with exercise, without changes in BMI or lipid profile has been noted in previously published studies [263]. Subjects with insulin resistance had lower exercise capacity, higher levels of systemic inflammation and poorer functional class. In those who improved with exercise therapy, lipid profile was more favourable at baseline however no other markers of metabolism were significantly different.

Despite the high prevalence of insulin resistance within this cohort and the benefits of exercise on metabolic function being well established in other conditions, exercise did not have a powerful impact on insulin resistance. It possible that there are other, more sensitive biomarkers that may more accurately reflect metabolism in PAH. Bioinformatics studies my help to identify the most appropriate biomarkers moving forwards.

9.6.2 **Muscle function and mechanistic pathways**

Exercise therapy improved clinical measures of muscle function, with improved strength and endurance in both quadriceps and hand grip.

From a mechanistic perspective, the most striking change in circulating and muscle miRs was in the down regulation of miR-126. In a post hoc analysis, a higher expression of miR-126 was noted in subjects with a poorer clinical course. In this exploratory analysis, miR-126 also negatively correlated with several prognostic markers such as mPAP, NTproBNP, IL-6 and positively correlated with gas exchange. This observation seems initially counter intuitive based on existing data suggesting increased miR-126 expression is beneficial in PAH muscle function through its angiogenesis promoting effects mediated by VEGF and SPRED[301]. Knock-out animal models of miR-126 have been studied in PAH[39], however no study to date has prospectively assessed serum and muscle expression of miR-126

following exercise therapy in PAH. There are several explanations for why this difference between this study and the existing literature exists:

1. In addition to its proangiogenic effects, miR-126 also has a pro-proliferative role; increased levels directly correlate with survival and tumour progression in cancers such as oral cancer[302]. Given the parallels between cancer and PAH biology, the downregulation of miR-126 observed in this study, may be reflect anti-apoptotic and anti-inflammatory effects of exercise[303].

2. miR-126 may have different roles in different scenarios; it is recognised that expression of specific miRs varies between “high” and “low” responders to exercise therapy and based on the duration, modality and intensity of exercise. miR126 expression is also likely to have different roles in different body tissues (skeletal muscle versus ventricular muscle).

3. The majority of the literature on miR126 in PAH pertains to animal models or invitro studies using miR126 mimic[304], it is possible that these changes do not translate over to in vivo human biology.

The changes in miR-1 and miR-21 were mirrored in serum and muscle, with a trend to down regulation following exercise, without statistical significance. Based on the roles of miR-1 and miR-21, one would hope for a reduction in miR-1 and miR-21 expression due to the association with myopathy, inflammation and vascular remodelling.

9.6.3 **IL-6 and inflammation**

Serum IL-6 concentration was elevated in a significant proportion of subjects, with a wide range in individual values. Subjects with very high IL-6 values had poorer exercise capacity and evidence of insulin resistance. These subjects were more likely to have connective tissue disease. Those who were alive without transplant at censoring, had a significantly lower IL-6 value than those who had died or been transplanted. This prognostic role was corroborated by the negative correlation of IL-6 with several markers of exercise capacity and positive correlation with NTproBNP.

Initial analysis of IL-6 revealed a paradox; high baseline levels were associated with poorer prognosis, but increased IL-6 concentration was seen following exercise therapy and was associated with improved exercise capacity and prognostic markers. Il-6 is known as a myokine, a cytokine produced by skeletal muscle, and is involved in the physiology of adaptation to exercise[305]. Exercise has been shown to acutely increase the levels of IL-6 and has been associated with reduction in visceral adipose, improved post-prandial glucose handling and suppression of pro-inflammatory mediators[306]. The increase in Il-6 seen following exercise therapy may therefore be seen as a positive response. To provide greater understanding of the role of IL-6 in relation to PAH pathogenesis and exercise, basal and post exercise levels would need to be studied over a period of time. A methodological limitation to this study is that it is not possible to say whether proximity to exercise sessions resulted in falsely high IL-6 levels in some cases, as the timing of venepuncture was not strictly controlled in relation to exercise.

NLR and PLR were raised in over half of the studied population, whilst CRP did not show any significant deviation from normal. The use of Epoprostenol in nearly half of this cohort and inclusion of subjects with liver disease may have reduced the efficacy of PLR due to the thrombocytopenic effect of both.

9.6.4 **Future research**

Further research to identify appropriate biomarkers will aid understanding of the processes involved in the development of metabolic dysfunction and inflammation in PAH and may help to identify potential therapeutic targets and provide clarity in the role of exercise therapy. Following on from this pilot study, further research is planned to assess histological changes in skeletal muscle, muscle cytokine expression and mitochondrial function.

9.7 **Conclusions**

Significant levels of metabolic dysfunction and inflammation were evident in this population in the face of clinical stability and optimal PAH therapy.

Metabolic dysfunction and inflammation were associated with markers of poorer prognosis and quality of life, particularly in relation to baseline serum IL-6. Composite measures of systemic inflammation, NLR and PLR, appeared to be more sensitive markers of systemic inflammation than CRP alone. Metabolic function was not strongly influenced by exercise therapy.

Exercise therapy improved muscle strength and endurance. miR-126 may be directly related to PAH myopathy and influenced by exercise therapy, however further research is required to investigate this.

It is clear that metabolic dysfunction and inflammation relate to prognosis in PAH, exercise therapy alone may not be sufficient in ameliorating these changes and further targets for intervention require investigation.

Chapter 10 Major findings and conclusions

The work in this thesis was undertaken to establish

- Whether PAH patients wished exercise therapy as part of their treatment and if so, what barriers may oppose their participation.

- Whether exercise therapy is effective in an optimally treated UK PAH population

- Explore potential mechanisms by which is exercise exerts its beneficial effects in PAH in order to

    - Further characterise persistent physiological and biological changes in those “optimally treated” prior to exercise therapy.

    - Understand factors that are modifiable with exercise therapy and therefore gain a better understanding of how exercise exerts its beneficial effects in PAH.

    - Identify key components of the programme to help develop and refine future exercise programmes and inform clinical service development.

Efficacy

- Overall, exercise therapy is a desired treatment option for patients with PAH in Scotland. It is highly effective in the majority, well tolerated and safe.

- Improvement with exercise therapy as defined by an increase in 6MWD of greater than 30m appears to be a suitable means of defining improvement to the intervention. 6MWD correlated strongly with quality of life and lack of change in walk distance following exercise therapy discriminated well those with poorer quality of life and prognosis.

- A small subset of patients did not improve with exercise therapy in a post hoc analysis. It appeared that these patients were more likely to have severe PAH, high levels of inflammation and connective tissue disease. It is unclear whether this sub-group derives benefit from exercise, it is possible the 6MWT is the wrong end point in this sub-population due to musculoskeletal comorbidities. Exercise therapy may slow the disease trajectory and improve function, alternatively it may exert no beneficial effects. This specific population requires further research and an appropriately powered study to address these points.

Disease insights

This small but highly phenotyped population provided interesting and useful insights into the role of exercise therapy in PAH and the underlying systemic manifestations of the disease.

Despite satisfactory improvements in haemodynamics with optimal drug therapy, and an acceptable mean baseline walk distance of 422m, the population studied displayed significant disease specific and systemic abnormalities. This highlights the ongoing need for new treatment strategies in this population.

- At rest PA haemodynamics appeared to be satisfactory, with low PVR, preserved cardiac index and relatively low mPAP. This was in keeping with the majority of patients displaying normal RV function and volumes on CMR. On exercise, significant rises were seen in mPAP and TPR.

- Gas exchange was significantly reduced and one third of the population had TLCO under 45%.

- Quality of life was impaired and the majority of patients were in WHO FC III.

- Excluding those with known diabetes, 43% of patients had insulin resistance, in the absence of other risk factors such as obesity.

- Over half of those included displayed elevated composite markers of inflammation (NLR and PLR), with a quarter having raised IL-6 values.

Mechanisms of improvement in exercise capacity

Key observations following treatment with exercise therapy

- Significant improvements occurred in peak aerobic exercise capacity and endurance exercise capacity.

- Cardiovascular efficiency improved, particularly on exercise, with increased pulmonary artery compliance, improved right ventricular oscillatory power fraction. Improvements in left ventricular function were seen in those who improved with exercise therapy.

- Tidal volumes, gas exchange and ventilatory efficiency improved.

- No clear change was seen in metabolic function.

- Muscle strength and endurance improved.

- Serum and muscle miR-126 appeared to be sensitive to change following exercise therapy, with down regulation in both. The clinical significance of this change is unclear due to miR-126s opposing roles of angiogenesis and abnormal proliferation.

Bringing these findings together, improvements in cardiovascular and ventilatory efficiency are to be strongly linked. These changes appear to be associated with treatment response and prognosis. The underlying mechanisms for these improvements may include improved alveolar blood flow due to higher stroke volume, improved endothelial health and release vasoactive mediators such as nitric oxide, lung recruitment, reduced atrial stretch and reduced hyperventilation.

Based on these data, respiratory muscle training appears to be a key component of the exercise programme both in terms of controlling hyperventilation and improving tidal volumes. Aerobic exercise also appears to be essential due to the improvements seen in cardiovascular efficiency and LV stroke volume. Inflammation and metabolic dysfunction are highly prevalent, however the signal for improvement in these abnormalities was not strong.

Future research and clinical implications

From a clinical perspective, it is clear that dedicated PAH specific exercise therapy is of benefit for those with PAH. The initial time intensive approach provides accelerated acquisition of fitness, independent exercise ability and motivation. It would be desirable to develop a more accessible and less time consuming rehabilitation programme in order to make this treatment available to all those who may benefit. Key components of this programme should include respiratory muscle training and aerobic exercise with initial face to face sessions with a health care professional experienced in PH and rehabilitation.

This research explored potential reasons for failure to improve with exercise therapy in a post-hoc analysis, therefore robust conclusions cannot be made from the data presented. Dedicated, controlled studies are required for "non-responders" to exercise therapy, those who are in higher risk disease categories and those with CTD-PAH. It is vital to understand whether exercise therapy may have a role in slowing disease progression in these populations. Time to clinical worsening may therefore be a more appropriate end point in this group.

Further research to characterise the improvements seen in vascular compliance, lung volumes and gas exchange would be of great interest. Exercise Cardiac MRI may provide useful insights into dynamic change in biventricular volume and function. Lung perfusion imaging and assessment of autonomic function pre and post exercise therapy could help to clarify whether the changes in improved compliance and reduced resistance on exercise are due to recruitment, improved autonomic function or other mechanisms. Measurement of vascular nitric oxide pre and post exercise therapy may help to understand the role of the endothelium in improved PA compliance. Finally, studies of respiratory muscle training in isolation

may allow assessment of the contribution this training modality provides to overall patient benefit and the degree to which it is responsible for the physiological changes observed.

The pathways involved in metabolic dysfunction, myopathy and inflammation in PAH remain poorly understood. Further studies of the metabolome in PAH may help identify suitable biomarkers for future clinical trials and potential targets for therapy.

Appendix 1: Patient survey letter

25th September 2014

Dear _____

We are writing to you because you attend the Scottish Pulmonary Vascular Unit for treatment of Pulmonary Hypertension. We would be grateful for your opinion on a new type of treatment. **Please find further details and a short survey below.**
Breathlessness is the most common symptom of Pulmonary Hypertension. People with Pulmonary Hypertension often feel their walking and activity is limited because of breathlessness. A programme of safe exercises specifically designed for patients with Pulmonary Hypertension is known to improve activity levels and quality of life. This programme is doing well in countries outside the UK.

These training programmes would be planned in order to meet the needs of each person. The main part of the programme would involve:

- A supervised training programme individually tailored to your abilities. (This will involve stationary cycling and walking)
- Gentle muscle strength training
- Breathing exercises

Exercises would be supervised by a physiotherapist and doctor. There would be 2 parts to the programme, which would last 14 weeks in total.

- First phase: 2 weeks of supervised exercise 5 days per week at the Golden Jubilee Hospital
- Second phase: 12 week training programme at home. You would be provided with equipment such as a stationary exercise bicycle. The physiotherapist or doctor would keep in regular contact by phone.

The aim of this training programme is to improve your breathlessness, increase the amount of activity you can do and improve your quality of life.
We would be grateful if you could complete this short survey and return it in the pre-paid envelope.

Yours sincerely

Dr Martin Johnson
Consultant Physician

Dr Alison MacKenzie
SPVU Fellow

On behalf of the Scottish Pulmonary Vascular Unit

Prof A Peacock
Director

Dr M Johnson
Consultant Physician

Dr C Church
Consultant Physician

Dr M Sproule
Consultant Radiologist

Dr N McGlinchey
Dr A Mackenzie
Dr G Jayasekera
Pulmonary Vascular Fellows

Dr D Welsh
Research Scientist

Sr A Crozier
Sr A Curran
Sr K Carson
Clinical Nurse Specialists

Sr V Pollock
Clinical Trial Nurse

Mr S Kerridge
Data Manager

Ms V Ferry
Ms K Menzies
Personal Secretaries

Name (optional)
Please tick the boxes that apply (YES or NO)

2. **The first phase of the programme would involve either**
   - **a.** Daily hospital visits for 2 weeks (excluding weekends) if you lived near the Golden Jubilee Hospital in Clydebank. Transport expenses would be covered.
   - **b.** A 2 week stay in the Beardmore Hotel, which adjoins the hospital to allow you to attend the daily sessions. Accommodation and meals would be provided.

   Would you find this acceptable?

   Yes☐ No ☐

3. The second phase would involve exercise at home with gentle cycling, muscle training and breathing exercises 5 days per week for a total of 12 weeks.

   Would you find this acceptable?

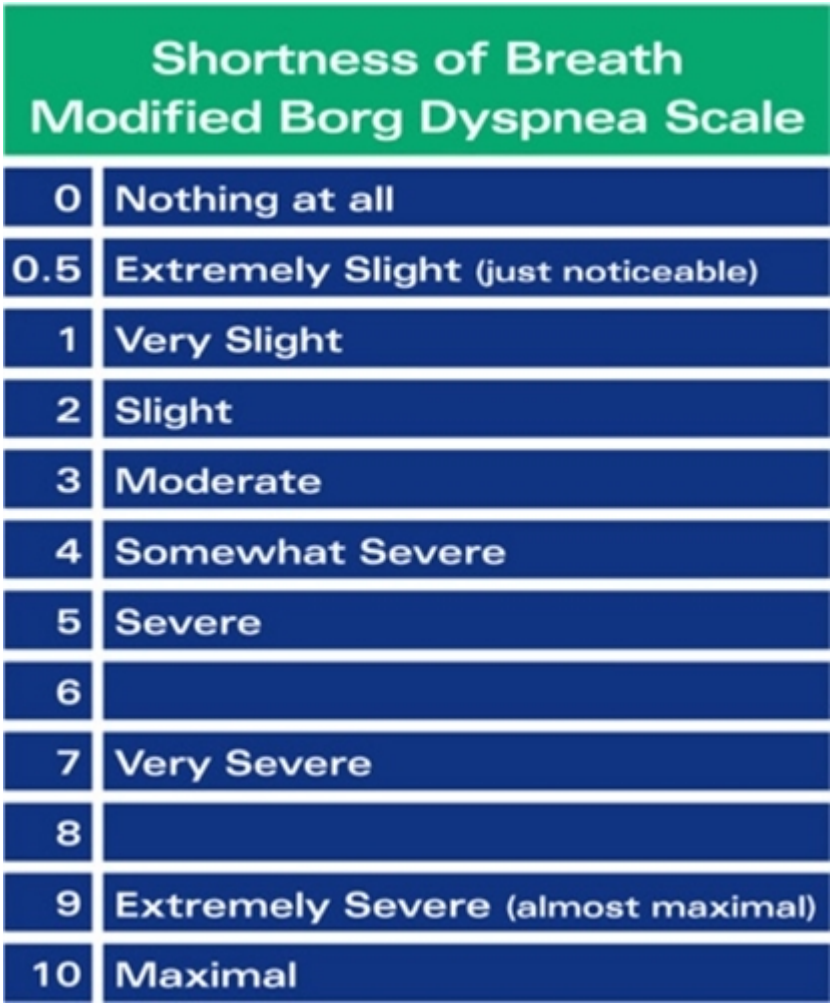
   Yes☐ No ☐

4. **Is this programme something you would be interested in taking part in?**

   Yes☐ No ☐

5. **If No what would put you off taking part?**
6. **Are there any other questions or comments you have regarding this programme?**

Appendix 2: Modified Borg dyspnoea scale



Appendix 3: Exercise Diary

SCOTTISH
PULMONARY
VASCULAR
UNIT

PERSONAL DIARY OF .....

"The effect of adding exercise training to optimal therapy in Pulmonary Arterial Hypertension"

Scottish Pulmonary Vascular Unit
Golden Jubilee National Hospital
Agamemnon Street
Clydebank
West Dunbartonshire
G81 4DY
Email: SPVUnit@gjnh.scot.nhs.uk

SCOTTISH PULMONARY VASCULAR UNIT

You can use it to:

- record personal information
- note personal goals
- write down thoughts and feelings
- detail progress
- keep a record to look back on and reflect on progress

Contents

During your stay in the hotel:
If you need to get in touch with us between the hours of 8.30am and 4.30pm please telephone the SPVU Secretaries on 0141 951 5497 who can pass on a message for you.

In the event of you feeling unwell out with these hours please contact hospital reception on 0141-951-5000 and ask them to contact a member of the SPVU medical team.

1. About your diary

This diary helps you to record and monitor your progress while you are here at the Golden Jubilee. It will provide guidance and help you to collect your thoughts and identify any personal goals you may have. There is space to record thoughts and feelings about your training programme and progress. You may want to discuss these thoughts with your rehabilitation team. It is important that you raise any questions or concerns at any stage of the study.

You can use this diary however you like, nevertheless, we do ask that you keep it safe and bring it with you to each session.

2. Your personal information

Name: .....
DOB: .....

Weight: .....
Height: .....

Diagnosis:
.....
.....

Oxygen therapy: Yes / No
Flow rate: ..... Litres/min
Pulsed / Continuous

Resting SpO2: ..... %
Minimum SpO2 during exercise: .....%

Resting heart rate: ..... bpm
Peak exercise heart rate: ..... bpm

6 minute walk test at baseline: ..... metres

Your present level of activity

.....

.....

.....

.....

.....

Your personal goals

1) .....

2) .....

3) .....

4) .....

3. What to expect

The aim of this study is to answer the following questions:

1. To determine whether exercise training as a therapy has the potential to improve exercise capacity, heart function and quality of life in Scottish patients who are on the best available treatment for Pulmonary Arterial Hypertension (PAH).
2. To understand how exercise training improves symptoms and exercise capacity in people with PAH in order to develop the most effective type of training programme.

During your stay at the Golden Jubilee you will attend a variety of tests to examine your heart, lungs and muscles and how they respond to the exercise programme. These tests will be performed at the beginning and end of your 3 week stay and at the end of the 12 week out-patient phase.

You will have daily exercise sessions. These will involve stationary cycling, walking, breathing exercises and light weight training. You can expect to be walking outdoors in the hospital grounds (remember your waterproofs!), performing

exercise in the gymnasium and attending relaxation sessions.

You will have several, short, supervised exercise sessions every week day with rest intervals in between. The intensity of exercise will be based on your initial fitness and will be gradually adjusted over the sessions by the Doctor or Physiotherapist. At the weekend you will perform lower intensity, unsupervised exercise.

You will be issued with a personalised timetable which the Doctor or Physiotherapist will discuss with you on your first day. This timetable will give you all the information that you will need.

When you go home you will be issued with a stationary exercise bike, weights, a pulse oximeter and a personalised training manual to follow to further improve your exercise capacity and function. You will be phoned by the Physiotherapist or Doctor at regular intervals over the 12 week out-patient period to check you are managing the training programme and make adjustments if needed.

Timeline	Assessment / Investigation	Walking	Respiratory training	Bicycle training	Weight training	Relaxation/ Education
Day 1-3	X					
Monday - Friday		X	X	X	X	X
Saturdays & Sundays		X	X		X	
Week 3	X	X	X	X	X	X

5. Your training programme explained

Walking programme

This will involve walking both indoors and outdoors (weather permitting). You will be walking at a pace that gets you breathing more deeply and slightly out of breath, but still allows you to keep a conversation going.

Each walking programme will start at a gentle pace on level ground. Gradually you will increase to a brisker pace and varied ground over the sessions. You will be taught breathing control and imagery techniques during these sessions which will help you manage your breathlessness and cope in more demanding situations e.g. walking uphill.

If you feel you are tiring or becoming uncomfortable during the walk, tell your therapist and you can stop and rest. Breathing control exercises will help you to gain control. Your oxygen level will be monitored while you are walking, if it falls significantly, you will be provided with supplementary oxygen.

Respiratory training

You will be taught basic breathing techniques, such as abdominal breathing and pursed lip breathing, which will help you to control your breathing rhythm during activity and strengthen your respiratory muscles. We will show you how to use these techniques during daily life and help you to cope with daily challenges.

Bicycle training

You will be instructed on how to undertake
safe and effective training on a stationary exercise bicycle. Bicycle training will initially be in 15 minute sessions. During this time your heart rate and oxygen levels will be monitored along with your own perception of your breathlessness. The sessions will then be adjusted to meet your individual training needs. These sessions will involve interval training. This means you will alternate between cycling at an easy resistance for 1 minute (like cycling on the flat), then at a higher resistance for 30 seconds (like cycling on a hill).

Weight training

You will be given a personal strengthening programme and taught how to use light weights efficiently. We will target specific muscle groups using an endurance programme to stimulate the most growth in muscle fibres. Lifting weights in this way will improve your muscle power and stamina. Once you are stronger we will slowly increase the weights you use to target your particular demands.

You can use this space to record the information you are given, your thoughts and feelings, or other questions you may have:

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Appendix 4: Sample outpatient timetable

Daily exercise Log

Day & Date	Walking practice	Heart rate (HR) & Oxygen (SaO2) during walk	Resp training	Bicycle training	Heart rate (HR) & Oxygen (SaO2) during cycle	Weight training Upper limb weight: 1.5kg Lower limb weight: Nil
Monday _____	Duration Terrain: BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Achieved Yes/No	Duration: Level: 1 /2 /3 BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Upper limb exercises performed: Yes/No Lower limb exercises performed: Yes/No
Tuesday _____	Duration Terrain: BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Achieved Yes/No	Duration: Level: 1 /2 /3 BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Upper limb exercises performed: Yes/No Lower limb exercises performed: Yes/No
Wed _____	Duration Terrain: BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Achieved Yes/No	Duration: Level: 1 /2 /3 BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Upper limb exercises performed: Yes/No Lower limb exercises performed: Yes/No
Thursday _____	Duration Terrain: BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Achieved Yes/No	Duration: Level: 1 /2 /3 BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Upper limb exercises performed: Yes/No Lower limb exercises performed: Yes/No
Friday _____	Duration Terrain: BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Achieved Yes/No	Duration: Level: 1 /2 /3 BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Upper limb exercises performed: Yes/No Lower limb exercises performed: Yes/No
Saturday _____	Duration Terrain: BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Achieved Yes/No	Duration: Level: 1 /2 /3 BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Upper limb exercises performed: Yes/No Lower limb exercises performed: Yes/No
Sunday _____	Duration Terrain: BORG at max:	HR max: __ bpm Sao2 min: __%	Achieved Yes/No	Duration: Level: 1 /2 /3 BORG at max:	HR max: ___ bpm Sao2 min: ___ %	Upper limb exercises performed: Yes/No Lower limb exercises performed: Yes/No

Appendix 5: Sample residential timetable

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
WEEK 1	1 Assessment 1. 1200-1300 Lunch CMR 6MWT1	2 Information session: Respiratory training and walking programme 1200-1300 Lunch CPET	3 Introduction to weight training 1200-1300 Lunch RHC at rest/ exercise	4 0930-1030 Bicycle training 1030-1130 Respiratory training 1200-1300 Lunch 1300-1400 Weight training 1500-1600 Walking	5 PFTS and mouth pressures 1200-1300 Lunch 1300-1400 Education session 1500-1600 Walking	6 0930-1030 Walking 1030-1130 Respiratory training 1200-1300 Lunch 1300-1400 Weight training	7 0930-1030 Weight training 1200-1300 Lunch 1300-1400 Walking 1500-1600 Respiratory training
WEEK 2	8 0930-1030 Bicycle training (30 mins) 1030-1130 Education session (30 mins) 1200-1300 Lunch 1300-1400 Respiratory training (30 mins) 1500-1600 Walking (60 mins)	9 0930-1030 Weight training (30 mins) 1030-1130 Respiratory training (30 mins) 1200-1300 Lunch 1300-1400 Bicycle training (30 mins) 1500-1600 Education session (30 mins)	10 0930-1030 Bicycle training (30 mins) 1030-1130 Education session 1200-1300 Lunch 1300-1400 Respiratory training 1500-1600 Walking	11 0930-1030 Weight training 1030-1130 Respiratory training 1200-1300 Lunch 1300-1400 Bicycle training 1500-1600 Education session	12 0930-1030 Bicycle training 1030-1130 Education session 1200-1300 Lunch 1300-1400 Respiratory training 1500-1600 Walking	13 0930-1030 Walking 1030-1130 Respiratory training 1200-1300 Lunch 1300-1400 Weight training	14 0930-1030 Weight training 1200-1300 Lunch 1300-1400 Walking 1500-1600 Respiratory training
WEEK 3	15 0930-1030 Bicycle training 1030-1130 Education session 1200-1300 Lunch 1300-1400 Respiratory training 1500-1600 Walking (60 mins)	16 0930-1030 Weight training (30 mins) 1030-1130 Respiratory training (30 mins) 1200-1300 Lunch 1300-1400 Bicycle training (30 mins) 1500-1600 Education session (30 mins)	17 0930-1030 Bicycle training (30 mins) 1030-1130 Education session (30 mins) 1200-1300 Lunch 1300-1400 Respiratory training (30 mins) 1500-1600 Walking (60 mins)	18 Assessment 2 6MWT1 1200-1300 Lunch	19 CPET PFTS 1200-1300 Lunch Issued with home exercise programme	20	21

Appendix 6: Walking training

SCOTTISH PULMONARY VASCULAR UNIT

Walking programme: Standard Operating Procedure

The walking programme will take place at two GJNH sites dependant on weather
1. In the hospital grounds under physiotherapy or doctor supervision.
2. Level 2 gym: indoor walking or walking on a treadmill

A maximum of 1 patient will undertake walking practice at any time.

The walking programme will be prescribed by the study physiotherapist or doctor and will be based on initial six minute walk test distance, heart rate, oxygenation saturation and Borg.

Mental imagery techniques should be adopted focusing on the movement, speed, breathing pattern in inner and outer perspective (section 1).

Heart rate, oxygen saturation and Borg will be assessed at 5 minute intervals (table 1).

Walking aids should be utilised as required by the patient.

Each walking session will begin with awareness of walking: movement; speed and breathing. Try different patterns and combinations of these three.

Commence on level ground at a gentle pace set by the patient.

Breathing control exercises should be adopted based on the patient's level of breathlessness and rate of perceived exertion. See respiratory training standard operating procedure for details.

Frequent standing rest stops should be encouraged based on the patient's breathlessness, heart rate, oxygen saturations and requirement.

Up-titration of exercise will be based on individual progress using the following parameters: If Heart rate < 120, SpO2 > 90 and Borg low / intermediate then it is safe to increase intensity.

Initial target exercise time will be 20 minutes. Once safely achieving the target duration, exercise can be up-titrated by adding more demanding situations such as uneven surfaces, inclines, stairs and variation of pace.

Specific circumstances:

- Steep incline: use a slow, zig zag route. This reduces the overall impact of the incline and enables better control of speed and breathlessness.
- Stairs: ascended at a slow pace. Small flights can be ascended at a continuous pace. Both feet should be brought to each step (rather than alternating pattern). Frequent rest stops should be taken on larger flights.

Exercise precautions:

- In the event of SpO2 < 90% supplementary oxygen will be provided via a home-fill portable oxygen system. If this occurs, ambulatory oxygen for home should also be organised.
- Breathing should be of a moderate intensity. The patient should be able to hold a conversation with ease.
- Walking on uneven ground may not be possible for those patients who require rollator or delta walking frames.
- Heart rate should not exceed 120 bpm for a sustained period of time, if this occurs, walking intensity will be reduced.

Contraindications / termination of exercise:

- Chest pain
- Syncope or pre-syncope
- Patient request
- Decompensated right heart failure
- Respiratory tract infection or other acute illness

Table 1: Parameters recorded during walking programme

Date & Time	Spo2 (%)		Heart Rate (bpm)		Comments
	Min	Max	Min	Max	

Section 1: Mental imagery techniques

The patient should imagine themselves in an environment performing a specific activity using all of their senses (hearing, sight, feel and sense of smell).

Familiarise the patient with a comfortable scenario. Ask the patient to close their eyes and recall the images of what they were doing or where they were going. Encourage the patient to focus on positive outcomes and success, imagining they have reached their desired goal.

Before mobilising ask the patient to perform a mental run through of their scenario. Use different imagery techniques for different paces (i.e. chasing dog in the park, walking faster to catch a bus etc).

Appendix 7: Bicycle ergometer protocol

Ergometer Training Standard Operating Procedure

SCOTTISH PULMONARY VASCULAR UNIT

- Ergometer training will take place in the physiotherapy gym, level 2, GJNH under physiotherapy or doctor supervision
- A maximum of 2 patients will undertake ergometer training at one time using the optibike rehabilitation ergometer
- Prescription of ergometer protocol by study doctor is based on initial CPET: 40-50% of peak WR
- HR and O2 saturations will be monitored throughout using a pulse oximeter
- Contraindications / termination of exercise
  - Chest pain
  - Syncope or pre-syncope
  - Patient request
  - Decompensated RHF
  - LRTI or other acute illness
- Total exercise time 17min (in fitter patients 21min, will be determined on an individual basis)
- Interval training will be undertaken (In fitter patients, constant load – individual patient basis)
  - 0/5W, 5/10W, 10/15W, 15/20W, 15/25W etc.
  - 1 min low WR, 30 sec high WR
  - Target 60 rpm
- HR / SpO2 / Borg legs / Borg breathing will be recorded as per table 1
- Up-titration of exercise will be based on individual progress using the following parameters: If HR < 120, SpO2 > 90 and Borg low / intermediate then ok to titrate up to the next step (5 watt increments).
- Supplementary O2 will be provided via a concentrator if SpO2 < 90%. If this occurs, ambulatory O2 for home should also be organised.
- HR should not exceed 120 bpm for a sustained period of time, if this occurs, the peak WR will be reduced.

Table 1: Parameters recorded during bicycle ergometer

Date:
Time:
Session number:

Time	BP	HR	SpO2	Borg Legs	Borg Breathing
Rest					
5min					
10 min					
15min					
17 min (End exercise)					
Recovery 30s					
Recovery 1min					
Recovery 1.5min					
Recovery 2min					
Recovery 2.5min					
Recovery 3 min					

Appendix 8: Respiratory muscle training

SCOTTISH PULMONARY VASCULAR UNIT

Respiratory training: Standard Operating Procedure

Respiratory training will take in the rehabilitation gym, level 2, GJNH under physiotherapy or doctor supervision.

A maximum of 4 patients will undertake respiratory training at any time.

Respiratory training will be delivered by the physiotherapist or study doctor.

The patient will be in a comfortable, upright seated position for the duration of the session. They will be advised to relax their neck, shoulders and upper chest muscle as able. Arms should be supported on the side arms of the chair or on their lap.

Patients should use their own oxygen if required. Supplementary oxygen will be used in the event of SpO2 < 90%. This will be provided via an oxygen concentrator.

Total exercise time should be 30 minutes.

Contraindications / termination of exercise

- Chest pain
- Syncope or pre-syncope
- Patient request
- Decompensated right heart failure
- Respiratory tract infection or other acute illness

Thoracic expansion exercises

"Take a long, slow breath in through your nose, first filling your lower lungs, then your upper lungs. Hold your breath to the count of three if possible. Exhale slowly through pursed lips, while you relax the muscles in your face, jaw, shoulders, and stomach."

Technique:

1) Place a clean towel around thorax.
2) Pull the towel tightly; crossing ends at the front of the thorax.
3) Using the towel, feel thoracic expansion on inspiration and expiration.
4) Repeat these steps for 10 breaths.
5) Next, assist expiration by pulling the towel tighter on expiration.
6) Repeat these steps for 10 breaths.

Up-titration of this exercise should begin week 2 through resisted inspiration.

1) Position as above with towel around thorax.
2) Pull the towel tightly; crossing ends at the front of the thorax.
3) Tighten towel further around your thorax.
4) Maintain pressure on both inspiration and expiration.
5) Repeat these steps for 10 breaths.

Breathing control exercises

The patient will be advised to:

"Breathe gently in and out. If possible, breathe in through your nose and out through your mouth in a steady slow rhythm. Try to keep your mouth closed when you breathe in through your nose. As you breathe out, pucker or 'purse' you lips. Try to make your breath out twice as long as your breath in. To do this you may find it helpful to count 'one, two' as you breathe in, and 'one, two, three, four' as you breathe out. Do not hold your breath between breathing in and out."

Technique:

1) Place hands on upper chest.
2) Feel chest rise and fall.
3) Repeat these steps for 10 breaths.
4) Next, place hands at mid thorax.
5) Feel ribs rise and fall.
6) Repeat these steps for 10 breaths.

Diaphragmatic breathing

Continue breathing control technique targeting diaphragm.

The patient will be advised to:

"Next, mainly use your lower chest muscle (diaphragm) to breathe. You can check if you are using your diaphragm by feeling just below your breastbone (sternum) at the top of your tummy (abdomen). If you give a little cough, you can feel the diaphragm push out here. If you hold your hand here you should feel it move in and out as you breathe."

Technique:

1) Place hands on lower abdomen.
2) Feel abdomen rise and fall.
3) Repeat these steps for 10 breaths.

Pelvic rocking with breathing control

Place hands on hips. Tuck head downward and round back up, making a curve with spine.

Use breathing control technique as described above.

1) Breathe in deeply
2) While slowly exhaling through pursed lips, pull in abdominal muscles, straighten lower spine, upper spine and then bring head into neutral position.
3) Relax lower abdominal muscles
4) Repeat these steps for 10 breaths.

Upper limb stretches with breathing control
Continue with breathing control technique.

1) Right hand on left knee/thigh – circle arm backwards into combined motion of shoulder flexion/elevation and abduction to right hand side.
2) Repeat 10 times.
3) Left hand on right knee/thigh – circle arm into backwards into combined motion of shoulder flexion/elevation and abduction to left hand side.
4) Repeat 10 times.
5) Left upper limb relaxed at side. Stretch right arm above head into left trunk lateral flexion.
6) Repeat 10 times.
7) Right upper limb relaxed at side. Stretch left arm above head into right trunk lateral flexion.

Alternatively, perform the below exercises with breathing control:

1) Shoulder shrugs.
2) Repeat 10 times.
3) Both upper limbs flexed to shoulder level and back to sides.
4) Repeat 10 times.
5) Both upper limbs abducted into shoulder elevation and hands above head then returned to sides.
6) Repeat 10 times.
7) Pelvic tilting with hands on hips.
8) Repeat 10 times.

Re-test thoracic expansion

1) Place towel around thorax.
2) Pull the towel tightly; crossing ends at the front of the thorax.
3) Using the towel, feel thoracic expansion on inspiration and expiration.
4) Repeat these steps for 10 breaths.
5) Next, assist expiration by pulling the towel tighter on expiration.
6) Repeat these steps for 10 breaths.

An improvement in thoracic excursion should be felt.

Appendix 9: Skeletal muscle training

SCOTTISH PULMONARY VASCULAR UNIT

Weight training: Standard Operating Procedure

Weight training will take place in the rehabilitation gym, level 2, GJNH under physiotherapy or doctor supervision.

A maximum of 4 patients will undertake training at any time.

Sessions will be delivered by the physiotherapist or study doctor.

Patients should use their own oxygen if required. Supplementary oxygen will be used in the event of SpO2 < 90%. This will be provided via an oxygen concentrator.

Patients should be advised to exhale when working against resistance during lifting and inhale as they release.

Total exercise time should be 30 minutes.

Weights
Light weight dumbbells will be used for upper limb exercises. Weight ranges from 0.5kg to 2kg. Less able patients may need to commence arm exercises without dumbbells.

Weights will only be used for lower limb exercises when the patient can competently manage ten repetitions of the exercise with ease. In these circumstances ankle weights will be used with weight ranging from 0.5kg to 2kg.

Starting weight should be chosen through individual patient assessment. Up-titration of exercise will be based on individual progress by slowly increasing weight.

The weight should fatigue the targeted muscle group by the last two repetitions while still allowing maintenance of good technique. If the patient cannot perform the last two repetitions, a lighter weight should be used. When the patient feels it is too easy to complete all ten repetitions weight should be increased by 0.5kg at a time.

If a patient can competently manage ten repetitions with the maximum weight of 2kg then exercise should be up-titrated by adding another set of repetitions. This should be in sets of ten repetitions at a maximum of three sets. The patient should be able to do all the repetitions with good technique and the targeted muscles should feel tired by the last two. If the patient struggles to complete the regime the repetitions should be reduced accordingly.

Considerations:

- Poor technique can cause injuries and reduce muscular gain
- Patients should not push past the point of fatigue
- Patients should rest briefly before resuming exercises if muscle spasm or discomfort occurs

- Arthritic, inflammatory or underlying musculoskeletal conditions may limit active range of motion. Altered technique may be required in these cases under the advice of the clinician.
- Some delayed onset muscular soreness should be expected

Contraindications / termination of exercise:

- Acute soft tissue injury
- Joint instability
- Chest pain
- Syncope or pre-syncope
- Patient request
- Decompensated right heart failure
- Respiratory tract infection or other acute illness

Warm up

A gentle warm up should be performed prior to strength training exercises. The warm up should consist of light physical activity, like walking, followed by static stretching. Walking will depend upon the ability and fitness level of the participant.

Static stretching should be performed during the warm up and cool down. This should include all major muscle groups to help prevent soft tissue injuries. Static stretching will be performed by placing the body in a position whereby the muscle groups to be stretched are under tension. Both the opposing muscle group and the muscles to be stretched should be relaxed. Then, slowly, the body is moved to increase the tension on the muscle group. The stretch should be static, not dynamic. The position should be held for 30 seconds for an effective stretch. Stretching should last for about five minutes in total.

Upper limb stretches

All upper limb stretches should be performed in an upright position in the chair with back straight. One repetition of each stretch should be performed. Each stretch should be held for 30 seconds.

Anterior shoulder stretch: *"Sit at the edge of your chair. Place the palms of your hands on your lower back. Try to bring your elbows together behind your back. A stretch should be felt across the front of your chest and shoulders. Relax."*

Posterior shoulder stretch: *"Bring one arm across your body. Using your opposite arm, push the elbow of the arm being stretched towards your opposite shoulder. A stretch should be felt across the back of your shoulder and shoulder blade. Relax. Now repeat this with your other arm."*

Triceps stretch: *"Place your hand on your upper back with your elbow bent towards the ceiling. Using your other hand, push your elbow towards your head. A stretch should be felt across the back of your arm. Relax. Now repeat this with your other arm."*

Biceps stretch: *"Sit at the edge of your chair. Lean backwards and place your hands flat on the chair close together behind your body. Position your fingers pointing away from your body. A stretch should be felt across the front of your arm. Relax."*

Lower limb stretches
All lower limb stretches should be performed in standing using the back of a chair for support if required. One repetition of each stretch should be performed. Each stretch should be held for 30 seconds.

Quadriceps stretch: *"Pull your foot up towards your bottom using your hand. Keep your knees close together. With your back straight, squeeze your buttock muscles and shift your body forward. A stretch should be felt at the front of your thigh. Relax. Now repeat this with your other leg."*

Or, if you the patient has difficulty with their balance:
Hip flexor and upper quadriceps stretch: *"Step one foot forward, keeping your other knee bent. Point the toes of your back foot forward. Squeeze your buttock muscles and shift your body forward bending your front knee. A stretch should be felt at your right hip and upper thigh. Relax. Now repeat this with your other leg."*

Calf stretch: *"Place one of your legs backwards with both of your feet facing forward. Keep your back knee straight and heel on the ground. Slowly bend your front knee until your knee is over your ankle. A stretch should be felt at the calf of your back leg. Relax. Now repeat this with your other leg."*

Hamstrings stretch: *"You should maintain a straight lower back while you are performing this stretch. Place one foot slightly forward. Bend forward at your hips until a stretch is felt at the back of your thigh. Relax. Now repeat this with your other leg."*

Upper limb exercises
The patient should be in a comfortable, upright seated position for the duration of the upper limb exercises. Each exercise should be repeated for ten repetitions.

1) Elbow flexion: Both upper limbs should be exercised at the same time.
   "Relax your arms at your sides. Grasp one weight in each hand with the palm of your hand facing forward. Squeeze your biceps muscles and bend your elbow, curling the weights up towards your shoulders. Slowly lower the weights, keeping a slight bend in your elbows at the bottom. Keep the tension on your muscle throughout. Do not lock out your joints."

2) Shoulder extension: One upper limb should be exercised at a time.
   "Grasp one weight at hip height with your elbow straight and your palm facing inwards. Starting just in front of your body, pull your arm backwards, keeping your elbow straight and squeezing your shoulder blades together.

Slowly lower the weight to starting position. Now repeat this with your other arm."

3) Lateral raises: Both upper limbs should be exercised at the same time. *"Relax your arms by your sides. Grasp one weight in each hand with the palm of your hand facing forward. From this position, raise your arms out to the sides until they are parallel to the floor, reaching shoulder height. Slowly lower the weight to starting position. Now repeat this with your other arm."*

Lower limb exercises

One lower limb should be exercised at a time. Each exercise should be repeated for ten repetitions.

The patient should be in a comfortable, upright seated position for the duration of the knee extension exercises.

1) Knee extension: *"Sit with your back resting against the back of the chair and your feet flat on the floor. Rest your hands on your thighs or on the sides of the chair. Take 3 seconds to extend your leg in front of you, parallel to the floor, until your knee is straight. With your leg in this position, flex your ankle so that your toes are pointing towards your head; hold foot in this position for 2 seconds. Slowly lower your leg back to the starting position, so that the ball of your foot rests on the floor again. Now repeat this with your other leg."*

Hip strengthening exercises should be performed in standing using the chair for gentle support. Stand with feet hip width apart.

2) Hip flexion: *"Take 3 seconds to bend your knee up towards your chest as high as possible. Keep your back straight and tighten your hip flexor muscles throughout. Hold for 2 seconds. Slowly lower your leg back to the starting position. Now repeat this with your other leg."*

3) Hip extension: *"Take 3 seconds to lift one leg straight back behind you. Hold your tummy in, and keep your knee and back straight. Do not bend your upper body any further forward. Hold this position for 2 seconds. Slowly lower your leg back to the starting position. Now repeat this with your other leg."*

4) Hip abduction: *"Keep your back and knee straight with your foot facing forwards. Take 3 seconds to raise your leg to the side, tightening your hip abductor muscles throughout. Hold this position for 2 seconds. Slowly lower your leg back to the starting position. Now repeat this with your other leg."*

Cool down

Complete training session by performing upper limb and lower limb stretches as before.

Appendix 10: EmPHasis-10

emPHasis10

NHS/Hospital number:

Name:

Date of birth:

This questionnaire is designed to determine how pulmonary hypertension (PH) affects your life. Please answer every question by placing a tick over the ONE NUMBER that best describes your recent experience of living with PH.

For each item below, place a tick (✓) in the box that best describes your experience.

I am not frustrated by my breathlessness	0 1 2 3 4 5	I am very frustrated by my breathlessness
Being breathless never interrupts my conversations	0 1 2 3 4 5	Being breathless always interrupts my conversations
I do not need to rest during the day	0 1 2 3 4 5	I always need to rest during the day
I do not feel exhausted	0 1 2 3 4 5	I always feel exhausted
I have lots of energy	0 1 2 3 4 5	I have no energy at all
When I walk up one flight of stairs I am not breathless	0 1 2 3 4 5	When I walk up one flight of stairs I am very breathless
I am confident out in public places/crowds despite my PH	0 1 2 3 4 5	I am not confident at all in public places/crowds because of my PH
PH does not control my life	0 1 2 3 4 5	PH completely controls my life
I am independent	0 1 2 3 4 5	I am completely dependent
I never feel like a burden	0 1 2 3 4 5	I always feel like a burden

Total:

Date:

pha UK pulmonary hypertension association

MANCHESTER 1824 The University of Manchester

Appendix 11: Camphor

CAMPHOR

Cambridge Pulmonary Hypertension Outcome Review

Please read this carefully

On the following pages you will find some statements that have been made by people who have Pulmonary Arterial Hypertension.

Please read each statement carefully.
We would like you to put a tick in the box ☑ next to **'Yes'** if you feel it applies to you and a tick in the box ☑ next to **'No'** if it does not

Please choose the response that applies best to you
at the moment

Please read each statement carefully and decide whether it applies to you ***at the moment***

1. My stamina levels are low — Yes ❒ No ❒
2. I have to rest during the day — Yes ❒ No ❒
3. I feel worn out — Yes ❒ No ❒
4. I get tired very quickly — Yes ❒ No ❒
5. I'm tired all the time — Yes ❒ No ❒
6. I feel very weak — Yes ❒ No ❒
7. I feel completely exhausted — Yes ❒ No ❒
8. I want to sit down all the time — Yes ❒ No ❒
9. I soon run out of energy — Yes ❒ No ❒

10. Everything is an effort — Yes ❒ No ❒

11. I get out of breath when I stand up — Yes ❒ No ❒

12. When I talk I get out of breath — Yes ❒ No ❒

Please read each statement carefully and decide whether it applies to you ***at the moment***

13. When I walk I get out of breath — Yes ❒ No ❒

14. I get breathless if I bend — Yes ❒ No ❒

15. I get breathless going up one step — Yes ❒ No ❒

16. I get breathless walking up a slight slope — Yes ❒ No ❒

17. I get breathless without doing anything — Yes ❒ No ❒

18. I get breathless climbing a flight of stairs — Yes ❒ No ❒

19. I have mood swings — Yes ❒ No ❒

20. I get very down

Yes ❒
No ❒

21. I seldom feel happy

Yes ❒
No ❒

Please read each statement carefully and decide whether it applies to you ***at the moment***

22. I've forgotten what it's like to enjoy myself

Yes ❒
No ❒

23. I feel hopeless

Yes ❒
No ❒

24. It does get me down

Yes ❒
No ❒

25. I often feel anxious

Yes ❒
No ❒

Activities

Please put a tick in the box ☑ under the response which best describes your abilities at the moment. Please respond to all 15 statements. **Please describe your ability without the use of aids or assistance. However, do describe your ability taking into account oxygen if you use it.**

	Able to do on own **without** difficulty	Able to do on own **with** difficulty	Unable to do on own
1. Cut your toenails	❒	❒	❒
2. Have an all over wash	❒	❒	❒
3. Get dressed	❒	❒	❒
Walk around inside the house (not including climbing stairs)	❒	❒	❒
5. Walk short distances on level ground	❒	❒	❒
6. Walk longer distances on level ground	❒	❒	❒
7. Walk up a slight incline	❒	❒	❒
8. Climb a flight of stairs	❒	❒	❒
9. Bend down to pick objects up from the floor	❒	❒	❒
10. Stand for a short time	❒	❒	❒
11. Stand for a long time	❒	❒	❒
12. Lift heavy items	❒	❒	❒
13. Carry heavy items	❒	❒	❒
14. Do light jobs around the house or garden	❒	❒	❒
15. Do heavy jobs around the house or garden	❒	❒	❒

Quality of Life

Please read each statement carefully and put a tick ☑ next to the response that applies best to you ***at the moment***

1. I have to talk very quietly — True ❒ Not True ❒

2. I can't stay away from home — True ❒ Not True ❒

3. I've lost interest in food — True ❒ Not True ❒

4. I can't put energy into my close relationships — True ❒ Not True ❒

5. Walking for pleasure is out of the question — True ❒ Not True ❒

6. My condition puts a strain on my close relationships — True ❒ Not True ❒

7. I feel very isolated — True ❒ Not True ❒

8. I can’t do things on the spur of the moment — True ❒ Not True ❒

9. I feel vulnerable when I’m on my own — True ❒ Not True ❒

10. It feels like my body has let me down — True ❒ Not True ❒

11. I feel as if I'm not in control of my life — True ❒ Not True ❒

12. I feel dependent on other people — True ❒ Not True ❒

Please remember to put a tick in ***only one*** of the alternative responses for each of the statements

13. .Sometimes it's too much effort to speak — True ❒ Not True ❒

14. I feel as if I am a burden to people — True ❒ Not True ❒

15. Travelling distances is a problem — True ❒ Not True ❒

16. I don't like to be seen like this — True ❒ Not True ❒

17. I feel that I'm losing my role in life — True ❒ Not True ❒

18. I worry that I neglect people close to me — True ❒ Not True ❒

Please read each statement carefully and decide whether it applies to you ***at the moment***

19. I feel guilty asking for help — True ❒ Not True ❒

20. My condition limits the places I can go — True ❒ Not True ❒

21. I dislike having to rely on other people — True ❒ Not True ❒

22. I don't want to talk to anybody — True ❒ Not True ❒

23. I feel as if I let people down — True ❒ Not True ❒

24. I am reluctant to leave the house — True ❒ Not True ❒

25. I'm unable to join in activities with my family and friends — True ❒ Not True ❒

Thank you for taking the trouble to fill in this questionnaire.

Please check all the pages to make sure that you have answered every statement.

Appendix 12: SF-36

Your Health and Well-Being

HEALTH STATUS SURVEY SF-36

Participant ID:_____ **Protocol** _____

Center:_____ **Visit Number:** _____

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. *Thank you for completing this survey!*

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. **In general, would you say your health is:**

Excellen	Very	Good	Fai	Poo
▼	▼	▼	▼	▼
☐1	☐2	☐3	☐4	☐5

2. **<u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?**

Much better now than one	Somewhat better now than one year ago	About the same as one year	Somewhat worse now than one year ago	Much worse now than one year
▼	▼	▼	▼	▼
☐1	☐2	☐3	☐4	☐5

3. **The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?**

	Yes, limited a	Yes, limited a	No, not limite
a Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐1	☐2	☐3
b Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐1	☐2	☐3
c Lifting or carrying groceries	☐1	☐2	☐3
d Climbing several flights of stairs	☐1	☐2	☐3
e Climbing one flight of stairs	☐1	☐2	☐3
f Bending, kneeling, or stooping	☐1	☐2	☐3
g Walking more than a mile	☐1	☐2	☐3
h Walking several hundred yards	☐1	☐2	☐3
i Walking one hundred yards	☐1	☐2	☐3
j Bathing or dressing yourself	☐1	☐2	☐3

4. **During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health</u>?**

	All of the time	Most of the time	Some of the	A little of the	None of the
a. Cut down on the <u>amount of time</u> you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. <u>Accomplished less</u> than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Were limited in the <u>kind</u> of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Had <u>difficulty</u> performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. **During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of any emotional problems</u> (such as feeling depressed or anxious)?**

	All of the time	Most of the time	Some of the	A little of the	None of the
a. Cut down on the <u>amount of time</u> you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. <u>Accomplished less</u> than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Did work or other activities <u>less carefully than usual</u>	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. **During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?**

Not at	Slightl	Moderatel	Quite a	Extremel
☐1	☐2	☐3	☐4	☐5

7. **How much bodily pain have you had during the past 4 weeks?**

Non	Very	Mild	Moderat	Sever	Very
☐1	☐2	☐3	☐4	☐5	☐6

8. **During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?**

Not at	A little	Moderatel	Quite a	Extremel
☐1	☐2	☐3	☐4	☐5

9. **These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	☐1	☐2	☐3	☐4	☐5
b. Have you been very nervous?	☐1	☐2	☐3	☐4	☐5
c. Have you felt so down in the dumps that nothing could cheer you up?	☐1	☐2	☐3	☐4	☐5
d. Have you felt calm and peaceful?	☐1	☐2	☐3	☐4	☐5
e. Did you have a lot of energy?	☐1	☐2	☐3	☐4	☐5
f. Have you felt downhearted and depressed?	☐1	☐2	☐3	☐4	☐5
g. Did you feel worn out?	☐1	☐2	☐3	☐4	☐5
h. Have you been happy?	☐1	☐2	☐3	☐4	☐5
i. Did you feel tired?	☐1	☐2	☐3	☐4	☐5

10. **During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?**

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐1	☐2	☐3	☐4	☐5

11. **How TRUE or FALSE is <u>each</u> of the following statements for you?**

	Definitely true	Mostly true	Don't kno	Mostly	Definitel
a. I seem to get sick a little easier than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

THANK YOU FOR COMPLETING THESE QUESTIONS!

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