Exercise testing and non-invasive haemodynamics in the assessment and monitoring of pulmonary hypertension: novel submaximal and peak exercise variables

A thesis submitted by

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Summary

Pulmonary hypertension is a disease characterised by progressive pulmonary vascular remodelling and obliteration with consequent development of right heart failure and ultimately death. First described many decades ago with a median survival of less than 3 years and no available treatments, the development of disease specific pulmonary vasodilator therapy has led to only modest improvements in survival and it remains an almost universally fatal disease.

One of the key symptoms of pulmonary hypertension is exercise intolerance, primarily a consequence of the underlying right ventricular failure and an inability to augment stroke volume on exercise. The gold standard diagnostic test is right heart catheterisation but this is unattractive as a tool for ongoing monitoring as it is invasive and not without risk, albeit that risk is small. As a result most monitoring of disease progression and of treatment response is carried out using surrogate markers, often exercise based such as the 6 minute walk test.

Increasing attention is focused on the role of exercise both in that monitoring of patients and also in helping to understand better the pathophysiology. The work presented in this thesis therefore aimed to explore novel exercise derived variables and noninvasive haemodynamic measurement as tools to improve our understanding of the disease limitation, to enhance our monitoring of treatment response and to give additional prognostic information.

In Chapter 3 the role of peripheral muscle oxygen extraction and exercise limitation was explored by performing right heart catheterisation on exercise with measurement of mixed venous oxygen saturation. This demonstrated that patients with pulmonary hypertension demonstrate no evidence of impaired oxygen extraction and that they appear to extract at least as much oxygen on exercise as healthy individuals have been shown to in other studies. This indicates that impairment of oxygen extraction is not a cause of exercise limitation in pulmonary hypertension.

Chapter 4 describes a series of studies evaluating the potential role of the oxygen uptake efficiency slope in pulmonary hypertension. This variable derived from the oxygen consumption and ventilation across an incremental cardiopulmonary exercise test has demonstrated promise as a potential submaximal measure of exercise performance and predictor of survival in left heart failure. The studies conducted demonstrated that this variable is a measure of peak exercise performance in pulmonary hypertension, that it can be measure on submaximal levels of exercise and that it predicts survival in patients with Group 1 and Group 4 disease.

The studies described in Chapter 5 investigated the rates of recovery of heart rate and oxygen consumption after exercise and found that both could predict survival. In particular the rate of recovery of heart rate after exercise was demonstrated to be a strong predictor of survival on multivariate analysis, thus providing a further method of assessing prognosis with exercise.

Finally the ability of noninvasive measures of stroke volume to predict outcome was explored in the studies detailed in Chapter 6. The underlying haemodynamic abnormalities are not assessed when surrogate measures such as exercise testing are employed in patient follow up. Standard practice is to review patients 3 to 4 months after any change in treatment and to assess them using these surrogate measures. Acute haemodynamic changes are able to be detected invasively immediately after administration of pulmonary vasodilator therapy. This study therefore investigated the ability of two noninvasive methods of measuring stroke volume, inert gas rebreathing and cardiac MRI, to detect treatment response after only 2 weeks and assess how this related to functional improvement at the standard 4 months. The study found that haemodynamic changes were able to be detected at 2 weeks and these appeared to relate to changes in 6 minute walk distance at the same time point but did not appear to relate to 6 minute walk distance at 4 months. This study however did not reach its recruitment target and therefore further work is needed in this area.

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Definitions/Abbreviations

6MWT	Six minute walk test
AT	anaerobic threshold
BNP	brain natriuretic peptide
BP	blood pressure
CaO2	arterial blood oxygen content
CcO2	pulmonary end-capillary blood oxygen content
CHF	chronic heart failure
CI	cardiac index
CO	cardiac output
CPET	Cardiopulmonary exercise test/testing
CTDPH	connective tissue disease associated pulmonary hypertension
CTEPH	chronic thromboembolic pulmonary hypertension
CTPA	computed tomography pulmonary angiography
DLCO	diffusing capacity of the lungs for carbon monoxide
ECG	electrocardiograph
EDTA	ethylenediaminetetraacetic acid
ET-1	endothelin 1
HRCT	high resolution computed tomography scan
HRR	heart rate recovery
HRR	heart rate
IGR	inert gas rebreathing
LV	left ventricular
LV	left ventricle
LVEDV	left ventricular end diastolic volume

NO	nitric oxide
NT-proBNP	N-terminal pro-brain natriuretic peptide
PA	pulmonary artery
PAH	Pulmonary arterial hypertension
PApp	pulmonary artery pulse pressure
PBF	pulmonary blood flow
PDE5	phosphodiesterase type 5
PH	Pulmonary hypertension
PVR	pulmonary vascular resistance
RCT	randomised controlled trial
RV	right ventricle/ventricular
RVEDV	right ventricular end diastolic volume
RVEF	right ventricular ejection fraction
RVESV	right ventricular end systolic function
RVSD	right ventricular systolic dysfunction
SPVU	Scottish Pulmonary Vascular Unit
SV	stroke volume
SvO2	mixed venous oxygen saturation
TPR	total pulmonary resistance
V/Q	ventilation/perfusion
VE/VCO2	ventilatory equivalent of carbon dioxide
VEGF	vascular endothelial growth factor
VO2	oxygen uptake
VTE	venous thromboembolism
WHO-FC	World Health Organisation functional class
WSPH	World Symposium on Pulmonary Hypertension

Publications and abstracts

Publications

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Abstracts

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Thomson S, Johnson M. Is systemic oxygen extraction on exercise impaired in pulmonary arterial hypertension?. Eur Respir J 2013; 42: Suppl. 57, 4833

Thomson S, Peacock AJ, Johnson M. Oxygen Uptake Efficiency Slope Is A Valid Submaximal Measure Of Exercise Performance In Precapillary Pulmonary Hypertension. Am J Resp Crit Care Med. 2014;189:A4752

Thomson S, Peacock AJ, Johnson M. P169 Rates of recovery of oxygen consumption and heart rate after cardiopulmonary exercise testing predict survival in patients with precapillary pulmonary hypertension. Thorax 2014;69:A148

1 Introduction

1.1 Definition and background

Pulmonary hypertension (PH) is defined as a resting mean pulmonary artery pressure (mPAP) ≥ 25mmHg[1, 2]. Although the upper limit of normal of mPAP is 20mmHg[3], the use of 25mmHg as the cut-off value to define PH has been used since the first World Symposium on Pulmonary Hypertension (WSPH) in 1973 and is now well established in both clinical guidelines[1, 4, 5] and epidemiological[6-8] and clinical trials[9-12]. The term PH refers not to a single disease but to this elevation in pulmonary artery pressure (PAP) when seen in association with a wide group of conditions, some of which affect the pulmonary vasculature directly and others which affect it indirectly. Key to the pathophysiology of PH is the development of progressive right ventricular (RV) failure which causes symptoms including increasing breathlessness, peripheral oedema and exertional presyncope and syncope.

Ultimately, unchecked PH results in premature death. Prior to the advent of disease specific pharmacological therapy, survival with PH was very poor. In 1991 in the United States of America a large multicentre registry study of 194 patients diagnosed with what was then known as primary PH demonstrated a median survival of only 2.8 years with 5 year survival of 34%[13]. Since that work was published an increasing number of targeted therapeutic options have proven successful in clinical trials and thereafter been incorporated into routine patient management with a resultant increase in survival however PH remains associated with significant morbidity and mortality. Data from the 2015 United Kingdom National Audit of Pulmonary Hypertension indicates that for a patient population similar to that described in the 1991 registry study outlined above, median survival remains poor at 4 years and 104 days despite the advances made in treatment[14].

This chapter will describe the background to and rationale for the work presented in this thesis. It explains the current clinical classification of PH, the pathophysiology of the disease process and the diagnostic and management strategies employed in the management of patients with PH. The pathophysiology of exercise limitation and the role of exercise testing in PH is described and consideration is given to noninvasive methods of measuring

haemodynamic variables in PH. The aims and hypotheses of the work presented in this thesis are then defined.

1.2 Clinical classification of pulmonary hypertension

An accepted structure for the clinical classification of PH was first agreed in 1998 at the second WSPH in Evian, France[15], grouping subtypes of PH by common features of pathology, clinical and haemodynamic features, and treatment. This classification has been modified over the subsequent WSPH meetings in Venice, Italy in 2003[16], Dana Point, California in 2008[17] and most recently in Nice, France in 2013[18], although the overall structure has remained. The Dana Point classification was the contemporary classification at the time the studies described in this thesis were carried out and this is described in Table 1.1. Although some changes were made at the 2013 WSPH[18] none of these would have affected the inclusion of the patients who participated in the studies. Patients with group 1 or group 4 PH were eligible for participation in the studies reported in this thesis while those with a diagnosis of group 2, 3 or 5 PH were excluded.

2008 Dana Point Clinical classification of PH

Group 1 **Pulmonary arterial hypertension**

- **1.1** Idiopathic PH
- **1.2** Heritable
  - **1.2.1** BMPR2
  - **1.2.2** ALK1, endoglin (with or without hereditary haemorrhagic telangiectasia)
  - **1.2.3** Unknown
- **1.3** Drug- and toxin-induced
- **1.4** Associated with
  - **1.4.1** Connective tissue diseases
  - **1.4.2** HIV infection
  - **1.4.3** Portal hypertension
  - **1.4.4** Congenital heart diseases
  - **1.4.5** Schistosomiasis
  - **1.4.6** Chronic haemolytic anaemia
- **1.5** Persistent pulmonary hypertension of the newborn

1' Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis

Group 2 **Pulmonary hypertension owing to left heart disease**

Group 3 **Pulmonary hypertension owing to lung diseases and/or hypoxia**

Group 4 **Chronic thromboembolic pulmonary hypertension**

Group 5 **Pulmonary hypertension with unclear multifactorial mechanisms**

- **5.1** Haematological disorders: myeloproliferative disorders, splenectomy
- **5.2** Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, vasculitis
- **5.3** Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
- **5.4** Others: tumoural obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

Table 1.1 2008 Dana Point clinical classification of pulmonary hypertension. PH: pulmonary hypertension; BMPR2: bone morphogenetic protein receptor type 2; ALK1: activin receptor-like kinase type 1; HIV: human immunodeficiency virus.

1.3 Pathogenesis of pulmonary hypertension

The healthy pulmonary circulation consists of thin walled blood vessels in a low pressure, high flow system[19] with significant vascular reserve[20]. The pathogenesis of pulmonary arterial hypertension (PAH) is multifactorial and not yet fully understood[21] but at its core consists of pulmonary vascular proliferation and remodelling, thrombosis and vasoconstriction causing a progressive rise in pulmonary vascular resistance (PVR) and consequent RV failure[4, 19, 20]. Primarily affecting vessels less than 500μm diameter the pathological changes seen in PAH include the development of smooth muscle in the wall of distal, usually nonmuscular, pulmonary arteries; neointima formation in which a layer consisting of extracellular matrix and myofibroblasts forms between the endothelium and the internal elastic lamina; and endothelial cell proliferation leading to the formation of complex vascular lesions known as plexiform lesions[20, 22]. Pulmonary veins are generally felt to be unaffected by the disease process in PAH although this is an area of current research interest[21].

Several cellular and molecular processes contribute to these pathological changes including upregulation of matrix metalloproteinases, disordered inflammation in favour of proinflammatory cytokines, a prothrombotic state leading to in situ thrombosis, induction of growth factors stimulating vascular remodelling such as vascular endothelial growth factor (VEGF), and endothelial dysfunction with a fall in production of endogenous vasodilators including nitric oxide (NO) and prostacyclin, and an increase in vasoconstrictor compounds such as endothelin 1 (ET-1) and thromboxane A2[19-21, 23, 24].

The pathogenesis of group 4 PH, i.e. chronic thromboembolic pulmonary hypertension (CTEPH) has a degree of overlap with that of group 1 PAH. Although as many as 50% of patients diagnosed with CTEPH have no identifiable prior episode of venous thromboembolism (VTE) it is widely accepted that the initial event in the pathogenesis is an episode of pulmonary embolism (PE)[25]. For reasons that have yet to be fully elucidated, there is aberrant thrombus resolution, resulting in pulmonary vascular narrowing and obliteration with the development of organised thrombus and vascular bands and webs[4, 26, 27]. This vascular obstruction diverts blood flow through unaffected pulmonary arteries, triggering the development of a similar vasculopathy to that seen in PAH with

increased shear stress, cytokine release and activation of proinflammatory pathways, and increased levels of ET-1, even in the absence of any further episode of PE[25, 28, 29].

While one prospective follow up single centre study suggested that CTEPH may develop in up to 3.8% of patients after an episode of PTE[30] the true rate is believed to be lower with international guidelines suggesting a rate of 0.5 – 2%[4]. Other factors associated with an increased risk of developing CTEPH include previous splenectomy, the presence of a ventriculo-atrial shunt for hydrocephalus and a history of chronic inflammatory disease[25, 26]. Although thrombophilia *per se* has not been associated with the development of CTEPH studies have demonstrated increased levels of factor VIII, von Willebrand factor and antiphospholipid antibody[26].

1.4 Diagnosis of pulmonary hypertension

Given the many possible causes for PH as described in Table 1.1 and that the treatment for each PH group is different, securing an accurate diagnosis is essential. Internationally recognised guidelines describing how best to achieve this have been published and were followed as standard practice in the Scottish Pulmonary Vascular Unit at the time of the studies described in this thesis.

1.4.1 Clinical presentation

Patients most commonly present with nonspecific symptoms of progressive exertional breathlessness and fatigue, having been symptomatic usually for several months or years before diagnosis, with a mean time from symptom onset to diagnosis in an early registry study of 2 years[31]. The gradual onset contributes to the long time to diagnosis as initial symptoms may be dismissed as a lack of fitness[32]. As the disease progresses and RV failure develops other symptoms of chest pain, peripheral oedema, presyncope and syncope may develop, with symptoms at rest indicating advanced disease[4]. There may be a history of illness with one of the conditions associated with Group I PH, e.g. connective tissue

disease, HIV infection or liver disease, or there may be a positive family history in a small number of cases.

On examination the most common and potentially only feature in the earlier stages of the disease may be a loud second heart sound in the pulmonary region. A pansystolic murmur at the left lower sternal edge consistent with tricuspid regurgitation may be heard and in the presence of RV failure a right ventricular heave may be felt in the left parasternal area[32]. On examination of the jugular veins there may be a prominent "a" wave as the right atrium contracts in the presence of a non-compliant RV and "v" wave as a consequence of tricuspid regurgitation. With the development of RV failure, the jugular venous pressure (JVP) will rise and peripheral oedema will develop, detectable in the legs and possibly also the sacrum and, in the form of ascites, in the abdomen. A third heart sound may be heard in advanced disease and central cyanosis develops. Examination of the lungs is usually unremarkable.

Clinical examination may reveal signs suggestive of an underlying cause of secondary PH, either in the form of left heart disease suggesting possible Group 2 disease or lung disease in keeping with a possible diagnosis of Group 3 disease. There may be stigmata of liver disease such as spider naevi, hepatomegaly or gynaecomastia, or clinical evidence of connective tissue disease, e.g. telangiectasia, Raynaud's, skin changes in the face and hands or digital ulceration.

1.4.2 Initial investigations

In PH the electrocardiogram (ECG) will frequently reveal evidence of RV hypertrophy and strain[31] however a normal ECG cannot be used to exclude the disease as it has insufficient sensitivity and specificity (55% and 70% respectively)[4]. Most patients with PH will be sinus rhythm but the development of atrial fibrillation or other supraventricular tachycardias will in the vast majority of cases precipitate clinical deterioration and worsening right heart failure[33], and such an event may trigger the initial assessment.

The chest x-ray is abnormal in as many as 90% of patients at diagnosis[31]. It may demonstrate enlarged central pulmonary arteries with "pruning" of the more

peripheral vessels and in more advanced disease may show evidence of RV and right atrial (RA) enlargement. It may in addition suggest the presence of lung disease or, in the presence of cardiomegaly, pulmonary oedema and/or bilateral pleural effusions, left heart disease.

1.4.3 Echocardiogram findings

The standard screening test for PH is the echocardiogram, used to provide an estimate of PA pressure, and to assess left ventricular (LV) and RV function[4]. Using the modified Bernoulli equation the PA systolic pressure (PASP) can be estimated as

$$PASP = TRPG + RAP$$

where PASP = pulmonary artery systolic pressure
TRPG = tricuspid regurgitation pressure gradient
RAP = right atrial pressure, estimated on the basis of the diameter and variation with respiration of the inferior vena cava

and

$$TRPG = 4 \cdot (TRV)^2$$

where TRV = peak velocity of the tricuspid regurgitant jet[34].

The estimated PASP measure at echocardiogram can in theory be used to estimate the mPAP but it is not sufficiently accurate for this to be relied on in clinical practice due to the frequency of overestimation or underestimation of the PASP[35]. It is however useful as a screening tool except in the mildest cases of PH[4].

The echocardiogram provides information on left ventricular function which may suggest pulmonary venous hypertension (PVH), i.e. Group 2 disease as the cause of PH. Evidence of septal or other congenital defects may be seen and the use of bubble contrast may indicate the presence of a right to left shunt.

In addition to the diagnostic information provided, the echocardiogram also provides prognostic information. The tricuspid annular plane systolic excursion (TAPSE) is a measure of apex-to-base RV shortening in systole and correlates with RV ejection fraction[36]. It has also been shown to be a significant predictor of survival in PAH[37, 38]. Right atrial size and the presence and severity of pericardial effusion have also been shown to associate with disease severity and to predict survival[39, 40].

1.4.4 Further investigations

If the clinical assessment and echocardiogram are suggestive of PH with no evidence of significant lung or left heart disease then further investigation is warranted. It is at this point that patients would usually be referred to the Scottish Pulmonary Vascular Unit (SPVU) (see Chapter 2.1) for more detailed assessment. The purpose of these further investigations is to confirm the pulmonary vascular haemodynamic measurements and to elucidate the cause if PH is confirmed.

1.4.4.1 Blood investigation

Routine haematological and biochemical blood investigations including assessment of liver and thyroid function, an autoimmune screen looking for evidence to suggest a diagnosis of connective tissue disease, and HIV and hepatitis C screening serology are checked in all patients. Although thrombophilia screening is recommended[4], local guidelines are that this should not be checked unless there is a clear family history as in the event that a diagnosis of CTEPH is made, lifelong anticoagulation will be required regardless of the presence or absence of an underlying clotting disorder.

1.4.4.2 Tests of respiratory function

Pulmonary function tests (PFTs) are performed in all patients suspected of having PH. The primary reason for conducting PFTs is to look for evidence of interstitial lung disease or airways disease and therefore suggest a possible diagnosis of Group 3 PH. However it also provides useful information in patients without lung disease as a cause of PH. In cases of PAH, spirometry is usually normal or close to normal but the diffusing capacity of the lungs for carbon

monoxide (DLCO) may be markedly reduced and correlate inversely with survival[41, 42]. Although a severely reduced DLCO can occur in patients with idiopathic pulmonary arterial hypertension (IPAH)[42], such a marked reduction should raise the possibility of connective tissue disease associated PH (CTDPH)[43]or pulmonary veno-occlusive disease (PVOD)[44] in the absence of left ventricular lung disease or lung disease.

Consideration should be given to whether obstructive sleep apnoea or obesity hypoventilation are present and if symptoms are suggestive, screening with overnight transcutaneous monitoring or polysomnography can be undertaken[4].

1.4.4.3 Ventilation/perfusion scanning

Ventilation/perfusion (V/Q) scanning should be performed in all patients with suspected PH to screen for possible CTEPH. Although possessing similar specificity, the V/Q scan has considerably greater sensitivity for the detection of pulmonary thromboembolic disease than does multidetector computed tomography pulmonary angiography (CTPA) with sensitivity 96 - 97.4% for V/Q scanning compared with 51% for CTPA[45]. Specificity is over 90% for V/Q and 99% for CTPA.

1.4.4.4 Cross sectional imaging

Cross sectional imaging in the form of high resolution computed tomography (HRCT) scanning and CTPA are undertaken in all patients. The HRCT is optimised for assessment of the lung parenchyma and therefore is used to examine for evidence of lung disease which could be the cause of PH, i.e. Group 3 disease. However features of PVOD may be seen, namely septal lines and ground glass opacities in keeping with interstitial oedema[46].

CTPA may demonstrate dilatation of the main pulmonary artery (PA) and right sided cardiac chambers in all forms of PAH but of particular note are the findings seen in CTEPH. Bilateral pleural effusions and mediastinal lymphadenopathy may be seen in PVOD and in left heart disease. Although less sensitive than V/Q scanning CTPA can provide information on the structural abnormalities affecting the pulmonary vasculature. Complete obstruction, eccentric laminated thrombus, stenosis in the form of bands or webs, and other intimal irregularities

may be seen[47]. Peripheral wedge shaped opacities representing areas of previous pulmonary infarction and mosaic perfusion may also be seen.

1.4.4.5 Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (MRI) provides a noninvasive mode of assessing RV structure and function and pulmonary vascular haemodynamics in the form of stroke volume (SV) and cardiac output (CO), and can add to the diagnostic assessment of patients with suspected PH, in terms of both the cause and the disease severity. Cardiac MRI is discussed in more detail in chapter 1.8.1.

1.4.4.6 Measurement of brain natriuretic peptide

Cardiac wall stress stimulates the mycocardium to release atrial and brain natriuretic peptides. These molecules increase natriuresis and also vasodilation. In PH, most research into natriuretic peptides has focused on brain natriuretic peptide (BNP). The molecular precursor of BNP is proBNP. BNP is formed when the N-terminal of proBNP (NTproBNP) is cleaved from the larger molecule, producing BNP. Both BNP and NTproBNP can be quantified in blood and both have been shown to have prognostic significance. NTproBNP is more stable in blood and after sampling and has a longer half-life. As a consequence it is used in preference to BNP in the assessment of patients attending the SPVU.

Work carried out in the SPVU demonstrated that NTproBNP at baseline correlated negatively with RV ejection fraction (RVEF) and was a highly sensitive and specific marker of RV systolic dysfunction (RVSD) [48]. This relationship between NTproBNP and disease severity is now well established. Higher levels are associated with higher mortality[49-51], increasing levels are associated with deteriorating RV systolic function[52] and falling levels have been seen in patients with improved haemodynamics on treatment[49].

1.4.4.7 Assessment of functional capability

With exercise limitation one of the key features of PH symptomatology, tests of exercise capacity have a role in the assessment of disease severity at baseline and on follow up. The most commonly used tests in PH re the 6 minute walk test

(6MWT) and the cardiopulmonary exercise test (CPET)[4] and these are discussed in Chapter 1.7.3.

The World Health Organisation Functional Class (WHO-FC) is a widely accepted method of stratifying patients with PH by the effect it has on their ability to perform activities. It is described in Chapter 2.2. Patients with poor WHO-FC have been demonstrated to have poorer survival than those in the best functional classes[13].

1.4.5 Right heart catheterisation

To confirm the diagnosis all patients must undergo right heart catheterisation which remains the gold standard method of clarifying the presence of PH, quanitifying the severity of the haemodynamic impairment and testing the degree of vasoreactivity of the pulmonary circulation[2, 4]. RHC is performed and measurements made as described in Chapter 2.7.1. When conducted in experienced centres such as the SPVU the rate of complications of RHC is low. In one multicentre retrospective and prospective study evaluating serious adverse events (SAEs) related to RHC in patients with PH found a rate of SAEs of 1.1%, the most frequent of which related to the initial venous puncture, with cardiac arrhythmia and hypotension occurring less frequently[53]. The overall procedure related mortality in that series was 0.059%.

The haemodynamic definition of different groups of PH is given in Table 1.2.

Haemodynamic definitions of pulmonary hypertension

Definition	Haemodynamics	PH Group
Pulmonary hypertension	mPAP ≥ 25 mmHg	All
Pre-capillary PH	mPAP ≥ 25 mmHg PAWP ≤15 mmHg CO normal or reduced	Group 1 Group 3 Group 4 Group 5
Post-capillary PH	mPAP ≥ 25 mmHg PAWP > 15 mmHg CO normal or reduced	Group 2

Table 1.2 Haemodynamic definitions of pulmonary hypertension. mPAP: mean pulmonary artery pressure; PH: pulmonary hypertension; PAWP: pulmonary artery wedge pressure; CO: cardiac output. PH groups are as described in Table 1.1.

In some patients with suspected Group 2 PH, particularly those in whom LV diastolic dysfunction may be suspected on the basis of history and other investigations, a normal pulmonary artery wedge pressure (PAWP) may still be seen, suggesting a diagnosis of precapillary PH. In such cases of raised suspicion the PAWP may be "challenged", either with a period of exercise on the cardiac catheterisation table or by giving a rapid fluid challenge of 0.9% saline and assessing for a disproportionate rise in the PAWP[54].

In cases of haemodynamically confirmed PAH, vasoreactivity testing should be undertaken[4]. In these patients an acute vasodilator is given following initial RHC measurements. As is the case in the SPVU, inhaled nitric oxide (NO) is the acute vasodilator used most often although intravenous epoprostenol may also be used. A positive response in defined as a reduction in mPAP of ≥ 10 mmHg to an absolute mPAP of ≤40 mmHg, with a stable or increased CO[4, 55]. The importance of this vasoreactivity testing is that patients who have a positive response are most likely to response to long term treatment with calcium channel blockers and have a better outlook than non-responders[55, 56].

1.4.6 Conventional pulmonary angiography

In patients in whom a diagnosis of CTEPH is suspected, generally those patients with a suggestive V/Q scan and/or CTPA, conventional pulmonary angiography should be performed at the time of RHC[4]. This provides detailed imaging of the extent and distribution of vascular defects, confirming the diagnosis, and enables planning of the optimum therapeutic strategy.

1.5 Features of prognostic significance

The various components of the assessment described in Chapter 1.4 should be taken together to give an overall view of prognosis. Those features which are of established prognostic significance are given in Table 1.3, adapted from McLaughlin and McGoon, 2009[57] and Galié *et al*, 2009[4].

Assessment of disease severity and prognosis

Better prognosis	Determinants of prognosis	Worse prognosis
No	**Clinical evidence of RV failure**	
Slow	**Rate of symptom progression**	
No	**Syncope**	
I/II	**WHO-FC**	
Longer, > 500m	**6 minute walk distance**	Shorter, <300m
Peak VO_2 > 15ml/min/kg	**Cardiopulmonary exercise test**	Peak VO_2 < 12ml/min/kg
Normal/near-normal	**BNP/NTproBNP**	Very elevated and rising
No pericardial effusion TAPSE > 2cm	**Echocardiographic findings**	Pericardial effusion TAPSE < 1.5cm
RAP < 8mmHg and CI ≥ 2.5l/min/m^2	**Haemodynamics**	RAP > 15mmHg or CI ≤ 2.0l/min/m^2

Table 1.3 Features associated with better or worse prognosis in patients with pulmonary arterial hypertension, adapted from McLaughlin and McGoon, 2009[57] and Galié *et al*, 2009[4]. RV: right ventricular; WHO-FC: World Health Organisation Functional Class; BNP: brain natriuretic peptide; NTproBNP: N terminal proBNP; TAPSE: tricuspid annular plane systolic excursion; CI: cardiac index.

1.6 Treatment of pulmonary arterial hypertension

1.6.1 General measures

1.6.1.1 The role of anticoagulation

There is a longstanding recommendation that patients with IPAH be therapeutically anticoagulated, originally with warfarin and more recently with novel oral anticoagulant agents. Post mortem examination of lung tissue from patients with IPAH and other studies in wider groups of PAH have demonstrated a high prevalence of thrombotic lesions[58] and in retrospective studies found anticoagulation to be associated with significantly better survival compared to those patients who were not anticoagulated[58]. A more recent study analysing data from the Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA) has confirmed the beneficial effect of anticoagulation in Group 1 PH but only in those patients with IPAH and no beneficial effect of treatment with anticoagulants was seen in patients with other types of PAH[59]. Clearly patients with CTEPH should have lifelong anticoagulation[4].

1.6.1.2 Diuretics

Patients with progressive RV failure are prone to becoming fluid overloaded with increasing right heart pressure and the accumulation of peripheral oedema and in some cases ascites. There are no randomised controlled trials (RCTs) of diuretic use in patients with PH but the symptomatic benefits of diuresis are widely accepted[4].

1.6.1.3 Oxygen

There is some evidence from the study of PH secondary to lung disease that administration of long term oxygen therapy can lead to improvements in the pulmonary vascular component of that disease process[60]. This and other evidence has been extrapolated to the management of PH and it is recommended that patients with a partial pressure of oxygen in arterial blood of less than 8 kPa receive oxygen at rates to maintain a level greater than 8kPA for at least 16 hours a day[4].

1.6.2 Specific pulmonary vasodilator therapy

1.6.2.1 Calcium channel blockers

As described in Chapter 1.4.5, patients with IPAH who have a significant response to an acute pulmonary vasodilator trial should be treated with calcium channel blockers[55, 56]. Patients meeting this criterion and who are started on calcium channel blockers should be monitored closely and if there is poor evidence of efficacy then additional or alternative PH therapy should be instituted[4, 61].

1.6.2.2 Phosphodiesterase type 5 inhibitors

Phosphodiesterase type 5 (PDE5) inhibitors are often used as first line therapy in Group 1 PH. PDE5 is expressed in high concentrations in the pulmonary vasculature. It enzymatically degrades cyclic guanosine monophosphate and inhibition of this causes vascular smooth muscle relaxation through the NO/cGMP pathway and thus cause vasodilation. It has been suggested from *in vitro* work that there may be an additional beneficial effect on pulmonary vascular remodelling by reducing proliferation[62, 63].

The beneficial effects of the PDE5 inhibitors sildenafil and tadalafil in PAH have been demonstrated in RCTs. SUPER-1 showed sildenafil to have a beneficial impact on exercise capacity, WHO-FC and haemodynamics compared with placebo[10] while SUPER-2, the long term uncontrolled extension study which followed SUPER-1, demonstrated the drug to be generally well tolerated[64]. The PHIRST study of tadalafil showed that compared with placebo, tadalafil increased 6 minute walk distance (6MWD), even in patients already on treatment with bosentan (see Chapter 1.5.2.3), and also improved time to clinical worsening and quality of life measures[65]. In a similar manner to SUPER-2, a tadalafil long term uncontrolled extension study was conducted, PHIRST-2, demonstrating that tadalafil was well tolerated and that the improvements seen in 6MWD in PHIRST appeared to be maintained after one year of treatment[66].

1.6.2.3 Endothelin receptor antagonists

Endothelin-1 is a powerful vasoconstrictor released primarily from vascular endothelium and as described in Chapter 1.3 plays a role in the pathogenesis of PH. It binds to both ETA and ETB receptors in the vasculature. ETA receptors are found in vascular smooth muscle cells with ETB receptors located on both vascular smooth muscle cells and vascular endothelial cells. When ET-1 binds to receptors of either type, vasoconstriction occurs. ET-1 binding to ETB receptors on endothelial cells however stimulates clearance of ET-1, stimulation of NO release and stimulation of prostacyclin release[67], thus promoting vasodilation.

Endothelin receptor antagonists (ERAs) were developed to counteract this increased expression of ET-1 in PAH. Bosentan is an oral dual ETA and ETB receptor antagonist. Its beneficial effects on 6MWD, WHO-FC, haemodynamic measurements and time to clinical worsening were demonstrated across a series of placebo controlled trials including the BREATHE series of studies and the EARLY trial[9, 68-70]. Longitudinal observational data has demonstrated that bosentan is safe and the improvements seen appear to be sustained long term[71] with improved survival[72]. Approximately 10% of patients develop raised liver aminotransferases. Although this is reversible on stopping the drug, patients on treatment with bosentan should have monthly liver function test monitoring[4, 61].

A second ERA, ambrisentan, has been accepted for use in patients with PAH. It is a selective ETA receptor antagonist and its efficacy was first demonstrated in two concurrent randomised double blinded placebo controlled studies, ARIES-1 and ARIES-2[73]. These trials found that therapy with ambrisentan was associated with improvements in 6MWD, WHO-FC, time to clinical worsening and BNP. Although increases in liver aminotransferases were less frequent and less marked than with bosentan treatment, patients on therapy with ambrisentan should also have monthly liver function test monitoring.

A third ERA has more recently been developed for use in PAH. Macitentan is a dual ETA and ETB receptor antagonist and has been demonstrated to improve morbidity and mortality in patients with PAH when compared with placebo in the SERAPHIN study[74]. It was not yet available for use at the time of the studies

described in this thesis. A further ERA, sitaxentan, was withdrawn in December 2010 due to associated liver toxicity[75-77].

1.6.2.4 Prostanoids

Prostacyclin is a potent endogenous vasodilator and is downregulated in PAH[78]. Synthetic analogues have therefore been developed for use in PAH. Epoprostenol is administered as a continuous intravenous infusion, usually via a Hickman line. No randomised double-blinded controlled trials of epoprostenol have been conducted but it has long been accepted as the treatment of choice, in combination with oral therapies, for patients with the most severe disease. Evidence of its benefit in PAH comes from unblinded randomised controlled trials demonstrating a sustained reduction in mPAP and improvements in exercise capacity and survival[79-81]. Flushing, headache, gastrointestinal upset and jaw pain may occur with increasing doses but the most severe complications come from the delivery system, including the possibility of pump failure and line infection or blockage. Due to the short half life of the drug there is a risk of rebound PH, potentially fatal, if the drug infusion is stopped or interrupted suddenly[4].

Another prostacyclin analogue, treprostinil, can be given either intravenously or subcutaneously, although inhaled and oral preparations have also been produced. It is not used routinely in the care of patients attending the SPVU but has been shown to cause a significant improvement in 6MWD, WHO-FC and breathlessness[82-84].

Inhaled iloprost was shown in a multicentre double blinded RCT to increase the number of patients meeting the primary endpoint, a composite of improvements in 6MWD and WHO-FC, compared with placebo[85]. It requires to be taken 6 to 9 times a day and has similar side effects to the other prostanoids of nausea, flushing and headache.

1.6.2.5 Emerging therapies

Patients have for several years been treated with PDE5 inhibitors, ERAs and prostanoids, either singly or in combination, and these were the drugs available for use at the time of the studies described in the following chapters. More

recently new drugs have become available which offer new approaches to PAH therapy. Briefly, riociguat, a stimulator of soluble guanylate cyclase works to increase cGMP in the NO/cGMP pathway and thus promote vasodilation. The PATENT and CHEST studies in patients with Group 1 and Group 5 PH respectively studied ricociguat in double blinded, placebo controlled trials and demonstrated a significant improvement in 6WMD, NTproBNP and WHO-FC with a longer time to clinical worsening and reduction in breathlessness also statistically significant in the Group 1 patients enrolled in PATENT[11, 86]. Riociguat is the first drug to be approved specifically for the medical management of patients with CTEPH.

Selexipag is an oral selective IP prostacyclin receptor agonist. In a randomised double blinded placebo controlled trial it significantly reduced the risk of the primary endpoint, a composite of death or a PAH-related complication although there was no significant benefit on mortality alone[12].

1.6.2.6 Pulmonary endarterectomy

If technically feasible, pulmonary endarterectomy (PEA) is the treatment of choice for patients with CTEPH as it is potentially curative therefore extensive assessment of the distribution and extent of organised thrombi, the functional and haemodynamic consequences of this and the general fitness and presence or absence of comorbid disease is undertaken prior to determining suitability for this surgery[87]. Consequently all patients with a diagnosis of CTEPH should be referred to an expert PEA centre for consideration of operability.

The operation involves a median sternotomy before the patient is put on cardiopulmonary bypass. The pulmonary arteries on each side are dissected sequentially to remove the inner core of organised thromboembolic material, including the intima and superficial media[88, 89].

In hospital mortality at the time of PEA is less than 5%. Haemodynamic variables may return to normal or near normal with a consequent improvement in functional parameters. The long term prognosis is excellent with 1 year and 10 year survival >90% and >70% respectively[90, 91].

1.7 Exercise and pulmonary hypertension

With exercise limitation a key symptom of PH, both at diagnosis and throughout the disease course, significant attention has focused on the role of exercise, both in helping to elucidate the pathophysiology and also as a mean of assessing disease severity and outcome.

1.7.1 Exercise limitation

It is reasonable to consider exercise the result of the process by which oxygen is transferred form the atmosphere, via the lungs and vasculature, to mitochondria in the peripheral muscle[92]. That oxygen consumption can be described by the Fick equation thus

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

where VO_2 = oxygen consumption

CO = cardiac output

CaO_2 = arterial oxygen content

CvO_2 = venous oxygen content.

This equation can be expanded to highlight individual contributors to a given VO_2 as follows

$$VO_2 = SV \cdot HR \cdot Hb \cdot (SaO_2 - SvO_2)$$

where SV = stroke volume

HR = heart rate

Hb = haemoglobin concentration

SaO_2 = arterial oxygen saturation

SvO_2 = venous oxygen saturation.

Therefore impairment of any one of stroke volume, heart rate, haemoglobin concentration, arterial oxygenation or oxygen extraction (and thus venous oxygen saturation) can cause a reduction in VO_2 and thus exercise capacity.

In PH the increased PA pressure and resultant RV failure lead to a reduction in the maximum achievable stroke volume[93, 94], thus limiting the maximum VO_2 which can be reached. In addition, patients with PH exhibit a steeply climbing HR response to exercise and often fail to reach the predicted peak HR[95] and iron deficiency anaemia is common[96, 97]. Furthermore a sharp decline in mixed venous oxygen saturation on exercise and ventilation-perfusion mismatch contribute to arterial desaturation on exercise[98, 99]. Thus, through several pathophysiological routes PH limits VO_2 and therefore exercise capacity.

The ventilatory response to exercise is also abnormal with markedly inefficient ventilation, most easily seen in the relationship between ventilation (V_E) and carbon dioxide production (VCO_2)[100, 101]. The V_E for a given VCO_2 on exercise is inversely related to the degree of physiological dead space and the "set point" at which CO_2 is regulated[102] as can be seen in the following equation

$$\left(\frac{V_E}{VCO_2}\right) = \frac{k}{PaCO_2 \cdot \left(1 - \frac{V_D}{V_T}\right)}$$

where V_E = ventilation

VCO_2 = carbon dioxide production

$PaCO_2$ = partial pressure of carbon dioxide

V_D = dead space volume

V_T = tidal volume.

The increased ratio of V_E to VCO_2 seen on exercise in PAH is even greater in patients with CTEPH[103], presumably due to the increased pulmonary vascular obstruction seen in that form of PH leading to an increased dead space fraction. It has been speculated that abnormalities of lung function may also contribute to an abnormal ventilatory response to exercise in patients with PAH. There is some evidence of peripheral airway obstruction in IPAH and a reduced inspiratory

capacity, which falls further with exercise, as a result of dynamic hyperinflation, has also been reported[104, 105] however despite these findings and the more marked abnormalities seen in ventilatory efficiency, true ventilatory limitation is rare in PAH patients without comorbid lung disease[106].

It has been established that the peripheral muscle of patients with PH differs from that of healthy controls with reduced capillarity and alterations in mitochondrial function and muscle fibre type[107-109]. It has been suggested that these alterations in peripheral muscle function may limit exercise capacity by reducing oxygen extraction and thus limiting peak VO_2 but evidence for this is limited in PAH[110].

1.7.2 The abnormal haemodynamic response to exercise

In PAH there are marked abnormalities of the haemodynamic response which can be detected at RHC. Pressure measurements on exercise are complicated by a large respiratory swing, particularly in measurement of PAWP but also seen in measurement of PA pressures[92, 111, 112]. Pressure tracings from a patient with IPAH undergoing resting and exercise RHC are shown in Figure 1.1, demonstrating both the sharp rise in PA pressures and the marked respiratory swing.

In health, mPAP and PAWP increase on exercise, with mPAP rising approximately 0.5 – 3 mmHg per litre per minute increase in CO[113] with a smaller rise seen in PAWP[114]. In PAH however there is a considerably steeper rise in mPAP with a smaller increment in CO than is seen in health, giving rise to a much steeper mPAP/CO relationship on exercise in PAH[92, 115].

The cardiac output response to exercise is blunted in PAH as a consequence of a failure to augment SV on exercise. In one study using cardiac MRI measurement of SV on submaximal exercise, healthy controls increased SV by approximately 25% while no change was seen in SV in patients with IPAH[93]. In contrast to the control subjects in whom left ventricular (LV) and RV end diastolic volume (EDV) were both stable, in the patients with IPAH there was an increase in RVEDV and decrease in LVEDV. The total cardiac EDV was constant suggesting that on exercise there was progressive failure of the RV leading to the increase in

RVEDV, with consequent bowing of the interventricular septum into the LV, thus reducing the LVEDV. A reduction in RV ejection fraction (RVEF) was also seen suggesting that impaired forward flow from the RV on exercise was reducing LV filling. Together these mechanisms contributed to a failure to augment SV on exercise in the IPAH patients. Similar results were seen in an earlier study which also found evidence of increased RV filling and reduced LV filling, and saw a reduction in SV on exercise in patients with what was then known as primary pulmonary hypertension[116].

While RHC is required to confirm the diagnosis of PH, serial measurements over time are impractical due to the invasive nature of the test. It is though helpful in our understanding of exercise limitation in PH to understand the contribution of this abnormal haemodynamic response.

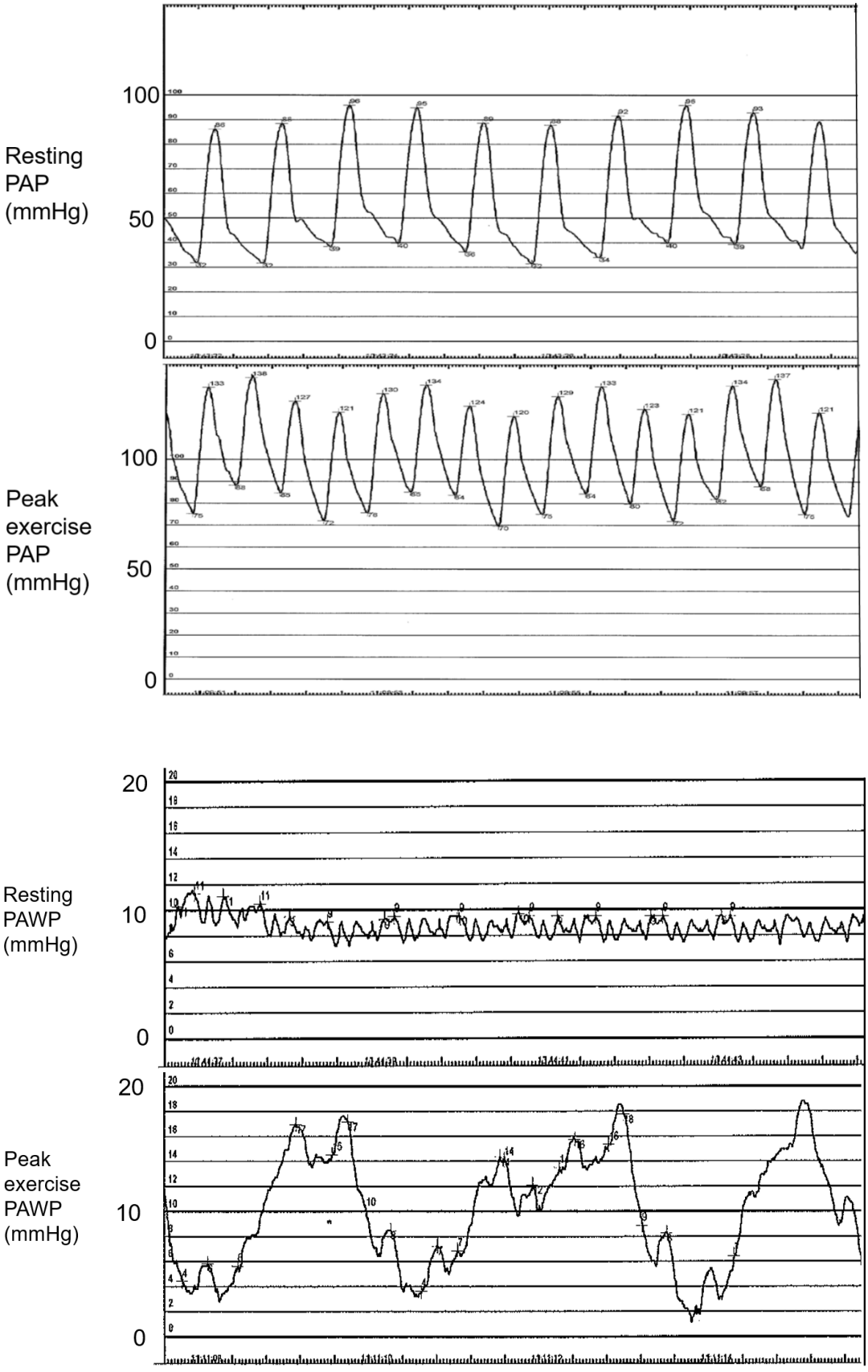


Figure 1.1 Right heart catheterisation pressure traces at rest and on exercise for a patient with idiopathic pulmonary arterial hypertension. PAP: pulmonary artery pressure; PAWP: pulmonary artery wedge pressure.

1.7.3 Noninvasive assessment of the exercise response

1.7.3.1 Cardiopulmonary exercise test response profiles in pulmonary arterial hypertension

Cardiopulmonary exercise testing (CPET) is the gold standard method of evaluating the cause of exercise limitation in patients with heart and lung disease[117]. It provides information not only on the degree of impairment but also on the relative contribution to that impairment of abnormalities of ventilation, gas exchange, and oxygen transport and delivery, enabling a more in depth diagnostic assessment. When performed in an experienced centre repeat testing demonstrates high reproducibility of measurements[118].

Pulmonary vascular disease is associated with a characteristic exercise response profile[101, 119-124] and this is demonstrated in Figure 1.2 in a series of selected panels taken from the 9 panel plot output of a CPET carried out in a patient with IPAH at the time of diagnosis, prior to commencement on pulmonary vasodilator therapy. Patients demonstrate a shallower than normal VO_2 / work rate (WR) slope and achieve significantly lower peak VO_2 levels compared with healthy individuals. A reduced peak oxygen pulse, i.e. VO_2 / HR, is also seen. Considering again the Fick equation as shown in Chapter 1.7.1,

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

and that CO is the product of SV and HR, dividing both sides by HR gives

$$\frac{VO_2}{HR} = SV \cdot (CaO_2 - CvO_2)$$

and thus oxygen pulse is closely related to stroke volume. The low oxygen pulse seen on CPET in patients with PAH therefore represents the failure to augment SV described in Chapter 1.7.2. Patients also show an accelerated HR response, seen as a steep VO_2 / HR slope. A high V_E / VCO_2 slope and high V_E /VCO_2 at anaerobic threshold are characteristic of the ventilatory inefficiency seen in PAH and a low end tidal CO_2 ($P_{ET}CO_2$) is also seen, indicative of the abnormal gas exchange occurring in PAH.

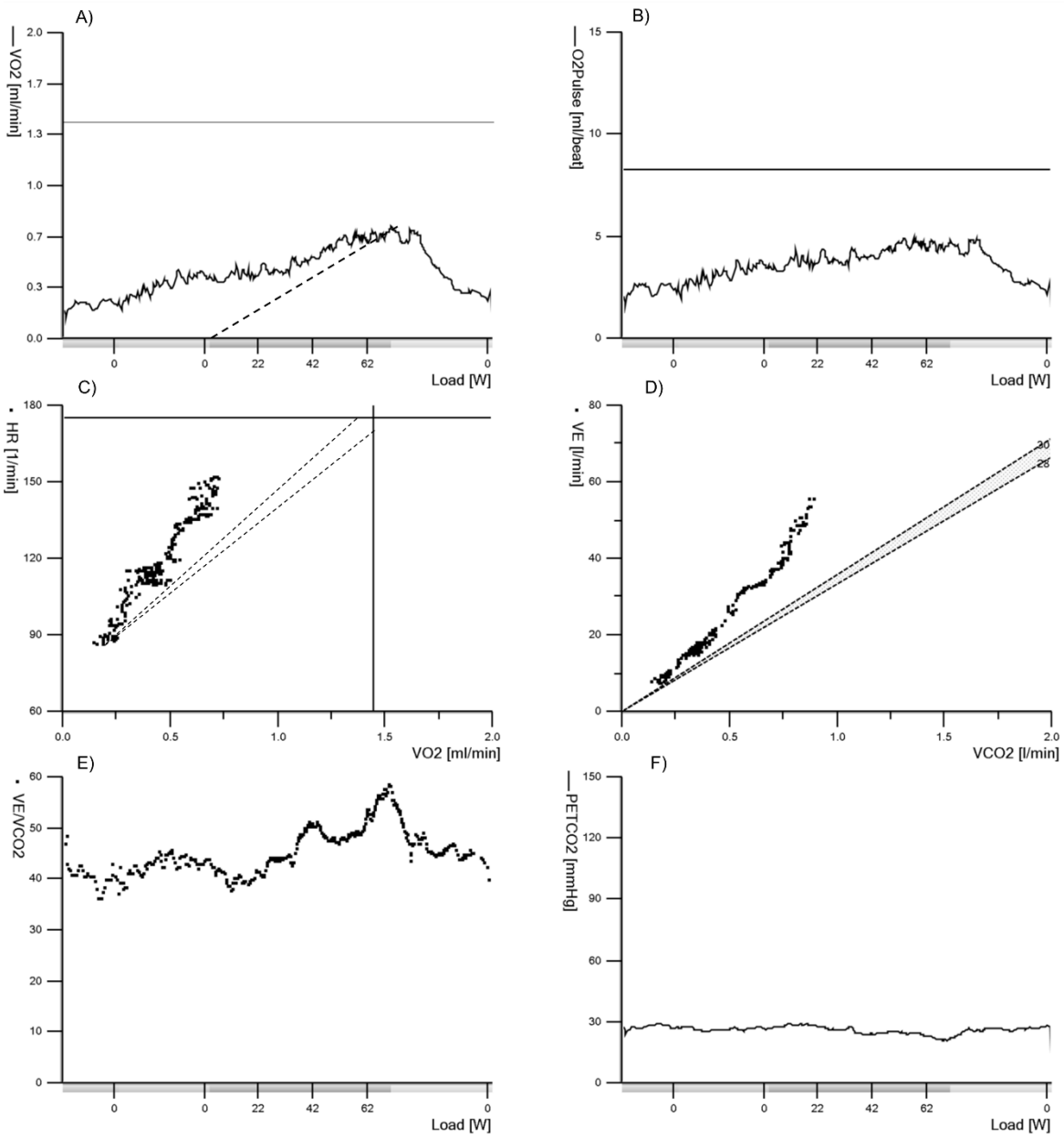


Figure 1.2 Typical CPET responses of a patient with PAH. The solid lines in A), B) and C) indicate the predicted peak values of the respective variables. The dashed line in A) represents a VO_2/WR slope of 10 ml/min/Watt – a healthy response would parallel this line. Note the more shallow VO_2/WR slope and reduced peak VO_2 in PAH. Reduced peak oxygen pulse is seen in B). Steep heart rate response and V_E/VCO_2 slope are evident in C) and D) respectively with the predicted response corridors indicated by dashed lines. E) displays a markedly elevated ventilatory equivalent of CO_2 while F) shows reduced end-tidal CO_2, demonstrating key elements of the abnormal gas exchange response in PAH.

1.7.3.2 Cardiopulmonary exercise testing and prognosis

CPET variables have in a number of studies been shown to predict outcome in patients with PAH. One study of incremental CPET in patients with PAH using either treadmill or cycle ergometer found that peak VO_2, $P_{ET}CO_2$, V_E/VCO_2 slope, peak systolic and diastolic blood pressure (BP) and peak HR were all predictive of survival on univariate analysis with peak VO_2 and peak systolic BP remaining significant on multivariate analysis[125]. A similar study found peak VO_2 and change in HR on exercise to be predictive of survival on multivariate analysis with those patients with lower peak VO_2 and a smaller increase in HR on exercise having a poorer survival[126]. Peak HR was also found to be associated with survival in a further study of patients with IPAH, which also reported that the V_E/VCO_2 slope was predictive of survival[127]

Patients with Group 1 PAH undergoing serial CPETs were assessed for survival in a prospective longitudinal study. The study authors found that the presence of a right to left shunt was predictive of mortality while in those patients without a right to left shunt, a higher V_E/VCO_2 ratio at anaerobic threshold (AT) was predictive of increased mortality[128]. A study of patients with IPAH and those with PAH associated with other conditions such as connective tissue disease, anorexigen use, liver cirrhosis and congenital left to right shunting found that although no clear predictor of survival was seen in the associated PAH group, peak VO_2 was predictive of time to clinical worsening and V_E/VCO_2 at AT was predictive of survival on multivariate analysis[129]. A retrospective analysis of patients diagnosed with PAH or CTEPH who had undergone CPET at baseline found that patients with a lower V_E/VCO_2 slope, higher peak VO_2 and greater increase in oxygen pulse from rest to peak exercise had significantly better survival on univariate analysis[130]. On multivariate analysis only the increase in oxygen pulse remained significant.

1.7.3.3 Cardiopulmonary exercise testing and treatment response

Studies of the use of CPET to detect a response to treatment have yielded mixed results. Small uncontrolled studies have shown an improvement in peak VO_2 on long term therapy with intravenous prostacyclin[131] and an increase in peak VO_2 and reduction in V_E/VCO_2 slope with nebulised iloprost[132]. A study of 28 patients

with PAH in which 14 continued on existing treatment and 14 were given additional treatment with sildenafil, found that there were improvements in V_E/VCO_2 at AT, $P_{ET}CO_2$ at AT and peak oxygen pulse in the group treated with sildenafil.

However in a considerably larger placebo controlled RCT of treatment with sitaxsentan, STRIDE-1, the ability of CPET to detect a treatment response was limited with only an increase in percent predicted peak VO_2 in the higher treatment dose group reaching statistical significance[133]. No improvements were seen in either of the two treatment dose groups compared with placebo for other CPET variables studied, namely VO_2 at AT and V_E/VCO_2 at AT. It has been suggested that these negative findings may be explained by the relatively complex nature of cardiopulmonary exercise testing and the multicentre nature of the study, perhaps leading to less accurate results when CPET was performed in centres with less experience[134].

1.7.3.4 The 6 minute walk test in pulmonary hypertension

The most common exercise test used in the assessment and monitoring of patients with PH is the 6minute walk test (6MWT)[92]. In contrast to incremental CPET the 6MWT is a submaximal test which relies mainly on aerobic rather than anaerobic metabolism[135]. Its popularity stems partly from the lack of specialist equipment required, with the only significant requirement a straight and quiet 30 metre corridor[136], and partly from the significant correlations seen between 6 minute walk distance (6MWD) and other measures of exercise performance, function and haemodynamic measurements.

In patients with IPAH the 6MWD has been shown to correlate significantly with peak VO_2, oxygen pulse and V_E/VCO_2 slope as measured on maximal CPET[137]. In the same study a modest but statistically significant correlation was also seen with baseline CO and total pulmonary resistance (TPR) but not with mPAP. The 6MWD was also seen to decrease in proportion with the severity of the functional class. In a separate study measuring gas exchange variables during 6MWT a correlation was seen between the 6MWD and the measured VO_2 achieved during the walk test[135]. Baseline 6MWD has been demonstrated to correlate with measures of quality of life and in addition the change in 6MWD on treatment has

been also been shown to correlate with change in quality of life[138]. The change in 6MWD has been shown separately to correlate significantly with cardiac index and PVR[139].

When measured at baseline, 6MWD has consistently been found to be predictive of survival in trials of pulmonary vasodilator treatment in PAH[140]. The strong predictive relationship with survival has also been seen in analyses of registry data and therefore baseline 6MWD has been included in risk scores from the French registry[6] and the North American REVEAL registry[141], and also in the Scottish Composite Score derived from UK data[142]. Although the prognostic strength is high in IPAH it is less strong and may be lost in patients with Group 1 PAH associated with other conditions such as connective tissue disease[129, 143].

Interestingly, although 6MWD improves with pulmonary vasodilator treatment, hence its inclusion in multiple clinical trials[140], and the change in 6MWD correlates with the change in haemodynamics as described above, the size of the change does not appear to have prognostic significance. Several studies have failed to demonstrate a prognostic effect of the change in 6MWD[140, 144-146]. It appears that the change in 6MWD accounts for only a minor part of the overall treatment effect[147]. The correlation between the change in 6MWD and the change in haemodynamics is stronger than that with change in RV function[148] but it is the change in RV function which is most strongly linked to outcome[149].

A further drawback of using 6MWD as an outcome measure is the ceiling effect, whereby it is more difficult to see an improvement in subjects with a higher baseline 6MWD than in those with a lower baseline 6MWD[150-153].

Despite these drawbacks however the 6MWT remains an integral part of the assessment and monitoring of patients with PAH, both in routine practice and in clinical trials.

1.8 Noninvasive assessment of the pulmonary circulation

The haemodynamic status of the RV and pulmonary circulation is key to the diagnosis and assessment of patients with PH. However while RHC is the gold standard method of this repeat procedures are unattractive due to the invasive nature of the test. Research is therefore ongoing into potential noninvasive modes of assessing the haemodynamic abnormalities. In addition to echocardiography as described in Chapter 1.4.3, cardiac MRI is increasingly being used in the noninvasive assessment of the pulmonary circulation of patients with PH. Inert gas rebreathing (IGR) has also provoked interest as a potentially useful measure of haemodynamic change which again can be measured noninvasively.

1.8.1 Cardiac magnetic resonance imaging

MRI is an attractive method of assessing the right heart as it is noninvasive, uses no ionising radiation and provides information on both the structure and the function of the heart. Despite being considerably more expensive than echocardiography and less widely available, cardiac MRI has evolved over recent years to become the gold standard method of assessing the structure and function of the heart in a range of diseases including pulmonary hypertension[154-159]. While some patients find MRI difficult to tolerate due to long scan times, claustrophobia and the need to perform repeated breath hold manoeuvres, newer technology has enabled scans to be performed more quickly and with real time acquisition[160, 161] thus improving patient tolerance.

In PH cardiac MRI can allow assessment of RV structure, size and function, and provide information on flow form the RV into the pulmonary circulation with measurements including SV, CO and PA distensibility[162]. Studies in patients with PAH have described impairment of RV systolic function, quantifiable by measurement of RVEF, SV and CO, with increased RVEDV and RV end systolic volume (RVESV) compared with control subjects[155, 163, 164]. MRI however is not able to measure actual PA pressures and therefore cannot replace RHC as a diagnostic tool. Correlations between PA pressure and cardiac MRI derived variables including the average velocity of pulmonary blood flow (PBF), septal curvature, relative area change of the main PA and the ventricular mass index (the ratio of RV mass to LV mass) have been seen raising the possibility that with

refinement cardiac MRI may have a role to play in the noninvasive diagnostic assessment of patients with PAH[165-168].

1.8.2 Inert gas rebreathing

Inert gas rebreathing (IGR) is a long established technique for the noninvasive measurement of CO. It is based on the principle that when rebreathing in a closed circuit, the rate at which a specified gas dissolves in blood is proportional to the blood flow through the pulmonary capillaries. The PBF is equal to the cardiac output in the absence of a significant intracardiac or intrapulmonary shunt. Initially studied using acetylene rebreathing[169] it was shown to have good agreement with more traditional techniques of measuring CO, namely the Fick and thermodilution methods, including in patients with Group 1 PH[170].

A modern refinement of this technique using a gas mixture of sulphur hexafluoride and nitrous oxide coupled to a rapid photoacoustic gas analyser has been shown to have good limits of agreement for SV measured by this method when compared with SV measured by cardiac MRI and by thermodilution in patients with PAH[171]. This method compares favourably with the conventional mass spectrometers previously used with IGR as it is more user friendly, lighter and less expensive[172]. Furthermore it can be used in the presence of fibrotic lung disease while maintaining good agreement with CO measured invasively by the indirect Fick method[173].

This method of IGR has been studied successfully in patients with chronic heart failure. In one study measurement of CO was seen to agree closely with measurements made by the thermodilution and direct Fick methods[174]. A separate study in patients with heart failure showed that this method of IGR could be used to measure CO successfully at rest and on exercise[175], while a further study demonstrated that such measurements of CO made at peak exercise can predict survival in patients with chronic heart failure[176]. In a study of 24 patients with Group 1 or Group 4 PH either starting treatment *de novo* or undergoing modification of existing treatment, PBF and SV were seen to increase significantly at 3 months[150]. The further role of IGR in the assessment and monitoring of patients with PAH remains to be explored.

1.9 Hypotheses and aims

This thesis aims to investigate the extent to which novel exercise derived variables and noninvasive haemodynamic measurements can enhance the assessment and monitoring of patients with pulmonary hypertension. The following hypotheses were examined in clinical studies:

1. that there is impairment of peripheral muscle oxygen extraction on exercise in patients with Group 1 and Group 4 pulmonary hypertension,

2. that the oxygen uptake efficiency slope is strongly correlated with peak VO_2 in PH, that it is valid as a submaximal measure of exercise performance in groups I and IV PH and that it predicts survival in patients within these disease groups,

3. that prolonged rates of recovery of heart rate and VO_2 after incremental CPET in patients with precapillary PH will be associated with poorer survival and

4. that inert gas rebreathing may detect early changes in stroke volume after institution or alteration of pulmonary vasodilator therapy and that this change will be associated with later functional improvement.

2 Materials and methods

2.1 The Scottish Pulmonary Vascular Unit

All patients recruited for the studies documented in this thesis were attending the Scottish Pulmonary Vascular Unit (SPVU) for diagnosis and treatment of PH. The SPVU was founded in 1990 and although based in Glasgow it is a tertiary referral centre for PH and is the sole centre in Scotland for managing adult patients with PH. Scotland has a population of 5.3 million of whom approximately 4.4 million are aged 16 years or over and therefore potentially served by the SPVU. The National Audit of Pulmonary Hypertension gathers data from all eight specialised pulmonary hypertension centres in the United Kingdom, seven of which treat adult patients and there is one paediatric centre. The most recent data from this audit, covering April 2014 to March 2015, demonstrates a referral rate to SPVU of 42 per million population, with a point prevalence of 82 cases of pulmonary hypertension per million population[14].

During the years 2012-2014 a total of 608 patients had referrals accepted to SPVU and 233 patients were diagnosed with group 1, 4 or 5 PH. At 31/12/14 there were 341 patients diagnosed with group 1, 4 or 5 PH with active ongoing follow up within SPVU. Due to the structure of the service in Scotland, with only the SPVU permitted to prescribe pulmonary vasodilator therapies, it is reasonable to assume that all patients in Scotland with a known diagnosis of precapillary PH are treated under the auspices of the SPVU.

2.2 World Health Organisation functional class

The World Health Organisation functional class (WHO-FC) for pulmonary hypertension was modified from the New York Heart Association classification for describing the functional impact of heart failure. The WHO-FC has been used since 1998 and is now well established in the assessment of patients with PH[4] (Table 2.1). The timing of WHO-FC assessment is documented in the chapters detailing the specific methods for each study.

WHO functional class	
Class I	Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnoea or fatigue, chest pain, or near syncope
Class II	Patients with pulmonary hypertension resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near syncope.
Class III	Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes undue dyspnoea or fatigue, chest pain, or near syncope.
Class IV	Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnoea and/or fatigue may even be present at rest. Discomfort is increased by any physical activity.

Table 2.1 WHO functional classification of pulmonary hypertension

2.3 Cambridge Pulmonary Hypertension Outcome Review score

The Cambridge Pulmonary Hypertension Outcome Review (CAMPHOR) score is a pulmonary hypertension specific patient-completed questionnaire and was used to assess health related and general quality of life[177] (Appendix 1). It asks questions across three domains: symptoms, activities and quality of life, with higher scores reflecting poorer quality of life.

2.4 N-terminal pro-brain natriuretic peptide

N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured on venous blood sampled by standard venepuncture and collected in vacuum tubes containing ethylenediaminetetraacetic acid (EDTA). In patients undergoing an exercise protocol as part of the studies blood for NT-proBNP analysis was sampled prior to the exercise component of the assessment.

2.5 Six minute walk test

Six minute walk tests (6MWT) were carried out by the respiratory physiologists at the Golden Jubilee National Hospital, by Ms Val Pollock, clinical trial nurse, or by Dr SD Thomson. All were conducted in accordance with American Thoracic Society guidelines[136].

2.6 Incremental cardiopulmonary exercise test

Maximal incremental cardiopulmonary exercise tests (CPETs) were conducted in accordance with established guidelines[178] using an electromagnetically braked cycle ergometer (ergoselect 200, ergoline GmbH, Bitz, Germany). CPETs were carried out by a trained respiratory physiologist with medical supervision (Dr SD Thomson). Patients wore a tight fitting face mask connected to a metabolic cart for breath by breath measurement of gas exchange variables (Medisoft, Sorinnes, Belgium; lovemedical, Manchester, United Kingdom). Oxygen saturations were measured transcutaneously 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.

Predicted values were calculated using published equations[179] and maximum voluntary ventilation (MVV) was estimated as 35 x forced expiratory volume in 1 second (FEV_1)[180]. CPET was contraindicated if the patients had a history of

exertional presyncope, syncope or arrhythmia, or the presence of a neurological or musculoskeletal deficit which would limit exercise performance.

Patients sat at rest on the bicycle for an initial period of a few minutes after fitting of the mask and connecting the monitoring equipment. Recording was commenced and a formal rest period of two minutes undertaken. The subjects then began a 3 minute unloaded cycling phase at a cadence of 60 revolutions per minute which was maintained throughout the test. After 3 minutes the load was gradually applied to the ergometer. The rate of increase of work rate through the loaded cycling phase was estimated by the respiratory physiology team prior to the test on the basis of each patient's description of their functional capabilities with the aim that the patient would be able to achieve 8 to 12 minutes of loaded cycling before reaching their symptomatic limit. Recordings were made of cardiac, gas exchange and ventilation variables continuously throughout the test. Recording continued in the recovery phase, usually for at least 2 minutes. Patients were encouraged to continue to cycle at a reduced cadence and with no load applied during this recover period.

2.7 Right heart catheterisation and exercise right heart catheterisation

2.7.1 Resting right heart catheterisation

Right heart catheterisation (RHC) is the gold standard method for diagnosing pulmonary hypertension and forms a routine part of each patient's initial diagnostic assessment. All the resting RHCs included in these studies were performed as part of routine clinical care at the time of first diagnosis of PH. None of the patients were on pulmonary vasodilator therapy at the time of the RHC. All RHCs were performed with the patient awake. On occasion some mild intravenous sedation with midazolam was used to improve tolerance but patients remained alert throughout the procedure. All RHCs were conducted supine.

To facilitate access to the central venous system an 8Fr venous introducer sheath was sited in the right internal jugular vein under direct ultrasound

visualisation with x-ray screening used to confirm the position. This was most often carried out the afternoon prior to the catheterisation session and the sheath was secured in place with a silk suture and an occlusive dressing. If it was not possible to site the sheath in the right internal jugular vein then it was placed instead in either the right femoral vein or left internal jugular vein, again using direct ultrasound visualisation and x-ray screening to confirm position.

A balloon-tipped flow-directed pulmonary arterial (Swan-Ganz) catheter was inserted through the introducer sheath and into the venous system. Under fluoroscopy screening the catheter was passed into the right atrium, right ventricle and pulmonary artery[181]. Measurements of right atrial pressure (RAP), right ventricular systolic pressure (RVSP), pulmonary arterial systolic, diastolic and mean pressures (PASP, PADP and mPAP respectively), and pulmonary artery wedge pressure (PAWP) were made using a pressure transducer zeroed externally at the level of the left atrium. This level was estimated as the point in the midaxillary line at the midthoracic level. PAWP was obtained by inflation of the balloon with the catheter in a pulmonary artery branch and confirmed by assessment of the resulting pressure wave trace. Measurements were captured and recorded using commercially available equipment and software (GE Healthcare, Buckinghamshire, United Kingdom). Pulmonary vascular resistance (PVR) was measured in Wood units and calculated as transpulmonary gradient (TPG) divided by cardiac output (CO) in litres/minute where TPG is the difference between mean pulmonary artery pressure and pulmonary artery wedge pressure in mm Hg, i.e.

$$PVR = \frac{(mPAP - PAWP)}{CO}$$

Cardiac output was quantified by the thermodilution method[182]. Although the direct Fick method of cardiac output measurement is the gold standard the thermodilution method has long been accepted as a more practical alternative, is standard practice within the SPVU 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[170]. 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.

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).

In patients not undergoing subsequent exercise RHC (described in 2.7.2) the catheter was removed under fluoroscopy screening. The introducer sheath was removed and the wound closed with direct pressure and the application of an airtight dressing.

2.7.2 Exercise right heart catheterisation

Exercise right heart catheterisation was performed immediately following the completion of the resting RHC (described in Chapter 2.7.1). The introducer sheath and pulmonary arterial catheter were left in situ. Patients in whom the introducer sheath had had to be inserted into the femoral vein did not participate in the exercise RHC due to the difficulty of pedalling with a venous access point in the groin. 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 a slight elevation of the legs compared with the fully supine resting RHC. After five minutes of rest in this position repeat measurements were taken of RAP, RVSP, PASP, PADP, mPAP, PAWP and CO (described in 2.7.1) and these were taken as the resting measurements for comparison with the subsequent exercise results.

In contrast to the upright CPET which used an incremental work rate protocol, the supine exercise test used a constant work rate protocol. Such protocols are ideally suited to measurements of gas exchange[178] and arterial blood gas measurements made after five minutes of constant work rate cycling at 70% of the peak work rate achieved on a maximal incremental CPET approximate those

measured at the peak of the incremental test[183]. Furthermore it has been shown in several studies that exercise capacity is significantly reduced in the supine compared to upright position during moderate to high intensity cycling and that this is most marked during constant work rate exercise[184-186]. Therefore the supine constant work rate was calculated as 50% of the total work rate achieved in the maximal upright cardiopulmonary exercise test which each patient had performed two days earlier.

The patients were asked to start cycling, initially with zero resistance, at a cadence of 60 revolutions per minute. The resistance was increased over approximately 30 seconds until the target work rate was reached. After 3 minutes cycling at this work rate repeat measurements of PASP, mPAP, PADP and PAWP were made. Cardiac output was then measured but in contrast to the resting CO measurements, this was carried out in duplicate rather than triplicate given the time needed between each CO thermodilution procedure for the blood temperature to stabilise and the measurement system to reset. Pressure measurements were then repeated before finally a further pulmonary arterial blood sample was withdrawn for end exercise blood gas analysis (described in 2.7.1).

Following the withdrawal of this blood sample patients were instructed to slow and then stop pedalling. Feet were unstrapped from the pedals and legs lowered onto the catheterisation table. The ergometer was removed from the table and the catheter was withdrawn from the pulmonary arterial system under fluoroscopy screening. The introducer sheath was removed (described in 2.7.1).

2.8 Oxygen uptake efficiency slope

2.8.1 Calculation of oxygen uptake efficiency slope

Oxygen uptake efficiency slope (OUES) is an index derived from breath by breath values of ventilation (V_E) and oxygen uptake (VO_2) measured over the course of an incremental cardiopulmonary exercise test[187]. CPETs were carried out as described in Chapter 2.6. For each subject the breath by breath measurements

of V_E and VO_2 from onset of loaded cycling until peak exercise were extracted from the CPET reporting software and entered into a spreadsheet (Excel, Microsoft, Redmond, Washington, USA).

The relationship between V_E and VO_2 during an incremental CPET is not linear (Figure 2.1).

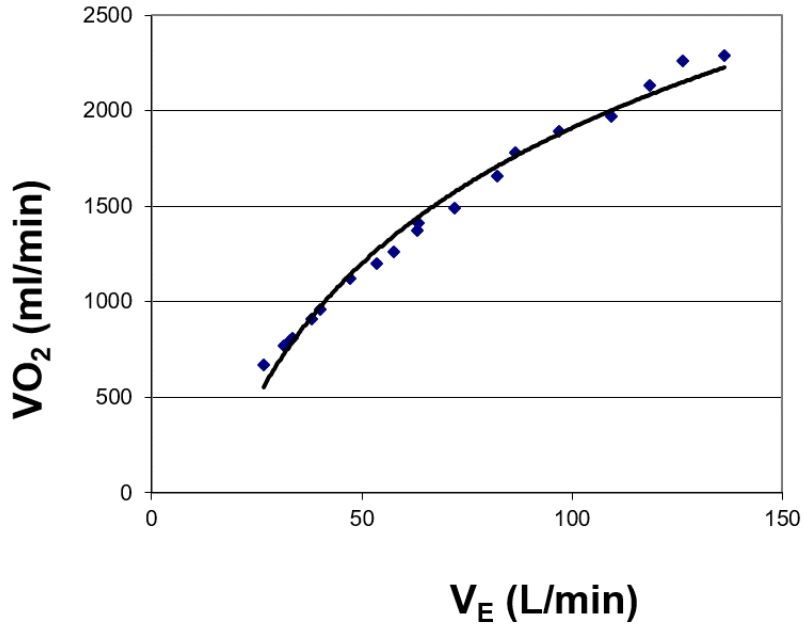


Figure 2.1 Relationship between minute ventilation and oxygen consumption during a maximal incremental cardiopulmonary exercise test. VO_2: oxygen consumption, V_E: minute ventilation

Logarithmic transformation of the minute ventilation data was therefore performed and the resulting values plotted against VO_2, producing a linear relationship with VO_2 where:

$$VO_2 = a\ logV_E + b$$

and *a* is defined as the OUES (Figure 2.2).

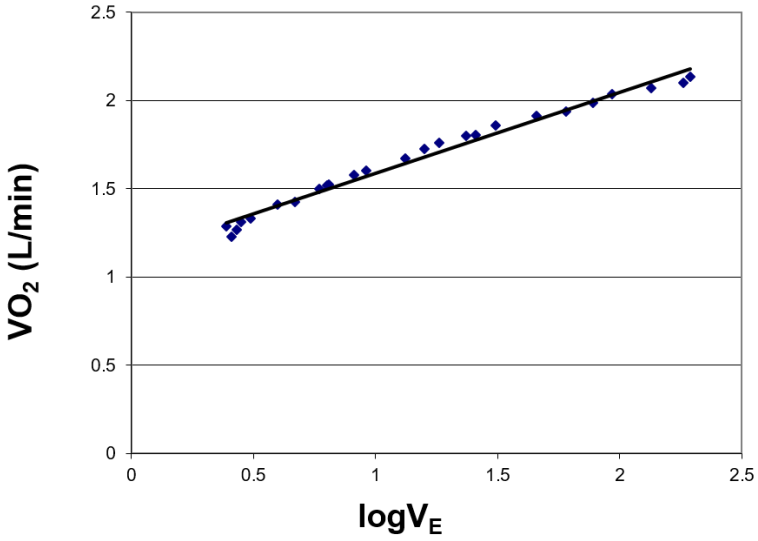


Figure 2.2 Oxygen uptake efficiency slope calculation. VO_2: oxygen consumption; $logV_E$: log transformed minute ventilation; OUES is the gradient of the line formed

2.8.2 Conversion of CPET plots into numerical data for OUES calculation

The value of OUES as a predictor of survival in PH was assessed (Chapter 4.3.3). Some of the earlier cardiopulmonary exercise tests had no data table available and therefore no breath by breath minute ventilation and oxygen uptake values were available for calculation of OUES. The original plots of V_E and VO_2 versus time were therefore converted back into numerical data using digitising software (GetData Graph Digitizer v2.26, http://getdata-graph-digitizer.com/).

The original paper plots were scanned then imported into PowerPoint (PowerPoint, Microsoft, Redmond, Washington, USA). An adjustable grid with 24 vertical lines was created in Word (Word, Microsoft, Redmond, Washington, USA) then also imported into PowerPoint and overlaid on the plot to be digitised with the first and last lines positioned over the five and fifteen minute time points on the x axis (Figure 2.3). The five minute time point was chosen as it represents the onset of loaded cycling. The resulting image was saved as a TIF file.

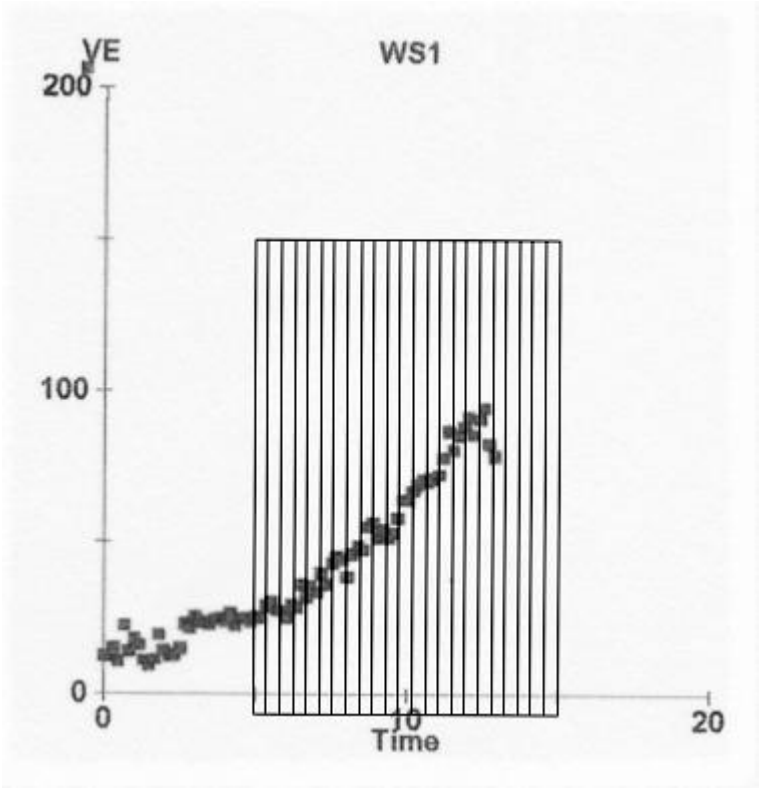


Figure 2.3 Plot of minute ventilation versus time with digitising grid overlaid and positioned between the 5 and 15 minute time points on the x axis. VE: minute ventilation in litres/minute, Time: exercise time in minutes

The TIF file was then opened in the digitising programme. Scales on the x and y axes were set by allocating minimum and maximum values along each axis. Using the manual digitising function the central point between each pair of gridlines was selected and its values on the x and y axes recorded in a table. The plot for the same patient as Figure 2.3 with digitising points selected is shown in Figure 2.4.

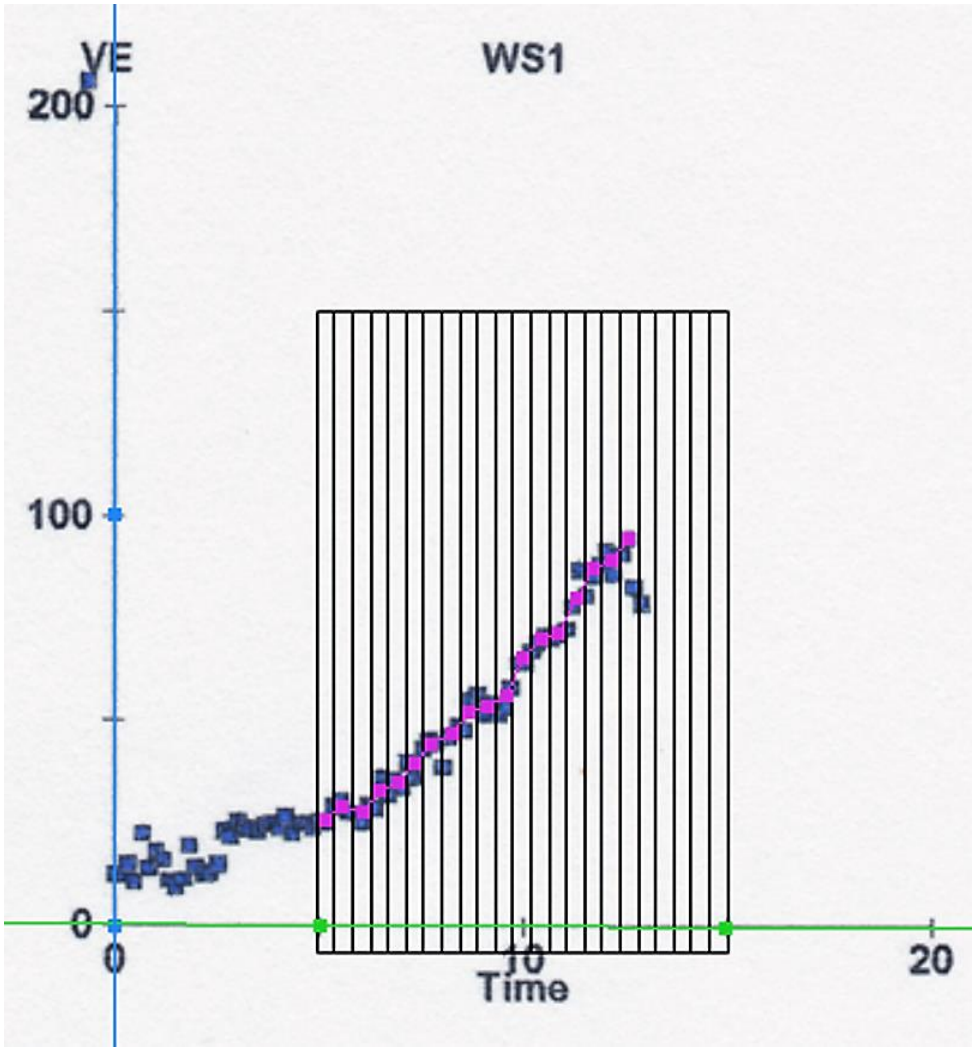


Figure 2.4 Plot of minute ventilation versus time with digitising grid overlaid and points manually selected shown in pink. VE: minute ventilation in litres/minute, Time: exercise time in minutes

The output table with values of the x (time) and y (minute ventilation) axes for each digitising point was then exported to Excel and OUES calculated as described in Chapter 2.8.1.

Due to the nature of the technique it is possible for there to be small errors in the values obtained by digitising individual data points. However any such errors would be random rather than systematic. Furthermore their effect would be minimised by the subsequent calculation of the OUES as a slope constructed of multiple data points. It was therefore felt that this technique was a valid method for the extraction of exercise test data and OUES calculation.

2.9 Measurement of heart rate and oxygen consumption recovery following incremental cardiopulmonary exercise test

Cardiopulmonary exercise tests for the patient group used for analysis of oxygen uptake efficiency slope and survival (Chapter 4.3.3) were reviewed and those with at least 60 seconds of technically acceptable recovery data recorded were selected. Only tests with available data tables were included. The values of both heart rate (HR) and oxygen consumption (VO_2) at peak exercise and at 30 seconds, 60 seconds and 120 seconds into recovery were recorded. The recovery was calculated as an absolute reduction from peak values and by taking the value at each time point as a percentage of the peak exercise value. Survival analysis was then undertaken as described in Chapter 2.10.

2.10 Survival analysis

2.10.1 Cox proportional hazards model

To identify predictors of all-cause mortality a Cox proportional hazards model was employed (IBM SPSS Statistics, International Business Machines Corp, Armok, New York, USA). Right censoring was used with survival time calculated from the date of diagnosis to the date of death or date of censor. Patients were censored at the time of death, transplantation, pulmonary endarterectomy, or loss to follow up (taken as the last date of clinical contact), or at the time of data cut-off (25th September 2013). Univariate analysis was performed on a series of candidate variables to determine which were predictors of mortality. These variables were then assessed for collinearity using either Pearson or Spearman correlation, depending on the distribution of each variable, and by calculation of the variance inflation factor (VIF). Those with a high correlation (r or $\rho > 0.7$) or a VIF > 3 were not included in the multivariate analysis.

Remaining variables with a p value less than a predefined limit in the univariate analysis were analysed in the multivariate model with backwards selection. One variable for every ten events (deaths) was included in the model to avoid

overfitting. A p value < 0.05 by likelihood ratio test was considered statistically significant.

2.10.2 Kaplan-Meier analysis

Kaplan-Meier analysis was used to compare survival rates for different groups with all-cause mortality the endpoint (IBM SPSS Statistics). Survival time was calculated and censoring performed as described in Chapter 2.10.1. Log rank tests were used to compare the groups and Kaplan-Meier survival curves produced. A p value < 0.05 was considered statistically significant.

2.11 Inert gas rebreathing in the assessment of treatment response in pulmonary hypertension

2.11.1 Operation of inert gas rebreathing with Innocor

Inert gas rebreathing (IGR) was used to determine pulmonary blood flow and therefore right ventricular stroke volume through rebreathing an oxygen enriched mix of nitrous oxide (N_2O) and sulphur hexafluoride (SF_6) (Innocor, Innovision A/S, Odense, Denmark). This is an established technique which has been validated for use in heart failure[172, 174, 175] and pulmonary hypertension[171, 172]. To perform the rebreathing manoeuvre patients wore a nose clip and breathed via a mouthpiece and filter connected to a valve either open to the ambient air or to a rebreathing Douglas bag containing a mix of oxygen, N_2O, SF_6, and room air (Figure 2.4).

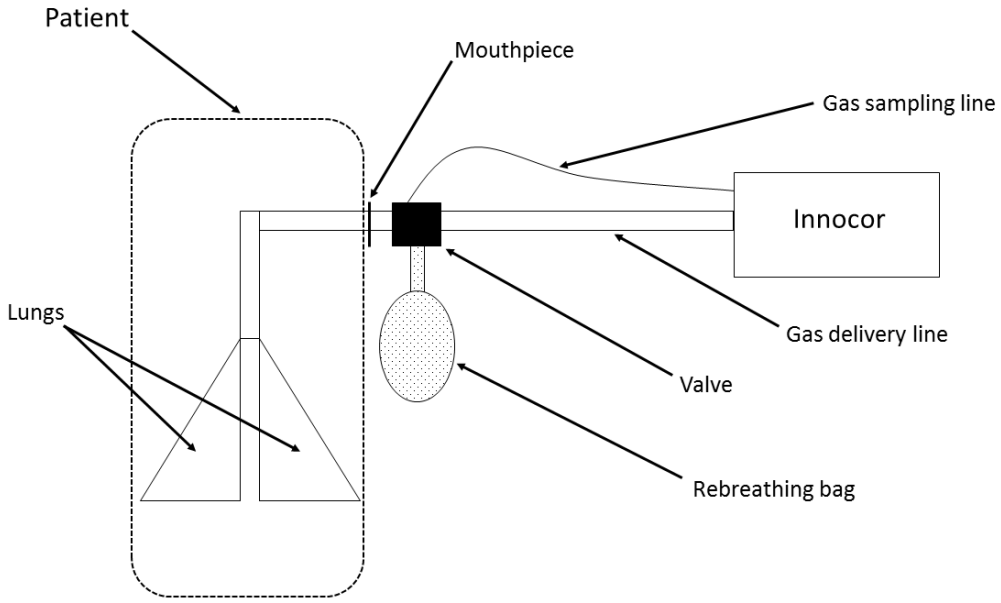


Figure 2.5 Innocor set-up for inert gas rebreathing manoeuvre.

Breath by breath respired gases were measured continuously at the mouthpiece via a sampling line connected to a rapid infrared photoacoustic gas analyser.

Before each IGR test a 3 litre Douglas bag was filled with a mix of ambient air via an air pump and a bolus of gas from the Innocor gas cylinder. The cylinder contained 94% oxygen, 5% N_2O and 1% SF_6 and a bolus fraction of 10% was used routinely to give a final concentration in the Douglas bag of 28.3% oxygen, 0.5% N_2O and 0.1% SF_6. The volume of the gas mixture in the bag before each IGR measurement was 200ml less than the highest volume of: 44% of the predicted vital capacity[188], the tidal volume during the preceding 5 breaths or the volume required to ensure the gas concentrations remained below the maximum CO_2 limit and above the minimum O_2 limit. These limits were set by default to a CO_2 less than 8% and an O_2 greater than 13%. A higher bolus fraction of 20% was used for IGR measurement on exercise to compensate for the increased ventilatory demand.

Patients breathed via the mouthpiece with the valve open to the air supply until the appropriate time for each IGR measurement. At that point the operator (Dr SD Thomson) then started each measurement by priming the valve to open at the end of the next expiration. From the point at which the valve opened the

patients then breathed in a closed circuit with the rebreathing bag. The patient was instructed to breathe deeply enough to empty the bag with each inspiration then to exhale normally with a target respiratory rate of 20-30 breaths per minute. Usually fewer than 8 breaths were required to complete the rebreathing measurement. Given that N_2O is highly soluble in blood it left the rebreathed gas mixture at a rate proportionate to the pulmonary blood flow (PBF). SF_6 is insoluble in blood and therefore remained in the closed circuit between alveoli and rebreathing bag and was used to determine the total volume from which the N_2O disappeared, known as the total systemic volume. Calculation of the total systemic volume and PBF was performed by the Innocor IGR machine by established methods[189] which are described in Sections 2.11.1 and 2.11.3 respectively.

2.11.2 Calculation of total systemic volume

The total systemic volume is the sum of the volumes of the lungs, the rebreathing bag and the deadspace of the mouthpiece and rebreathing valve. During each rebreathing manoeuvre the concentration of SF_6 declined from the initial concentration in the rebreathing bag until fully mixed with the air in the lungs and an equilibrium reached. The difference between maximum and minimum SF_6 concentrations within each breath was analysed continuously and a mixing level calculated as the difference between these maximum and minimum concentrations divided by their mean. When this level fell below a threshold value of 15% the gases were considered adequately mixed and estimation of pulmonary blood flow could begin (Chapter 2.11.3). This usually occurred within 3-5 breaths. A maximum limit of 30 seconds was allowed for each IGR measurement to ensure there was no recirculation of dissolved N_2O back into the pulmonary circulation as this would have created an error within the measurements.

Given the volumes of the rebreathing bag and deadspace were known the total systemic volume could be determined from the dilution of insoluble SF_6, i.e. from the ratio of the initial concentration of SF_6 in the rebreathing bag, taken as the peak concentration measured during the first inspiration, to the concentration of SF_6 at equilibrium.

The total systemic volume was subject to change throughout the rebreathing measurement due to changes in CO_2 excretion while O_2 uptake remained constant. The IGR manoeuvre required patients to breathe a little more deeply and with slightly increased respiratory rate compared with normal ventilation. This increased ventilation caused a lower alveolar partial pressure of CO_2 as PBF remained constant. The resulting increased diffusion gradient between the capillaries and the alveoli led to increased CO_2 excretion into the lungs and therefore an increase in the total systemic volume. However as the CO_2 level rose, the gradient lessened and the rate of CO_2 excretion fell causing the volume to shrink. For the purpose of calculation of PBF the total systemic volume was taken at time "zero", actually the midpoint of the first inspiration. At this point the SF_6 would not yet have adequately mixed and therefore the SF_6 concentration at time zero was calculated by back extrapolation from the point at which adequate mixing was achieved (Figure 2.6).

The total systemic volume at time zero was calculated as

$$V_{tot} = \frac{[SF6]_i}{[SF6]_{eq}} \cdot V_{RB}$$

where

V_{tot}	=	the total systemic volume at time zero
$[SF_6]_i$	=	the initial concentration of SF_6 in the rebreathing bag
$[SF_6]_{eq}$	=	the concentration of SF_6 once equilibrium achieved, back extrapolated to time zero
V_{RB}	=	the volume of the rebreathing bag

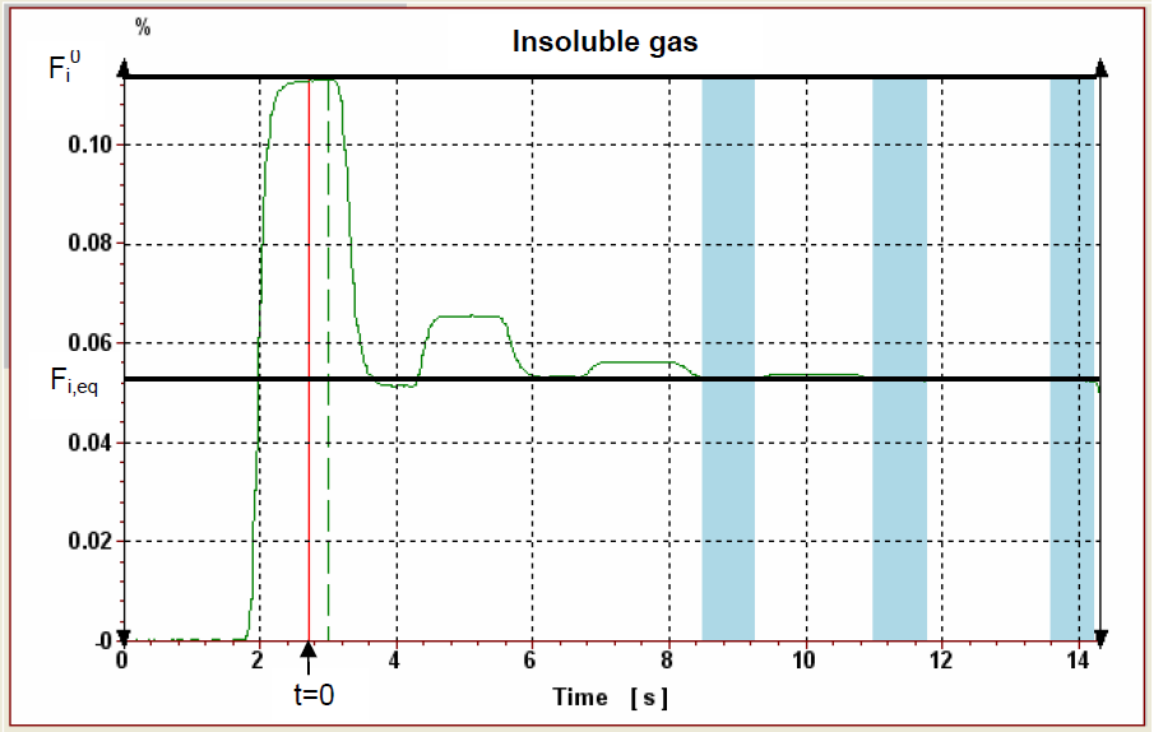


Figure 2.6 Concentration of insoluble SF_6 during rebreathing. The black line through the expiratory points once adequate mixing was achieved (represented by vertical blue bars) allowed back extrapolation to determine the SF_6 concentration at time zero (t=0).

2.11.3 Calculation of pulmonary blood flow

Pulmonary blood flow is that which perfuses the ventilated parts of the alveoli. It was calculated during inert gas rebreathing by measuring the rate at which blood soluble N_2O disappeared from the rebreathed gas mixture. An initial, almost instant, fall in N_2O was attributed to dissolution of the gas in the tissue of the lung while a subsequent more gradual decline occured due to dissolution in the perfusing pulmonary blood flow. This rate of decline was proportional not only to the PBF but also to the alveolar concentration of N_2O and therefore the disappearance of N_2O was described by a monoexponentially decreasing function of time. As described in chapter 2.11.2 the actual total systemic volume changed during each IGR manoeuvre. To account for this and for any incomplete mixing the N_2O concentrations were “normalised” by adjusting for changes in the SF_6 concentration. Representing the decay of the normalised N_2O concentration in a semilogarithmic plot produced a linear relationship, the slope of which was proportionate to the PBF (Figure 2.7).

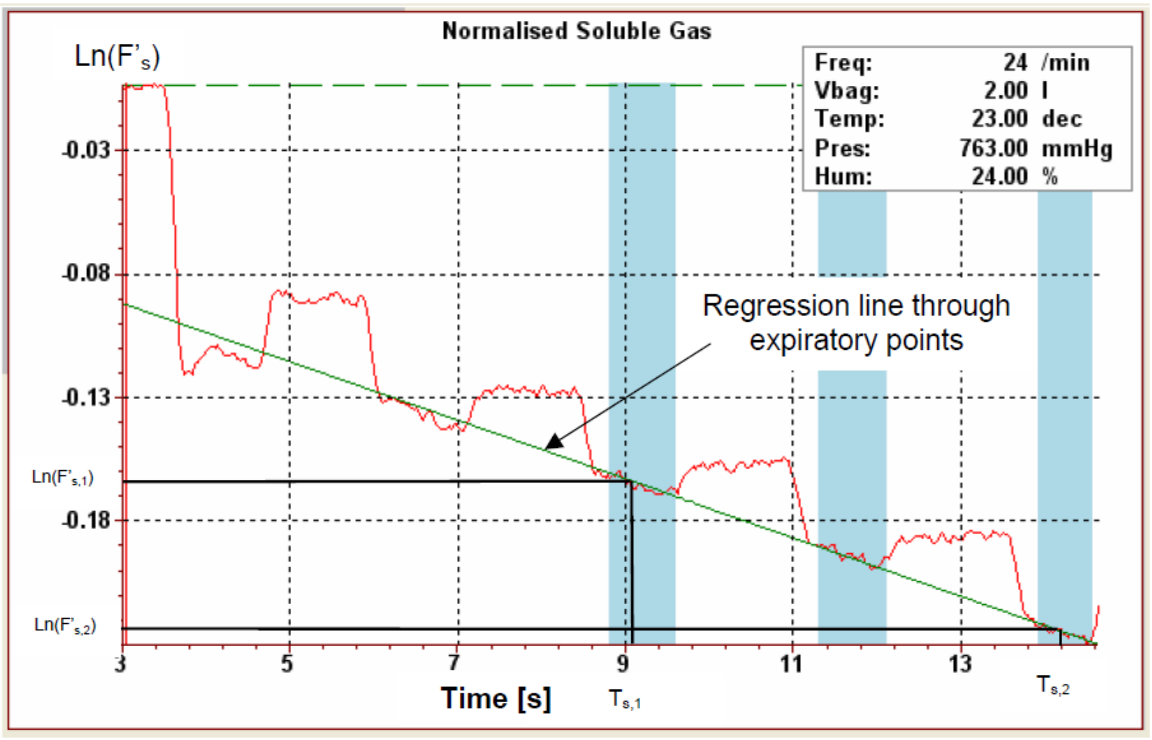


Figure 2.7 Semilogarithmic plot of normalised N_2O on the y axis and time on the x axis. The regression line through the end-expiratory points once adequate gas mixing was achieved (vertical blue bars) was proportionate to the pulmonary blood flow.

PBF was then calculated using the formula:

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

where

β	=	slope of the regression line through the expiratory points of the semilogarithmic normalised N_2O concentrations
V_{tot}	=	total systemic volume (STPD) (Chapter 2.11.2)
C_1	=	760/(ambient pressure in mmHg – 47)
C_2	=	$\alpha_t \cdot V_t$, constant to account for the dissolution of N_2O in lung tissue
α_t	=	Bunsen solubility coefficient in tissue of N_2O = 0.407 (STPD)
V_t	=	lung tissue volume (default 600ml)
α_b	=	Bunsen solubility coefficient in blood of N_2O = 0.412 (STPD)

In the absence of a significant intracardiac or intrapulmonary shunt pulmonary blood flow is equivalent to the right ventricular cardiac output and therefore the stroke volume was calculated by dividing PBF by heart rate. The Innocor software does have the ability to estimate and correct for shunting using the following formula:

$$CO = \frac{1}{\left(\frac{1}{PBF}\right) + \frac{CaO_2 - CcO_2}{VO2}}$$

where

CO	=	cardiac output
PBF	=	pulmonary blood flow
CaO_2	=	arterial blood oxygen content (0.000139 × haemoglobin concentration (g/dL) × arterial oxygen saturation (%) by pulse oximetry)
CcO_2	=	pulmonary end-capillary blood oxygen content (0.000139 × haemoglobin concentration (g/dL) × pulmonary end-capillary oxygen saturation (%))
VO_2	=	oxygen uptake (L/min)

The algorithm assumes a pulmonary end-capillary saturation of 98%. The validity of this assumption in pulmonary hypertension has been questioned due to the increased ventilation/perfusion mismatch and lower mixed venous oxygen saturations seen in PH with previous work using the Innocor equipment in PH advocating against use of the shunt correction algorithm[190]. Therefore PBF was used for the IGR work included in this thesis without application of the shunt correction algorithm.

2.11.4 Resting inert gas rebreathing protocol

The first set of resting IGR measurements were made with the patient lying supine. 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. For most patients only 2 or 3 practice attempts were required. Patients at future visits who had

previously performed the IGR test were given a brief verbal reminder and a practice attempt was undertaken to ensure the IGR test could still be performed without difficulty.

Patients lay at rest in the supine position for 10 minutes to ensure the recorded measurements would be a true reflection of the resting supine state. The first IGR manoeuvre was performed and the data recorded. In undertaking serial measurements it is necessary to ensure that the SF_6 and N_2O have washed out of the lungs and circulating blood. The N_2O concentration had to be less than 0.002% and the SF_6 concentration less than 0.001% prior to each test and in practice at least 5 minutes was used between tests to ensure washout occurred. The IGR manoeuvres were repeated in the supine position until 3 technically acceptable results were obtained.

Patients were then asked to sit on an upright cycle ergometer (ergoselect 200, ergoline GmbH, Bitz, Germany) and feet were strapped into the pedals. The Innocor was repositioned to enable comfortable upright measurements at rest and on exercise and ECG leads were connected for the later exercise component of the test. A further 10 minute rest period was required before measurement of PBF by IGR was performed. As with the supine measurements the manoeuvres were repeated until 3 technically acceptable readings were obtained, ensuring adequate gas washout between each test.

2.11.5 Exercise inert gas rebreathing protocol

Following the resting supine and erect measurements described in chapter 2.11.4 patients then undertook a period of constant load exercise on the upright cycle ergometer with measurement of PBF by IGR performed in the final 30 seconds of exercise. Patients exercised at 40% of the peak work rate achieved during baseline cardiopulmonary exercise testing. The IGR test was always performed at least one day after the CPET to ensure full recovery from the maximal exercise test. After 5 minutes of exercise and while continuing to cycle at the same workload the IGR manoeuvre was performed. Due to the difficulties of sustaining exercise at that level for a longer period and the need for gas washout between tests only one measurement of PBF took place at end-exercise.

2.12 Cardiac magnetic resonance imaging

Cardiac MRI scanning was performed by specialist radiographers at the Golden Jubilee National Hospital, Clydebank, and interpreted by Dr Melanie Brewis, Scottish Pulmonary Vascular Unit. All scans were performed on a 1.5T MRI scanner (Sonata Magnetom, Siemens Healthcare, Germany) using an established protocol[191]. Fast cine imaging with steady state free precession (SSFP) sequences (TrueFISP Siemens) was used for functional imaging. All cine imaging was acquired during a 5-8 second breath hold and the total MRI scanning duration was approximately 45 minutes per scan.

Analysis of CMR images was performed using Argus software (Siemens Healthcare, Germany). RV and LV volumes were determined by planimetry, manually determining the endocardial and epicardial borders of each ventricle on each slice of the end diastolic and end systolic images. From this the software was able to calculate the RV and LV end diastolic and end systolic volumes. Stroke volumes for each ventricle were calculated from the difference between the respective ESV and EDV. Measurements could then be indexed to each patient's body surface area. RV ejection fraction (RVEF) was calculated as RV SV / RVEDV * 100. LV ejection fraction (LVEF) was calculated in a similar manner with corresponding LV measurements. Flow mapping of the main pulmonary artery and aorta was used to provide additional measures of SV from the PA and aorta, with and without phase correction. LV SV and aortic SV have previously been shown to reflect more accurately than the corresponding right sided measurements the SV measured invasively at RHC in patients with PAH[192].

3 Measurement of mixed venous oxygen saturation on exercise in pulmonary hypertension

3.1 Introduction

The reduced exercise capacity seen in PH is predominantly a consequence of abnormalities in oxygen transport and delivery[92] with a lower maximum stroke volume[93], accelerated heart rate response[95] and often a degree of anaemia[96] contributing. It has been postulated that abnormalities in the exercising peripheral muscle may also contribute to the reduction in exercise capacity.

Compared with control subjects the skeletal muscle of patients with PAH has been demonstrated to have lower capillarity and an impaired *in vitro* angiogenic response[108, 193]. This reduced muscle capillarity has been shown to correlate with exercise capacity in patients with PAH[108]. In addition to these changes in the muscle microvasculature patients with PH have an alteration in muscle fibre type with a relative decrease in type I and increase in type II fibres when compared with controls[107, 109], and abnormalities in mitochondrial morphology and number have also been demonstrated[109]. These structural changes are accompanied by functional abnormalities. An altered metabolic profile with differential enzyme expression favouring a more glycolytic metabolism has been demonstrated in patients with PAH[107] and molecular changes suggestive of a switch in signalling toward muscle proteolysis and away from hypertrophy have been identified[109]. Furthermore a reduction in skeletal muscle strength and endurance has been identified in these and other studies[107-109, 193, 194]. Similarly, in patients with chronic obstructive pulmonary disease, a respiratory disease where the primary abnormality is within the lungs, limb muscle atrophy and weakness are well characterised and are known to play a role in exercise limitation[195].

Patients with mitochondrial myopathies have been demonstrated to have reduced oxygen extraction on exercise, manifesting as abnormally high venous oxygen saturation[196, 197], lower peak systemic arteriovenous oxygen difference[198] and a smaller increase in deoxygenated haemoglobin and myoglobin during exercise[199]. Given the documented abnormalities of skeletal muscle in patients with PAH it is reasonable to consider whether these changes cause a similar impairment of oxygen extraction as that seen in patients with myopathies and in turn whether this limits exercise capacity in PAH. Evidence in support of this comes from a retrospective review of cardiopulmonary exercise tests carried out

in patients either undergoing assessment for heart or lung transplantation, or presenting with unexplained dyspnoea[110]. Each CPET was conducted with invasive monitoring in the form of radial and pulmonary arterial catheters. The study divided subjects into those with PAH, left ventricular systolic dysfunction (SD) and left ventricular diastolic dysfunction (DD) and found that those in the PAH group had reduced oxygen extraction compared with those in the DD and SD groups. However the PAH group included patients with normal resting pulmonary artery pressure who developed elevated PAP on exercise and therefore differs from the population of patients with resting PAH who suffer the greatest morbidity and mortality.

In this study we performed right heart catheterisation on exercise in patients with true resting PAH to evaluate the hypothesis that there is impairment of oxygen extraction in the peripheral muscles on exercise in this patient group.

3.2 Methods

3.2.1 Study subjects

Patients undergoing baseline diagnostic assessment (see Chapter 1.4) at the SPVU were invited to participate in this study. All subjects were treatment naïve and were undergoing routine right heart catheterisation as part of their diagnostic evaluation. All had performed a maximal CPET two days prior to the RHC. Patients were eligible to participate if they were able to give informed written consent, had no musculoskeletal or neurological impediment to exercise and had resting haemodynamic measurements at RHC compatible with a diagnosis of precapillary pulmonary hypertension by standard criteria as described in Chapter 1.4.5. All patients received a diagnosis of group 1 or group 4 PH. This study was descriptive, aiming to explore whether there appeared to be a limit to oxygen extraction by demonstrating the absolute level of mixed venous oxygen saturation on maximal exercise in this patient group and therefore no power calculation was employed.

3.2.2 Study design

Two days prior to exercise RHC patients all performed a maximal symptom-limited incremental CPET on an upright cycle ergometer as described in Chapter 2.6. Resting RHC was undertaken as described in Chapter 2.7.1 before proceeding to exercise RHC at a constant work rate of 50% the maximum work rate achieved in the preceding incremental CPET (see Chapter 2.7.2).

3.2.3 Ethical approval

The West of Scotland Research Ethics Committee (Glasgow, United Kingdom) approved the study. All subjects gave informed written consent.

3.3 Results

19 patients participated in the study of whom 16 produced acceptable results which were included in the analysis. Of the 3 patients excluded in the analysis, in one the pulmonary arterial catheter position was lost during exercise RHC and could not be repositioned within the pulmonary artery prior to cessation of exercise; one on review had performed a clearly submaximal incremental CPET and the workload selected for exercise RHC was therefore too low; and one patient was diagnosed at multidisciplinary team review with PH secondary to sarcoidosis and therefore was classified as having group 5 disease and thus no longer met the inclusion criteria. Baseline characteristics of the 16 included patients are given in table 3.1.

Baseline characteristics

Diagnosis (n)	
Group 1	9
IPAH	6
CTDPH	1
PPH	2
Group 4	7
Gender (n)	
Female	7
Male	9
Age (years)	53 (20)
WHO FC (n)	
II	9
III	7
6MWD (metres)	386 (103)
Workload for exercise RHC (W)	40 (13)

Table 3.1 Baseline characteristics. IPAH: idiopathic pulmonary arterial hypertension; CTDPH: connective tissue disease associated pulmonary hypertension; PPH: portopulmonary hypertension; WHO FC: functional class; 6MWD: 6 minute walk distance; RHC: right heart catheterisation. Data given as number (n) or median (interquartile range).

The resting and exercise RHC measurements are described for the whole group in table 3.2 and for individual patients in table 3.3. The relative change in haemodynamic measurements is demonstrated in figure 3.1. For almost all patients the PASP rose to a greater degree than the PADP, leading to an increase in pulmonary artery pulse pressure (PApp, the difference between PASP and PADP) on exercise. In only one patient did the PApp fall (patient 7 in table 3.3), a consequence of a more marked increase in PADP and a relatively more modest increase in PASP than seen in the other patients in the group.

Resting and exercise right heart catheterisation

	Resting	Exercise
PASP (mmHg)	80 (18)	114 (37)
PADP (mmHg)	32 (13)	41 (10)
mPAP (mmHg)	50 (14)	77 (20)
PAWP (mmHg)	6 (2)	10 (2)
CO (l/min)	3.9 (1.3)	5.7 (2.8)
PVR (Wood units)	11.1 (6.7)	10.8 (4.6)
HR (beats/min)	74 (21)	126 (17)
SV (ml)	46 (21)	49 (32)
SvO_2 (%)	62 (8)	22 (11)
pO_2 (kPa)	4.3 (0.3)	2.5 (0.5)

Table 3.2 Resting and exercise right heart catheterisation. PASP: pulmonary arterial systolic pressure; PADP: pulmonary artery diastolic pressure; mPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; CO: cardiac output; PVR: pulmonary vascular resistance; HR: heart rate; SV: stroke volume; SvO_2: mixed venous oxygen saturation; pO_2: partial pressure of oxygen in mixed venous blood.

Individual haemodynamic responses to exercise

Patient	Age	M/F	Diag.	Resting								Exercise							
				PASP	PADP	mPAP	PAWP	CO	PVR	HR	SV	PASP	PADP	mPAP	PAWP	CO	PVR	HR	SV
1	71	M	IPAH	79	34	50	2	3.8	12.6	94	40	**	**	**	**	4.8	**	120	40
2	43	M	CTEPH	58	12	29	5	4.4	5.5	58	76	**	**	**	**	11.3	**	135	84
3	29	F	IPAH	94	54	70	5	2.5	26	111	23	**	**	**	**	3.1	**	141	22
4	51	M	CTEPH	90	40	63	*	3.2	*	71	45	170	50	100	*	4.6	*	**	**
5	64	M	IPAH	80	29	47	5	3.9	10.8	74	53	145	47	86	22	6.1	12	128	48
6	55	F	CTEPH	101	38	59	7	3.5	14.9	79	44	178	58	100	10	4.1	22	**	**
7	71	M	CTEPH	66	13	51	7	4.5	5.3	62	73	83	41	63	*	7.6	*	75	101
8	45	F	IPAH	81	33	50	4	2.9	15.9	94	31	110	37	66	6	4.2	14.3	126	33

Table 3.3, continued overleaf

Individual haemodynamic responses to exercise

Patient	Age	M/F	Diag.	Resting								Exercise							
				PASP	PADP	mPAP	PAWP	CO	PVR	HR	SV	PASP	PADP	mPAP	PAWP	CO	PVR	HR	SV
9	48	M	CTEPH	74	30	47	6	3.2	12.8	69	46	113	40	70	9	5.0	12.3	141	35
10	60	F	CTDPH	80	23	43	3	4.3	9.3	74	58	114	32	68	3	6.0	10.8	120	50
11	76	M	CTEPH	74	20	40	6	4.9	6.9	87	56	102	38	66	8	7.5	7.7	125	60
12	39	M	PPH	92	34	55	6	6.4	7.7	83	77	137	45	84	10	9.8	7.6	137	72
13	47	F	PPH	58	23	36	6	2.7	11.1	66	41	98	28	60	9	5.3	9.7	126	42
14	66	M	CTEPH	77	34	55	7	3.2	13.1	73	44	108	52	77	11	6.3	10.6	120	53
15	56	F	IPAH	68	20	37	10	5.9	4.1	62	95	134	33	78	10	11.6	5.9	108	107
16	45	F	IPAH	129	44	75	9	4.2	15.2	108	39	155	47	86	14	4.8	15	140	34

Table 3.3 Individual haemodynamic responses to exercise. M: male, F: female; Diag: diagnosis; IPAH: idiopathic pulmonary arterial hypertension; CTEPH: chronic thromboembolic pulmonary hypertension; CTDPH: connective tissues disease associated pulmonary hypertension; PPH: portopulmonary hypertension; PASP: pulmonary artery systolic pressure (mmHg); PADP: pulmonary artery diastolic pressure (mmHg); mPAP: mean pulmonary artery pressure (mmHg); PAWP: pulmonary artery wedge pressure (mmHg); CO: cardiac output (l/min); PVR: pulmonary vascular resistance (Wood units); HR: heart rate (beats/min); SV: stroke volume (ml). * unable to obtain reliable pressure trace to measure PAWP accurately; ** unrecorded data: peak pressures not recorded for patients 1-3; HR (and therefore SV) not recorded for patients 4 and 6.

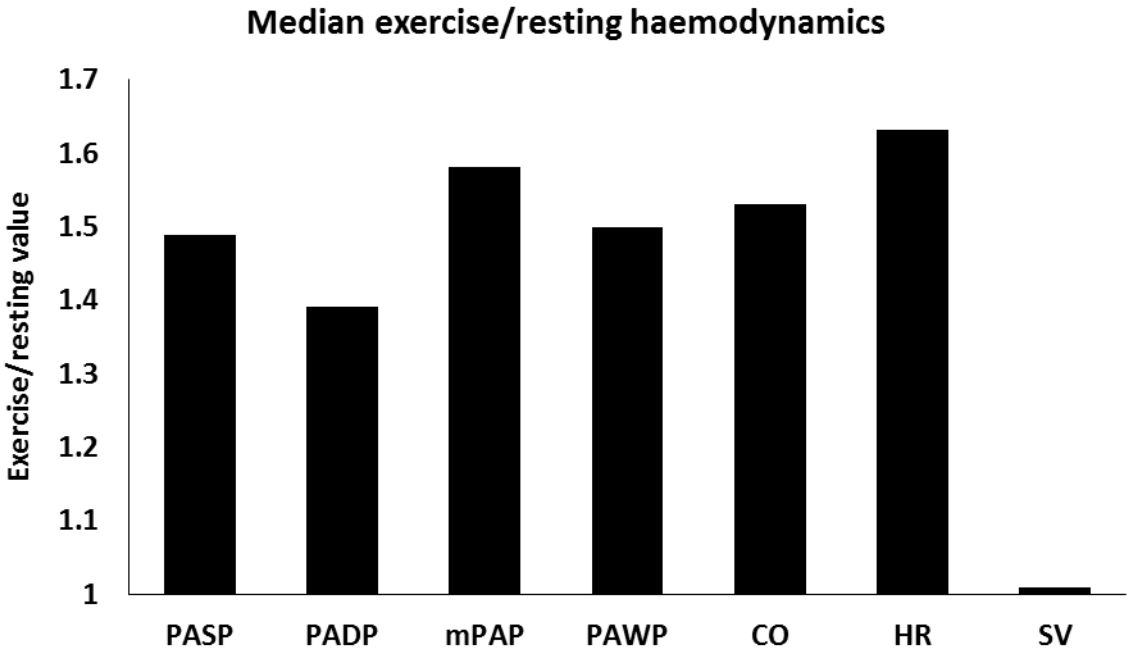


Figure 3.1 Peak exercise haemodynamics as a proportion of their resting values, expressed here as the median ratio of peak exercise to resting measurements for all patients. PASP: pulmonary arterial systolic pressure; PADP: pulmonary artery diastolic pressure; mPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; CO: cardiac output; PVR: pulmonary vascular resistance; HR: heart rate; SV: stroke volume.

Comparing the increase in mPAP with the increase in CO demonstrates a range of values as shown in table 3.4, giving a median mPAP-CO slope on exercise of 12 mmHg/l/min (IQR 9 mmHg/l/min).

The median mixed venous oxygen saturation (SvO_2) fell from 66% at rest to 22% at peak exercise. The measurements at rest and at peak exercise for each patient are shown in Figure 3.2. Patients with lower resting SvO_2 had generally lower peak exercise SvO_2 (Figure 3.3) although the reduction in SvO_2 on exercise was greater in patients with higher resting SvO_2 (Figure 3.4).

For patient 6 the blood gas analyser was unable to calculate the oxygen saturation from pO_2 using its standard equation due to the very low level achieved (pO_2 = 1.84 kPa) and therefore a separate equation[200] was used to estimate the SvO_2 for this patient to facilitate comparison across the patient group as 5 of the 16 patients had only the SvO_2 recorded and not the corresponding pO_2 value.

Relative increases of mPAP and CO on exercise

Patient	Δ mPAP	Δ CO	mPAP-CO slope
4	37	1.4	26.4
5	39	2.2	17.7
6	41	0.6	68.3
7	12	3.1	3.9
8	16	1.3	12.3
9	23	1.8	12.8
10	25	1.7	14.7
11	26	2.6	10.0
12	29	3.4	8.5
13	24	2.6	9.2
14	22	3.1	7.1
15	41	5.7	7.2
16	11	0.6	18.3

Table 3.4 Increases of mean pulmonary artery pressure and cardiac output on exercise. Δ mPAP: change in mean pulmonary artery pressure on exercise (mmHg); Δ CO: change in cardiac output on exercise (l/min); mPAP-CO slope: Δ mPAP / Δ CO (mmHg/l/min).

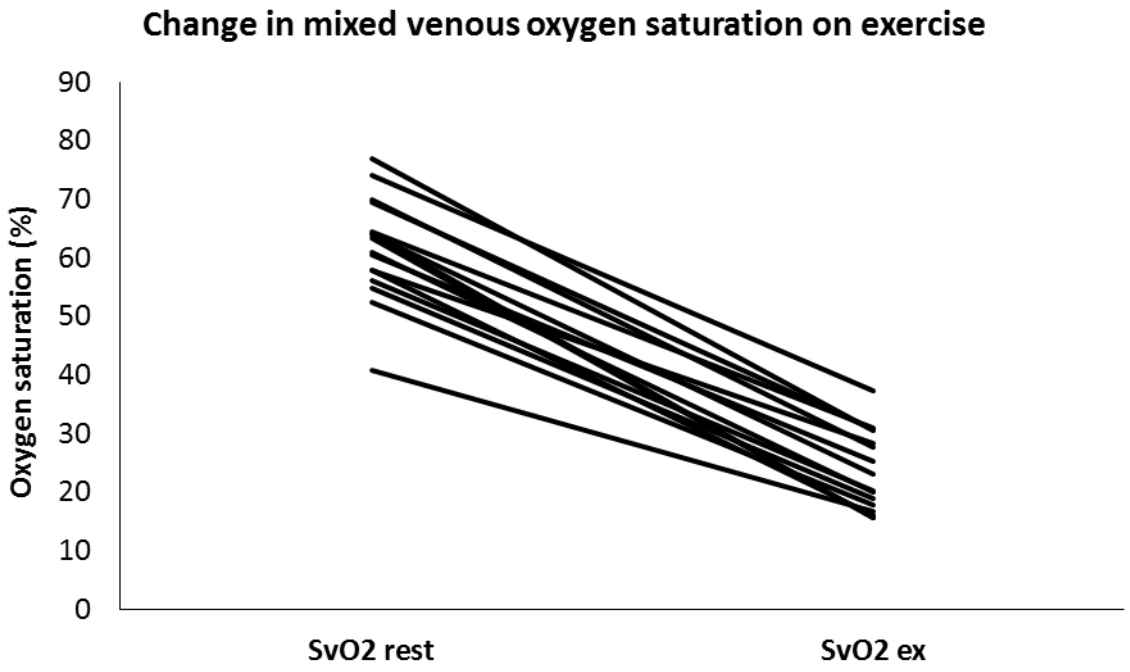


Figure 3.2 Mixed venous oxygen saturation at rest (SvO_2 rest) and peak exercise (SvO_2 ex) for each patient.

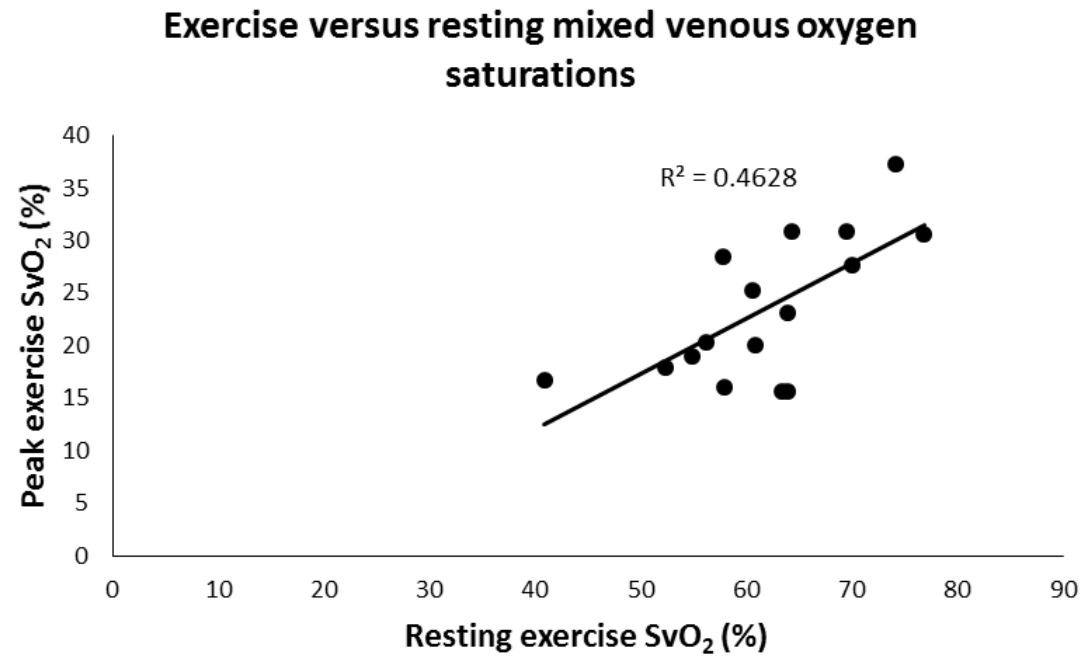


Figure 3.3 Peak exercise versus resting mixed venous oxygen saturations (SvO_2).

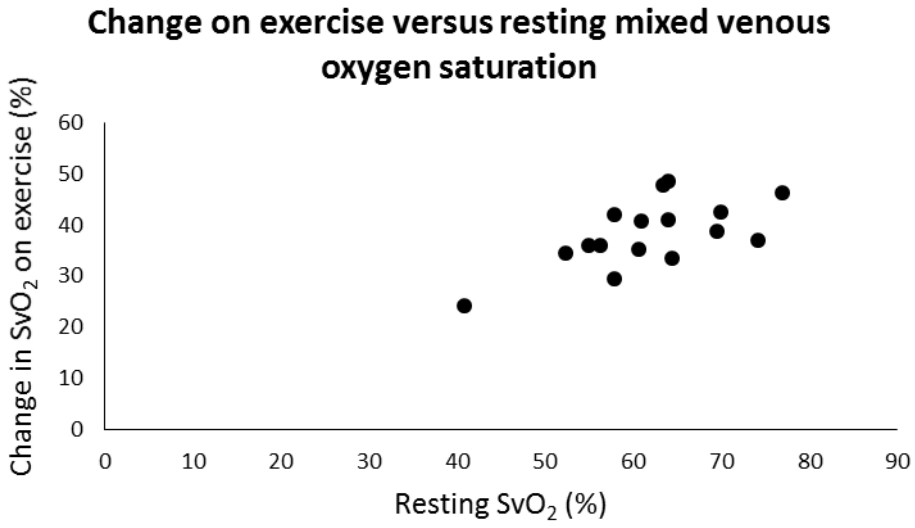


Figure 3.4 Change on mixed venous oxygen saturation (SvO_2) on exercise versus resting SvO_2. The change in SvO_2 is given as the absolute reduction in percentage saturation from rest to peak exercise.

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 at peak exercise. As no systemic arterial blood sampling was undertaken as part of this study the peripheral arterial

oxygen saturation as measured by pulse oximetry was used. The results are given in Table 3.5.

Oxygen extraction at peak exercise

Patient	SaO_2 (%)	SvO_2 (%)	$(SaO_2-SvO_2)/SaO_2$
4	79	19	0.76
5	98	16	0.84
6	93	17	0.82
7	88	23	0.74
8	99	25	0.74
9	95	20	0.79
10	79	31	0.61
11	*	31	*
12	96	37	0.61
13	*	28	*
14	79	16	0.80
15	*	31	*
16	96	20	0.79

Table 3.5 Oxygen extraction at peak exercise. Quantification of oxygen extraction at peak exercise taken as the difference between arterial oxygen saturation measured by pulse oximeter and mixed venous oxygen saturation measured by blood gas analysis, divided by the arterial oxygen saturation. SaO_2: arterial oxygen saturation; SvO_2: mixed venous oxygen saturation. * no peak exercise arterial oxygen saturation recorded.

3.4 Discussion

In this study the response to exercise in treatment naïve patients at the point of diagnosis with group I and IV pulmonary hypertension was studied at right heart catheterisation to determine the haemodynamic response profile and in particular to measure the mixed venous oxygen saturation at maximal exercise to investigate the hypothesis that there is impairment of peripheral muscle oxygen extraction in PH. All patients exhibited a rise in PA pressures and cardiac output with an essentially flat stroke volume response. The mPAP-CO slope was abnormal in all patients, reflecting the underlying pathophysiology. Mixed venous oxygen saturations fell markedly and suggest that there was no significant impairment of oxygen extraction limiting exercise capability in the studied patients.

3.4.1 The haemodynamic response

This study demonstrated a sharp increase in PA pressures on exercise with a more modest increase in CO. The relative increases in mPAP and CO are in keeping with data published in a study of similarly treatment naïve patients newly diagnosed with PAH or distal CTEPH also undergoing supine exercise[201] although the absolute rises in both measurements were less marked than seen in the results presented above. That study employed a stepwise incremental protocol and the median maximum work rate was lower at 30W compared to 40W which will have contributed in part to the lower absolute rises seen in their work however the patients appear to have had less severe disease with lower resting median mPAP (34 mmHg versus 50 mmHg), higher median CO (5.2 l/min versus 3.9 l/min, lower median resting PVR (4.6 Wood units versus 11.1 Wood units) and higher median 6MWD (445m versus 386m) than in the study presented in this chapter.

The steep mPAP-CO slope is a reflection of the abnormal nature of the pulmonary vasculature and its impaired ability to adapt to the stress of exercise. Several studies in healthy individuals have demonstrated the normal mPAP-CO slope to be less than 3 mmHg/l/min[202-205] and this value has been accepted as the upper limit of normal[111, 113]. Resistance is considered the ratio of driving

pressure to flow and therefore the standard equation for PVR describes it in terms of mPAP, PAWP and CO thus[111, 113]

$$PVR = \frac{(mPAP - PAWP)}{CO}$$

which can be rewritten for mPAP as

$$mPAP = PVR \cdot CO + PAWP$$

In healthy subjects PVR is seen to decrease on exercise and although the fall is smaller in supine exercise it still occurs in the normal pulmonary circulation[113, 202]. The reduction in PVR is primarily due to the distensibility of the pulmonary vasculature and *in vitro* modelling has demonstrated the profound difference in mPAP a small change in distensibility can cause at higher levels of cardiac output[113] such as might be seen with exercise. This study demonstrates that in a group of patients with precapillary pulmonary hypertension there is minimal change in PVR in response to exercise and this occurs presumably largely due to a pathological loss of pulmonary vascular distensibility although there may be a contribution from exercise induced pulmonary vasoconstriction occurring secondary to sympathetic nervous system activation and a lower oxygen saturation in the returning venous blood[206]. The inability to reduce sufficiently the PVR in response to an increasing CO on exercise is the cause of the steep mPAP-CO slope seen in this study and in other groups of patients with PH[115, 201, 207, 208].

In this study there was no significant change in stroke volume on exercise, adding to the evidence describing the stroke volume exercise response in patients with precapillary PH. There is a large body of evidence demonstrating that in healthy individuals stroke volume increases on exercise in the upright position[209] but the evidence is mixed when exercise is undertaken supine with studies variously describing an increased[210], decreased[211] or unchanged[212, 213] stroke volume on exercise. Previous imaging studies in PAH have demonstrated a stable or falling SV on exercise with one study showing all but one subject with IPAH failing to augment SV on exercise[93] and a separate study finding a fall in SV on exercise[116]. It is likely that the increased venous return at rest in the supine

compared with erect position due to elevation of the legs maximises right ventricular filling and, via the Frank-Starling mechanism, stroke volume. Given the RV is already under strain in PH due to its increased afterload it is likely that as mPAP increases rapidly on exercise the RV struggles to adapt and thus is unable to augment SV in the supine position.

3.4.2 Oxygen extraction on exercise

This study investigated the extent to which an impairment of peripheral muscle oxygen extraction might contribute to the exercise limitation in PH by measuring the mixed venous oxygen saturation at end exercise, demonstrating a median level of 22%. While those patients achieving the lowest exercise SvO_2 tended to have the lowest resting SvO_2, those patients with a higher resting SvO_2 demonstrated a greater absolute reduction in SvO_2 than those with lower resting SvO_2.

If there were a significant myopathic type component causing exercise limitation then it would be expected that due to the impairment of oxygen extraction the mixed venous oxygen level would be higher than in healthy individuals. There were no comparator subjects included in this study, either healthy individuals or patients with myopathy. While acknowledging the difficulties of comparing studies performed with differing exercise protocols it may nevertheless be helpful to put the results of this study into context by considering the oxygen levels seen in other states of health and disease at maximal exercise as shown in Figure 3.5. This would suggest that the current results are not in keeping with a myopathic picture and that in patients in this study oxygen was extracted down to a similar level to that seen in healthy individuals.

A further method of considering oxygen extraction is to use the systemic oxygen extraction ratio (SER).

$$SER = \frac{Ca - \tilde{v}O_2}{CaO_2}$$

where Ca - $\tilde{v}O_2$ is arteriovenous oxygen content difference and CaO_2 is arterial oxygen content.

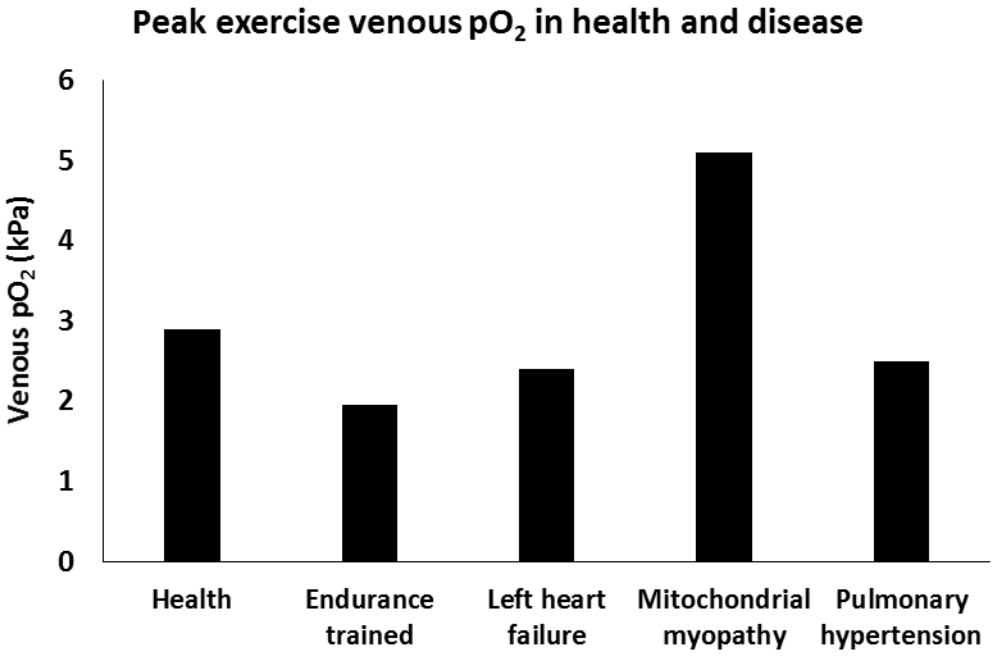


Figure 3.5 Peak exercise venous pO_2 levels from different studies in health and disease: healthy individuals[214]; after endurance training[215]; in left heart failure[216]; in mitochondrial myopathy[197] and patients from the current study with pulmonary hypertension.

Calculation of SER would have required arterial sampling which was not included as part of this study. As a surrogate measure however extraction was estimated as $(SaO_2\text{-}SvO_2)/SaO_2$. One study employing SER in healthy individuals undertaking leg exercise found a mean SER of approximately 0.7 in this group[214]. In comparison with that value for a normal SER the surrogate measure employed in this study and presented in Table 3.5 demonstrated a ratio of greater than 0.7 in all but 2 of the patients in whom it could be calculated.

Together with the comparatively low peak exercise mixed venous oxygen levels achieved on exercise, these two elements of the analysis do not support the hypothesis that there is a myopathic component to exercise limitation in PH. While the abnormalities seen in peripheral muscle are well established[107-109, 193, 194] they do not appear to have had an impact on oxygen extraction in the patient group studied. There is a level below which further oxygen extraction is not possible, occurring as a combination of a reducing diffusion gradient and mitochondrial limit to oxygen uptake and usage[217, 218] and the sigmoid shape of

the oxyhaemoglobin dissociation curve, with high affinity for oxygen at low saturations[94]. The results of this study would suggest that the exercising peripheral muscle is extracting oxygen to a level approaching this limit and that the primary limiting factor is therefore the amount of oxygen which is being delivered to the muscle, i.e. limitation is central rather than peripheral. It is reasonable to consider given the known muscle abnormalities that an increase in oxygen delivery to the exercising muscle might "unmask" an impairment of oxygen extraction but in this group of treatment naïve patients with true pulmonary arterial disease there was no evidence of abnormal extraction limiting exercise performance.

These results are in contrast to those seen in the study by Tolle *et al* which investigated a group of patients who developed PAH on exercise and found a reduction in SER[110]. The significance of patients with normal resting PA pressure who develop PH on exercise remains controversial[113, 219] and the current consensus view is that there is insufficient evidence to define clinically significant exercise induced PH[2]. It may be that the patients in that study had early disease and that perhaps the muscle abnormalities manifest earlier than the central limitation. It is possible that as the disease process advances the progressive pulmonary vascular remodelling and consequent rise in PA pressure and onset of RV failure come to predominate and by the time of diagnosis with resting PH the central limitation is such that the peripheral muscle abnormalities have a minimal influence. Differences in the exercise protocol may also have played a role while their methodology has also been questioned[220].

There are limitations to this study. Firstly, there was no comparator group. The inclusion of a control group of healthy individuals and a further group of patients with mitochondrial myopathy would have allowed a clear comparison between their results and the results of the patients with PH. Secondly, the study protocol involved sampling of venous blood from the main pulmonary artery and thus by the time it was sampled, blood returning from the exercising muscles would have mixed with venous blood returning from the rest of the body. In order to isolate the exercising muscle and sample blood from just proximal to the confluence of the left and right common iliac veins while also taking central haemodynamic measurements the insertion of a second venous catheter would

have been required. This was not felt to be appropriate. Sampling from the main PA will have led to a venous saturation higher than would have been recorded if sampling solely the blood draining from the exercising legs and therefore the outcome of the study was not affected. Finally, it would ideally have been feasible to take muscle biopsy samples at baseline to demonstrate that the muscle abnormalities documented by other groups were present in the group studied but that they did not affect oxygen extraction.

3.5 Conclusions

This study has shown no evidence that there is an impairment of oxygen extraction in patients with PH which contributes to exercise limitation. Indeed it appears that the exercising muscles are extracting oxygen to a similar level seen in healthy individuals. The study provides further evidence that the cause of exercise limitation in PH is a central impairment of oxygen delivery to the muscles rather than a primary myopathic impairment.

4 Oxygen uptake efficiency slope in pulmonary hypertension

4.1 Introduction

Interest in using CPET as part of the assessment and monitoring of patients with PH has increased in recent years[92, 101]. Maximal oxygen uptake is the gold standard measure of exercise performance with peak VO_2 used as a more practical surrogate[117, 178]. Peak VO_2 has been shown to predict survival in PAH[125, 126, 130] but requires patients to perform a maximal exercise test. It is therefore dependent on patient motivation[221] and although CPET has a good safety record in cardiopulmonary disease including PH[95, 118, 222], concerns have nevertheless been expressed regarding the performance of maximal exercise testing in patients with cardiopulmonary disease[92, 223]. Attention has therefore focused on measurements which can be made at submaximal levels of exercise. The most commonly studied of these in PH is the relationship between minute ventilation and CO_2 output with both the slope of that relationship over the course of the exercise test[100] and the value of the V_E/VCO_2 ratio at anaerobic threshold[129] being predictive of survival. However these variables are not measures of exercise performance.

Oxygen uptake efficiency slope (OUES) is an index derived from breath by breath values of ventilation (V_E) and oxygen uptake (VO_2) measured over the course of an incremental cardiopulmonary exercise test[187] (see Chapter 2.8.1). The OUES is recognised as describing the combined functional performance of the cardiovascular, pulmonary and peripheral skeletal muscle systems during exercise[221]. It was initially developed in a population of young healthy volunteers and patients with congenital or acquired cardiac disease (mean age 11.7 years, range 5.8 to 29 years)[187] and was notable for two key reasons. Firstly, OUES was found to correlate strongly with peak VO_2 and therefore it could be considered a surrogate measure of exercise performance. Secondly, the value of OUES determined from the first 90% of maximal exercise tests was not significantly different to the OUES value calculated from the full exercise tests. Although the value of OUES calculated from the first 75% of tests was on average 3.5% lower, OUES determined from both 90% and 75% of tests retained a strong correlation with peak VO_2. It was therefore suggested that OUES could be used to provide a measure of exercise performance from submaximal exercise. This finding was replicated in a group of 998 healthy adults in a study which also demonstrated less variability in OUES on repeat testing than was seen with peak VO_2[221]. A

correlation between OUES calculated from 100% of test data and from tests truncated on the basis of respiratory exchange ratio rather than exercise time in a further group of healthy volunteers was also shown, particularly at higher exercise levels[223].

The role of OUES in the assessment of patients with cardiac disease has been investigated in several studies. It has been shown to be correlated with exercise capacity in older patients with ischaemic heart disease[224], to be reduced in patients with left heart failure both with and without a reduced left ventricular ejection fraction[225], and to correlate significantly with peak VO_2 in a similar group of patients with cardiac disease regardless of the presence or absence of left ventricular impairment[226]. That study also demonstrated stability of OUES measured across the exercise period. A separate study demonstrated that in patients with clinical evidence of heart failure peak VO_2 was significantly affected by changes in lung function whereas OUES was not, suggesting that perhaps OUES was isolating the cardiac component of exercise limitation[227].

OUES has also been shown to predict outcome in cardiac disease[228]. A study of cardiopulmonary exercise testing in patients with chronic heart failure demonstrated that not only did OUES predict survival on univariate analysis alongside peak VO_2, V_E/VCO_2 slope and ventilatory anaerobic threshold but also it was the only exercise variable predictive of survival on multivariate analysis[229]. That study also demonstrated little change in OUES when it was calculated from only the first 50% of the exercise tests. A further study investigating the effect of both OUES and percentage predicted OUES in comparison to other exercise derived variables on survival in patients with left ventricular failure found that both expressions of OUES were retained in final multivariate models although V_E/VCO_2 slope was the strongest predictor[230].

The studies described in this chapter therefore assessed the potential role of OUES in PH, both in terms of assessment of function and as a predictor of survival, to investigate the hypotheses that OUES is strongly correlated with peak VO_2 in PH, that it is valid as a submaximal measure of exercise performance in groups I and IV PH and that it predicts survival in patients within these disease groups.

4.2 Methods

4.2.1 Comparison of OUES and peak VO_2

To assess the validity of using OUES as a measure of peak exercise performance the slope values were compared to the measured peak VO_2 for a series of CPETs. Only CPETs meeting recognised criteria for a maximal test were included[178]. All patients were treatment naïve at the time of the test and all CPETs were conducted within 48 hours of confirmation of pulmonary hypertension at right heart catheterisation (Chapter 2.7.1). Patients diagnosed in accordance with international guidelines[2, 4] with idiopathic pulmonary arterial hypertension (IPAH), connective tissue disease associated pulmonary hypertension (CTDPH), chronic thromboembolic pulmonary hypertension (CTEPH) and pulmonary venous hypertension (PVH) were included.

The value of the OUES was calculated for each subject as described in Chapter 2.8.1 and plotted against peak VO_2. Linear regression analysis was performed to describe the relationship between peak VO_2 and OUES for each group and tested for a statistically significant difference (IBM SPSS Statistics, International Business Machines Corp, Armok, New York, USA).

4.2.2 Calculation and analysis of submaximal oxygen uptake efficiency slope

To assess the validity of OUES as a submaximal measure of exercise performance, data from maximal incremental cardiopulmonary exercise tests was analysed from patients diagnosed with group I and group IV pulmonary hypertension, to calculate OUES as described in Chapter 2.8.2. The breath by breath exercise data was then truncated at 90%, 75% and 50% of the incremental exercise time and OUES calculated from the onset of loaded cycling to each truncated time point. The slope values obtained at each level were plotted against OUES calculated from the corresponding full test and the strength of the relationship between these was assessed by Pearson correlation (IBM SPSS Statistics, International Business Machines Corp, Armok, New York, USA).

4.2.3 Oxygen uptake efficiency survival analysis

Data from all available cardiopulmonary exercise tests performed on treatment naïve patients at the time of initial diagnosis with group I and group IV PH and who were subsequently commenced on specific pulmonary vasodilator therapy was collected and used to calculate the OUES for each patient. Survival analysis was then undertaken as outlined in Chapter 2.10.

For some of the older CPETs the original data tables were not available and therefore the breath by breath data for oxygen uptake and minute ventilation could not be extracted to calculate OUES directly. The original plots of VO_2 and V_E versus time were however available and graph digitising software was used to convert the plots back into numerical data to be used to calculate the OUES as described in Chapter 2.8.5.

4.2.4 Ethical approval

The studies described in this chapter were discussed with the West of Scotland Research Ethics Service who felt that as the core data was collected routinely as part of clinical practice in SPVU and that the data was fully anonymised prior to analysis, no research ethics approval was required.

4.3 Results

4.3.1 Oxygen uptake efficiency slope as a measure of peak exercise performance in pulmonary hypertension

59 patients with groups I, II and IV PH had performed maximal CPETs and fulfilled the criteria described in Chapter 4.3.1. Their tests were therefore used in the assessment of OUES as a measure of peak exercise performance in PH. The group's baseline demographics, haemodynamic results and peak VO_2 and OUES are described in Table 4.1. Regression analyses for the prediction of peak VO_2 from OUES for each PH diagnosis group are shown in Figure 4.1 with R squared values given in Table 4.2. In addition to the OUES derived from maximal tests being a strong predictor of peak VO_2 in each group of PH studied, the relationship between OUES and peak VO_2 was not significantly affected by the diagnosis group (p = 0.13).

Baseline characteristics

	IPAH	CTDPH	CTEPH	PVH
Gender (n)				
Male	9	3	19	4
Female	13	4	1	6
Age (years)	54 (23)	54 (16)	63 (13)	71 (13)
WHO FC (n)				
II	13	5	11	3
III	8	2	9	7
IV	1	0	0	0
6MWD (metres)	356 (79)	300 (87)	410 (122)	346 (134)
mPAP (mmHg)	50 (17)	41 (6)	39 (11)	34 (12)
PAWP (mmHg)	7 (5)	6 (6)	8 (4)	21 (7)
CO (l/min)	3.7 (2.2)	4.3 (2.1)	4.3 (1.5)	5.2 (2.6)
PVR (Wood units)	10.0 (10.0)	8.3 (4.1)	6.9 (3.9)	3.3 (2.3)
Peak VO_2 (l/min)	0.85 (0.32)	0.57 (0.30)	1.07 (0.40)	0.84 (0.52)
OUES	0.88 (0.54)	0.66 (0.29)	1.23 (0.40)	1.06 (0.88)

Table 4.1 Baseline characteristics. IPAH: idiopathic pulmonary arterial hypertension; CTDPH: connective tissue disease associated pulmonary hypertension; CTEPH: chronic thromboembolic pulmonary hypertension; PVH: pulmonary venous hypertension; WHO FC: functional class; 6MWD: 6 minute walk distance; mPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; CO: cardiac output; PVR: pulmonary vascular resistance; VO_2: oxygen uptake; OUES: oxygen uptake efficiency slope. Data given as number (n) or median (range).

OUES as a predictor of peak VO_2 for different groups of PH

PH group	R squared value	*p* value
IPAH	0.750	<0.001
CTDPH	0.613	0.037
CTEPH	0.887	<0.001
PVH	0.945	<0.001
Whole group	0.873	<0.001

Table 4.2 Oxygen uptake efficiency slope (OUES) as a predictor of peak oxygen uptake (VO_2) for different groups of PH. PH: pulmonary hypertension; IPAH: idiopathic pulmonary arterial hypertension; CTDPH: connective tissue disease associated pulmonary hypertension; CTEPH: chronic thromboembolic pulmonary hypertension; PVH: pulmonary venous hypertension.

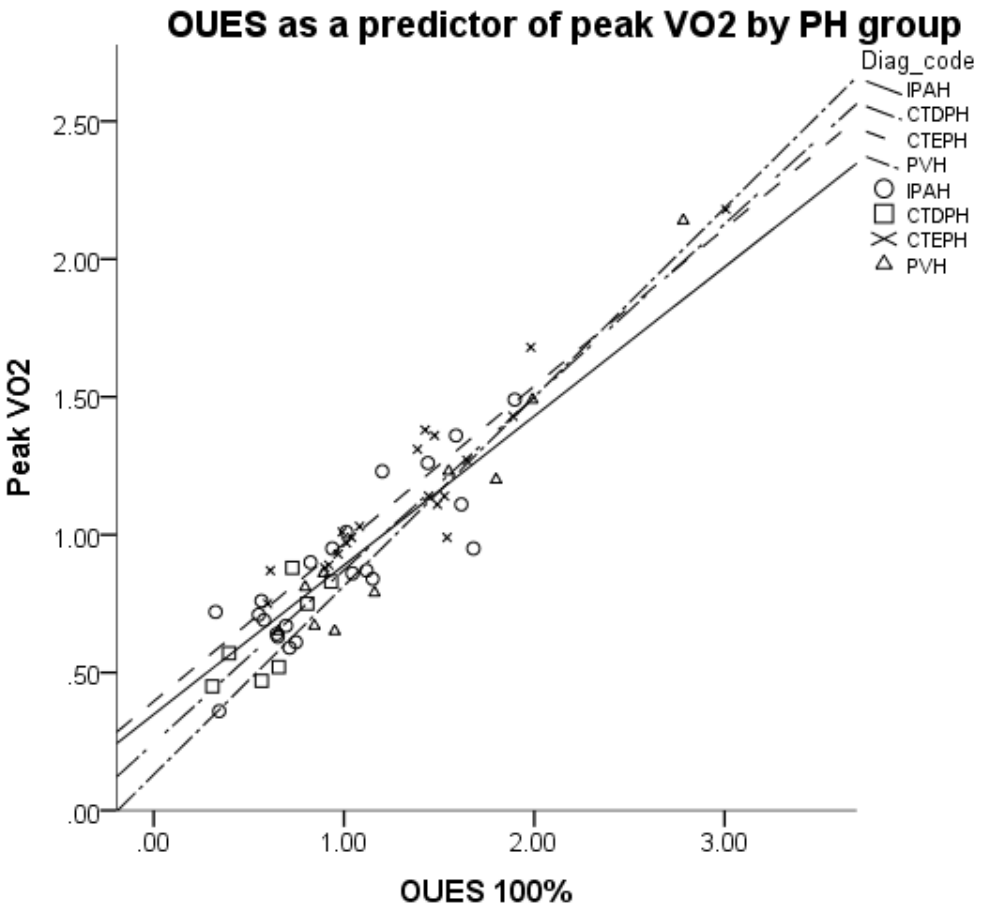
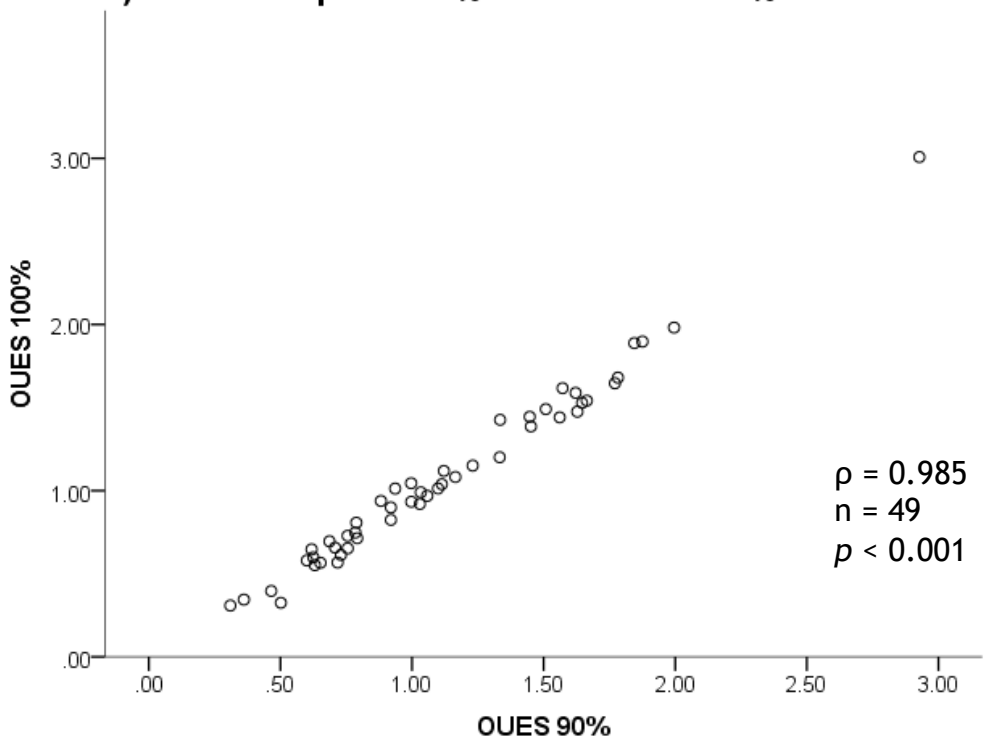


Figure 4.1 Relationship between oxygen uptake efficiency slope (OUES) derived from complete maximal incremental cardiopulmonary exercise tests and peak oxygen uptake (VO_2). Solid and broken lines are regression lines for each diagnostic group. PH: pulmonary hypertension; Diag_code: diagnosis group; IPAH: idiopathic pulmonary arterial hypertension; CTDPH: connective tissue disease associated pulmonary hypertension; CTEPH: chronic thromboembolic pulmonary hypertension; PVH: pulmonary venous hypertension.

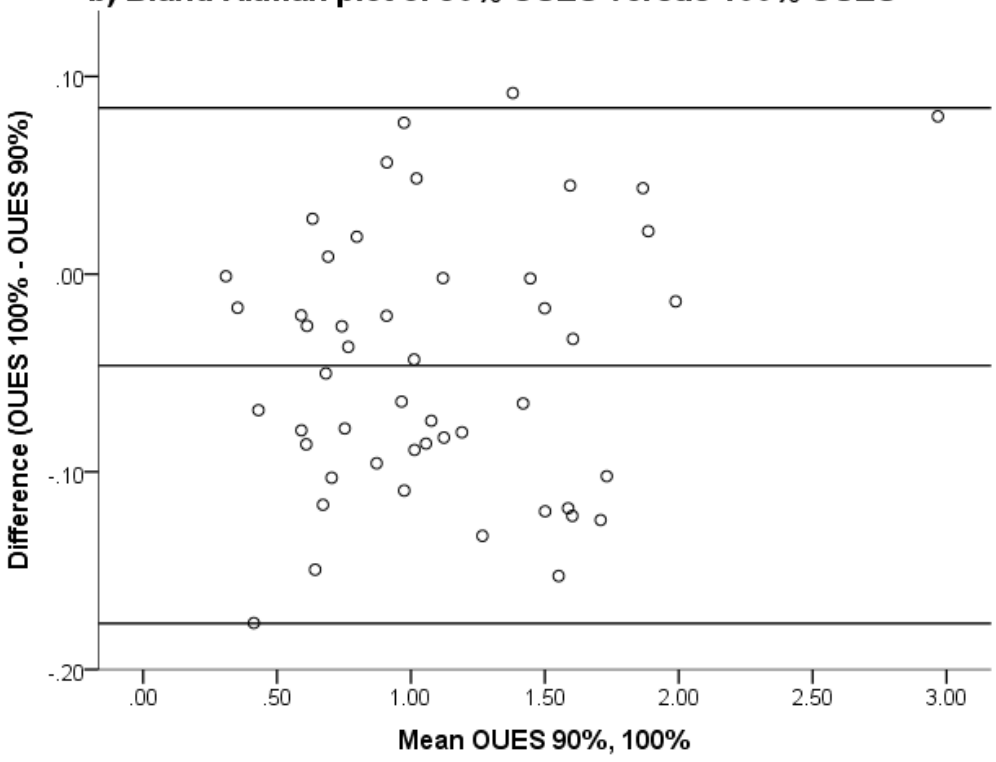
4.3.2 Oxygen uptake efficiency slope as a submaximal measure of exercise performance in pulmonary hypertension

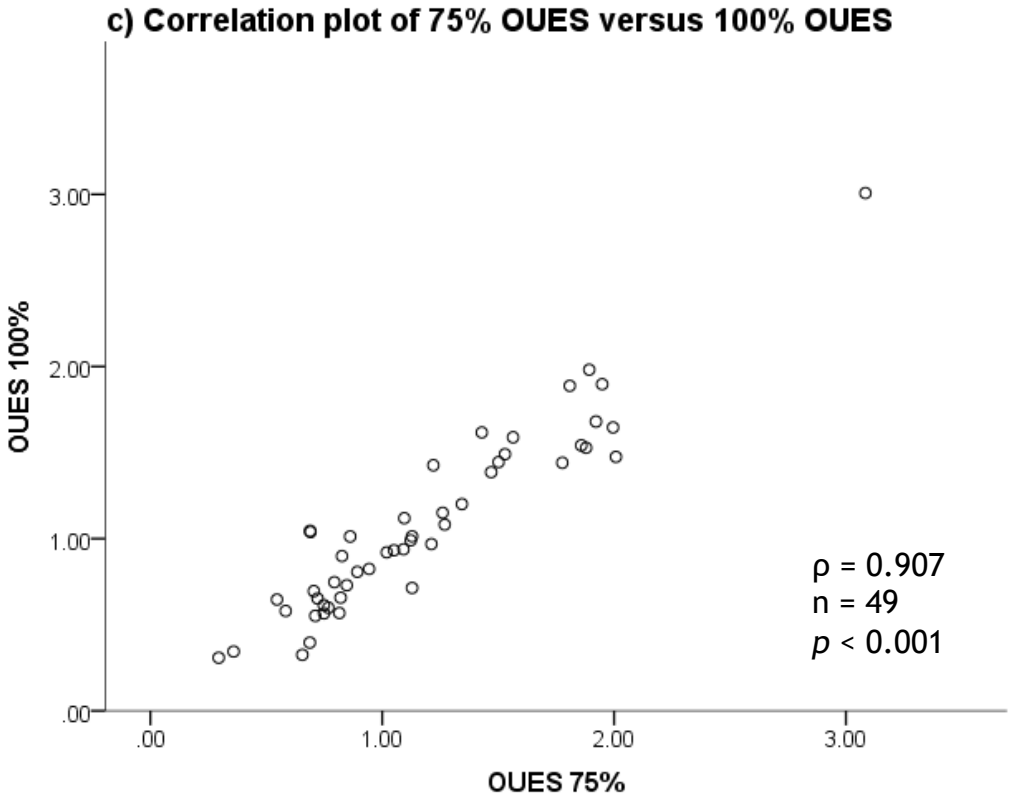
CPETs for the patient group described in Chapter 4.4.1, excluding those for patients with a diagnosis of PVH, were used to assess the use of OUES as a submaximal measure of exercise performance in PH. The correlations between OUES calculated from each truncated exercise test level and OUES calculated from the full test data are demonstrated in Figure 4.2 together with Bland-Altman analyses of agreement.

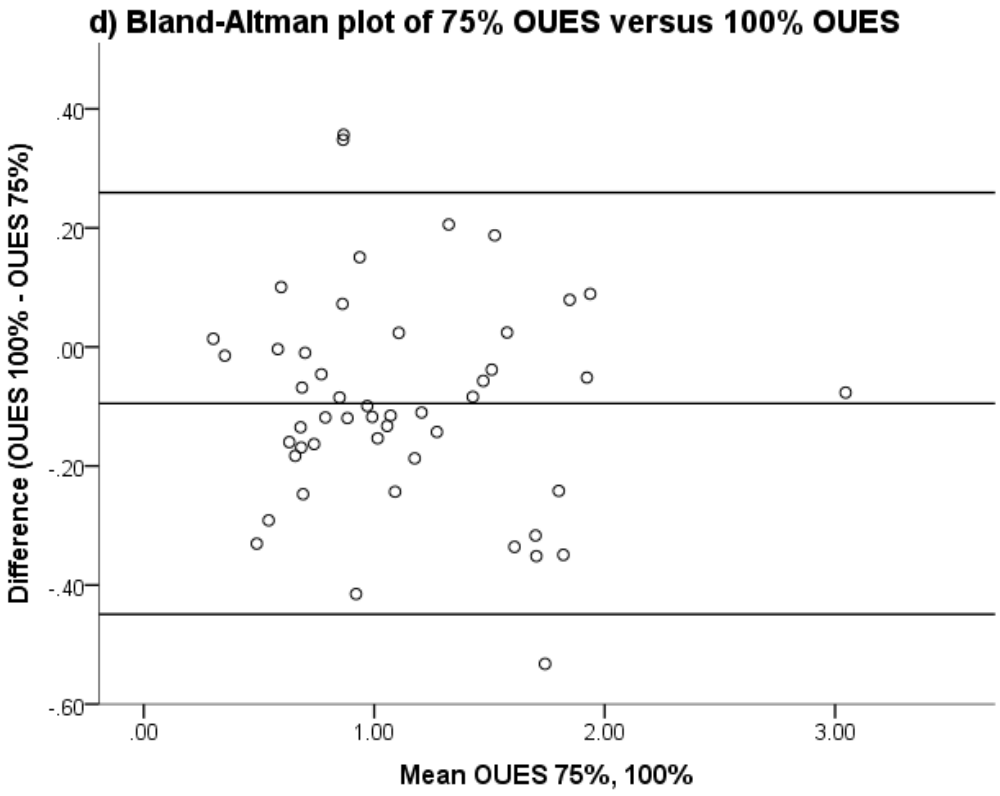
a) Correlation plot of 90% OUES versus 100% OUES

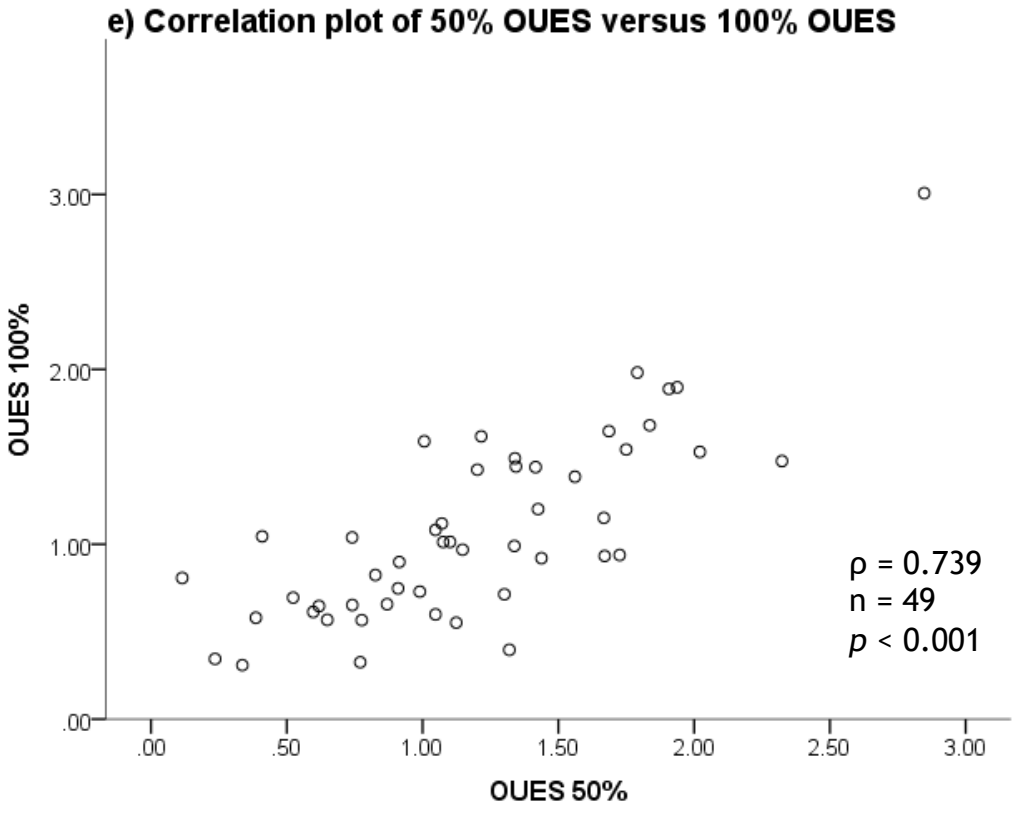


b) Bland-Altman plot of 90% OUES versus 100% OUES




c) Correlation plot of 75% OUES versus 100% OUES
OUES 100%
3.00
2.00
1.00
.00
.00
1.00
2.00
3.00
OUES 75%
ρ = 0.907
n = 49
p < 0.001


d) Bland-Altman plot of 75% OUES versus 100% OUES
Difference (OUES 100% - OUES 75%)
.40
.20
.00
-.20
-.40
-.60
.00
1.00
2.00
3.00
Mean OUES 75%, 100%



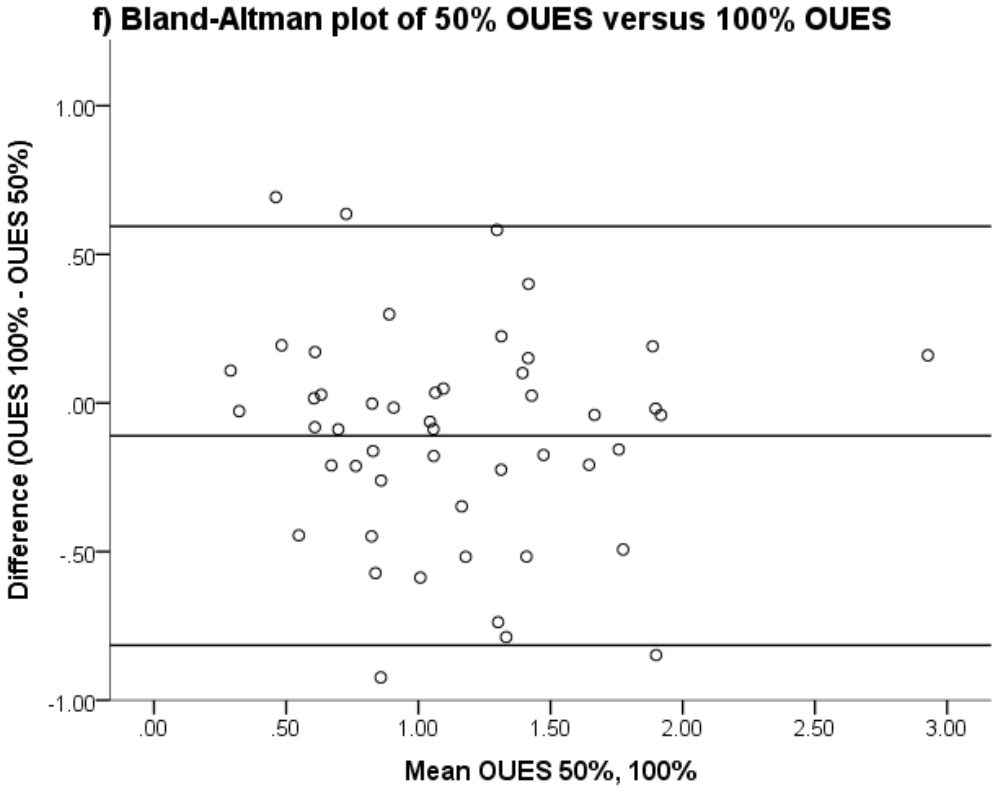


Figure 4.2 Correlation and Bland-Altman plots for OUES calculated from 100% of maximal cardiopulmonary exercise tests and OUES calculated from a), b): 90%; c), d): 75%; and e), f): 50% of test data. OUES: oxygen uptake efficiency slope, r: Pearson correlation coefficient.

The ability of OUES calculated from each truncated test level to predict peak VO_2 and therefore exercise capacity was assessed by linear regression and the results presented in Table 4.3. There was a strong predictive relationship for OUES calculated from the first 90% and first 75% of test data (R^2 = 0.845 and 0.756 respectively) but this was weaker when OUES was calculated from the first 50% of test data (R^2 = 0.551).

OUES from submaximal exercise levels as a predictor of peak VO_2

OUES exercise level	R squared value	*p* value
90%	0.845	<0.001
75%	0.756	<0.001
50%	0.551	<0.001

Table 4.3 Oxygen uptake efficiency slope (OUES) calculated from maximal cardiopulmonary exercise tests truncated to the first 90%, 75% and 50% of test data as a predictor of peak oxygen uptake (VO_2).

4.3.3 Oxygen uptake efficiency slope as a predictor of survival

Data from 108 patients with group I and group IV PH was used for the assessment of OUES as a predictor of survival (4 with PAH secondary to congenital heart disease, 20 with CTDPH, 4 with familial PAH, 1 with HIV associated PAH, 37 with IPAH, 5 with portopulmonary hypertension and 37 with CTEPH). Their baseline characteristics are given in Table 4.4. The median duration of follow up was 580 days with a range from 13 to 5942 days. During the follow up period there were 33 deaths from all causes, 0 patients received a lung transplant, 17 patients underwent pulmonary endarterectomy and 3 patients were lost to follow up.

OUES correlated weakly with other measures of disease severity, namely with RAP (ρ = -0.234, *p* = 0.015), CO (ρ = 0.512, *p* = <0.001), SvO_2 (ρ = 0.47, *p* = <0.001), 6MWD (ρ = 0.263, *p* = 0.006) and log NTproBNP (ρ = -0.437, *p* = <0.001). It also correlated weakly with age (ρ = -0.192, *p* = 0.047).

Baseline characteristics

Gender (n)	
Male	55
Female	53
Age (years)	56 (24)
WHO FC (n)*	
I	1
II	44
III	60
IV	2
6MWD (metres)	332 (101)
mPAP (mmHg)	46 (14)
PAWP (mmHg)	8 (5)
CO (l/min)	4.2 (1.8)
PVR (Wood units)	8.4 (6.3)
SvO_2 (%)	64 (13)
DLCO (% predicted)	57 (27)
NTproBNP	807 (2146)
Peak VO_2 (l/min)	0.87 (0.46)
OUES	0.99 (0.85)

Table 4.4 Baseline characteristics. WHO FC: functional class; 6MWD: 6 minute walk distance; mPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; CO: cardiac output; PVR: pulmonary vascular resistance; SvO_2: mixed venous oxygen saturation; DLCO: diffusing capacity of the lungs for carbon monoxide; NTproBNP: N-terminal pro-brain natriuretic peptide; VO_2: oxygen uptake; OUES: oxygen uptake efficiency slope. * WHO FC not recorded for one patient.

4.3.3.1 Oxygen uptake efficiency slope Cox proportional hazards analysis

On Cox proportional hazards analysis OUES, peak VO_2, age, percent predicted diffusing capacity of the lungs for carbon monoxide (DLCO) and V_E/VCO_2 at anaerobic threshold were all demonstrated to predict all cause mortality (Table 4.5).

Univariate Cox proportional hazards analysis

Variable	Hazard ratio	*p* value
OUES	0.401 (0.179 – 0.897)	0.026
Peak VO_2	0.259 (0.08 – 0.834)	0.024
Age	1.047 (1.021 – 1.073)	<0.001
DLCO % predicted	0.967 (0.946 – 0.988)	0.002
V_E/VCO_2 at AT	1.036 (1.003 – 1.070)	0.032
Gender		
Male	0.696 (0.348 – 1.393)	0.306
Female (reference)	--	--
WHO FC		
I/II	0.438 (0.057 – 3.378)	0.428
III/IV (reference)	--	
RAP	1.031 (0.96 – 1.106)	0.401
mPAP	0.992 (0.967 – 1.017)	0.532
CO	0.810 (0.606 – 1.081)	0.152
PVR	0.994 (0.933 – 1.059)	0.857
SvO_2	0.972 (0.939 – 1.007)	0.115
6MWD	0.997 (0.992 – 1.002)	0.205
logNTproBNP	2.168 (0.957 – 4.915)	0.064

Table 4.5 Univariate Cox proportional hazards analysis for prediction of all cause mortality. Hazard ratios expressed as hazard ratio (95% confidence interval). OUES: oxygen uptake efficiency slope; VO_2: oxygen uptake; DLCO: diffusing capacity of the lungs for carbon monoxide; V_E/VCO_2 at AT: ventilatory equivalent for carbon dioxide at anaerobic threshold; WHO FC: functional class; RAP: right atrial pressure; mPAP: mean pulmonary artery pressure; CO: cardiac output; PVR: pulmonary vascular resistance; SvO_2: mixed venous oxygen saturation; 6MWD: six minute walk distance; logNTproBNP: log transformed N-terminal pro-brain natriuretic peptide.

On multivariate analysis age was the sole remaining covariate in a model including OUES and percent predicted DLCO. Only three covariates were included due to the number of events occurring (30) to avoid overfitting (Table 4.6).

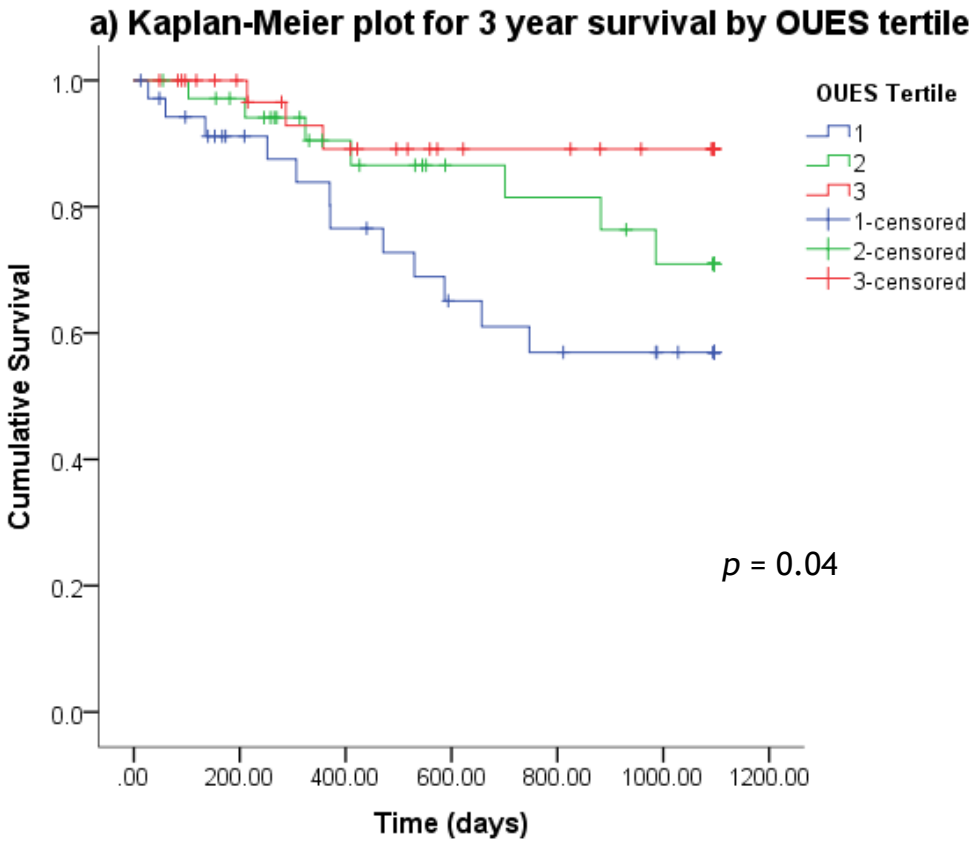
Multivariate Cox proportional hazards analysis

Variable	Hazard ratio	*p* value
Age	1.040 (1.014 – 1.067)	0.002
OUES	0.513 (0.222 – 1.184)	0.118
DLCO % predicted	0.999 (0.998 – 1.001)	0.331

Table 4.6 Multivariate Cox proportional hazards analysis for prediction of all cause mortality. Hazard ratios expressed as hazard ratio (95% confidence interval). OUES: oxygen uptake efficiency slope; DLCO: diffusing capacity of the lungs for carbon monoxide.

4.3.3.2 Survival by oxygen uptake efficiency slope

Subjects were stratified by OUES tertiles. Kaplan-Meier analysis was undertaken and the results for 3 and 4 year survival are presented in Figure 4.3



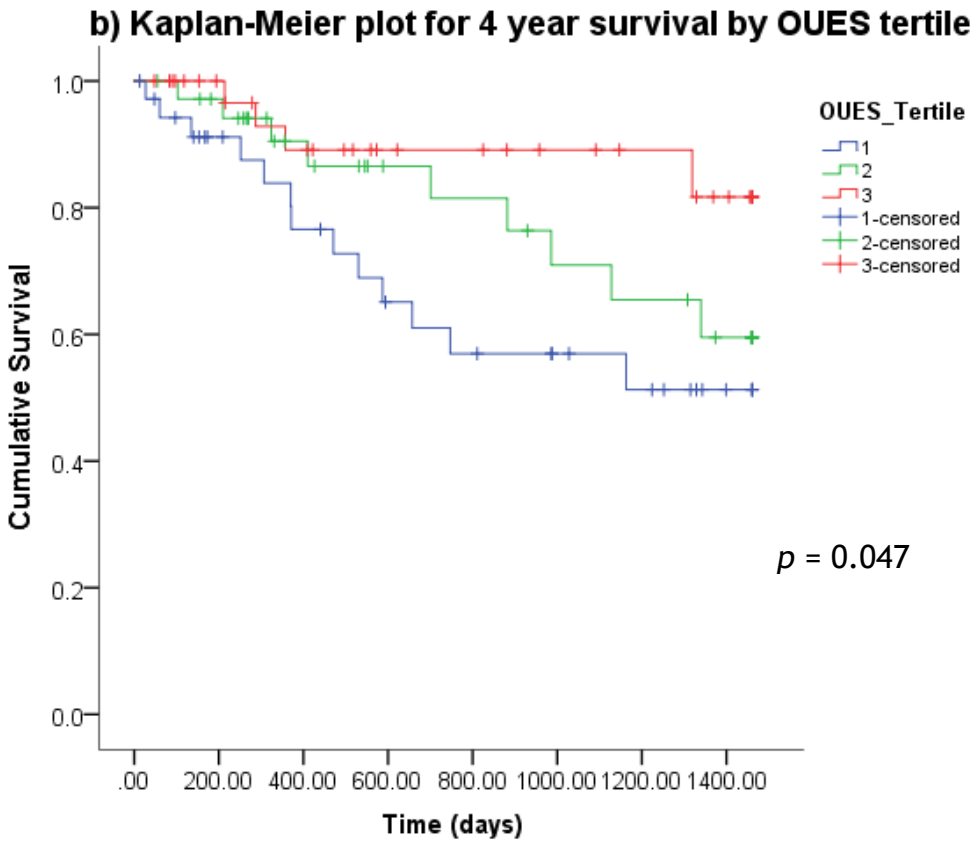


Figure 4.3 Kaplan-Meier survival plots for a) 3 year and b) 4 year survival by OUES tertile in the study cohort. Tertile 1 represents the subjects with the lowest tertile values and tertile 3 the highest tertile values for OUES.

4.4 Discussion

The potential role of the oxygen uptake efficiency slope in the assessment of patients with precapillary pulmonary hypertension was investigated in this series of studies. OUES was shown to be a valid measure of exercise performance in patients with PH through its strong ability to predict peak VO_2. The relationship between OUES and peak VO_2 was the same across different groups of PH and in precapillary PH OUES calculated from submaximal levels of exercise was shown to predict peak VO_2, thus demonstrating that it may be considered a submaximal measure of exercise performance in this patient group. OUES at diagnosis was also shown to predict all cause mortality in patients with precapillary PH.

Taken together these results suggest that OUES has a role in the assessment of patients with PH, both as a submaximal measure of exercise performance and as a predictor of survival.

4.4.1 Oxygen uptake efficiency slope and peak exercise performance

The potential role of OUES as a measure of exercise performance stems from its ability to predict peak VO_2. The study results presented in Chapter 4.4.1 demonstrates that this relationship is maintained in patients with PH and therefore OUES can be considered a surrogate measure of exercise performance in such patients. OUES was a strong predictor of peak VO_2 in subjects with IPAH, CTEPH and PVH (R squared values of 0.75, 0.887 and 0.945 respectively) and although the strength of prediction seen was lower in patients with CTDPH (R squared value of 0.613) this is likely to reflect the small number of patients in this group (7 patients versus 22, 20 and 10 patients for the IPAH, CTEPH and PVH groups respectively).

On exercise the minute ventilation (V_E) for a given VCO_2 is dependent on the arterial partial pressure of CO_2 ($PaCO_2$) and the physiological dead space fraction (V_D/V_T) as follows

$$\frac{V_E}{V_{CO_2}} = \frac{1}{PaCO_2\left(1 - \frac{V_D}{V_T}\right)}$$

Patients with CTEPH have been shown to have a steeper V_E/VCO_2 slope than patients with IPAH, driven by an increase in physiological dead space[103]. Given this increased ventilatory inefficiency or "excess exercise ventilation"[102] in CTEPH compared with IPAH it might have been expected that the OUES, as the relationship between VO_2 and log transformed minute ventilation on exercise, would have been lower for a given VO_2 in the CTEPH group compared with IPAH however no significant difference was seen. One possible explanation for this is that the number of patients in each group was too small to detect a difference and this could be explored by repeating the study in a larger cohort of patients, ideally alongside a control group of healthy individuals.

The potential advantage of OUES over peak VO_2 as a measure of exercise performance is that studies in healthy individuals and in patients with cardiac disease have shown that it can be calculated from submaximal levels of exercise[187, 221, 223, 226, 231, 232] and therefore can be used as a submaximal measure

of exercise performance in such subjects. This study is the first to investigate this key aspect of the OUES in pulmonary hypertension. The results presented in Chapter 4.4.2 demonstrate that OUES calculated from the first 90% and first 75% of maximal cardiopulmonary exercise tests was strongly correlated with OUES calculated from the full test data, with strong agreement seen on Bland-Altman analyses. There remained a statistically significant correlation between OUES calculated from the first 50% of test data and the full test OUES but the strength of this correlation was weaker than that seen at the higher exercise test levels. Similarly, OUES measured at the 75% and 90% levels was a strong predictor of peak VO_2 while OUES calculated from the 50% level was weaker. It is therefore appropriate to consider OUES valid as a submaximal measure of exercise performance in precapillary PH, at least as far as the 75% level.

One weakness of this study is that it used only exercise time as a percentage of the maximal test to delineate the different submaximal exercise levels. Limited work has been carried out in healthy individuals using respiratory exchange ratio as a secondary criterion in the reporting of OUES[223]. Further work should explore the potential of RER and other markers of exercise intensity to characterise better the level of submaximal exercise beyond which OUES can be considered a valid measure of exercise performance.

4.4.2 Oxygen uptake efficiency slope and survival

The results presented in Chapter 4.4.3 demonstrate that in the studied cohort of patients OUES was significant predictor of all cause mortality both on univariate Cox proportional hazards analysis and on Kaplan-Meier analyses for three and four year survival. OUES did not remain in the model when multivariate analyses including age were conducted. Indeed, age was the only remaining variable in the models. One previous study in healthy individuals showed age, sex and body surface area to be statistically significant predictors of OUES and equations taking account of these factors were developed to give reference values for OUES[233], raising the possibility of using percent predicted OUES as a candidate variable. However the subjects in that study were aged 20-60 years and given the median age of patients included in the presented survival analysis was 56 years, extending the reference values to the older patient group studied was not felt to be appropriate. One study which developed reference equations for older

healthy individuals used treadmill rather than cycle ergometer exercise[221] and therefore could not be extrapolated to the current patient cohort. A subsequent study has also investigated the impact of OUES on survival in a group of patients with idiopathic and associated PAH[234]. That work showed that OUES was a significant predictor of poor outcome in the form of death or atrial septostomy on multivariate analysis. However, that model did not include age and the age of the patient cohort was not stated.

Interestingly, some variables generally accepted as predictors of survival in PH were not seen to be significant predictors in this patient cohort and this could be considered a weakness of this study. RAP, CO, SvO_2 and logNTproBNP were found to be non-significant. However other established predictors, namely peak VO_2, percent predicted DLCO and V_E/VCO_2 at anaerobic threshold, were shown to be statistically significant predictors of survival in the study and this provides reassurance that the significant results for OUES could be replicated in other patient cohorts. Investigation of that is a clear next step in the further evaluation of OUES in PH.

4.5 Conclusions

The presented studies are the first to demonstrate that OUES is valid as a submaximal measure of exercise performance in precapillary pulmonary hypertension. Furthermore they have demonstrated that OUES is a significant predictor of survival in this patient group. Take together these results suggest that OUES offers potential benefits over the gold standard peak VO_2 and these should be explored in future work.

5 Rates of recovery of heart rate and oxygen consumption after incremental exercise and survival in pulmonary hypertension

5.1 Introduction

As described in Chapter 4.2 the role of exercise, and in particular CPET, in the assessment and risk stratification of patients with PH has been increasing. Attention has generally focused on variables derived from and measurements made during the active period of exercise, in the case of CPET from the onset of loaded cycling to peak exercise. Increasingly in chronic heart failure and other cardiovascular diseases the recovery period after exercise is providing insights into both the pathophysiology of these diseases and their associated morbidity and mortality[235]. Of greatest interest is the rate of recovery of heart rate (HR) following exercise, notable because it reflects the degree of underlying cardiac autonomic dysfunction which in turn is associated with morbidity and mortality.

Recovery of HR following exercise demonstrates an exponential decay pattern governed by the balance of changes in sympathetic and parasympathetic activity[235, 236]. Parasympathetic vagal activity has been shown to increase gradually in recovery[237] with parasympathetic activity predominating in the early phase[238]. One study in sedentary normal individuals measured heart rate recovery (HRR) and noradrenaline concentration during recovery from cycle ergometer exercise at three different levels of intensity, finding that restoration of vagal tone was responsible for the immediate decline in HR in the first minute following the cessation of exercise but that from the second minute after exercise noradrenaline concentrations, a marker of sympathetic activity, declined linearly with heart rate, suggesting that sympathetic withdrawal was responsible for this later period of HRR[239]. Similar results were seen in a group of normal subjects in whom sympathetic blockade with propranolol and parasympathetic blockade with atropine were used singly and together to elucidate the autonomic changes underlying HRR, demonstrating that the recovery in the first 30 seconds after exercise was a result of vagal reactivation while the recovery at 2 minutes was a consequence of both vagal reactivation and sympathetic withdrawal[240].

Lower cardiac vagal activity is associated with increased morbidity[241] and all cause mortality[241, 242]. In a study of 605 subjects aged 50-75 years taken from the general population, autonomic dysfunction was demonstrated to be associated with all cause and cardiovascular mortality, an association which was strongest

in those with a history of diabetes mellitus, systemic hypertension or cardiovascular disease[243]. In studies of patients with recent myocardial infarction, increased autonomic dysfunction was associated with increased mortality[244, 245] and, reflecting the underlying pathophysiology, was highest in those with inferior myocardial infarction, 3 vessel versus single vessel coronary artery disease and episodes of ventricular tachycardia[244]. In patients with PH, sympathetic hyperactivity[246] and increased cardiac sympathetic activation[247] have been demonstrated compared with control subjects, and plasma noradrenaline concentrations have been shown to correlate with PAP, cardiac index (CI) and PVR[248]. Increased sympathetic nervous system activation in PAH has been shown to be associated with disease severity and to predict clinical deterioration on multivariate analysis[249].

Given the impact of autonomic dysfunction on morbidity and mortality in health and in a range of cardiopulmonary diseases, and that the rate of HRR is governed by alterations in the autonomic nervous system, HRR will reflect the degree of underlying autonomic dysfunction and may thus be a predictor of morbidity and mortality. Analysis of treadmill testing in approximately 3000 subjects from the Framingham Heart Study who were free of cardiovascular disease demonstrated that those individuals with the fastest HRR had a lower risk of coronary arterial and cardiovascular disease than those with slower HRR[250]. In healthy individuals slower HRR has been associated with an increased risk of sudden death[251] and has been shown to predict all cause[252, 253] and cardiovascular[253] mortality. In a study comparing the HRR in the first 30 seconds after exercise in normal volunteers with patients with CHF and with endurance trained athletes, a faster decay was seen in the trained group and a slower decay noted in the CHF group[240]. Further studies in patients with CHF demonstrated a significantly attenuated HRR in this patient group[254] and also that CHF patients with faster HRR had significantly better survival[255].

One study compared the exercise response of patients with CHF with those with PAH and found that in both groups there was a lower than expected HRR at 1 minute[123]. In a small case control study in PAH, cases were noted to have a significantly lower absolute reduction in HR at 1 minute after exercise compared to controls, despite a significant proportion of patients being on active

treatment with pulmonary vasodilator therapy[256]. A further similar case control study comparing patients with PAH, 36% of whom were taking specific pulmonary vasodilator therapy, with age and gender matched controls found that HRR was significantly slower in patients compared with controls as far as the fifth minute after CPET[257].

The pattern of recovery of oxygen consumption after exercise is similar to that of heart rate in that there is a rapid early decline followed by a longer, more gradual return to resting values[258-262]. The initial rapid decline represents the period during which levels of the phosphagens adenosine triphosphate and creatine phosphate are replenished in the exercised muscle(s) and haemoglobin and myoglobin are reloaded with oxygen[259, 263, 264]. Most VO_2 recovery research has tended to focus on the more prolonged, slower phase of excess postexercise oxygen consumption which lasts for periods up to several hours after exercise however there is evidence of detectable differences in the rate of early VO_2 decline between groups in health and disease.

In a comparison of trained versus untrained healthy individuals, VO_2 recovery after cycle ergometer exercise was faster in the trained group from 30 seconds post-exercise onwards[265]. VO_2 recovery in the first 3 minutes after exercise has been demonstrated to be slower in severe heart failure compared with healthy individuals but not different in groups with less severe CHF[266]. Two further small studies both found evidence of slower early phase VO_2 recovery in patients with CHF compared with normal subjects[267, 268] while a separate larger study assessed the time taken for VO_2 to fall to 50% of its peak value and showed that this early recovery was not only prolonged in CHF compared with healthy volunteers but also that it increased with increasing severity of CHF[269]. One study comparing the response to constant work rate cycle ergometer exercise of 9 patients with primary pulmonary hypertension, what would be termed IPAH under the current classification, with 9 matched normal control subjects, showed significantly slower VO_2 recovery in patients compared with controls[270].

The study presented in this chapter sought to assess the rates of recovery of heart rate and VO_2 after incremental CPET in patients with precapillary PH and to investigate how these relate to survival, to test the hypothesis that prolonged early phase recovery will predict survival in PH.

5.2 Methods

5.2.1 Heart rate recovery

Data from all available cardiopulmonary exercise tests performed in the SPVU on treatment naïve patients at the time of initial diagnosis with group I and group IV PH (Chapter 2.7.1) and who were subsequently commenced on specific pulmonary vasodilator therapy was collected. Tests which did not include at least 2 minutes of recorded recovery data were excluded. CPETs were performed as described in Chapter 2.6. None of the patients with PH secondary to CHD had had atrial or other cardiac surgery which could have affected their HR responses.

HRR was described at 30 seconds, 60 seconds and 120 seconds from onset of recovery, both as the absolute reduction in beats per minute at each time point and as the heart rate at each time point as a percentage of the peak value. Given HRR was calculated from the values recorded at discrete time points it was felt that unlike for calculation of the OUES as described in Chapter 4.3.3, only tests with complete data tables should be included and therefore no digitising software was used to convert CPET plots back into numerical data (Chapter 2.8.5) to determine the HRR, minimising the potential for error in this measurement.

5.2.2 Oxygen consumption recovery

VO_2 recovery was calculated in the same manner as that described for HRR in Chapter 5.3.1.

5.2.3 Heart rate and oxygen consumption recovery survival analysis

Survival analysis was undertaken as described in Chapter 2.10.

5.2.4 Ethical approval

The studies described in this chapter were discussed with the West of Scotland Research Ethics Service who felt that as the core data was collected routinely as part of clinical practice in SPVU and that the data was fully anonymised prior to analysis, no research ethics approval was required.

5.3 Results

Data from 87 patients with group I and group IV PH was included in the assessment of HRR and VO_2 recovery as predictors of survival (3 with PAH secondary to congenital heart disease, 13 with CTDPH, 3 with familial PAH, 1 with HIV associated PAH, 32 with IPAH, 4 with portopulmonary hypertension and 31 with CTEPH). Their baseline characteristics are given in Table 5.1.

Baseline characteristics

Gender (n)	
Male	44
Female	43
Age (years)	56 (23)
WHO FC (n)*	
I	1
II	41
III	44
IV	1
6MWD (metres)	340 (105)
mPAP (mmHg)	45 (14)
PAWP (mmHg)	8 (5)
CO (l/min)	4.2 (1.9)
PVR (Wood units)	8.4 (6.7)
SvO_2 (%)	64.5 (12.9)
DLCO (% predicted)	57 (26)
NTproBNP	809 (2224)
Peak HR (1/min)	131 (30)
Peak VO_2 (l/min)	0.88 (0.47)

Table 5.1 Baseline characteristics. WHO FC: functional class; 6MWD: 6 minute walk distance; mPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; CO: cardiac output; PVR: pulmonary vascular resistance; SvO_2: mixed venous oxygen saturation; DLCO: diffusing capacity of the lungs for carbon monoxide; NTproBNP: N-terminal pro-brain natriuretic peptide; HR: heart rate; VO_2: oxygen uptake.

The median duration of follow up was 531 days with a range from 13 to 1868 days (interquartile range 941 days). During the follow up period there were 22 deaths from all causes, 0 patients received a lung transplant, 14 patients underwent pulmonary endarterectomy and 0 patients were lost to follow up.

5.3.1 Univariate Cox proportional hazards analysis

The results of univariate Cox proportional hazards analysis for both heart rate and VO_2 recovery are given in Table 5.2.

Recovery univariate Cox proportional hazards analysis

Variable	Hazard ratio	*p* value
HRR 30	0.953 (0.869 – 1.045)	0.302
HR 30 (% peak)	1.068 (0.944 – 1.208)	0.297
HRR 60	0.957 (0.915 – 1.000)	0.048
HR 60 (% peak)	1.058 (0.998 – 1.121)	0.056
HRR 120	0.936 (0.893 – 0.981)	0.005
HR 120 (% peak)	1.101 (1.035 – 1.171)	0.002
VO_2R 30	0.098 (0.001 – 16.669)	0.375
VO_2 30 (% peak)	0.995 (0.950 – 1.042)	0.822
VO_2R 60	0.066 (0.004 – 1.032)	0.053
VO_2 60 (% peak)	1.024 (0.997 – 1.052)	0.082
VO_2R 120	0.101 (0.010 – 0.995)	0.05
VO_2 120 (% peak)	1.031 (1.005 – 1.058)	0.021

Table 5.2 Univariate Cox proportional hazards analysis of heart rate and VO_2 recovery after CPET for prediction of all cause mortality. Hazard ratios expressed as hazard ratio (95% confidence interval). HRR 30, 60, 120: absolute reduction in heart rate at 30 seconds, 60 seconds and 120 seconds of recovery; HR 30 (% peak), HR 60 (% peak), HR 120 (% peak): heart rate at 30 seconds, 60 seconds and 120 seconds of recovery expressed as a percentage of the peak heart rate achieved. VO_2R 30, 60, 120: absolute reduction in VO_2 at 30 seconds, 60 seconds and 120 seconds of recovery; VO_2 30 (% peak), VO_2 60 (% peak), VO_2 120 (% peak): VO_2 at 30 seconds, 60 seconds and 120 seconds of recovery expressed as a percentage of the peak VO_2 achieved.

The absolute reduction in HR at 60 and 120 seconds, and the HR at 120 seconds expressed as a percentage of the peak HR, were significant predictors of all cause mortality on univariate analysis (p = 0.048, 0.005 and 0.002 respectively).

The absolute reduction in VO_2 at 120 seconds was seen to predict mortality with $p = 0.05$ while the VO_2 at 120 seconds expressed as a percentage of the peak VO_2 was also a significant predictor ($p = 0.021$).

The results of univariate Cox proportional hazards analysis for other candidate variables in given in Table 5.3.

Univariate Cox proportional hazards analysis

Variable	Hazard ratio	*p* value
OUES	0.446 (0.177 – 1.123)	0.087
Peak VO_2	0.240 (0.056 – 1.019)	0.053
Age	1.041 (1.011 – 1.073)	0.008
DLCO % predicted	0.990 (0.973 – 1.007)	0.267
V_E/VCO_2 at AT	1.010 (0.989 – 1.031)	0.366
Gender		
Male	0.756 (0.323 – 1.770)	0.520
Female (reference)	--	--
WHO FC		
I/II	1.027 (0.675 – 1.561)	0.902
III/IV (reference)	--	--
RAP	1.010 (0.932 – 1.095)	0.803
mPAP	0.984 (0.949 – 1.020)	0.378
CO	0.843 (0.624 – 1.139)	0.266
PVR	0.978 (0.910 – 1.051)	0.543
SvO_2	0.991 (0.969 – 1.014)	0.461
6MWD	0.996 (0.991 – 1.002)	0.166
logNTproBNP	2.151 (0.952 – 4.860)	0.065

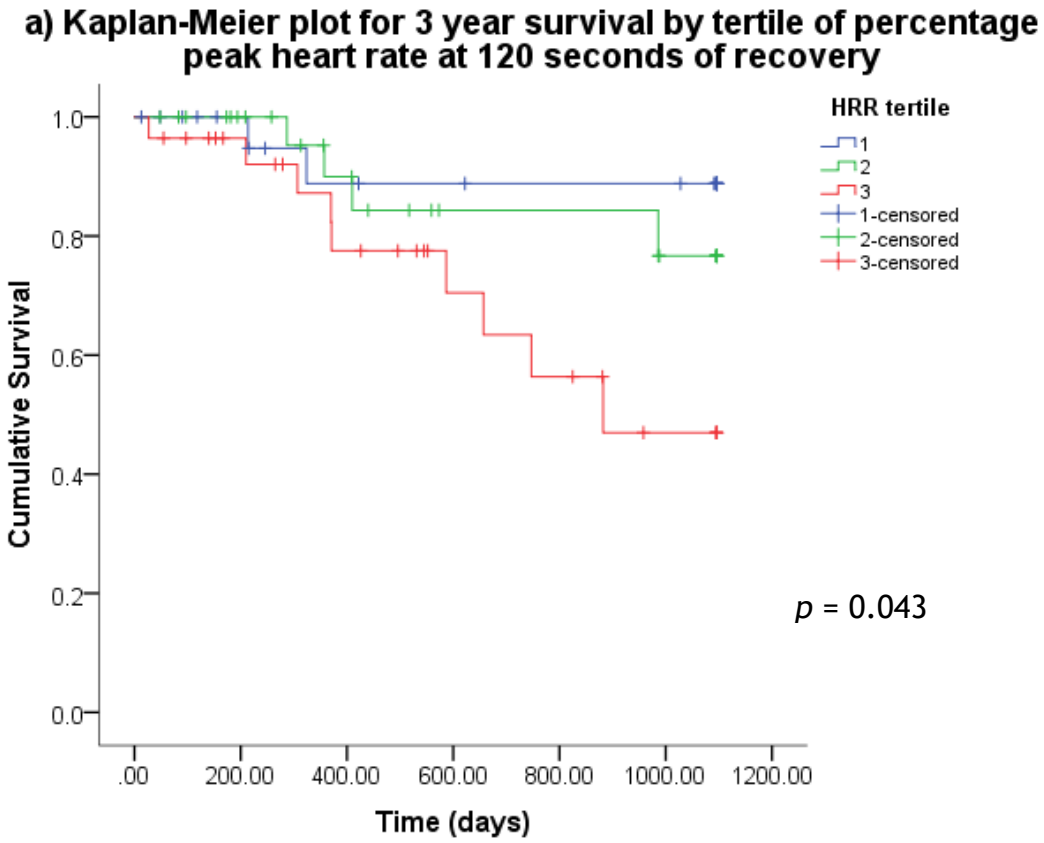
Table 5.3 Univariate Cox proportional hazards analysis for prediction of all cause mortality by non recovery candidate variables. Hazard ratios expressed as hazard ratio (95% confidence interval). OUES: oxygen uptake efficiency slope; VO_2: oxygen uptake; DLCO: diffusing capacity of the lungs for carbon monoxide; V_E/VCO_2 at AT: ventilatory equivalent for carbon dioxide at anaerobic threshold; WHO FC: functional class; RAP: right atrial pressure; mPAP: mean pulmonary artery pressure; CO: cardiac output; PVR: pulmonary vascular resistance; SvO_2: mixed venous oxygen saturation; 6MWD: six minute walk distance; logNTproBNP: log transformed N-terminal pro-brain natriuretic peptide.

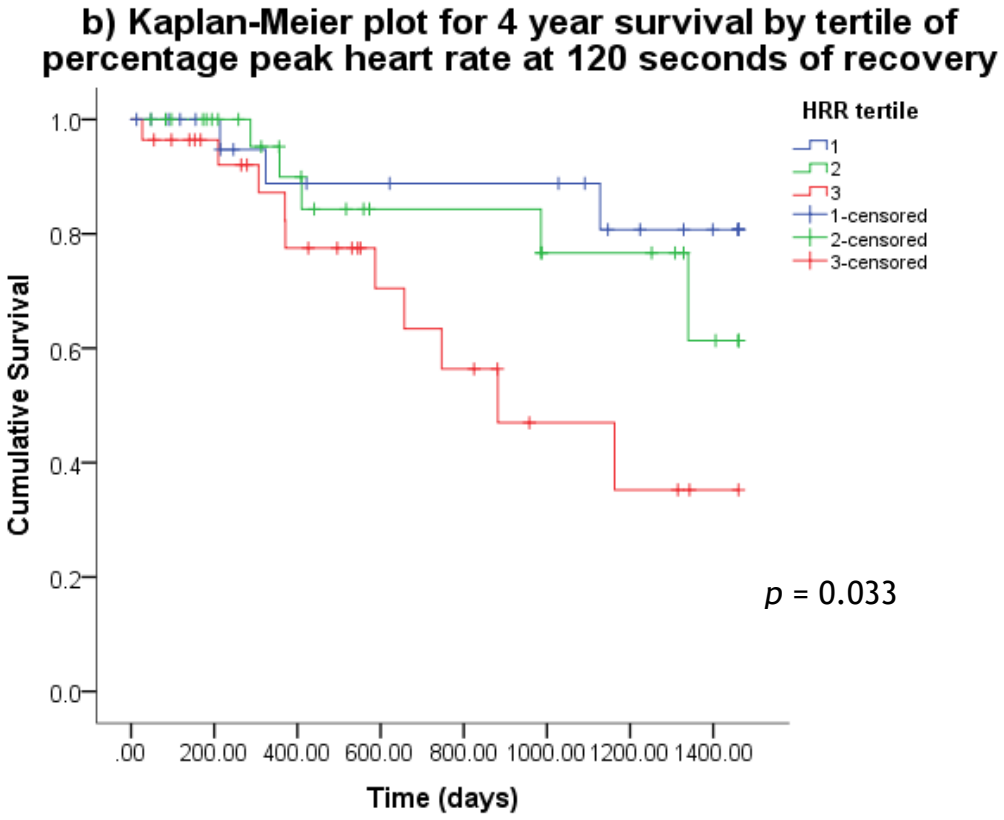
5.3.2 Multivariate Cox proportional hazards analysis

On multivariate analysis the absolute reduction in heart rate at 120 seconds was the sole remaining covariate in a model including age and logNTproBNP (hazard ratio 0.918, 95% confidence interval 0.870 – 0.970, p = 0.002). Similar results were seen when HRR was expressed as the heart rate at 120 seconds as a percentage of the peak HR (hazard ratio 1.115, 95% confidence interval 1.041 – 1.194, p = 0.002). However HRR at earlier time points and VO_2 recovery at all time points were not significant predictors of mortality on multivariate analysis.

5.3.3 Kaplan Meier analysis of heart rate and VO_2 recovery

Subjects were stratified by tertiles of HR and VO_2 recovery. Kaplan-Meier analysis was performed for 3 and 4 year survival. The significant results are given in Figure 5.1.





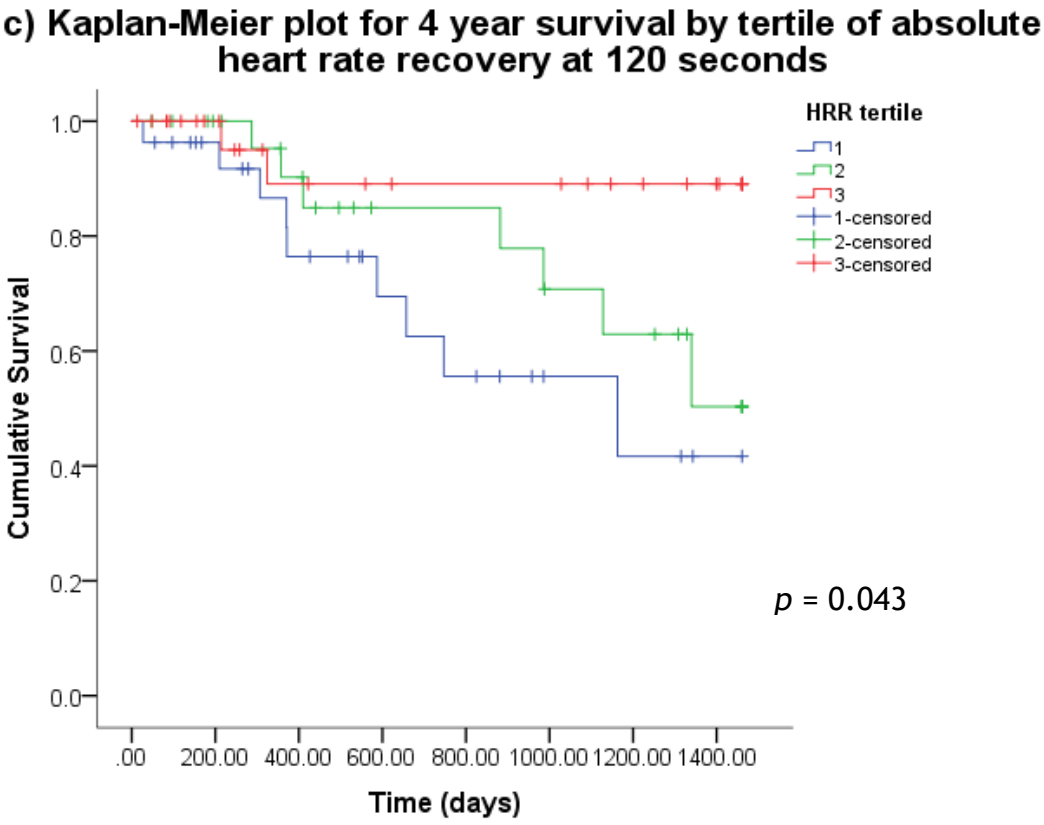


Figure 5.1 Kaplan-Meier plots for a) 3 year and b) 4 year survival by tertile of percentage peak heart rate at 120 seconds recovery and c) 4 year survival by tertile of absolute heart rate recovery at 120 seconds. HRR: heart rate recovery.

5.4 Discussion

These studies explored the rates of recovery of heart rate and VO_2 in the first two minutes of recovery after incremental CPET to test the hypothesis that prolonged recovery would be associated with an increase in all cause mortality. The results demonstrate that the absolute reduction in HR at both 60 seconds and 120 seconds of recovery and the HR at 120 seconds expressed as a percentage of the peak HR were all significant predictors of mortality on univariate analysis, with slower HRR associated with an increased risk of death in the patient cohort studied. On multivariate analysis the absolute reduction in HR at 120 seconds remained a significant predictor of all cause mortality after controlling for age and logNTproBNP.

These results add to the small body of emerging evidence linking delayed HRR with poor outcome in patients with PAH and is the first to do so in treatment naïve patients. One study employed a composite endpoint of clinical worsening, defined as any one of escalation of PH drug therapy, admission to hospital due to PH, lung transplantation or death, and studied the HRR at 60 seconds after 6MWT[271]. In contrast to the treatment naïve patient cohort used in the recovery analysis presented in this chapter, all but 4 of the 75 subjects in that study were on treatment with specific pulmonary vasodilator therapy, including 47 receiving parenteral prostanoid therapy, either alone or in combination with oral treatment. The authors demonstrated that an absolute fall in HR of less than 16 beats per minute at 60 seconds of recovery was associated with a higher likelihood of clinical worsening events and a shorter time to clinical worsening.

The same group conducted a similar study of HRR after 6MWT in a cohort of patients with CTDPH and again found that an absolute reduction in HR of less than 16 beats per minute at 60 seconds of recovery was the strongest predictor of hospitalisation, death and time to clinical worsening[272]. A separate study in a cohort of 72 patients with PAH, 26 of whom were on treatment with pulmonary vasodilator therapy, found that an absolute reduction in HR of 18 beats per minute or less after the first 60 seconds of recovery was associated with poorer survival[257].

In contrast to the results presented in this chapter which showed that HRR at 120 seconds was a stronger predictor of mortality than HRR at 60 seconds, in the study of recovery after 6MWT in patients with CTDPH the HRR at 60 seconds was a better predictor than that seen at 120 seconds[272]. One possible explanation for this is that in that study the recovery was passive with the participants sitting at rest for the duration of recovery whereas in the presented work patients continued cycling at a reduced work rate for at least part of the recorded recovery period, providing an ongoing stimulus to subjects to maintain an elevated heart rate in the initial recovery period regardless of underlying disease severity. It has been shown that HRR is slower in active recovery compared to assisted recovery in which the subjects' legs are moved by an assistant[273] and that furthermore it is slower in assisted recovery than passive recovery, albeit over a timescale of several minutes[274]. These results have been taken as suggesting that the rate of the early phase of HRR is affected both by cessation of central drive and by feedback from mechanoreceptors in the exercising muscle.

One weakness of the presented study is that no fixed work rate or duration of active recovery was set as part of the CPET protocol. It may be considered that patients with more severe disease might cease cycling earlier in recovery, leading to a faster rate of recovery and therefore to a potential source of systematic bias. That would be expected to reduce the difference between patients with more and less severe disease however despite that possible influence, in this study slower rates of recovery were still found to be significant predictors of survival.

The study of VO_2 recovery after exercise presented in this chapter is the first in PAH to study early phase VO_2 recovery and its relationship to survival. While slower rates of VO_2 recovery were associated with increased mortality when assessed at 120 seconds after exercise, VO_2 recovery was not as strong a predictor as HRR and was not a significant predictor of mortality on multivariate analysis.

6 The use of inert gas rebreathing to measure early treatment response in pulmonary hypertension

6.1 Introduction

As described in Chapter 1.1 PH is a disease defined by its haemodynamic abnormalities and while invasive RHC is required to make the initial diagnosis, most ongoing assessment of disease severity and treatment response is made by noninvasive surrogate measures such as 6MWD and NTproBNP. Exercise limitation is one of the most common features of PH and a large component of this limitation is attributable to a failure to augment SV on exercise[93, 94, 116, 119]. In considering the assessment of treatment response it would therefore be of value to measure the underlying haemodynamic change but serial measurements with RHC are unattractive due to the invasive nature of the test and associated potential for complications[53].

Cardiac MRI provides information on the structure and function of the right heart and the pulmonary circulation, and has been demonstrated to provide evidence of response to treatment. Relief of PH by either PEA or lung transplantation is associated with improvements in RV function and PBF[275-277]. The more subtle improvements seen with drug treatment can also be detected with cardiac MRI with improvements in RV SV, RV mass (RVM), RVEF and CI seen in a selection of trials of oral and intravenous pulmonary vasodilator therapy[163, 278-280]. However this improvement in cardiac MRI variables has not been universally seen with two studies of ERAs finding no benefit on either RVEF or RV volumes in response to treatment[281, 282].

With exercise limitation one of the key symptoms of PH it is important to consider how the underlying haemodynamic abnormalities change on exercise. While cardiac MRI has been used to assess changes on exercise the narrow bore of an MRI scanner makes exercise while scanning difficult to achieve and studies have tended to involve a period of exercise followed by movement into the MRI scanner for measurements to take place, thus not achieving the goal of assessing patients during exercise[93, 283].

Inert gas rebreathing (IGR) has been shown to allow noninvasive measurement of SV on exercise in both chronic heart failure and PH[150, 175, 176, 284]. In addition it has been demonstrated that in patients with Group 1 and Group 4 PH IGR can detect changes in SV in response to institution of treatment both at rest and on

exercise[150]. Patients underwent measurement of PBF and SV by IGR at rest in the supine and erect positions, and on erect exercise at 40% of the peak work rate (WR) achieved on a prior CPET, and in all 3 positions there was an increase in SV seen 3 months after commencing treatment. There was a trend to increase in 6MWD but changes in WHO-FC, NTproBNP and CAMPHOR quality of life assessment were not seen. Interestingly that study suggested that measurements made by IGR may be more sensitive than 6MWD in detecting treatment change in patients with a higher baseline 6MWD.

It is standard clinical practice to assess the impact of institution or alteration of specific pulmonary vasodilator therapy after 3-4 months, primarily by measures of function such as 6MWT, CPET and WHO-FC[4]. However it has been demonstrated that acute haemodynamic changes occur in response to treatment with each class of disease targeted therapy used in PH: PDE5 inhibitors[285-288], ERAs[289] and prostacyclin analogues[81, 207, 287, 288, 290]. It is not known if this early haemodynamic change predicts clinical response at 3-4 months. If such a relationship exists it may be possible to assess patients haemodynamically shortly after commencing disease targeted therapy and alter therapy at that point if a poor clinical response is predicted.

This study therefore aimed to explore the feasibility of using IGR to assess the early haemodynamic change and explore how it relates to later functional improvement to investigate the hypotheses that haemodynamic improvement is detectable by IGR, that it is seen earlier that functional improvement and that this earlier response is predictive of later change in exercise capacity.

6.2 Methods

6.2.1 Patient recruitment

Potential subjects were identified by the clinical team at the SPVU with the following inclusion and exclusion criteria.

6.2.1.1 Inclusion criteria

Subjects with Group 1 or Group 4 PH

Newly diagnosed patients being commenced on therapy *de novo* or patients already established on pulmonary vasodilator therapy undergoing a change in treatment as planned when undergoing routine review in the outpatient clinic.

6.2.1.2 Exclusion criteria

Subjects who were unable to perform the 6MWT due to comorbid neurological or musculoskeletal limitation

Subjects with exercise-induced syncope, chest pain or cardiac arrhythmia

Subjects who were pregnant

Subjects who were unable to give informed consent

Subjects who were unable to undergo MRI scanning

6.2.2 Power calculation

The study was powered to show a significant change in 6MWD at 4 months. On the basis of α of 0.05 and power of 0.8, the required sample size was calculated. The effect size of change in 6MWD was taken as 22 metres with a standard deviation of the change of 28 metres[150]. The required sample size was 13.

6.2.3 Study protocol

Subjects were assessed at baseline and at 2 weeks, 4 months and 1 year after starting or changing disease targeted therapy. Each assessment involved two visits and, except for the 2 week assessment, coincided with routine clinical review. Visit 2 occurred 1 day after Visit 1 at all four assessments. At each time point the subjects underwent the assessment outlined in Figure 6.1.

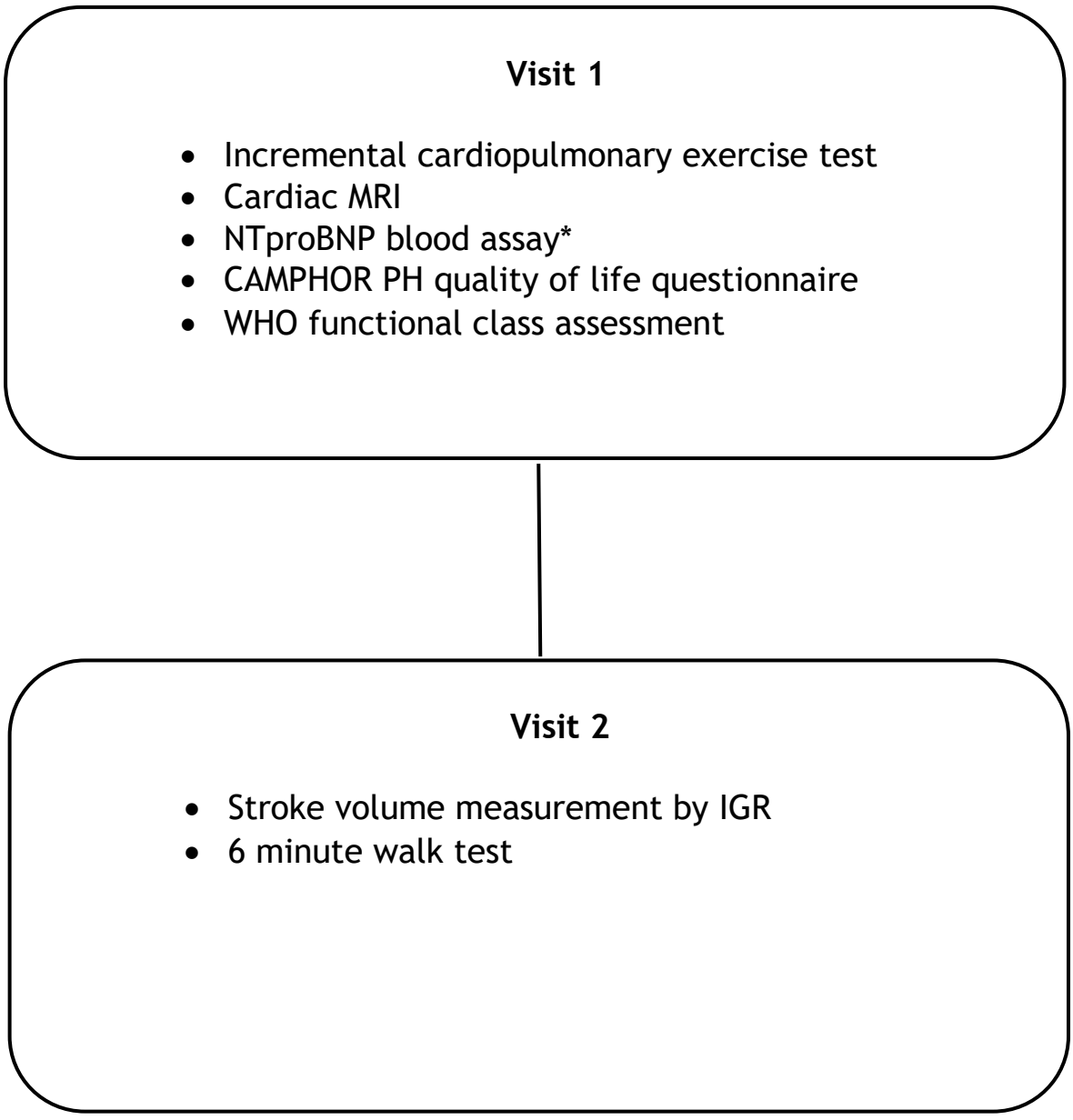


Figure 6.1 Protocol for assessment at each timepoint, baseline, 2 weeks, 4 months and 1 year. MRI: magnetic resonance imaging, NTproBNP: N terminal pro-brain natriuretic peptide: CAMPHOR: Cambridge Pulmonary Hypertension Outcome Review; PH: pulmonary hypertension; WHO: World Health Organisation; IGR: inert gas rebreathing. *NTproBNP performed at baseline, 4 months and 1 year only.

All study procedures were performed as described in Chapter 2.

6.2.4 Ethical approval

Ethical approval for this study was granted by the West of Scotland research Ethics Committee.

6.2.5 Analysis of results

This study was largely exploratory to assess the feasibility of using IGR to detect changes in SV at 2 weeks and if present, to assess their relationship to later functional improvement. As described in Chapter 6.3 recruitment to this study was lower than expected, thus limiting the statistical analysis given the small number of subjects. Significance tests have therefore not been performed.

6.3 Results

A total of 8 patients were recruited to this study, short of the original recruitment target of 13 patients.

The baseline characteristics for the 8 subjects are given in Table 6.1. The RHC data for 6 patients is from RHCs undertaken within a fortnight of study entry, 5 of whom were at the point of diagnosis and one was undergoing invasive reassessment prior to treatment escalation. For one patient the RHC data was from 4 months earlier when the diagnosis was made and treatment first instituted. For another patient the RHC had last been performed as a child and therefore was not included in the figures used for Table 6.1.

Baseline characteristics

Diagnosis (n)		
	IPAH	4
	CTDPH	3
	CTEPH	1
Age (years)		54 (14)
Gender		
	Male	4
	Female	4
WHO-FC		
	I	1
	II	5
	III	2
mPAP (mmHg)		47 (5)
PAWP (mmHg)		6 (6)
CO at last RHC (l/min)		4.3 (0.4)
SV at last RHC (ml)		50 (12)
PVR at last RHC (Wood units)		9.3 (2.2)

Table 6.1 Baseline characteristics of participants in early treatment response study. IPAH: idiopathic pulmonary arterial hypertension; CTDPH: connective tissue disease associated pulmonary hypertension; CTEPH: chronic thromboembolic pulmonary hypertension; WHO-FC: WHO functional class; mPAP: mean pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; CO: cardiac output; RHC: right heart catheterisation; SV: stroke volume; PVR: pulmonary vascular resistance.

Absolute values of resting supine SV measured by cardiac MRI and IGR are given in Figure 6.2 and Figure 6.3. The comparison of the changes seen in supine SV at 2 weeks when measured by both IGR and cardiac MRI is given in Figure 6.4. The aortic SV measured at MRI was used for the comparison. The direction and magnitude of change in SV was similar by both methods for all but one patient in whom the mean supine SV increased by 2ml measured by IGR but fell by 7ml measured by cardiac MRI. These results suggest that both IGR and cardiac MRI area able to detect early haemodynamic changes.

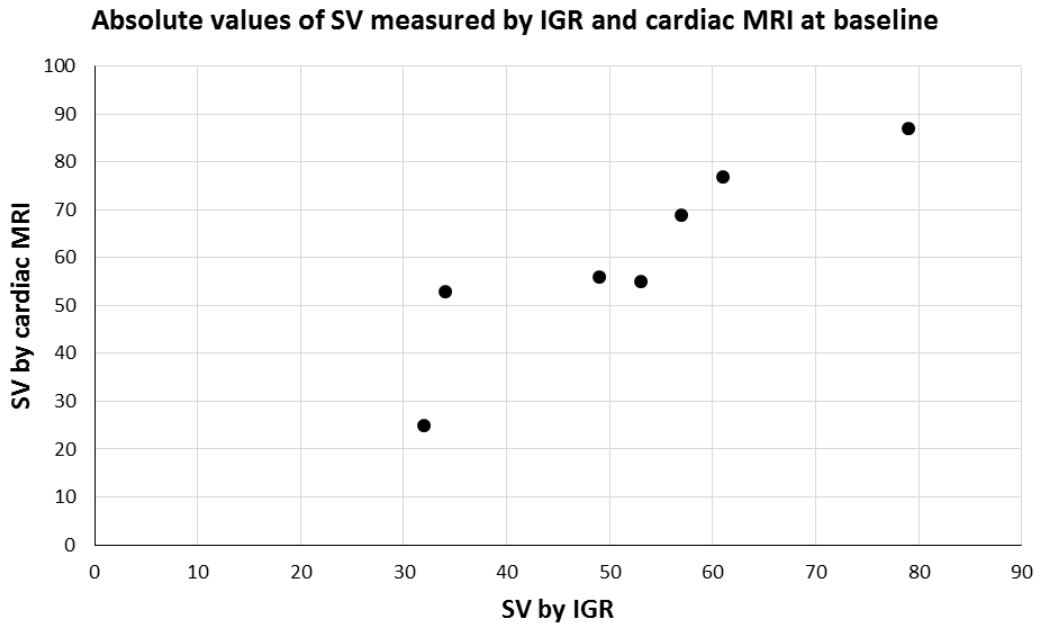


Figure 6.2 Absolute values of supine resting stroke volume measured by cardiac MRI (aortic SV) and inert gas rebreathing at baseline. SV: stroke volume (ml); MRI: magnetic resonance imaging; IGR: inert gas rebreathing.

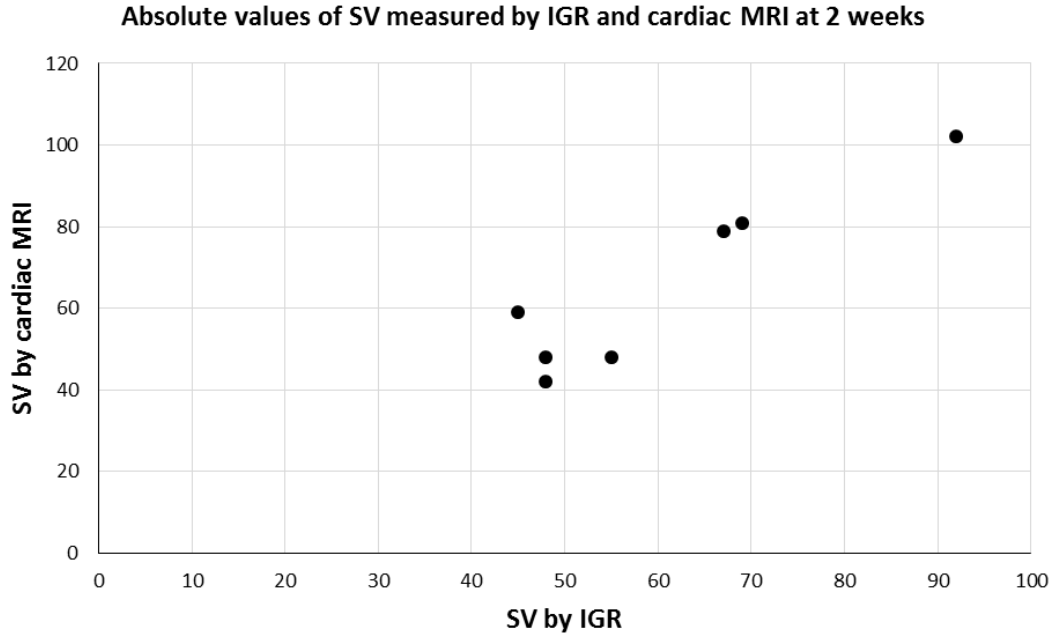


Figure 6.3 Absolute values of supine resting stroke volume measured by cardiac MRI (aortic SV) and inert gas rebreathing at 2 weeks. SV: stroke volume (ml); MRI: magnetic resonance imaging; IGR: inert gas rebreathing.

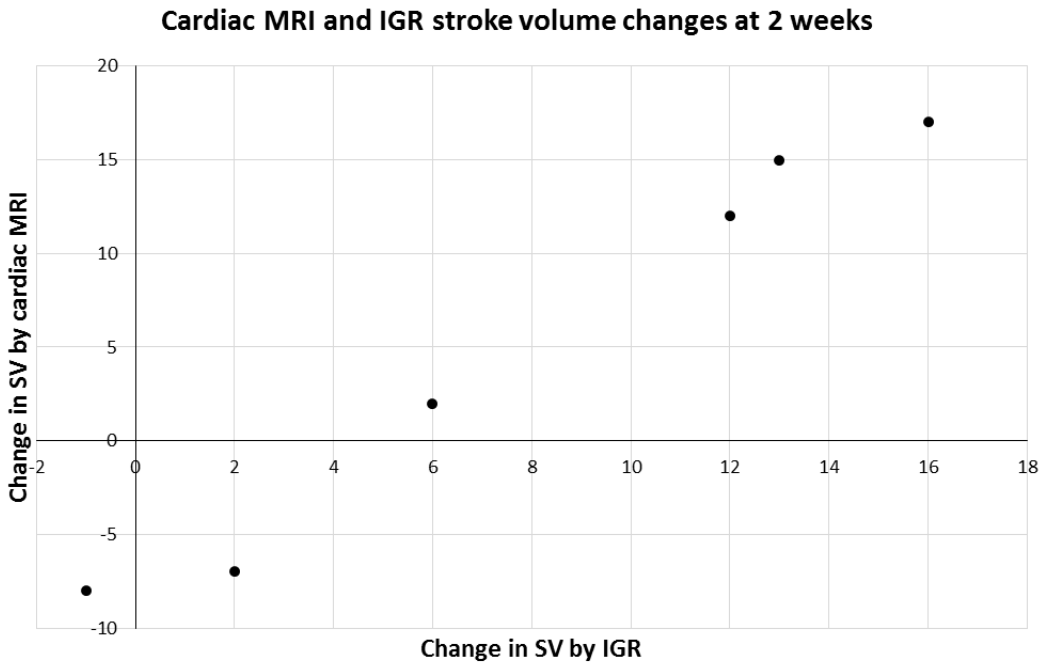


Figure 6.4 Comparison of change in supine stroke volume at 2 weeks as measured by cardiac MRI (aortic SV) and IGR. SV: stroke volume (ml); MRI: magnetic resonance imaging; IGR: inert gas rebreathing.

The changes in SV by IGR in the supine and erect positions at 2 weeks are given in Figure 6.5. The direction of change in SV was the same for all patients whether measured supine or erect but the magnitude of the change for some patients appeared to be lower with the erect measurements suggesting that perhaps the erect measurement may be less responsive to treatment change.

The relationship between the change in supine SV at 2 weeks and the change in 6MWD at 2 weeks is given in Figure 6.6. It appears to show a positive relationship between the change in SV and the change in 6MWD with those patients with the largest increase in SV at 2 weeks also having the largest increase in 6MWD at 2 weeks.

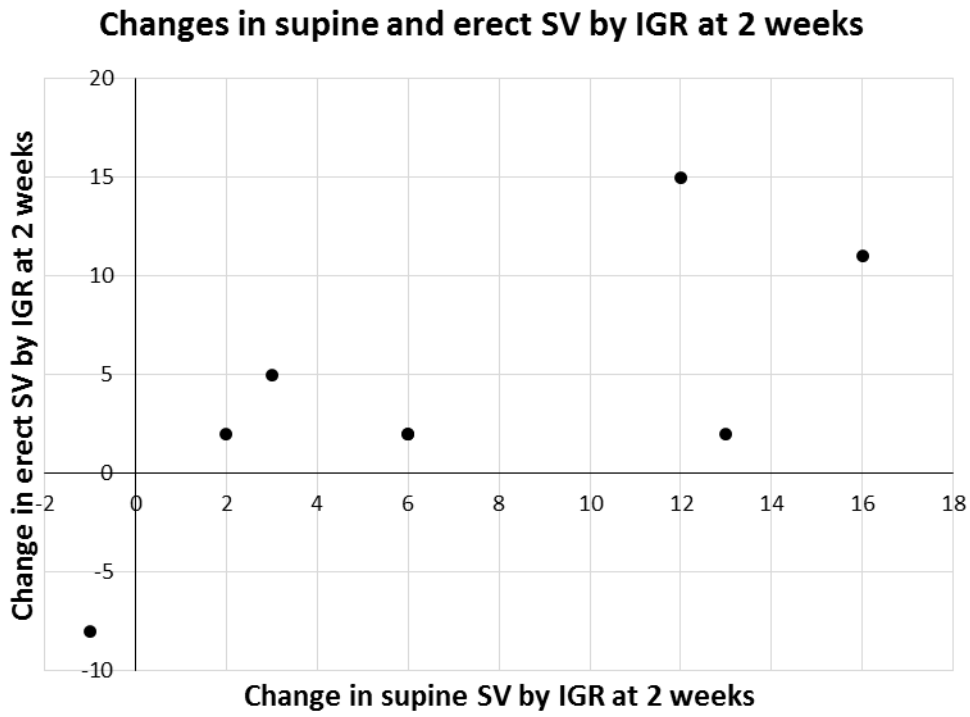


Figure 6.5 Relationship between the change in stroke volume measured by inert gas rebreathing at 2 weeks in the supine and erect positions. SV: stroke volume; IGR: inert gas rebreathing.

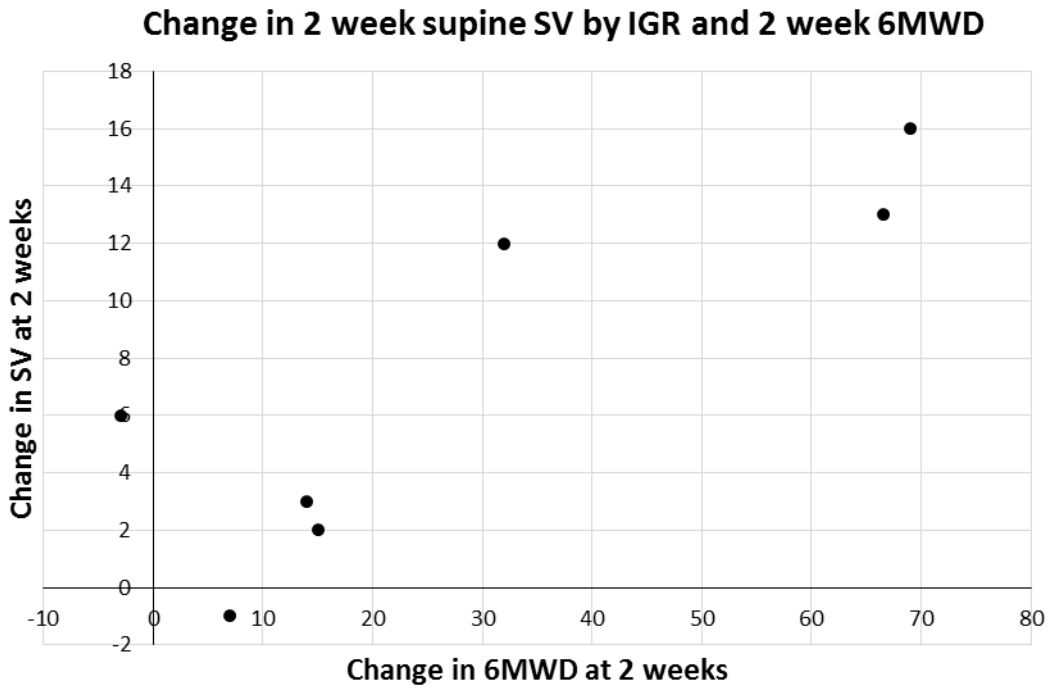


Figure 6.6 Relationship between the change in supine stroke volume measured by inert gas rebreathing at 2 weeks and the change in 6 minute walk distance at 2 weeks. SV: stroke volume (ml); IGR: inert gas rebreathing; 6MWD: 6 minute walk distance (metres).

A less clear relationship is seen between by the change in supine SV by IGR at 2 weeks and the change in 6MWD at 4 months as demonstrated in Figure 6.7.

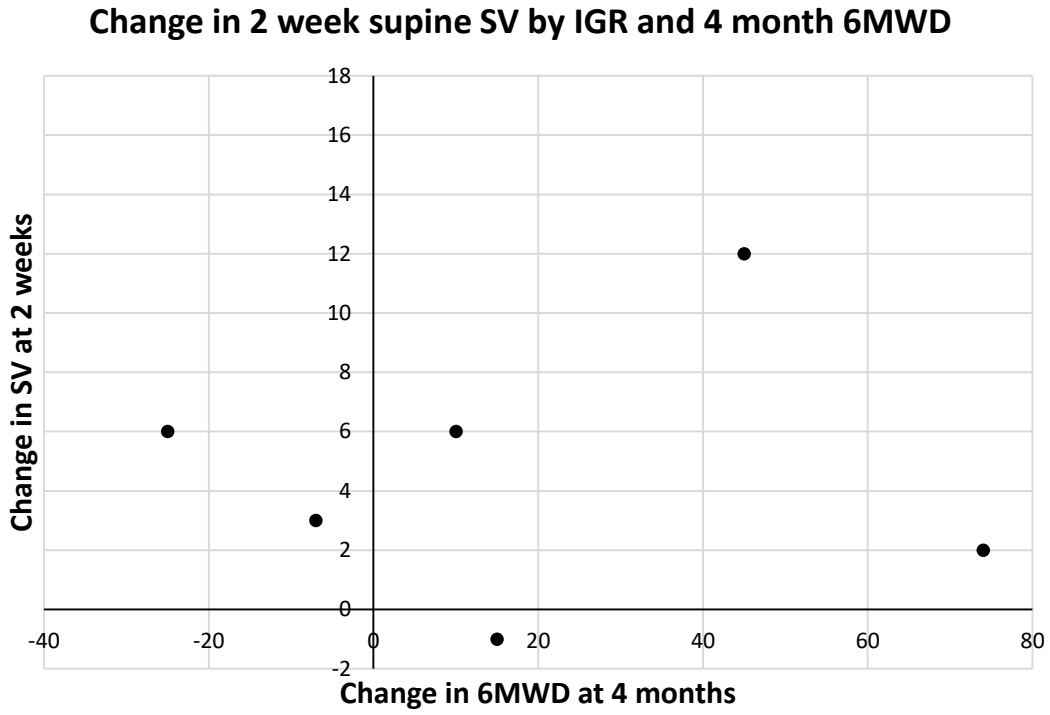


Figure 6.7 Relationship between the change in supine stroke volume measured by inert gas rebreathing at 2 weeks and the change in 6 minute walk distance at 4 months. SV: stroke volume (ml); IGR: inert gas rebreathing; 6MWD: 6 minute walk distance (metres).

Similarly no clear relationship is seen between the change in supine SV by IGR at 4 months and the change in 6MWD at 4 months as demonstrated in Figure 6.8.

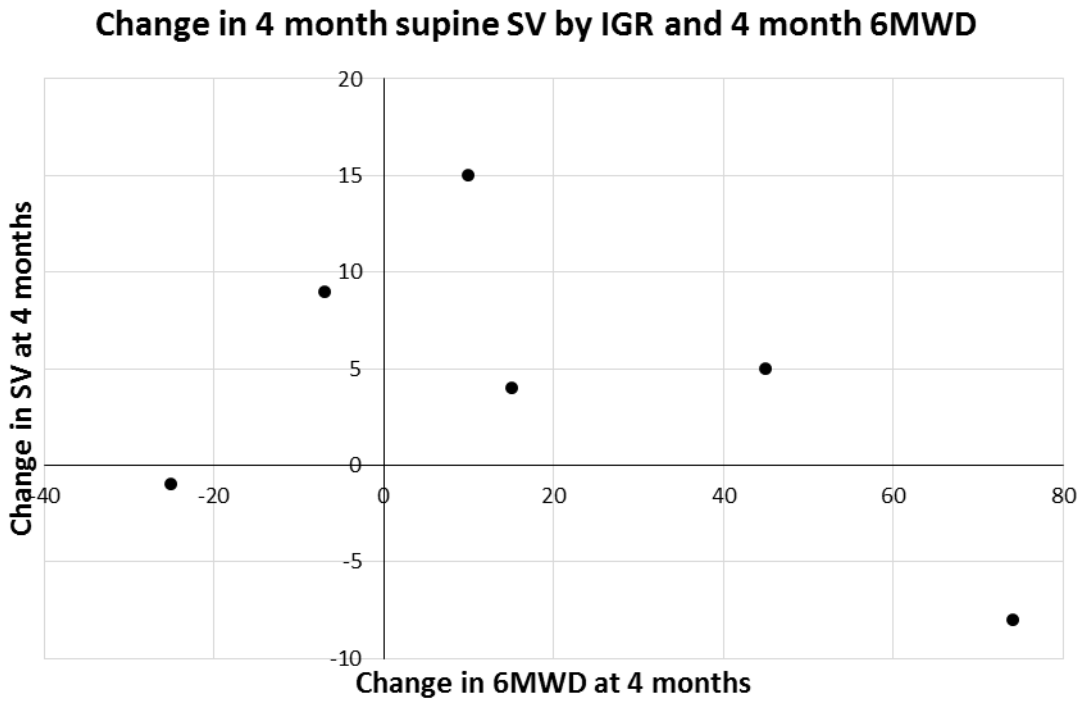


Figure 6.8 Relationship between the change in supine stroke volume measured by inert gas rebreathing at 4 months and the change in 6 minute walk distance at 4 months. SV: stroke volume (ml); IGR: inert gas rebreathing; 6MWD: 6 minute walk distance (metres).

6.4 Discussion

Although a large volume of data was collected as part of this study, given the smaller than expected number of participants it was decided to concentrate on the main focus of the study, i.e. if haemodynamic changes can be detected at 2 weeks after starting or altering disease targeted pulmonary vasodilator therapy and whether these changes relate to changes in function at the standard follow up time of 4 months.

SV measured by IGR appears to be able to detect haemodynamic changes 2 weeks after starting or altering treatment and these changes appear comparable to those seen by cardiac MRI. On the basis of the data obtained this change in supine SV measured by IGR relates to the change in 6MWD at the same time point. Interestingly however the change in 6MWD at 4 months does not appear to relate to the change in SV either at 2 weeks or at 4 months. Given the close

relationship between impaired stroke volume and exercise limitation it is perhaps surprising that the changes seen in 6MWD at 4 months do not appear to relate to changes in SV. It is possible however that this is a consequence of the small number of patients included in the study.

The main weakness of this study is the small number of patients who participated. Difficulties encountered recruiting to the trial centred on two main issues. Firstly, the study protocol was relatively intense with repeated exercise testing occurring alongside the other investigations over two visits at each time point. Given the large geographical spread of patients attending the SPVU for assessment the protocol necessitated an overnight stay for the majority of patients and while this could be offered without charge it added to the perceived intensity of the visits. Secondly, the recruitment from prevalent patients undergoing a change of treatment after assessment in the outpatient clinic was less than anticipated. This again stemmed partly from the intensity of the study protocol and that in addition to their current clinic review they would need to return for a further four days of assessment over a two week period.

6.5 Conclusions

The limited data from this study appears to show that both IGR and cardiac MRI, noninvasive surrogate measures of the underlying haemodynamics, can detect changes as early as 2 weeks after a change in treatment. These changes seem to relate to changes in 6MWD seen at the 2 week time point but there does not appear to be any relationship between the SV changes and the changes observed in 6MWD at 4 months. This study was small and further assessment of the role of IGR and cardiac MRI in assessing early haemodynamic change and the relationship between that and future functional change should be assessed in a larger study with a more streamlined protocol to enhance recruitment and lend more certainty to these provisional results.

7 Major findings and conclusions

The work in this thesis was undertaken to investigate the extent to which novel exercise derived variables and noninvasive haemodynamic measurements can enhance the assessment and monitoring of patients with pulmonary hypertension.

Right heart catheterisation on exercise in patients with resting Group 1 and Group 4 PH was carried out to test the hypothesis that there is impairment of oxygen extraction in the peripheral muscles on exercise in these patient groups. The major findings were:

- On exercise patients with PAH can achieve very low levels of mixed venous oxygen saturation, lower than has been previously suggested

- Oxygen extraction does not appear to be reduced in patients with PH and may be higher than in healthy individuals

- Impairment of oxygen extraction does not appear to be a limiting factor in the exercise capacity of patients with PH.

The potential role of the oxygen uptake efficiency slope in the assessment and monitoring of patients with PH was investigated. The major findings were:

- The relationship between OUES and peak VO_2 is constant across different groups of PH

- OUES calculated from submaximal levels predicts peak VO_2 and therefore OUES can be considered a submaximal measure of exercise performance in PH

- OUES is a significant predictor of survival in patients with Group 1 and Group 4 PH

The rates of recovery of heart rate and VO_2 after incremental CPET were studied to investigate their relationship with survival in patients with Group 1 and Group 4 PH. The major findings were:

- The absolute reduction in HR at 60 and 120 seconds, and the HR at 120 seconds expressed as a percentage of the peak HR, were significant predictors of all cause mortality on univariate analysis

- The absolute reduction in heart rate at 120 seconds was the sole remaining covariate on multivariate analysis in a model including age and logNTproBNP. Similar results were seen when the HR at 120 seconds was expressed as a percentage of the peak HR

- The absolute reduction in peak VO_2 at 120 seconds and the VO_2 at 120 seconds expressed as a percentage of the peak VO_2 were both significant predictors of survival on univariate analysis

The ability of noninvasive measurement of stroke volume by inert gas rebreathing and by cardiac MRI to detect early haemodynamic changes and the relationship of these changes to later functional improvement was investigated. The study did not meet its recruitment target but did appear to show on the basis of the limited data:

- Both IGR and cardiac MRI are able to detect haemodynamic changes 2 weeks after starting or altering pulmonary vasodilator therapy

- The change in SV at 2 weeks as measured supine by IGR appears to relate to changes in 6MWD seen at 2 weeks

- The change in SV at 2 weeks does not appear to relate to the change in 6MWD seen at 4 months and similarly the change in SV at 4 months does not appear to relate to the change in 6MWD at 4 months

In conclusion, the aims of this thesis, to investigate the role of novel exercise derived variables and noninvasive haemodynamic measurements in enhancing the assessment and monitoring of patients with PH has been achieved. The work on oxygen extraction has suggested that while pathological changes do occur in the muscles of patients with PH, these do not seem to cause impairment of oxygen extraction and consequent exercise limitation. This could be explored further through a rehabilitation trial involving exercise right heart catheterisation, muscle biopsies and cardiopulmonary exercise testing both before and after a course of rehabilitation to clarify in more detail the impact of the peripheral muscle abnormalities.

OUES has demonstrated promise as a submaximal measure of exercise performance and predictor of survival in PH and further work on this measure and its role in assessing treatment response with serial measurements should be explored.

The rates of heart rate and VO_2 recovery after CPET have been shown to predict survival. In particular the rate of recovery of HR at 120 seconds after exercise has been demonstrated to be a powerful predictor of survival. Work investigating how this might combine with other exercise derived and haemodynamic variables may lead to enhanced prediction of patient outcome.

Finally inert gas rebreathing and cardiac MRI both appear able to detect a haemodynamic response 2 weeks after treatment. The number of participants was too small to draw conclusions on how this change may or may not relate to later functional improvement. A larger more streamlined study focusing on this issue in isolation may yield interesting results which could change the current strategy for assessing treatment response in PH.

Appendix 1 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

Symptoms

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 ***<u>at the moment</u>***

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 ❒

please turn over

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.

Please mark only one box.

	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	☐	☐	☐
4. 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 stat_____

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