Physiological Adaptations to Chronic Hypoxemia in Eisenmenger Syndrome

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ABSTRACT

Eisenmenger syndrome is characterised by severe, lifelong hypoxaemia and pulmonary hypertension. Despite this, patients do surprisingly well and report a reasonable quality of life. This thesis describes a series of experiments investigating the adaptations that occur in these patients in response to the chronic hypoxaemia.

Patients with Eisenmenger syndrome have severely limited exercise tolerance when assessed using cardiopulmonary exercise testing. However, they appear to maintain aerobic metabolism until late on in exercise. Studies using skeletal muscle ^{31}P MRS during and throughout recovery from exercise showed that these patients have similar mitochondrial oxidative capacity compared to healthy controls.

Echocardiography showed that patients with Eisenmenger syndrome have preserved right and left ventricular systolic function. However they have evidence of right ventricular diastolic dysfunction as evidenced by impaired early diastolic relaxation. The cardiac ^{31}P MRS study demonstrated that despite the normal systolic function shown on echocardiography, there is impairment of septal energetics as revealed by a reduction in PCr/ATP ratio.

The results presented in this thesis indicate that adult patients with Eisenmenger syndrome have undergone beneficial adaptations to the severe hypoxaemia that they are exposed to from infancy.

To Rob and Isabel, this is for you.

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ABBREVIATIONS

A4C	Apical four chamber
ACHD	Adult congenital heart disease
ADP	Adenosine diphosphate
ASD	Atrial septal defect
ATP	Adenosine triphosphate
AVSD	Atrioventricular septal defect
Ca	Calcium
CaO_2	Arterial oxygen content
CHF	Chronic heart failure
CHO	Carbohydrate
CK	Creatine kinase
CO	Cardiac Output
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise test
Deoxy-Hb	Deoxygenated haemoglobin
EDV	End diastolic volume
EF	Ejection fraction
ESV	End systolic volume
FAC	Fractional area change
Hb	Haemoglobin
HbT	Total haemoglobin
HCM	Hypertrophic cardiomyopathy
IPAH	Idiopathic pulmonary arterial hypertension
IVA	Isovolumic acceleration
IVCT	Isovolumic contraction time
IVRT	Isovolumic relaxation time
K^+	Potassium
LV	Left ventricle
LVEDD	Left ventricular end diastolic diameter
LVH	Left ventricular hypertrophy

MPI	Myocardial performance index
MRS	Magnetic resonance spectroscopy
mVO_2	Maximal oxygen uptake (VO_2)
NO	Nitric oxide
NO_3	Nitrate
NO_2^-	Nitrite
O_2	Oxygen
OUES	Oxygen uptake efficiency slope
PAH	Pulmonary arterial hypertension
PCr	Phosphocreatine
PVR	Pulmonary vascular resistance
VSD	Ventricular septal defect
NIRS	Near-infrared spectroscopy
Oxy-Hb	Oxygenated haemoglobin
Pi	Inorganic phosphate
PLAX	Parasternal long axis
PSAX	Parasternal short axis
pVO_2	Peak oxygen uptake
RER	Respiratory exchange ratio
RQ	Respiratory quotient
ROS	Reactive oxygen species
RV	Right ventricle
RVEDD	Right ventricular end diastolic diameter
SNS	Sympathetic nervous system
SV	Stroke volume
TAPSE	Tricuspid annular plane systolic excursion
TDi	Tissue Doppler imaging
TGA	Transposition of the great arteries
ToF	Tetralogy of Fallot
UCP	Uncoupling proteins
VE	Minute ventilation
VEGF	Vascular endothelial growth factor

VO_2	Oxygen uptake

1. INTRODUCTION

1.1 Eisenmenger Syndrome

1.1.1 Background

In 1897 Victor Eisenmenger described the post mortem findings of a large ventricular septal defect (VSD) and pulmonary hypertension in a patient with a history of cyanosis from infancy who died of haemoptysis aged 32 years[1]. However, Eisenmenger attributed the cyanosis to poor systemic circulation and inadequate ventilation rather than shunt reversal. It was not until 1924, that Abbot and Dawson attributed the cyanosis to shunt reversal[2] and 1947, before pulmonary hypertension was suggested as the cause for the shunt reversal by Bing et al[3]. Subsequently the term Eisenmenger complex was used to describe "pulmonary hypertension at the systemic level of arterial pressure, due to a high pulmonary vascular resistance, with a reversed or bidirectional shunt through a large ventricular septal defect"[4].

Paul Wood subsequently noted that the site of the shunt matters little and, in his Croonian lectures in 1958, coined the term Eisenmenger syndrome to describe the pathophysiology of cyanotic patients with pulmonary hypertension at systemic levels with high pulmonary vascular resistance due to a shunt at atrial, ventricular or arterial level, associated with reversed or bidirectional shunting and cyanosis[4]. He described 12 different anatomical abnormalities that may all develop an Eisenmenger physiology (table 1.1).

1.1.2 Pulmonary Hypertension in Congenital Heart Disease

Some degree of pulmonary arterial hypertension (PAH) is found in 5-10% of adults with congenital heart disease[5] with Eisenmenger syndrome being at the severe end of the

spectrum of pulmonary hypertension. There is a considerable degree of heterogeneity within pulmonary hypertension associated with congenital heart disease, both in the level of pulmonary vascular resistance and in the outcome. Thus patients with similar underlying conditions may develop PAH of variable severity; this stresses the need for accurate classification of ACHD patients with PAH. One method of classification is summarised in table 1.2.

1.1.3 Aetiology of Eisenmenger Syndrome

Progressive histological changes are seen in the pulmonary vasculature in patients with pulmonary hypertension secondary to congenital heart disease; Heath and Edwards described 6 different grades in 1958[6]. Since then it has become clear that increased pulmonary blood flow due to a significant left to right shunt causes endothelial damage by shear stress and circumferential stretch[5] with subsequent release of growth factors. Various growth factors have been linked with the pathophysiological changes seen in PAH including transforming growth factor β. This induces vascular smooth muscle cell (SMC) proliferation and hypertrophy and SMCs are seen extending into the vascular bed and also neointima formation[5;7]. The endothelial damage also causes activation of platelets and leukocytes, increased levels of endogenous vasoconstrictors including endothelin I and thromboxane and decreased levels of vasodilators such as prostacyclin, nitric oxide and vasoactive intestinal peptide[7-9]. These changes eventually lead to vasoconstriction and pulmonary vascular remodelling with occlusion of medium and small pulmonary arteries by excessive cellular proliferation and in-situ thrombi[5;8]. These histological findings are present in all forms of pulmonary hypertension.

With progressive remodelling of the pulmonary vasculature, the pulmonary vascular resistance (PVR) increases and thus the pulmonary artery pressure also rises. Eventually, the pulmonary artery pressure reaches suprasystemic levels and the shunt is reversed (right to left) leading to cyanosis and Eisenmenger syndrome[4;10].

Currently, the abnormalities resulting from endothelial damage that have been translated into clinical practice as targets for intervention are down-regulation of the prostacyclin axis, down-regulation of the NO/cGMP axis and up-regulation of the endothelin axis[11;12]. Future therapies are likely to focus on reversal of the pulmonary vascular remodelling and may involve the final common pathways e.g. tyrosine kinase.

1.1.4 Epidemiology of Eisenmenger Syndrome

In the 1950s Eisenmenger syndrome accounted for approximately 8% of all congenital heart disease[4]. Due to the advent of cardiopulmonary bypass, advances in paediatric cardiology and improved diagnosis, Eisenmenger syndrome is now largely preventable in most children with congenital defects; as a result the incidence has fallen to <4% in modern cohorts in developed countries[13;14]. However, Eisenmenger syndrome is likely to continue to occur at high rates in developing countries where there may be restricted access to paediatric surgery or limited health care sources.

1.1.5 Natural History of Eisenmenger Syndrome

Patients with Eisenmenger syndrome are chronically hypoxaemic with resting oxygen saturations of <90% in room air and with further desaturation on exercise[5]. They are prone to the multi-organ complications of chronic hypoxaemia. These effects are cardiac

(progressive heart failure, endocarditis, paradoxical emboli), haematopoietic (erythrocytosis, hyperviscosity syndrome, neutropenia, thrombocytopenia and bleeding disorder), pulmonary (haemoptysis, pulmonary artery thrombosis), neurological (strokes, cerebral abscesses), renal (proteinuria, progressive renal failure) and metabolic (hyperuricaemia, pigment gallstones and renal calculi)[15].

These complications might be expected to adversely affect the quality of life of patients with Eisenmenger syndrome. However, Daliento et al suggested otherwise when they assessed the natural history of 188 patients with Eisenmenger syndrome [16]. A standardised Ability Index questionnaire was used for functional assessment (appendix 1) which assesses the patient's ability to lead a normal life[17]. In this study, the majority of patients reported a satisfactory Ability Index (1 and 2) suggesting a good quality of life at initial presentation, with one third being in full-time employment. Increasing age is associated with clinical deterioration with 84% of patients reporting a significant reduction of effort tolerance at a mean age of 28± 13.5 (SD) years.

Despite the many effects that chronic hypoxaemia and pulmonary hypertension has on patients with Eisenmenger syndrome, it is also notable that these patients actually do surprisingly well with a reasonable long term prognosis. From the same study by Daliento et al, a survival of up to 55% at 55 years was seen[16]. Further, Diller et al noted that these patients have a good short term prognosis, but overall a reduced life expectancy with a survival at 40, 50 and 60 years of age of 94, 74 and 52% respectively[18]. This is in contrast to patients with idiopathic pulmonary arterial hypertension (IPAH) who have comparable levels of pulmonary hypertension to that seen in Eisenmenger syndrome, but who have an

estimated median life expectancy of three years following diagnosis[19]. Similarly patients with chronic lung disease and persistent hypoxaemia have a 50% three year mortality[20]. Factors associated with a worse prognosis in Eisenmenger syndrome included poor right ventricular function, complex underlying cardiac anomaly, elevated creatinine level and non-cardiac surgery.

1.1.6 Purpose of Work

The purpose of the studies described in this thesis was to investigate the physiological adaptations that have occurred in response to chronic hypoxaemia and pulmonary hypertension in adult patients with Eisenmenger syndrome. Specifically we have looked at the exercise performance, skeletal muscle adaptations and cardiac function in these patients. Whilst these areas have previously been investigated extensively in both patients with different forms of congenital heart disease and other groups with profound hypoxaemia, little is known as to how these patients adapt so well to survive often into their fifth or sixth decade, with a reported good quality of life.

1.2 Exercise Intolerance in Eisenmenger Syndrome

Despite the generally reasonable Ability index score described by patients with Eisenmenger syndrome[16], this group do have severely impaired exercise tolerance when measured objectively with cardiopulmonary exercise testing (CPEX) with peak VO_2 being markedly lower than healthy controls[21-23]. Diller et al compared the exercise capacity of patients with different forms of congenital heart disease[24]. Overall cyanotic patients performed worse than non-cyanotic and those with Eisenmenger syndrome performed the worse of all

with peak VO_2 11.5±3.6ml/kg/min. They found that low peak VO_2 was associated with a poor outcome including increased hospitalisation and death.

Patients with Eisenmenger syndrome are also noted to have an impaired ventilatory efficiency during exercise, as shown by a high VE/VCO_2 slope, with an inverse correlation between resting O_2 saturations and the VE/VCO_2 slope[25;26]. Thus, cyanosis is thought to be a powerful stimulus for the abnormal ventilatory pattern observed during exercise. Whilst a high VE/VCO_2 slope is again known to be associated with a poor outcome and predicts increased hospitalisation rates and mortality in patients with chronic heart failure and non-cyanotic congenital heart disease, Dimopoulos et al did not find this in cyanotic patients[26]. This may be due to different causes of the ventilatory inefficiency in this group of patients such as a significant hypoxic contribution to the ventilatory control.

1.3 The Right Ventricle

1.3.1 Right Ventricular Structure

The right ventricle (RV) is triangular in shape and is formed of three anatomical and functional subunits; the inlet portion, the body and the infundibulum. In normal circumstances it is thin walled with the free wall 3-5mm in thickness[17;27].

The myocardium is composed of a mesh of myocytes within fibrous tissue. The myocytes have a predominantly longitudinal orientation and join with other myocytes to form myofibres[28]. It is these bundles of myofibres that generate the contractile force of the ventricles. The RV differs from the LV in the orientation of the layers of the myofibres. It has

a superficial layer of circumferential or transverse fibres that are arranged parallel to the atrioventricular groove and a deep layer that lie longitudinally from apex to base[28].

The interventricular septum is now known to be the central component of the relationship between the RV and LV[29] and also has an important role in determining RV function, with Saleh et al describing the septum as "the lion of RV function"[30]. The septum is made up of oblique / helical fibres compared to the predominantly transverse orientation of the RV free wall[31]. The septum's resultant twisting action was described by Borelli in 1681 as "like wringing a cloth"[32]. It is the orientation of these myofibres that dictates the contractile force produced by the ventricle[29]. Rushmer et al demonstrated that contraction of only circumferential fibres results in an ejection fraction of 30%[33]. Contraction of oblique fibres, however, results in an ejection fraction of 60%[34].

In normal conditions of low pulmonary vascular resistance (PVR), the inward bellows-like activity of the circumferential fibres, with some contribution from the septum, is able to generate sufficient force to maintain cardiac output[35]. However, as PVR increases, helical fibres assume an increased importance to contract effectively against the increased afterload[30].

1.3.2 Right Ventricular Function in Eisenmenger Syndrome

Patients with severe pulmonary hypertension (PHT) due to Eisenmenger syndrome have a better survival when compared to patients with idiopathic or acquired forms of PHT, despite an associated cardiac anomaly and chronic hypoxaemia[36;37]. Hopkins hypothesised that the key to their relative longevity lies in the adaptations undergone by the RV[37]. In

Eisenmenger syndrome, RV function is typically preserved and clinical heart failure uncommon[38-40]. The majority of deaths are secondary to intrapulmonary haemorrhage or rupture of a major vessel[41].

Throughout gestation the fetal heart has equal systolic pressures in the aorta, pulmonary artery, left ventricle (LV) and RV due to a non-restrictive ductus arteriosus. Thus, there is equal RV and LV wall thickness in utero[42]. In normal hearts, changes begin shortly after birth with regression of the RV wall due to the closure of the ductus arteriosus and reduction in right sided pressures[37]. However, in the presence of a persistent and large left to right shunt, the RV appears to be "primed" in a pre-Eisenmenger phase and adapts for a lifetime of functioning at systemic pulmonary pressures[43]. The hearts of adults with Eisenmenger syndrome resemble fetal hearts rather than normal adult hearts with equal RV and LV wall thickness. This is in comparison to IPAH, for example, in which normal regression of RV wall thickness occurs in infancy.

1.3.3 Right Ventricular Function in Other Conditions with High RV Afterload

The RV structure and function in other conditions associated with elevated RV pressures have been previously studied. Sanchez-Quintana et al compared the ventricular myoarchitecture of hearts from patients with unrepaired tetralogy of Fallot (ToF) and normal hearts. They found that the RVs of the patients with ToF have a more oblique arrangement of their superficial fibres and also an extra middle layer of circumferential fibres[44]. They hypothesise that these adaptations reduce radial wall stress and may preserve short axis function. Pettersen et al demonstrated that the systemic RV in patients with a previous atrial switch for transposition of the great arteries (TGA) have a predominant circumferential over longitudinal systolic

function[45]. This is in contrast to normal circumstances when the majority of the force generated from the RV is through longitudinal deformation. This again may be due to increased circumferential fibres in the RV myocardium. Tan et al studied RV function in patients with TGA who have undergone an atrial switch, congenitally corrected TGA (ccTGA) and IPAH[46]. They show that patients with ccTGA had overall better RV function due to the RV adapting to the increased afterload from birth and having faced no surgical insults.

In Eisenmenger syndrome, RV failure has been shown to be associated with a poor outcome[16;16] and is the main cause of death in PAH from all causes[47].

1.4 Skeletal Muscle

1.4.1 Skeletal Muscle Structure and Function

The principal roles of skeletal muscle are to allow skeletal movement and give support to the skeleton to maintain posture. Skeletal muscle is made up of multiple cylindrical muscle fibres, each containing multiple nuclei and mitochondria, arranged close to the sarcolemma in the longitudinal axis of the muscle[48]. Within skeletal muscle there are both thick filaments, containing predominantly myosin, and thin filaments, containing mainly actin[48]. These filaments are arranged into bundles, known as myofibrils, and it is these myofibrils that give the characteristic striated appearance of skeletal muscle.

Myosin has projections, known as cross bridges, which bridge the space between adjacent thick and thin filaments[48;49]. These cross bridges generate force during contraction of the

muscle by making contact with the actin of the thin filaments. The resulting movement of the thick and thin filaments over one another is termed the sliding filament mechanism[48].

Blood vessels run between the fibres with arterioles supplying blood to a capillary network with the capillaries running alongside and occasionally over the muscle fibres[48;49]. The vascular bed functions to oxygenate muscle fibres and the muscle fibre's oxidative capacity correlates strongly with the capillary density[50].

Different fibre types exist within skeletal muscle and can be distinguished by their ATP forming pathways and maximum velocity of shortening[51]. Type I fibres are "slow" fibres and have numerous mitochondria with a high capacity of oxidative phosphorylation (thus termed slow-oxidative). The fibres are surrounded by numerous capillaries supplied by a dense network of arterioles that deliver O_2 and fuel at high blood flow. Type I fibres contain high levels of myoglobin, an O_2-binding protein that binds O_2 very effectively at low PO_2 values. Type I fibres resist fatigue and are thus useful in endurance exercise. Type II fibres are "fast" and larger in diameter. They have fewer mitochondria, increased concentrations of glycolytic enzymes as well as significant glycogen stores. They have the capacity to use both oxidative and anaerobic metabolism depending on the subtype. They have a less dense arteriolar and capillary supply and the fibres have little myoglobin, which reflects their limited use of O_2. In humans the main subtypes are type IIa (fast-oxidative) which have an intermediate resistance to fatigue and type IIb fibres (fast-glycolytic) which fatigue rapidly.

1.4.2 Energy Metabolism in Striated Muscle

Muscle contraction is an active process requiring energy and this energy is generated predominantly by adenosine triphosphate (ATP) hydrolysis which breaks the high energy bond to release energy as shown in the following equation:

$$ATP + H_2O \rightarrow ADP + Pi + H^+ + energy$$

Each contracting muscle fibre requires vast amounts of ATP (approximately 2500 ATP molecules per second per thick filament)[49]. However, resting fibres have only small reserves of ATP and therefore, further mechanisms are required to generate the required ATP during contraction. The generation of ATP within the mitochondria and muscle fibre is shown in figures 1.1 and 1.2.

1.4.2.1 Phosphorylation of Adenosine Diphosphate (ADP) by PCr Hydrolysis

At the onset of muscle contraction PCr is rapidly hydrolysed and as a result ADP is phosphorylated to form ATP, a reversible reaction catalysed by creatine kinase (CK).

$$PCr + ADP \underset{CK}{\rightleftharpoons} ATP + Creatine$$

This transfer of energy is very rapid as it is a single enzyme reaction. Levels of ATP in the muscle fibre therefore change little whereas PCr concentrations drop quickly. This process is limited by cellular PCr concentrations.

1.4.2.2 Oxidative Phosphorylation of ADP in Mitochondria

This is the final stage in the aerobic metabolism of glucose, fatty acids and amino acids and occurs at the inner mitochondrial membrane, only in aerobic conditions. It provides up to 95% of the ATP demands of the resting cell[52]. Hydrogen atoms are transferred through a series

of oxidation and reduction reactions known as the electron transport chain. The net result is the combining of protons and electrons with O_2 to form water and the release of ATP.

1.4.2.3 Substrate Level Phosphorylation of ADP by Glycolysis in the Cytosol

Glycolysis is the pathway that generates pyruvate from glucose. It is an anaerobic process and generates just 2 molecules of ATP. In aerobic conditions the pyruvate enters the TCA cycle and subsequently undergoes oxidative phosphorylation. In conditions when the availability of O_2 is exceeded by the rate of glycolysis however, the conversion of pyruvate to acetyl CoA is inhibited, through the inhibition of the pyruvate dehydrogenase enzyme complex, and the pyruvate is converted to lactic acid. Glycolysis is important when the mitochondrial production of ATP is limited by the availability of O_2. The production of lactate has a low yield of ATP per molecule of glucose, with just 2 ATP molecules being produced compared to 34 when oxidative phosphorylation can proceed.

1.4.2.4 Fuel for ATP Production

From the above it can be seen that the dominant fuels used in human skeletal muscle are glucose (both blood borne and from the breakdown of glycogen stores) and fatty acids (from muscle triglyceride stores and from plasma) through β-oxidation. Although fatty acids have a higher ATP yield per molecule compared to glucose (129 moles ATP per mole of palmitate versus 38 moles per mole of glucose), they do so at a higher O_2 cost. The ATP synthesised/ O_2 consumed ratio for glucose is 3.19 whereas for palmitate, it is 2.80[53].

1.4.3 Skeletal Muscle Metabolism and Exercise

1.4.3.1 Skeletal Muscle ATP Generation During Exercise

At the start of exercise the rapid hydrolysis of PCr provides the required ATP, allowing time for the slower pathways of glycolysis that utilise multiple enzymes and also for oxidative phosphorylation to increase ATP production, and because this hydrolysis does not require O_2 there is an initial lag of around 45 seconds before O_2 consumption reaches steady state during fixed rate exercise [54]. The PCr stores become depleted rapidly with sustained contraction with levels depleted by >60% after 9 seconds of intense exercise[55]. Thus with longer duration exercise, there is an increased demand for ATP from other sources along with an increase in O_2 consumption. In health, O_2 availability is not a limiting factor and in steady-state exercise, ATP production relies primarily on aerobic metabolism through oxidative phosphorylation[56-58]. However, at peak exercise, the maximal mitochondrial ATP production is limited by O_2 availability and increasing levels of ATP are produced by anaerobic glycolysis, with recruitment of fast-glycolytic fibres[59].

Immediately following exercise the O_2 demand is still elevated. This is termed the O_2 debt and refers to the amount of O_2 needed to restore pre-exertion conditions including PCr, ATP and glycogen concentrations[49]. In healthy subjects, O_2 is now readily available and PCr recovery is primarily aerobic[60;61]. Thus, PCr resynthesis reflects mitochondrial oxidative capacity with PCr resynthesis being faster in slow fibres with high oxidative capacity, than in fast glycolytic fibres[62]. It is also faster, following submaximal exercise, in exercise-trained subjects than in sedentary subjects, due to their increased muscle oxidative capacity[63-65]. Haseler et al also showed that in trained athletes, the PCr recovery rate was limited by O_2 delivery and can be increased by giving supplementary O_2. This contrasted with the situation

in sedentary subjects in whom supplementary O_2 did not change PCr recovery indicating that in them the rate of recovery is limited by the mitochondrial oxidative capacity[63;66].

1.4.3.2 Fuel Utilisation during Exercise

At rest and during moderate exercise (up to 60% of mVO_2), fats are the primary source of fuel for skeletal muscle[59;67;68]. With increasing exercise intensity and duration, glycogenolysis and glycolysis increase and there is increased recruitment of fast-glycolytic fibres. Thus, there is an increased reliance on the more oxygen-efficient use of carbohydrates[59].

The rate of skeletal muscle oxidation is related to the mVO_2, oxidative power, fibre type composition, insulin sensitivity, size of fuel stores, exercise intensity and duration, sympathetic nervous system activity and substrate availability[69].

It is well known that phenotypical adaptations of skeletal muscle occur in endurance athletes. These include an increase in fatty acid oxidation with less lactate production and less utilisation of glycogen and blood glucose with matching of glycolysis to TCA cycle-turnover, during exercise of a given intensity[59;70]. Muscle biopsies from endurance trained athletes show a higher mitochondrial content, increased capillary density and increased oxidative enzymes[69;71].

1.4.4 Control of Mitochondrial Respiration

Under conditions of aerobic work, oxygen consumption and major ATP production occur in the mitochondria. The main parameters that control oxidative metabolism are oxygen and

substrate supply and the intracellular transfer of energy, most importantly the creatine / phosphate (Pi) system[72-74].

Working muscle needs to regulate the steps of ATP production so that production is equal to utilisation and homeostasis is obtained when this occurs[75;76]. The sum of the concentrations of PCr and Pi is constant during exercise and, providing pH is constant, [Pi]/[PCr] is proportional to [ADP][60]. Chance et al showed that the principle role in the control of oxidative metabolism is by ADP rather than ATP or phosphate. ADP exhibits a negative feedback on ATP synthesis[74;75] with ATP synthesis having a hyperbolic dependence on [ADP][60]. PCr recovery following exercise, which reflects the mitochondrial oxidative capacity, is also limited by oxygen availability and intracellular acidosis with pH being responsible for altered states of the CK reactions[77].

1.4.5 Skeletal Muscle Fatigue

Fatigue is defined as "any decline in muscle performance associated with muscle activity and may manifest as a decreased peak force or a decreased ability to maintain a force"[78]. There is both central and peripheral fatigue arising proximal and distal to the neuromuscular junction respectively[79]. For the purpose of this discussion only peripheral fatigue is considered below as our study has looked at skeletal muscle adaptations.

The development of peripheral fatigue is considered to be mediated by metabolic by-products that accumulate during muscle contraction, the rate of their accumulation being O_2-dependent[79]. That at least part of the mechanism was O_2-dependent is also shown by Fordy and Marshall who demonstrated that breathing 40% O_2 rather than air during the recovery

from a period of maximum voluntary forearm contraction to exhaustion greatly improved the time to exhaustion during a second period of forearm contraction[80]. The substances currently implicated in the development of fatigue are potassium ions (K^+), inorganic phosphate (Pi) and hydrogen ions (H^+). Lactate, produced as a result of anaerobic metabolism, was also traditionally implicated in the development of fatigue. However, muscle lactate levels have been shown to poorly correlate with fatigue and so is an unlikely contributor[81;82]. Reactive oxygen species (ROS) have also been linked to fatigue and their role is also briefly discussed below in 1.4.5.4.

1.4.5.1 K^+ and Fatigue

Increasing concentrations of K^+ ions are seen both within muscle interstitium[83] and arterial and venous blood[84] during exercise. K^+ is released from muscle fibres during exercise as a result of the K_{IR} channels remaining open in response to decreasing ATP levels. K^+ is then taken back up by the cell via Na^+/K^+-ATPase[85;86]. A number of human studies have linked a rise in extracellular K^+, as evidenced by increased interstitial K^+ or plasma concentrations, with a decreased time to fatigue[83;87;88]. Further, Clausen and Nielsen demonstrated that pre-incubation of rat skeletal muscle with 10mM K^+ caused a decrease in initial force and an increased rate of force decline in muscle relative to that seen when incubation in 4mM K^+ solution was performed[89].

1.4.5.2 Inorganic Pi and Fatigue

Pi is produced during muscle contraction due to the breakdown of PCr, via ATP hydrolysis. Pi accumulation occurs when the supply of ATP from the breakdown of PCr exceeds the consumption of ATP[78;90].

Pi is implicated in both phase I and III of fatigue (see Allen DG 2009 for full review of the phases of fatigue)[91]. In phase I there is an initial reduction in force to 80-90% of control due to the inhibitory effect of the increasing Pi concentrations on the myofibril cross bridges. In phase III, a further reduction in force is caused by the attenuation of the contractile proteins' sensitivity to calcium (Ca^{2+}) as well as a decrease in the release of Ca^{2+} from the sarcoplasmic reticulum, both due to the elevated Pi concentration[78].

The association between Pi and fatigue is well documented. Cooke et al demonstrated that injecting increasing concentrations of Pi to rabbit psoas muscle fibres resulted in a decreased isometric force. Further to this, studies on CK knockout (CK -/-) mice by Dahlstedt et al showed that, although the CK-/- mice had higher resting Pi levels, Pi did not increase with exercise and these mice, on exercise, showed reduced fatigability.

1.4.5.3 H^+ and Fatigue

H^+ accumulates in muscle when ATP hydrolysis exceeds the rate of mitochondrial respiration due to the following reaction [90]:

$$ATP + H_2O \rightleftharpoons ADP + Pi + H^+$$

Traditionally, accumulation of H^+ during muscle contraction was implicated in the development of fatigue with single fibre studies showing a decreased shortening velocity and

isometric tension with decreasing pH[92;93]. However, more recent studies have shown poor temporal correlation between H^+ concentration and fatigue and, as a result, H^+ are considered unlikely to be directly responsible for peripheral fatigue at physiological temperatures[94;95]. H^+ may play an indirect role by stimulating group III and IV nerve afferents to cause discomfort during exercise[96].

1.4.5.4 ROS and Fatigue

ROS are produced in active muscle and there is increasing support that they contribute to fatigue[78]. ROS produced in the muscle include superoxide, hydrogen peroxide, hydroxyl radicals and peroxynitrate, formed by the interaction between nitric oxide and superoxide. Their production is increased with increasing exercise intensity[97] and also temperature[98-100].

The infusion of ROS scavengers, including N-acetyl cysteine (NAC) and superoxide dismutase (SOD), into isolated muscle preparations have demonstrated a smaller reduction in force and slower fatigue with intermittent contractions[101;102]. Moopanar et al demonstrated that the sensitivity of the muscle to fatigue increased with temperature, in a way that is consistent with increased ROS production[103]. These findings have been confirmed in human studies with Medved et al showing an increased time to volitional fatigue in subjects infused with NAC during submaximal cycling[104].

Despite the growing evidence for ROS and fatigue Allen et al note that uncertainties remain including the source of the ROS, which particular ROS is responsible and the mechanism by which they cause fatigue[78].

To date, the substances implicated in peripheral fatigue have not been investigated in Eisenmenger syndrome.

1.4.6 Skeletal Muscle Changes in Disease

As indicated above, production of cellular energy by oxidative tissues occurs primarily by oxidative phosphorylation. Therefore, it follows that there must be a constant supply of both fuels and O_2 for normal cellular function to occur. O_2 supply may be impaired in different disease states and this may be due to hypoxaemia and / or a decreased delivery of O_2 to the tissue. Hypoxaemia occurs in pulmonary diseases, for example chronic obstructive pulmonary disease, due to ventilation – perfusion (V/Q) mismatching and hypoventilation, or in the presence of a right to left shunt such as that seen in Eisenmenger syndrome. Reduced O_2 delivery may be caused by decreased O_2 carrying capacity in anaemia or impaired perfusion of the tissue in heart failure or shock. It may also occur in the healthy individual at high altitudes due to the reduced barometric pressure of the atmosphere. Skeletal muscle changes have been well documented in chronic heart failure (CHF) and chronic obstructive pulmonary disease.

1.4.6.1 Chronic Heart Failure

Exercise intolerance in patients with CHF is common, however it does not correlate well with central haemodynamics or left ventricular ejection fraction[105-107] suggesting peripheral abnormalities. Both reduced skeletal muscle blood flow and skeletal muscle mass have been demonstrated in patients with CHF[108], while biopsies show reduced mitochondrial density, fewer capillaries per fibre and an increase in type-IIb glycolytic fibres with a corresponding

decrease in type I oxidative fibres[109-111];. ^{31}P-MRS studies have shown a greater magnitude of PCr depletion, a decreased pH and a slower PCr recovery time following regional exercise in these patients[112-114]. These changes correspond with the systemic exercise intolerance shown by Okita et al[112]. Further, Hanada et al showed that PCr resynthesis was not significantly limited by haemoglobin re-saturation following exercise, which suggests that O_2 utilisation has a bigger role in recovery than O_2 delivery[113]. It has also been demonstrated that in skeletal muscle of CHF patients there are decreased levels of aerobic mitochondrial enzymes involved in terminal oxidation eg citrate synthetase but normal levels of glycolytic enzymes[110;115;116]. These results suggest a myopathy of metabolic origin in CHF with the decreased aerobic activity playing an important role in mediating the premature anaerobic metabolism and lactate accumulation seen during exercise[115].

1.4.6.2 Chronic Obstructive Pulmonary Disease

COPD is a chronic debilitating condition that is also characterised by exercise intolerance. Traditionally the cause of the exercise limitation was considered to be primarily ventilatory, but more recently skeletal muscle dysfunction has been reported[117] with the true cause likely to be more complex with both ventilatory limitation and a peripheral component contributing[118]. Similar changes have been described as seen in CHF with muscle atrophy and decreased muscle strength[119], increased fatigability[117], a change in fibre type composition favouring glycolytic type-IIb fibres[120], reduced capillaries per fibre[121], a greater lactate accumulation on exercise[122] and a decrease in oxidative enzyme capacity[123]. ^{31}P-MRS studies have also shown a similar increased magnitude of PCr depletion with an increased PCr recovery time and reduced pH[124].

1.4.6.3 Eisenmenger Syndrome

Skeletal muscle of patients with Eisenmenger syndrome has not previously been studied. It is known that these patients have similar degrees of exercise intolerance to patients with end stage heart failure[24] but whether they have the same metabolic myopathy is unknown. Also, as well as having abnormal central haemodynamics like in CHF, they also have profound hypoxaemia. The potential effects of this are discussed below.

1.5 Hypoxia

1.5.1 Background

Hypoxia is defined as "a decrease in tissue O_2 supply below normal levels"[71]. Hypoxaemia denotes a decreased oxygen tension in the blood, that is a PaO_2 less than normal[71;125]. The term dysoxia is used to define a deficiency at a cellular level and the PO_2 at which this occurs must be defined[71]. Below this is a region of PO_2 in which VO_2 is limited by the availability of O_2 and metabolic adaptations must occur to support mitochondrial ATP production. P_{Crit0} is the lowest PO_2 that can be considered normal and is tissue specific. Below this ATP can only be produced by glycolysis with a resultant increase in lactate production. Mitochondria require PO_2 of 0.13-0.4kPa to generate ATP to maintain aerobic cellular function[20].

Hypoxia is an adverse environmental condition that organisms need to adapt to [126]. Chronic and systemic hypoxia can be considered physiological, that is not as a result of disease, in both the fetus and at high altitude[127].

Grocott et al hypothesise that "hypoxic adaptive processes are likely to be common to tissue hypoxia whatever the cause", such as that occurring in critical illness[128]. Much of the work

into how the body adapts to hypoxia has been performed using healthy individuals who are exposed to hypoxia through altitude. With increasing altitude the barometric pressure falls and the partial pressure of O_2 (PO_2) decreases linearly with the PO_2. At the summit of Everest this is just 30% of that at sea level[129]. With falling inspired FiO_2 resting arterial O_2 saturations also decrease, down to 92% at 3000m and to 80% or lower at 5000m with an ambient PaO_2 85mmHg[130]. However, other stressors such as low ambient temperature and exercise, must be taken into account when looking at the effects of altitude[127].

Groups studied include those living at altitude and low land dwellers visiting altitude. The World Health Organisiation, in 1996, estimated that there were approximately 140 million people living at altitudes over 2500m including several areas at over 4000m[131]. Hoppeler et al used the following nomenclature to describe the hypoxic exposure of different groups[132]. Native to hypoxia implies that the resident population has been exposed to hypoxia for many generations and may have genetic modifications to improve performance at altitude. This includes the Tibetan Sherpas and the Quechas from the Andes, Peru. Permanent hypoxia is used for subjects who live under hypoxic conditions from birth until death, for example residents of La Paz, Bolivia, parts of which are 4000m above sea level. Long term hypoxia is used for individuals who are brought into hypoxic environments for several weeks or months and thus may acclimatise, such as on mountaineering expeditions. Short term hypoxia describes short bouts of exposure to hypoxia, lasting minutes to hours, with normoxic conditions between.

1.5.2 Hypoxaemia in Eisenmenger Syndrome

Patients with Eisenmenger syndrome are permanently cyanotic from infancy until death. They are characterised by persistence of a relatively intact overall physiological performance despite the presence of PO_2 and SaO_2 values that must be close to the limits of survival. In addition they have apparently normal brain function, moderate exercise capacity and good long-term survival suggesting that this group of patients must have adapted to the marked hypoxaemia[16]. The sections below outline some of the physiological adaptations to hypoxia in humans.

1.5.3 Physiological Adaptations to Chronic Hypoxia

Adolph defined adaptations as "modifications of organisms that occur in the presence of particular environments and circumstances" and were not necessarily limited to those that seem favourable to the individual[133]. In this thesis I have used the term "adaptation" to mean physiological changes that result from chronic exposure to hypoxia and have not covered responses resulting from acute hypoxia.

1.5.4 Oxygen Kinetics

O_2 must be extracted from the air at the alveoli and delivered to the cells for metabolism. O_2 delivery is the product of cardiac output and the O_2 content (CaO_2)[134]. CaO_2 can be altered by changes in the ventilation, pulmonary blood flow and Hb concentration. Cardiac output is discussed further below and is not covered here.

1.5.4.1 Oxygen Content

Ventilation Ventilation increases progressively with altitude and there is a concomitant decrease in arterial PCO_2 and alveolar CO_2 i.e. increased alveolar ventilation [135;136]. Sutton et al demonstrated a fourfold increase in ventilation during a simulated climb of Mount Everest and this was predominantly due to an increased frequency (2.4 fold increase) rather than tidal volume which only increased by 1.3 fold[135]. Increased ventilation is a response to decreased PaO_2 not O_2 saturations or content and is mediated by peripheral arterial chemoreceptors in the carotid bodies[125]. This hypoxic ventilatory drive declines with acclimatisation to altitude, ageing and is also blunted in cyanotic congenital heart disease[125]. Chua et al have also demonstrated a blunted hypoxic chemosensitivity in chronic hypoxaemia secondary to cyanotic congenital heart disease[137].

Haemoglobin Concentration Hypoxia activates sensors within the kidneys which increases the expression of erythropoietin, augmenting the red blood cell mass and Hb through an increase in erythropoiesis[138;139]. The majority of O_2 in the blood is carried bound to Hb and there is only a small amount dissolved in plasma, each gram of Hb carrying 1.31ml O_2 of when fully saturated[134]. The increase in Hb therefore helps to normalise the O_2 content (O_2 carriage), augmenting peripheral O_2 delivery.

Cerretelli et al demonstrated a 40% increase in Hb and RBC count with a concomitant increase in haematocrit after an expedition to Everest base camp (altitude 5350m)[140]. This increase in Hb was also shown by Radgran et al who showed that Danish lowland dwellers at altitude increased their Hb to similar level as Aymara natives who live in the Andes at altitude

of 5260m and he concluded that O_2 delivery to periphery is secured primarily by increase in Hb[141].

Pulmonary circulation In response to alveolar hypoxia, small "resistance" pulmonary arteries actively vasoconstrict [142;143]. This results in blood being diverted away from poorly ventilated regions of the lung. This results in improved ventilation – perfusion matching, decreased shunt fraction and an improved systemic PO_2. This process is modulated upstream by the endothelium and downstream by Ca sensitisation of the contractile apparatus, in turn controlled by rho kinase[143]. The mitochondria likely act as sensors in this process[142]. The vasoconstriction is rapid, occurring within seconds of moderate hypoxia and results in an elevated pulmonary vascular resistance. In the acute setting it is immediately reversed by breathing O_2. However, Groves et al, in their simulated Everest expedition "Everest II", showed that lowlanders at altitude for 2-3 weeks developed a degree of pulmonary hypertension, not fully reversed by breathing O_2, suggesting that vascular remodelling has occurred[144]. The physiological effects of this pulmonary vascular remodelling was shown by Penaloza et al who demonstrated increased smooth muscle cells in the distal pulmonary artery branches and associated right ventricular hypertrophy in healthy high altitude dwellers [145].

1.5.4.2 Oxygen Delivery

On initial exposure to altitude a decrease in arterial O_2 delivery to muscle during submaximal exercise is observed but, whilst SaO_2 and erythropoiesis increase with acclimatisation, the overall delivery of O_2 to tissues does not increase accordingly[130]. After acclimatisation at 5260m, Calbet et al demonstrated that the haemoglobin concentration had increased by 36%

with a 21% increase in resting CaO_2 thus resting O_2 delivery to muscle can reach similar levels as seen prior to exposure[146]. However, during maximal exercise the systemic O_2 delivery was 10% lower than at sea level and this was thought to be due, at least in part, to a reduction in cardiac output (CO)[147].

Increased arterial O_2 content and SaO_2 are offset by a decrease in the CO during exercise and decreased muscle blood flow[148;149]. Calbet et al demonstrated that blood flow to exercising muscles reduced from 6.61l/min to 4.81l/min after 8 weeks of exposure to 5250m[150]. A higher vasoconstriction may contribute to the observed decrease in muscle blood flow; mean arterial pressures, systemic vascular resistance and leg vascular resistance are all elevated and there is a parallel increase in plasma noradrenaline levels[148;149]. Other reasons for the reduction in blood flow to muscles include a reduction in CO, a lower proportion of the CO supplying the exercising muscles, an increased haematocrit and decreased vascular reactivity to endogenous vasodilator substances[150].

1.5.4.3 Oxygen Extraction

The reduction in O_2 delivery and cardiac output is compensated for by an increase in tissue O_2 extraction as shown by an increase in the arteriovenous O_2 differential[130;135;151]. O_2 extraction is not impaired with altitude exposure with the offloading and diffusion of O_2 from the Hb to the mitochondria being helped by a shift to the right of the O_2 dissociation curve and an increase in the capillary density[150;152]. This allows cellular O_2 delivery to match demand despite the reduction in systemic O_2 delivery. Thus, the exercising muscles are capable of extracting the required O_2 during submaximal exercise[130]. However, as exercise

intensity increases and maximum extraction levels have been reached, a reduction in VO_2 will be seen.

1.5.5 Oxygen Consumption and Exercise

In the presence of hypoxia, adaptations of both physiological and metabolic processes must occur to allow adequate oxygenation of tissues in the event of further stresses, such as that imposed by exercise, occur. Hypoxia and exercise will have an accumulative effect that will influence the maximal exercise capacity and performance.

Exercising at altitude, compared to sea level, results in an increase in the relative exercise intensity, as well as a more pronounced disturbance of homeostasis[153]. The primary reason is that although a given submaximal exercise task results in a similar VO_2 at both sea level and altitude, the decline in VO_2 at increasing altitudes means that it represents a higher proportion of the maximal VO_2[130]. Thus, exercise performed at sea level will represent a higher proportion of the maximal exercise intensity when performed at altitude.

At sea level there is a tight relationship between CaO_2 and mVO_2 and, in normoxia, mVO_2 is set by O_2 transport predominantly (75%) and peripheral factors in the remaining 25%[154]. This relationship is diminished at altitude with the capacity for exercise decreasing progressively with increasing altitude[155] and the mVO_2 changes little after acclimatisation[140]. This is despite a marked increase in O_2 carrying capacity.

Cerretelli et al demonstrated a decreased mVO_2 in acclimated low land dwellers and high altitude natives[140]. They then showed that the mVO_2 improved slightly by giving FiO_2 1.0

but not to sea level values. They concluded that it is not due to cardiovascular O_2 transport but changes in peripheral factor including the attenuation of effective blood flow to working muscles.

It is now generally accepted that mVO_2 is not limited by a single factor, as previously believed, but by multiple factors[156] including cardiovascular O_2 transport, lungs (such as ventilation and / or diffusion) and peripheral factors including reduced muscle blood flow, O_2 conductance[152] and reduced oxidative capacity of the skeletal mass[157;158].

1.6 Skeletal Muscle and Hypoxia

1.6.1 Skeletal Muscle Adaptations

Alterations in the delivery of O_2 to skeletal muscle can result in tissue hypoxia, leading to a wide range of metabolic abnormalities and ultimately culminating in contractile failure[159]. Skeletal muscle is able to show marked phenotypic plasticity in response to hypoxia; its key problem being able to sustain sufficient levels of aerobic metabolism despite reduced O_2 availability[160].

One of the first papers looking at skeletal muscle changes in hypoxia was by Reynafarjee in 1962. This reported an increase in oxidative enzyme activity and myoglobin concentration in Andean altitude natives (Peruvian miners) compared to low-land dwellers[161]. This led to the assumption that muscle oxidative capacity is increased in the presence of altitude hypoxia thus compensates for the environmental hypoxia[161]. These finding of high oxidative enzymes and myoglobin are now known to be likely due to the training status of the subjects as exercise is also known to be a stimulator of mitochondrial enzyme activity[132].

Subsequent studies have shown a decrease in the oxidative capacity with decreased concentrations of oxidative enzymes in skeletal muscle of low land dwellers exposed to hypoxia for prolonged periods[159;162;163]. In 1990 Hoppeler et al also demonstrated a 20% reduction of mitochondrial volume density in acclimatised lowlanders[164]. However, as the same capillary bed length remained, the O_2 supply for the remaining mitochondria is likely to be improved. A similar decrease in oxidative capacity, along with a corresponding fall in capillarity, is seen in permanent high altitude residents[165;166]. Compared to lowland dwellers, however, they have low intracellular lipid deposits that did not increase with training[166;167]. The authors suggest that this may represent a shift towards favouring carbohydrates for metabolism over lipids with permanent exposure to high altitude (see below)[168].

Studies from lowland dwellers exposed to high altitude have also demonstrated reductions in cross-sectional area of muscle fibres. Hoppeler et al described a 20% reduction in the vastus lateralis muscle after 8 weeks [164]. Further, MacDougall et al in the simulated Everest II expedition showed 25% and 26% reductions in type II and type I fibre cross-sectional areas respectively[163]. A corresponding 9-12% increase in capillary density has also been demonstrated however the capillary-to-fibre ratio remains unchanged suggesting this increased density is due to the muscle fibre atrophy rather than capillary neo-formation[159;163;164]. This increased capillary density results in an improved O_2 diffusion as the same size capillary bed serves a smaller muscle volume which may, in part, compensate for the reduction in oxidative capacity. Increased skeletal muscle lipofuscin inclusions were

also found after return from high altitude expeditions[169]. Lipofuscin is a mitochondrial degradation product and prevails under conditions of high radical formation[170].

Sherpas and Quechas, who have phylogenetic adaptations to altitude, display defence mechanisms to hypoxia that have arisen, both independently and by positive selection[171]. These include a preponderance of slow type fibres[172;173], a reduction in muscle oxidative capacity as measured by mitochondrial density and oxidative enzymes [156;173] and up regulation of oxidative rather than glycolytic contributions to metabolism[174]. Kayser et al also demonstrated that, despite a low mitochondrial density, Sherpas actually have a higher maximal O_2- consumption-to-mitochondrial volume than lowland dwellers. Possible reasons for this include tighter coupling between ATP supply and demand and better metabolite homeostasis[132]. This suggests that, whilst lowlanders do not tolerate spending long times at high altitude, native high altitude dweller seem to have adapted allowing them to function more successfully in hypoxic environments.

1.6.2 Metabolic Adaptations

Hypoxia-inducible factor 1(HIF-1) is a key regulator involved in the response to hypoxia in most mammalian cells[175]. It is a heterodimer with a dominant α subunit[132]. HIF-1α is required for a variety of physiological responses to chronic hypoxia. On exposure to hypoxia HIF-1α is rapidly stabilised and accumulates in the nucleus[176;177]. It induces the expression of a variety of hypoxia-inducible genes including those that enhance glucose metabolism, such as glucose transporters including GLUT-1[178] and glycolytic enzymes[179]. Its targets also include non-metabolic targets including erythropoietin and the angiogenic factor VEGF, both of which will increase O_2 delivery to tissue through increases

in O_2 carrying capacity and vascular density[132;180]. Thus, HIF-1α expression may play a key role in the adaptation to hypoxaemia in patients with Eisenmenger syndrome which to date has not been investigated.

Glucose utilisation is more O_2 efficient than fatty acid utilisation in that it produces 6-6.3moles of ATP per mole of O_2 oxidised compared to 5.6mole for fat[181]. Thus, increased utilisation of CHO over fats, would aid the maintenance of homeostasis by optimising the energy produced per mole of O_2. A switch to an increased reliance on CHO in hypoxic conditions would appear to be advantageous and previous work has shown an increased reliance on glucose for metabolism both during rest and during exertion after acclimatisation to altitude[182-184]. These earlier studies, however, did not consider the possible impact that the intensity of the exercise preformed may have. The effect of exercise intensity on substrate utilisation is well documented being first reported in 1896 by Chaveau[185] and confirmed in 1939 by Christensen and Hansen[186]. This led to the suggestion that the switch to an increased utilisation of glucose was related to increasing exercise intensity not hypoxia[187;188].

In 2002 Lundby et al studied the effect of submaximal exercise, of different intensities, in low land dwellers[189]. The initial studies were performed during acute hypoxia and subsequently after acclimatisation to >4000m of altitude. Their results show that substrate utilisation, during submaximal exercise, is similar during both acute and chronic hypoxia, if the work rate is adjusted for the relative sea level maximal VO_2 i.e. hypoxia per se does not increase carbohydrate utilisation. They concluded that the previous increased CHO oxidation reported by others was most likely secondary to higher relative exercise intensity. Similar results were

obtained on rats by McClelland et al, who also suggested that the O_2 saving advantages of CHO is outweighed by limited CHO stores[190].

A difference between sexes was reported by Braun et al in 2000. It is known that at sea-level, women show increased preference for lipid metabolism and decreased preference for CHO and protein, as compared to men of similar training status[191]. This is due to the metabolic regulatory effects of oestrogen and progesterone and is most noticeable in the mid-luteal phase. Braun studied substrate utilisation during sub-maximal exercise in women at an altitude of 4300m. He found that blood glucose utilisation rates were lower at rest but did not differ from those recorded at sea-level during sub-maximal exercise [192].

The adaptations of skeletal muscle in patients with Eisenmenger syndrome have not previously been studied. It is feasible though that they have undergone similar adaptations as described above which may account, in part, for their known severe exercise intolerance.

1.7 Cardiovascular System and Hypoxia

1.7.1 Cardiac Adaptations

Prolonged exposure to hypoxia can trigger cardiovascular defence mechanisms, such as increased erythropoiesis and angiogenesis to increase O_2 delivery as discussed above. It can also promote adaptive cardiac metabolic remodelling[180]. This includes an increased reliance in carbohydrates for metabolism and an augmented mitochondria respiratory capacity[193]. HIF-1 plays a central role in this switch to increased glucose metabolism in cardiac muscle. As indicated above, in hypoxia it becomes stabilised to the heterodimer HIF-1α that induces the expression of GLUT1 and other glycolytic genes[194]. Alongside this

there is also activation of other factors to induce a "fetal gene programme" with increased reliance on carbohydrates and a reduction of fat metabolism[180]. Thus, there is increased efficiency of energy production and an improved capacity of the mitochondria to sustain cardiac contractile function despite hypoxia.

Hypoxia becomes pathophysiological, however, when it exceeds the host organism's defence apparatus. This leads to disruption of the homeostatic balance leading to maladaptive signalling cascades and myocardial damage. It is known that hypoxia plays a major role in cardiac pathophysiology, including acute coronary syndromes and cor pulmonale.

Myocardial performance in hypoxic environments is dependent on the acclimation state[195] with previous biochemical studies on Sherpas showing evidence of increased glucose preference by the heart[196]. This was confirmed in an in vivo and non-invasive study using cardiac ^{31}P MRS[197]. This showed that the steady state concentration ratio of PCr: ATP was maintained at about half that of lowlander hearts. The authors suggested this reflected a shift to carbohydrate oxidation to meet the myocardium's metabolic needs. Holden et al in 1995 performed positron emission tomography (PET) on high altitude natives (Sherpas and Quechuas) and compared the regional cardiac glucose uptake rates to that of sea-level residents[196]. They showed that the high altitude dwellers' hearts have a higher glucose uptake in response to the chronic hypoxia. To date no studies have been performed looking at the cardiac metabolism in patients with Eisenmenger syndrome.

1.7.2 Fetal Circulation

The fetal circulation is more resistant to hypoxia than the adult heart as it is adapted to the relatively hypoxic conditions found in utero[198]. Despite the lower arterial O_2 content in the fetus compared to the adult, animal studies have shown that the myocardial O_2 consumption is the same suggesting an increased blood flow and O_2 extraction in the fetal circulation[199].

Unlike adult hearts, fetal hearts are more dependent on carbohydrates for metabolism, with a decreased capacity to utilise fatty acids[200]. Bartelds et al showed that glucose and lactate account for 84% of myocardial O_2 consumption in fetal lambs, decreasing to 12% following birth[201]. Developmental maturation leads to a shift towards fatty acid oxidation with rapid maturation of fat metabolism and increased free fatty acid levels occurring soon after birth[198]. However, in hypoxic environments, the neonatal heart remains able to maintain anaerobic energy production, thus protecting cardiac function[202].

Patients with Eisenmenger syndrome are not born hypoxic, however they become cyanotic in early infancy due to the presence of a large right to left intracardiac shunt. A possible mechanism for adaptation to the hypoxaemia is that these patients revert to a fetal form of cardiac metabolism with a preference of carbohydrates over fats.

1.7.3 Haemodynamic Adaptations

On initial exposure to altitude there is an increase in cardiac output ensuring an adequate supply of O_2 to tissues[130;131]. This increase is due to a rise in heart rate[203] which in turn is predominantly due to activation of the cardiac β-adrenoreceptors by increased levels of circulating adrenaline[204]. A reduction in stroke volume, and thus CO, is seen with

prolonged exposure to high-altitude but stabilises after 1-2 weeks[130]. The cause of this is not fully understood but a reduction in plasma volume[205] and an increased afterload secondary to sympathetic driven elevated systemic vascular resistance are likely to contribute[131].

Previous studies have demonstrated that the sympathetic nervous system (SNS) is activated during both acute and chronic exposure to high altitude, with acclimatisation being accompanied by maintained sympathoexcitation[130;206]. Mazzeo et al demonstrated a significantly increased muscle sympathetic activity, as measured by elevated plasma noradrenaline levels, both at rest and during submaximal exercise in individuals exposed to chronic high altitude[153]. Hansen et al measured sympathetic response directly using peroneal microneurography and showed an increased response in acclimatised individuals[206]. Interestingly they also showed that the elimination of the chemoreflex by supplemental O_2 and the baroreflex with additional saline had little effect on the increased SNS response, thus the major underlying mechanisms remain unexplained. The lower maximal heart rate despite the elevated catecholamine levels suggests a down-regulation of the β-adrenoreceptors. This was confirmed by Voelkel et al in 1981 who demonstrated a halving in density of β-adrenoreceptors in rats after five weeks at a simulated altitude of 4250m[207].

1.8 Adaptations to Hypoxaemia in Eisenmenger Syndrome

As discussed above, the adaptations to the chronic and severe hypoxaemia found in Eisenmenger syndrome have not previously been investigated. A further understanding to

these adaptations may help us in the future to develop new therapeutic strategies in managing patients with hypoxaemia from other causes, such as those acutely unwell on Intensive Care.

Table 1.1. Causes and frequency of Eisenmenger syndrome (adapted from Wood [4])

Anatomical abnormality	Frequency of Eisenmenger reaction (%)
PDA	16
AP window	60
Truncus arteriosus	100
TGA with VSD	58
Corrected TGA with VSD	100
Single ventricle	100
VSD	16
Common AV canal or persistent ostium primum	43
Single atrium	None seen in series
ASD	6
Hemianomalous pulmonary venous drainage	0
Total anomalous pulmonary venous drainage	17

Table 1.2 Classification of PAH associated with congenital heart disease (adapted from Diller at al [5])

Type of lesion	Simple
	Complex
Lesion dimensions	Small to moderate (ASD Ò2cm. VSD & PDA Ò1cm)
	Large (ASD >2cm, VSD & PDA >1cm)
Associated extracardiac anomalies	
Repair status	Unoperated
	Palliated
	Repaired

Appendix 1.1 **Ability Index questionnaire (adapted from Warnes et al[17]**

Ability Index 1	Normal life and full-time work or in school
Ability Index 2	Able to work, with intermittent symptoms, interference with daily life (socio / community impositions because of cardiac anomaly)
Ability Index 3	Unable to work and limited in all activities
Ability Index 4	Extreme limitation, dependent, almost housebound

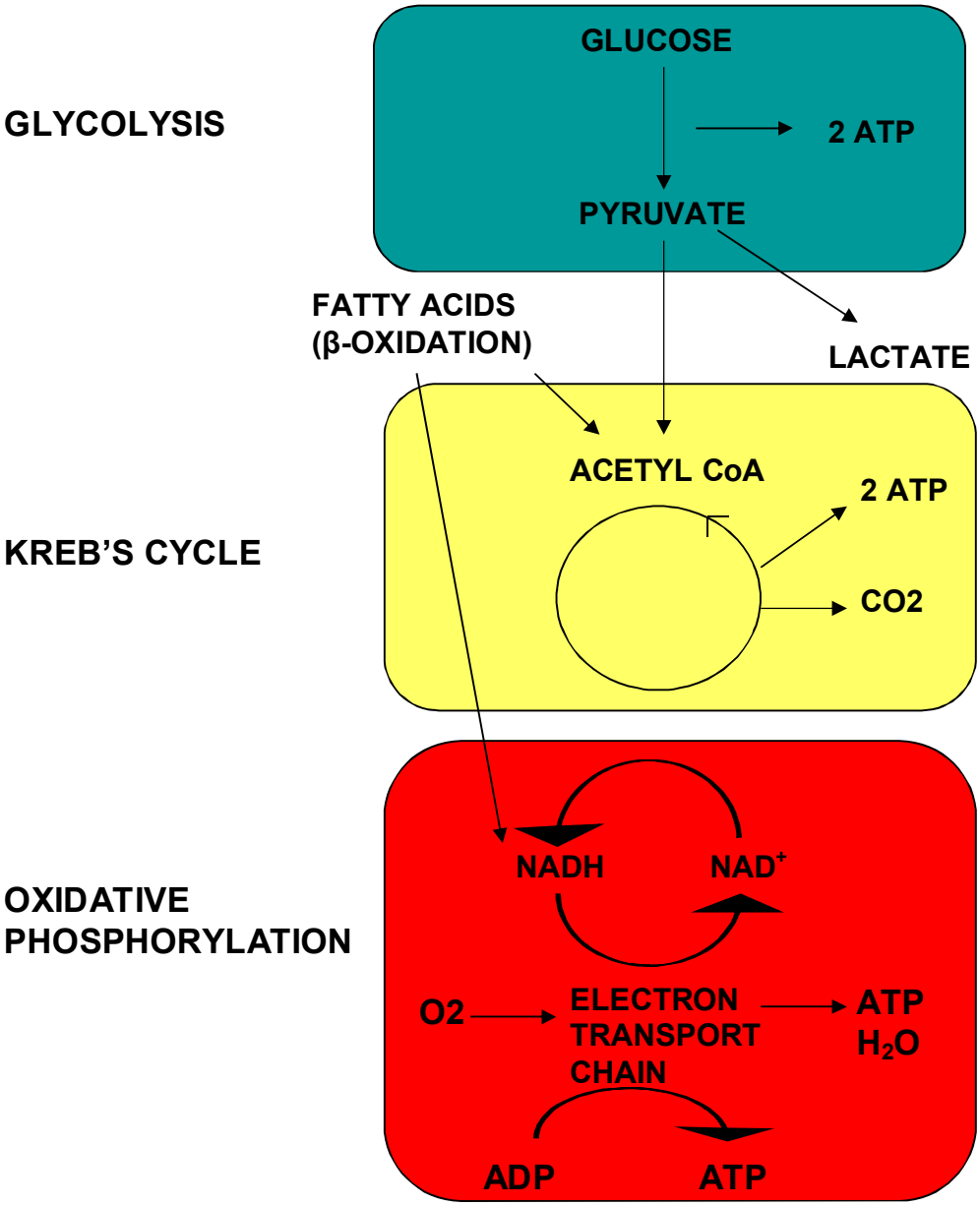

Figure 1.1 Generation of ATP in the mitochondria

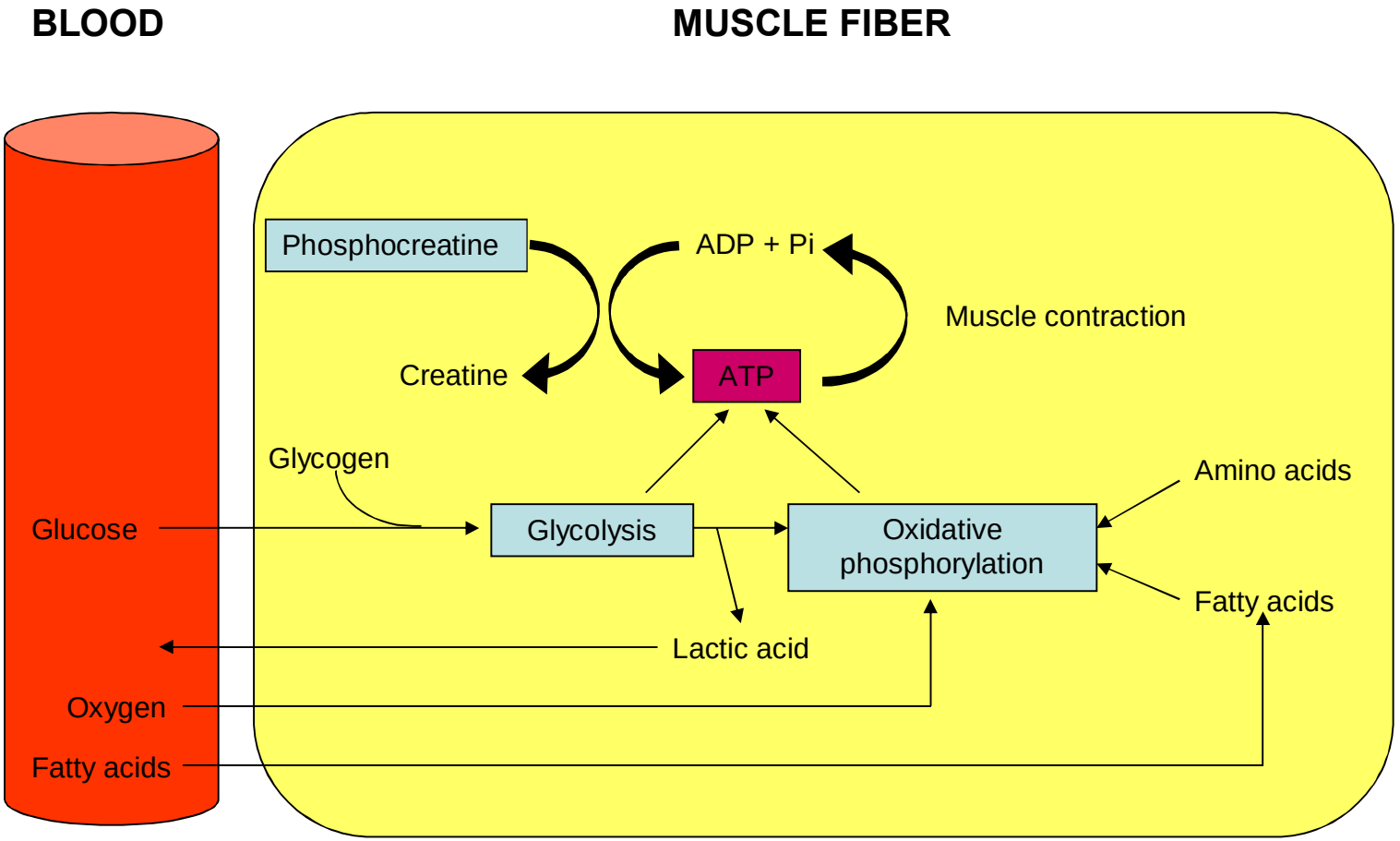

Figure 1.2 Generation of ATP during muscle fibre contraction

2. Objectives

The primary objective of this work was to investigate how the patient with Eisenmenger syndrome has adapted physiologically to a life time of severe hypoxaemia and pulmonary hypertension. Our aim was to gain an understanding of how these patients manage to function relatively well and have better than expected survival despite these adverse factors. A greater understanding of how these adaptive changes have conferred protection in these patients may, in the future, be applied in other hypoxic patients who do less well. They may also serve as potential targets for future therapeutic strategies.

2.1 Hypotheses

1. Patients with Eisenmenger syndrome have severe exercise intolerance with an early shift to anaerobic metabolism.

2. There are alterations in skeletal muscle mitochondrial metabolism to account for the exercise intolerance, as assessed by the PCr recovery.

3. They have maintained cardiac systolic function but right ventricular diastolic dysfunction as measured by echocardiography.

4. There is preservation of the septal energetics allowing maintenance of the contractile function.

2.2 Patient Selection

Patients with Eisenmenger syndrome under the care of the Adult Congenital Heart Disease unit at the Queen Elizabeth hospital, Birmingham, were invited to participate in this study. Patients with any diagnosis listed in table 1.1 were included. Patents who had undergone previous cardiac surgery were excluded. Patients were also excluded if they had limiting musculoskeletal abnormalities or peripheral vascular disease, or if they were unable to give informed consent. This included patients with co-existent Downs's syndrome which excluded approximately half of our Eisenmenger population. Patients were required to be clinically stable with no deterioration in the previous three months.

Healthy controls who were age and sex matched to our patients were recruited. They were excluded if they had limiting musculoskeletal abnormalities or peripheral vascular disease and must have no history of cardiac or other systemic illness.

2. 3 Experimental Studies

We performed a series of non-invasive clinical studies looking at four specific areas as outlined below in section 2.4. Studies were carried out in the department of Cardiovascular Medicine research department, Queen Elizabeth hospital, Birmingham. The MRI scans were performed in the Birmingham University Imaging Centre. Ethical approval for the study was obtained from the Warwickshire Local Research Ethics Committee.

2.4 Questions Investigated

2.4.1 Exercise

As discussed in section 1.2, patients with Eisenmenger syndrome have severe exercise intolerance when measured objectively. We therefore investigated this intolerance in greater detail with the aim of identifying the factors that limits exercise by examining the cardiorespiratory response to exercise and the VO_2 kinetics using cardiopulmonary exercise testing.

2.4.2 Skeletal Muscle Adaptations

In addition, we used ^{31}P MR spectroscopy to investigate skeletal muscle energetics in patients with Eisenmenger syndrome, specifically the PCr recovery time, and compared them with our healthy control subjects. We have also examined the pH drop in the exercising muscle to gain further insight into the balance of aerobic vs anaerobic metabolism in severe hypoxaemia. In a subset of patients we assessed the resting O_2 consumption of skeletal muscle by using near infra-red spectroscopy with the aim of establishing whether this differs in the setting of severe hypoxaemia. Finally, we investigated peripheral fatigue and the substances linked to it in Eisenmenger syndrome to assess whether they are affected by a low PO_2.

2.4.3 Cardiac Function

We performed transthoracic echocardiography to investigate the ventricular function in Eisenmenger syndrome. As the interventricular septum is known to have an increased importance in pulmonary hypertension, we specifically investigated its role as well as the interaction between the ventricles in presence of a large intracardiac shunt. Whilst previous studies have demonstrated that systolic function is relatively preserved in Eisenmenger, no

study to date has investigated diastolic function thus this was assessed in the left and right ventricles.

2.4.4 Cardiac Energetics

We used 31P MR spectroscopy to investigate the energetics of the interventricular septum in Eisenmenger syndrome. We then examined whether there are correlations between this and echo parameters in order to investigate whether abnormalities in the energetics precede contractile dysfunction.

3. INVESTIGATION OF THE FACTORS RESPONSIBLE FOR EXERCISE LIMITATION IN EISENMENGER SYNDROME

3.1 Introduction

3.1.1 Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing (CPET) offers significant advantages over conventional methods of assessing performance, as it provides an integrated assessment of cardiopulmonary function and reflects underlying metabolic processes[208]. In doing so it assesses O_2 delivery, via the lungs, O_2 transport via the cardiovascular system, and O_2 uptake by the end organ, with further information being obtained by the analysis of CO_2 production. It is an objective and reproducible approach for determining true functional capacity by combining both performance and physiological parameters to underlying metabolic processes[209;209]. It can be used to assess the response to both submaximal and maximal exercise and provides prognostic information[210;211].

CPET directly measures O_2 uptake (VO_2), CO_2 production (VCO_2) and airflow (minute volume, tidal volume and respiratory rate) using a non-breathing valve attached to a metabolic cart. From these measurements, other clinically relevant parameters may be derived.

Exercise capacity is determined by gas exchange within the lungs, cardiovascular performance and skeletal muscle metabolism[212].

The foundation of the studies described in this chapter was that patients with Eisenmenger syndrome may have abnormalities of any of these and the use of CPET may allow us to

establish the predominant limiting factor. The discussion below considers some of the issues we particularly wished to address.

3.1.2 Oxygen Uptake

Oxygen uptake (VO_2) occurs at the alveoli in proportion to the pulmonary blood flow and the O_2 saturation of the haemoglobin in the pulmonary capillary blood[213]. The VO_2 is the product of cardiac output and the O_2 content differential between pulmonary artery and vein and is defined by the Fick equation:

$$VO_2 = (SV \times HR) \times (CaO_2 - CvO_2)$$

It is determined by the maximum cardiac output, arterial oxygen content, fractional distribution of the cardiac output to skeletal muscle and the ability of muscle to extract oxygen. Muscle extraction of oxygen is dependent on oxygen delivery and peripheral circulatory factors such as muscle capillary density, mitochondrial density and oxidative enzyme levels (as discussed in section 1.5.4).

The VO_2 plateaus as one of the above determinants approaches its threshold. The maximal VO_2 (mVO_2) was originally defined as "the VO_2 at which performance of increasing levels of constant work fails to increase VO_2 by 150ml/min despite increasing work rate"[214]. As 150ml/min is a significant fraction of that obtained by some patients, it may also be determined by observing that the VO_2 fails to increase normally relative to an increase work rate just prior to fatigue[213]. This plateau may not be achieved by some subjects prior to symptom limitation and the peak VO_2 (pVO_2) is then used, defined as "the highest VO_2 averaged over a 20-30 second period at maximal effort"[209]. For this study we will refer to pVO_2.

Reduced VO_2 is seen in many acquired cardiovascular diseases including chronic heart failure and has been shown to be associated with a poor outcome[105;210;215;216]. Diller et al studied pVO2 in cohorts of patients with congenital heart disease, again showing that a low pVO_2 is associated with poor clinical outcomes[24].

3.1.3 Ventilatory Efficiency

Ventilatory efficiency, as measured by the VE/VCO_2 slope, is a measurement of the amount of air required to eliminate 1 litre of CO_2. A high VE/VCO_2 slope has again been shown to be associated with a worse outcome, with higher rates of hospitalisation and mortality, in both patients with acquired heart failure[210;211;217] and non-cyanotic cardiac defects[26]. However, this did not hold for patients with cyanotic congenital heart disease[26].

3.1.4 Anaerobic Threshold

Exercise capacity can also be estimated using the anaerobic threshold (AT), an index that is independent of motivation. It is defined as "the VO_2 during exercise at which aerobic energy production is supplemented by anaerobic mechanisms such that it is reflected by an increase in lactate concentration"[218]. A reduced AT of <40% of the predicted peak VO_2 is considered abnormal and is suggestive of cardiovascular disease[219].

There are two main methods of estimating the VO_2AT using CPET[212]. In the V-slope method, the AT occurs when the VCO_2 accelerates relative to VO_2[212] (figure 3.1a). The ventilatory equivalent method defines the AT as "the VO_2 at which the ventilatory equivalent for O_2 (VE/VO_2) begin to increase without an immediate increase in the ventilatory equivalent for CO_2 (VE/VCO_2)"[212](figure 2.1b). However, the use of CPEX to assess the AT is open

to considerable inter- and intraobserver variability[220], therefore in this study we used both methods.

3.1.5 Respiratory Exchange Ratio

The respiratory exchange ratio (RER) is the ratio of CO_2 produced and O_2 consumed and equals the respiratory quotient in the steady state[212]. It is often used as a marker of effort with a peak RER <1.1suggesting submaximal exercise[209;219].Patients with cyanotic congenital heart disease have been shown to achieve a significantly lower RER than non-cyanotic patients[24;26] and it was suggested that the usual benchmarks for interpreting gas exchange in this group may not have the same meaning. [26].

3.1.6 Oxygen Uptake Efficiency Slope

The oxygen uptake efficiency slope (OUES) is a further method used to assess exercise capacity and is a measurement of the relationship between the VO_2 and a given VE during exercise, that is the efficiency by which the body takes in and then extracts O_2 [209]. It is calculated by the following equation where "a" is the OUES and "b" the intercept (figure 2.2)[221]:

$$VO_2 = a \log_{10} VE + b$$

Whilst mVO_2 and VE/VCO_2 slope are both effort-dependent and may not be accurate for submaximal exercise, the OUES uses the whole exercise data and is thus useful in patients who only complete a submaximal test[222]. Hollenberg et al demonstrated that there was only a 1.9% difference in the OUES calculated during the first 75% of exercise data from the data

obtained from the complete test in patients with CHF[223]. It is also highly reproducible with low interprotocol variability[224].

OUES has been shown to be significantly reduced (indicating greater ventilatory effort for a given VO_2) in patients with CHF[222;224] and significant coronary artery disease coronary artery[225]. Like mVO_2 and VE/VCO_2 slope it has also been shown to be a powerful prognostic marker in CHF. Giardini et al has looked at the accuracy of the OUES in adults with congenital heart disease[226]. He noted that in cyanotic patients with a Fontan circulation, the OUES at 50% maximal exercise capacity ($OUES_{50}$) was different to that over the second half of exercise. However it is not clear whether this was due to the cyanosis or the abnormal haemodynamics found in Fontan patients. One study has looked at the effect of acute hypoxia on the OUES showing that there was no difference in the $OUES_{100}$ or $OUES_{80}$ and concluded that is an accurate submaximal index to assess cardiorespiratory fitness in patients with arterial hypoxaemia[227]. To date no studies have specifically examined the OUES in patients with Eisenmenger syndrome.

3.1.7 The Exercise Paradox in Eisenmenger Syndrome

In patients with Eisenmenger syndrome there appears to be a profound paradox between the objective exercise indices, as demonstrated in previous studies, and patient survival and functional ability[16;24;26]. The true cause of the exercise limitation is not fully understood and likely to be multifactorial. Therefore the primary aim of the studies described below was to investigate the cause of the exercise limitation in this patient group.

3.2 Methods

3.2.1 Subjects

Nine patients with Eisenmenger syndrome and 8 age and sex matched healthy volunteers (HV) underwent a symptom –limited erect treadmill test in the department of Cardiovascular Medicine, University of Birmingham. Prior to exercise the functional class of all subjects was assessed by ability index[17] and Borg dyspnoea score[228]

3.2.2 Monitoring and Equipment

A face mask was fitted tightly to cover mouth and nose and ensuring no air leak. The mask was attached to a non-breathing valve to prevent mixing of inspired and expired air. Simultaneous gas analysis was performed throughout exercise with a Schiller CS-200 Ergo-Spiro exercise machine. Blood pressure, ECG and oxygen saturations were monitored throughout exercise and for 5 minutes of recovery using an automatic sphygmanometer, ECG cables and pulse oximeter all connected through the exercise machine.

3.2.3 Protocol

A standardised ramp protocol was used as previously described[229]. In brief, this uses simultaneous increases in speed and inclines to produce a ramp-wise increase in work rate. The "Ramp 2" protocol begins with a belt speed of 1km/h and an incline of 1%. It then increases each minute by 1km/h and 1% inclines. A "ramp 1" protocol was used in subjects with self-reported poor exercise tolerance. This starts at 0.5km/h at 0.5% and increases by 0.5km/h and 0.5% at one minute intervals.

Exercise was preceded by a resting period of at least five minutes to allow steady state gaseous exchange to be reached. During this time the resting VO_2, VE, O_2 pulse and HR were calculated as the mean value during the last minute prior to exercise starting.

All subjects were encouraged to continue until fatigued. Medical reasons to terminate the test were as per the ACC/AHA guidelines for exercise testing[230]. Reason for stopping was recorded for each subject.

3.2.4 Data Analysis

Directly measured variables were VO_2, VCO_2, minute ventilation (VE), respiratory rate and heart rate. Directly calculated from these values were RER (VCO_2/VO_2) and O_2 pulse (VO_2/HR) which gives an estimate of stroke volume[209]. After completion of the test the data was transferred to a Microsoft Excel programme for off-line analysis. The pVO_2, VE/VCO_2 slope, and OUES were calculated as previously described[210;213;222]. The AT was assessed by both V-slope method and ventilatory equivalent methods and expressed as an average of both. The breathing reserve relates the ventilatory response during maximal exercise to the maximal ability to breathe and is estimated by the ratio of the maximal VE and maximal voluntary ventilation (MVV).

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3.2.5 Statistical Analysis

Continuous data are expressed as mean ± SEM. The data was normally distributed, as determined by Kolmogorov-Smirnov test of normality, and thus groups were compared using independent-samples t-test. A $p<0.05$ was taken as being statistically significant. All

statistical operations in this and subsequent chapters were performed using SPSS (version 15.0. SPSS Inc, Chicago, Illinois).

3.3 Results

3.3.1 Baseline Data

Nine subjects (six female) with Eisenmenger syndrome aged 31-50 years (mean 42 years) underwent CPET. Their individual underlying diagnoses are presented in table 2.1. Two patients were on bosentan and one on sildenafil. Their mean haemoglobin was 20.4 ± 1.4g/dl with baseline oxygen saturations of 79.7 ± 2.1%. The ability score was I in three patients, II in five patients and III in one patient. Three patients were in full time employment, one in part time and five not working (one of whom had recently retired). Their mean Borg dyspnoea score at rest was two. All patients were in sinus rhythm during this study and had a normally reactive heart rate.

The control group consisted of eight healthy volunteers (six female) aged 33-53 years (mean 42years). All controls were free from cardiovascular, pulmonary or other systemic disease. Trained athletes were excluded. Their mean haemoglobin was 14.2 ± 1.2 with baseline oxygen saturations of 98.3 ± 0.3%. The ability index was I in all control subjects and all were in full time employment. Mean Borg score at rest was one.

All eight control subjects exercised using the ramp 2 protocol. Four of them stated shortness of breath as the reason for terminating exercise and four were unable to keep with the speed and incline. Only two of the patients with Eisenmenger syndrome attempted ramp 2 with the other seven using ramp one. All nine patients stated shortness of breath as their reason to stop.

3.3.2 Resting Data

There was no difference in the resting O_2 consumption (VO_2) between healthy controls (0.38 ± 0.03ml/kg/min) and patients (0.33 ± 0.02 ml/kg/min, p = 0.2). Resting O_2 pulse, as an index of stroke volume, was also similar in controls and patients (4.2 ± 0.4ml/beat and 3.9 ± 0.3 ml/beat, p = 0.42).

3.3.3 Exercise Data

The mean pVO_2 was significantly lower in patients with Eisenmenger syndrome (13.2 ± 1.2ml/lg/min) than in the healthy controls (37.3 ± 2.1ml/kg/min, p < 0.001) (figure 2.3). The VE/VCO_2 slope was higher in patients (54.2 ± 2.8) compared to the controls (29.8 ± 1.1, p < 0.001) (figure 2.4). The OUES was also lower in the Eisenmenger patients (1.0 ± 0.4l/min) compared to the healthy controls (2.7 ± 0.8l/min, p < 0.001) (figure 2.5).

During exercise the patient group achieved a lower maximum heart rate than controls (132 ± 6.8 vs 179 ± 4.6, P < 0.05) despite there being no difference in resting heart rate (85.4±3.7 vs 91.1 ± 7.1, p = 0.47). The O_2 pulse at peak exercise within the patient group was lower at peak exercise compared to the controls (6.7 ± 0.7 ml/beat vs 16.7 ± 1.3 ml/beat, p < 0.001).

3.3.4 RER and Anaerobic Threshold

Only 3 in the patient group reached an RER > 1.0 despite exercising to exhaustion, whereas all control subjects did. The control group had a significantly higher AT compared to the Eisenmenger group (23.0 ± 3.8 vs 12.5 ± 3.4ml/kg/min, p<0.01), however 3 Eisenmenger patients again did not reach their AT. When expressed as a percentage of maximum exercise,

the patients with Eisenmenger syndrome reached their AT at a later stage in exercise than the healthy controls did.

3.3.5 Ventilatory Response to Exercise

Patients with Eisenmenger syndrome had a significantly higher resting ventilation, as measured by minute volume (VE), compared to healthy controls (13.7 ± 0.6l/min vs 11.4 ± 0.8l/min, $p < 0.05$). However, during exercise they achieved much lower peak VEs (48.3 ± 7.5 l/min vs 80.5 ± 6.4 l/min, $p < 0.05$) and lower maximal respiratory rates (38.5 ± 4.3 vs 46.5 ± 2.3, $p<0.05$). Both groups had normal breathing reserves indicating that they were not limited by restrictive or obstructive airway disease. The breathing reserve was actually significantly higher in the patient group (68 ± 5.7%) than in the healthy controls (36.8 ± 7.1%, $p < 0.05$).

3.4 Discussion

Whilst other groups have looked at the exercise capacity of patients with cyanotic heart disease, they have included only small proportion of patients with Eisenmenger syndrome. Cyanotic congenital heart disease includes a very heterogeneous group of patients whose haemodynamic response to exercise will vary between patients depending on the underlying diagnosis and physiology. Whilst our numbers are also small, our study is the first study to look solely at adult patients with Eisenmenger syndrome.

Patients with Eisenmenger syndrome have severely limited exercise capacity as shown by a markedly impaired pVO_2 compared to age matched healthy control subjects. The pVO_2 can be decreased by either decreased O_2 utilisation by the cells or reduced supply of O_2 to active tissue. Whilst CPET does not give specific information regarding the former, information

regarding the latter can be determined. We have documented a reduction in peak VE, respiratory rate, heart rate and O_2 pulse during maximal exercise in patients with Eisenmenger syndrome; all of which would be expected to lead to impaired supply of O_2 to the tissues.

In our study patients with Eisenmenger syndrome had a similar resting VO_2 to healthy controls. This differs from the findings of Theodore at al who documented that their resting VO_2, measured invasively, was actually double that of normal subjects[21]. They attributed this increase to the increase cardiac work and work of breathing. They also noted that the patients could increase their VO_2 to approximately double the resting value during exercise. They hypothesised that this indicates that the basic mitochondrial function is not impaired despite the profound hypoxaemia in Eisenmenger syndrome.

Despite having higher resting ventilation than healthy controls, their VEs and respiratory rates at peak exercise were significantly lower. However, previous studies investigating the effects of hypoxia during exercise have shown that ventilation increases with decreasing arterial PaO_2 due to the hypoxic ventilatory drive mediated by peripheral chemoreceptors[135]. Patients with Eisenmenger syndrome therefore appear to have acclimatised to the severe hypoxaemia present from an early age and it is possible that their hypoxic ventilatory drive may be suppressed, similar to that of native high altitude dwellers. However, all patients in this study stated severe shortness of breath as the reason for terminating exercise. One reason may be that they are unused to exerting themselves to this degree. Another may be alterations in their perception of breathless due to the longstanding severe hypoxaemia.

Our patients have a markedly abnormal ventilatory efficiency during exercise as shown by elevated VE/VCO_2 slopes. A decreased ventilatory efficiency in patients with Eisenmenger syndrome and other cyanotic heart disease has been demonstrated previously[26] and correlates well to the degree of cyanosis and thus size of shunt[231]. This inefficiency is due to both the increase in physiological dead space and alveolar hypoventilation. During exercise the right to left shunt increases, with up to 60% of the cardiac output not being exposed to alveolar gas exchange during exercise. This leads to pulmonary hypoperfusion, VQ mismatching and an increase in physiological dead space[26]. The dependence of ventilatory efficiency and physiological dead space has also been reported in CHF and idiopathic pulmonary hypertension[232;233]. In these conditions the increase in right to left shunting also leads to alveolar hyperventilation, reflected by alveolar hypocapnia, in order to normalise the systemic $PaCO_2$[234].

A further contributory factor to the ventilatory inefficiency may be the severe pulmonary hypertension present in Eisenmenger syndrome. A high VE/VCO_2 slope has also been demonstrated in other causes of pulmonary hypertension (PHT) including idiopathic PHT[235], congestive cardiac failure complicated by PHT[236] and COPD associated with PHT[237]. Lewis et al have demonstrated a significant correlation between VE/VCO_2 slope and both exercise PVR and poor RV performance[236]. Furthermore they showed that the administration of sildenafil, a selective pulmonary vasodilator, for 12 weeks significantly reduced the VE/VCO_2 slope. In our study, only three patients were taking selective pulmonary vasodilators thus it is not possible to assess for a difference within our group.

We also demonstrated an abnormal ventilatory response to exercise using OUES. Our results show that patients with Eisenmenger syndrome had markedly low OUES compared to the control group, indicating that increased ventilatory effort was required for a given VO_2. This study is the first to use OUES as a measure of ventilatory response to exercise in this group of patients. It has previously been shown, in other groups of patients, to be a non-effort dependent and useful submaximal index of cardiorespiratory reserve. The effect of hypoxia on the OUES was investigated by Mollard et al who investigated the response of the OUES to hypoxia by exercising both trained and untrained subjects at increasing levels of simulated altitudes[227]. They found no difference between the $OUES_{80}$ and $OUES_{100}$ and that the $OUES_{80}$ was influenced to the same extent as the peak VO_2 during exercise performed in hypoxia. Thus, OUES could be considered a reasonable sub-maximal index in patients with arterial hypoxaemia such as those with Eisenmenger syndrome.

In our study 3 patients with Eisenmenger syndrome failed to reach their anaerobic threshold (AT). As discussed above in section 2.1.4 there are two methods of assessing the anaerobic threshold; the V-slope method and the ventilatory equivalent method. Glaser et al determined the VO_2AT in 25 patients with cyanotic congenital heart disease, including five with Eisenmenger syndrome[231] and found that they were unable to accurately determine the VO_2AT in almost one third of patients using the V-slope method and they comment that it was not clear if these patients really did not reach AT or if it was obscured by alterations in their ventilatory patterns. As measurement of AT by the V slope method assumes a strictly CO_2-controlled ventilatory drive and these patients may have a significant hypoxic contribution to ventilatory controls, they conclude that the V slope method may not be appropriate in this cohort. However, in our study we used both methods and used the average

of the two. In those patients who appeared not to reach their AT we were unable to measure it by either method.

In the present study, the patients with Eisenmenger syndrome who did reach their AT did so at a relatively later stage in exercise, as measured by percentage of maximal exercise, than healthy controls. A similar finding was made by Sun et al who investigated the exercise response of patients with primary PHT and showed that AT becomes a higher fraction of peak VO2 as disease severity increases[235]. They suggested that this reflects a decreasing cardiovascular reserve as PHT worsens. Conversely Glaser et al report that patients with cyanotic heart disease in their study showed early anaerobic metabolism [231]. However, his cohort of 27 patients only included 5 with Eisenmenger syndrome thus other factors, such as single ventricle physiology, may have contributed to their result.

On the other hand, Theodore et al examined the venous lactate concentrations during exercise in patients with Eisenmenger syndrome[21]. They noted that the increase in lactate levels during exercise were modest and suggested that the term "anaerobic threshold" is not appropriate for these patients who function above an AT during the course of normal activity.

In conclusion, patients with Eisenmenger syndrome have severely impaired exercise tolerance with marked ventilatory inefficiency. Despite this they appear to have a reliance on aerobic pathways as evidenced by reaching their anaerobic threshold late in their exercise or not reaching it at all. Further information regarding the energetic status of the exercising skeletal muscle using ^{31}P MRS may give further insight into the mechanism of the exercise intolerance.

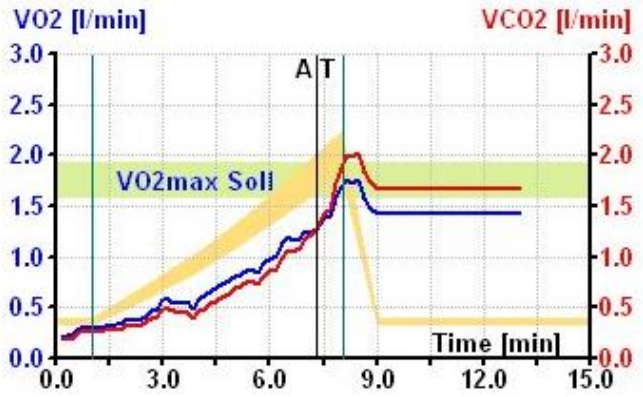


Figure 3.1a Original graph demonstrating the V-slope method of determining anaerobic threshold. The y-axes plot the VO_2 (blue line) and VCO_2 (red line) during exercise against the duration of exercise eon the x-axis. The anaerobic threshold (AT) is calculated from the point the VCO_2 increases relative to the VO_2.

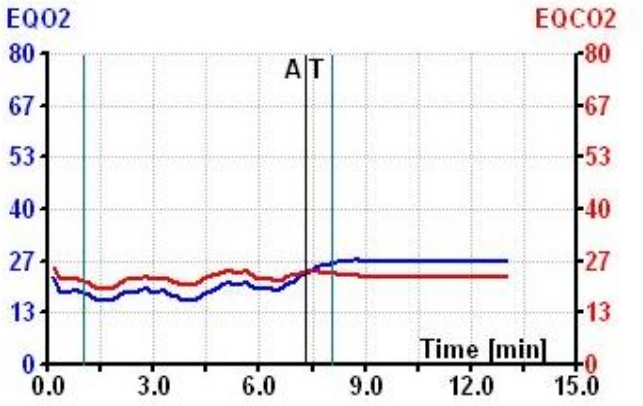


Figure 3.1b Original graph demonstrating the ventilatory equivalent method for determining anaerobic threshold. The y-axes show the ventilatory equivalents for O_2 (VE/VO_2, blue line) and CO_2 (VE/VCO_2, red line) and the x-axis plots the exercise time. The anaerobic threshold is the VO_2 at which the EQO_2 increases without an increase in ECO_2.

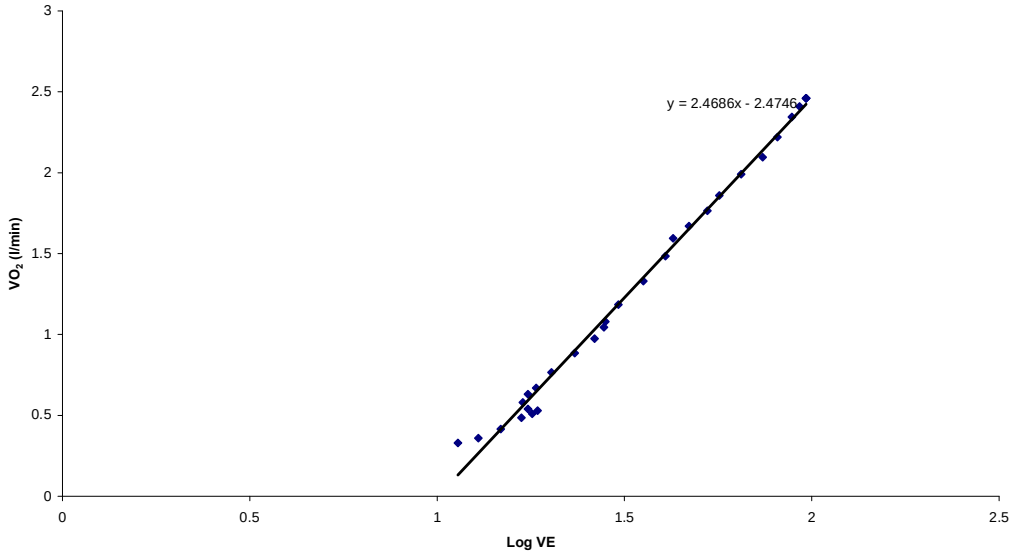


Figure 3.2 An example of the oxygen uptake efficiency slope of a healthy control subject. The y-axis shows the VO2 in ml/kg/min and the x-axis shows logVE in l/min

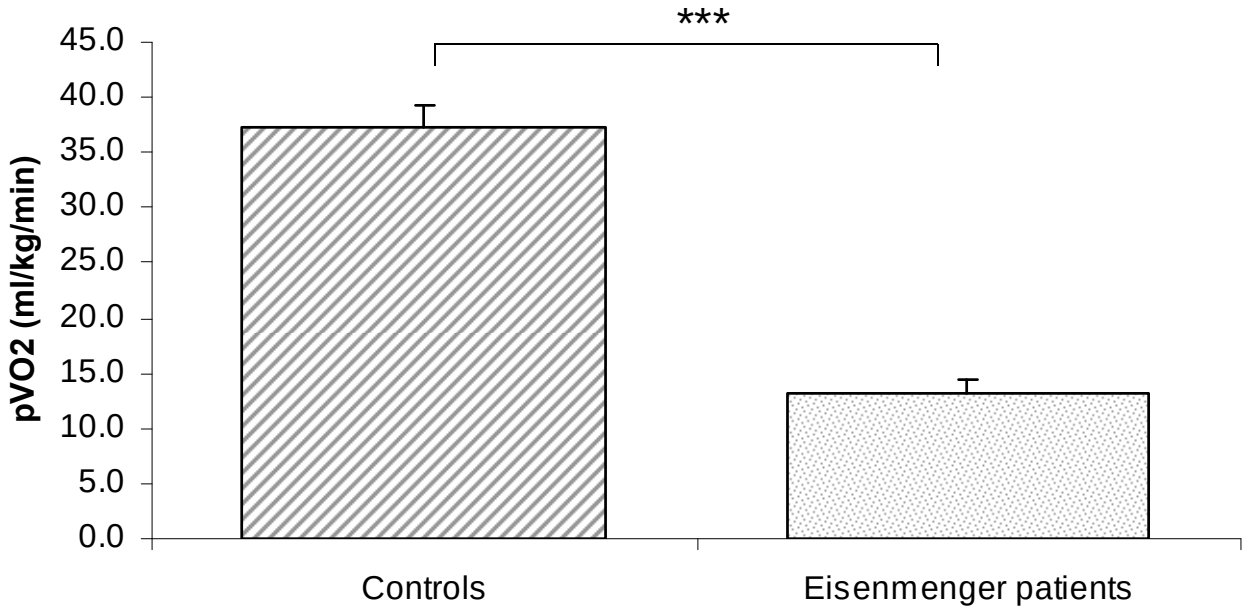


Figure 3.3 Comparison of pVO2 in healthy controls and Eisenmenger patients. Each column shows mean ± SEM; ***: P<0.001

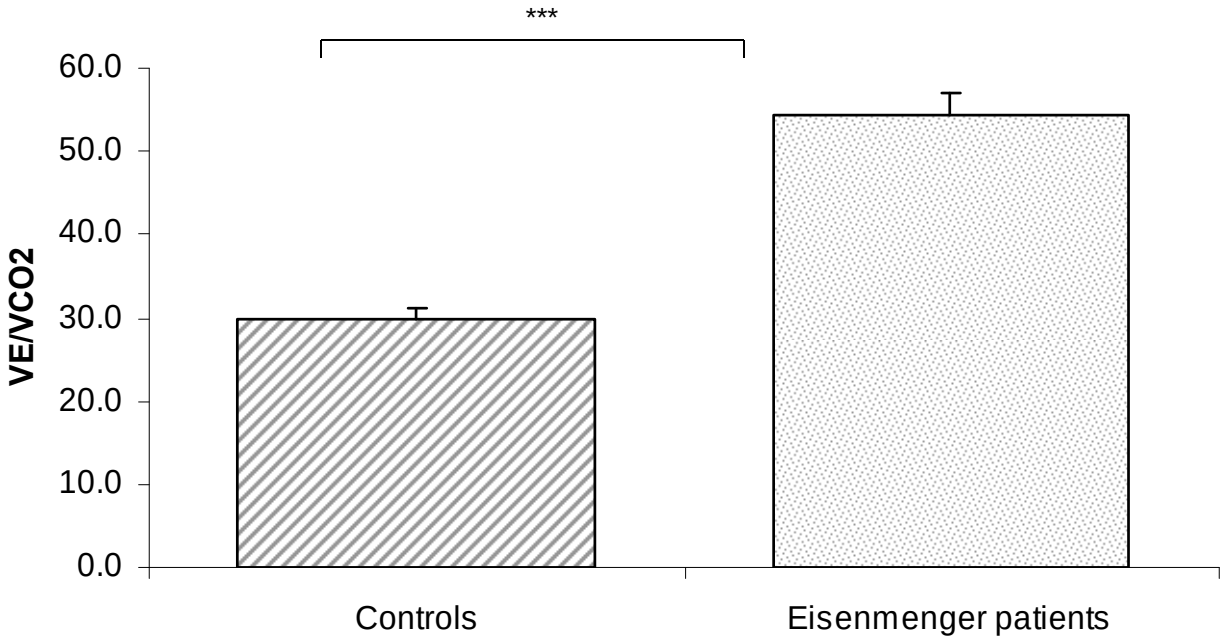


Figure 3.4 Comparison of VE/VCO2 in healthy controls and Eisenmenger patients. Each column shows mean ± SEM; ***: P<0.001

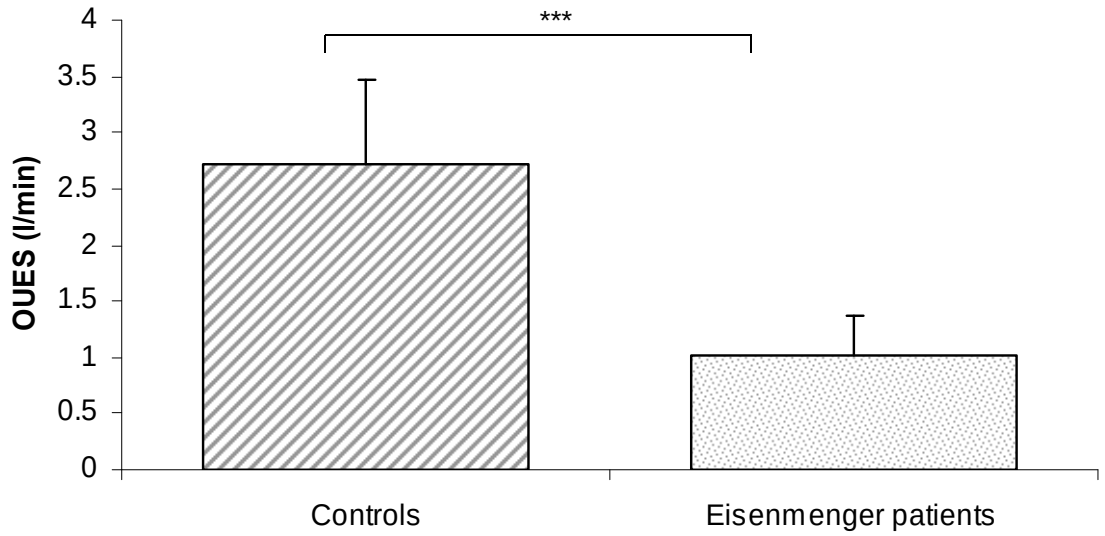


Figure 3.5 Comparison of OUES in healthy controls and Eisenmenger patients. Each column shows mean ± SEM; ***: P<0.001

	Age (y)	Underlying cardiac defect
1	50	VSD
2	41	VSD
3	42	VSD, PDA
4	49	VSD
5	50	VSD
6	48	VSD
7	31	VSD
8	38	AVSD
9	38	ASD

Table 3.1 Characteristics of patients with Eisenmenger syndrome

VSD = ventricular septal defect, PDA = patent ductus arteriosus, AVSD = atrioventricular septal defect, ASD = atrial septal defect

4. INVESTIGATION OF SKELETAL MUSCLE ADAPTATIONS IN PATIENTS WITH EISENMENGER SYNDROME

4.1 Introduction

As indicated in the introduction, very little is known of skeletal muscle energetics or mechanisms underlying fatigue in patients with Eisenmenger syndrome. Thus, in the present studies, ^{31}P MRS was used as means of following high energy phosphates, PCr and pH during and following exercise and NIRS was used as way of following tissue oxygenation. In addition, separate studies were performed to assess venous efflux of some of the substances that may be involved in fatigue. The discussion below provides the background to these studies.

4.1.1 Basis of MRS

Magnetic resonance spectroscopy (MRS) provides metabolic information from nuclei with magnetic spin including ^{31}P, ^{13}C, ^{1}H and ^{123}Na, each nucleus type having a characteristic frequency when placed in the same magnetic field[238]. This technique allows non-invasive assessment of the biochemical composition and metabolic changes of tissue without the need for a tracer.

A radiofrequency (rf) pulse, emitted from a surface coil, causes spin excitation of the nuclei followed by relaxation when the rf pulse is switched off. This results in a MR signal, termed the free induction decay, which is then subjected to Fourier transformation into a frequency domain spectrum[238;239]. The resonance frequency of the individual nuclei, termed the chemical shift, allows the differentiation of single metabolites within one molecule[240]. Once correction factors have been applied, the area under each spectral peak is directly

proportional to the metabolite's relative concentration[239]. Of note, the spectrum obtained gives the concentrations of metabolites relative to each other, expressed as parts per million (ppm), not absolute concentrations.

4.1.2 Skeletal Muscle ^{31}P MRS

^{31}P MRS allows the non-invasive assessment of skeletal muscle high energy phosphate use in vivo. In normal skeletal muscle, there are peaks for PCr, Pi and the 3 types of ATP (γ, α and β), thus their relative concentrations may be determined. ADP concentration cannot be quantified as it is predominantly protein bound and thus free concentrations are too low to be detected[77].

4.1.3 Skeletal Muscle ^{31}P MRS and Exercise

As discussed in section 1.4.2, high energy phosphate molecules are important in both cellular energy and storage, with ATP providing the energy for muscle contraction. However, whilst muscle performance and O_2 consumption can vary greatly, ATP levels remain virtually constant[241]. ATP levels are maintained during exercise by hydrolysis of PCr, oxidative phosphorylation and anaerobic glycogenolysis.

PCr hydrolysis occurs 10 times quicker than oxidative phosphorylation and therefore represents most important initial energy source in skeletal muscle at at the start of exercise[77;241]. PCr is dephosphorylated by the following equation:

$$PCr + ADP + H^+ \underset{CK}{\rightleftharpoons} ATP + Creatine$$

High energy phosphate metabolism within skeletal muscle during exercise and recovery from exercise can be measured using ^{31}P MRS[242]. The rate of PCr recovery following the cessation of exercise closely reflects mitochondrial oxidative capacity[77]. Submaximal exercise is associated with low levels of stress and thus it may be more appropriate in those subjects unused to more strenuous activity[243].

^{31}P MRS may also be used to calculate intracellular pH within the exercising skeletal muscle. The ionisation constant (pKa) of PCr is significantly different from physiological pH and thus the PCr peak on the spectrum differs little throughout exercise and recovery[77]. The pKa of Pi however is approximately equal to its pH and its position on the spectrum, therefore, is pH dependent. The difference between the two peaks is termed the chemical shift and this can be used to determine pH using the following equation[242]:

$$pH = 6.75 + \log_{10}[(\delta Pi - 3.27) / (5.69 - \delta Pi)$$

4.1.4 ^{31}P MRS and PCr Recovery Following Exercise

At the end of exercise glycolysis is switched off and studies have shown that no metabolic recovery is seen if the muscle is made ischaemic at the end of exercise[61;244] with resynthesis only occurring if there is an intact blood supply[245;246]. Recovery is therefore entirely oxidative and the use of ^{31}P MRS to assess the PCr recovery following exercise, has been recognised as a valuable and reliable method in the quantification of the rate of oxidative ATP production in intact muscle [60;61;74;244;247].

This method of measuring skeletal muscle oxidative capacity is useful as it is independent of the intensity of exercise, provided that pH does not fall severely[61;248] and also for active muscle mass[249].

4.1.5 PCr Recovery in Other Disease States and Hypoxia

The use of ^{31}P MRS to measure PCr recovery does not discern between limitations caused by O_2 supply from that of mitochondrial demand. Thus, Haseler et al have shown that in sedentary subjects, it is the mitochondrial capacity and not O_2 availability that determines the maximal oxidative rate [250]. On the other hand, they have also demonstrated that PCr recovery is prolonged in severe hypoxia due to the decreased availability of O_2, whilst in normoxia and moderate hypoxia the maximal oxidative rate is dependent on training status[243]. They conclude that until the O_2 levels fall well below that of normal air, the O_2 availability does not affect the maximal oxidative rate in sedentary subjects.

Interestingly, PCr recovery has also been shown to be prolonged in some disease states characterised by impaired O_2 delivery to the skeletal muscle, either by reduced flow or hypoxaemia. These include COPD, CHF, diabetes mellitus and peripheral vascular disease[113;251-253].

Only two studies have previously used ^{31}P MRS to investigate skeletal muscle energetics in congenital heart disease. Miall-Allen et al studied children and found a non-significantly prolonged PCr recovery in cyanotic children when compared to those with acyanotic conditions[254]. Adatia et al studied 10 subjects, median age 17.3years, with cyanotic congenital heart disease[255]. They found that the PCr recovery time was 2-3 times longer

than in healthy control subjects. They also describe a greater PCr depletion during exercise and a bigger fall in pH. Both of these groups commented on a higher resting pH in cyanotic patients that correlated with Hb concentration.

To date no study has investigated the skeletal muscle energetics in adults with Eisenmenger syndrome. Thus, it is not known whether a reduced skeletal muscle oxidative capacity secondary to the severe hypoxaemia, a decreased rate of oxidative metabolism or even a sedentary lifestyle, contributes to the known exercise limitation seen in these patients.

4.1.6 Assessment of Tissue Oxygenation

Unlike ^{31}P MRS, near-infrared spectroscopy (NIRS) is able to discern the limitation caused by O_2 supply rather than that of mitochondrial demand. NIRS is a non-invasive and continuous technique that measures tissue oxygenation in skeletal muscle. It can be used to estimate the concentrations of oxy- (oxy-Hb), deoxy- (deoxy-Hb) and total haemoglobin (HbT) within the region being investigated.

In the absence of blood flow, for example during arterial occlusion, the O_2 is consumed by the mitochondria thus its content within the tissue will decrease as it. Therefore O_2 consumption, which reflects resting muscle metabolic rate, can be assessed by the reduction in oxy-Hb during arterial occlusion, providing the duration of occlusion does not allow a significant shift to anaerobic metabolism.

NIRS has been used to demonstrate abnormal tissue oxygenation due to insufficient delivery in several conditions associated with skeletal muscle abnormalities including CHF[256],

peripheral vascular disease[257] and diabetes mellitus[253]. To date, skeletal muscle NIRS has not been used in cyanotic congenital heart disease to investigate tissue oxygenation abnormalities.

4.1.7 Fatigue

Early skeletal muscle fatigue during exercise has been demonstrated to be one cause of exercise intolerance in patients with CHF and is due to intrinsic muscle factors rather than cardiac reserve or central control[258]. It has also been shown to occur in patients with hypoxaemia secondary to COPD[259].

As discussed in section 1.4.5 peripheral fatigue is mediated by metabolic by-products that accumulate during muscle contraction. This mechanism is at least partly O_2 dependent[260]. Thus, it is reasonable to propose that early fatigue may underlie exercise intolerance in the severe hypoxaemia found in Eisenmenger syndrome.

Thus, against this background, the aims of this part of the study were to investigate whether abnormal production of O_2 dependent substances contribute to the fatigue and exercise intolerance seen in patients with Eisenmenger syndrome.

4.2 Methods

4.2.1 MRS

10 adults with Eisenmenger syndrome and 10 age matched controls were studied. The subject was positioned supine and legs first in the MRI scanner (Phillips Achieva 3T scanner). A linearly polarised transmitter and receiver ^{31}P coil with a diameter of 14cm was secured over

the posterior aspect of the left calf and the left leg was placed in the midline with the centre of the coil at the isocentre of the magnet.

Survey images were obtained to check the position of the coil and ISIS (image-selected *in vivo* spectroscopy) technique was used to select a voxel of interest (VOI). The VOI was moved to include the maximum amount of muscle and minimum bone. 2D shimming on the tissue water proton signal was used to optimise the magnetic field homogeneity. Manual fine adjustment of the centre frequency (F_0) was performed if the automatic F_0 determination was not correct.

Spectra were obtained with a repetition time of 3 seconds with two averages per spectrum. Thus the acquisition time for each spectrum was 6 seconds.

4.2.1.1 MRS Exercise

After one minute of resting data subjects performed plantar flexion of their left foot against a resistance pump. Contribution from the quadriceps during the exercise was minimised by securing the upper leg to the table. An investigator present in the scan room encouraged subjects to maintain a constant pace (approximately 30 extensions per minute) and to work to volitional fatigue, that is they felt unable to exercise further. On fatigue they were asked to stop, refrain from moving the leg further and recovery data was collected for 5 minutes.

4.2.1.2 MRS Data Analysis

The spectra were analysed using a time domain fitting programme (AMARES) on jMRUI (java-based Magnetic resonance User Interface). Post-processing was performed with Fourier

transformation and 5Hz Gaussian line broadening. The PCr peak was used for phase correction and quantification was carried out using AMARES[261]. A prior knowledge file was used to pre-select the peaks[262].

The relative concentrations of PCr and Pi were then calculated from the area under the peaks for each spectrum throughout rest, exercise and recovery and were plotted against time (figure 4.1). PCr recovery, expressed as the PCr recovery halftime ($t_{½}$) was calculated, by graphical interpolation, as the time taken to reach 50% of the initial PCr concentration after cessation of exercise. Intracellular pH was calculated as previously described in section 4.1.3 and peak drop in pH during exercise was calculated.

4.2.2 NIRS

NIRS was used to measure skeletal muscle oxygenated Hb concentration ([oxy-Hb]), deoxygenated Hb (deoxy-Hb]) and total Hb ([HbT]). The probe, an OxiplexTS Near Infrared tissue oximeter (ISS Inc., Champaign, IL, USA), is a frequency domain multi-distance NIRS. It consists of four light sources and one detector. The light sources are emitted at two separate wavelengths, 692nm and 834nm. The NIRS probe limits contribution by skin and subcutaneous tissue by using a source-detector distance of 3-4.4cm. The degree of absorption of the light source represents both the concentration of Hb within the microvasculature and also the myoglobin in the myocytes. It is not possible to distinguish between Hb and myoglobin due to their identical spectral characteristics. NIRS therefore uses the differences in the absorption of the light between oxy-Hb and deoxy-Hb. Similar absorption of both oxy- and deoxy-Hb is seen at a wavelength of 834mm thus absorption is proportional to the [HbT]. However, at the shorter wavelength of 692nm, the absorption is predominantly by deoxy-Hb

and thus differences in absorption at this wavelength reflect changes in [deoxy-Hb]. [Oxy-Hb] can thus be calculated by the difference between [HbT] and [deoxy-Hb]. Furthermore, alterations of the blood volume within the tissues can be used to reflect changes in the [HbT][256].

The subject was positioned in a comfortable semi-supine position. The dominant arm was supported at heart level so that circulation was completely unrestricted and the NIRS probe was secured over the flexor digitorum superficialis muscle. A manual pneumatic cuff was applied around the upper arm, proximal to the probe.

Continuous measurements of [oxy-Hb], [deoxy-Hb] and [HbT] were recorded for 3 minutes of rest. The cuff was then inflated to 200 mmHg for two minutes to induce forearm arterial occlusion. Continuous recordings were made throughout this period and for a further two minutes after release of the cuff.

During arterial occlusion, the fall of [oxy-Hb] – [deoxy-Hb] was used to calculate the O_2 consumption rate, VO_2, of resting muscle. The O_2 consumption rate was expressed per 100g of muscle as previously described[263]. The ratio of [oxy-Hb] and [HbT] was used to calculate muscle O_2 saturations.

4.2.3 Fatigue

Five patients with Eisenmenger syndrome and five healthy volunteers took part in this study. This was carried out on a separate day to other forms of exercise testing. The subject was positioned in a semi-supine position with their dominant arm extended and supported and the

wrist. The hand rested on a handgrip dynamometer. A venous cannula was inserted into this arm in the anterior cubital fossa and rest samples for [K^+], [Pi], [lactate] and [H^+] were taken.

After a rest period of five minutes, the subject was asked to perform a handgrip as hard as they could and to sustain this at maximal voluntary effort (MVE) for as long as they were able to. Peak force and duration of handgrip were recorded. On release of the handgrip the timer was started and bloods were taken immediately for [K^+], [Pi], [lactate] and [H^+]. Further bloods were taken at 3 minutes and 6 minutes. After seven minutes they were asked to repeat the handgrip at MVE, again for as long as they were able to. On release, bloods were repeated at identical time points to before.

Bloods taken during the experiment were placed on ice in an insulated container. The samples for [K^+], [lactate] and [H^+] were analysed using a pHox Plus L analyser (Nova Biomedical, Deeside, UK) within 20 minutes of completing the experiment. A separate sample was spun down in a centrifuge and the serum was snap frozen using liquid nitrogen. These samples were stored in a -70°C freezer for later analysis of Pi. Unfortunately these samples were lost and so never analysed.

4.2.4 Statistical analysis

Continuous data are expressed as mean ± SEM. Groups were compared using independent-samples t-test. The relationship between two variables was assessed by Pearson correlation coefficient. A p value of <0.05 was taken as being statistically significant.

4.3 Results

4.3.1 MRS Baseline Data

Ten subjects with Eisenmenger syndrome and eight healthy control subjects underwent ^{31}P MRS of their skeletal muscle. There was no significant difference between the groups in age, sex or body mass index (Table 4.1). Patients with Eisenmenger syndrome had a higher ability index score compared to healthy controls and higher haemoglobin due to lower arterial O_2 saturations. The calculated O_2 content was higher in the Eisenmenger group than the controls (22.3± 0.8 mlO_2/100ml vs 19.4±0.5 mlO_2/100ml, $P<0.05$).

4.3.2 Exercise Data

Figure 4.1 shows a typical example of a spectrum at baseline and at end exercise in a healthy control subject. All subjects were able to exercise as instructed until fatigue. Fatigue occurred at a similar level of PCr depletion (figure 4.2) in both healthy volunteers (55.6 ± 5.2%) and patients with Eisenmenger syndrome (57.3 ± 6.6%, p = 0.8) and there was a similar increase in Pi (252.1 ± 16.5% vs 237.6 ± 17.6%, p = 0.5) (figure 4.3), suggesting that patients with Eisenmenger syndrome did not stop early. There was no difference in exercise times between healthy controls (218 ± 24s) and Eisenmenger patients (208 ± 12s, p = 0.7) however the exercise protocol was not standardised for effort.

4.3.3 pH

Resting intracellular pH was higher in patients with Eisenmenger syndrome (7.05 ± 0.02) than in healthy controls (7.0 ± 0.02) although this did not quite meet statistical significance, p=0.08. Whilst there was a positive correlation between resting intracellular pH and

haemoglobin concentration in control subjects (r = 0.374, p = 0.03, figure 4.4a), this was not seen in patients with Eisenmenger syndrome (r = 0.29, p = 0.5, figure 4.4b).

The decrease in intracellular pH from baseline during exercise (figure 4.5) was not different between the two groups (0.3 ± 0.06 vs 0.28 ± 0.04, p = 0.7).

4.3.4 Recovery Data

All subjects had recovered their PCr to the baseline value after the five minutes of rest following exercise. There was no significant difference in the PCr recovery $t_{1/2}$ between the healthy controls (34.9 ± 2.9s) and patients with Eisenmenger syndrome (35.2 ± 1.7s, p = 0.9) (Figure 4.6). There was a weak negative correlation between age of subject and PCr recovery $t_{1/2}$ within the patient group but this did not reach significance (r = -0.42, p = 0.08).

4.3.5 NIRS Results

NIRS assessment of muscle O_2 consumption was made in five subjects with Eisenmenger syndrome and five healthy controls. There was no difference in age between groups (44 ± 3.8 vs 42 ± 4.3, p = 0.7). The patients with Eisenmenger syndrome however had a significantly higher haemoglobin (20.1 ± 0.6g/dl) compared to healthy controls (13.5 ± 0.9g/dl, p = 0.001) calculated from a venous blood sample.

Figure 4.7 shows typical graphs obtained from NIRS recording. Resting muscle O_2 saturations were non-significantly higher in the controls (72 ± 4.6%) than the Eisenmenger patients (64 ± 6.3%, p = 0.3). Concentrations of oxy-Hb, deoxy-Hb and HbT were all higher in the Eisenmenger group than the healthy controls although this did not reach statistical

significance, possibly due to the small group size (table 3.2). On the basis of changes seen following arterial occlusion, there was a trend towards higher resting O_2 consumption in the subjects with Eisenmenger syndrome (0.23 ± 0.06) compared to healthy controls (0.15 ± 0.04, p = 0.3) but again this did not reach significance.

4.3.6 Fatigue Results

Five patients with Eisenmenger syndrome and five healthy control subjects took part in this study. There was no difference in the ages of the two groups (44.0 ± 3.0 vs 39.4 ± 3.0, p = 0.37).

The patients with Eisenmenger syndrome were able to generate similar peak forces to the healthy controls during both handgrips (handgrip 1 20.0 ±1.2N vs 23.9 ± 3.7N, p = 0.37, handgrip 2 18.0 ± 1.5N vs 22.0 ± 2.7N, p = 0.27). The control subjects however were able to sustain the MVE for significantly longer than the patients (handgrip 1: 163.0 ± 35.7s vs 89.0 ± 16.8s, p < 0.05, handgrip 2: 170.0 ± 35.6s vs 77.6 ± 8.5s, p <0.05) indicating early fatigue of Eisenmenger patients.

The $[H^+]$, expressed as pH, was the same for both groups at rest and at each time point with no difference in the fall in pH immediately after each handgrip (figure 4.8a). Patients with Eisenmenger syndrome had a trend towards higher $[K^+]$ at all time points (figure 4.8b) but these values did not reach statistical significance, possibly due to the small sample size. The increase in $[K^+]$ after both handgrips was again non-significantly higher in the patients compared to the controls (figure 4.8b). The resting [lactate] was significantly higher in patients with Eisenmenger syndrome compared to healthy controls. There was a trend for a

higher lactate in the patient group at other time points but this did not reach significance (figure 4.8c). The increase in lactate after handgrip was similar for both groups.

4.4 Discussion

This is the first study to specifically investigate the skeletal muscle adaptations in patients with Eisenmenger syndrome. It is known that skeletal muscle mitochondria require a constant supply of fuels and O_2 to generate energy in the form of ATP. Thus, if hypoxia occurs, such as in Eisenmenger syndrome, it seems probable that adaptations must occur to maintain cellular energy haemostasis.

The findings of the present study actually showed by using ^{31}P MRS, that patients with Eisenmenger syndrome showed a similar fall in PCr at fatigue as controls. Moreover, mitochondrial oxidative capacity, as measured by the PCr recovery, was the same in our patients with Eisenmenger syndrome as in the group of age matched healthy controls. These results differ from previous studies on other groups of patients with cyanotic congenital heart disease[254;255] which both showed significantly prolonged PCr recovery in the cyanotic patients. Both of these studies also showed a bigger PCr depletion at peak exercise and larger falls in pH during exercise compared to the non-cyanotic controls, which again is in contrast with the present findings on Eisenmenger patients. One possible reason for the differences may be that the other studies investigated much younger patients than we did. Miall-Allen et al looked only at children[254] and Adatia et al, whilst including some adults, states a mean age of 17.3 years[255]. This contrasts to the mean age of 42.6 ± 2.1years for our Eisenmenger patients who underwent MRS. Thus, it could be that the beneficial adaptations to the chronic hypoxaemia continue to develop with increasing age; certainly, we showed a weak negative

correlation with PCr recovery and age. A further explanation may lie in the heterogeneous group of conditions studies in the two earlier studies. They studied a number of conditions including those with single ventricle physiology and other factors, including an inability to increase cardiac output during exercise, which may account for the abnormally low oxidative capacity they reported.

In healthy individuals, mitochondrial oxidative capacity is in part dependent on O_2 delivery. In response to hypoxia there is an increase in O_2 delivery to the tissues by increasing ventilation, cardiac output and then an increase in haemoglobin and capillarity[135;264]. In the longer term there is also a decreased O_2 utilisation, for example by improved substrate utilisation. Whilst chronic hypoxia in natives at high altitude is associated with a decreased mitochondrial density within the skeletal muscle[156;169], in lowlanders who are acclimatised to high altitude ATP production during exercise is maintained by improved metabolic efficiency. Thus, more ATP is synthesised per molecule of O_2 consumed[187]. This may be achieved by the preferential use of carbohydrates during exercise as has also been demonstrated in high altitude dwellers[265]. Indeed, a recent study investigating climbers at extreme altitude showed preserved skeletal muscle function with no changes in exercising metabolites and PCr recovery despite significant muscle atrophy[266].

Considering patients with Eisenmenger syndrome, they certainly have a significantly higher Hb concentration compared to controls. Similarly, the Caudwell Xtreme group of high altitude mountaineers also demonstrated a very elevated Hb concentration which maintained O_2 content or above sea levels up to an altitude of 7100m[267]. In the present study we have also shown that Eisenmenger patients have a higher calculated arterial O_2 content. This might

be expected to aid the local delivery of O_2 to the cells even at rest and it might be anticipated that delivery may also be enhanced during exercise by augmented local vasodilatation.

In fact, at rest, we demonstrated that the patients with Eisenmenger syndrome had a trend for higher O_2 delivery, as measured by HbT, and O_2 consumption than healthy controls, despite there being lower O_2 saturations within the muscle. This is consistent with the idea of improved O_2 delivery to the muscle in the face of marked hypoxaemia and at least part of this will be due to the markedly elevated Hb concentration and O_2 content of the blood. An unchanged O_2 consumption in skeletal muscle would be consistent with our results from the CPET study, which demonstrated a similar whole body VO_2 in both Eisenmenger patients and healthy controls (see chapter 2).

During exercise we showed, in both the ^{31}P MRS study and by assaying venous blood in the fatigue study, that patients with Eisenmenger syndrome have a similar fall in intracellular and extracellular pH to the healthy control group. The pH decrease during exercise is due in part to the production of lactate. In acute hypoxia, for example in unacclimatised individuals at high altitude, there is a much greater increase in lactate for a given exercise task due to an increase in anaerobic metabolism[188]. However, in individuals who are acclimatised to hypoxia there are depressed levels of blood lactate[59] and this has been termed the lactate paradox. Hochachka describes the functional advantages of this as faster recovery from exercise and maintenance of the more energy efficient aerobic metabolism during hypoxia[268]. Allen et al also showed that Sherpas, who are phylogenetically adapted to hypoxia, have a stable intracellular pH during exercise on ^{31}P MRS[269]. Thus, it appears that ATP production becomes more efficient in the hypoxic state. The similar fall in pH on

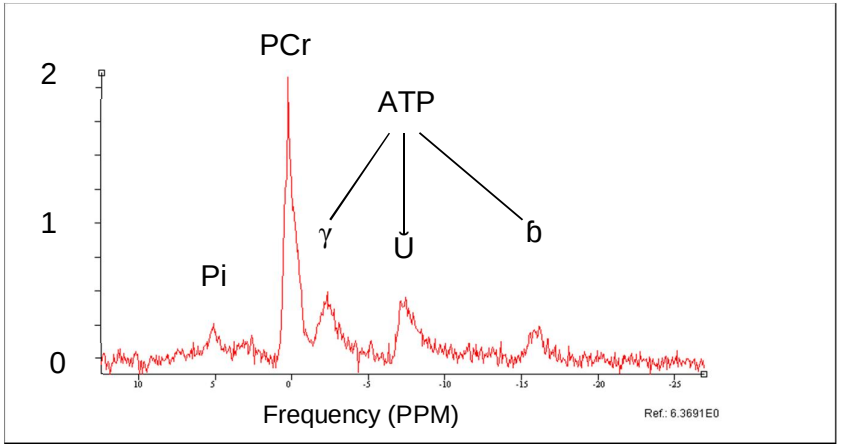
exercise in patients with Eisenmenger syndrome compared to the control group may indicate a decreased reliance on anaerobic metabolism. This would be consistent with our finding from the CPET study (chapter 2), which showed that they reach their anaerobic threshold at a proportionally later stage of exercise compared to controls. On the other hand, if you consider that the interstitial H^+ increases during exercise when ATP usage exceeds oxidative phosphorylation[90], the fact that H^+ was not raised in Eisenmenger patients may be evidence that the efficiency of oxidative phosphorylation is increased.

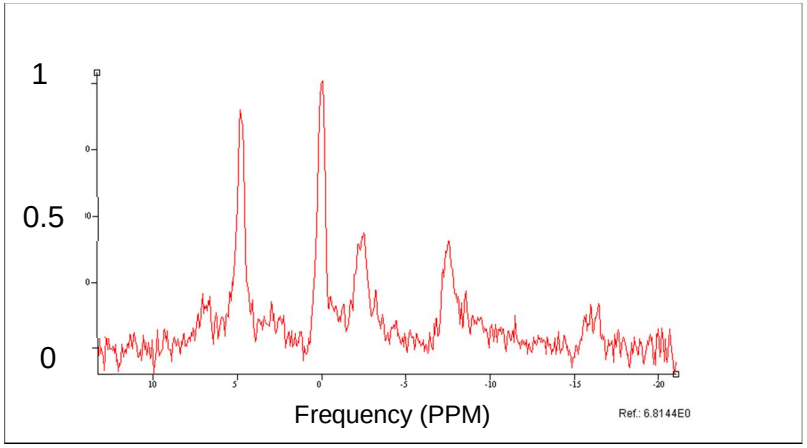
Whilst our patients with Eisenmenger syndrome were able to generate similar peak forces using a handgrip dynamometer, we demonstrated that they fatigue more quickly compared to healthy controls. The venous blood samples indicated that K^+ and pH, which have been considered to be associated with fatigue[78], failed to show any significant differences between the patient group and controls. However, there was a clear trend for higher $[K^+]$ at all time points in the Eisenmenger patients and this may have become significant if we had studied a greater number of subjects. Thus, it still may be that K^+ does contribute to fatigue in Eisenmenger syndrome. It is unfortunate that the phosphate samples were lost prior to analysis and this gives a further reason to expand this part of the study in the future.

Amann et al investigated the effects of hypoxia severity, from normoxia to severe hypoxia, on both the peripheral and central determinants of exercise fatigue in healthy subjects[270]. They demonstrated that with increasing hypoxia they switch from a principally peripheral origin of fatigue in normoxia, to a more centrally driven origin with increasing levels of hypoxia. Thus, patients with Eisenmenger syndrome may also have a significant central drive for their fatigue which to date has not been investigated.

Muscle biopsies from patients with Eisenmenger syndrome may also give further information regarding the cause for fatigue. For example, in CHF there is a marked increase in fast twitch fibres[109-111] which have high concentrations of glycolytic enzymes and fatigue early, whereas high altitude natives have increased slow twitch fibres which have greater oxidative capacity and are more resistant to fatigue[173]. These have a greater oxidative capacity and are more resistance to fatigue.

In summary, from the results described in this chapter, it is clear that the skeletal muscle of patients with Eisenmenger syndrome has under gone marked adaptations to become "hypoxia tolerant", with similar mitochondrial oxidative capacities, as measured by PCr recovery and similar falls in pH during exercise compared to healthy controls. Our studies have backed up previous work showing that hypoxia-tolerant systems are not reliant on anaerobic metabolism despite severe hypoxaemia. The following two chapters investigate whether there are further adaptations in the cardiac muscle.





Rest

End exercise

Figure 4.1 Comparison of skeletal muscle ^{31}P spectra from a healthy control subject at rest and at end exercise. It is clear that PCr concentrations fall with a concomitant increase in Pi concentration.

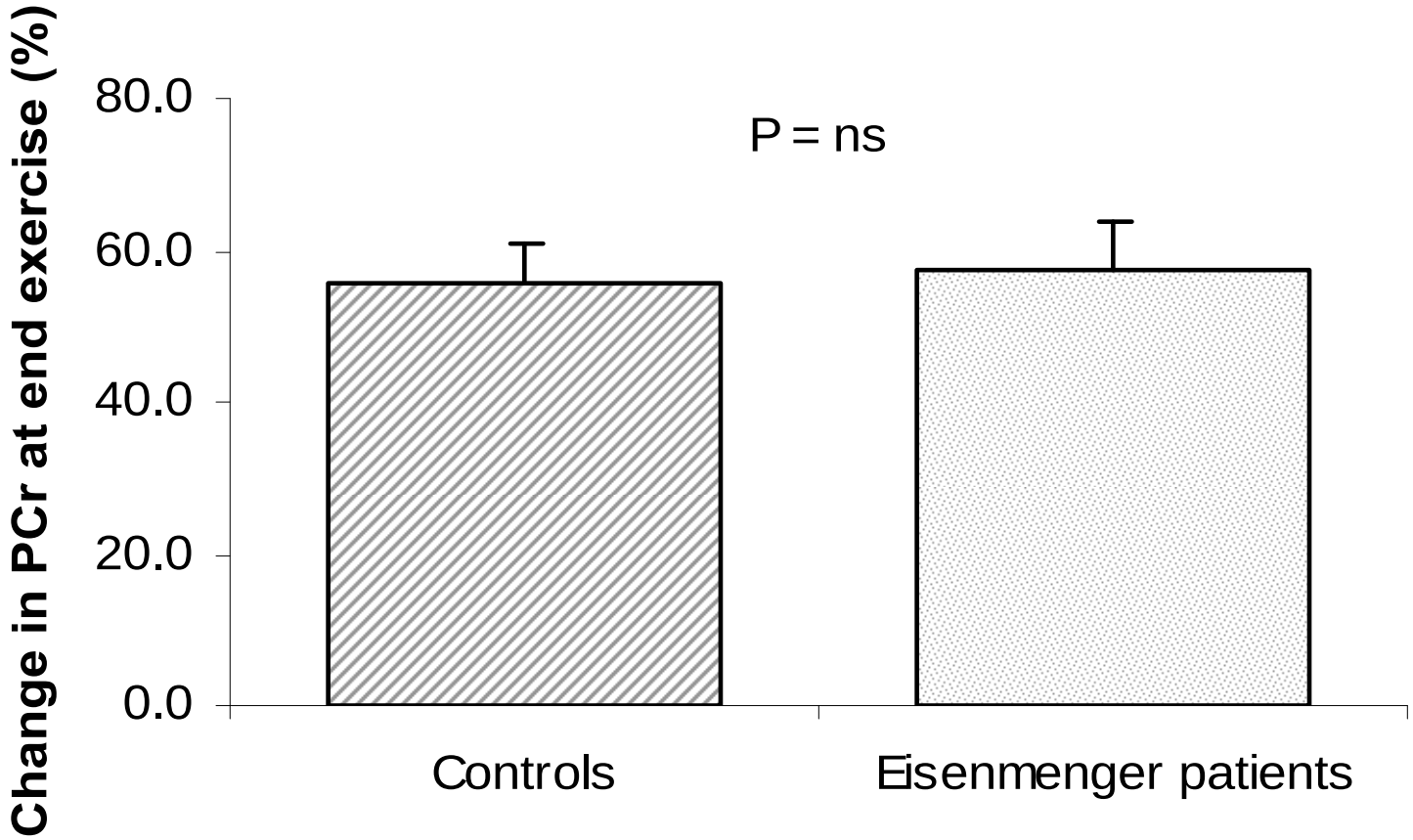


Figure 4.2 Percentage change in relative PCr concentrations at end exercise compared to rest for healthy controls and patients with Eisenmenger syndrome. Columns show mean±SEM; (ns = non significant).

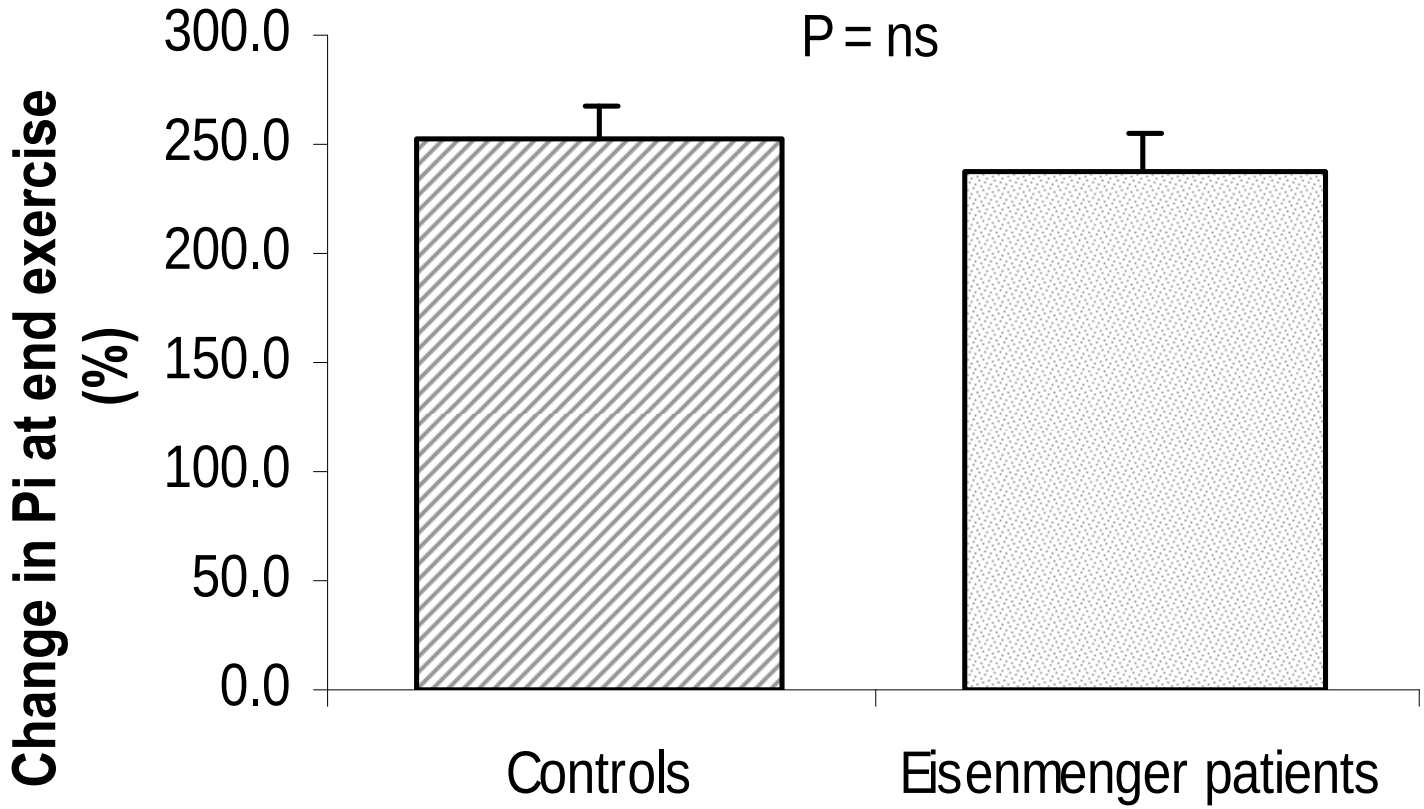
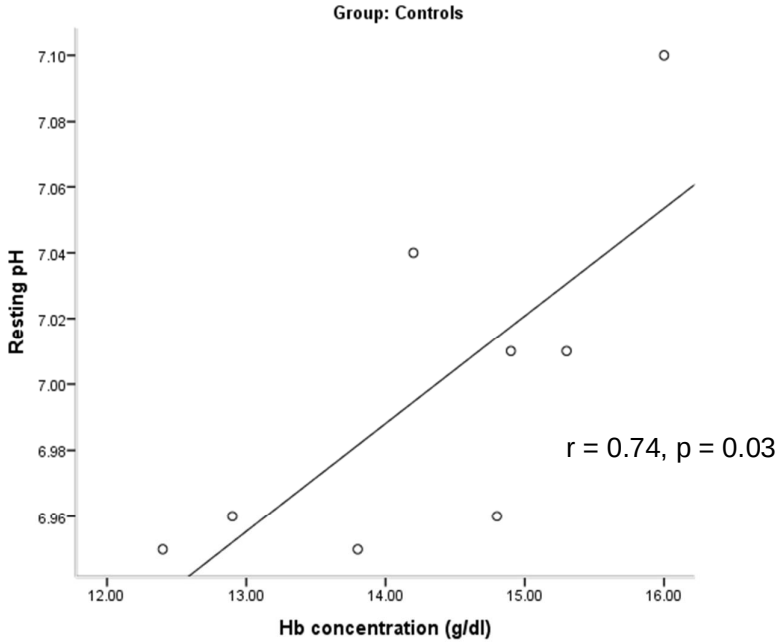


Figure 4.3 Percentage change in relative Pi concentrations at end exercise compared to rest for healthy controls and patients with Eisenmenger syndrome. Columns show mean±SEM; (ns = non significant).

4.4a



4.4b

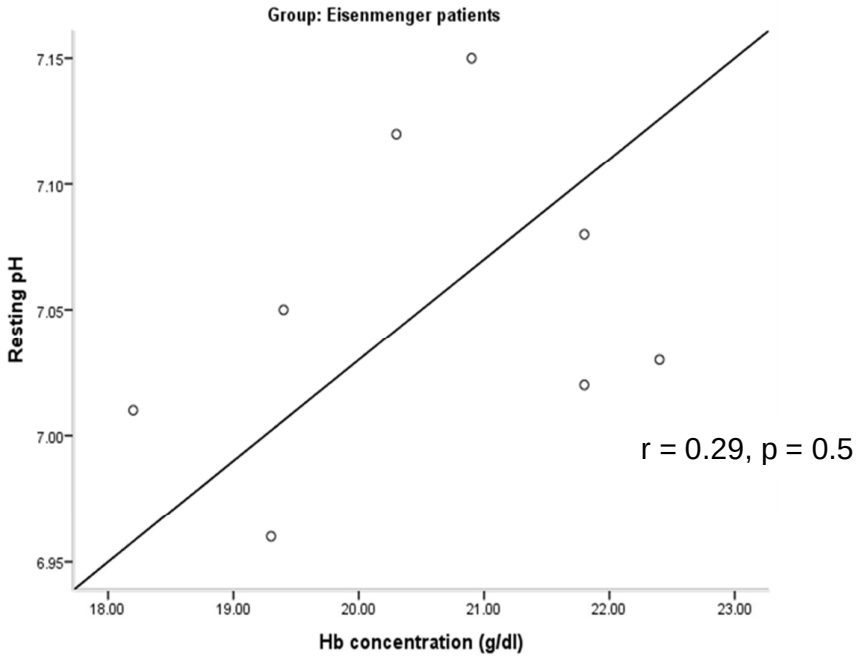


Figure 4.4 Scatter plot showing relationship between haemoglobin concentration (x-axis) and resting intracellular pH (y-axis) in control subjects (4.4a) and Eisenmenger patients (4.4b)

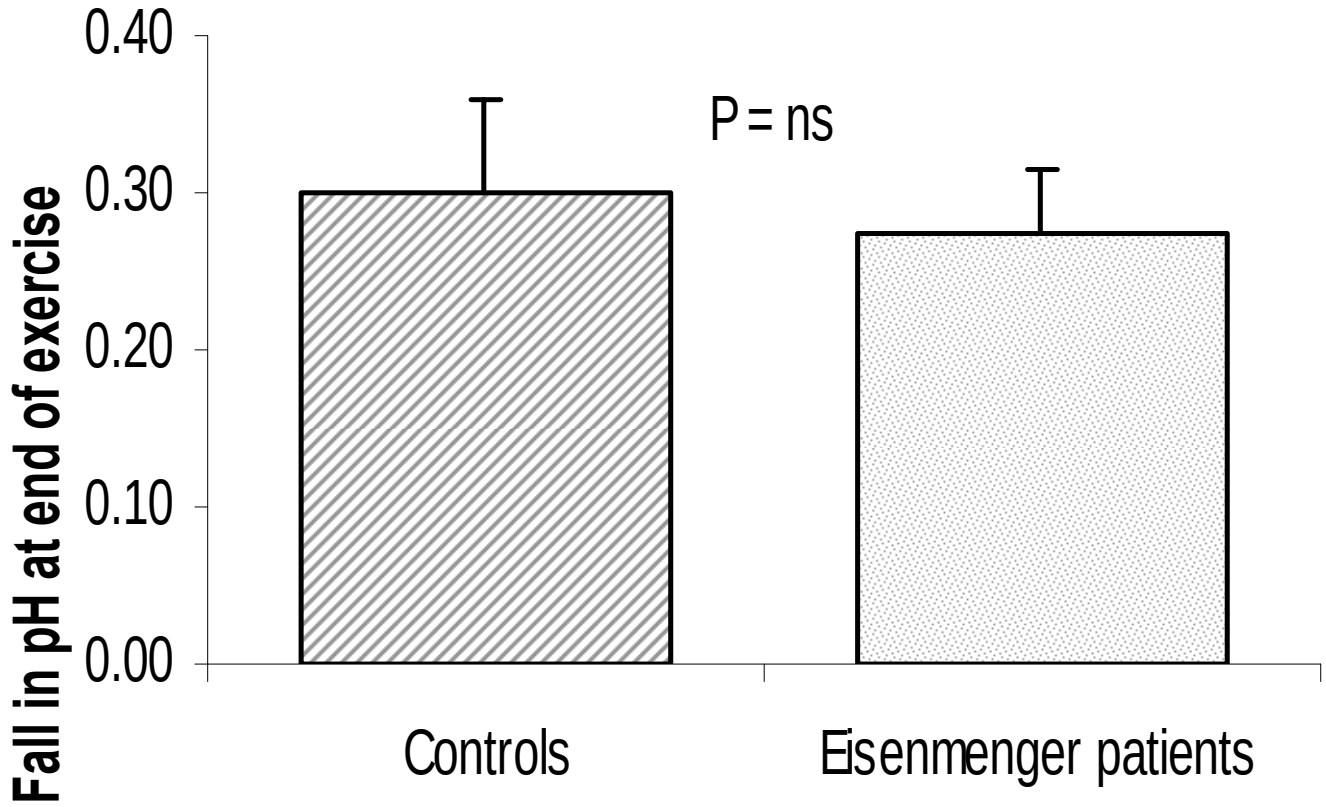


Figure 4.5 Decrease in intracellular pH from baseline resting value at end of exercise for healthy controls and patients with Eisenmenger syndrome. Columns show mean±SEM; (ns = non significant).

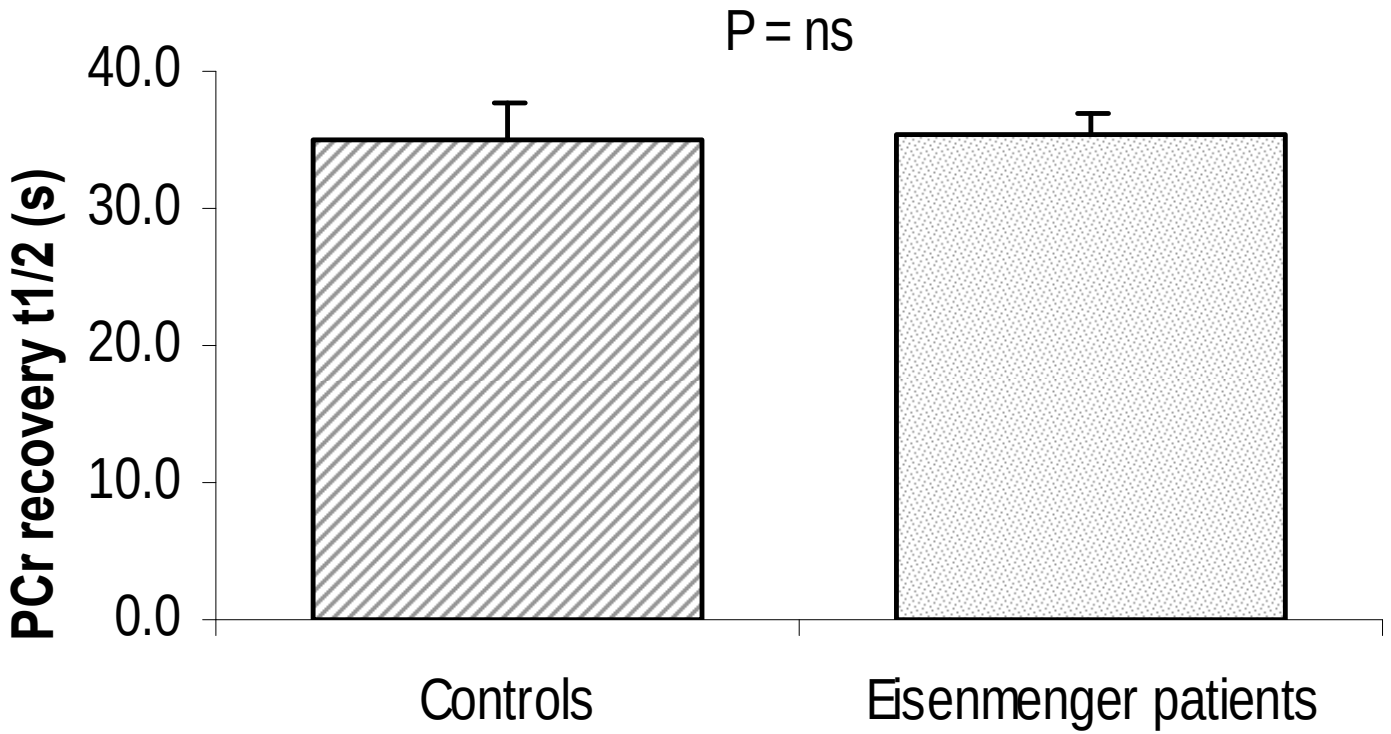
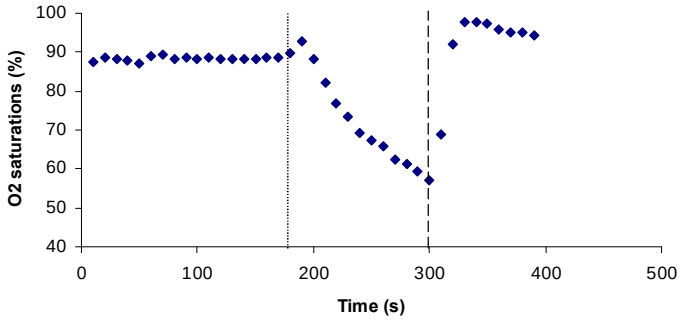


Figure 4.6 PCr recovery half-times for healthy controls and patients with Eisenmenger syndrome. Columns show mean±SEM; (ns = non significant).

4.7a



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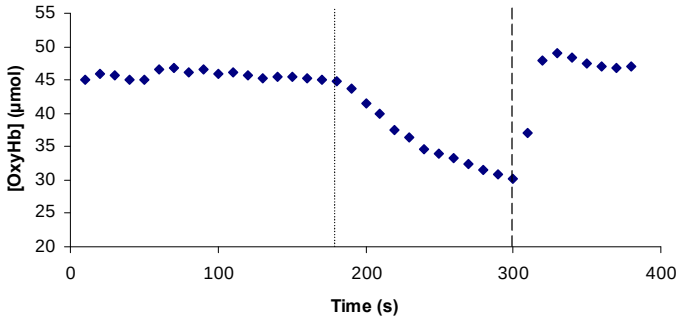

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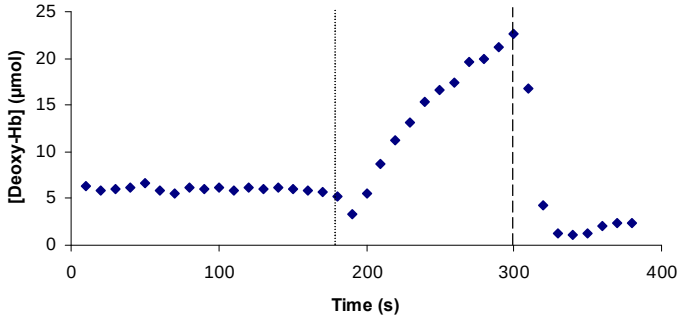

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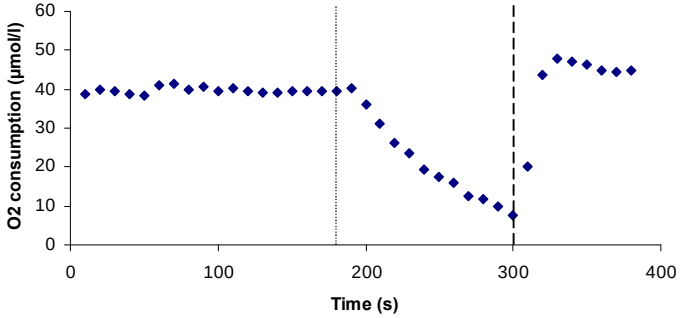


Figure 4.7 Typical graphs from NIRS during arterial occlusion in a healthy control subject showing a) muscle O_2 saturations b) [Oxy0Hb] c) [Deoxy-Hb] d) O_2 consumption.
Dotted line represents start of arterial occlusion; dashed line represents cessation of arterial occlusion.

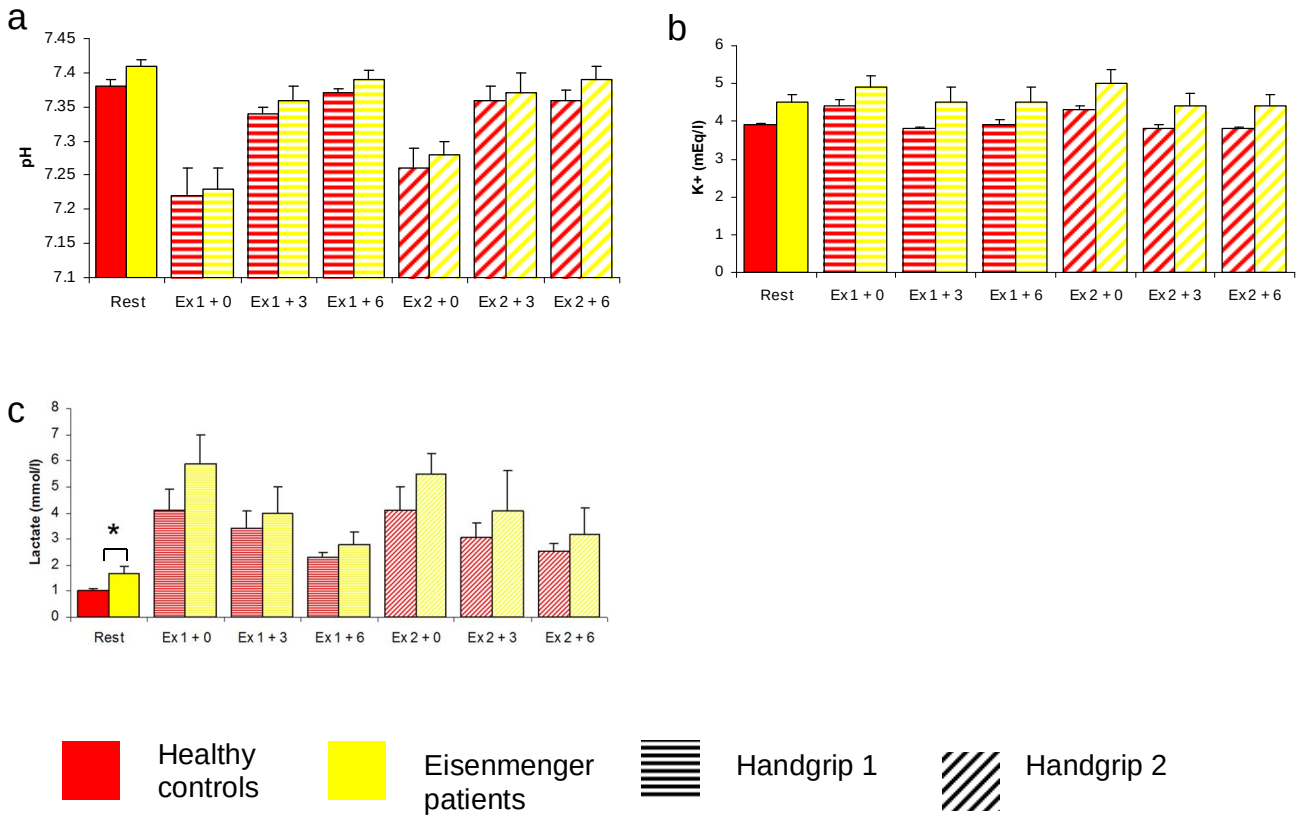


Figure 4.8 Effect of static handgrip at 100% MVE on venous efflux of a) pH b) K+ concentration c) Lactate. Columns represent mean±SEM. In each case, values are shown at rest and at 1, 3 and 6 min after contraction 1 and contraction 2 as indicated below.

	Healthy control subjects (n = 8)	Eisenmenger syndrome patients (n = 10)
No of women, (% of n)	6 (75)	6 (60)
Age (y)	42.6 ± 2.1	41.9 ± 2.3
Ability index	1.0 ± 0	1.7 ± 0.2*
Haemoglobin (g/dl)	14.3 ± 0.4	20.4 ± 0.4†
Baseline O_2 saturations (%)	98.3 ± 0.3	79.7 ± 2.1†
Calculated O_2 content (mlO_2/100ml)	19.4 ± 0.5	22.3 ± 0.8*
Body mass index (kg/m^2)	25.8 ± 1.3	23.1 ± 0.6
Pulmonary vasodilator therapy, n (% of n)	0 (0)	3 (33)

Data are expressed as mean ± SEM. *P<0.05; †P<0.001 vs control

Table 4.1 Baseline characteristics of patients undertaking skeletal muscle ^{31}P MRS study

	Healthy control subjects (n = 5)	Eisenmenger syndrome patients (n = 5)	p value
Muscle O_2 saturations (%)	72 ± 4.6	64 ± 6.3	0.30
[Oxy-Hb] (µmol)	54.6 ± 4.2	71.8 ± 8.3	0.10
[Deoxy-Hb] (µmol)	23.3 ±6.0	40.8 ± 9.5	0.16
[HbT] (µmol)	78.0 ± 10.1	103.1 ± 10.3	0.12

Table 4.2 Resting NIRS measurements in Eisenmenger syndrome patients and healthy controls

5. INVESTIGATION OF RIGHT AND LEFT VENTRICULAR FUNCTION WITH ECHOCARDIOGRAPHY

5.1 Introduction

As discussed in the introduction, patients with Eisenmenger syndrome have preserved right ventricular function and clinical heart failure is uncommon. That the RV function remains preserved indicates that it must have adapted to maintain contractility in the face of pulmonary hypertension. This present study uses echocardiography to investigate the systolic and diastolic function of both the right and left ventricles and the role of the interventricular septum. The discussion below provides the background to this study.

5.1.1 Echocardiography and the RV

Transthoracic echocardiography is the most commonly employed method for the assessment of the RV in the routine clinical setting. However, echocardiographic assessment of the RV is difficult due to its complex geometry, poor endocardial definition and the load dependency of most conventional echo parameters[35]. The American Society of Echocardiography recommend that echocardiographic assessment of the RV should use "multiple acoustic windows" and the assessment should be based on qualitative and quantitative parameters[271]. The parameters they advise are outlined in table 5.1 along with reference limits for abnormal results.

5.1.2 Indices of RV Function

The various indices that can be followed are considered below.

FAC The percentage fractional area change (FAC) is defined as (end-diastolic area – end-systolic area)/end-diastolic area x 100. It is better correlated to RV ejection fraction derived from cardiac MRI than other 2D derived indices[272]. Nass et al demonstrated that, in patients with a large pulmonary embolism, it can be used as an independent predictor of a poor clinical outcome[273]. However, poor endocardial definition can limit its accuracy and reproducibility.

TAPSE Tricuspid annular plane systolic excursion (TAPSE) is a simple and reproducible measure of RV function. Although it predominantly assesses longitudinal function, it does have a reasonable correlation with radionuclide derived RVEF[274]. TAPSE does not assess the septal contribution to RV function and ignores the outlet portion of the RV[275;276]. It is also load dependent and thus its accuracy in the presence of severe TR is unclear[35].

TDi Tissue Doppler imaging (TDi) of the RV free wall at the lateral tricuspid annulus is a simple, reproducible and quantitative method of assessing RV function. The peak systolic excursion velocity (Sm) has good correlation with radionuclide angiography and is good at discriminating between normal and abnormal RV function[271].

MPI The myocardial performance index (MPI) is a global assessment of RV function, incorporating both systolic and diastolic function. It is independent of geometric assumptions and is relatively independent of heart rate and loading conditions[35]. It has been shown to be a useful predictor of adverse outcome in IPAH[277] and has demonstrated to be accurate in congenital heart disease; Eidems et al showing a higher MPI with increasing RV

dysfunction in patients with Ebstein's anomaly, a condition characterised by a complex RV anatomy[278;279].

IVRT Isovolumic relaxation time (IVRT) is the period of the cardiac cycle after the aortic valve closes and before the mitral valve opens. During this period tension of the myocardial fibres reduces without any lengthening thus the volume remains unaltered. In diastolic dysfunction there is a deterioration in active relaxation with a prolonged IVRT[280]. It has been demonstrated to be prolonged in patients with PAH and improves with a reduction in the RV afterload[281].

IVA Isovolumic acceleration (IVA) is a TDi-derived parameter that reliably assesses the intrinsic contractility of the RV[282]. It has been shown to be less load dependent than other indices under a range of physiological conditions[282;283] and correlates with the severity of disease in both repaired tetralogy of Fallot[284] and in patients with an atrial switch for transposition of the great arteries whose RV is characterised by pressure overload[285].

5.1.3 Echocardiographic Assessment in Eisenmenger Syndrome

The majority of studies using echocardiography to assess RV function in Eisenmenger syndrome describe the RV function in mostly qualitative terms. For example Daliento et al describe a visual assessment of global RV function[16]. This limits our understanding of the pathophysiological process leading to any RV dysfunction.

Kalogeropoulos et al used both M-mode and Doppler derived indices, including RV strain, to compare RV function in patients with Eisenmenger syndrome, PAH not due to congenital heart disease and subjects with a structurally normal hearts[36]. They demonstrated a preserved short axis function in patients with Eisenmenger syndrome compared to PAH but longitudinal function was depressed in both groups. They comment that conventional assessment of RV function which is heavily dependent on longitudinal function may not be suitable for patients with Eisenmenger syndrome.

On the other hand, Moceri et al carried out a longitudinal echocardiographic study of Eisenmenger syndrome patients over a six year period[286]. They report that most patients have maintained or only mildly impaired RV function as described by TAPSE or TDi derived systolic velocities. They also found that a decreased TAPSE is a predictor of poor outcome. Similarly, Van de Bruaene et al demonstrate that patients with a TAPSE of <16mm have a lower event-free survival and a greater all-cause mortality[287]. TAPSE has also been shown to be strongly related to survival in IPAH although the authors do comment that TAPSE may be overestimated in the presence of significant TR[288].

To date no studies have examined the contribution of septal function or diastolic dysfunction to the known exercise intolerance and dyspnoea in Eisenmenger syndrome. We also examined the left ventricular systolic and diastolic function and looked for correlation between RV and LV function in the presence of a large intracardiac shunt.

5.2 Methods

5.2.1 Data Acquisition

10 patients with Eisenmenger syndrome and 9 age matched healthy controls were studies. All subjects were scanned using a Vivid 7 cardiac ultrasound system and a standard adult 2D probe (GE Healthcare). Data was analysed off line using a dedicated workstation and EchoPAC software (version 108.1.4, GE Healthcare). Patients were positioned in a left decubitus position with their left arm raised to spread the ribs and optimise their acoustic window. Images were normally obtained during quiet respiration. If images were obtained in held expiration then valsalva was avoided. ECG gating was used in all subjects.

The frame rate was optimised to a minimum of 40 frames per second by reducing the depth and sector width as appropriate. The image was focused on the structure of interest. The loop length was set at five beats. Ectopic beats and beats post ectopics were avoided. Measurements were made on three consecutive beats and the final value was the mean of these.

Chamber quantification of the left and right ventricles, doppler quantification and tissue Doppler imaging were performed as described by the American Society of Echocardiography[271;289;290].

5.2.2 Assessment of Left Ventricle

Dimensions Left ventricular dimensions were measured using M Mode in the parasternal long axis view (PLAX). The cursor was positioned through the tip of the mitral valve leaflets. Septal and posterior wall thickness and LV cavity diameter were obtained at

end diastole and end systole. If it was not possible to position the cursor perpendicular to the long axis, measurements from 2D were performed.

Ejection fraction The left ventricular ejection fraction (LVEF) was calculated using the modified Simpson's biplane method as previously described[291]. In brief, the LV end diastolic volume (EDV) and end systolic volume (ESV) were calculated from the summation of a stack of elliptical discs. The endocardial borders were manually traced, avoiding the papillary muscles, in both the apical four chamber (A4C) and apical two chamber (A2C) views. End diastole was taken as either the onset of the QRS or one frame after mitral valve closure. End systole was taken as the frame preceding mitral valve opening. In the A2C the mitral valve was not always clearly visualised and the frames at which the ventricle appeared the biggest or smallest were used. The ventricle is then divided along its long axis into a series of discs of equal height. The stroke volume (SV) is calculated as EDV – ESV. The ejection fraction is then calculated as SV/EDV.

Mitral inflow Pulsed wave (PW) Doppler was used with the sample volume at the tips of the mitral valve leaflets in the A4C view. Peak forward velocities (E wave and A wave) and deceleration time of the A wave were recorded,

Isovolumic relaxation time Continuous wave (CW) Doppler cursor was positioned in an intermediate position between the inflow and outflow signals in the apical five chamber view. The IVRT was calculated as the interval from the closure of the aortic valve to the opening of the mitral valve.

Stroke volume LV stroke volume (SV) was calculated as the product of the left ventricular out flow tract (LVOT) cross sectional area and LVOT velocity. The LVOT area was derived from the diameter in the PLAX view, making the assumption that it is circular in systole (area = πr^2). LVOT velocity was measured using PW Doppler in the apical five chamber view with the sample volume 5mm proximal to the aortic valve. The SV was indexed for body surface area.

Tissue Doppler imaging A high frame rate was obtained (>100 frame/sec) by reducing the depth and sector width. The A4C view was used but the views were modified to keep the angle of alignment to <15° from the motion direction to minimise error. TDi velocities were obtained for the lateral wall and septum by placing a 3-5mm pulsed Doppler sample volume at the junction of the LV wall with the mitral annulus of the lateral and septal myocardial segments. Peak velocities during systole (Sm), early diastole (E') and late diastole (A) were measured.

Myocardial performance index LV MPI was calculated using values derived from PW Doppler. The LV ejection time, IVRT and IVCT were all measured and the MPI was calculated using the following equation:

$$MPI = \frac{(IVCT + IVRT)}{ET}$$

5.2.3 Assessment of Right Ventricle

Dimensions The basal RV end diastolic diameter was measured using 2D in a RV focused A4C view.

Fractional area change The RV endocardial border was traced in the A4C view from the tricuspid annulus, along the free wall to the apex, and back to the annulus along the septum at end systole and end diastole. Trabeculations were traced under.

The FAC was then calculated as:

$$FAC = \frac{\text{(end-diastolic area – end-systolic area)}}{\text{end-diastolic area}} \times 100$$

Tricuspid inflow Pulsed wave (PW) Doppler was used with the sample volume at the tips of the tricuspid valve leaflets in the A4C view. Peak forward velocities were recorded. The IVRT and IVCT were calculated from the TV inflow.

TAPSE The TAPSE was acquired by placing the M mode cursor through the lateral aspect of the tricuspid valve annulus in the A4C view. The longitudinal distance moved by the annulus during systole is then measured.

Tissue Doppler Imaging The image was optimised as for the LV. A 3-5mm pulsed Doppler sample volume was placed approximately 1cm towards the RV apex from the lateral TV annulus in the A4C view, ensuring the alignment was <15° to the RV free wall. Peak systolic velocity (Sm) was measured. The isovolumic acceleration time was measured as the time to peak velocity in systole at the lateral TV annulus.

Myocardial performance index (MPI) The RV MPI was assessed using the PW Doppler method as for the LV.

5.2.4 Statistical Analysis

Continuous data are expressed as mean ± SEM. The data was normally distributed and thus groups were compared using independent-samples t-test. The relationship between two variables was assessed by Pearson correlation coefficient. A $p<0.05$ was taken as being statistically significant.

5.3 Results

5.3.1 Baseline Data

Ten subjects (six female) with Eisenmenger syndrome aged 31 – 50 years (mean 43 ± 1.9 years) underwent echocardiography. Seven had an underlying isolated VSD, one had a VSD and a PDA, one had an ASD and one had an AVSD. Three patients were on selective pulmonary vasodilators (two bosentan, one sildenafil). Their mean Hb was 20.5 ± 0.4g/dl with resting O_2 saturations of 80.6 ± 1.9%.

Nine healthy age matched controls (seven female) aged 33-53 years (mean 42 ± 2.2 years) also underwent echocardiography. All were free from cardiovascular, pulmonary and other systemic disease. Their mean Hb was significantly lower (14.2 ± 0.4g/dl, $p < 0.001$) and their resting O_2 saturation higher (98.3 ± 0.2%, $p < 0.001$).

5.3.2 Left Ventricular Indices

The left ventricular size, as measured by the left ventricular end diastolic diameter (LVEDD), was normal in both patients with Eisenmenger syndrome (4.1 ± 0.15cm) and healthy controls (4.4 ± 0.2cm, $p = 0.2$). The global systolic function was also normal with no difference between the Eisenmenger group and control group (LVEF 57 ± 4.1% vs 65 ± 2.4%, $p = 0.12$).

There was a trend towards a higher indexed stroke volume in the Eisenmenger group (42.7ml/m^2 vs 33.2ml/m^2, p = 0.07) reflecting the large intracardiac shunt. The longitudinal function, assessed by the systolic motion of the lateral MV annulus towards the apex (Sm), was similar and within the normal range for the Eisenmenger group (9.5 ± 1.2cm/s) and control group (10.5 ± 1.1cm/s, p = 0.5). The longitudinal function of the septum however, measured by the Sm of the septum, was significantly reduced in the Eisenmenger group (6.0 ± 0.9cm/s vs 9.6 ± 0.6cm/s, p = 0.006).

Left ventricular diastolic function also appeared to be similar between the Eisenmenger group and control group (table 5.2) with no significant difference in MV E/A ratio, peak E velocity, deceleration time or IVRT. The E/E' ratio, which correlates with the pulmonary capillary wedge pressure and thus left atrial pressure, was also similar in subjects with Eisenmenger syndrome and healthy controls (8.1 ± 2.1 vs 5.7 ± 0.8, p = 0.34).

The MPI was higher in the patients with Eisenmenger syndrome than the healthy controls although this did not reach statistical significance (0.48 ± 0.05 vs 0.36 ± 0.03, p = 0.08). The mean value for the Eisenmenger group was higher than the accepted normal range (<0.4) suggesting mild ventricular impairment. The MPI is an index of global function and may reflect the abnormal septal motion.

5.3.3 Right Ventricular Indices

The RV was mildly dilated in patients with Eisenmenger syndrome, as measured by right ventricular end diastolic diameter (RVEDD) and was significantly larger than the RV of healthy controls (basal RVEDD 4.3 ± 0.2cm vs 3.3 ± 0.14cm, p = 0.015).

The RV systolic indices are shown in table 5.3. The RV fractional area change was lower in the Eisenmenger group than the control group although this did not quite meet statistical significance. (47.4 ± 4.2% vs 56.8 ± 2.2%, p = 0.08) (figure 5.1). The TAPSE was also significantly lower in the Eisenmenger group (17.4 ± 0.7mm vs 24.7 ± 1.0, p < 0.001) (figure 5.2) However, the values obtained in the patients with Eisenmenger syndrome are still within the normal range previously discussed in section 5.1.

The RV longitudinal function, as measured by the TDI of the RV free wall, was lower in the Eisenmenger group than the control group (Sm (12.4 ± 0.8cm/s vs 16.2 ± 1.1cm/s, p < 0.001) (figure 5.3) but this again does fall below the normal range.

There was no difference in the IVA between the Eisenmenger group and control group (4.3 ± 0.3 vs 4.4 ± 0.4, p= 0.8) indicating that the intrinsic contractility is not impaired in Eisenmenger syndrome.

The RV diastolic indices are shown in table 5.4. The IVRT was prolonged in the patients with Eisenmenger syndrome compared to healthy controls (72.6 ± 2.2 vs 62.3 ± 5.3ms, p = 0.05) (figure 5.4) suggesting an abnormality of diastolic relaxation. The E/E' was also significantly higher in the Eisenmenger patients than the control subjects (6.3 ± 1.0 vs 3.3 ± 0.3, p = 0.012) (figure 5.5) as was the A'/E' ratio (2.0 ± 0.5 vs 1.0 ± 0.1, p = 0.04) (figure 5.6). This parameter has been shown to correlate with an elevated RV end diastolic pressure[292;293].

The RV MPI was significantly elevated in patients with Eisenmenger syndrome compared to healthy controls (0.65 ± 0.04 vs 0.34 ± 0.04, $p < 0.001$) (figure 5.7). This value suggests that the global function of the RV was mildly impaired. However, the RV ejection time was lower in the Eisenmenger group compared to controls (252 ± 12ms vs 281 ± 13ms, $p = 0.11$), although this did not meet statistical significance; and this may account for the higher MPI in this group.

There was a trend towards a positive correlation between left ventricular and right ventricular MPI (figure 5.8) in patients with Eisenmenger syndrome ($r = 0.4$, $p = 0.24$) but no correlation between these indices was seen in healthy control subjects ($r = 0.2$, $p = 0.63$).

5.4 Discussion

In pulmonary hypertension the survival and disease progression is, at least in part, related to the ability of the RV to adapt to the significantly elevated pulmonary artery pressures rather than the degree of vascular injury[294]. Accordingly, RV failure has been shown to be associated with a poor outcome in Eisenmenger syndrome [16] and is the main cause of death in PAH from all causes[47].

In the present study we showed that right ventricular systolic function in patients with Eisenmenger syndrome, as measured by FAC and TAPSE, is preserved and there is normal intrinsic contractility. This preservation of function may, in part, help to explain their better than expected survival when compared to PAH from other causes. The exact reason for the relatively maintained RV function is not known but there are several different possibilities.

Ventriculoarterial (VA) coupling is a simultaneous assessment of ventricular performance and arterial elastance. It is expressed as the ratio of effective arterial elastance to end systolic elastance. Sanz et al used right heart catheterisation and cardiac MRI to look at the RV VA coupling in PAH[295]. They demonstrate that in early disease there is preservation of VA coupling by an increase in contractility in proportion to the increased afterload. However, with disease progression, the RV is unable to increase contractility adequately resulting in VA uncoupling and RV failure.

In high right ventricular afterload states, such as in TGA with an atrial switch or cTGA, it has been suggested that one way the RV adapts is by alterations in the geometry and myocyte fibre architecture[45;46]. Thus, the RV starts to resemble a normal LV and enhances ventriculoarterial coupling through preserved contractility. Thus, our results, indicating a normal RV contractility with a preserved IVA in patients with Eisenmenger syndrome, may may lead to maintained VA coupling and thus preserved RV function.

We did find that the longitudinal RV systolic function, as measured by the systolic velocity of the lateral tricuspid valve annulus using TDi, was decreased when compared to our healthy controls. An increased systemic arterial stiffness, such as that arising with advancing age or hypertension, has been demonstrated to lead to a reduction of the subendocardial fibres of the left ventricle resulting in a reduction of longitudinal function[296]. Thus, it is plausible that a similar effect occurs in the right heart. In other words, an increased pulmonary arterial stiffness, arising from pulmonary vascular remodelling, may lead to a reduction in the RV longitudinal function. However, the decrease in longitudinal function observed in our study

was only mild and may have reflected a shift in the RV free wall from longitudinal to circumferential shortening in response to pressure overload[45].

Tan et al demonstrated that the RV is adapted less well in IPAH patients than in those with TGA or cTGA[46]. This is likely to be due to the ability of the RV to adapt when faced with high pressures from birth or in the early neonatal period, as opposed to de novo PAH in adulthood. In Eisenmenger syndrome, the right ventricle has been exposed to systemic pressures from infancy and it is feasible that this time has allowed it to adapt, possibly in a similar way to the RV in TGA or cTGA.

Whilst our results show that the RV systolic function is maintained in patients with Eisenmenger syndrome, there was evidence of diastolic dysfunction and elevated RV end diastolic pressures. Thus the problem appears to be one of abnormal early relaxation of the ventricle. Tan et al reported a similar pattern in patients with IPAH with abnormal early diastolic function, as shown by prolonged IVRT with a secondary fall in filling time[46]. Diastolic dysfunction has also been demonstrated in chronic hypoxia in healthy individuals investigated during expeditions to altitude with decreased early filling[297;298]. This may represent a failure to meet the energy requirements of the active relaxation that occurs in early diastole.

A further explanation for the RV diastolic dysfunction may be progressive fibrosis of the myocardium. Myocardial fibrosis is known to occur in diseases associated with left ventricular hypertrophy, such as hypertension and hypertrophic cardiomyopathy, and leads to increased stiffness and thus impaired relaxation of the ventricle[299]. Congenital conditions

associated with pressure or volume overloaded RVs, including TGA and tetralogy of Fallot, have also been demonstrated to have late gadolinium enhancement, suggestive of fibrosis, on cardiac MRI[300;301]. Whilst patients with Eisenmenger syndrome have not been previously investigated in this way, Blyth et al have demonstrated fibrosis affecting the septum in patients with IPAH with the degree of fibrosis correlating well to the RV function[302]. Areas of fibrosis in the RV of patients with Eisenmenger syndrome have also been reported in post mortem studies[303].

In contrast to the RV, our study has shown normal diastolic function of the LV in patients with Eisenmenger syndrome. This differs from PAH from other causes in which diastolic dysfunction of the LV, with a delayed pattern of relaxation, is common[35]. Further, Kitahori et al used an animal model with pulmonary artery banding to create pressure overload and subsequent RVH in infancy[304]. This also resulted in left ventricular diastolic dysfunction with preservation of ejection fraction. They describe septal and LV apoptosis, pathological remodelling of the LV and a reduction in capillary density.

Even though LV diastolic function appears normal in Eisenmenger syndrome in the resting state, it is possible that it becomes impaired during exercise. This has been demonstrated to occur in patients with well treated systemic hypertension with normal resting echocardiography in whom exercise results in widespread diastolic dysfunction[305].

It is also possible that systolic dysfunction of both the RV and LV occurs during exercise in patients with Eisenmenger syndrome. Tan et al looked at the effects of exercise on patients with heart failure with normal ejection fraction (HFNEF) who are characterised by resting

diastolic abnormalities[306]. During exercise the authors describe widespread abnormalities of both systolic and diastolic function and conclude that it is not an isolated disorder of diastole as previously thought. Systolic dysfunction during exercise may contribute towards the exercise intolerance in patients with Eisenmenger syndrome and this warrants further investigation.

The relative preservation of RV systolic function may also be related to the presence of a large right to left shunt. During exercise, patients with Eisenmenger syndrome are unable to increase their cardiac output by reducing their PVR. Instead they increase it by increasing the size of the shunt, at the expense of increasing cyanosis. Thus the shunt may protect the right ventricle from some of the effects of pressure overload.

The use of conventional echocardiographic parameters to assess the RV function in Eisenmenger syndrome has been debated, in particular those that measure longitudinal function such as TAPSE.

Whilst TAPSE is easily measured and highly reproducible, it ignores the outlet portion of the RV and also the septal contribution. In the normal heart it has been validated as the longitudinal displacement of the RV base accounts for most of the RV function. However, as discussed in section 1.3.1, the septum accounts for a greater proportion of the RV contraction in severe PAH suggesting that TAPSE may not accurately reflect the RV function in this situation. TAPSE is also volume dependent and may be overestimated in the presence of significant tricuspid regurgitation such as is found in PAH. Two recent studies, however, have found that a reduced TAPSE is a predictor of poor outcome in Eisenmenger

syndrome[286;287]. In the present study the mean TAPSE was lower than that of the healthy controls, although still above the normal range given by the ASE[271].

The MPI is a relatively load-independent measure of global ventricular function. Our results showed that the RV MPI was higher in the Eisenmenger group than controls. However, this may be due to the diastolic dysfunction or the shortened ejection time resulting from reduced pulmonary artery compliance[307]. To date there has not been a study looking at the association with the MPI and outcome in this patient group. The LV MPI also tended to be higher in Eisenmenger patients than in controls, although this did not reach significance. This may reflect the dyssynchronous septal motion in severe pulmonary hypertension as the septum assumes greater importance for RV contraction. This interaction between the ventricles was also demonstrated by the trend towards a positive correlation between the RV and LV MPI in patients with Eisenmenger syndrome.

In summary the present results show that the overall RV systolic function in Eisenmenger syndrome is maintained at rest, as suggested by previous studies. In addition, the present study results are the first to demonstrate diastolic dysfunction of the RV in these patients; may be due to myocardial fibrosis as a result of years of high afterload. The reason for the maintained systolic function is not clear and newer echocardiographic techniques such as strain rate, speckle tracking and exercise echo, may provide further answers.

Table 5.1 Recommended measures of RV assessment and their reference limits. Adapted from Rudski et al[271]

Variable	Units	Abnormal
Right ventricular size		
• Basal (A4C)	cm	>4.2
• Mid cavity (A4C)	cm	>3.5
Right ventricular systolic function by at least 1 of:		
• FAC	%	<35
• Tissue doppler velocity at annulus	cm/s	<14
• TAPSE	mm	<16
• MPI	-	<0.4
Pulmonary artery systolic pressure	mmHg	>36

A4C, apical four chamber; FAC, fractional area change; TAPSE, tricuspid annular plane systolic excursion; MPI, myocardial performance index.

The reference limits for both tissue Doppler velocity and MPI are for pulse spectral technique of data acquisition.

Table 5.2 Indices of left ventricular diastolic function in Eisenmenger syndrome patients and healthy controls. All values are given as mean±SEM, NS; not significant

	Eisenmenger syndrome patients	Healthy control subjects	p value
Peak E velocity (m/s)	0.67 ± 0.06	0.71 ± 0.05	NS
DT (ms)	184 ± 27.9	247 ± 16.7	NS
E/A ratio	1.23 ± 0.25	1.14 ± 0.1	NS
IVRT (ms)	80 ± 3	76 ± 4	NS
E/E’	8.1 ± 2.1	5.7 ± 0.8	NS

DT, deceleration time; IVRT, isovolumic relaxation time

Table 5.3 Indices of right ventricular systolic function in Eisenmenger syndrome patients and healthy controls. All values are given as mean±SEM, NS; not significant

	Eisenmenger syndrome subjects	Healthy control subjects	p value
FAC (%)	47.4 ± 4.2	56.81 ± 2.2	NS
TAPSE (mm)	17.4 ± 0.7	24.7 ± 1.0	0.001
TDi Sm (cm/s)	1.23 ± 0.25	1.14 ± 0.1	<0.001
IVA (m/s)	4.3 ± 0.3	4.4 ± 0.4	NS

FAC, fractional area change; TAPSE, tricuspid annular plane systolic excursion; TDi, tissue Doppler imaging; IVA, isovolumic acceleration

Figure 5.4 Indices of right ventricular diastolic function in Eisenmenger syndrome patients and healthy controls. All values are given as mean±SEM, NS; not significant.

	Eisenmenger syndrome subjects	Healthy control subjects	p value
IVRT (ms)	72.6 ± 2.2	62.3± 5.3	0.05
E/E'	6.3 ± 1.0	3.3 ± 0.3	0.012
A'/E'	2.0 ± 0.5	1.0 ± 0.1	0.04

IVRT, isovolumic relaxation time

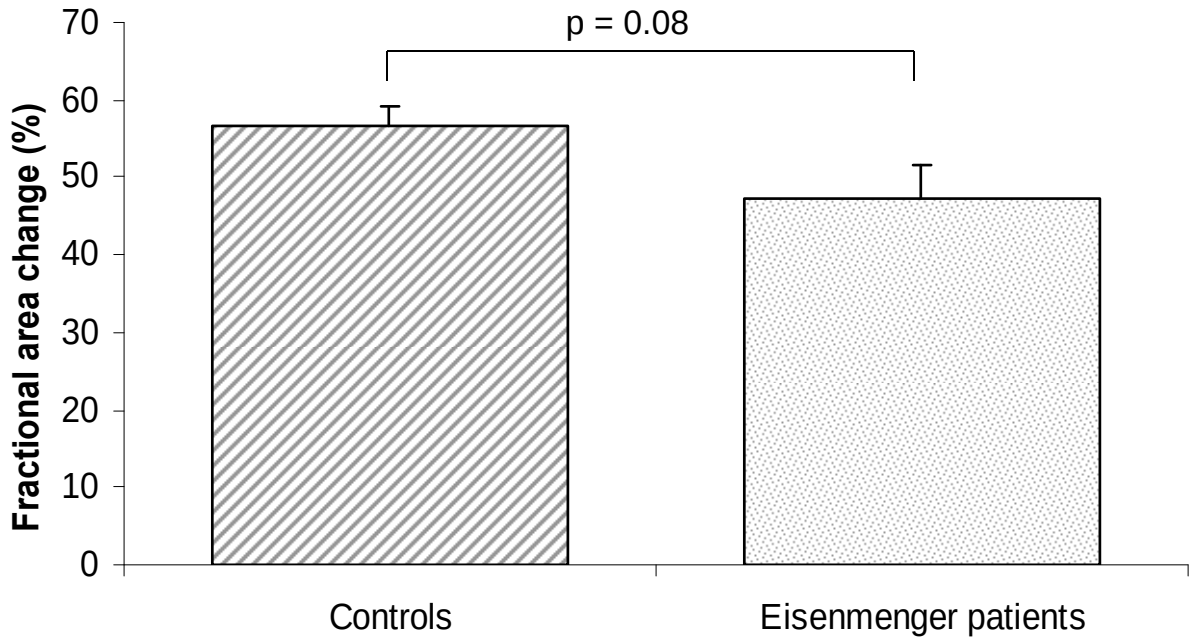


Figure 5.1 Right ventricular fractional area change in healthy controls and patients with Eisenmenger syndrome

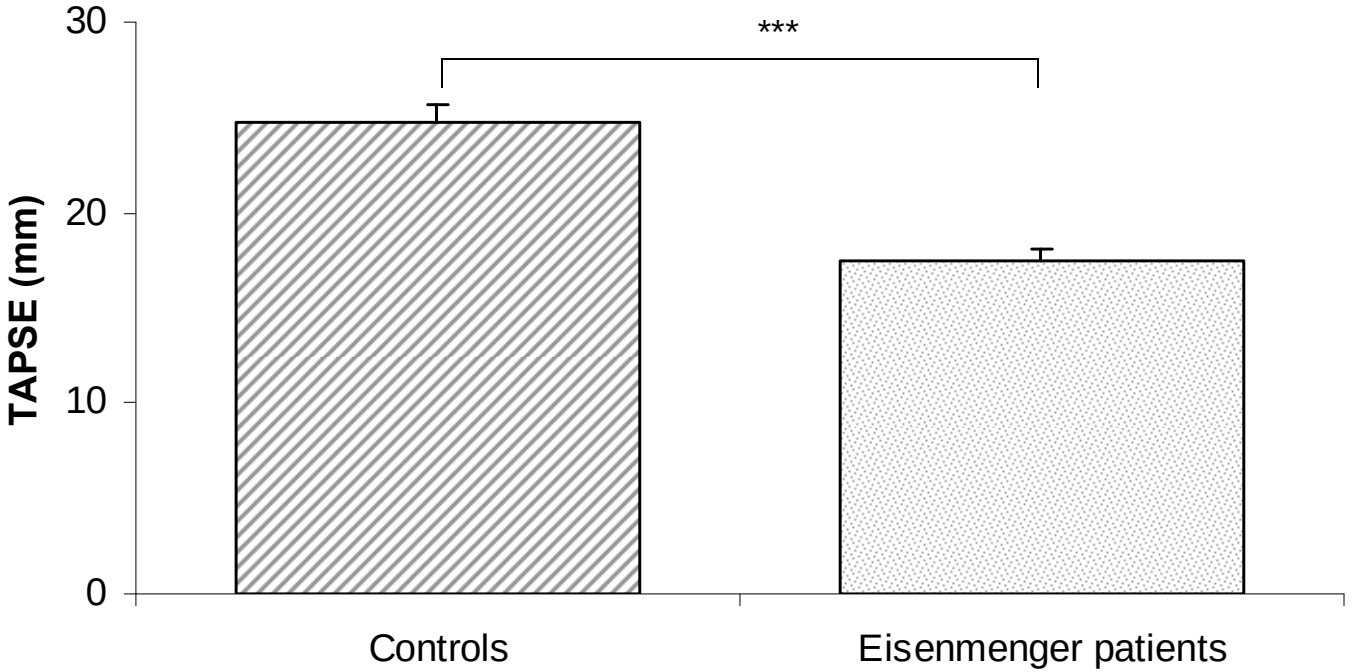


Figure 5.2 Tricuspid annular plane systolic excursion in healthy controls and patients with Eisenmenger syndrome. *** p<0.001

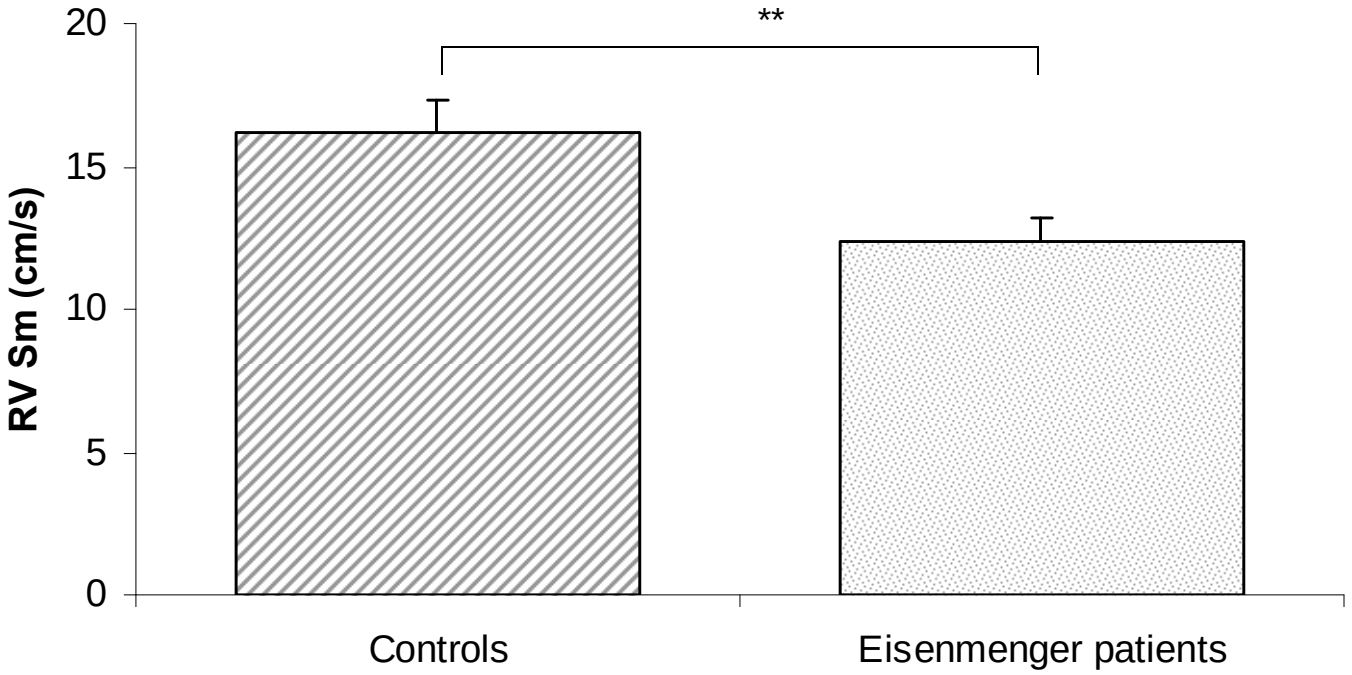


Figure 5.3 Right ventricular free wall peak systolic velocity (Sm) in healthy controls and patients with Eisenmenger syndrome. ** $p<0.01$

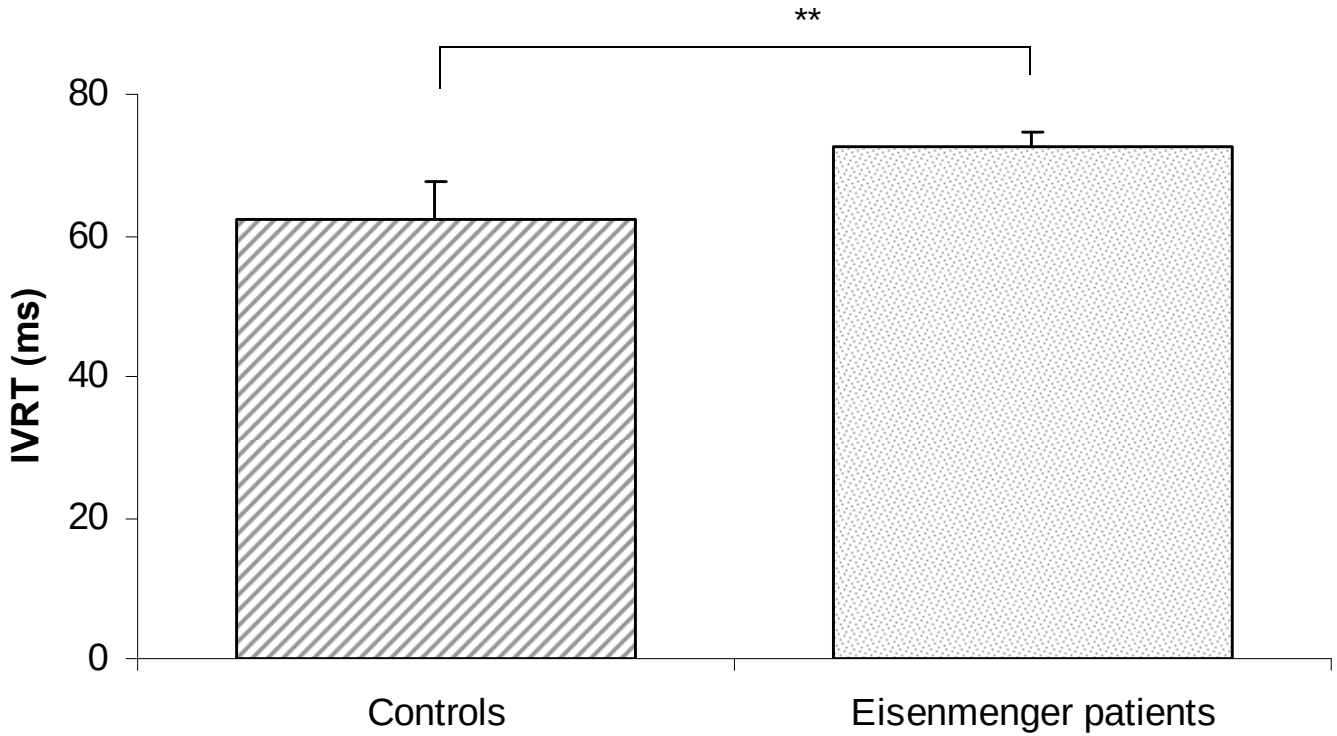


Figure 5.4 Right ventricular isovolumic relaxation time in healthy controls and patients with Eisenmenger syndrome. ** p<0.01

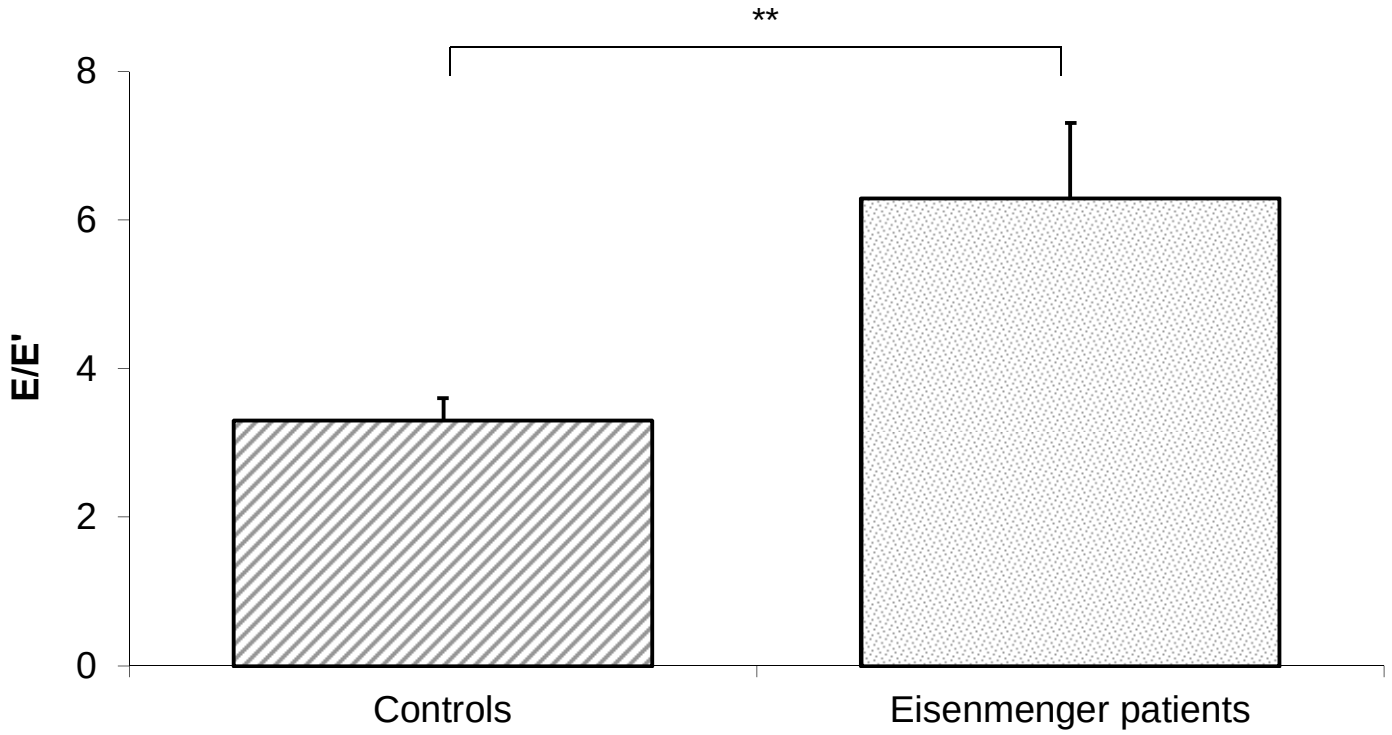


Figure 5.5 Ratio of peak E wave velocity through tricuspid valve (E) and tissue Doppler derived early diastolic velocity (Eð) in healthy controls and patients with Eisenmenger syndrome. ** p<0.01

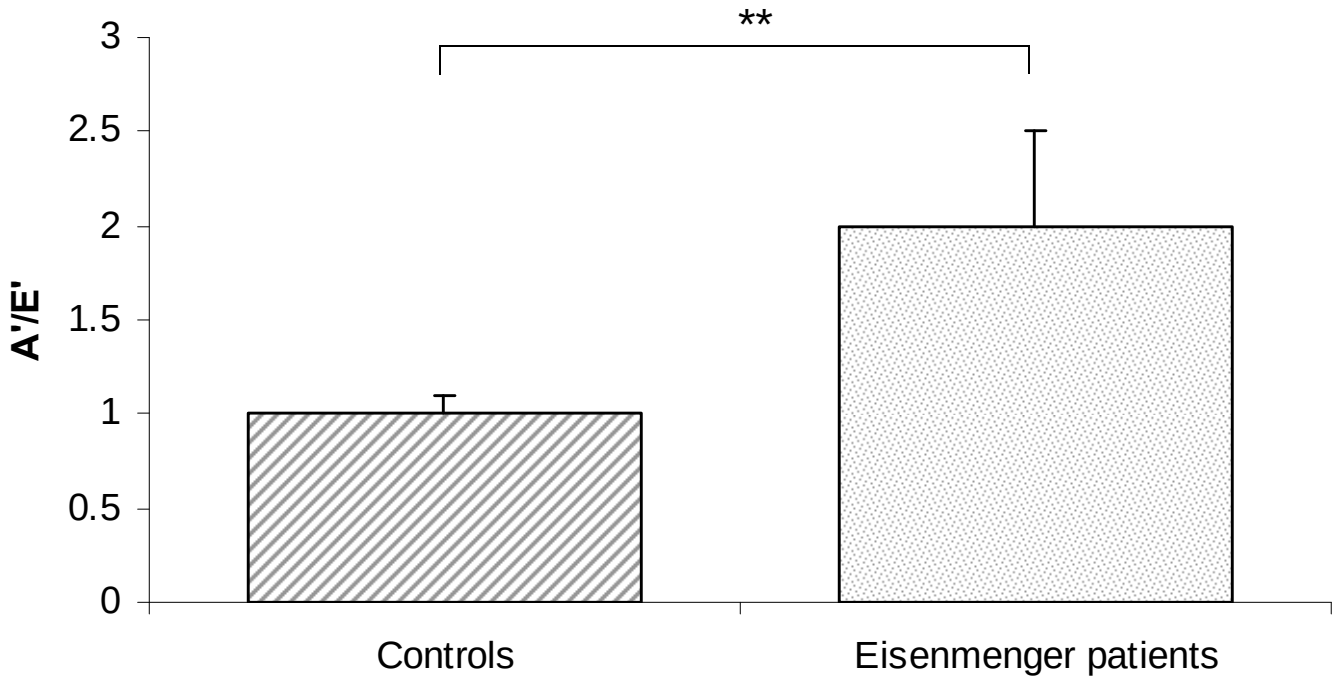


Figure 5.6 Ratio of right ventricular late diastolic (Aʹ) and early diastolic velocity in healthy controls and patients with Eisenmenger syndrome. ** p<0.01

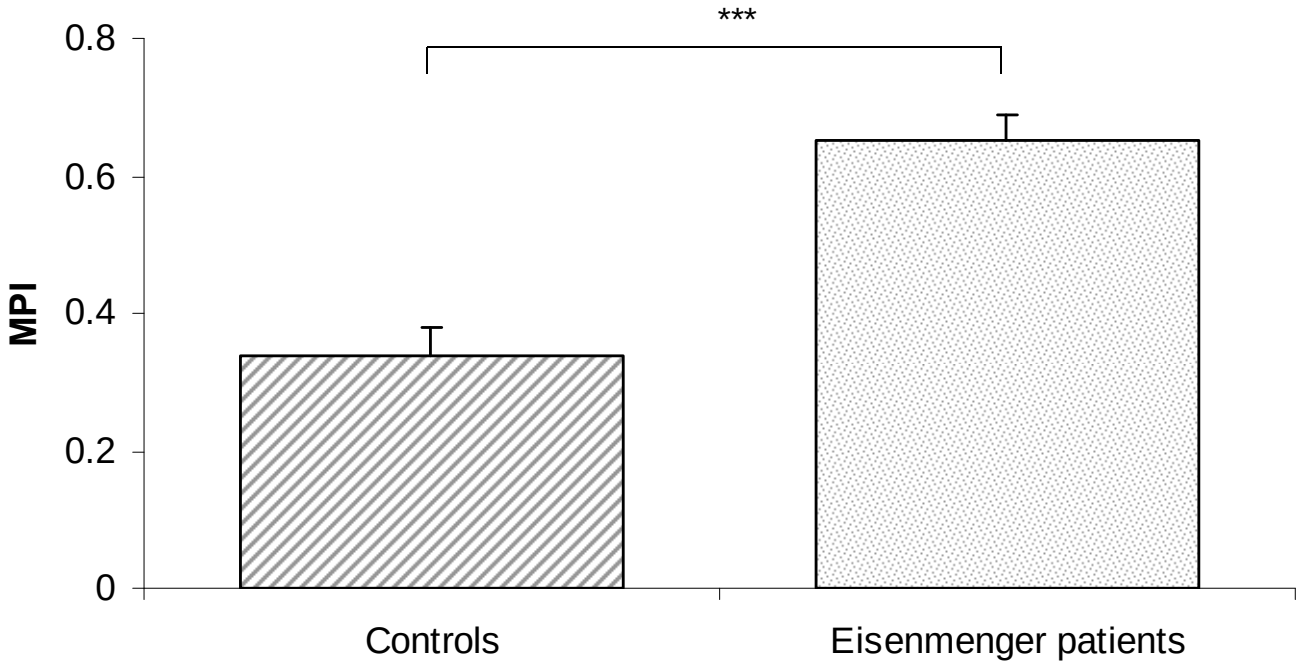
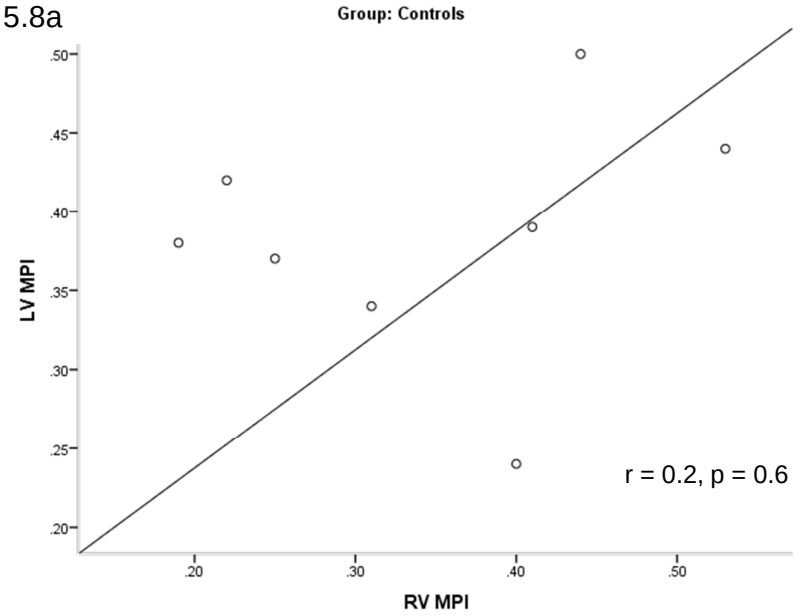


Figure 5.7 Right ventricular myocardial performance index in healthy controls and patients with Eisenmenger syndrome. *** p<0.001

5.8a



5.8b

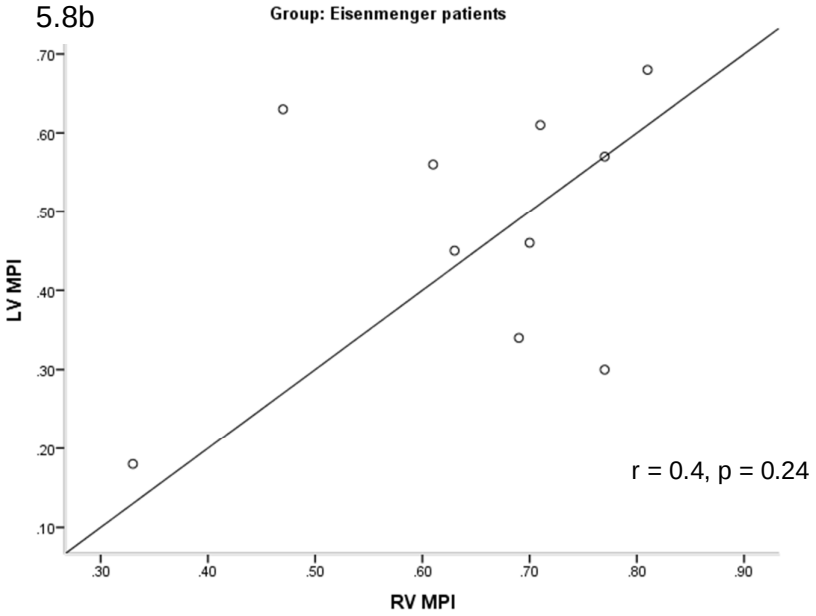


Figure 5.8 Relationship between left and right ventricular myocardial performance index in control subjects (5.8a) and Eisenmenger patients (5.8b)

6. COMPARISON OF CARDIAC ENERGETICS IN PATIENTS WITH EISENMENGER SYNDROME AND CONTROLS

6.1 Introduction

As indicated in the introduction, very little is known of cardiac muscle energetics in patients with Eisenmenger syndrome. Thus, in the present studies, ^{31}P MRS was used as means of assessing PCr and ATP. The discussion below provides the background to these studies.

6.1.1 Cardiac ^{31}P MRS

Magnetic resonance spectroscopy (MRS), as discussed in section 4.1, is a non-invasive technique that allows the *in-vivo* assessment of myocardial energetics. ^{31}P MRS measures the high energy components of PCr, adenosine triphosphate (ATP), 2,3 Diphosphoglycerate (2,3-DPG) and phosphodiester (PDE) compounds with the PCr / ATP ratio reflecting the energetic state of the myocardium. A typical spectrum obtained from cardiac ^{31}P MRS is shown in figure 6.1.

6.1.2 Myocardial Energetics and Metabolism

The myocardium relies on ATP production for both contractile function and cell viability. It is capable of consuming 20-30 times it own weight in ATP per day[308]. Whilst it is able to respond to large variations in energy demand and substrate availability, with ATP production being loosely coupled to myofibrillar contraction, it has limited capacity for energy storage[309]. Thus, the myocardial cell must continually re-synthesise ATP to maintain normal myocardial function with even small variations in the efficiency of ATP production or utilisation having a significant impact on cellular energy levels[310].

In aerobic conditions, β-oxidation of free fatty acids account for 60-90% of the energy generated by ATP re-synthesis with carbohydrate metabolism, in normal circumstances, only contributing 10-40%[311]. As discussed previously in section 1.4.2.4, whilst fatty acid oxidation has a higher ATP yield per mole compared to glucose, it does so at a higher O_2 cost, requiring 10-15% more O_2 to generate an equivalent amount of ATP when compared to glucose[53]. High rates of fatty acid oxidation also suppresses glucose oxidation by directly inhibiting pyruvate dehydrogenase thereby increasing lactate concentrations, reducing pH and further impairing the myocardial contractile function[312].

Not surprisingly, suppression of fatty acid uptake or oxidation by any means increases myocardial glucose substrate utilisation and this has been a recent focus for therapeutic agents used in both ischaemic heart disease and chronic heart failure such as perhexiline, trimetazidine and ranolazine[53;313].

6.1.3 Abnormal Energetics in Cardiovascular Disease States

Even small changes in the efficiency of energy production by the myocardium can have a significant affect on the cell's energy levels. The creatine kinase equilibrium strongly favours ATP synthesis over PCr synthesis. Therefore, when the demand for ATP outweighs the synthesis of ATP, there is an initial fall in PCr, with a resultant low PCr / ATP ratio[239]. Abnormal myocardial energetics, demonstrated by a low PCr / ATP ratio using ^{31}P MRS, have been described in several cardiac disease states including left ventricular failure[314], valvular heart disease[315] and hypertrophic cardiomyopathy[316]. Modulation of the energetics is the focus for several new therapeutic approaches.

Considering congenital heart disease, Miall et al investigated at the myocardial energetic status of babies and children. They showed that the PCr / ATP ratios were similar in controls and those who were hypoxaemic only; however they were significantly lower in those with heart failure and hypoxaemia[254]. An impaired high energy phosphate metabolism has also been described by Spindler et al in idiopathic pulmonary arterial hypertension[317]. They demonstrated a reduced PCr / ATP ratio in a patient with rapidly deteriorating symptoms. After administering bosentan, a selective pulmonary vasodilator, for six months the PCr / ATP ratio had significantly increased and this was associated with improved functional class and six minute walk distance.

To date, there are no studies investigating the myocardial energetic status of adults with cyanotic heart disease including Eisenmenger syndrome. Thus, we have investigated the energetics of the septum, focusing on this region because of the increased importance it assumes in RV contraction in severe pulmonary hypertension (see section 1.3.1).

6.2 Methods

6.2.1 Acquisition

Eight patients with Eisenmenger syndrome and six age matched healthy volunteers (HV) were studied. ^{31}P cardiac magnetic resonance spectroscopy was carried out using a Phillips Achieva 3T scanner (Philips Healthcare, Best, the Netherlands) as previously described and validated[261]. A ^{31}P linearly polarized transmitter and receiver coil with a diameter of 14 cm (figure 6.2) was used to acquire the data and was performed using ISIS (image-selected *in vivo* spectroscopy) volume selection[318].

Subjects were positioned in the supine position and the coil was placed on the chest wall, directly over the heart, and secured with straps. Once in the magnet, the position of the coil was checked using survey images (figure 6.3) and the patient and / or coil were repositioned if necessary so that the distance between the coil and the septum was kept to a minimum[319]. Subjects were to be excluded if the distance of the coil to the region of interest was greater than 9cm to decrease any potential artefacts from chemical shift that are known to be present with a 3T scanner[261]. Minimising this distance and decreasing artefacts ensures that the chemical shift displacement of PCr and ATP is <10%[261]. In practice, no subjects from either group were excluded.

An image-guided shim volume was used for the shimming and this was positioned to include the whole myocardium. The shimming quality was subsequently assessed using a short axis stack and from this the trigger delay was also calculated for ECG gating. To ensure that the spectra were acquired during diastole, when cardiac movement was minimal, a trigger delay was set by subtracting 250-300ms from the total cardiac cycle[261].

The 3-D voxel of interest (VOI) was subsequently planned to include as much of the septum and apex as possible. The right ventricular cavity was avoided to minimise contamination from blood. In all subjects, the voxel size was 89.54 ml (44 x 55 x 37 mm^3) thus allowing comparisons between subjects[316].

^{1}H spectra were acquired first from this VOI using a repetition time of 2000ms. This gave a total scan time of 16 s. This further ensured shim quality and correct F0 determination. F0 was manually adjusted using tuning rods if required. Following this the ^{31} P spectra were acquired with a repetition time of 10000 ms, which has been shown to be optimal to adequately reduce saturation effects whilst

keeping the scan time to a minimum. 136 averages were taken with 512 sample giving a total scan time for the acquisition of ^{31}P spectra of 23 minutes.

6.2.2 Analysis

The spectra were analysed using a time-domain fitting programme (AMARES) on jMRUI (java-based Magnetic resonance User Interface). Fourier transformation and 15Hz Gaussian line broadening were used to perform post-processing[261]. Phase correction was performed with PCr as the reference peak. The peaks of 2,3-DPG, PDE, PCr, γ-ATP, α-ATP and β-ATP were identified and their concentrations calculated as the area under the peaks.

6.2.2.1 Correction for Blood Contamination

The PCr/ATP ratio may be artificially reduced if the VOI includes blood, which contains ATP but not PCr[240]. Blood also contains 2,3 DPG, observed between 5.4 and 6.3 on the spectra. The degree of contamination can thus be calculated by the ratio between ATP and DPG. Conway et al describe one method to correct the value of the ATP peak for blood contamination[315]. The size of the γ-ATP was reduced by an amount equal to one sixth of the area of the combined peaks representing 2,3 DPG. This correction factor was applied if the area from the combined 2,3 DPG resonances were equal or greater than the γ-ATP peak or if the amplitude of 3 DPG was less or equal to 2 DPG.

6.2.2.2 Cramer-Rao Lower Bounds

The quality of the signal to noise ratio (SNR) of the spectra was further assessed using coefficient of variation in the measured PCr/ATP value and was based on the Cramer-Rao lower bounds (CRLB)[320]. Their calculated coefficient of variation denotes a 95% Student's –t interval for the

measurement of PCr/ATP from the acquired spectrum and a low CRLB indicates the spectra were of good quality and with good reproducibility[321].

6.2.3 Statistical analysis

Continuous data are expressed as mean ± SEM. Groups were compared using independent-samples t-test. The relationship between two variables was assessed by Pearson correlation coefficient. A p value of <0.05 was taken as being statistically significant.

6.3 Results

6.3.1 Baseline Data

Eight patients with Eisenmenger syndrome (five female) aged 31-50 years,(mean 41.5 ± 2.7 years), underwent cardiac ^{31}P MRS. Seven of these subjects had an underlying VSD, one with a co-existent PDA, and one had an AVSD. One patient was on a selective pulmonary vasodilator (bosentan). Their mean Hb was 20.5 ± 0.5g/dl with resting O_2 saturations 80 ± 2.1%. The ability score was I in four patients, II in three patients and III in one.

The control group consisted of six healthy volunteers (four female) aged 30-48 years, (mean 40.3 ± 3.0 years). All controls were free from cardiovascular and pulmonary disease. Their mean Hb was significantly lower than the Eisenmenger group (14.1 ± 0.65g/dl, $p<0.001$) and their resting O_2 saturations higher (98.2 ± 0.17%, $p<0.001$). All reported an ability index of I.

6.3.2 PCr/ATP Ratio

Adjustment for blood contamination as described above was applied to the cardiac spectra of seven patients with Eisenmenger syndrome and all six healthy volunteers.

The PCr / ATP ratio in patients with Eisenmenger syndrome was significantly lower than the healthy control group (1.55 ± 0.10 vs 2.17 ± 0.15, p = 0.004) (Figure 6.4). There was no correlation between PCr / ATP ratio and age (r = 0.12, p = 0.66).

6.3.3 Cramer Rao Lower Bounds

The CRLBs for the PCr peak were 5.3 ± 0.5% in the Eisenmenger syndrome group and 5.8 ± 0.7% in the control group. The CRLBs for the γ-ATP peak were 6.5 ± 0.4% in the Eisenmenger group and 8.8 ± 0.5% in the controls. These values are all less than 10% indicating that the spectra were of good quality and reproducibility.

6.3.4 Correlations with Echo Parameters

There was no significant relationship between the PCr / ATP ratio and the radial systolic function of the RV, as measured by fractional area change, (r = 0.214, p = 0.5). There was, however, a weakly positive correlation between the longitudinal systolic function of the RV, assessed by TAPSE, and the PCr / ATP ratio, although this did not quite reach statistical significance(r = 0.48, p = 0.08). There was also a significant negative correlation between the right ventricular myocardial performance index, a measure of global RV function, and PCr / ATP ratio (r = -0.57, p = 0.04).

6.4 Discussion

This is the first study to investigate the myocardial energetics in patients with Eisenmenger syndrome and we have demonstrated that the myocardial energetic status of the interventricular septum, as expressed by the PCr/ATP ratio, is impaired when compared to healthy control subjects. This reduction in PCr/ATP ratio is present despite a maintained

biventricular systolic function as demonstrated by echocardiography (see section 4) which may suggest that the energetic abnormality precedes contractile dysfunction. Interestingly, Holloway et al demonstrated an 18% reduction in PCr/ATP ratio in lowlanders returning from a period at altitude, with a maintained systolic function, and hypothesised that a reduction in this ratio may be a universal response to hypoxia[322].

The active myocardial relaxation that occurs early in diastole is highly energy dependent[323]. Thus, processes that affect the energetic status of the myocardial would be expected to result in diastolic dysfunction. The impaired energetic status we have demonstrated in Eisenmenger syndrome, therefore, may contribute to the right ventricular diastolic dysfunction that we described in section 5.3.3. This is also suggested by the inverse relationship between the PCr/ATP ratio and MPI, an indicator of global ventricular performance.

Other conditions associated with diastolic dysfunction, including hypertrophic cardiomyopathy (HCM), have also been shown to have abnormal energetic status. Abozguia et al demonstrated a PCr/ATP ratio of 1.28 in patients with HCM compared to a mean of 2.26 in age matched healthy controls[316]. Whilst systolic function was normal in HCM patients, there was significant diastolic dysfunction that normalised after the administration of perhexiline along with an increase in PCr/ATP ratio. The hypertrophy in HCM is associated with mitochondrial dysfunction with subsequent impairment of contractile *and* relaxation reserves[324]. There is also microvascular dysfunction with inadequate myocardial blood flow in relation to the degree of hypertrophy[325;326]. Our results raise the question as to

whether patients with Eisenmenger syndrome may benefit from the administration opf perhexiline.

It is well established that impaired energetics occurs in left ventricular failure of different aetiologies[314-316]. However, much less is known regarding the energetics of the right ventricle in RV failure. Daicho et al investigated the mitochondrial energy-producing ability of the RV myocardium in an animal model of pulmonary hypertension and subsequent RV failure[327]. They showed a reduction in the O_2 consumption of the right ventricular myocardium that was inversely related to the MPI on echo, and associated with a decrease in the myocardial high energy phosphates. Seen in this context, the relative preservation of the RV contractile function in Eisenmenger syndrome raises the possibility that there is a further factor that protects the myocardium from failing. One possible factor may be altered substrate utilisation.

Certainly, Jung et al demonstrated abnormal phosphate metabolism in HCM patients[328]. They hypothesise that one reason for this may be altered energy sources with a shift from fatty acid to glucose metabolism even in asymptomatic patients. The preferential use of glucose by the hypertrophied heart as its main energy substrate was also shown previously by Allard et al in an animal model of HCM[329]. This may be due to a down regulation of PPARα in hypertrophied myocardium which results in a switch in substrate utilisation from fats to glucose[323].

Sherpas have also been demonstrated to utilise glucose preferentially to fatty acids as a result of the lifelong hypoxia, and despite the absence of hypertrophy[196]. Moreover, Hochachka

et al reported a low PCr/ATP ratio in Sherpas[197]. They suggest that, in the absence of myocardial ischaemia or increased workload, this may be due to an accelerated glycolysis; the increased ADP concentration that results from the low PCR/ATP ratio leading to increased activity of the enzymes involved in aerobic glycolysis. Thus, it is possible that patients with Eisenmenger syndrome also utilise glucose in preference to fatty acids due to both the chronic hypoxaemia and the myocardial hypertrophy. The eventual failure of the RV that occurs in some patients with Eisenmenger syndrome may then be due to a progressive insulin resistance which eventually inhibits this preferential glucose utilisation, similar to the changes that occur in left ventricular failure[323].

A further reason suggested by Jung et al to explain the low PCr/ATP ratio found in HCM patients is O_2 limitation, resulting from low blood flow through narrowed intramural coronary arteries[328]. This is unlikely to be a factor in the heart in Eisenmenger syndrome as chronic hypoxia is known to induce coronary vasodilatation and remodelling of the coronary microcirculation resulting in an elevated basal coronary blood flow[330;331]. Thus it is reasonable to assume that the coronary circulation at rest is not a limitation in patients with Eisenmenger syndrome. This may explain in part why the PCr/ATP ratio that we have demonstrated in patients with Eisenmenger syndrome is higher than that shown in HCM by others in our group[316].

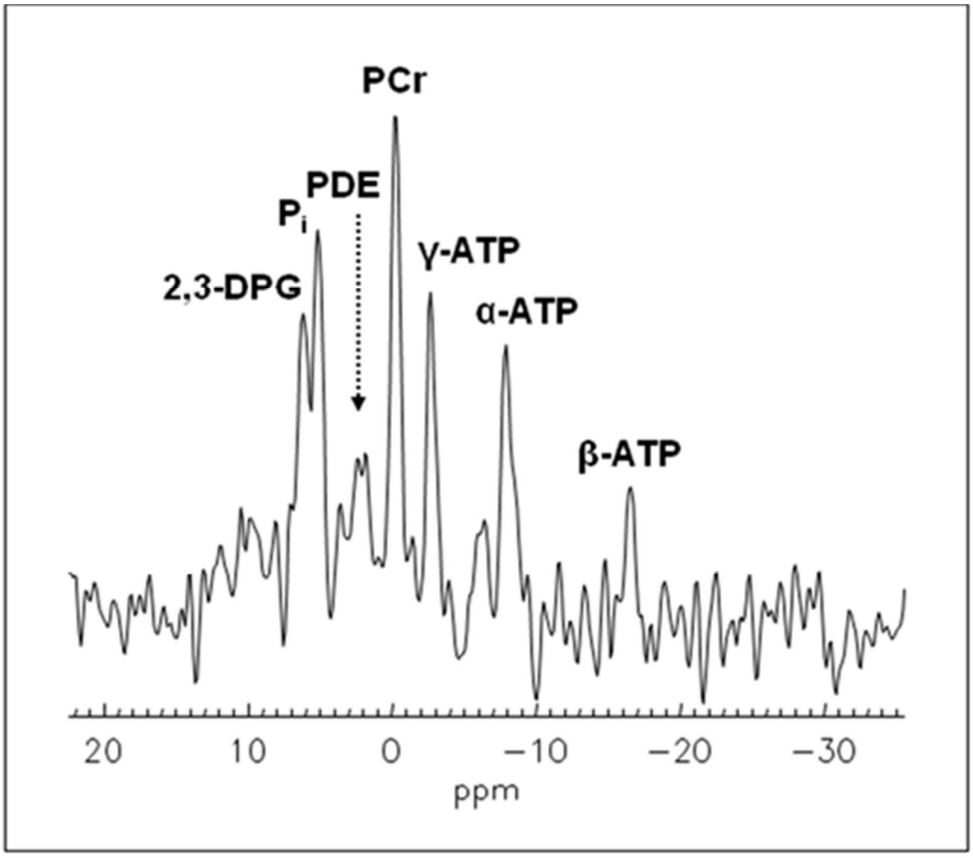


Figure 6.1 An example of a typical ^{31} P cardiac spectra in a healthy control subject.

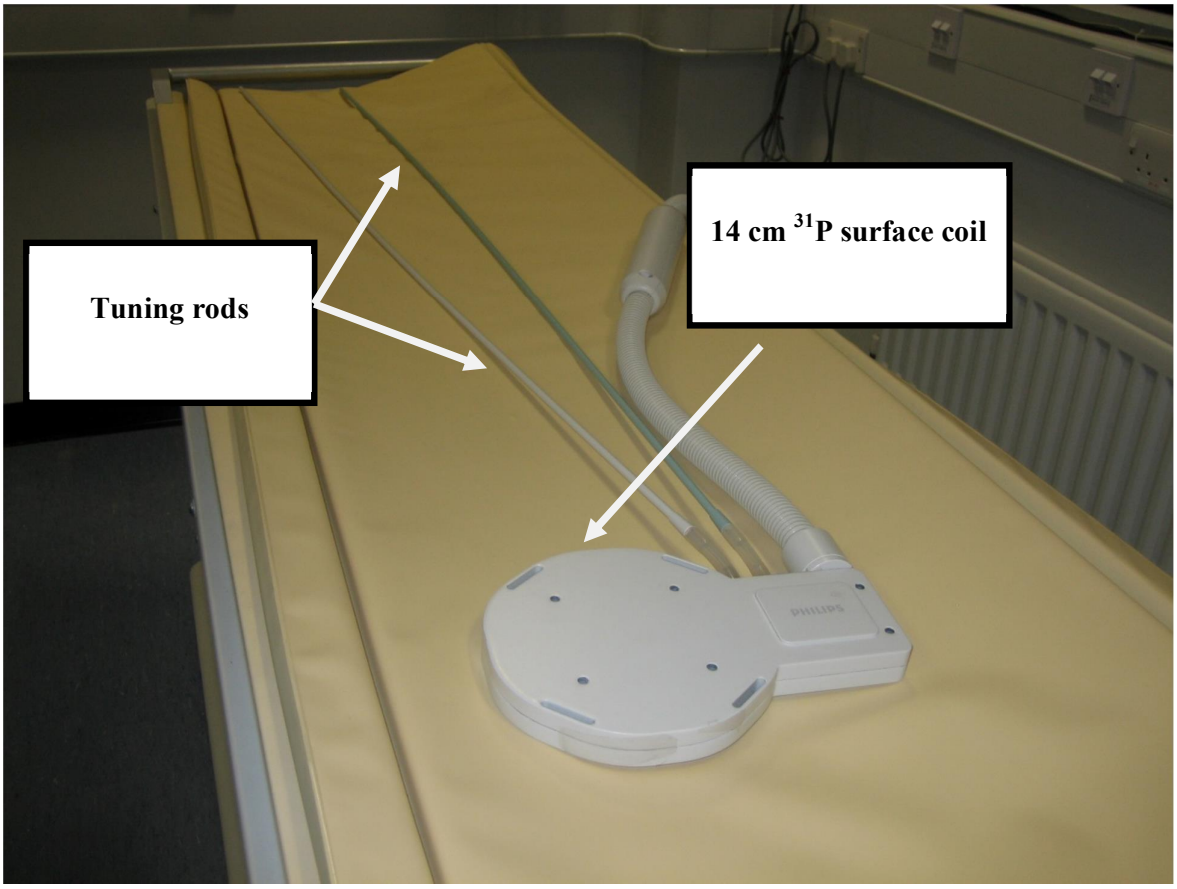


Figure 6.2 Transmitter and receiver ^{31}P surface coil used in present study

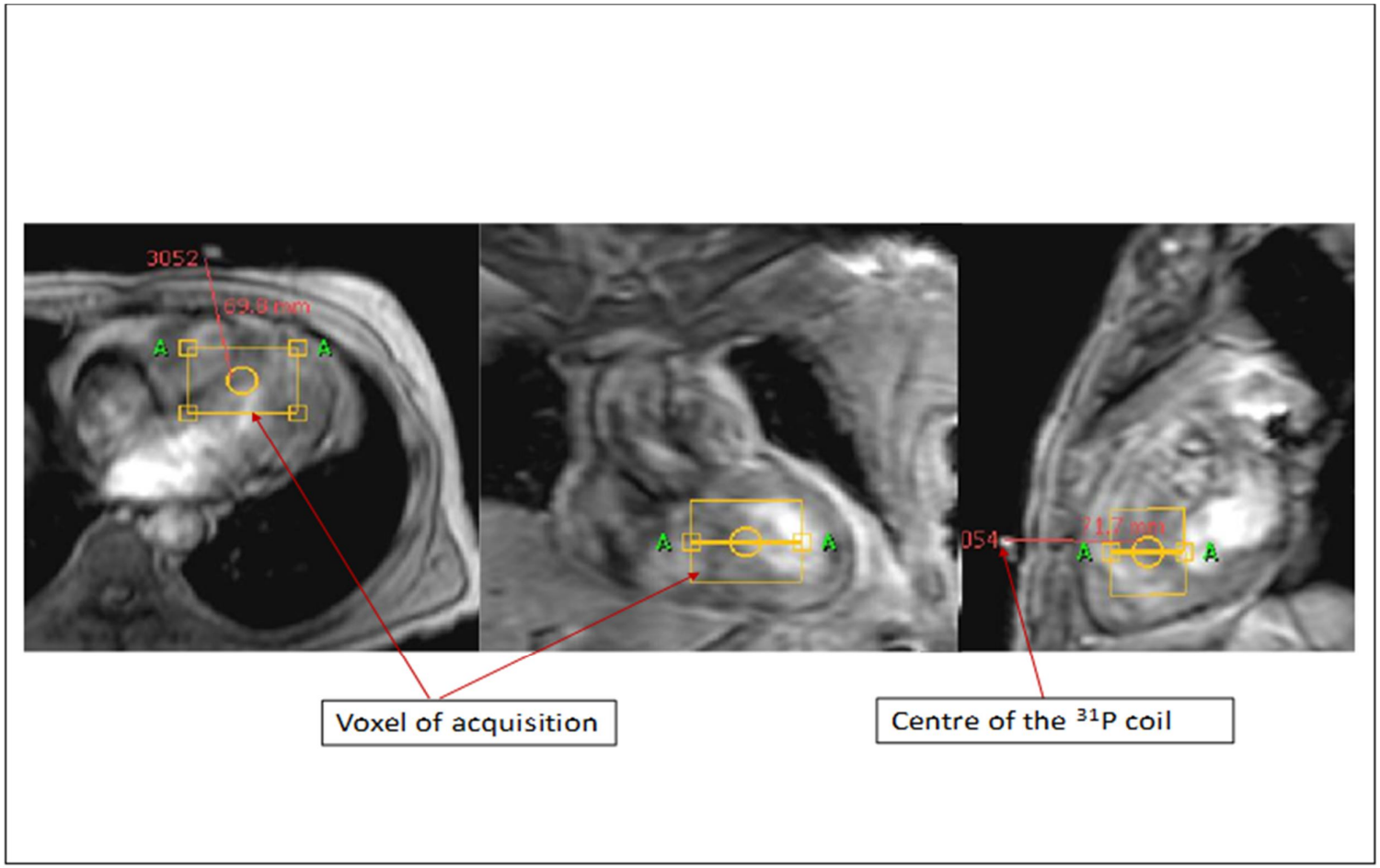


Figure 6.3 Survey images showing the position of the voxel of interest (VOI) and centre of the ^{31}P coil

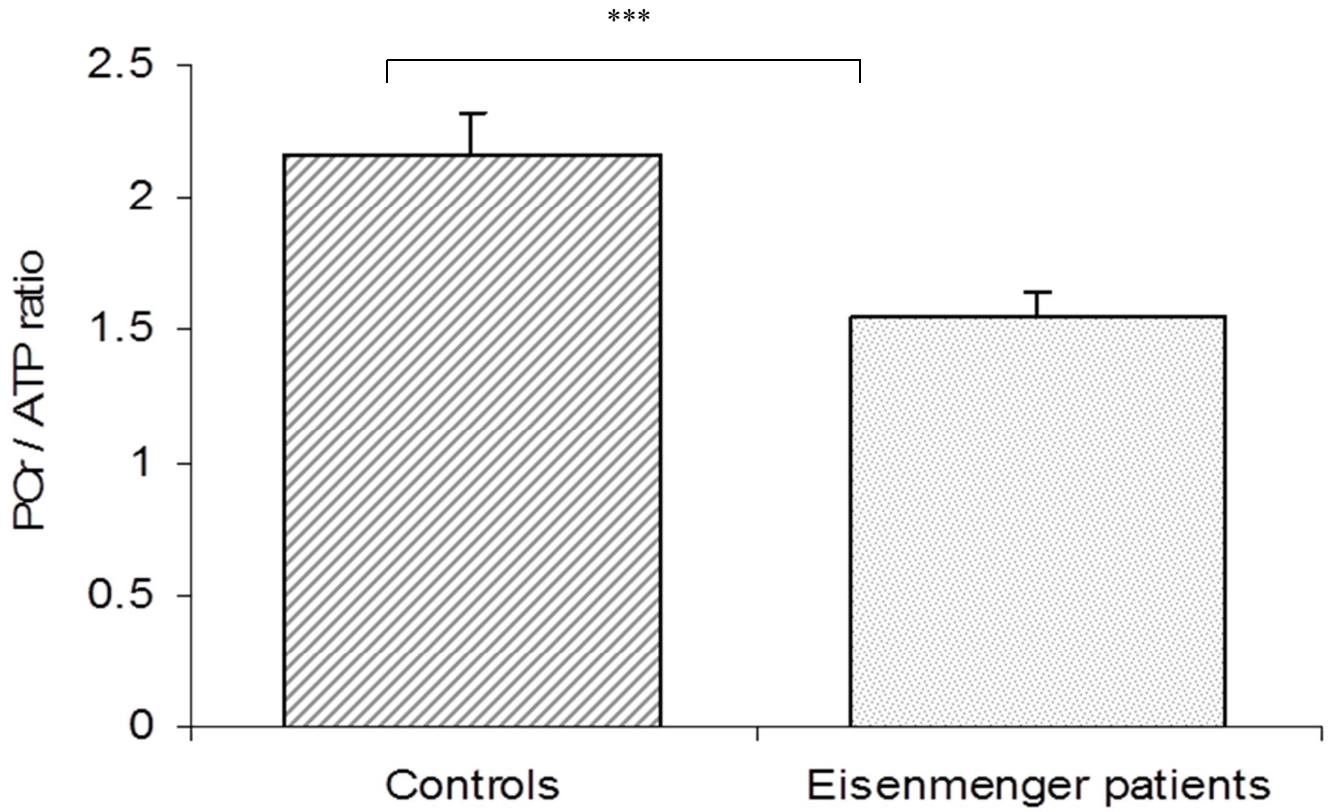


Figure 6.4 PCr / ATP ratios in healthy controls and patients with Eisenmenger syndrome.
*** P<0.001

7. CONCLUSIONS

7.1 Hypotheses

1. *Patients with Eisenmenger syndrome have severe exercise intolerance with an early shift to anaerobic metabolism (chapter 3).*

We have shown that patients with Eisenmenger syndrome have a severely limited exercise capacity. However, in contrast to our hypothesis, they do not appear to show an early shift to anaerobic metabolism despite the severe hypoxaemia, as judged by the point at which the anaerobic threshold occurs relative to maximal exercise. This ability to maintain aerobic metabolism occurs despite reductions at maximal exercise of determinants of O_2 delivery including minute volume, respiration rate, heart rate and O_2 pulse (stroke volume). This finding therefore suggested that in adults with Eisenmenger syndrome, the mitochondria in skeletal muscle have optimised their O_2 utilisation.

2. *There are alterations in skeletal muscle mitochondrial metabolism to account for the exercise intolerance as assessed by the PCr recovery (chapter 4).*

Our results on lower leg skeletal muscle have demonstrated, in accord with our hypothesis, that patients with Eisenmenger syndrome have a maintained mitochondrial oxidative capacity, relative to control subjects, as measured by PCr recovery time following exercise to fatigue. We also showed that they have a similar reduction in intramuscular pH during exercise to healthy controls, again suggesting that they do not have an early reliance on anaerobic metabolism.

3. *They have maintained cardiac systolic function but right ventricular diastolic dysfunction as measured by echocardiography (chapter 5).*

The present findings with echocardiography demonstrated that patients with Eisenmenger syndrome have preserved right and left ventricular systolic function. However, in agreement with our hypothesis, we provide evidence of right ventricular diastolic dysfunction as evidenced by impaired early diastolic relaxation.

4. *There is preservation of the septal energetics allowing maintenance of the contractile function (chapter 6).*

In agreement with our hypothesis, the results we obtained with ^{31}P MRS demonstrated that despite the normal systolic function shown on echocardiography, there is impairment of septal energetics as revealed by a reduction in PCr/ATP ratio. From our results it is not clear if this reduction in PCr/ATP ratio represents sub-clinical ventricular dysfunction or is due to an increased reliance on glucose over fatty acids for metabolism.

7.2 Adaptations to Hypoxaemia

Overall, the results presented in this thesis indicate that adult patients with Eisenmenger syndrome have undergone beneficial adaptations to the severe hypoxaemia that they are exposed to from infancy. As with lowlanders, such as mountaineers who acclimatise to hypobaric hypoxia, it is likely that time has allowed these adaptive processes to occur and allow them to survive despite PO_2s that, if exposed to acutely, may be fatal[128;332]. The long term nature of their exposure may also explain why some of our results differ to previous studies carried out in children with cyanotic congenital heart disease[254;255].

The present findings demonstrated that patients with Eisenmenger syndrome, like other individuals exposed chronically to hypoxia, have increased haematocrit and increased ventilation at rest, both of which will help to increase the O_2 delivery to the contracting muscle, providing these patients show adequate exercise hyperaemia; in future studies, measurements of blood flow during exercise are required to fully test this proposition. Notwithstanding, our MRS results indicated a preserved mitochondrial oxidative capacity in skeletal muscle as evidenced by the finding that from a similar level of PCr depletion caused by exhaustive exercise, they showed a preserved PCr recovery following exercise as compared to healthy controls. Further, although Eisenmenger patients showed a low cardiac PCr/ATP ratio relative to controls, it was preserved relative to other patient groups with HCM or left ventricular dysfunction. Thus, patients with Eisenmenger syndrome appear to have, through exposure to longstanding hypoxaemia, adapted metabolically to maintain their cellular energetics and function.

As discussed in previous chapters, one mechanism by which patients with Eisenmenger syndrome may adapt to chronic hypoxaemia is by switching cardiac and skeletal muscle metabolism to a greater reliance on glucose for fuel rather than free fatty acids to maximise the generation of ATP per mole of O_2 consumed. Hoppeler et al describe how long term or permanent exposure to profound hypoxia results in a reduced mitochondrial content of muscle fibres, a move towards increased metabolism of carbohydrates for oxidative metabolism and decreased intramyocellular lipid substrate stores[132]. Despite this reduction of mitochondria, there is improved high altitude performance which they argue is due to better coupling between ATP supply and demand pathways.

An important factor in the cell's adaptive responses to hypoxia is through hypoxia-inducible factor 1(HIF-1) which acts as the "master regulator", as discussed in chapter 1[168;333]. In normoxia the heterodimer HIF-1α is hydroxylated and targeted for proteasomal degradation[334]. However, in hypoxia it becomes stabilised and plays a central role in regulating the expression of numerous adaptive genes. These include those that enhance glucose metabolism, such as GLUT-1 and other glycolytic enzymes[179], and non-metabolic targets including erythropoietin and VEGF which lead to an increased O_2 carrying capacity and the potential for increased O_2 delivery.

The switch from fatty acid to carbohydrate metabolism in cardiac muscle has been termed a switch to the fetal gene programme which maximises the production of ATP per mole of O_2 consumed[335]. Thus, Sharma et al, who followed the expression of the genes involved in glucose metabolism in rat hearts in response to hypobaric hypoxia, showed that the right ventricle showed an early switch to a fetal pattern of glucose predominance[335]. They suggested that since only the RV showed the switch, that the stimulus was the hypoxia induced increase in RV workload.

Whilst the role of HIF-1α in chronic hypobaric hypoxia has been extensively investigated, only two studies to date have looked at the expression of HIF-1α and its target genes in cyanotic congenital heart disease (CHD). Lemus-Varela et al examined the mRNA transcripts for HIF-1α and its targets, VEGF and Epo, in newborn infants with cyanotic CHD and persistent pulmonary hypertension of the newborn, a condition also characterised by hypoxaemia[336]. They demonstrated increased expression of all of these substances in the hearts of cyanotic subjects compared to healthy non cyanotic newborns. Further, Qing et al

compared the expression and activity of HIF-1α, VEGF and eNOS in the ventricular myocardium of infants with cyanotic congenital heart disease and again found up-regulation of these substances compared to infants with non-cyanotic heart disease[337]. They also showed that the response was proportional to the degree of hypoxaemia. Both of these studies were performed in infants, but it may be that this up regulation of HIF-1α continues with time and may explain, in part, our findings of preserved mitochondrial oxidative capacity.

However, a complete switch to glucose metabolism is unlikely and would not be sustainable over time. A further mechanism to hypoxia may be the down regulation of mitochondrial uncoupling proteins. Uncoupling proteins (UCP) 2 and 3 are found in human heart and skeletal muscle cells and act to decrease the proton gradient across the inner mitochondrial membrane and thus decrease the ATP yield[338]. Up regulation of these is seen in acquired heart failure and contributes to the abnormal energetics status found and a shift towards less O_2 efficient fatty acid metabolism [339].

The expression of UCP2 and 3 is controlled by peroxisome proliferator-activated receptor (PPARα)[339]. The expression of PPARα and its target genes, including UCP2 and 3, has been demonstrated to be reduced in chronic hypoxia with Levett et al recently demonstrating a decreased expression of UCP3 in skeletal muscle in climbers exposed to sustained hypoxia[333]. The expression of PPARα is again mediated by HIF1-α[340;341].

Thus, exposure to chronic hypoxia appears to allow a switch away from fatty acid metabolism plus improved mitochondrial coupling with a subsequent preservation of cardiac and skeletal muscle contractile function despite low O_2 availability.

A further potential mechanism in the adaptation to hypoxia may be through alterations in concentrations of nitric oxide and its metabolites. Nitric oxide (NO) is an important signalling molecule that acts as an endogenous regulator of cell and tissue function[342]. Amongst its functions are effects on vascular tone as a vasodilator, thus affecting blood pressure and blood flow, and regulation of intermediary metabolism and mitochondrial energy production by competing with O_2 for the same binding site on cytochrome oxidase[343]. NO is produced within the cell from L-arginine by NO synthases (NOS), an O_2 dependent process, and is stabilised in the blood by oxidation to nitrate (NO_3^-) and nitrite (NO_2^-). NO can also be produced through the reduction of dietary $NO_3^-$and NO_2^-.

Whereas NO_3^- and NO_2^- were previously considered to be inert by-products of NO metabolism it is now known that under hypoxic conditions, when O_2 dependent NOS activity is compromised, NO_2^- is an important source of NO and maintains NO production through the reduction of NO_2^- by heme proteins[344]. Indeed, Maher et al demonstrated the hypoxic augmentation of the dilator action of NO_2^- on blood vessels in healthy individuals[345]. They showed that under hypoxic conditions, infusion of NO_2^- led to a significant increase in forearm blood flow compared to infusion during normoxia.

In accord with this, Erzurum et al demonstrated that Tibetan highlanders have more than ten fold higher levels of active NO products within their circulation, including plasma NO_2^-, red cell NO_3^- and nitroso proteins, with a resultant increase in forearm blood flow and O_2 delivery when compared to lowlanders[346]. Further to this, Levett et al in the Caudwell Xtreme group have shown an increase in plasma biomarkers and changes in microcirculatory blood flow in skeletal muscle of lowlanders acclimatising to altitude in the Caudwell Xtreme group[347].

Further beneficial effects of increased levels of NO_3^- and NO_2^- have been shown by recent studies showing a decreased O_2 cost of exercise following dietary supplementation with nitrates[348;349]. Thus, Larsen et al demonstrated a profound effect of dietary NO_3^- on mitochondrial function as well as whole body O_2 consumption during exercise[349]. However, they also found a decreased expression in skeletal muscle of ATP/ADP translocase, a protein involved in proton conductance, and thus suggested that the mechanism for the improved function was through improved mitochondrial coupling between respiration and oxidative phosphorylation. Further to this they also found a trend towards down regulation of UCP3.

Our results show a small increase in O_2 delivery, as shown by total Hb in the NIRS study, at rest in patients with Eisenmenger syndrome compared to healthy controls. Whilst initially it was hypothesised in chapter 4 that this was likely due to an elevated O_2 content of the blood secondary to an increase in haemoglobin, it is feasible that these patients also have elevated levels of NO and its metabolites with subsequent increase blood flow within the microcirculation. Furthermore, elevated levels of circulating NO metabolites in these patients may have a further beneficial effect through improved mitochondrial coupling and more efficient metabolism.

Whilst it is clear that patients with Eisenmenger syndrome have adapted in many beneficial ways to a life time of severe hypoxaemia, it cannot be overlooked that they remain severely limited in exercise when objectively assessed.

The cause of their limitation is probably multifactorial, with an inability to increase their cardiac output sufficiently, as shown by a reduced peak O_2 pulse (stroke volume), and decreased O_2 delivery to exercising muscle likely to be important contributing factors. However, in the experiments described in chapter 3, all of the patients stated shortness of breath as the primary reason for stopping exercise during cardiopulmonary exercise testing. This was despite them achieving a lower respiratory rate and tidal volume compared to healthy controls at peak exercise. Some of the subjects with Eisenmenger syndrome also failed to reach maximal exercise, not reaching their anaerobic threshold or an RER>1.0. This suggests a further mechanism limiting their exercise capacity.

Whereas the traditional theory of exercise limitation, suggested by Hill et al in 1924, is that mVO_2 is limited by the cardiovascular system, with the heart limiting the amount of blood pumped to the exercising muscle resulting in anaerobic metabolism and lactic acidosis[350], an alternative centrally driven model has more recently been suggested and may partly explain our observations.

The central governance model states that the extent of skeletal muscle recruitment is determined by the magnitude of central neural drive, in turn determined by feedback from peripheral factors[351]. Thus, exercise can be affected by physiological and psychological factors before exercise, such as the physiological state of the subject, expectations of duration and level of self belief, and during exercise there is continuous feedback to the brain from all organs including state of fuel reserves and hydration state. This has been described as a model of anticipatory regulation of exercise performance.

Whilst patients with Eisenmenger syndrome undoubtedly may have physiological reasons for their severe exercise intolerance, this centrally driven limitation would help explain why they stop before reaching their maximal level of exercise and, as shown by our skeletal muscle MRS data, do not appear to rely on anaerobic metabolism. Patients with Eisenmenger syndrome have lived with severe hypoxaemia and pulmonary hypertension all of their life and, whilst the majority report a good Ability index, have probably modified their activity to cope within their known limits, thus they become "contented invalids". Therefore, exercising on a treadmill was likely to be a significant challenge for them and it is plausible that psychological factors, such as a pre-conceived perception of not being able to exercise for long or a low level of self-belief, may have led to a centrally driven premature cessation of exercise.

A relationship between centrally-mediated exercise limitation and hypoxaemia has been previously reported in healthy subjects. Thus, Kayser et al demonstrated a decrease in EMG activity and lactate concentration during maximal exercise by acclimatised subjects at an altitude of >5000m compared to values obtained at sea level[352]. Furthermore, Annan et al showed that in acute severe hypoxia, exercise was terminated at lower levels of peripheral fatigue than in normoxia or moderate hypoxia[270;353]. These authors concluded that in acute severe hypoxia, the decision to terminate exercise is due to decreased central motor output and this precedes the development of significant peripheral muscle fatigue. This has led them to suggest that the lactate paradox, as discussed in chapter 4, may result from altered behavioural responses with in the brain rather than due to altered skeletal muscle metabolism[354].

Further to the adaptations shown by skeletal muscle, our studies have looked at adaptations by cardiac muscle. Our results show that patients with Eisenmenger syndrome have overall preserved systolic ventricular function. Whilst they have a reduced PCr/ATP ratio at rest it is not clear from our results if this is due to an increased reliance on carbohydrates for metabolism or represents sub-clinical ventricular dysfunction. It is known however that the onset of RV failure in these patients is associated with a poor outcome. Therefore, understanding the underlying causes of the deterioration in RV function, and being able to correctly predict its onset, may lead to the development of therapeutic options to treat it.

A recent study by Drake et al used a mouse model of pulmonary artery banding, to induce RV hypertrophy, with and without hypoxia to compare the gene expression pattern that distinguishes between adaptive RVH and RVF[355]. They showed that the genes that discriminate between these two phenotypes include those for growth and angiogenesis factors and enzymes involved in energy metabolism. Whilst confirmation of these changes in human studies is awaited, these may allow the future prediction of the development of RVF in Eisenmenger syndrome.

Rondelet et al used a pig model to look at the effects of prolonged systemic to pulmonary shunting to investigate the effects on right ventricular function[356]. They demonstrated right ventriculoarterial uncoupling, as expressed by the ratio of end-systolic to pulmonary arterial elastance, with accumulation of the extracellular matrix, activation of apoptotic pathways and an increase in the expression of pro-inflammatory cytokines in the right ventricular myocardium. This maladaptive process led to decompensated RV failure. Whilst the authors state that this may have been due to the inability of the porcine heart to sustain a prolonged

increase in cardiac output and afterload, it is feasible that subjects with Eisenmenger syndrome are protected from these maladaptive changes until late on in the disease process. Further studies looking at the ventriculoarterial coupling in these patients, such as by the method described by Sanz et al using cardiac MRI and right heart catheterisation[295], may help predict the onset of RV decline.

In conclusion, the studies described in this thesis indicate that the surprisingly good survival of patients with Eisenmenger syndrome, despite adverse cardiopulmonary haemodynamics and a lifetime of severe hypoxaemia, is due, in part, to beneficial physiological adaptations of both skeletal and cardiac muscle. However, whilst they appear to be well adapted to life at rest, sustained exercise is not feasible.

Other studies have shown similar adaptations occur in healthy adults during acclimitisation to high altitude. The normal cardiac anatomy that exists in these individuals eliminates the confounding effects of disease and therapeutic interventions that may complicate interpretation of findings made in Eisenmenger patients. However, the results obtained in acclimatised lowlanders may themselves be confounded by the effects of the hypobaric atmosphere at altitude and also the known effects of endurance training on muscle metabolism[65]. We have shown, by the results presented in this thesis that these adaptations can also occur in the presence of severe cardiopulmonary disease. This gives support to lending these findings to critically ill patients.

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7.3 Limitations of Study

All of the studies described in this thesis were non-invasive with the exception of the venous blood samples taken in chapter 3. Our planned methodology initially included taking a skeletal muscle biopsy from these patients. However, we subsequently decided this carried an unacceptable risk to these patients due to the risk of bleeding[15;357]. A further proposed experiment was the calculation of the respiratory quotient (RQ) within the exercising muscle. The RQ is the ratio of CO_2 produced and O_2 consumed and gives further information regarding substrate utilisation. A RQ of 1.0 being indicative of glucose being the predominant fuel whilst a RQ of 0.7 suggesting fatty acids. However, this would have required obtaining a blood sample from an artery which again was felt to carry an unacceptable risk to the patient.

Thus, the interpretations of our findings have been drawn on largely non-invasive measurements. Some of our conclusions are therefore based on assumptions with respect to exercise physiology in these complex cardiac patients.

The numbers of patients with Eisenmenger syndrome who agreed to participate in this study was small. The study was initially powered for 13 subjects to enable to us to detect a 20% difference in PCr/ATP ratio, but just 7 for a 20% difference in PCr recovery half time. The small numbers recruited reflects a relatively small cohort of patients with Eisenmenger syndrome in the modern era of ACHD from which to draw, which is made smaller when patients with Down syndrome are excluded.

7.4 Future Work

Whilst our work demonstrated that beneficial physiological adaptations have indeed occurred in adult patients with Eisenmenger syndrome, future studies would be required to determine the exact mechanism underlying these adaptations. However, due to the risk to the patient of invasive procedures as outlined above, future studies would similarly need to avoid these and would therefore need to focus on non invasive experiments. The specific areas that warrant further investigations are outlined below.

7.4.1 HIF-1α Activity

The expression of HIF-1α and its target genes such as GLUT-1 and other glycolytic genes as well as non-metabolic genes such as EPO and VEGF, would help to give further information regarding their roles in the long term adaptation to the chronic hypoxaemia. It may also give further information as to the substrate utilisation in these subjects. This would be performed by isolating mRNA from white blood cells in peripheral blood as described by Lemus-Varela et al[336].

7.4.2 Nitric Oxide and its Metabolites

To further assess the NO availability in these patients we would assess the circulating biomarkers of NO availability in venous blood including nitrite, nitrate and S-nitrosothiols at rest and following exercise.

7.4.3 Effects of Exercise on Cardiac Function

All of our cardiac data was obtained at rest. Exercise echo would allow investigation of systolic and diastolic function as well as pulmonary artery pressures during exercise. Exercise

echo has previously been demonstrated to reveal widespread systolic and diastolic abnormalities in both hypertensive patients and patients with heart failure with preserved ejection fraction[306;358;359]. Therefore, this may give further insight into the cause of the exercise limitation.

7.4.4 Ventriculo-arterial Coupling

To further assess the proposal made above that maintenance of ventriculo-arterial coupling in part explains the preservation of ventricular systolic function, and it is the uncoupling that leads to RV failure, this could be further investigated to see if it is possible to predict the onset of RV failure. Whilst assessment of this has previously required invasive cardiac catheterisation, Sanz et al describe a non-invasive technique using cardiac MRI to approximate the degree of right ventriculo-arterial coupling[295].

ABSTRACTS AND PRESENTATIONS ARISING FROM THIS WORK

- Fatigue and Exercise Intolerance in Eisenmenger syndrome
  Oral presentation in "Young Investigator" competition at BCCA 2009

- Resting Skeletal Muscle Oxygen Consumption and Maximal Oxidative Capacity in Eisenmenger Syndrome
  Poster presentation at BCCA 2009 (winner of "best poster")

- Right Ventricular Function and Cardiac Energetic Status in Eisenmenger Syndrome
  Poster presentation at BCCA 2009

- Exercise Intolerance and Skeletal Muscle Adaptations in Eisenmenger Syndrome
  Oral presentation in "State of the Art" session at ESC 2009

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