Imperial College London

National Heart & Lung Institute

PATHOPHYSIOLOGIC CORRELATES OF EXERCISE INTOLERANCE IN ADULTS WITH PULMONARY HYPERTENSION AND CONGENITAL HEART DISEASE

PhD thesis

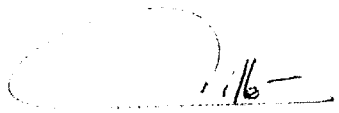
by

Gerhard-Paul Diller, MD

2009

I hereby declare that the work presented in this thesis is my own.

Contributions of others are mentioned in the acknowledgement section.



Gerhard-Paul Diller

London, 22 June 2009

ABSTRACT

Adult congenital heart disease (ACHD) patients have markedly depressed exercise capacity. This thesis examined (i) the prevalence of chronotropic incompetence, its relationship to symptoms and exercise capacity and its prognostic value in ACHD patients; (ii) investigated exercise capacity in patients with Eisenmenger syndrome and assessed survival prospects in this cohort as well as risk factors for mortality. In addition, clinical effects of Bosentan (a pulmonary vasodilator) were examined during longer-term follow-up. (iii) Mathematical modelling studies were employed to assess the impact of intracardiac shunting on oxygen delivery and tissue oxygenation potentially affecting exercise capacity. (iv) As pulmonary and systemic endothelial dysfunction are integral features of pulmonary arterial hypertension (and are related to exercise intolerance in this setting), this thesis examined the number and function of endothelial progenitor cells (EPC) in patients with idiopathic pulmonary hypertension and Eisenmenger syndrome. The main findings are: (i) chronotropic incompetence is prevalent in ACHD patients, relates to exercise intolerance and is an important prognostic marker across the spectrum of ACHD. However, chronotropic incompetence appears not to be causally related to exercise intolerance in many ACHD patients and artificially augmenting heart rate during exercise does not improve exercise capacity in many patients with a systemic right ventricle and a pre-existing pacemaker. (ii) Exercise intolerance is prevalent in patients with Eisenmenger syndrome and treatment with Bosentan improves objective exercise capacity in this cohort. (iii) This thesis shows how "unphysiologic" circulatory conditions and right-to-left shunt lesions limit

exercise capacity. It also emphasises the limitations of physiologic concepts such as the concept of arterial oxygen delivery in ACHD patients with complex conditions. (iv) Finally, this thesis shows that patients with Eisenmenger syndrome have significantly lower EPC numbers and impaired EPC function and that the number of EPCs correlates directly with symptoms and objective exercise capacity.

Table of Contents

List of tables

Chapter II Chronotropic Incompetence

Chapter V Inflammation and Endothelial Progenitor cells

List of figures

Figure 5.6: Plasma levels of tumor necrosis factor-α, interleukin-6, monocyte chemotactic protein-1 and C-reactive protein. Plasma levels of asymmetrical dimethylarginine, cyclic GMP, total plasma nitrate/nitrite levels and vascular endothelial growth factor in control, Eisenmenger with and without Down syndrome and idiopathic pulmonary arterial hypertension subjects.

Figure 5.7: Correlation between inflammatory mediators or asymmetrical dimethylarginine and numbers of circulating $CD34^+/KDR^+$ cells.

Figure 5.8: Correlation between brain-type natriuretic peptide (BNP) and cyclic GMP (cGMP) levels, $CD34^+$, $CD34^+$-$AC133^+$ or $CD34^+$-$AC133^+$-KDR^+ progenitor cells in patients with Eisenmenger syndrome.

Figure 5.9: Relationship between plasma cGMP levels and circulating $CD34^+/KDR^+$ EPCs in control, Eisenmenger and idiopathic pulmonary hypertension subjects.

Figure 5.10: Correlation between haemodynamic parameters and cGMP levels or circulating $CD34^+/KDR^+$ cells in patients with idiopathic pulmonary hypertension.

Figure 5.11: Interplay between immune inflammation, oxidative stress, reduced nitric oxide-bioavailability and endothelial progenitor cell numbers and function, endothelial dysfunction and ultimately exercise intolerance.

Chapter VI Conclusions

Figure 6.1: Summary slide illustrating the mechanisms limiting exercise capacity in adult patients with congenital heart disease.

List of abbreviations

6MWT, six minute walk test distance

AA, arachidonic acid

AC133, prominin/cluster of differentiation (CD)133

ACE-Inhibitors, angiotensin converting enzyme inhibitor

ACHD, adult congenital heart disease

ADMA, asymmetric dimethylarginine

ALT, alanine aminotransferase

Ao, Aorta

APC, allophycocyanin

AST, aspartate aminotransferase

ATP, adenosine-5'-triphosphate

BNP, brain natriuretic peptide

CaO_2, arterial oxygen content

Cap_{O2}, oxygen carrying capacity of blood

$C(a\text{-}v)O_2$, arterio-venous oxygen difference

CCB, calcium channel blocker

CCL-2, monocyte chemotactic protein-1

ccTGA, congenitally corrected transposition of the great arteries

CD34, cluster of differentiation molecule 34

CD45, cluster of differentiation molecule 45

CFDA SE, carboxyfluorescein diacetate, succinimidyl ester tracer

CFU, colony forming unit

cGMP, cyclic guanosine monophosphate

CHD, congenital heart disease

CI, confidence interval

CMV_{O2}, mixed venous oxygen content

CO, cardiac output

COX-1, cyclo-oxygenase 1

CRP, C-reactive protein

CvO_2, venous oxygen content

DaO_2, arterial oxygen delivery

Δ-avO2, arterio-venous oxygen difference

dil-acLDL, 1,1'-dioctadecyl-3,3,3',3-tetramethyl-indocarbocyanine perchlorate labelled acetylated LDL

EBM-2, endothelial growth medium-2

ECE, endothelin converting enzyme

ECG, electrocardiogram

EDTA, ethylenediaminetetraacetic acid,

ELISA, enzyme-linked immuno sorbent assay

EM, Eisenmenger syndrome

EMEA, European Agency for the Evaluation of Medicinal Products

eNOS, endothelial nitric oxide synthase

EPC, endothelial progenitor cells

ET-1, endothelin 1

FCS, fetal calf serum

FDA, Food and Drug Administration

FITC, fluorescein

Hb, haemoglobin concentration

HR, heart rate

HRR, heart rate reserve

HUVEC, human umbilical vein endothelial cells

IL, interleukin

IQR, interquartile range

IPAH, idiopathic pulmonary arterial hypertension

IVC, inferior vena cava

k, proportion of oxygen consumption in the upper body

K, oxygen carrying capacity of blood ($1.38 \cdot 10^{-3}$ liter O_2/gram Hb)

kDa, Kilodalton

KDR, vascular endothelial growth factor receptor-2

LA, left atrium

LV, left ventricle

MCP, monocyte chemotactic protein-1 (=CCL-2)

NO, nitric oxide

NOx, combined concentrations of nitrite and nitrate

NYHA, New York Hear Association class

O_2, oxygen

PA, pulmonary artery

PAH, pulmonary arterial hypertension

PAP, pulmonary arterial pressure

PBMC, peripheral blood mononuclear cells

PGI_2, prostacyclin

PGI_2S, PGI2-synthase

pCO_2, carbon dioxide partial pressure

PDE-5, phosphodiesterase type 5

PE, phycoerythrin

PKC, protein kinase C

PVR, pulmonary vascular resistance

Q_{IVC}, inferior vena cava blood flow

Qp, pulmonary blood flow

Qs, systemic blood flow

Q_{SVC}, superior vena cava blood flow

QRL, right-to-left shunt volume

RV, right ventricle

Sa_{O2}, arterial oxygen saturation

Sat, oxygen saturation

$Sat_{MVO2min}$, minimal mixed venous saturation

Sat_{PVO2}, pulmonary venous oxygen saturation

sGC, soluble guanylate cyclase

$SIVC_{O2}$, oxygen saturation in the inferior vena cava

SpO_2, transcutaenous oxygen saturation

SPV_{O2}, pulmonary venous oxygen saturation

SRV, systemic right ventricle

$SSVC_{O2}$, oxygen saturation in the inferior vena cava

SV, stroke volume

SVC, superior vena cava

t-FT, total filling time

TGA, transposition of the great arteries

t-IVT, total isovolumic time

TNF, tumor necrosis factor

UEA, ulex europeus agglutinin

UVH, univentricular heart

VCO_2, carbon dioxide production

VE,ventilation

VEGF, vascular endothelial growth factor

VO_2, oxygen consumption

VSD, ventricular septal defect

VTI, velocity time integral

VE/VCO_2 slope, ventilation-VCO_2 – slope.

vWF, von Willebrand factor

WBC, white blood cell count

Acknowledgements

I would like to thank Professor Michael A. Gatzoulis for giving me the opportunity to perform this PhD thesis, his ongoing support and the multiple fruitful discussions. Without his help and support this thesis had not been possible. I am also very thankful for the help and scientific advice I received from Dr. John Wharton, Professor Martin R. Wilkins, and especially Dr. Darrel P. Francis.

I would like to thank my parents for their love and support. I dedicate this PhD thesis to my beloved wife Astrid and to our baby daughter Liv Sophie.

I am thankful to the following people who helped a lot during my PhD thesis:

Chapter II Chronotropic Incompetence

Anselm Uebing helped performing transthoracic echocardiograms and Mark Maskell helped with pacemaker reprogramming in patients with a systemic right ventricle and a pre-existing cardiac pacemaker.

Chapter III Pulmonary vascular disease

Utpal Singh Naghotra helped collecting data on Eisenmenger patients under follow-up at the Royal Brompton Hospital, London and Kostas Dimopoulos helped with data analysis.

Chapter V Inflammation and Endothelial Progenitor cells

I thank Sharon Meehan and Carl Harries for their invaluable help in recruiting patients with pulmonary hypertension. Thomas Thum measured ADMA levels and Adrian J.Hobbs performed measurements of stable nitric oxide oxidation products in plasma samples of patients with pulmonary hypertension. Darlington O Okonko helped with the initial setup of the FACS assay. Sven van Eijl helped with incorporation into tube-like structure assays and performed the staining of cultured cells.

Chapter I Introduction

The work described in this thesis has been performed between February 2005 and December 2007 at the Adult Congenital Heart Centre and Centre for Pulmonary Hypertension, Royal Brompton Hospital, London and the Section on Experimental Medicine and Toxicology, Imperial College London, Hammersmith Campus, London.

1.1. Exercise Intolerance in Adult Patients with Congenital Heart Disease

Adult congenital heart disease (ACHD) patients have markedly depressed exercise capacity (1-5). The presence and severity of symptoms are associated with a worse objective exercise capacity in these patients and relate to increased short-term mortality (6). Exercise intolerance in adults with congenital heart disease is multi-factorial as illustrated in Figure 1.1. In a previous study chronotropic response to exercise, pulmonary arterial hypertension and cyanosis were found to be the most important correlates of exercise intolerance (1). In addition to central haemodynamic factors the ability of the periphery to utilise oxygen may limit oxygen uptake in patients with congenital heart disease (7).

The physiologic components contributing to exercise capacity

During exercise energy in the form of ATP is continuously produced in skeletal muscles predominantly by aerobic metabolism. This requires adequate coupling of oxygen delivery to mitochondrial oxygen consumption. Other mechanisms such as anaerobic glycolysis and hydrolysis of phosphocreatine contribute to ATP production only to a minor extent if oxygen delivery is inadequate or at the beginning of exercise, respectively. As a consequence normal exercise capacity is dependent on the cardio-respiratory chain comprised of ventilation, pulmonary perfusion and the cardiovascular system (including blood vessels and the microvasculature). Malfunction in any of these components (lungs, heart, blood, endothelial function) can limit exercise capacity. Congenital heart disease can affect all components of the cardio-respiratory chain, resulting in significant impairment of exercise capacity.

Cardiac function during exercise plays a central role in the cardio-respiratory chain. As illustrated by the Fick principle peak oxygen consumption increases in proportion to increased cardiac output. Cardiac output, in turn, represents the product of stroke volume and heart rate. In patients with congenital heart disease numerous factors may limit stroke volume during exercise. Increased afterload to the subpulmonary ventricle may limit ventricular output during exercise in patients with pulmonary arterial hypertension. In addition, valvular dysfunction is prevalent in patients with congenital heart disease and may also limit stroke volume during exercise. In addition, various studies have demonstrated the importance of the interaction

between both ventricles. In patients with right ventricular volume overload (e.g. tetralogy of Fallot patients with severe pulmonary regurgitation) right ventricular enlargement and dysfunction impairs left ventricular function thus inducing biventricular dysfunction (i.e. ventriculo-ventricular interaction) (8). Shunt lesions such as atrial and ventricular septal defects may impose volume overload on the right and left ventricle, respectively (9). Furthermore, previous studies have demonstrated abnormal myocardial perfusion in subgroups of patients with congenital heart disease. Hauser et al. demonstrated perfusion abnormalities in patients with transposition of the great arteries after atrial switch operation (10). These perfusion abnormalities correlated to impaired ventricular function and to older patient age. Unfortunately stroke volume during exercise is particularly difficult to assess in patients with congenital heart disease due to abnormal anatomy and physiology. Several non-invasive techniques of assessing stroke volume during exercise (e.g. impedance cardiography, Doppler echocardiography and photoplethysmography) have been proposed but none of these have been validated in this cohort. Assessing stroke volume according to the Fick principle represents an invasive technique requiring measurement of central venous oxygen saturation (e.g. by placing a pulmonary catheter during exercise). As a consequence there are ethical concerns regarding the application of this technique for research purposes, only. Therefore, changes in stroke volume during exercise are largely unknown in adults with congenital heart disease.

Chronotropic incompetence

While stroke volume increases predominantly at the beginning of exercise, further increase in cardiac output occurs mainly through increase in heart rate. Chronotropic incompetence represents an inappropriate increase in heart rate to meet the metabolic demands of exercise. Chronotropic incompetence is an established predictor of mortality in various cardiovascular cohorts as well as in healthy populations (11-14). An attenuated heart rate response to exercise has been reported across the spectrum of adult congenital heart disease (1,2). In a recent study peak heart rate was found to be the strongest physiologic determinant of exercise capacity in ACHD patients (1). Despite the importance of chronotropic incompetence no generally accepted definition of chronotropic incompetence is available. Currently used definitions are based on the heart rate response to exercise (15), comparative (16,17) and correlational (2,18) statistics.

Cyanosis, pulmonary vascular disease and the Eisenmenger physiology

Cyanosis and pulmonary hypertension significantly affect exercise capacity. Cyanosis reduces arterio-venous oxygen difference, while pulmonary arterial hypertension – representing an increased right ventricular afterload – limits stroke volume during exercise. Not surprisingly Eisenmenger patients, afflicted by a combination of both factors have a significantly impaired exercise tolerance as shown in previous studies (1,19). In addition to reducing arterio-venous oxygen difference, thereby limiting oxygen uptake, right-to-left shunting and cyanosis lead to an increased dead space ventilation (as venous blood is bypassing the lungs) inducing an exaggerated

increase in ventilation and a reduced ventilatory efficiency as suggested by high values of the VE/VCO_2 slope in cyanotic patients (6). It has been argued that despite the apparent "inefficiency" of the ventilatory response to exercise in cyanotic patients leading to the early onset of dyspnoea and exercise limitation in these patients, the exaggerated ventilatory response is appropriate from a "chemical" point of view (7). In fact, it has been reported that arterial pH and pCO_2 values are maintained at a normal or near normal value during exercise in patients with significant right-to-left shunting during exercise (20).

Anaemia and secondary erythrocytosis

As illustrated by the Fick equation (21) adequate haemoglobin concentration is closely linked to oxygen uptake and thus exercises capacity:

$$VO_2 = SV \bullet HR \bullet (SatO_2art - SatO_2ven) \bullet K \bullet Hb$$

VO_2 = oxygen uptake; SV = stroke volume ; HR = heart rate ; $SatO_2art$ = arterial oxygen saturation ; $SatO_2ven$ = venous oxygen saturation; K = oxygen carrying capacity of blood ($1.38\ 10^{-3}$ L O_2 per gram haemoglobin) ; Hb = haemoglobin concentration .

In fact, maximal oxygen uptake is directly proportional to haemoglobin concentration. As a consequence anaemia results in inadequate oxygen delivery to tissue during exercise, a premature shift to anaerobic metabolism during exercise and reduced exercise tolerance. The association between the presence of anaemia and reduced exercise capacity as well as impaired prognosis in patients with heart failure due to acquired heart disease is well established (22-25). Anaemia – as conventionally defined (26) – is occasionally encountered in non-cyanotic adult patients with congenital heart

disease. In contrast many cyanotic adult congenital heart disease patients have secondary erythrocytosis as reflected by elevated mean haemoglobin concentrations (27). Secondary erythrocytosis represents an adaptation to cyanosis and relates to arterial oxygen saturations thus helping to maintain oxygen carrying capacity of the blood (28). Patients who cannot maintain adequate haemoglobin concentrations due to frequent phlebotomies, iron deficiency or vitamin deficiency (29) have reduced exercise capacity and increased morbidity and mortality compared to patients with adequate haemoglobin levels (30,31).

Endothelial dysfunction and Immune Inflammation

The endothelium forms the interface between circulating blood and the rest of the vessel wall. In addition to forming a physical and metabolic barrier, the endothelium is an important endocrine organ (32). Numerous humoral factors released by the endothelium and influencing vascular tone have been identified. This area of research has received considerable clinical interest as some of these factors are now amenable to pharmacological therapy (33). These factors include nitric oxide, endothelin and prostacyclin (Figure 1.2).

Nitric oxide (formerly known as endothelium-derived relaxing factor, EDRF) is a potent vasodilator (34,35). Nitric oxide is produced by nitric oxide synthase (NOS) (36). Different isoforms of the enzyme have been identified (NOS I-III). Nitric oxide synthase III (eNOS) is mainly expressed in endothelial cells, while NOS I and NOS III are found in neural tissue and inflammatory cells, respectively (32). Endothelial NOS is activated by

increased intracellular calcium concentrations through calcium-calmodulin interaction. Endothelial NOS generates nitric oxide from L-arginine with NADPH as a co-substrate and flavin adenine dinucleotide, flavin mononucleotide, and tetrahydrobiopterin as cofactors (37). Nitric oxide diffuses to neighbouring smooth muscle cells where it leads to increased intracellular levels of cyclic guanylate monophosphat (cGMP), thus inducing vasodilation and inhibiting cell proliferation (36). Cyclic GMP in turn is degraded by phosphodiasterases (PDEs). Pharmacological inhibition of PDEs, therefore, provides a way to augment intracellular cGMP levels. Sildenafil represents such a PDE-5 inhibitor currently employed for example in the treatment of pulmonary arterial hypertension (38). Asymmetric dimethylarginine (ADMA) is a metabolic by-product of continual protein methylation, a common mechanism of post-translational protein modification. It is closely related to L-arginine and represents a physiologic inhibitor of NOS. Increased levels of ADMA have been linked to various cardiovascular disorders (39).

Endothelin was first described in 1988 and represents one of the most potent vasoconstrictors identified (40,41). Endothelin-1 (ET-1), the predominant form produced by endothelial cells, is synthesized as pre-pro-endothelin (pre-pro-ET-1) following translation from mRNA and is subsequently processed by furin-linke enzymes to big-ET-1. Big-ET-1 is cleaved by endothelin converting enzymes to ET-1 (42). Regulation of ET-1 production occurs mainly at the level of gene expression (43). Endothelin-1 activates endothelin A and B receptors on vascular smooth cells leading to calcium

release and activation of protein kinase C. Endothelin-1 is a potent vasoconstrictor with mitogenic, pro-fibrotic and pro-inflammatory properties (44). Endothelin receptor antagonists (ERAs) have been demonstrated to improve pulmonary haemodynamics in patients with pulmonary arterial hypertension (45). Their efficacy in improving 6 minute walk test distance as well as time to clinical worsening in patients with idiopathic pulmonary hypertension has been confirmed by several randomised controlled trials (46-52). Preliminary results also suggest that Bosentan may prolong survival in patients with advanced pulmonary arterial hypertension (53). As a consequence ERAs have become the mainstay of pulmonary arterial hypertension therapy.

Reduced production of prostacyclin (PGI2) is an additional hallmark of endothelial dysfunction. Prostacyclin is formed by cyclo-oxygenase-1 and prostacyclin synthase from arachidonic acid liberated from membrane-bound lipids by phospholipase A2. Activation of prostacyclin receptors on smooth muscle cells induces stimulation of adenylate cyclase thus increasing intracellular cAMP levels, leading to vasodilation and antiproliferative effects. Different PGI2-analoga (Epoprostenol, Iloprost, Treprostinil) have been developed and are commonly used in patients with pulmonary arterial hypertension. Intravenous Epoprostenol is generally regarded as first line treatment in patients with pulmonary arterial hypertension presenting in NYHA class IV (54). Intravenous prostacyclin administration is technically challenging and prolonged intravenous therapy is associated with frequent complications such as sepsis and line dislocation. Therefore, continuous i.v.

prostacyclin administration requires special expertise and extensive patient training. Treprostinil has been demonstrated to improve functional capacity and pulmonary haemodynamics and can be delivered subcutaneously. However, pain at the infusion site (in up to 85% of patients) prompting discontinuation of therapy in 8% of patients limit its use (55). Inhaled iloprost has been demonstrated to improve 6 minute walk test distance and NYHA class in patients with idiopathic pulmonary arterial hypertension, pulmonary arterial hypertension associated with connective tissue disease and thrombembolic pulmonary arterial hypertension and may represent an alternative to intravenous or subcutaneous prostacyclin analogues in selected patients (56).

Endothelial dysfunction is ubiquitous in patients with heart failure and adults with congenital heart disease and relates to exercise intolerance and symptoms (57-63). In addition, endothelial dysfunction is a predictor of outcome in patients with advanced heart failure (64,65). Furthermore, endothelial dysfunction has been found to improve after heart failure treatment (61,66). It has been postulated that endothelial dysfunction may have a detrimental effect on myocardial and skeletal muscle function (7). Endothelial dysfunction has been linked to reduced availability of vasoactive mediators (Figure 1.2), shear stress, oxidative stress, the presence of inflammation and reduced number of bone marrow derived endothelial progenitor cells (67-70) as illustrated in Figure 1.3.

This thesis aimed to elucidate some of the mechanisms limiting exercise tolerance in adult patients with congenital heart disease by performing retrospective and prospective clinical studies, employing mathematic modeling as well as using laboratory experiments to investigate endothelial progenitor cell numbers and function in patients with congenital heart disease. Patients with congenital heart disease represent a heterogeneous group and different mechanisms limit exercise capacity to a different degree in individual patients. As a consequence and for logistic reasons different sub-studies focused on different diagnostic groups such as patients with a systemic right ventricle, univentricular circulation or Eisenmenger syndrome.

1.2. Figures

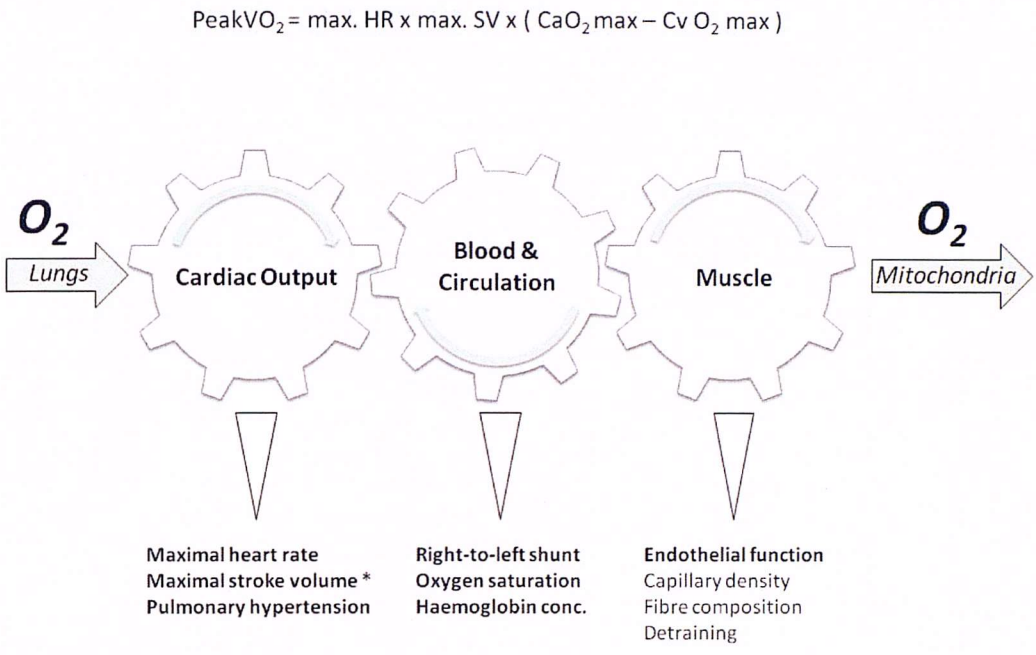


Figure 1.1: Factors related to reduced peak oxygen consumption and exercise intolerance in patients with congenital heart disease. The Equation illustrates the Fick principle showing that oxygen uptake equals heart rate x stroke volume (SV) x the difference between arterial (CaO_2) and venous (CvO_2) oxygen content.

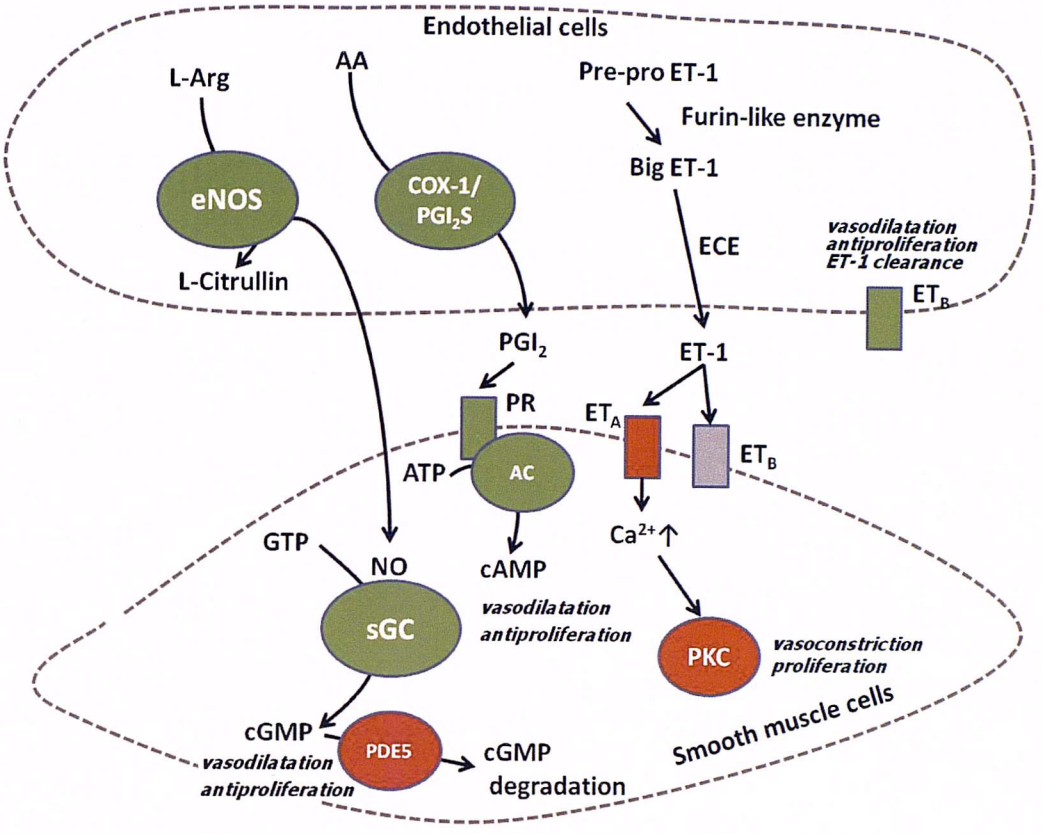
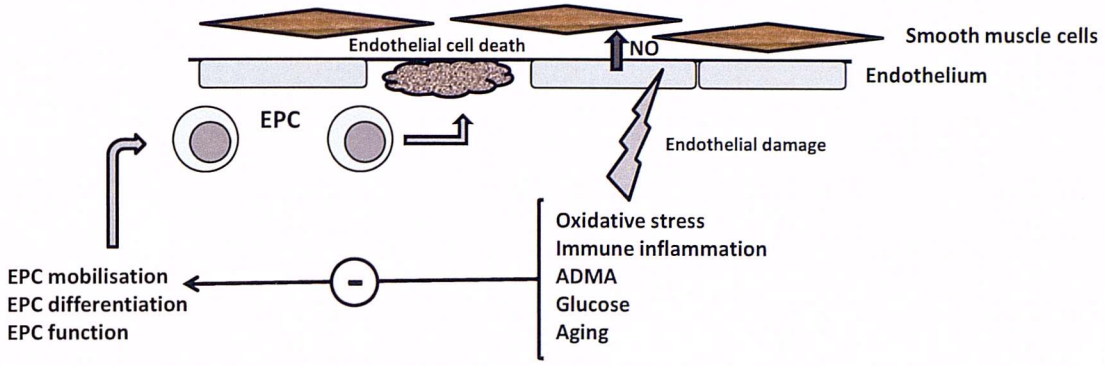


Figure 1.2: Components of the nitric oxide (NO), prostacyclin (PGI_2) and endothelin pathway regulating pulmonary vascular tone and pulmonary vascular remodelling. Nitric oxide is synthesized by endothelial NO-synthase (eNOS) from L-arginine. NO stimulates soluble guanylate cyclase (sGC) in smooth muscle cells resulting in increased production of cyclic GMP (cGMP) inducing vasodilation and antiproliferatory effects. Cyclic GMP in turn is degraded by phosphodiesterase 5 (PDE5). Prostacyclin (PGI_2) is produced by cyclo-oxygenase-1 (COX-1) and PGI_2-synthase (PGI_2S) from arachidonic acid (AA). Activation of prostacyclin receptors (PR) on smooth muscle cells induces stimulation of adenylate cyclase thus increasing intracellular cAMP levels leading to vasodilation and antiproliferative effects. Pre-pro-endothelin (pre-pro ET-1) is formed following translation from mRNA and is subsequently processed by furin-like enzymes to big-ET-1. Big-ET-1 is cleaved by endothelin converting enzymes (ECEs) to ET-1. ET-1 induces vasoconstriction and leads to smooth muscle cell proliferation via activation of ET-A and ET-B receptors with subsequent calcium release and activation of protein kinase C (PKC). Factors inducing vasodilation/antiproliferation are in green, those leading to vasoconstriction/proliferation in red.



ADMA = asymmetric dimethylarginine; EPC = endothelial progenitor cell; NO = nitric oxide

Figure 1.3: Effect of various physiologic factors on endothelial function and endothelial progenitor cell (EPC) mobilisation from bone marrow, EPC differentiation and EPC function.

Chapter II Chronotropic Incompetence

2.1. Background of the study

Chronotropic incompetence — a blunted increase in heart rate during exercise — is an established predictor of mortality in various cardiovascular cohorts as well as in healthy populations (11-14). An attenuated heart rate response to exercise has been reported across the spectrum of adult congenital heart disease (ACHD)(1,2). In a recent study peak heart rate was found to be the strongest physiologic determinant of exercise capacity in ACHD patients (1). Despite the importance of chronotropic incompetence, its prevalence, association and causal relation to exercise intolerance are poorly understood. Furthermore little is known about the prognostic implications of chronotropic incompetence in ACHD patients. Similar to chronotropic incompetence, attenuation of heart rate recovery — the rate of decrease in heart rate after cessation of exercise — has been found to be associated with poor prognosis in patients being assessed for coronary artery disease (71).

Despite the reported strong statistical association between peak heart rate and exercise capacity, attributing exercise limitation directly to chronotropic incompetence in an individual patient is difficult as no generally accepted definition or non-interventional method of assessing the functional impact of chronotropic incompetence exists. Currently used definitions are based on the heart rate response to exercise (15), comparative (16,17) and correlational (2,18) statistics. These methods, however, are based on large

numbers of patients, and cannot be directly applied to individual patients to determine whether a reduced peak heart rate during exercise is causally responsible for exercise intolerance.

2.2. Association between chronotropic incompetence and exercise capacity

Exercise testing with metabolic monitoring measures oxygen consumption (VO_2) and heart rate simultaneously and has the potential to identify patients with an abnormal cardiovascular response to exertion. In healthy individuals, VO_2 increases linearly in proportion to heart rate (72). Departure from linearity indicates the presence of a pathological condition, and provides information on the factors limiting exercise tolerance (72). Factors that impair cardiac output or arterio-venous oxygen difference (e.g. ventricular dysfunction or cyanosis) cause a disproportionately greater increase in heart rate than VO_2. In contrast, factors that impair heart rate (e.g. chronotropic incompetence) lead to a disproportionately greater increase in VO_2 than heart rate. The usefulness of such oxygen pulse kinetics (VO_2 / heart rate) analyses in discriminating between underlying causes of exercise limitation in ACHD patients is unknown.

This study tested the hypothesis that formal cardiopulmonary exercise testing with analysis of oxygen-pulse kinetics would identify individual patients in whom chronotropic incompetence was causally related to exercise intolerance.

2.2.1. Methods

Study population

All cardiopulmonary exercise tests performed in patients with a systemic right ventricle or univentricular circulation at the Adult Congenital Heart Disease Unit, Royal Brompton Hospital, London between January 2003 and May 2004 were analysed.

Patients with implanted pacemakers who were paced during exercise were excluded. Patients were referred for exercise testing as part of routine clinical follow-up. All patients underwent symptom limited exercise testing on a treadmill according to a modified Bruce protocol (73,74), with the addition of a "stage 0" in which the patient walked at a velocity of 1 mile/hour and a gradient of 5% for 3 minutes. Subjects were encouraged to exercise to exhaustion regardless of the maximal heart rate achieved. Ventilation, oxygen uptake and carbon dioxide production were measured continuously using a respiratory mass spectrometer (Amis 2000, Innovision, Odense, Denmark). Heart rate was measured continuously by electrocardiography and arterial blood pressure was assessed manually by sphygmomanometry. All patients gave informed consent prior to exercise testing. Anti-arrhythmic drug use was recorded at the time of exercise testing. Patients with resting oxygen saturations <90% were classified as cyanotic. In addition, 10 age-matched healthy volunteers who underwent cardiopulmonary exercise testing using the same exercise protocol and measurement equipment as for the congenital heart disease patients were recruited.

Chronotropic incompetence

Resting heart rates were recorded after a minimum of 30 seconds rest in a seated position. Peak heart rate was measured as the maximal heart rate achieved during exercise. Heart rate reserve was calculated as the difference between the peak and resting heart rate. The chronotropic index, (peak heart rate – resting heart rate) / (220 – age – resting heart rate) (13), is derived by applying the chronotropic metabolic relationship concept described by Wilkoff and colleagues to a symptom limited exercise test. This allows definition of the normal chronotropic response independently of age, resting heart rate and functional state (72). In a group of 410 healthy adults Wilkoff et al. (72) reported 95% limits of normality of chronotropic index to be 0.8 – 1.3. Based on this finding, chronotropic incompetence was defined for the purpose of this study as failure to achieve a chronotropic index of 0.8 (i.e. falling below 97.5% of healthy adults).

Oxygen uptake kinetics

In a subgroup of patients (n=43), who underwent exercise testing more recently, raw heart rate and gas exchange measurement data were available and were analysed prospectively using custom-written computer software (MatLab 6.5, Mathworks, Cambridge, UK). Oxygen pulse (O_2pulse) was calculated as the instantaneous ratio of oxygen consumption (VO_2) and heart rate (HR), representing the product between stroke volume and arterio-venous oxygen difference (c(a-v)O_2) as shown in *Equation 2.1* Therefore, any change in oxygen pulse is due to a change in one or both of these variables:

$$O_2\text{pulse} = VO_2 / \text{heart rate} = SV \cdot C(a\text{-}v)O_2 \quad \textit{Equation 2.1}$$

$C(a\text{-}v)O_2$ = oxygen content difference between arterial and mixed central venous blood. SV = stroke volume.

In healthy individuals, oxygen consumption increases linearly in proportion to heart rate (72). Departure from linearity indicates the presence of a pathological condition and the nature of the departure provides information on the factors limiting exercise tolerance. Thus, linearity between heart rate and oxygen consumption was assessed using polynomial regression analysis. A quadratic equation (heart rate = $a \cdot VO_2^2 + b \cdot VO_2 + c$) was fitted to the second half of the exercise test. The quadratic coefficient *a* provides information about how far the regression line departs from linearity. Graphs are used to illustrate the relationship between heart rate and oxygen consumption during exercise in individual subjects. In addition, isopleths (lines of equal value) of oxygen pulse are superimposed to the graph to show changes in oxygen pulse during exercise.

Statistical analysis

All values are presented as mean ± standard deviation. Comparisons between groups were made using Student's t test, Mann-Whitney U test or Chi-squared test as appropriate.

Influence variables were studied on univariate analysis. Significant parameters were subsequently included into a multivariate regression model in a stepwise forward procedure. For all analyses a *P*-value <0.05 was considered significant. Areas under curve (AUC) for sensitivity and specificity

were calculated using receiver-operating characteristics (ROC) to compare the predictive accuracy of different parameters. Statistical analyses were performed using MedCalc for Windows, version 7.5 (MedCalc Software, Mariakerke, Belgium).

2.2.2. Results

Patient characteristics

Sixty-four adult congenital heart disease patients (mean age 28.1±11.3 years, 37 male) with a systemic right ventricle or univentricular circulation were included. There were 12 patients with congenitally corrected transposition of the great arteries (ccTGA; 2 without associated lesions), 20 patients after Mustard-type atrial switch operation for transposition of the great arteries (TGA), and 32 patients with univentricular circulation (24 with atriopulmonary-Fontan, 4 with total cavopulmonary connection, 1 with classical Glenn, and 3 with bidirectional Glenn anastomosis).

Patient baseline characteristics including parameters of chronotropic incompetence, peak oxygen consumption, presence of sinus rhythm and use of anti-arrhythmic drugs are listed in Table 2.1. There was no significant difference in age, gender, presence of cyanosis, NYHA functional class or anti-arrhythmic drug therapy between patients with and without chronotropic incompetence. Patients with chronotropic incompetence had lower values of chronotropic index, peak heart rate, heart rate reserve and percentage age predicted heart rate than those without chronotropic incompetence (Table 2.1).

Exercise capacity and ventilatory efficiency

Peak oxygen consumption was significantly reduced in patients compared with healthy control subjects (21.2±6.2 versus 39.4±3.4 ml/kg/min, *P*=0.04) as illustrated in Figure 2.1. Indeed, none of the patients included in the current study had normal peak oxygen consumption and only 5 patients had a peak oxygen consumption between 90 and 100% of the predicted value. Peak oxygen consumption in patients with ccTGA, univentricular circulation and Mustard operation was 20.0±7.1, 20.3±5.6, and 23.3±6.4 ml/kg/min, respectively.

Ventilatory inefficiency (VE/VCO_2 slope) was greater in the patients than the controls (41.6±17.3 versus 26.4±5.4, *P*=0.006) and was highest in those who were cyanotic (52.3±21.3).

Relationship between chronotropic incompetence, symptoms and exercise capacity

There was a non-significant trend towards a higher NYHA class in patients with chronotropic incompetence (49% NYHA class I, 38% NYHA class II, 13% NYHA class III) compared with patients without chronotropic incompetence (56% NYHA class I, 44% NYHA class II, 0% NYHA class III, *P*=NS). While none of the patients with chronotropic competence were in NYHA class III, 5 patients with chronotropic incompetence reported severe exercise limitation. In contrast to subjective exercise tolerance, clear evidence of an association between impaired objective exercise capacity and chronotropic incompetence was found.

Patients with chronotropic incompetence had lower peak oxygen consumption (19.8 ± 5.5 versus 24.6 ± 6.8 ml/kg/min, P=0.005) as shown in Figure 2.1. Heart rate reserve (r=0.43, P<0.001) and peak heart rate (r=0.42, P<0.001) correlated with peak oxygen consumption. These results suggest that peak heart rate accounted for 18% (r^2=18%) of the variation in peak oxygen consumption. On multivariate regression analysis, functional class (r=-0.34, χ^2=-3.1, P=0.003), peak heart rate (r=0.30, χ^2=2.9, P=0.006) and the presence of cyanosis (r=-0.27, χ^2=-2.6, P=0.01) were found to be independent predictors of peak oxygen consumption.

Oxygen pulse and oxygen uptake – heart rate relationship

Oxygen pulse at peak exercise was significantly lower in patients with ccTGA (10.3±3.5 ml/beat), Mustard operation (10.5±3.7 ml/beat) or univentricular circulation (8.4±2.4 ml/beat) compared with healthy individuals (15.3±3.5 ml/beat, P<0.001 for each). In contrast, there was no significant difference in oxygen pulse between patients with chronotropic incompetence (9.2±2.9 ml/beat) and those without (9.9±3.8 ml/beat, P=0.44).

Oxygen pulse kinetics during exercise was assessed visually and by calculation of quadratic coefficients to determine the degree of non-linearity between VO_2 and heart rate. On visual assessment 8 of 43 patients (19%) were found to have kinetics compatible with "functional" chronotropic incompetence (i.e. an inappropriate increase in heart rate compared with VO_2) as illustrated in Figure 2.2D. In contrast, 13 of 43 patients (30%) had kinetics suggestive of exercise limitation due to an inappropriate oxygen

pulse, either due to an inappropriate stroke volume (e.g. due to ventricular failure) and/or arterio-venous oxygen difference (usually due to right-to-left shunting and arterial desaturation) as illustrated in Figure 2.2B and 2.2C. Visually, the remaining patients (51%) had a linear relationship between VO_2 and heart rate, thus, providing no further information on the aetiology of exercise limitation (Figure 2.2A).

A good agreement between calculated quadratic coefficients and visual assessment of oxygen pulse kinetics was found (AUC on ROC analysis 0.96 [95% confidence interval 0.84 – 0.99]). On ROC analysis a quadratic coefficient below –46 was identified as the optimal cut-off value discriminating between patients with and without oxygen pulse kinetics suggestive of chronotropic incompetence. A quadratic coefficient above 52 ml/beat was associated with an oxygen pulse pattern suggestive of an inappropriate stroke volume and/or arterio-venous oxygen-content-difference as illustrated in Figure 2.3 (AUC 0.97 [95% CI 0.86-0.99]), sensitivity 100%, specificity 93%).

Patients with a linear relationship between heart rate and peak VO_2 had a significantly higher exercise capacity compared with those with a non linear response ("linear relationship" 24.6±6.1 versus 20.0±5.5 and 17.9±3.4 ml/kg/min for inappropriate heart rate and inappropriate oxygen pulse, respectively, ANOVA P=0.003). A similar result was found when patients were stratified by a quadratic coefficient below –50, between -50 and 50, and above 50, respectively (ANOVA P=0.001).

The respiratory exchange ratio at peak exercise was not significantly different between patients with and without a linear oxygen pulse response and indeed was highest in patients with a linear response (1.07±0.09). This suggests that a linear response is not due to poor exertional effort by patients.

2.3. Impact of optimized rate responsive pacing on exercise capacity in adults with a systemic right ventricle

To further elucidate the association between heart rate response to exercise and peak oxygen consumption and to obtain further information on the factors limiting exercise tolerance in this setting 9 patients with systemic right ventricle (SRV) and pre-existing pacemakers were studied prospectively using Doppler echocardiography and treadmill exercise testing. This sub-study investigated whether an artificial heart rate increase by reprogramming a pre-existing pacemaker during exercise leads to an improved exercise capacity. Furthermore, to assess the impact of heart rate increase on the physiology and filling of the systemic right ventricle, echocardiography was performed at rest and at higher heart rates.

2.3.1. Methods

This was a prospective exercise and echocardiographic study. From the institutional database of the Adult Congenital Heart Disease Unit, Royal Brompton Hospital, London all patients with a systemic right ventricle (n=180; congenitally corrected transposition of the great arteries (n=81), transposition

of the great arteries after Mustard (n=88) or Senning repair (n=11)) were identified. Of these, 19 had a permanent pacemaker implanted and were considered candidates for the study. Three patients could not be contacted as they had moved abroad. The remaining 16 patients were contacted and invited to take part in the study. Unfortunately, 5 refused to participate and 2 were unable to perform an exercise test due to severe exercise limitation. Therefore, 9 patients with a systemic right ventricle could be examined. Control subjects matched for age without a history of heart disease were also studied with treadmill exercise testing. This sub-study was approved by the local ethics committee and all subjects gave written informed consent for this study.

Cardiopulmonary Exercise Testing

In patients with a systemic right ventricle two symptom limited cardiopulmonary exercise tests on a treadmill were performed on a single day. Recovery time between the tests was at least 3 hours. One test was carried out with the patient's pre-existing pacemaker settings. In those patients fitted with a rate responsive pacemaker, rate responsiveness was not switched off for the baseline test. The other test was carried out while the pacemaker was actively reprogrammed to simulate a normal heart rate response to exercise. Tests were performed in a randomized order with the patients blinded to the actual test protocol. Healthy volunteers underwent a single exercise test using to the same treadmill protocol.

All tests were performed according to a ramp protocol that uses a linear increase in walking speed coupled with a curvilinear increase in treadmill grade yielding a linear increase in work rate (75).

The treadmill exercise protocol was individually adjusted for each patient by predicting peak work rate according to patient's height, age and gender. Peak work rate was calculated according to these equations (76):

Males:

WR_{peak} [Watts] = 1506 x Height(m)2.70 x Age(yrs)-0.46 / 6.12 *Equation 2.2*

Females:

WR_{peak} [Watts] = 969 x Height(m)2.80 x Age(yrs)-0.43 / 6.12 *Equation 2.3*

WR_{peak}= predicted peak work rate in Watts

Subsequently, the treadmill velocity range was determined. Starting speed was set at 0.5 mph; maximum speed after 10 minutes of exercise was set at 3.5-4 mph to allow the subject to walk comfortably during the entire test period and to avoid change in gait pattern leading to a steeper rise of metabolic rate per calculated work rate than established over a slower speed range. Changes in work rate and speed were spread linearly over 20 stages to reach the target work rate after 10 minutes of exercise.

The inclination of the treadmill was calculated based on the subjects body weight, the desired initial and 10 minute speeds, the initial grade and the predicted 10 minute work rate according to the formula provided by Porszasz (75):

$$Grade(t) = \frac{\left[\left[\left(WR_{peak} / m \times g \times V_0\right) - grade_0\right] \times t + 10 \times grade_0\right]}{\left[\left[\left(V_{max} / V_0\right) - 1\right] \times t + 10\right]} \quad \text{Equation 2.4}$$

WR_{peak}= predicted peak work rate in Watts, m=body mass in kilograms, g=gravitational acceleration (9.81m/s^2), V_0 and V_{max}=desired initial and final treadmill speeds, grade0=initial treadmill grade

This treadmill ramp protocol was chosen as it provides a near linear increase of work rate and allows prediction of a normal heart rate response as heart rate relates in a predictable linear fashion to work rate. The pacemaker was programmed to simulate a normal heart rate response according to the data provided by Kindermann et al. based on the relationship between work rate and heart rate response (77).

All subjects were encouraged to exercise to exhaustion regardless of the maximal heart rate achieved. Ventilation, oxygen uptake and carbon dioxide production were measured continuously using a respiratory mass spectrometer (Amis 2000, Innovision, Odense, Denmark). Heart rate was recorded by continuous electrocardiography and arterial blood pressure was recorded manually by sphygmomanometer.

Resting heart rate measured after 30 seconds in a seated position and maximal heart rate achieved during exercise testing were recorded. Exercise duration, peak oxygen consumption, ventilatory response to exercise expressed as ventilation per unit of carbon dioxide production (VE/VCO_2 slope), arterial oxygen saturation at rest and at maximum exercise where also recorded.

Echocardiography

Transthoracic echocardiography was performed in all patients with SRV using a standardized transthoracic approach with a Philips Sonos 7500 echocardiograph interfaced with a multifrequency MHz transducer. Transtricuspid (systemic atrioventricular valve) pulsed wave Doppler recordings were obtained from the apical 4-chamber view and aortic pulsed wave Doppler recordings were acquired with anterior angulation of the transducer with the sample volume at the tips of the respective valve leaflets (78). All recordings were made simultaneously with ECG and phonocardiogram and were stored digitally for offline analysis using Medcon software (Medcon Telemedicine Technology, Whippany, NJ).

The timing of the systemic right ventricular cardiac cycle was measured from aortic and tricuspid valve pulsed wave Doppler recordings at resting heart rate (68±8 bpm) as well as 106±10 bpm and 140±9 bpm. Systemic ventricular ejection time (ET) was measured as the interval from the onset to the end of forward flow across the aortic valve. Filling time (FT) of the systemic ventricle was measured from the transtricuspid flow recording as the interval from the onset of the E wave to the end of the A wave. Time intervals were multiplied by heart rate and expressed as seconds per minute as these values are independent of the heart rate. Total isovolumic time (t-IVT), the time of the cardiac cycle were the ventricle is neither filling nor ejecting, also expressed in seconds per minute was calculated as: 60-(t-ET + t-FT), where t-ET and t-FT represent total ejection and filling time expressed in seconds per minute. The aortic velocity time integral (VTI) was calculated from the aortic valve Doppler tracings.

To assess ventricular long axis function M-mode and tissue Doppler recordings of the lateral wall of the systemic (right) ventricle, the ventricular septum, and the free wall of the subpulmonary (left) ventricle were obtained from the apical four chamber view with the sample volume positioned at the right and septal angles of the mitral valve ring and the left angle of the tricuspid valve ring as described previously (78,79). The maximal diastolic inlet width of the systemic right ventricle has been reported a good correlate of end diastolic ventricular volume from cardiovascular magnetic resonance imaging and was, therefore, obtained from the apical four chamber view to quantify systemic right ventricular size (79). A qualitative, subjective assessment of systemic right ventricular size and function from multiview 2-dimensional echocardiography was also employed as previously described (80). Systolic ventricular function was graded as normal, mildly, moderately, or severely impaired. Ventricular size was graded similarly as normal, mildly, moderately, or severely enlarged. A corresponding numerical grading from 1 to 4 was for calculations.

Statistical Analysis

For all measured variables, values are expressed as mean ± SD or median and range. Comparisons between groups were made using Mann-Whitney U test (unpaired samples), Wilcoxon test (paired samples) and Chi-squared test as appropriate. Statistical analysis was performed using MedCalc for Windows, version 9.2.0.0 (MedCalc Software, Mariakerke, Belgium).

2.3.2. Results

Patient characteristics

There was no significant difference between patients (n=9) and controls (n=8) regarding age (36.4±9.4 versus 32.7±5.0, *P*=0.37) and gender distribution (*P* =0.74). Individual patients' characteristics are outlined in Table 2.2.

Exercise capacity and heart rate response to exercise

Complete exercise data with two tests at different pacemaker settings could be obtained in 7 of 9 patients. One patient refused repeat testing after an uneventful first test without explanation. A second patient developed symptomatic atrial tachycardia after two minutes of the initial test and therefore no repeat testing was performed.

Peak heart rate, exercise duration and peak oxygen consumption were significantly reduced and VE/VCO_2 slope was abnormally elevated in patients with SRV compared to controls (Table 2.3).

Pacemaker reprogramming resulted in an increase of peak heart during exercise from 135±32 to 149±22 beats per minute. However, due to large inter-individual variability and the development of atrial tachyarrhythmia during the exercise test with the pre-existing pacemaker settings in one patient this increase was not statistically significant. Overall, active reprogramming did not result in an increase of peak VO_2 (12.6±6.8 versus 12.4±4.9 ml/kg/min, *P*=0.44). In fact, in none of the patients a clinically relevant improvement of peak VO_2 was achieved with active pacemaker reprogramming (Figure 2.4). Interestingly, even in the patient with the largest

improvement of heart rate response (82 to 162 bpm), peak VO_2 decreased from 10.7 to 8.9 ml/kg/min. While in all control subjects VO_2 increased linearly with heart rate, a markedly non-linear VO_2 to heart relationship was observed in 6 of 8 patients as illustrated in Figure 2.5.

Physiology of systemic right ventricular cardiac cycle and heart rate

The timing of the systemic right ventricular cardiac cycle was markedly altered by increase in heart as a result of pacemaker reprogramming. There was a significant increase in t-IVT accompanied by a marked reduction in t-FT and aortic VTI with increasing heart rate (Figure 2.6 and 2.7). In fact, these two parameters decreased in all patients studied. In addition, t-ET increased significantly from baseline to maximum heart rate (18.3±2.3 versus 21.9±2.3, P=0.008 at 68±8 and 140±9 bpm, respectively) whereas peak aortic velocity remained unchanged (82.7±16.1 versus 76.8±16.1 cm/s, P=0.55 at 68±8 and 140±9 bpm, respectively).

2.4. Prognostic value of chronotropic incompetence in ACHD patients

The aims of this study were to evaluate the prevalence of an abnormal heart rate response to exercise (chronotropic incompetence) in ACHD patients, to assess the relationship between heart rate response and exercise capacity, and to evaluate whether chronotropic incompetence is a prognostic marker in ACHD patients after accounting for exercise capacity and use of anti-arrhythmic medication.

2.4.1. Methods

Study population

Data from all cardiopulmonary exercise tests performed in ACHD patients at the Adult Congenital Heart Unit, Royal Brompton Hospital London between February 1999 and April 2005 were analysed retrospectively. Patients were referred for exercise testing as part of clinical follow-up for ACHD patients. This study was approved by the local ethics committee. Almost all patients underwent only one test during the study period. Any repeat test was not included in the analysis. A main diagnosis was determined for every patient from the hospital records. Patients with multiple, complex cardiac lesions substantially affecting haemodynamic function were classified as complex anatomy (comprising mainly patients with single ventricle physiology). The New York Heart Association (NYHA) functional class was determined by physician assessment of patients' self-reported symptoms before the date of the exercise test. Anti arrhythmic drug use was recorded at the time of exercise testing.

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed on a treadmill according to a modified Bruce protocol (73,74) with the addition of a "stage 0" in which the patient walks at a velocity of 1 mile/hour and a gradient of 5% for 3 minutes. All subjects were encouraged to exercise to exhaustion regardless of the maximal heart rate achieved. Ventilation, oxygen uptake and carbon dioxide production were measured continuously using a respiratory mass spectrometer (Amis 2000, Innovision, Odense, Denmark) as described previously (81). Heart rate was assessed by continuous electrocardiography and arterial blood pressure was recorded manually by sphygmomanometry. Resting heart rate was measured after at least 30 seconds in a seated position and peak heart rate was defined as the maximal heart rate achieved during exercise. Predicted maximum heart rate was estimated according to the formula 220-age (82), and percentage of maximum age-predicted heart rate was calculated as the ratio between peak heart rate and age-predicted maximum heart rate 220-age.

Calculation of heart rate reserve

Heart rate reserve was calculated as the difference between peak and resting heart rate. The chronotropic index, (peak heart rate – resting heart rate) / (220 – age – resting heart rate), is derived by applying the chronotropic metabolic relationship concept introduced by Wilkoff to a symptom limited exercise test as described previously (13,72). This allows definition of the normal chronotropic response independently of age, resting heart rate and functional state (72). In a group of 410 healthy adults Wilkoff

et al. reported 95% limits of normality of chronotropic index to be 0.8 – 1.3. Based on this finding, we defined chronotropic incompetence as failure to achieve a chronotropic index of 0.8 (i.e. falling below 97.5% of healthy adults) (72).

Calculation of heart rate recovery

Heart rate was also recorded 1, 2, 3 and 5 minutes after the cessation of exercise and heart rate recovery calculated as the difference between peak heart rate and the heart rate at these recovery time points. In addition, the relative decrement in heart rate was calculated as heart rate recovery divided by the heart rate at peak exercise.

Follow-up

Follow-up was complete for all patients. Survival status and time to death was assessed through the health service computer system, which is linked to a national database held by the Office of National Statistics. The study was planned to use all-cause mortality as the end-point in order to eliminate any possibility of bias arising from incorrect classification of cause of death.

Statistical analysis

All values are presented as mean ± standard deviation. Comparisons between groups were made using Student's t test, Mann-Whitney U test or Chi-squared test as appropriate. Variables were assessed on univariate analysis. Significant parameters were subsequently included into a multivariate regression model in a stepwise forward procedure. Univariate

Cox proportional-hazards analysis was used to assess the association between variables and the endpoint of all cause mortality. Parameters significantly predicting prognosis in univariate analysis were subsequently tested in a multivariate Cox proportional-hazards analysis by the stepwise forward method to assess the independent effect of these variables. Areas under curve (AUC) for sensitivity and specificity were calculated using receiver-operating characteristics (ROC) analysis to compare prognostic accuracy of different parameters. Statistical analyses were performed using the StatView 5.0 package (Abacus Concepts, Berkeley, CA, U.S.A.).

2.4.2. Results

Patient characteristics

Characteristics of the 727 consecutive ACHD patients (mean age 33 ±13 years, 52% male) included in this analysis are presented in Tables 2.4 and 2.5. Patients with chronotropic incompetence were more likely to be cyanotic, female or treated with anti-arrhythmic drugs. In addition, by definition, such patients had lower values of peak heart rate and heart rate reserve as shown in Table 2.4.

Prevalence of chronotropic incompetence

Chronotropic incompetence was present in 62% of the patients. The prevalence was highest in patients after Fontan palliation (84%) and in patients with Eisenmenger physiology (90%), while it was lowest in patients with repaired tetralogy of Fallot (52%), repaired ventricular septal defect (52%) and isolated valvar disease (47%) as shown in Table 2.5. On

considering only those patients who reached anaerobic threshold (i.e. those with a respiratory quotient above 1.0) the frequency of chronotropic incompetence was found to be 54% overall. Once again it was most frequent in the Eisenmenger patients (96%) and least frequent in patients with repaired ventricular septal defects (35%).

Relationship to symptoms and exercise capacity

Patients with chronotropic incompetence were more likely to be in a higher NYHA class (34% NYHA class I, 47% NYHA class II, 19% NYHA class III) compared to the other patients (64% NYHA class I, 30% NYHA class II, 6% NYHA class III, $P<0.0001$). Patients with chronotropic incompetence also had lower peak oxygen consumption (20.4±8.2 vs. 28.0±9.9 ml/kg/min, $P<0.0001$), and shorter exercise duration (541±196 vs. 732±196 seconds, $P<0.0001$). In addition, heart rate reserve ($r=0.53$, $P<0.0001$) and peak heart rate ($r=0.49$, $P<0.0001$) correlated with peak oxygen consumption.

Prognostic value of parameters of chronotropic incompetence

During a median follow-up of 851 days after cardiopulmonary exercise testing (range 60 - 2254 days) 38 patients died. Patients who died had the following diagnoses: Fontan physiology (n=7), complex anatomy (n=10), congenitally corrected transposition of the great arteries (n=2), atrial switch procedure for transposition of the great arteries (n=1), tetralogy of Fallot (n=5), isolated valvar disease (n=2), single ventricle physiology (n=1), aortic coarctation (n=1), Ebstein anomaly (n=3), repaired atrial (n=1) and atrio-ventricular (n=2) septal defects and others (n=2).

On univariate analysis heart rate reserve ($\chi 2 = 26.1$), chronotropic index ($\chi 2 = 26.9$), peak heart rate ($\chi 2 = 20.9$), percentage predicted heart rate ($\chi 2 = 21.0$), use of anti-arrhythmic drug therapy ($\chi 2 = 24.0$), peak oxygen consumption ($\chi 2 = 19.3$) and NYHA class ($\chi 2 = 17.2$) were significant predictors of survival. In addition amiodarone ($\chi 2 = 30.1$) or digoxin ($\chi 2 = 6.0$) use was related to survival as shown in Table 2.6. Age, gender, cyanosis and treatment with sotalol, beta-blocker, calcium-antagonist, class I anti-arrhythmic drugs or ACE-inhibitors were not related to survival.

Measures of chronotropic response correlated strongly with each other (r-value between 0.81 and 0.96, $P<0.001$ for each). Therefore, for multivariate analysis the parameter with the highest predictive value on univariate Cox proportional-hazard analysis and the greatest area under curve on ROC analysis was chosen. In both analyses heart rate reserve ($\chi 2 = 26.1$, AUC = 0.74) and chronotropic index ($\chi 2 = 26.9$, AUC = 0.74) were superior to peak heart rate ($\chi 2 = 20.9$, AUC = 0.72) and percentage age predicted peak heart rate ($\chi 2 = 21.0$, AUC = 0.72) in predicting prognosis. As a consequence heart rate reserve, representing a much simpler parameter than chronotropic index was used in subsequent analyses.

To investigate the predictive power of heart rate reserve independently of other prognostic variables, bivariate analyses were performed. Heart rate reserve predicted mortality independently of peak oxygen consumption, anti-arrhythmic therapy and NYHA class (Table 2.7). On multivariate analysis,

heart rate reserve, NYHA class and therapy with anti-arrhythmic drugs jointly predicted mortality, independent of peak oxygen consumption, as shown in Table 2.7. These results remained unchanged when patients who did not reach the anaerobic threshold during exercise (i.e. those with a respiratory quotient below 1.0) were excluded from the analyses.

Prognostic value of heart rate reserve in individual diagnostic groups

Heart rate reserve predicted mortality in patients after Fontan palliation ($\chi 2$ = 7.5, hazard ratio=1.48 per 10 bpm; 95% CI 1.10-2.16, P<0.05), complex anatomy ($\chi 2$ = 3.8, hazard ratio=1.21 per 10 bpm; 95% CI 1.10-1.48, P<0.05) and repaired tetralogy of Fallot ($\chi 2$ = 6.3, hazard ratio=1.48 per 10 bpm; 95% CI 1.10-2.16, P<0.05) on univariate analysis. No significant association between low heart rate reserve and mortality was found in patients with simple lesions, systemic right ventricles or Ebstein anomaly of the tricuspid valve.

Comparative and combined prognostic value of heart rate reserve and peak oxygen consumption

Heart rate reserve was at least as good as peak oxygen consumption in predicting mortality, both on univariate Cox analysis ($\chi 2$ = 26.1 vs. 19.3) and on ROC analysis (AUC 0.74 vs. 0.68). Combining these two variables was also helpful: patients with both heart rate reserve and peak oxygen consumption within the lowest quartile had the worst prognosis, patients with only one in the lowest quartile had an intermediate prognosis, and those with neither in the lowest quartile had the best prognosis (P<0.0001). Kaplan-

Meier-Estimates of death from any cause among adult congenital heart disease patients with stratified by quartiles of heart rate reserve are shown in Figure 2.8.

Prognostic value of heart rate recovery

Data on heart rate recovery was available in 505 patients (those patients who underwent exercise testing after March 2001). Of these, 16 patients died during follow-up. Heart rate recovery at 1, 2, 3 and 5 minutes was significantly lower in patients who died compared to surviving patients ($P<0.05$ for each) as was heart rate recovery expressed as percentage of peak heart rate (Figure 1.9). Heart rate recovery at 1, 2, 3 and 5 minutes after exercise was significantly related to mortality on univariate Cox proportional hazards analysis ($P<0.05$ for each). After adjustment for anti-arrhythmic drug therapy heart rate recovery at 1, 2, 3 and 5 minutes after exercise remained independently predictive of survival in bivariate Cox analysis ($P<0.05$ for each).

2.5. Tables

Characteristic	All patients (n=64)	Failed * (n=46)	Reached * (n=18)	*P Value*
Age (years)	28 ± 11	27 ± 8	30 ± 14	0.26
Gender (%male)	58 %	54 %	67%	0.41
NYHA I / II / III	51%/ 40%/ 9%	49%/ 38%/ 13%	56%/ 44%/ 0%	0.29
Cyanosis	22%	26%	11%	0.31
Sinus rhythm	90%	82%	94%	0.31
Class I-IV anti-arrhythmic drugs †	35%	39%	29%	0.75
Anti-arrhythmic drugs incl. digoxin	40%	39%	41%	0.89
Digoxin	4%	0%	12%	0.12
Amiodarone	23%	26%	18%	0.72
Sotalol	10%	13%	6%	0.64
Betablocker	4%	3%	6%	0.75
Heart rate reserve (bpm)	71 ± 27	61 ± 23	97 ± 19	<0.001
Chronotropic index	0.66 ± 0.32	0.52 ± 0.19	1.02 ± 0.33	<0.001
Peak VO_2 (ml/kg/min)	21.2 ± 6.2	19.8 ± 5.5	24.6 ± 6.8	0.005
% age-predicted heart rate	0.79 ± 0.18	0.71 ± 0.12	0.99 ± 0.13	<0.001
Peak pulse (bpm)	151 ± 32	137 ± 24	185 ± 22	<0.001
Resting pulse (bpm)	80 ± 14	77 ± 12	89 ± 15	0.001

P-values (unpaired t-test or χ^2 / Fisher's exact test) for comparison between patients achieving and patients failing to reach a chronotropic index of at least 0.8. NYHA = New York Heart Association class. bpm = beats per minute. Peak VO_2 = peak oxygen consumption.
† Classification of anti-arrhythmic drugs according to the Vaughan Williams classification.

Table 2.1: Selected baseline characteristics according to the ability to reach a chronotropic index of at least 0.8*. Plus-minus values are mean ± standard deviation

Sex (M/F)	Age (yrs)	Diagnosis	PM Indication	PM Mode at Baseline	UTR (bpm)	Notes
M	35	TGA, Mustard	sympt. Bradycardia	DDDR	140	
M	35	TGA, Mustard	sympt. Bradycardia	VVI	-	
F	27	TGA, Mustard	sympt. Bradycardia	DDIR	120	
M	50	ccTGA	sympt. Bradycardia	DDDR	150	AT 2nd test
F	50	ccTGA	complete heart block	VVIR	150	AT 1st test, no 2nd test
F	39	TGA, Mustard	sympt. Bradycardia	DDDR	120	
M	39	TGA, Mustard	complete heart block	DDDR	145	
F	23	TGA, Mustard	complete heart block	DDDR	130	refused 2nd test
M	30	TGA, Mustard	complete heart block	DDDR	140	

Diagnosis: TGA, transposition of the great arteries (=D-TGA); ccTGA, congenitaiiy corrected TGA. PM, pacemaker; bpm, beats per minute; AT, atrial tachycardia. UTR = upper tracking rate

Table 2.2: Baseline characteristics

	Patients with SRV	Controls	*P*
Exercise Duration, sec	494 ± 179	758 ± 125	0.005
Peak HR, bpm	135 ± 32	186 ± 11	0.0006
Peak VO_2, ml/kg/min	12.6 ± 6.8	31.4 ± 6.6	0.0006
VE/VCO_2 slope	57.4 ± 12.3	36.6 ± 7.7	0.002

Data from patients with systemic right ventricle (SRV) obtained from tests with pre-existing pacemaker settings; bpm, beats per minute; HR, heart rate; VO_2, oxygen consumption; VE, ventilation; VCO_2, carbon dioxide production.

Table 2.3: Parameters of cardiopulmonary exercise testing in SRV patients and control subjects.

Characteristic	All patients (n=727)	Failed * (n=453)	Reached * (n=274)	*P* Value
Age (years)	33 ± 13	33 ± 13	33 ± 13	0.87
Gender (%male)	52	47	61	0.001
Deaths	38	34	4	0.0004
NYHA I / II / III (%)	45 / 40 / 15	34 / 47 / 19	64 / 30 / 6	<0.0001
Cyanosis (%)	17	24	5	<0.0001
Sinus rhythm (%)	96	96	97	0.34
Class I-IV † anti-arrhythmic drugs (%)	28	39	10	<0.0001
Anti-arrhythmic drugs incl. digoxin (%)	26	37	7	<0.0001
Digoxin (%)	5	6	4	0.18
Amiodarone (%)	13	19	3	<0.0001
Sotalol (%)	4	5	2	0.02
ACE inhibitors (%)	19	21	16	0.14
Heart rate reserve (bpm)	71 ± 29	57 ± 24	95 ± 18	<0.0001
Chronotropic index	0.70 ± 0.28	0.54 ± 0.20	0.96 ± 0.20	<0.0001
Peak VO_2 (ml/kg/min)	23.3 ± 9.6	20.4 ± 8.2	28.0 ± 9.9	<0.0001
Percentage age-predicted heart rate	83 ± 16	74 ± 13	97 ± 8	<0.0001
Peak pulse (bpm)	154 ± 30	138 ± 26	181 ± 14	<0.0001
Resting pulse (bpm)	83 ± 14	82 ± 16	85 ± 15	0.004

P-values (t-test or Mann-Whitney U-test) for comparison between patients achieving and patients failing to reach a chronotropic index of at least 80%. ACE = angiotensin converting enzyme. NYHA = New York Heart Association class. bpm = beats per minute. Peak VO_2 = peak oxygen consumption.
* Plus-minus values are mean ± standard deviation
† Classification of anti-arrhythmic drugs according to Vaughan Williams.

Table 2.4: Selected baseline characteristics according to the ability to reach a chronotropic index of at least 0.8 *

	Low CI	HRR (bpm)	Peak Pulse (bpm)	peak VO_2 (ml/kg/min)	Sinus rhythm	AAD treatment
ASD (n=42)	60%	69±30	154±34	21·6±7·9	89%	32%
ccTGA (n=25)	68%	70±33	151±38	21·8±9·6	95%	43%
CoA (n=23)	59%	73±24	156±27	28·9±7·9	95%	30%
Complex (n=75)	81%	56±28	138±32	20·2±7·7	88%	35%
Ebstein (n=32)	53%	77±28	158±31	21·5±5·2	90%	35%
Eisenmenger (n=53)	90%	51±23	136±24	12·8±5·7	100%	40%
Fontan (n=58)	84%	59±27	140±33	20·9±6·1	96%	53%
Mustard (n=56)	58%	77±26	160±28	25·8±6·9	94%	29%
TOF (n=228)	52%	80±26	162±26	25·7±8·4	98%	19%
Valvar (n=78)	47%	76±31	159±29	26·9±12·8	97%	17%
VSD (n=25)	52%	70±27	157±26	22·2±7·1	100%	13%

ASD = atrial septal defect, ccTGA = congenitally corrected transposition of the great arteries (TGA=L-TGA), CoA = aortic coarctation, Complex = complex anatomy including mostly single ventricle physiology (excluding Fontan type patients), Mustard = patients after Mustard type atrial switch operation for TGA, TOF = tetralogy of Fallot, VSD = ventricular septal defect.
bpm = beats per minute, Low CI = percentage of patients with a low chronotropic index (<0·8), HRR = heart rate reserve (beats per minute), peak pulse (beats per minute), peak VO_2 = peak oxygen consumption (ml/kg/min), Sinus rhythm = percentage of patients in sinus rhythm at the time of the exercise testing, AAD treatment = percentage of patients treated with at least one anti-arrhythmic drug (including type I-IV or digoxin).
* Plus-minus values are mean ± standard deviation

Table 2.5: Distribution of parameters of chronotropic incompetence, peak oxygen consumption, presence of sinus rhythm and use of antiarrhythmic drugs by underlying anatomy

Variable	Hazard Ratio * (95% confidence interval)	*P* value
Parameters of chronotropic incompetence		
Heart rate reserve (bpm)	1.03 (1.02 – 1.04)	<0.0001
Chronotropic index	1.03 (1.02 – 1.04)	<0.0001
Peak heart rate (bpm)	1.02 (1.01 – 1.03)	<0.0001
Percentage predicted peak heart rate	1.04 (1.02 – 1.05)	<0.0001
Heart rate recovery 1 minute (bpm)	1.04 (1.01 – 1.06)	0.002
Heart rate recovery 2 minutes (bpm)	1.04 (1.02 – 1.06)	0.0002
Heart rate recovery 3 minutes (bpm)	1.03 (1.01 – 1.04)	0.001
Heart rate recovery 5 minutes (bpm)	1.03 (1.01 – 1.04)	0.002
Other univariate predictors		
Peak VO_2 (ml/kg/min)	0.90 (0.86 – 0.94)	<0.0001
NYHA class	2.7 (1.7 – 4.4)	<0.0001
Anti-arrhythmic therapy (excl. digoxin)	5.4 (2.7 – 11.0)	<0.0001
Anti-arrhythmic therapy (incl. digoxin)	3.0 (6.3 – 13.2)	<0.0001
Amiodarone therapy	3.4 (6.6 – 12.9)	<0.0001
Digoxin therapy	3.3 (1.3 – 8.4)	0.01

NYHA class = New York Heart Association class, peak VO_2 = peak oxygen consumption (ml/kg/min).
* Hazard ratios for heart rate reserve and peak oxygen consumption are per beat per minute and one ml/kg/min, respectively.

Table 2.6: Univariate predictors of mortality

Variable	Hazard Ratio * (95% confidence interval)	*P* value	χ^2 Value
Bivariate Analyses			
Heart rate reserve (bpm)	1.02 (1.01 – 1.03)	0.0005	12.0
Anti-arrhythmic therapy (incl. digoxin)	4.2 (1.9 – 9.0)	0.0003	13.4
Heart rate reserve (bpm)	1.02 (1.01 – 1.04)	0.0003	13.1
Anti-arrhythmic therapy (excl. digoxin)	3.5 (1.7 – 7.3)	0.0007	11.4
Heart rate reserve (bpm)	1.02 (1.01 – 1.04)	0.0001	14.5
Amiodarone therapy	4.5 (2.2 – 8.9)	<0.0001	18.0
Heart rate reserve (bpm)	1.02 (1.004 – 1.031)	0.01	6.7
Peak VO_2 (ml/kg/min)	0.94 (0.89 – 0.99)	0.02	5.4
Heart rate reserve (bpm)	1.02 (1.005 – 1.031)	0.008	7.0
NYHA class	2.1 (1.2 – 3.5)	0.006	7.7
Multivariate Analysis			
Heart rate reserve (bpm)	1.02 (1.001 – 1.031)	0.04	4.3
Anti-arrhythmic therapy (incl. digoxin)	3.7 (1.7 – 8.0)	0.0009	11.0
NYHA class	2.0 (1.2 – 3.4)	0.007	7.3
Peak VO_2 (ml/kg/min)	–	*NS*	-
Heart rate reserve (bpm)	0.98 (0.97 – 0.996)	0.01	6.3
Amiodarone therapy	4.7 (2.4 – 9.5)	<0.0001	19.1
NYHA class	2.1 (1.3 – 3.5)	*0.002*	9.4
Peak VO_2 (ml/kg/min))	–	*NS*	-

NYHA class = New York Heart Association class, peak VO_2 = peak oxygen consumption (ml/kg/min).
* Hazard ratios for heart rate reserve and peak oxygen consumption are per beat per minute and one ml/kg/min, respectively.

Table 2.7: Bivariate and multivariate predictors of mortality

2.6. Figures

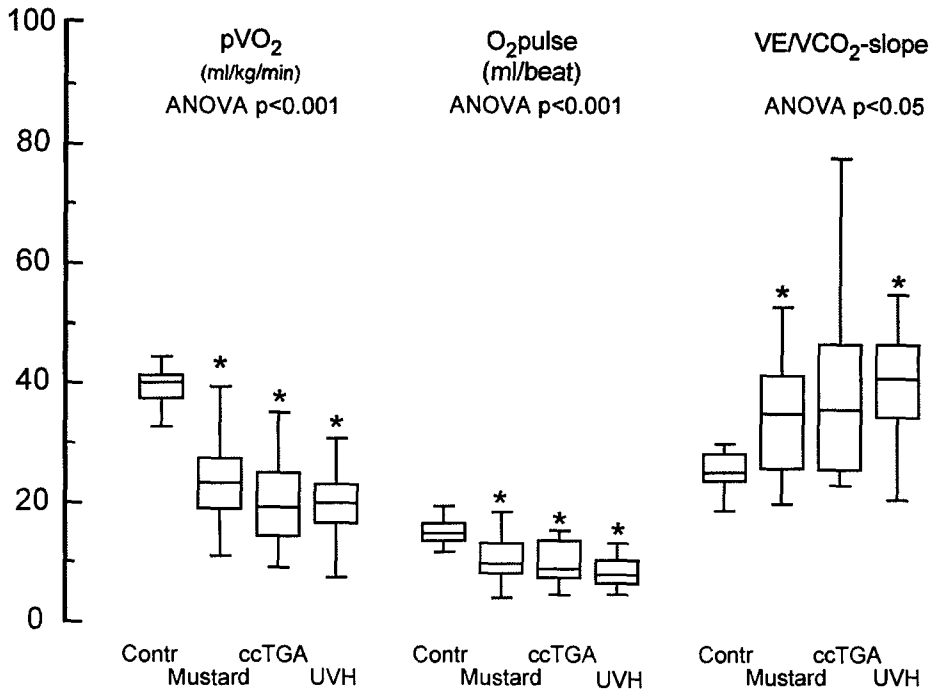


Figure 2.1: Comparison between peak oxygen consumption (pVO_2), oxygen pulse (O_2pulse) and ventilatory efficiency (VE/VCO_2-slope) between control subjects (Contr) and patients with transposition of the great arteries (TGA) after Mustard operation, patients with congenitally corrected TGA (ccTGA) and patients with univentricular heart (UVH). * $P<0.05$ compared with control subjects.

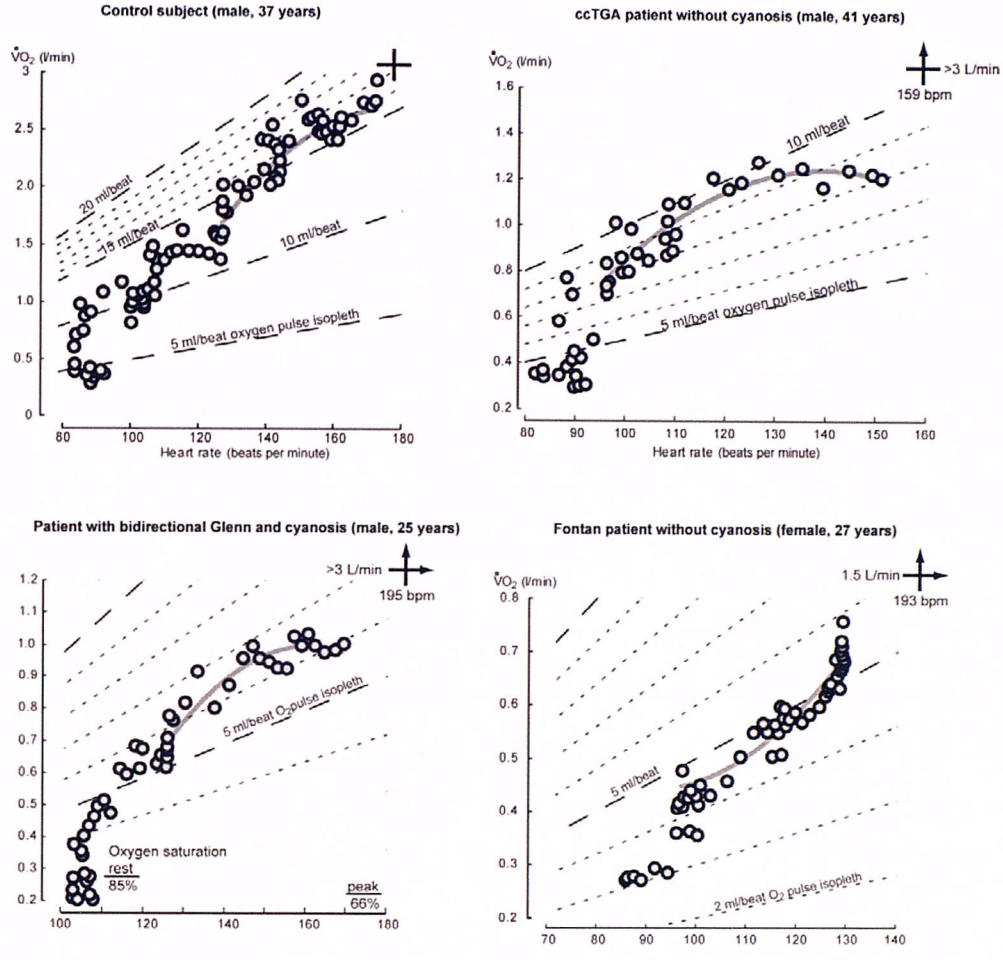


Figure 2.2: Examples of normal (A) and pathological (B-D) heart rate-oxygen uptake kinetics. The cross indicates predicted peak oxygen consumption (VO_2) and peak heart rate.

Panel A Normal (expected) linear relationship between heart rate and oxygen consumption in a healthy individual (Quadratic coefficient 7.8).
Panel B 41 year-old male patient with congenitally corrected transposition of the great arteries (ccTGA) and markedly abnormal heart rate-oxygen consumption kinetics. Despite increase in heart rate, oxygen pulse is decreasing above a heart rate of 125 bpm. In addition, ST depression on ECG was noted at this stage (Quadratic coefficient 155).
Panel C 25 year-old patient with bi-directional Glenn anastomosis. Resting saturations were 85% in room air. During exercise arterial oxygen saturations

dropped to 65%. This is reflected in an abnormal heart rate-oxygen consumption pattern, due to decreased arterio-venous oxygen difference during exercise (Quadratic coefficient 230).

Panel D 27-year-old female-patient with classical Fontan for tricuspid atresia treated with Amiodarone for paroxysmal atrial arrhythmia. The heart rate does not increase above 130 beats per minute, however there is evidence of an increase in oxygen pulse. This pattern suggests exercise limitation due to impaired heart rate response to exercise (Quadratic coefficient -224).

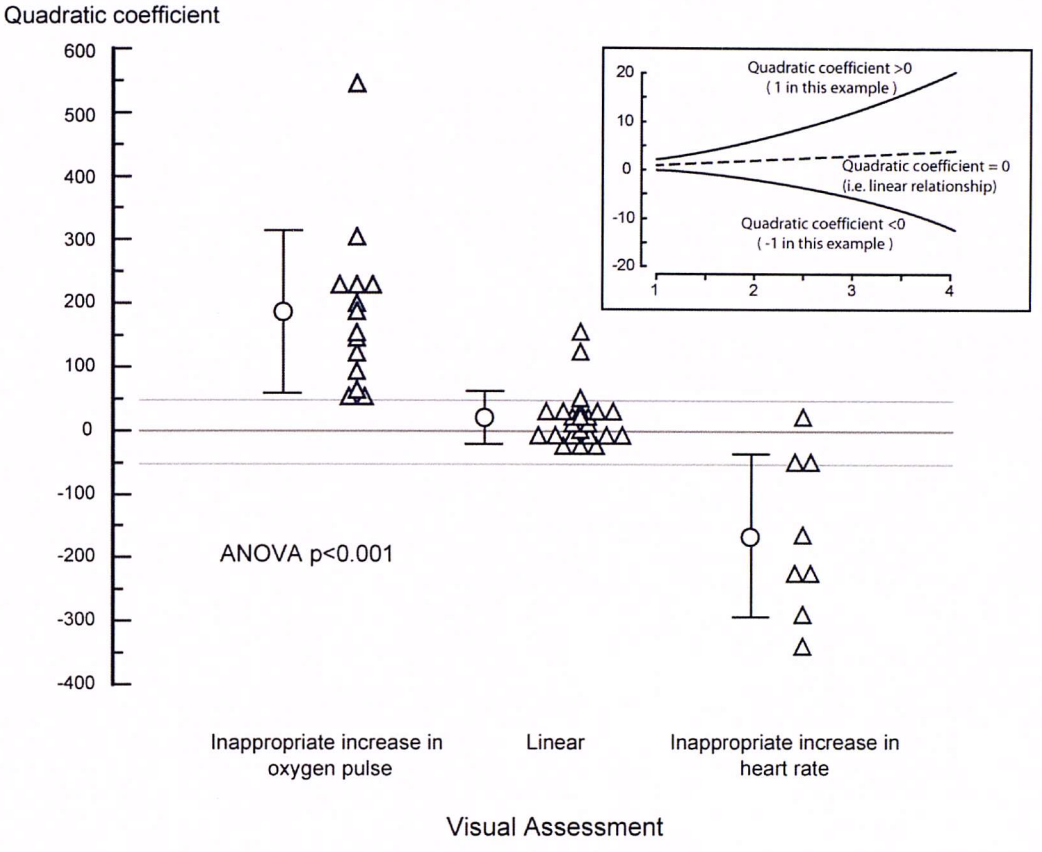


Figure 2.3: Relationship between visual assessment of heart rate-oxygen uptake kinetics and calculated quadratic coefficient. The insert illustrates that a quadratic coefficient of 0 is equivalent with a linear relationship, whereas a negative or positive quadratic coefficient describe the curvature of the graph.

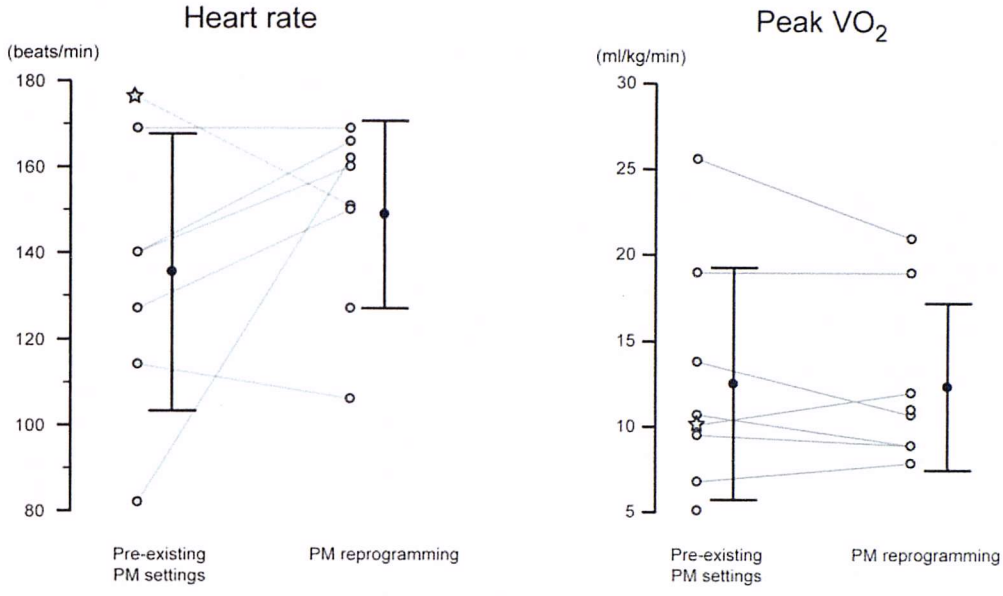


Figure 2.4: Heart rate and peak oxygen consumption (peak VO_2) with pre-existing pacemaker (PM) settings and after active reprogramming.
One patient (marked with an asterisk) developed atrial tachycardia at the test with pre-existing pacemaker settings.

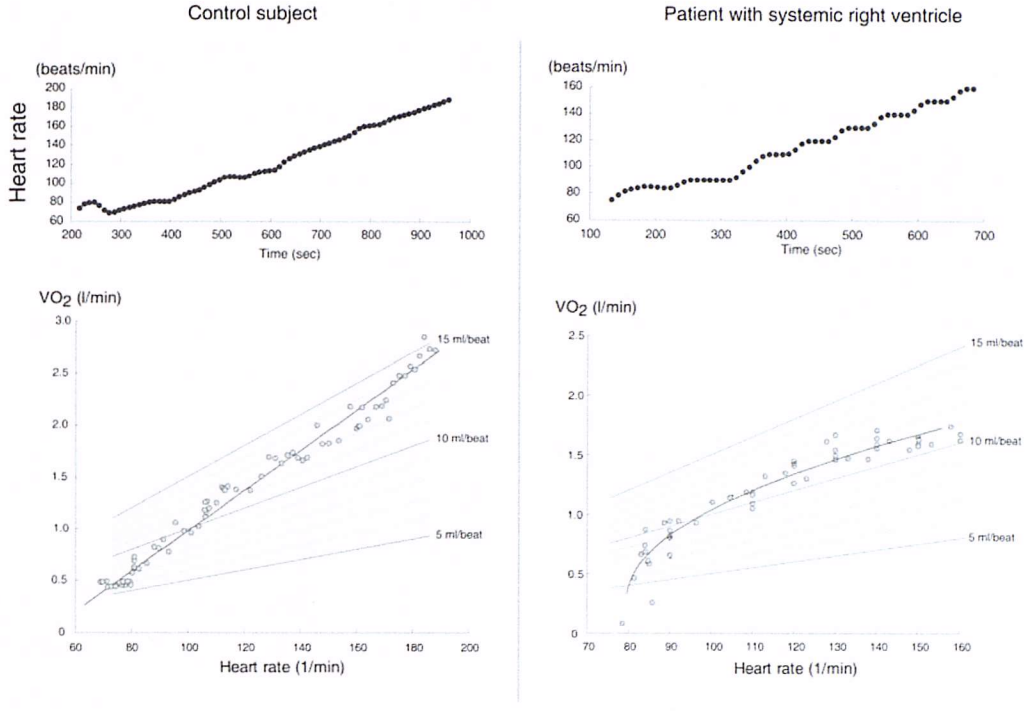


Figure 2.5: Heart rate and oxygen consumption (VO_2) versus heart rate plots (including oxygen pulse isopleths with corresponding oxygen pulse values given on the right) recorded from a healthy control subject (left panel) and a patient with systemic right ventricle (right panel). The plots illustrate the normal (expected) linear relationship between heart rate and oxygen consumption in the control subject and pathological heart rate-to-oxygen-uptake kinetics in the patient. Despite increase in heart rate the patient is unable to increase oxygen uptake above a heart rate of approximately 130 beats per minute resulting in a curvilinear heart rate-to-oxygen- uptake kinetics.

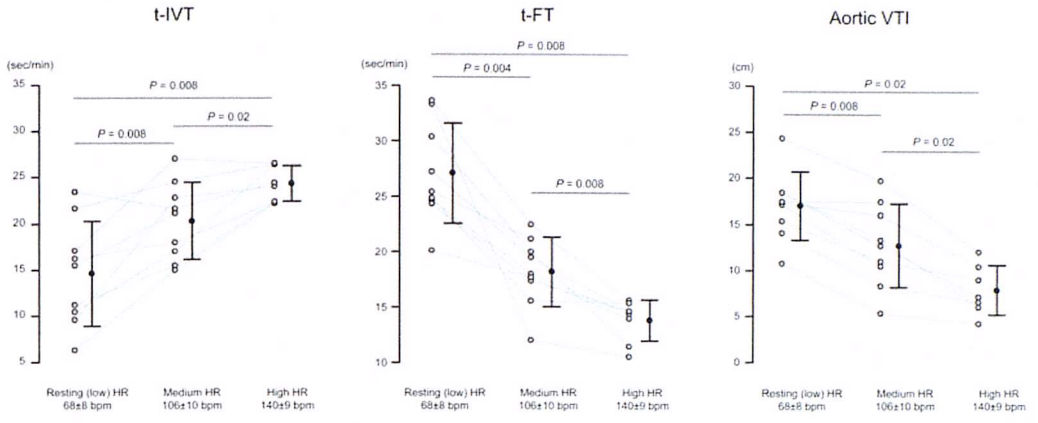


Figure 2.6: Comparison between systemic right ventricular total isovolumic time (t-IVT), total filling time (t-FT) and aortic velocity time integral (VTI) at 3 different heart rates (HR). Non-parametric Wilcoxon tests (paired samples) were used to compare data.

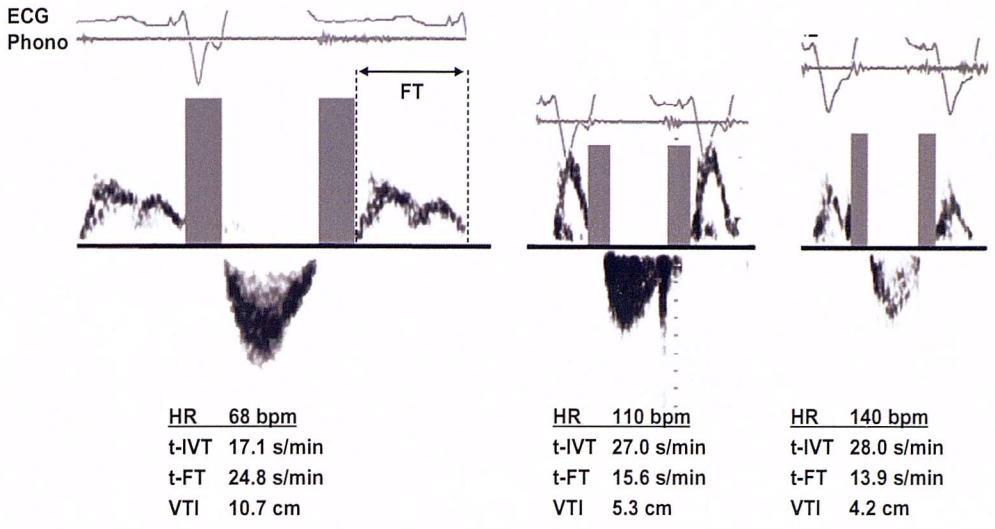
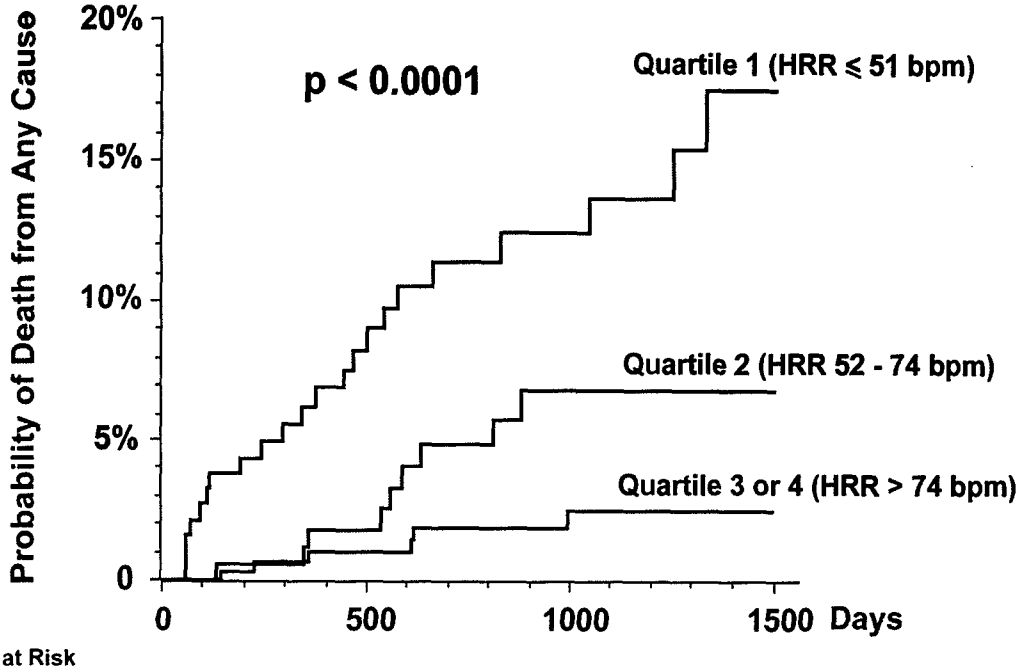


Figure 2.7: Superimposed aortic and tricuspid Doppler recordings from a patient with a systemic right ventricle at 3 different heart rates. The 2 components of total isovolumic time (t-IVT) are shaded. ECG indicates electrocardiogram, Phono, phonocardiogram; t-FT, total filling time, VTI, aortic velocity time integral.



No. at Risk				
Quartile 1	186	124	72	33
Quartile 2	187	137	83	40
Quartile 3 or 4	354	259	154	71

Cumulative Mortality	1 year	2 years	5 years
Quartile 1	6.2 % (2.6 - 9.7 %)	11.4 % (6.3 - 16.4 %)	17.5% (9.9 - 25.2 %)
Quartile 2	1.8 % (0 - 3.8 %)	4.9 % (1.3 - 8.4 %)	10.1 % (2.4 - 17.8%)
Quartile 3 or 4	1.0 % (0 - 2.0 %)	1.8 % (0.2 - 3.4 %)	2.4 % (0.2 - 3.4 %)

Figure 2.8: Kaplan-Meier-Estimates of death from any cause among adult congenital heart disease patients stratified by quartiles of heart rate reserve.

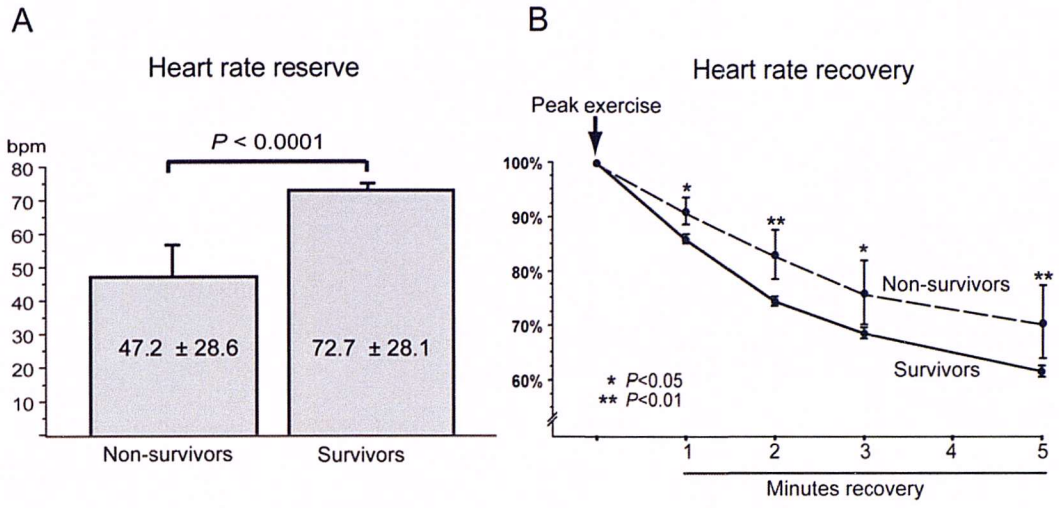


Figure 2.9: **A)** Increase in heart rate during exercise – heart rate reserve, and **B)** decrease in heart rate at the end of exercise – heart rate recovery – in surviving and non-surviving patients. Error bars indicate 95% confidence intervals.

2.7. Discussion

Given that increase in heart rate during exercise mirrors increase in cardiac output, heart rate at maximal exertion has received considerable interest. Astrand and Rhyming (83) described a close correlation between heart rate during submaximal work and maximal oxygen uptake. Other authors have expanded upon this work providing a number of formulas to predict maximal heart rate during exercise (16,17,84,85). The term chronotropic incompetence - implying that the heart's response to exercise is not physiological - was introduced in 1975 by Ellestad and colleagues (86). The authors analysed follow-up results of 2700 patients who had undergone treadmill testing. They found that those with a blunted heart rate response to exercise were at greater risk for cardiac events compared to those with ischemic ST depression (87). In 1991 the American College of Cardiology and the American Heart Association recommended implantation of rate-responsive pacemakers in patients who fail to reach a heart rate of 100 beats per minute at maximal exertion (88). However in subsequent guidelines the definition of chronotropic incompetence was revised and chronotropic incompetence vaguely defined as the inability to increase heart rate "appropriately with exercise" (89). Recently chronotropic incompetence has been defined as the inability to achieve a maximal predicted heart rate (MPHR) or reaching a given percentage (e.g. 85%) of MPHR during exercise. This approach is mainly limited by the lack of a formula that provides an accurate prediction of MPHR. Depending on the formula used errors in MPHR estimation may be in excess of 11 beats per minute (90). Wilkoff and colleagues (72) described the mathematical relationship between

heart rate and oxygen uptake termed the metabolic-chronotropic relation. Based on these findings Lauer and colleagues (13) derived the chronotropic index, (peak heart rate – resting heart rate) / (220 – age – resting heart rate) by applying the metabolic-chronotropic relation concept to a symptom limited exercise test. This allowed definition of the normal chronotropic response independently of age, resting heart rate and functional state (72). In a group of 410 healthy adults Wilkoff et al. (72) reported 95% limits of normality of chronotropic index to be 0.8 – 1.3. Based on these findings, chronotropic incompetence can be defined statistically as failure to achieve a chronotropic index of 0.8 (i.e. falling below 97.5% of healthy adults).

The reported prevalence of chronotropic incompetence ranges between 30% in patients with chronic heart failure (18) to 60% in patients with chronic atrial fibrillation (91). In the current study chronotropic incompetence was found to be prevalent, affecting 62% of ACHD patients. The prevalence of chronotropic incompetence was lowest in patients with simple lesions such as repaired ventricular septal defect and was highest in patients with complex, uncorrected and cyanotic lesions. This increase in prevalence of chronotropic incompetence parallels the decline in peak oxygen consumption across the spectrum of ACHD.

Previous studies have suggested that a blunted heart rate response may in part account for the reduced exercise capacity seen in ACHD patients (2). However, consistent with studies in various cardiovascular cohorts (2,18), the correlation between heart rate and exercise capacity is modest,

explaining only a quarter of the variation in peak oxygen consumption. This study demonstrates that cardiopulmonary exercise testing with measurement of oxygen pulse kinetics identifies a subgroup of patients with distinctively abnormal pattern of cardiovascular response to exertion and may allow the recognition of patients in whom chronotropic incompetence is causally related to exercise intolerance. Assessing oxygen uptake kinetics in individual patients, the results of this study suggest that a blunted heart rate response to exercise is responsible for reduced exercise tolerance in approximately 20% of ACHD patients. It therefore appears that heart rate response during exercise does not account for 1/5 of peak oxygen consumption in each patient, but in fact determines exercise capacity almost entirely in subgroup of patients, while it may be largely unrelated to peak oxygen consumption in others. As a consequence additional parameters such as age, sex, pulmonary function, cyanosis and peripheral factors determine exercise capacity in ACHD patients.

The notion that blunted heart rate response may not be causally related to reduced oxygen uptake in many patients could be confirmed mechanistically in a subgroup of ACHD patients (those with a systemic right ventricle and a pre-existing pacemaker). Active reprogramming of the pre-existent pacemaker did not result in improved exercise capacity in these patients. Artificially increasing peak heart rate in itself was complicated in some patients and even in those in whom peak heart rate could be successfully augmented this intervention did not lead to a clinically relevant increase in peak oxygen consumption. The echocardiographic assessment of the timing

of the cardiac cycle at baseline and during higher heart rates provides an physiologic insight into the mechanisms potentially responsible for the discrepancy between high heart rates and low VO_2 in these patients: Heart rate increase leads to a significant reduction in total filling time and a significant increase in total isovolumic time accompanied by a marked reduction of aortic VTI (a surrogate parameter of stroke volume). This is in contrast to data published in healthy individuals showing an increase in total filling time and a reduction in total isovolumic time with increasing heart rate during dobutamine stress (92).

Total isovolumic time - the time where the ventricle neither ejects nor fills - has been shown to be prolonged in patients with myocardial ischemia and has been linked to mechanical incoordination (93). Both, limited coronary flow reserve and mechanical incoordinaton during pharmacological stress have been reported previously in patients with a systemic right ventricle (10,78). It appears likely, therefore, that the increase in total isovolumic time with increasing heart rate in these patients reflects myocardial ischemia and mechanical incoordination. This notion is supported by the fact that increasing heart rate during exercise was not accompanied by an increase in VO_2 in the majority of patients (Figure 2.5), a well recognized feature of myocardial ischemia in acquired heart disease (94). The increase in t-IVT in turn leads to a reduction in total filling time finally resulting in a reduction in stroke volume as indicated by reduced aortic VTI (Figure 2.6). Derrick and colleagues reported a limitation in stroke volume augmentation during exercise and dobutamine stress resulting from impaired atrio-ventricular transport potentially reflecting anatomical and functional abnormalities of the

atrio-ventricular pathways after the Mustard operation (95). This suggests that there may be two mechanisms limiting stroke volume during heart rate increase in patients with a systemic right ventricle.

The aetiology of chronotropic incompetence in patients with cardiovascular disorders is not fully understood. It appears likely that chronotropic incompetence is a multifactorial phenomenon. Colluci and colleagues (96) reported that impaired chronotropic response to exercise in patients with chronic heart failure is, at least in part, due to postsynaptic desensitization of β-adrenergic receptors. Others have demonstrated that autonomic function is significantly decreased in patients with acquired heart disease who are chronotropically incompetent (97,98). In addition, conduction disease as part of the natural history or as a consequence of previous surgery is common in ACHD patients and anti-arrhythmic drug therapy is commonly required in this population.

The prognostic value of an inappropriate increase in heart rate during exercise has been well established in various cardiovascular cohorts (11) and in healthy individuals(12,14,99).

This study demonstrates that chronotropic incompetence predicts an enhanced mortality risk independently of anti-arrhythmic medication in ACHD patients. Moreover, even an attenuated rate of recovery of heart rate after exercise testing carries important prognostic information. Heart rate reserve - a simple and easy to obtain parameter – was found to be a powerful prognostic marker in ACHD independently of anti-arrhythmic medication and

peak oxygen uptake. Stratifying patients by diagnostic groups revealed that a lower heart rate reserve was also associated with a greater risk of death in patients with complex anatomy, Fontan circulation and tetralogy of Fallot. Furthermore, a combination of heart rate reserve and peak oxygen consumption identifies a subpopulation of ACHD patients with a 3.8 fold increase in mortality.

2.8. Limitations

This work cannot identify why chronotropic incompetence predicts poor prognosis in ACHD patients. It is also possible that the low chronotropic index in some patients included in the current study was due to inadequate effort. However, reference values are based on comparable cohorts of healthy subjects undergoing symptom limited exercise testing.

Of necessity, the number of patients with a systemic right ventricle and a pre-existing pacemaker in this single-centre study was small. Moreover, two patients with congenitally corrected transposition of the great arteries were included who in contrast to the 7 patients with atrial switch procedure did not have atrial surgery potentially limiting their atrio-ventricular transport capacity. However, when these patients were excluded the results of the study did not change.

Echocardiographic assessment was performed at resting heart rate and after artificially increasing heart rate by pacemaker reprogramming. It is likely that the results of the study do not fully reflect the physiologic changes occurring during physical exercise. These include inotropic activation, peripheral vasodilatation and increased venous return. Nevertheless, the findings of the

current study findings are consistent with previous data in patients with systemic right ventricle studied during pharmacological stress and exercise showing comparable abnormalities. Finally, conventional ventricular pacing may – in itself - induce incoordination and thereby limiting total isovolumic time especially at higher heart rates and this requires further studies.

2.9. Conclusion

An abnormal heart rate response to exercise is prevalent across the spectrum of adult congenital heart disease, and is associated with an enhanced risk of death. Even on its own, heart rate reserve has the potential as a simple means to identify ACHD patients at elevated risk. In combination with formal measurement of peak oxygen consumption, it identifies a subpopulation with a 3.8 fold elevated risk of death in the midterm. Assessing oxygen pulse kinetics visually or by polynomial regression analysis may be valuable in identifying patients in whom an attenuated heart-rate response is responsible for poor exercise capacity. Furthermore, this study suggests that despite inappropriate heart rate response to exercise (chronotropic incompetence) optimized rate responsive pacing does not improve objective exercise capacity in patients with a systemic right ventricle. The results of this study rather indicate that higher heart rates may be detrimental in these patients by prolonging isovolumic time and reducing diastolic filling time. These findings may have clinical implications when considering implantation of a permanent pacemaker in a patient with a systemic right ventricle.

Chapter III Pulmonary vascular disease

3.1. Background of the study

Pulmonary arterial hypertension (PAH) — an elevated mean pulmonary arterial pressure of more than 25 mmHg at rest or 30 mmHg on exercise — is commonly associated with congenital heart disease (100). It has been estimated that approximately 5-10% of patients with congenital heart disease develop PAH (101-103). Eisenmenger syndrome represents the extreme manifestation of PAH in adult congenital heart disease (ACHD) patients and has become the epitome of severe PAH in this setting (104-107). It has been recently demonstrated that patients with Eisenmenger syndrome as a group exhibit the lowest exercise tolerance among adult patients with congenital heart disease (Figure 3.1) (1). Furthermore, on uni- and multivariate analysis the presence of PAH and cyanosis emerged as important determinants of peak oxygen consumption (Cyanosis r=-0.41, 95% confidence interval [CI] -0.31 - -0.50, $P<0.0001$; PAH r=-0.38, 95% CI -0.28- -0.47, $P<0.0001$). Perhaps not surprisingly the combination of PAH and cyanosis – as in patients with Eisenmenger physiology – severely limits exercise capacity (108).

This series of studies examined the prevalence of Eisenmenger syndrome amongst patients followed-up at a tertiary centre for adult congenital heart disease, provided a contemporary overview of adult patients with Eisenmenger physiology, examined their exercise capacity and assessed survival prospects as well as risk factors for mortality. In addition the impact of Bosentan (a pulmonary vasodilator) on exercise capacity was examined

during longer-term follow-up in patients with pulmonary arterial hypertension associated with congenital heart disease.

3.2. Impact of Eisenmenger syndrome on exercise tolerance and survival in adults with congenital heart disease

This study aimed to provide an overview of adult congenital heart disease patients with Eisenmenger syndrome followed at the Adult Congenital Heart Unit, Royal Brompton Hospital, London, to assess symptoms and exercise capacity in this cohort as well as to investigate survival prospects and risk factors for mortality. To provide an overview of the patient characteristics a cross-sectional study was performed. This retrospective analysis was combined with a case-control study to investigate risk factors for mortality. Despite utilizing a smaller number of patients overall, case control studies include all patients with adverse outcome and may be adjusted for known confounders such as age and underlying diagnosis.

3.2.1. Methods

Retrospective analysis

This was a retrospective study. All patients with Eisenmenger physiology under regular follow-up were identified from the computerized database of patients with adult congenital heart disease followed-up at the Adult Congenital Heart Disease unit, Royal Brompton Hospital, London. Inclusion criteria were evidence of pulmonary hypertension (confirmed by echocardiography and/or cardiac catheterisation) in the presence of a large unrestrictive atrial or ventricular septal defect or aorto-pulmonary

communication as previously described (105). Patients who had undergone shunt closure previously and those with restrictive shunts unlikely to be related to pulmonary hypertension were excluded. For the retrospective analysis the data of the first full assessment within the study period was included.

Case control study

The patient population was screened for deaths occurring in Eisenmenger patients between 2000 and 2005. Each patient who died was matched with a surviving control patient of similar age (within 7 years) and underlying anatomy. Medical records were reviewed and underlying characteristics, including diagnosis, ECG parameters, echocardiographic findings and laboratory results were recorded. For the case control study data from the last clinical visit were recorded for mortality cases, whereas for surviving control patients data around the time of the last clinical visit of the corresponding mortality case (within 6 months) were acquired. Risk factors considered included: functional (NYHA) class, deterioration in NYHA class (deterioration of at least one functional class within the last year before the clinical visit), need for more aggressive drug therapy (diuretics and anti-arrhythmic drugs) within the last year before the clinical visit, presence of clinical signs of heart failure (peripheral oedema or ascites), history of documented clinical arrhythmia, ECG parameters (such as underlying rhythm, QRS duration, QT and QTc interval), laboratory parameters (such as serum electrolytes, creatinine, albumin, liver function tests), cardiac medication, history of pulmonary or systemic emboli, history of pulmonary

infections and/or haemoptysis as well as history of near-syncope or syncope. In addition, systemic and subpulmonary systolic ventricular function and dimensions were assessed semiquantitatively from transthoracic echocardiograms, and quantified as previously described. Iron deficiency was defined as a ferritin level ≤ 15 ng/ml or a transferrin saturation ≤ 15%. In patients where there was discrepancy between these values, transferrin saturation was considered paramount.

Statistical analysis

Values are presented as mean ± standard deviation. Comparisons between groups were made using Student's t test, Mann-Whitney U test or Chi-squared test as appropriate. Kaplan-Meier survival curves were constructed to illustrate the survival of Eisenmenger patients. Survival prospects were compared to those predicted for an age and gender matched healthy cohort of UK residents using life table data (2001-2003 Interim life tables) published by the Government Actuary's Department (http://www.gad.gov.uk) as described previously (109).

Risk factors for death were identified by univariate conditional regression analysis. Odds ratios and 95% confidence intervals for significant predictors were reported. R version 2.1.1. and MedCalc 8.1 (MedCalc Software, Mariakerke, Belgium) were used for statistical analysis. For all analyses, a 2-sided *P*-value of less than 0.05 was considered statistically significant.

3.2.2. Results

Patient population

Overall, 171 patients (115 female, 56 male) fulfilled inclusion criteria and were included. The mean age at the last visit was 37±12 years (range 14-72 years). Ninety-seven patients (57%) had simple anatomy (12 atrial septal defect, 63 ventricular septal defect, 22 patent arterial duct) while 74 patients (44%) had complex anatomy, comprising atrioventricular septal defects, "univentricular heart", transposition of the great arteries and common arterial trunk. There were 48 patients with Down's syndrome (28%). Down patients were significantly younger compared to the remaining patients (30±9 vs. 40±12 years, $P<0.001$). The majority of the patients were symptomatic (94%), whereas only 6% of patients reported no symptoms. Classifying symptoms according to the NYHA classification revealed that 6% of patients were in functional class I, 48% in class II, 42% in class III and only 4% were in class IV. When patients were stratified according to the location of shunting, no significant difference in symptoms could be found: symptoms corresponding to NYHA class III or above were found in 54% of patients with atrial shunt, 54% of patients with a shunt at ventricular level and 57% of patients with aorto-pulmonary shunt ($P=0.96$).

Mean oxygen saturation in room air was 84±8%, lower in patients with higher NYHA class (81±7% in patients with NYHA class III or IV vs. 86±8 in patients with NYHA class I or II, $P<0.001$) and in those with complex anatomy (82±8% in patients with complex lesions vs. 85±8% in patients with simple lesions, $P=0.006$).

The majority of patients were in sinus rhythm (98%) at the last visit, whereas only 2% of patients were in atrial fibrillation (n=2) or had a pacemaker rhythm (n=2, for complete heart block). A clinical history of arrhythmia requiring cardioversion or anti-arrhythmic therapy was documented in a minority of patients (13%). Palpitations, however, were more common, and were present in 44 patients (26%). Furthermore, 23 patients (13%) experienced at least one syncopal episode during the follow-up period.

Dizziness was reported by 25 patients (15%) and was related to history of syncope, independent of age and survival status ($P<0.001$). Thirty-five patients had a history of haemoptysis. Patients with haemoptysis were more likely to be anticoagulated with warfarin ($P=0.04$). In addition, patients in a higher functional class were more likely to have clinical signs of heart failure, independent of age and survival status ($P=0.003$).

Medication

Twenty-three patients were treated with a beta-blocker, 19 patients with an ACE-inhibitor, 7 with a calcium-antagonist and 17 patients with digoxin. Forty-six patients received diuretics. Twenty patients were treated with potassium-sparing diuretics such as spironolactone or amiloride. Only one patient (with the lowest potassium level) was treated in our hospital with potassium supplements. Forty-nine patients were anti-coagulated with warfarin, while 17 patients were treated with anti-arrhythmic drugs. In addition 39 patients had home oxygen therapy and 20 patients were treated with advanced therapies for pulmonary hypertension (18 with Bosentan - an

endothelin antagonist, 1 patients with Sildenafil - a phosphodiesterase-5-inhibitor, and 1 with a combination of both).

Laboratory measures, haematology and iron deficiency

Laboratory results were available in 129 patients. Mean potassium level was 4.3±0.5 mmol/L (range 3.1-5.7 mmol/L). Mean haemoglobin concentration was 19.2±3.2 g/dl (range 10.1 – 26.1 g/dl). Iron deficiency was found In 24 patients. Twenty-nine patients had a history of phlebotomy. Phlebotomy was strongly associated with the presence of iron deficiency (relative risk 4.1, 95% confidence interval 2.2 – 7.7, $P<0.0001$). There was an inverse relationship between resting oxygen saturations and haemoglobin levels in patients without iron deficiency as shown in Figure 3.2. However, no such relationship could be found in patients with iron deficiency.

Survival analysis

During a median follow-up period of 67 months (range 8 – 68), 20 patients died. Nine patients died in progressive heart failure (45%), while 11 patients (55%) died unexpected, presumably suddenly. These numbers are comparable with those reported by Niwa et al (110), where 63% of patients died suddenly. Survival at 30, 40, 50 and 60 years of age was 98%, 94%, 74%, and 52%, respectively (Figure 3.3). Patients with complex anatomy had a significantly worse prognosis compared to those with simple anatomy ($P=0.02$). While 50% of patients with simple anatomy were still alive at 58 years of age, 50% of those with complex anatomy had died by the age of 42 years, as demonstrated in Figure 3.4. In addition, this figure also shows the

estimated survival curve for a gender-matched cohort of healthy individuals based on Life Tables for England and Wales (2001-2003) published by the Government Actuary's Department. Survival was significantly worse for Eisenmenger patients than expected for a matched healthy cohort (standardised mortality ratio 3.8; 95% confidence interval 2.0 – 7.0; $P<0.0001$). While 20 patients Eisenmenger patients died during follow-up, the expected mortality in the matched population was only 5.3 deaths.

Predictors of mortality

Twenty patients died (8 male) and were included into the case control study. Underlying cardiac anatomy was a non-restrictive ventricular septal defect (VSD) in 5, atrial septal defect in 4, common arterial trunk in 4, transposition of great arteries with VSD in 3 and patent arterial duct in 4. Mean age at death was 43.2±10.5 years.

On conditional logistic regression analysis, functional (NYHA) class (odds ratio = 3.37, 95% confidence interval [CI] 1.05 – 10.8, $P=0.041$) and presence of signs of heart failure (odds ratio = 9.00, 95% CI 1.14 – 71.04, $P=0.037$) were found to be predictive of death. On ECG, longer QRS duration (odds ratio = 1.10/ms, 95% CI 1.002 – 1.199/ms, $P=0.044$) and longer QTc interval (odds ratio = 1.07, 95% CI 1.001 – 1.151/ms, $P=0.047$) were associated with a worse outcome. Low serum albumin (odds ratio = 0.84/gL-1, 95% CI 0.71 – 0.99/ gL-1, $P=0.034$) and low potassium levels (odds ratio = 0.06/ mmolL-1, 95% CI 0.004 – 0.960/mmolL-1, $P=0.047$) were also predictive of death.

In addition, a history of clinical arrhythmia (odds ratio = 9.00, 95% CI 1.14 – 71.03, *P*=0.037), deteriorating NYHA class (odds ratio = 11.00, 95% CI 1.42 – 85.20, *P*=0.022) as well as need for more aggressive drug therapy (odds ratio 9.00, 95% CI 1.14 – 71.03, *P*=0.037) within one year before the last clinic visit were all predictive of poor outcome.

In contrast, neither medication nor semi-quantitative echocardiographic assessment of right and left ventricular size and function were predictive of death. Furthermore, previous history of pulmonary or systemic emboli, recurrent pulmonary infections or haemoptysis and near-syncope or syncope was not significantly different between the two outcome groups.

3.3. Impact of Bosentan therapy on exercise capacity in patients with congenital heart disease and pulmonary arterial hypertension

Exercise capacity is greatly diminished in patients with Eisenmenger syndrome. In practice, >90% of patients are in New York Heart Association class 2 or above, and approximately 50% report severe limitation (New York Heat Association class 3 or 4). Bosentan, a dual-receptor endothelin antagonist and pulmonary vasodilator, has an established role in the management of patients with idiopathic pulmonary arterial hypertension (PAH). This study aimed to assess safety profile and clinical effects of Bosentan in adults with PAH and congenital heart disease during longer-term follow-up.

3.3.1. Methods

Patient characteristics

All adult patients with significant pulmonary hypertension (as defined by current guidelines(54)) associated with congenital heart disease or with Eisenmenger syndrome who were treated with Bosentan at the Adult Congenital Heart Disease Program, Royal Brompton Hospital, London were included in this retrospective analysis. A subgroup of patients (n=10) was included in a previous prospective short-term (16 weeks) safety and tolerability study of Bosentan (111) and these patients were subsequently continued on Bosentan on compassionate grounds. Medical records were reviewed and underlying demographics as well as clinical characteristics were recorded. Additional parameters analysed at baseline and during follow-up included: functional class, oxygen saturation in room air, 6-minute walk test distance, ECG related parameters and liver function tests. Treatment with Bosentan as part of clinical studies was approved by the local ethics committee and these patients provided informed consent before commencement of therapy.

Medication

At the Adult Congenital Heart Unit, Royal Brompton Hospital, London a policy of commencing therapy with 62.5 mg twice daily, increased to 125 mg twice a day after 4 weeks, as tolerated is operated. Bosentan therapy is initiated in hospital with close monitoring of oxygen saturations as well as blood pressure as described previously (111).

Electrocardiographic parameters and six-minute walk test

Electrocardiograms were reviewed and a voltage criterion for right ventricular hypertrophy was derived as the sum of the R-wave amplitude in V_1 and the maximum amplitude of the S-wave in either V_5 or V_6. Amplitudes on ECG were measured by two independent investigators blinded towards date of the ECG (baseline vs. therapy).

Statistical analysis

Values are presented as mean ± standard deviation. Unless stated otherwise, comparisons between baseline and follow-up groups were made using non-parametric tests (Wilcoxon's paired rank test or McNemar test). MedCalc Version 8.1 (MedCalc, Mariakerke, Belgium) was used for statistical analysis. For all analyses a *P*-value of less than 0.05 was considered statistically significant.

3.3.2. Results

Demographics

Overall, 18 (14 female) patients fulfilled inclusion criteria and were included in the study. Average age at commencement of Bosentan therapy was 41±9 years (range 23 – 69 years). Median follow-up was 29 months (range 1-39 months) as shown in Figure 3.5. Underlying diagnoses were: simple lesions in 12 patients (large atrial septal defect in 2, non-restrictive ventricular septal defect in 7, persistent arterial duct in 2, aortopulmonary window in 1), and complex lesions in 6 patients (atrio-ventricular septal defect in 2, "single ventricle" physiology in 3, and double discordance with non-restrictive VSD in

1). Fifteen patients did not undergo any previous corrective surgery and constituted the Eisenmenger group, while three patients had undergone corrective surgery late in infancy and subsequently developed secondary pulmonary arterial hypertension.

Safety and tolerability

All but one patient received 125 mg Bosentan twice a day after the initial up-titration period. One patient had noticed subjective improvement with the initial dose of 62.5 mg Bosentan twice daily, however she experienced lightheadedness after uptitration to 125 mg Bosentan and was therefore downtitrated to 62.5 mg Bosentan twice daily. None of the patients needed discontinuation of Bosentan therapy due to untoward effects. One patient was hospitalized early after commencement of Bosentan treatment due to a chest infection that resolved with appropriate antibiotic therapy.

No significant rise in liver transaminases was observed during follow-up (Figure 3.6). None of the patients exhibited elevation of transaminase values exceeding three times the upper limit of the reference range. Furthermore, transaminase values did not further increase during Bosentan therapy even in patients with elevated liver enzyme levels at baseline.

One patient died during follow-up. This was a 69-year-old female patient with "single ventricle" physiology and unprotected pulmonary circulation (situs solitus, double inlet left ventricle, rudimentary right ventricle, discordant ventriculo-arterial connection and a non-restrictive inlet ventricular septal

defect) who was commenced on Bosentan on compassionate grounds following marked deterioration in functional class. The patient who reported symptomatic improvement on Bosentan therapy, died 11 months later due to respiratory insufficiency and progressive heart failure.

Oxygen saturations

There was no significant drop in oxygen saturations, neither within the first six months of therapy, nor during longer-term follow up. Arterial saturations in Eisenmenger patients increased compared to baseline early during therapy (81.1±4.9% vs. 84.7±2.6%, P=0.014). At 1 and 2 years of follow-up oxygen saturations were higher compared to baseline, however the difference was not significant (81.1±4.9% vs. 84.5±2.8, P=0.054; and, 81.1±4.9% vs. 84.2±4.8, P=0.078, respectively) as shown in Figure 3.7.

Six-minute walk test distance

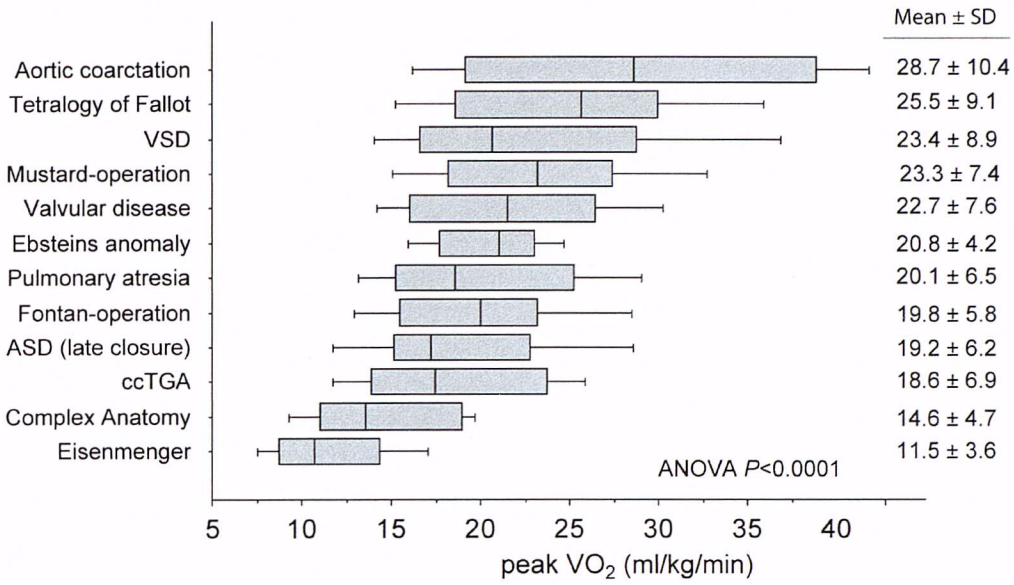
Six-minute walk test distance improved significantly during Bosentan therapy compared to baseline (284±144 vs. 363±124, 380±91, and 408±114 m at baseline, 0-6 months, 6-12 months and 1-2 years of therapy, respectively; P<0.05 for each) as shown in Figure 3.8. In addition, during the first 6 months of therapy 70% of patients improved their 6 minute walk distance by more than 54m, an improvement associated with a noticeable clinical difference in the patients' perception of exercise capacity as recently defined by the American Thoracic Society.

Consistent with this objective improvement in exercise capacity, patients' perceived functional class improved during follow-up (P=0.001). In fact, 11 patients improved in their functional class during therapy, while the remaining 7 patients remained stable. None of the patients felt subjectively worse on Bosentan therapy.

Electrocardiographic findings

Voltage criteria for right ventricular hypertrophy (sum of the R-wave amplitude in V1 and the maximum amplitude of S in either V5 or V6) improved significantly during Bosentan therapy (21.9 ± 6.9 vs. 19.7 ± 6.1 mV; P=0.01; median time between baseline and follow-up ECG of 11 months).

3.4. Figures



ASD = atrial septal defect; ccTGA = congenitally corrected transposition of the great arteries; SD = standard deviation; VSD = ventricular septal defect.

Figure 3.1: Distribution of peak oxygen consumption (peak VO2) in different diagnostic groups among 335 adult congenital heart disease patients.

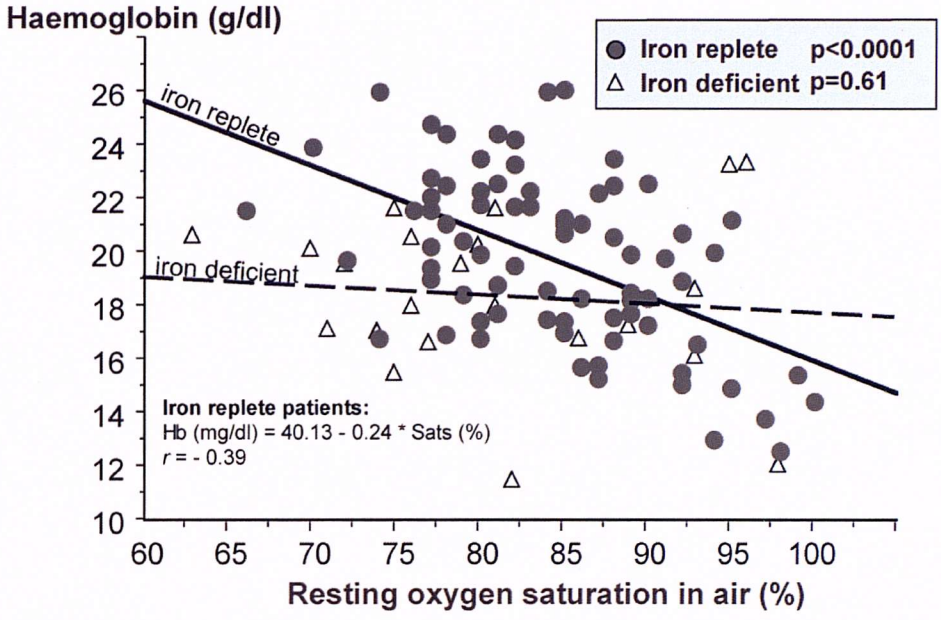


Figure 3.2: Association between resting oxygen saturation in air (%) and haemoglobin concentration stratified by iron deficient and iron replete patients. While no relationship between arterial saturations and haemoglobin concentrations was found in iron deficient patients, arterial saturations and haemoglobin concentrations were inversely related in iron replete patients.

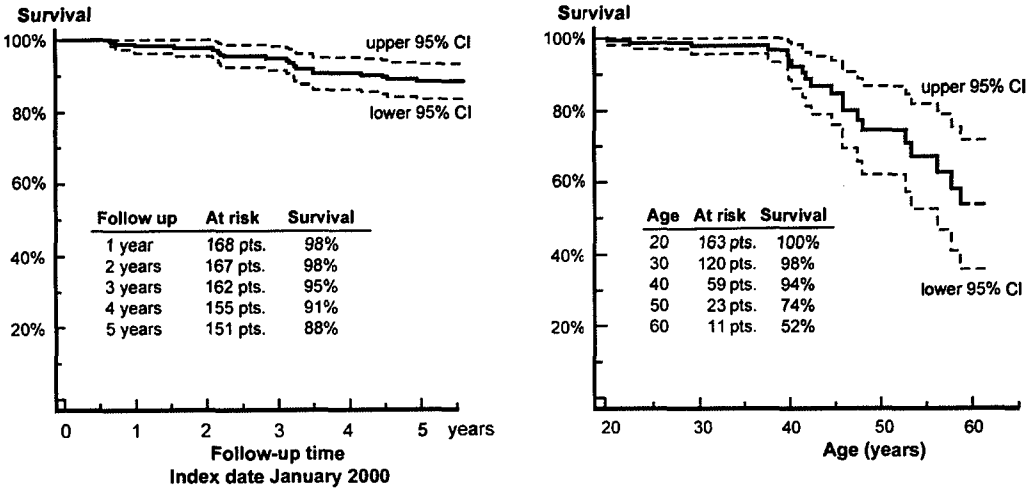
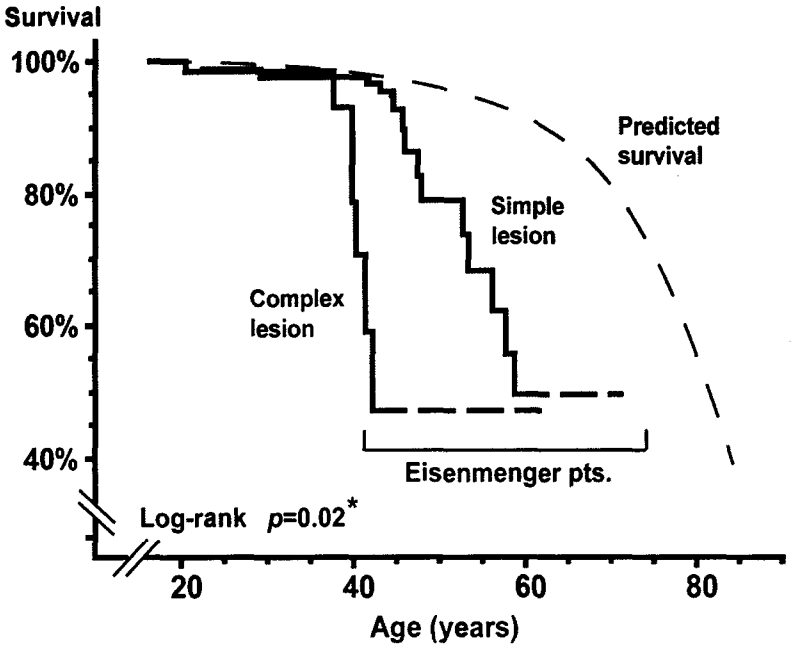


Figure 3.3: Survival as a function of follow-up time (left) and as a function of age (right) in patients with Eisenmenger physiology, illustrating that while short-term prognosis is relatively good, overall life expectancy is reduced in Eisenmenger patients.



† Predicted survival is based on life tables for England and Wales (2001-2003 interim life tables) published by the Government Actuary's Department;
* Comparison between Eisenmenger patients with simple and complex lesions. Patients with complex lesions had a significantly worse outcome compared to those with simple lesions.

Figure 3.4: Survival prospects of patients with Eisenmenger physiology compared to an age and gender matched healthy population showing reduced life expectancy in patients with Eisenmenger syndrome. The survival curve appears to be shifted leftwards by approximately 20 years in patients with simple underlying lesions and approximately 40 years in those with complex lesions.

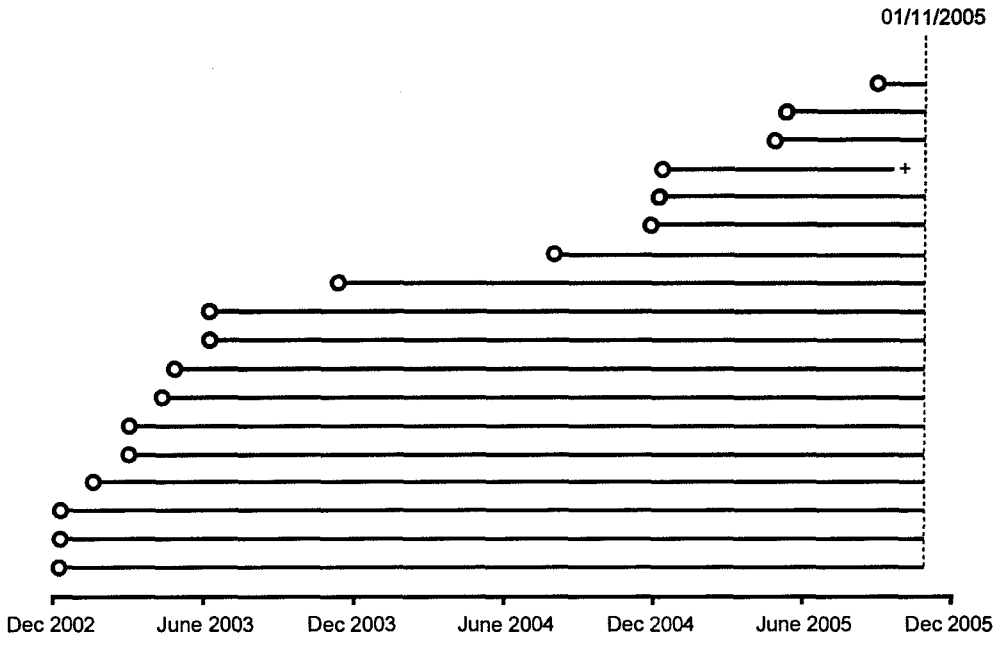


Figure 3.5: Date of commencement and duration of Bosentan therapy. One patient (+) aged 69 years with complex congenital heart disease died due to combined respiratory and right heart failure. Follow-up was complete for all patients.

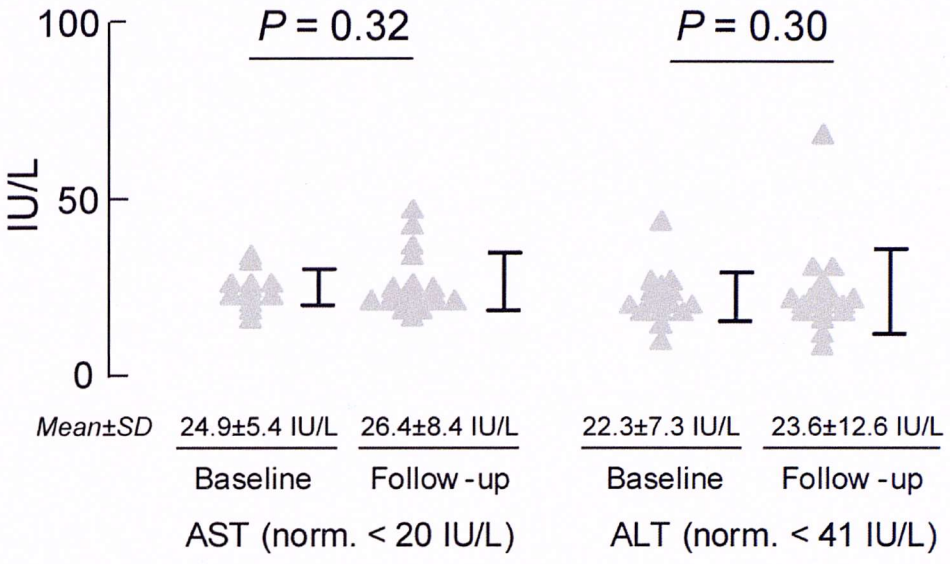


Figure 3.6: Serum levels of aspartate (AST) or alanine (ALT) aminotransferase as markers of hepatic function at baseline and during follow-up.

Comparisons were made between baseline and follow-up values using a non-parametric test (Wilcoxon paired rank test).

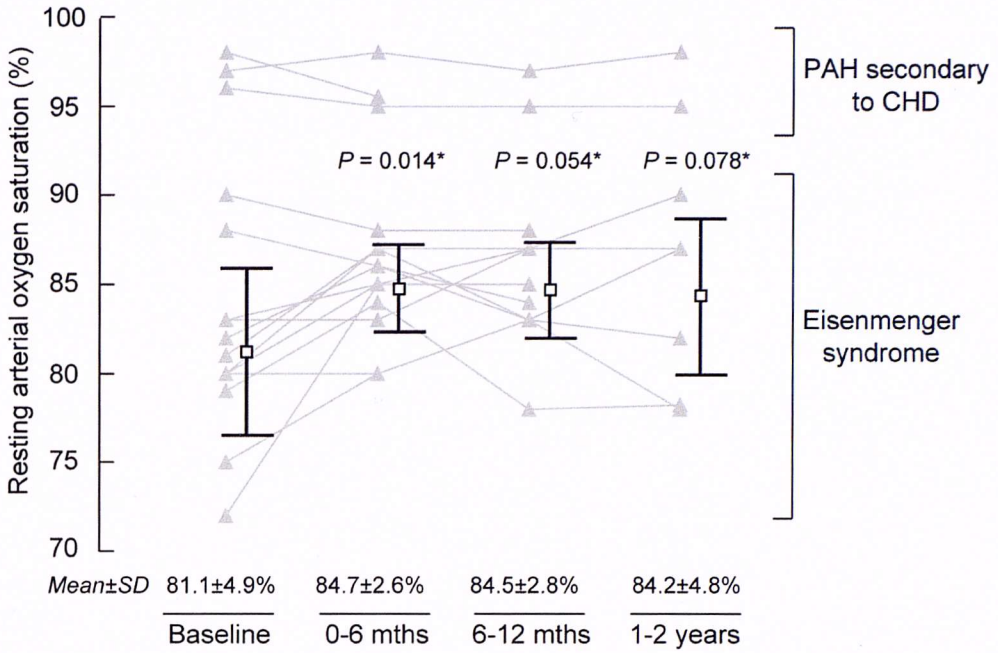


Figure 3.7: Oxygen saturation at rest in room air at baseline and during follow-up. * Comparisons were made to baseline using a non-parametric test (Wilcoxon paired rank test). CHD = congenital heart disease. PAH = pulmonary arterial hypertension.

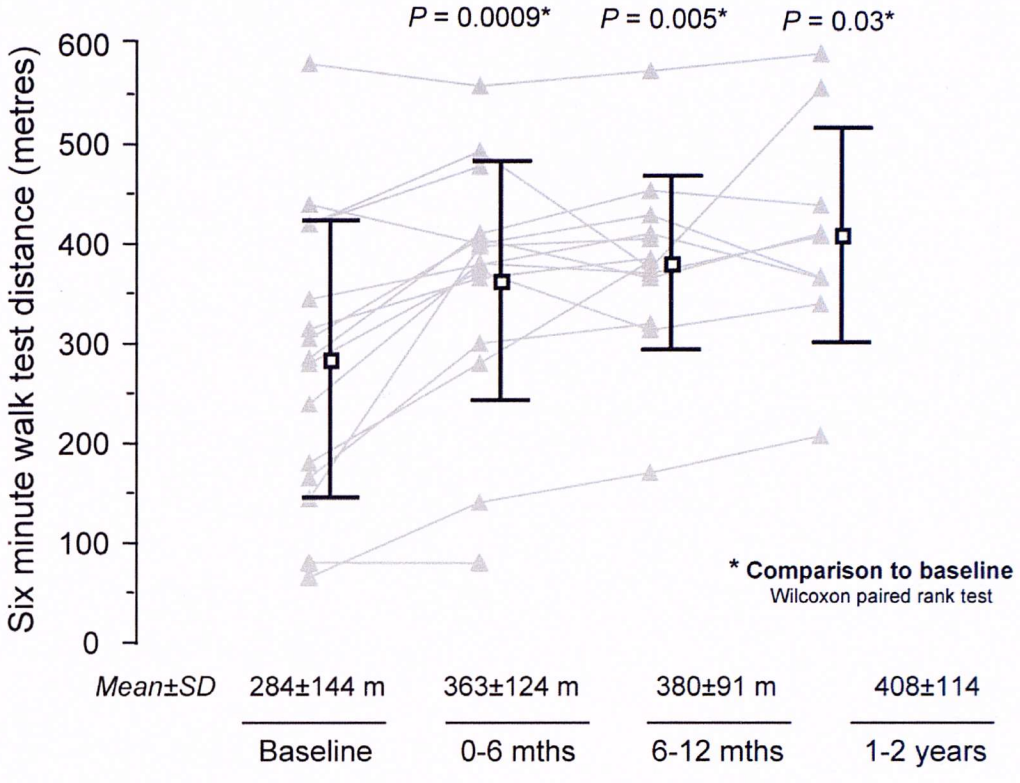


Figure 3.8: Six minute walk test distance at baseline and during follow-up. * Comparisons were made to baseline using a non-parametric test (Wilcoxon paired rank test).

3.5. Discussion

Information on characteristics and prognostic variables is limited in contemporary Eisenmenger patients. In a retrospective study, Cantor et al. (1999) reported predictors of death in Eisenmenger patients referred for lung transplantation, while Daliento et al (1998) characterised 188 Eisenmenger patients followed-up at 3 different European centres over 3 decades (112,113). The current study was performed at a single centre, thus avoiding differing management strategies at different centres, included only contemporary patients - reflecting current practice - and is the first case control study in this population.

Exercise capacity is markedly depressed in adult patients with Eisenmenger syndrome. This study shows that >90% of Eisenmenger patients had symptoms equivalent to New York Heart Association class II or above. A higher functional class was related to lower resting arterial oxygen saturations and a higher incidence of ascites or peripheral oedema. In contrast to previous reports (106), this study could not confirm a relationship between the location of shunting and degree of physical limitation. Overall, less than 10% of Eisenmenger patients were treated with standard heart failure medication such as beta-blockers, ACE inhibitors or calcium antagonists. This is likely to reflect the lack of evidence for a beneficial effect of these therapies in this cohort as well as the fear that these drugs may induce systemic vasodilation with subsequent increase in right-to-left shunting. In contrast, 19 patients were treated with disease targeting therapies for pulmonary hypertension (endothelin receptor or

phosphodiesterase-5 antagonists) as part of ongoing clinical studies or on a compassionate basis. Twenty-three percent of patients were treated with home oxygen. Data regarding the chronic use of oxygen in patients with Eisenmenger syndrome is inconsistent (114,115), however some patients benefit and home oxygen should be used in individual patients.

Formal exercise testing provides information on objective exercise capacity and insight into the factors limiting exercise capacity (such as change in arterial oxygen saturation during exercise). Exercise capacity is either assessed by measurement of 6-minute walk test distance or cardiopulmonary exercise testing with measurement of parameters of gas exchange. Both modalities have been used successfully to assess exercise intolerance in patients with PAH of various aetiology (1). Impaired peak oxygen uptake and reduced 6-minute walk test distance have been reported to be related to impaired prognosis in this setting (116-118). In most centres the 6-minute walk test represents the preferred modality to assess patients with PAH. The 6-minute walk test is robust, cheap and unlike cardiopulmonary exercise testing with measurement of parameters of gas exchange does not require sophisticated equipment or special expertise. For reasons discussed in detail elsewhere the 6-minute walk test is the only exercise modality approved by the US Food and Drug Administration as an end point in clinical trials in the setting of PAH (119-121).

The vast majority of Eisenmenger patients in this study had secondary erythrocytosis as reflected by elevated mean haemoglobin concentrations.

This represents an adaptation to cyanosis. In the present study a strong association between arterial oxygen saturations and haemoglobin values was found in iron replete patients. In contrast no such association could be found in iron deficient patients suggesting that iron deficient patients were unable to increase their haemoglobin concentrations in proportion to reduced arterial oxygen saturations, thus failing to maintain oxygen carrying capacity of the blood (Figure 3.2). Consistent with previous studies the presence of iron deficiency was linked to routine phlebotomies (relative risk approximately 4). Routine phlebotomy is therefore discouraged at most centres in patients with Eisenmenger syndrome due to well recognized complications such as iron deficient anaemia and increased risk for cerebrovascular accidents (31). In addition, iron deficiency should be promptly treated with oral iron supplementation (or intravenous, if oral therapy fails).

Haemoptysis is a well recognized complication of Eisenmenger syndrome. In the current study a history of haemoptysis was found in 20% of patients. This is consistent with previously reported incidence rates, ranging between 11% and 33% (106,110,112). In the current study haemoptysis was not related to anaemia or an increased risk of death. This is consistent with the results published by Daliento in 1998 (113). In this study an association between haemoptysis and anticoagulation therapy was present. However, some patients are anticoagulated for pulmonary arterial thrombosis, which in itself is often associated with haemoptysis due to pulmonary infarction (105,122).

Therefore, the relationship between anticoagulation and haemoptysis may be confounded in this population.

Life expectancy in contemporary patients with Eisenmenger physiology was found to be reduced by approximately 20 years and mortality risk increased 3.8 fold compared to the general population. In agreement with previous studies, however, short-term mortality was found to be low (12.5% at 5 years) (112,113), supporting the notion that survival prospects in Eisenmenger patients are better than previously appreciated and are far superior to those observed in patients with idiopathic pulmonary hypertension (123). Survival prospects were significantly poorer in patients with complex anatomy compared to those with simple underlying cardiac lesions. While 50% of patients with simple anatomy were still alive at the age of 58 years of age, 50% of those with complex anatomy had died by the age of 42 years.

Signs and symptoms of heart failure (functional class and presence of oedema) were found to predict mortality in Eisenmenger patients. In addition, laboratory parameters related to impaired liver function – potentially linked to congestive heart failure - were also predictive of mortality. Furthermore a history of clinical arrhythmia was predictive of death. Low potassium concentrations, potentially associated with a greater risk of malignant tachy-arrhythmias also carried prognostic information. In agreement with previous reports a higher functional class was found to be predictive of mortality (112,113). In contrast, data on the predictive value of arrhythmias is

conflicting: while Cantor and colleagues (112) found an association between history of arrhythmia and mortality, another report could not identify a link between history of arrhythmia and death (113). None of these previous studies describe an association between signs of heart failure and risk of death. The current study suggests that a history of syncope is not related to poor outcome. This is consistent with data published by Cantor et al. and Daliento et al. (112,113), the current study suggests that a history of syncope is not related to poor outcome in Eisenmenger patients. In contrast, earlier studies found syncope to be an predictor of mortality (105). Furthermore, in this study ECG parameters (QRS duration and QTc) were identified to be predictive of poor outcome in Eisenmenger patients.

Traditionally treatment options for patients with PAH associated with congenital heart disease were limited to palliative measures and heart-lung transplantation in selected patients. In addition, as pointed out by Oechslin the 'mainstay of care is not to destabilize the balanced physiology' (27). Recently, however, this therapeutic nihilism has been challenged by the advent of effective drugs for the treatment of pulmonary hypertension (46,124,125), These therapies may also be applicable to patients with PAH with congenital heart disease and preliminary results are promising (126,127).

Endothelin-1 – a potent vasoconstrictor and mitogen for vascular smooth muscle cells - is produced primarily by endothelial cells (128). Elevated systemic endothelin levels have been reported in patients with Eisenmenger

syndrome (129). In addition, endothelin-1-like immunoreactivity was reported in endothelial cells of pulmonary arteries of patients with primary and secondary PAH (130). Bosentan is a competitive endothelin receptor antagonist that inhibits binding of endothelin to its type A and B receptors. Bosentan has been demonstrated to reduce pulmonary arterial pressure, decrease pulmonary vascular hypertrophy and to attenuate pulmonary fibrosis and inflammation (131). Its efficacy in patients with idiopathic pulmonary hypertension has been confirmed by two randomized controlled trials (46,47). Recently, the results of the first randomized study using Bosentan in patients with Eisenmenger syndrome (BREATHE-5) have been reported (132). BREATHE-5 was a multicenter, double-blind, randomised, placebo-controlled study safety study over 16 weeks that provided evidence that Bosentan is safe in this cohort and improves six-minute walk distance. Due to the relatively short follow-up period employed in this study, the long-term effects of Bosentan remained largely unknown.

The current study demonstrates that Bosentan is safe and well tolerated in patients with pulmonary hypertension associated with congenital heart disease not only during short- but also medium to long-term therapy. Exercise capacity, as assessed formally by six-minute walk test distance improved significantly and to a degree that is likely to be clinically relevant early during Bosentan therapy, and this effect was maintained throughout the study period. Furthermore, electrocardiographic evidence suggesting regression of right ventricular hypertrophy was found.

There was no evidence that oxygen saturations deteriorates during Bosentan therapy. In fact, a modest but statistically significant increase in oxygen saturation early during follow-up was found. These findings are consistent with results of previous studies (126,127,133). In none of the studies – including the current one – the theoretical concern of an aggravated right-to-left shunt induced by a fall in systemic vascular resistance as a consequence of vasodilator therapy was substantiated. Endothelin antagonists have been reported to cause dose-dependent hepatic dysfunction. In addition, Eisenmenger syndrome represents a multiorgan disease and thus hepatic injury may be of even greater concern in this cohort compared to patients with pulmonary arterial hypertension of different aetiologies. Monthly monitoring of aminotransferase levels is, thus a licensing requirement by both the European Agency for the Evaluation of Medicinal Products (EMEA) and the Food and Drug Administration (FDA) for these drugs. In the current study, no evidence of a clinically relevant elevation in serum levels of aspartate or alanine aminotransferase as markers of hepatic function was found. In addition, in none of the studied patients did these parameters exceed three-fold the upper reference value on Bosentan therapy. In previous studies hepatic dysfunction was primarily observed in patients treated with higher Bosentan doses (250 mg twice daily) compared to the doses routinely employed at our institution (134). Furthermore, none of the patients from the current study had to be discontinued from Bosentan therapy due to untoward effects.

A significant increase in six minute walk test distance early during therapy was found and this effect was sustained up to two years of Bosentan treatment as shown in Figure 3.8. It is noteworthy that the observed improvement in six minute walk test distance is likely to be clinically relevant in these very limited patients (1). In 70% of patients an increase in six minute walk test distance of > 54 meters was observed within 6 months of treatment. A recent guideline suggested that an improvement of this magnitude is associated with a perceivable improvement in exercise capacity by the patient (119). The findings of the current study concur with studies in patients with idiopathic pulmonary arterial hypertension, in which initial improvement in six minute walk test distance was sustained during long-term Bosentan treatment (135). Moreover, in agreement with the observed improvement in objective exercise capacity, symptomatic status improved during therapy. Recently, Schulze-Neick and colleagues reported improved six minute walk test distance in adult patients with Eisenmenger syndrome and pulmonary arterial hypertension associated with congenital heart disease followed-up at six different German centres (133). The current study confirms these results and expands on these findings by providing a detailed overview over the time-course of improvement in objective exercise capacity during long term follow-up.

Eisenmenger syndrome and pulmonary arterial hypertension associated with congenital heart disease are associated with right ventricular hypertrophy and subsequent right ventricular dysfunction. Endothelin antagonism may attenuate these adaptive right ventricular changes by reducing right

ventricular afterload. Indeed, ECG derived voltage criteria for right ventricular hypertrophy improved compared to baseline. This is consistent with experimental data demonstrating attenuated right ventricular hypertrophy after treatment with endothelin antagonists in various animal models of pulmonary hypertension (131,136). However, patients with Eisenmenger physiology represent a unique and heterogeneous patient group. Especially in patients with non-restrictive ventricular communication the right ventricle is not only exposed to elevated pulmonary arterial pressures but also to systemic pressures. Therefore, further studies incorporating comprehensive imaging techniques are required to elucidate whether endothelin antagonism may indeed have the potential to reverse long-standing right ventricular hypertrophy in these patients.

3.6. Limitations

This was a descriptive and retrospective study in which the presence of confounding elements and a placebo effect cannot be excluded. Nevertheless, previous studies in patients with pulmonary arterial hypertension have consistently demonstrated that improvements in six minute walk test distance were not observed beyond the first 12 weeks of therapy (46,47), indicating that the early improvement in the six minute walk test as seen in the current study is due to a genuine treatment effect of Bosentan. No invasive haemodynamic data is available as serial cardiac catheterization in this setting provides limited information and maybe associated with considerable risks for the patient. Further studies, including a larger number of patients and robust imaging modalities such as cardiac

magnetic resonance imaging are required to assess the potential of endothelin antagonism to induce right ventricular remodeling in patients with pulmonary arterial hypertension associated with congenital heart disease.

3.7. Conclusion

The vast majority of Eisenmenger patients have limited exercise capacity. There is a four-fold increase in mortality amongst contemporary adult patients with Eisenmenger syndrome followed up in a tertiary specialized centre. Poor functional class, clinical and laboratory signs of heart failure and a history of arrhythmias were all predictive of death. These data may be helpful in guiding clinicians and assisting targeting therapies in patients with Eisenmenger physiology. Bosentan - a competitive endothelin receptor antagonist - appears to be safe and well tolerated in adults with pulmonary arterial hypertension associated with congenital heart disease or Eisenmenger syndrome during mid to long-term follow-up. In addition functional class and the six-minute walk test distance improved and this effect was maintained up to 2 years of Bosentan therapy.

Chapter IV Cyanosis and Impaired Tissue Oxygen Delivery

4.1. Background of the study

Shunt lesions are common in adult congenital heart disease (ACHD) patients and affect exercise capacity in this population. Not surprisingly, right-to-left shunting with cyanosis was found to be an important determinant of oxygen consumption in ACHD patients (1). Right-to-left shunting reduces arterial oxygen content thereby impairing oxygen delivery to tissue. In physiologic terms, reduced arterial oxygen content decreases arterio-venous oxygen difference thus impairing peak oxygen consumption according to the Fick principle (21) as illustrated in Equation 4.1:

$$VO_2 = CO \cdot \Delta\, avO_2 \qquad \textit{Equation 4.1}$$

VO_2 = oxygen consumption; CO = cardiac output; $\cdot\, \Delta\, avO_2$ = arterio-venous O_2 difference

To quantify the amount of oxygen available to tissue the concept of oxygen delivery has been developed (Equation 4.2) (137):

$$\text{Oxygen delivery} = \text{cardiac output} \times \text{arterial } O_2 \text{ content} \qquad \textit{Equation 4.2}$$

This concept combines systemic blood flow, oxygen carrying capacity and oxygen saturation into a single parameter by multiplying them together (137). Increasing any of these parameters will invariably increase their product. Therefore it is widely used in quantifying oxygenation status for example in the intensive care unit (138). However, in situations where increases in

systemic flow are achieved at the expense of decreases in systemic oxygen saturation – such as in patients with shunt lesions (e.g. bidirectional cavopulmonary shunt and univentricular circulation) - this simple approach of multiplying flow and arterial saturation can easily be misleading. In principle venous parameters of oxygenation may be better suited to characterise tissue oxygenation in this setting. Venous blood has returned from tissues with which it has recently been in equilibrium: its oxygen saturation offers useful information about tissue oxygenation not available from arterial parameters (139).

In this series of studies equations defining the mandatory relationship between physiologic variables and oxygen delivery were formulated in patients with right-to-left shunting and fixed pulmonary blood flow as well as patients with bidirectional cavopulmonary shunt and univentricular circulation. Using calculus and computer modeling the effects of blood flow distribution, different metabolic rates, oxygen-carrying capacity and pulmonary blood flow on oxygen delivery and markers of tissue oxygenation were considered.

4.2. Oxygen Delivery in Patients with Bidirectional Cavopulmonary Shunt and Univentricular Circulation

The bidirectional cavopulmonary shunt is used in the staged palliation of patients with univentricular circulation. As illustrated in Figure 4.1. in this circulation pulmonary blood flow is supplied exclusively by venous return from the upper body via the superior vena cava. This represents an inherently inefficient circulation uncoupling lower body perfusion from

pulmonary blood flow. This study aimed to explore the complex relationship between systemic blood flow, arterial and venous oxygen content and tissue oxygenation in patients with a bidirectional cavopulmonary shunt.

4.2.1. Methods

A standard model of the univentricular circulation after the bidirectional cavopulmonary anastomosis was used, as shown in Figure 4.1. and explained in the legend.

Cardiac output is represented by CO. This is considered to have two components, Q_{SVC} and Q_{IVC}, with coronary sinus flow considered, to be part of inferior vena cava flow. Ca_{O2} represents the oxygen carrying capacity of blood. A steady state is assumed. When blood flowing at Q l/min gaining oxygen at a rate of V l/min while passing through an organ (e.g. the lungs), its oxygen concentration must rise by V/Q. Its saturation, being the ratio between oxygen content and carrying capacity Cap_{O2}, must therefore rise by $(V/Q)/Cap_{O2}$. This (Fick) principle applies to all locations where oxygen is transferred. Across the lung bed,

$$Ssvc_{O_2} = Spv_{O_2} - VO_2/(Qsvc \cdot Cap_{O_2}) \qquad \textit{Equation 4.3}$$

Across the upper part of the systemic circulation,

$$Sa_{O_2} = Ssvc_{O_2} + kVO_2/(Qsvc \cdot Cap_{O_2}) \qquad \textit{Equation 4.4}$$
$$= Spv_{O_2} + (k-1)VO_2/(Qsvc \cdot Cap_{O_2})$$

Across the lower part of the systemic circulation,

$$S_{IVC_{O2}} = Sa_{O_2} - (1\text{-}k)VO_2/(Q_{IVC} \cdot Cap_{O_2}) \qquad \textit{Equation 4.5}$$

$$= Spv_{O_2} - ((1\text{-}k)VO_2/Cap_{O2}) \left(\frac{1}{Q_{SVC}} + \frac{1}{Q_{IVC}} \right)$$

Mathematical analysis

The ultimate aim is to maximise tissue oxygen tension. An increase in cardiac output, a decrease in total body metabolism, or an increase in oxygen carrying capacity would clearly increase oxygen tension. However, the effect on oxygenation of changes in metabolic balance between upper and lower parts of the body, and changes in blood flow to these two parts, is less obvious.

Differential calculus provides simple and well-validated methods for analysing the behaviour of such complex systems of equations without a computer. Differential calculus was applied to the formulae above, to study the effect of changes in the proportion of metabolism occurring in the upper body (k) and changes in the proportion of blood flow to the upper body (Q_{SVC}/CO) on total oxygen delivery to the systemic circulation, and on saturation in the systemic arteries and superior and inferior vena cava.

The effect on the conventional parameters of oxygen status (140): total oxygen delivery, upper-body oxygen delivery, lower-body oxygen delivery, and the oxygen saturations SaO_2, S_{SVCO2} and S_{IVCO2} was examined. Of these, the last two may best represent tissue oxygen status, since they refer to blood that has been in equilibrium with tissues. However, choosing between them is not straightforward. S_{SVCO2} is the lower of the two in some

circumstances, and S_{IVCO2} in others. Therefore a novel parameter $Smin_{O2}$, which we defined as the lower of these two values was considered.

Graphical display

As an alternative method of displaying the results to the symbolic mathematics, a computer was used to generate graphs showing the effect of changes in metabolic and flow balance on oxygen status. Computer calculations are not as general as the analytical approach because specific values must be chosen. For the purpose of this study 0.2 l/kg/min for cardiac output and 0.009 l/kg/min for oxygen consumption, which are suitable approximations for the post-surgical situation in neonates (140,141) were used. Oxygen carrying capacity of blood was taken as 0.207 l O_2/l blood, based on conventional values (140) of 1.38•10-3 l O_2 per gram haemoglobin, and a haemoglobin concentration of 150 g/l. Pulmonary venous saturation was taken as 98% (142). It should be remembered that these specific values were used only in order to obtain specific oxygen saturations for the purposes of graph plotting. The conclusions derived from the calculus analysis apply, in contrast, to all possible values of these four variables.

4.2.2. Results

Effect of changes in metabolic and flow balance on arterial parameters

The four conventional arterial parameters of oxygenation are SaO_2, total oxygen delivery ($CO \cdot CapO_2 \cdot SaO_2$) (140) and its two components upper body oxygen delivery and lower body oxygen delivery. Since total oxygen delivery is SaO_2 multiplied by a constant factor ($CO \cdot CapO_2$), studying the

effect on SaO_2 is sufficient to determine the effect of flow distribution and metabolic balance on total oxygen delivery.

Inspection of equation 4.4 reveals that SaO_2 is a linear function of $(k-1)/Q_{SVC}$. As k is always ≤1, a rise in k or a rise in Q_{SVC} always causes a rise in SaO_2. For any given distribution of metabolism (k), SaO_2 is therefore maximal when Q_{SVC} is maximal (Figure 4.2). Therefore complete diversion of all the blood through the upper part of the body, with none to the lower part, achieves the greatest SaO_2 (and thus the greatest total oxygen delivery).

Upper body oxygen delivery ($Q_{SVC} \cdot CapO_2 \cdot SaO_2$) equals, by application of equation 4.4, $Q_{SVC} \cdot CapO_2 \cdot SpvO_2 - (1-k)\ O_2$. This expression increases linearly with Q_{SVC} and with k, and so again for any k, it is maximal when Q_{SVC} is maximal (see Figure 4.4), i.e. when all the flow goes to the upper body, and no blood to the lower.

Lower body oxygen delivery ($Q_{IVC} \cdot CapO_2 \cdot SaO_2$) equals, by application of equation 4.4, $(CO - Q_{SVC}) \cdot CapO_2 \cdot SpvO_2 - (1\ k)\ O_2 \cdot (CO/Q_{SVC} - 1)$. Applying calculus, the derivative of this expression with respect to Q_{SVC} is $(1-k)\ O_2 \cdot CO/Q_{SVC}^2 - CapO_2 \cdot SpvO_2$. For maximal lower body oxygen delivery, this derivative must be zero, i.e. the proportion of blood flowing to the upper body is:

$$\frac{Q_{SVC}}{CO} = \sqrt{\frac{(1-k)\cdot \dot{V}_{O_2}}{CO \cdot Cap_{O_2} \cdot Spv_{O_2}}} \qquad \textit{Equation 4.6}$$

The dependence of lower body oxygen delivery on the distribution of flow and metabolism is shown in Figure 4.4. The highest achievable lower-body oxygen delivery (for a given k) is marked with a dot. These dots form a

parabola, which corresponds to equation 4.6. As k increases, the Qs/CO fraction required falls, until it reaches zero when k=1.

Effect of changes in metabolic and flow balance on venous parameters

There are two conventional venous oxygenation parameters: S_{SVCO2} and S_{IVCO2}. S_{SVCO2} represents blood that has recently been in equilibrium with the tissues of the upper part of the body: its value therefore gives useful information about tissue oxygenation in that region. Equation 4.3 shows that S_{SVCO2} is independent of k, and increases with increasing Q_{SVC}. It is therefore maximal when all the blood flows through the upper body (see Figure 4.3), regardless of any other physiological variables.

S_{IVCO2} represents blood that has recently been in equilibrium with the tissues of the lower body: its value therefore provides information about lower-body tissue oxygenation. From equation 4.5, we can see that S_{IVCO2} is a non-linear function of k and blood flow distribution, whose maximum point may not be obvious by inspection. By calculus, its first derivative with respect to Q_{SVC} is

$$(1-k)\frac{\dot{V}_{O_2}}{Cap_{O_2}}\left(\frac{1}{Q_{SVC}^{\ 2}}-\frac{1}{Q_{IVC}^{\ 2}}\right) \qquad \textit{Equation 4.7}$$

For S_{IVCO2} to be maximal, this derivative must be zero; this occurs when $Q_{SVC} = Q_{IVC}$, i.e. half the flow passes to each part of the circulation. Again, this is true for any value of k and of the other parameters (see Figure 4.3).

Since S_{SVCO2} and S_{IVCO2} peak at different flow balances, the question might arise: which of them is more important? Both are important, and that in any scenario the lower of the two values represents the greater degree of tissue hypoxia. Therefore a parameter $SminO_2$ was defined which takes the value

of S_{SVCO2} when $S_{SVCO2} < S_{IVCO2}$, and of $S_{IVCO}2$ when $S_{IVCO2} \leq S_{SVCO2}$. From equations 4.3 and 4.5, it can be seen that the former situation occurs when

$$\frac{\dot{V}_{O_2}}{Cap_{O_2}}\left(\frac{1}{Q_{SVC}}\right) \geq (1-k)\frac{\dot{V}_{O_2}}{Cap_{O_2}}\left(\frac{1}{Q_{SVC}}+\frac{1}{Q_{IVC}}\right) \qquad \textit{Equation 4.8}$$

i.e.

$$\frac{1}{Q_{SVC}} \geq (1-k)\frac{Q_{SVC}+Q_{IVC}}{Q_{SVC}\cdot Q_{IVC}} \qquad \textit{Equation 4.9}$$

or $\frac{Q_{IVC}}{CO} \geq (1-k)$, which means $Q_{SVC}/CO \geq k$. In other words, where the fraction of blood perfusing the upper body is less than the fraction of metabolism occurring in the upper body, $SminO_2$ is S_{SVCO2}, otherwise it is S_{IVCO2}.

Thus the situations are markedly different depending on whether k is above or below 0.5. For $k \leq 0.5$, $SminO_2$ is maximised when flow is equally distributed to the upper and lower parts of the body, while for $k \geq 0.5$, $SminO_2$ is maximised by distributing flow in proportion to metabolism (Figure 4.4).

4.3. Atrial Right-to-Left Shunting in Pulmonary Arterial Hypertension and its Effect on Tissue Oxygenation and Systemic Blood Flow

Atrial right-to-left shunting occurs in patients with an atrial septal defect and pulmonary hypertension (e.g. Eisenmenger syndrome) and it has been argued that the interatrial communication may function as a "pop-off" valve, allowing to decompress the right ventricle and to maintain systemic blood flow when metabolic demand is increased (143), for example during exercise and pulmonary arterial hypertensive crises, albeit at the expense of cyanosis

(144). In addition, the artificial creation of an "atrial septal defect" – i.e. balloon atrial septostomy - has been proposed as a therapy option for highly symptomatic patients with pulmonary arterial hypertension refractory to advanced vasodilator therapy (145-147). The artificially created, atrial septostomy has been shown to improve haemodynamics and quality of life and prolong survival (145,148-152).

It is not clear whether the benefits from atrial right-to-left shunting in the setting of pulmonary arterial hypertension arise entirely from increased blood flow (i.e. preservation of an adequate perfusion pressure), or whether improved oxygen transport contributes. There is no doubt that systemic blood flow increases and arterial oxygen saturation falls after atrial septostomy. It is unclear whether the increase in blood flow is adequate to compensate for the decrease in oxygen saturation: it is therefore not known whether tissue oxygen status improves, stays the same, or deteriorates. Clinical studies have observed an increase in calculated arterial oxygen delivery (145), and this is currently widely accepted as evidence of improved tissue oxygen status (145,150,153). However, oxygen delivery is potentially flawed in patients with abnormal circulatory connections.
Therefore a modeling study into the effect of right-to-left shunting on useful oxygen delivery, mixed venous oxygen content and systemic cardiac output in patients with pulmonary arterial hypertension and fixed pulmonary blood flow was performed.

4.3.1. Methods

A standard model of the circulation in the presence of a right to left shunt on atrial level was used, as shown in Figure 4.5 and explained in the legend. In the presence of a right-to-left shunt systemic blood flow (Q_S) represents the sum of pulmonary blood flow (Q_P) and right-to-left shunt volume (Q_{RL}). As a consequence of admixture of venous blood bypassing the lungs to pulmonary venous blood, arterial oxygen content (Ca_{O2}) is the weighted mean of mixed venous oxygen content and pulmonary venous oxygen content::

$$C_{aO2} = \frac{C_{MVO2} \cdot Q_{RL} + C_{PVO2} \cdot Q_P}{Q_{RL} + Qp} \qquad \textit{Equation 4.10}$$

C_{aO2} = arterial oxygen content; C_{MVO2}=mixed venous oxygen content; C_{PVO2} = pulmonary venous oxygen content; Q_p = pulmonary blood flow; Q_{RL} = right to left shunt.

Mixed venous oxygen saturation represents the difference between arterial oxygen content and the amount of oxygen removed at tissue level:

$$C_{MVO2} = Ca_{O_2} - \frac{VO_2}{Q_S} \qquad \textit{Equation 4.11}$$

C_{MVO2}=mixed venous oxygen content; C_{PVO2} = pulmonary venous oxygen content; Q_p = pulmonary blood flow; VO_2 = total body oxygen consumption

By combining the equations for C_{aO2} and C_{MVO2} given above systemic venous oxygen content (C_{MVO2}) can equally be expressed as:

$$C_{MVO2} = Cpv_{O_2} - \frac{VO_2}{Qp} \qquad \textit{Equation 4.12}$$

Arterial oxygen C_{aO2} content was calculated by combining *Equation 4.10* and *4.11* to eliminate C_{MVO2} from the equation and solving for C_{aO2} as follows:

1. $C_{aO2}(Q_{RL}+Q_P) = \left(C_{PVO2} - \frac{VO_2}{Qp}\right) \cdot Q_{RL} + C_{PVO2} \cdot Q_P$

2. $C_{aO2}(Q_{RL}+Q_P) = Cpv_{O_2} \cdot Q_{RL} - \frac{VO_2}{Qp} \cdot Q_{RL} + C_{PVO2} \cdot Q_P$

3. $C_{aO2}(Q_{RL}+Q_P) = Cpv_{O_2} \cdot (Q_{RL} + Q_P) - \frac{VO_2}{Qp} \cdot Q_{RL}$

after dividing by $(Q_{RL}+Q_P)$:

4. $C_{aO2} = Cpv_{O_2} - \left(\frac{Q_{RL} \cdot VO_2}{Qp \cdot (Q_{RL} + Qp)}\right)$ *Equation 4.13*

C_{aO2} = arterial oxygen content; C_{PVO2} = pulmonary venous oxygen content; Q_p = pulmonary blood flow; Q_{RL} = right to left shunt; VO_2 = total body oxygen consumption

Oxygen content represents the product between oxygen saturation, haemoglobin concentration and oxygen carrying capacity of blood ($\kappa = 1.38 \times 10^{-3}$ L of O_2 per gram of haemoglobin) (140).

Therefore, arterial oxygen saturation can be calculated by dividing *Equation 4.13* by ($\kappa \cdot$ haemoglobin concentration):

$$Sat_{aO2} = Sat_{PVO2} - \left(\frac{Q_{RL} \cdot VO_2}{\kappa \cdot Hb \cdot Qp \cdot (Q_{RL} + Qp)}\right) \quad \textit{Equation 4.14}$$

Sat_{aO2} = arterial oxygen saturation; Sat_{PVO2} = pulmonary venous oxygen saturation; Q_p = pulmonary blood flow; Q_{RL} = right to left shunt; Hb = haemoglobin concentration; □ = oxygen carrying capacity of blood; VO_2 = total body oxygen consumption

To illustrate the effect of changes in distribution of pulmonary blood flow, total body oxygen consumption and left ventricular output on arterial and systemic mixed venous oxygen content, a computer model was developed using commercial mathematical modeling software (Matlab 6.5, Mathworks, USA and R version 2.6.1, R Foundation for Statistical Computing, Vienna, Austria). If not otherwise stated oxygen carrying capacity of blood was taken

as 0.207 l O_2/l blood, based on conventional values of $1.38 \cdot 10^{-3}$ L O_2 per gram haemoglobin, and a haemoglobin concentration of 150 g/l (140).

4.3.2. Results

Effect of Changes in Right-to-Left Shunting and Pulmonary Blood Flow on Arterial Oxygen Saturation

The impact of different combinations of pulmonary blood flow and right-to-left shunting on arterial oxygen saturations was examined. For any given combination of pulmonary blood flow and VO_2, arterial oxygen saturation decreases with increasing degree of right-to-left shunting as illustrated in Figure 4.6. Figure 4.6B also demonstrates that for low values of pulmonary blood flow even small increases in shunt volume drastically reduce arterial oxygen saturation, whereas at higher levels of pulmonary blood flow arterial oxygen saturations are relatively independent of changes in shunt volume.

Impact of Changes in Right-to-Left Shunt Volume on Hemodyanamic Parameters and Arterial Oxygen Delivery

The effects of varying right-to-left shunt fractions on systemic cardiac output, arterial blood pressure, arterial oxygen saturation and oxygen delivery in the presence of fixed pulmonary blood flow were studied. As systemic blood flow represents the sum of pulmonary blood flow and right-to-left shunt volume any increase in shunt volume will lead to a linear increase in systemic cardiac output. If total systemic vascular resistance remains constant, arterial blood pressure (representing the product between systemic blood flow and

systemic vascular resistance) will increase in proportion to increased systemic blood flow.

Exploring the relationship between shunt volume and arterial oxygen delivery reveals that arterial oxygen delivery (D_{aO2}) increases in a linear fashion with increasing degree of right-to-left shunting (*Equation 4.15)*:

$$Da_{O2} = \underbrace{(Cpv_{O_2} - \frac{VO_2}{Qp})}_{} \cdot Q_{RL} + Q_P \cdot Cpv_{O_2}\text{; as } (Cpv_{O_2} - \frac{VO_2}{Qp}) = C_{MVO2} \text{ this}$$

is identical to

$$Da_{O2} = (C_{MVO2} \cdot Q_{RL}) + (Q_P \cdot Cpv_{O_2})$$

Da_{O2} = arterial oxygen delivery; C_{PVO2} = pulmonary venous oxygen content; Q_p = pulmonary blood flow; Q_{RL} = right to left shunt; VO_2 = total body oxygen consumption; ≡ identical to

The relationship between right-to-left shunt volume and arterial oxygen content is illustrated in Figure 4.7.

Impact of Changes in Right-to-Left Shunt Volume on mixed venous oxygen content

This modeling study indicates that in contrast to arterial oxygen delivery mixed venous oxygen content (which mirrors tissue oxygen tension) is independent of the degree of right-to-left shunting and therefore is unaffected by an increased shunt fraction (as demonstrated by *Equation 4.12* and illustrated in Figure 4.7).

Impact of right ventricular dysfunction on oxygen status

Adequate right ventricular function is critical in maintaining pulmonary blood flow. If the right ventricle is unable to increase its output to match pulmonary blood flow then this in itself can become the limiting factor to oxygenation. As shown in Figure 4.8 any reduction in right ventricular output impairs arterial oxygen delivery and mixed venous oxygen saturation.

Lower limit of pulmonary blood flow for any given oxygen consumption and oxygen carrying capacity

To maintain adequate tissue oxygenation a minimal pulmonary blood flow is required for any given combination of oxygen consumption, oxygen carrying capacity and the ability of peripheral organs to extract oxygen. As illustrated in *Equation 4.16,* minimal required pulmonary blood flow ($Q_{P\ Limit}$) is independent of the degree of right-to-left shunting. In fact, the minimal required pulmonary blood flow is determined by the ratio between oxygen consumption, the product of haemoglobin concentration, κ (=1.38 · 10-3 L of O_2 per gram of haemoglobin) and the difference between pulmonary venous saturation and the minimal mixed venous oxygen saturation achievable ($Sat_{MVO2min}$). Reducing oxygen consumption, augmenting the ability of tissue to utilize oxygen (i.e. reducing $Sat_{MVO2min}$) and increasing haemoglobin concentration will reduce minimal required pulmonary blood flow.

$$Qp_{Limit} \geq \frac{VO_2}{Hb \cdot \kappa \cdot (Sat_{PVO2} - Sat_{MVO2min})} \qquad \textit{Equation 4.16}$$

$Sat_{MVO2min}$ = minimal mixed venous saturation; Sat_{PVO2} = pulmonary venous oxygen saturation; Q_p = pulmonary blood flow; Hb = haemoglobin concentration; κ = oxygen carrying capacity of blood; VO_2 = total body oxygen consumption

Impact of Haemoglobin Concentration on Arterial Oxygen Delivery and Tissue Oxygen Status

Exploring the relationship between haemoglobin concentration and arterial oxygen delivery reveals that arterial oxygen delivery (D_{aO2}) increases in a linear fashion with increasing haemoglobin concentration (Hb). Re-arranging *Equation 4.15* and replacing C_{PVO2} by $Sat_{pvO_2} \cdot \kappa \cdot Hb$ yields:

$$Da_{O2} = [(Sat_{pvO_2} \cdot \kappa \cdot (Q_{RL} + Q_P)] \cdot Hb - \frac{Q_{RL} \cdot VO_2}{Qp} \qquad \textit{Equation 4.17}$$

Da_{O2} = arterial oxygen delivery; Sat_{PVO2} = pulmonary venous oxygen saturation; Q_p = pulmonary blood flow; Q_{RL} = right to left shunt; VO_2 = total body oxygen consumption; Hb = haemoglobin concentration; κ = oxygen carrying capacity of blood

Therefore, for any given combination of pulmonary venous oxygen saturation (Sat_{PVO2}), oxygen carrying capacity (κ) and systemic blood flow (Q_P+Q_{RL}), arterial oxygen content increases in proportion to haemoglobin concentration with a slope of $[(Sat_{pvO_2} \cdot \kappa \cdot (Q_{RL} + Q_P)]$.

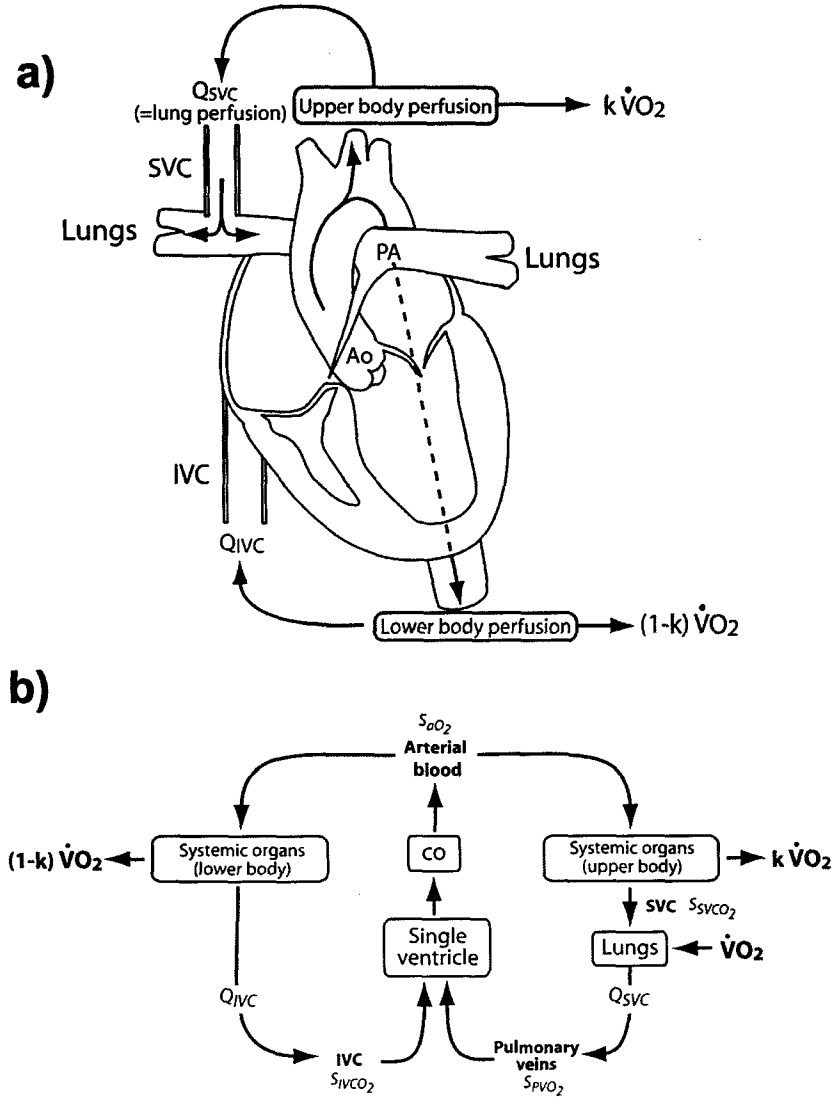
In addition, mixed venous oxygen concentration increases with increasing haemoglobin concentration:

$$Sat_{MVO2} = Satpv_{O_2} - \frac{VO_2}{\kappa \cdot Hb \cdot Qp} \qquad \textit{Equation 4.18}$$

Sat_{MVO2} = mixed venous saturation; Sat_{PVO2} = pulmonary venous oxygen saturation; Q_p = pulmonary blood flow; Hb = haemoglobin concentration; κ = oxygen carrying capacity of blood; VO_2 = total body oxygen consumption

As a consequence decreases in Qp can be offset by a proportional increase in haemoglobin concentration (*Equation 4.18)* as shown in Figure 4.9:

4.4. Figures



VO_2 is the total body oxygen consumption. Systemic organs of the upper part of the body consume a proportion k of this (k VO_2), and their blood flow Q_{SVC} drains into the superior vena cava (SVC) whose oxygen saturation is S_{SVCO2}. Organs of the lower part of the body are responsible for the remainder, (1–k) VO_2, of the body's oxygen consumption and their blood flow Q_{IVC} drains into the inferior vena cava (IVC) whose saturation is S_{IVCO2}. SaO_2 and $SpvO_2$ represent the saturations in systemic arteries and pulmonary veins respectively.

Figure 4.1: Model of the circulation in patients with functionally univentricular hearts with a bidirectional cavopulmonary anastomosis shown as a cartoon (upper panel) and as a mathematical schematic (lower panel).

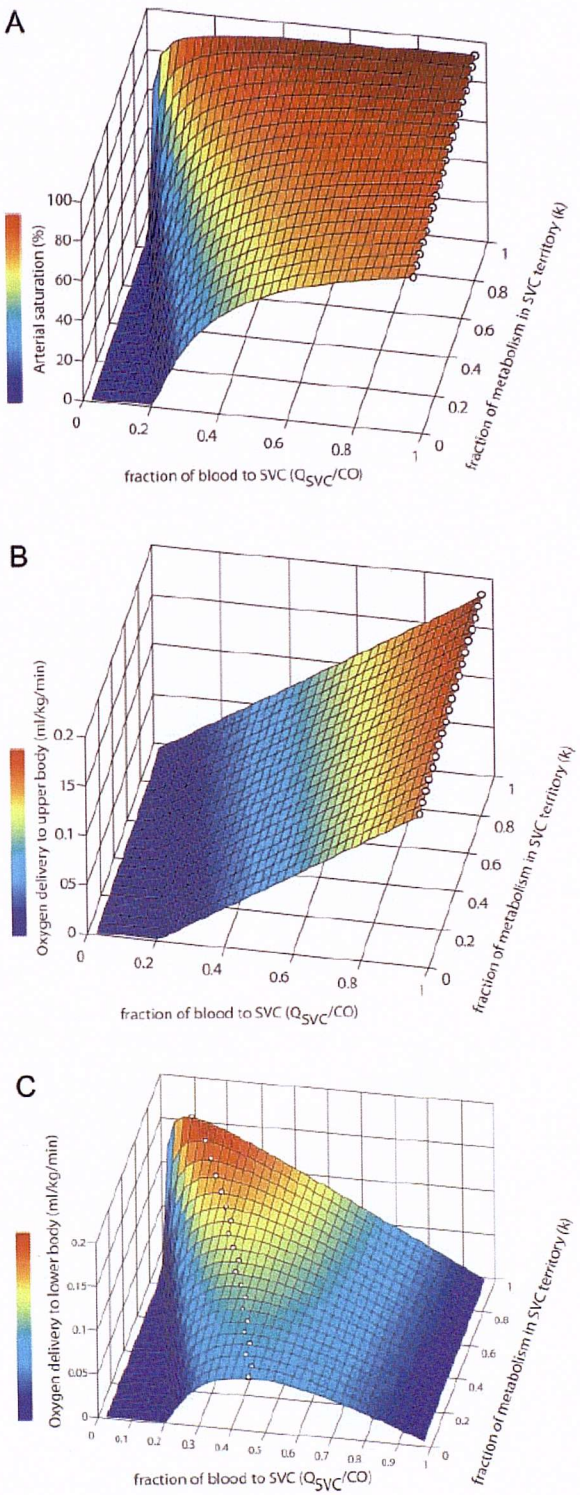


Figure 4.2: Effects of changes in metabolic and flow balance on arterial parameters: a) arterial saturation; b) upper-body oxygen delivery; c) lower body oxygen delivery

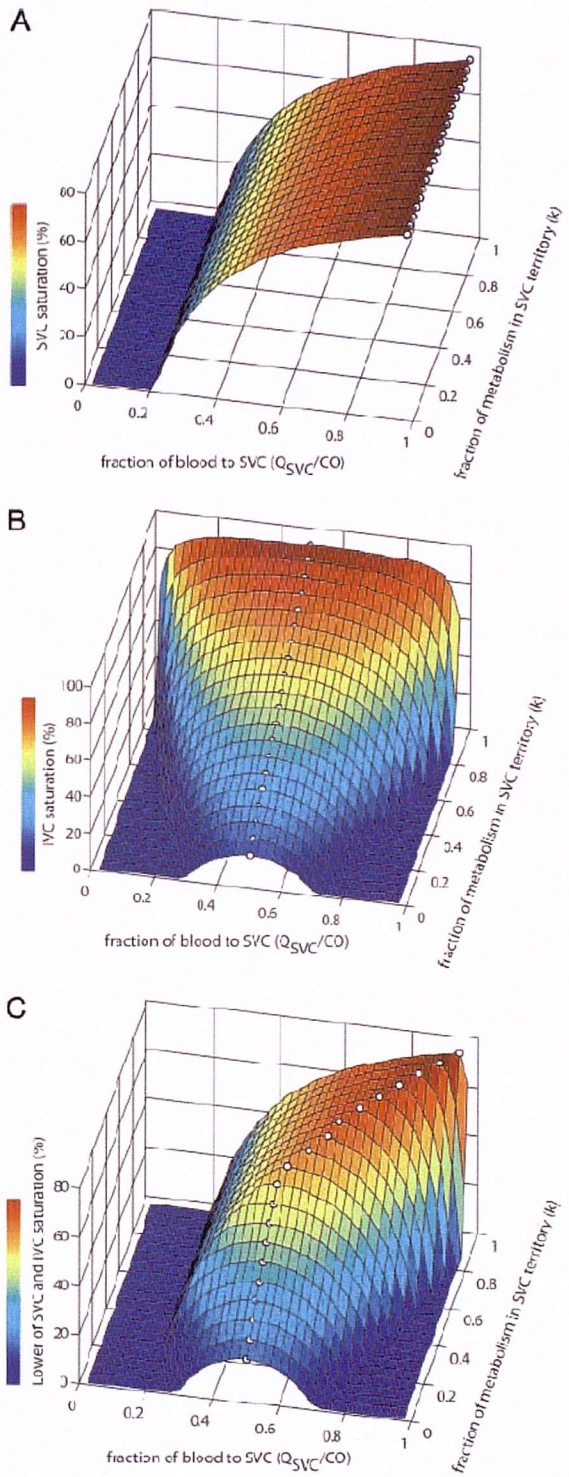


Figure 4.3: Effects of changes in metabolic and flow balance on venous oxygenation parameters: a) superior vena cava saturation; b) inferior vena cava saturation; c) So_2min, defined as the lower of these two.

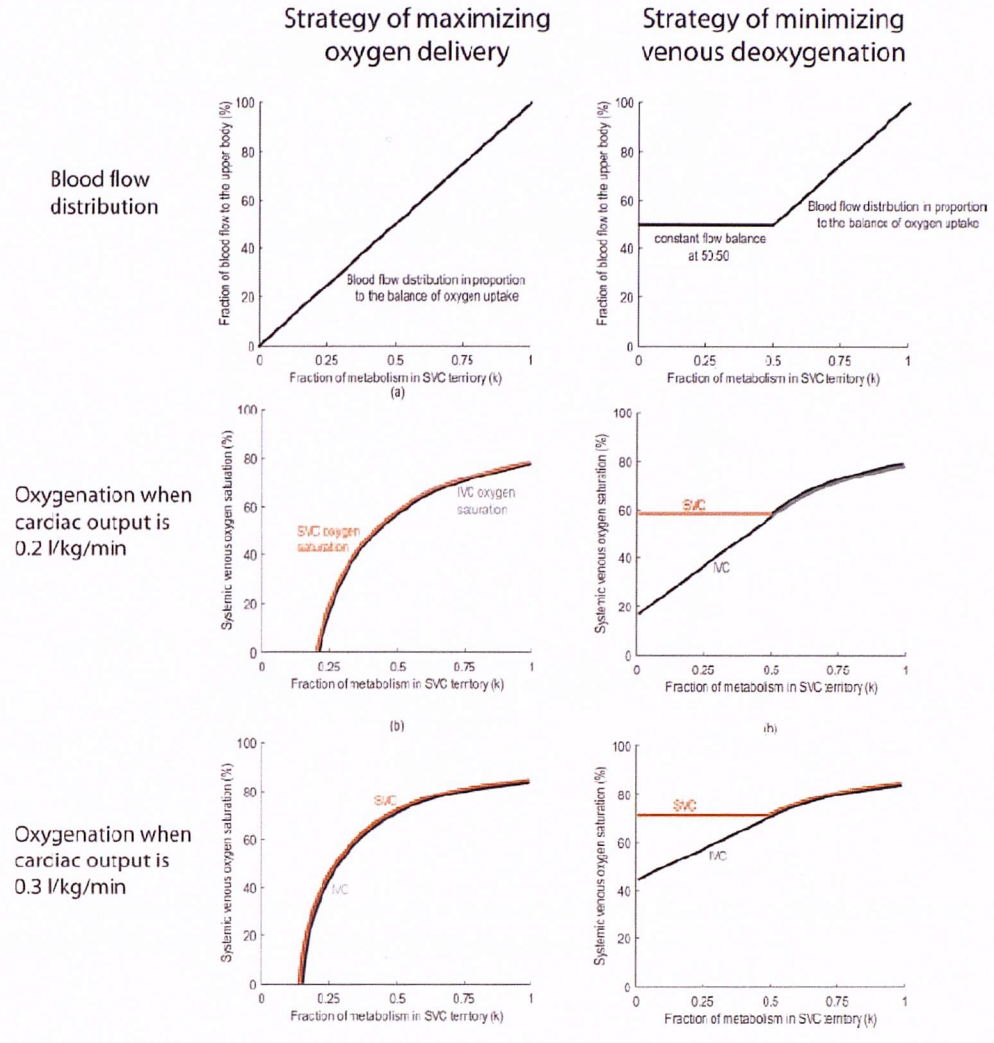
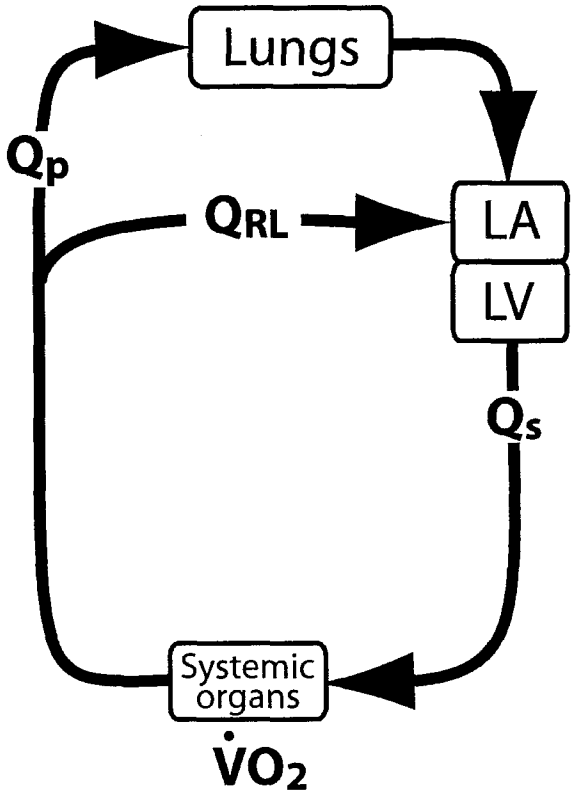


Figure 4.4: Difference between two strategies for optimising tissue oxygenation with a cardiac output of 0.2 l/kg/min (middle panels) and with a cardiac output of 0.3 l/kg/min (lower panels). In each panel, the lower curve represents the effect of a distribution of blood flow in proportion to oxygen uptake in the upper and lower body; the straight lines represent the effect of maintaining equal flows to upper and lower body whenever k<0.5.



LA = left atrium, LV = left ventricle; Q_p = pulmonary blood flow; Q_{RL} = right to left shunt; Q_S = systemic blood flow; VQ_2 = total body oxygen consumption.

Figure 4.5: Model of the circulation in the presence of a right to left shunt on atrial level.

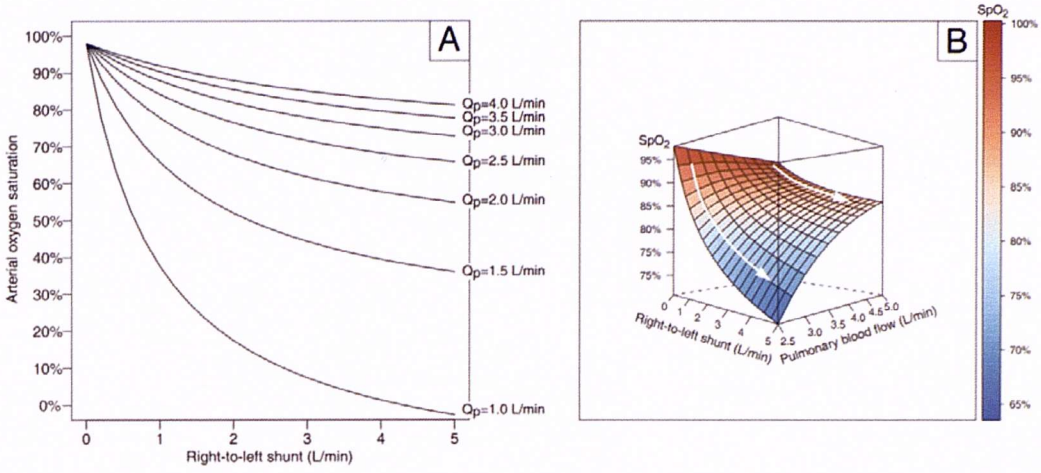


Figure 4.6: A) Arterial oxygen saturation (%) as a function of right-to-left shunt (QRL) for a given total body oxygen consumption (taken as 300 ml/min for this example) and different values of pulmonary blood flow (QP). **B)** Arterial oxygen saturation for different combinations of pulmonary blood and right-to-left shunt. The arrows demonstrate that the same increase in right-to-left shunting causes a bigger decrement in oxygen saturations when pulmonary blood flow is lower.

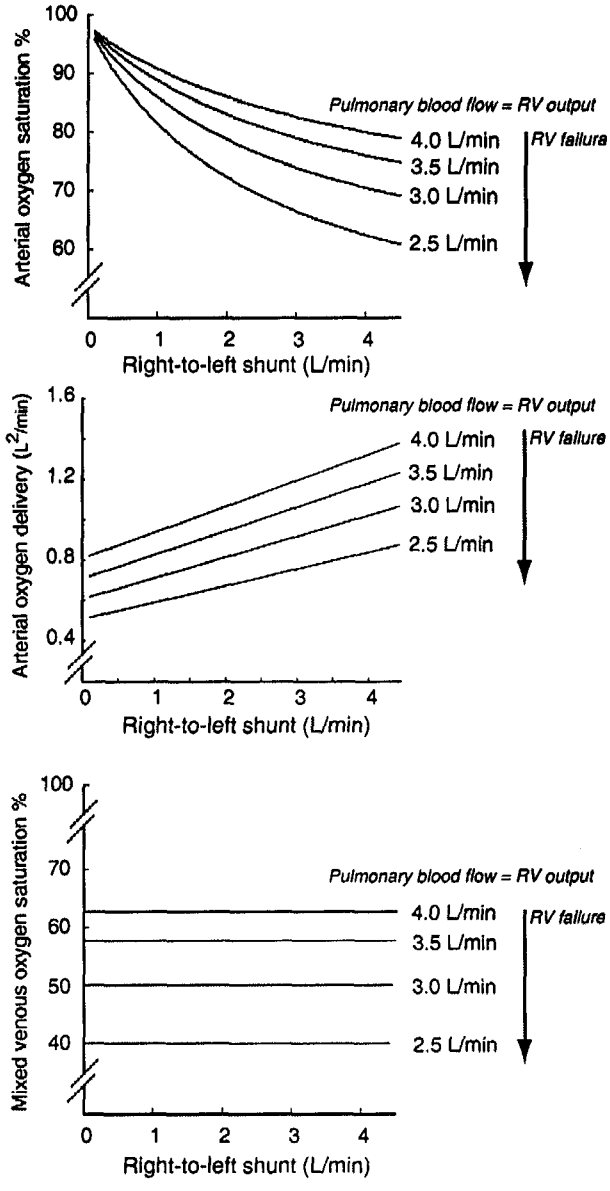


Figure 4.7: Arterial oxygen saturation, arterial oxygen delivery and mixed systemic venous oxygen saturation as a function of right-to-left shunt for a given total body oxygen consumption (taken as 300 ml/min in this example) and different values of pulmonary blood flow. Note that despite a drop in arterial oxygen saturation, arterial oxygen delivery is augmented and mixed venous oxygen content is unaffected as right-to-left shunt increases. RV = right ventricle.

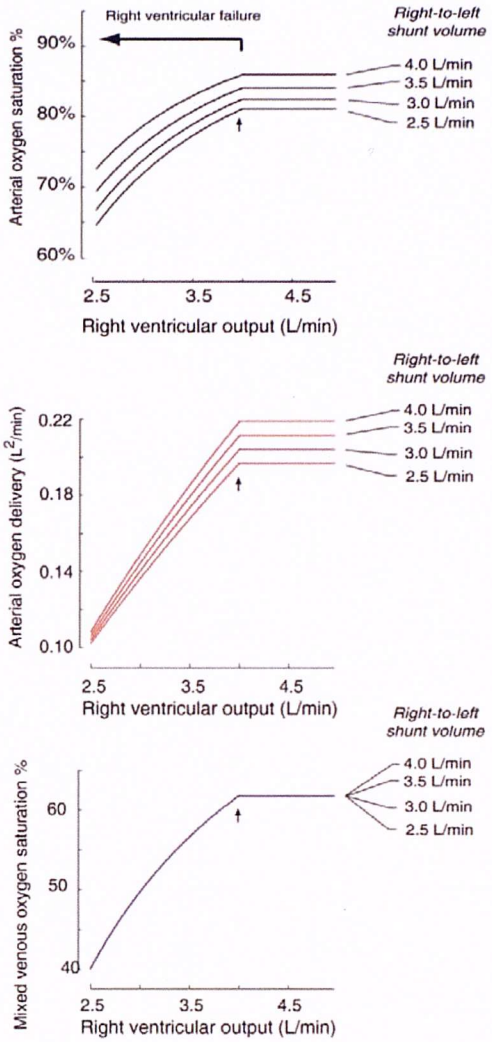


Figure 4.8: Effect of right ventricular output on oxygenation response. Arterial oxygen saturation (black), arterial oxygen delivery (red) and systemic venous oxygen saturation (blue) as a function of right ventricular output for a given total body oxygen consumption (taken as 300 ml/min in this example) and different values of right-to-left shunt volume. If the right ventricle is unable to increase its output to match pulmonary blood flow then this in itself can become the limiting factor to oxygenation: In the figures above, to the right of the arrows it is the pulmonary blood flow that is limiting, and to the left of the arrow it is right ventricular output that is limiting oxygen status.

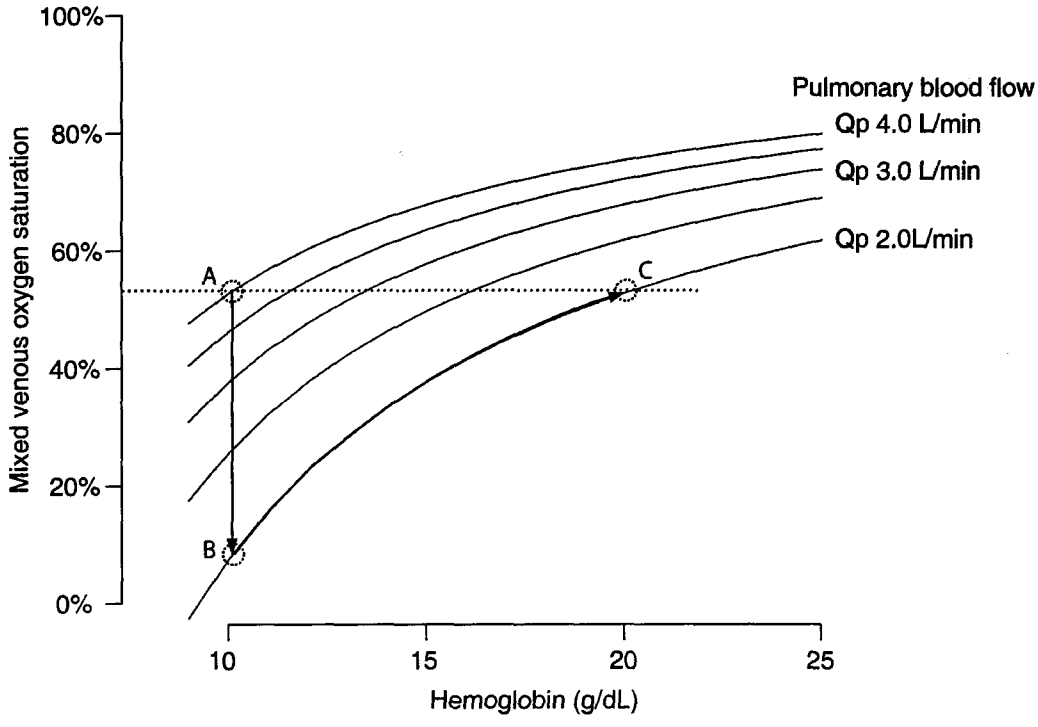


Figure 4.9: Effect of haemoglobin concentration on mixed venous oxygen saturation. A reduction (from 4 L/min to 2 L/min in this example [segment AB]) in pulmonary blood flow is offset by a proportional increase in haemoglobin concentration (from 10 g/dL to 20 g/dL in this example [segment BC]).

4.5. Discussion

Oxygen delivery to tissue represents a complex and poorly understood phenomenon in various forms of congenital heart disease such as patients with univentricular circulation or shunt lesions. This is due to multiple interacting parameters affecting oxygen transport in this setting. Therefore, simple intuition cannot determine the effect that a change in one physiological measure will have on another. Mathematical models provide a way of assessing the independent effect of individual parameters. A mathematical model is a simplified abstract view of the complex reality. Such models are typically used when it is impractical to create experimental conditions to measure outcomes directly. As no adequate animal models of complex cyanotic heart disease exist and it is unethical to subject patients with these conditions to potentially dangerous interventions to manipulate and measure parameters of oxygen transport, we contend that mathematical modeling represents an ideal way to study the complex pathophysiologic changes underlying impaired oxygen delivery and ultimately exercise intolerance in this cohort.

The bidirectional cavopulmonary anastomosis represents a modification of the classical Glenn shunt introduced 50 years ago and is commonly used in the staged palliation of patients with univentricular circulation (154). In this circulation pulmonary blood flow is supplied exclusively by venous return from the upper body via the superior vena cava. Both the classical and the bidirectional cavopulmonary shunt improve systemic arterial oxygen saturation by augmenting the effective pulmonary blood flow without

increasing total pulmonary blood flow and without volume loading the ventricle (155). The bidirectional cavopulmonary anastomosis was chosen for this modeling study because it is at the extreme end of the spectrum of "unphysiologic" circulatory connections and represents an inherently inefficient circulation uncoupling lower body perfusion from pulmonary blood flow.

This study examined the effect of the bidirectional cavopulmonary anastomosis on oxygenation using a quantitative model of the circulation and oxygen saturation. It developed the known relationships between physiological variables into equations suitable for analytical solution by differential calculus, which allowed consideration of all conditions of cardiac output, metabolic rate, oxygen carrying capacity and pulmonary venous saturation simultaneously.

The main findings of this study are: (1) conventional arterial parameters (arterial oxygen saturation, total oxygen delivery, upper and lower body oxygen deliveries) are poor markers of tissue oxygenation in these patients; (2) venous saturation, which represents tissue oxygenation most closely, differs between the upper and lower body. Since neither is consistently lower than the other, a parameter $SminO_2$ can be defined as the lower of the two, representing the degree of hypoxia in whichever part of the body is more hypoxic; (3) when the majority of the metabolism is in the upper body, $SminO_2$ is maximised when the flow distribution matches the metabolic distribution (and the two venous saturations are equal). In contrast, when the majority of the metabolism is occurring in the lower part of the body, $SminO_2$

is optimised by an equal distribution of blood flow between the upper and lower body regardless of the exact ratio of metabolic rates.

Difficulties with arterial parameters of oxygenation

Arterial parameters are an initially attractive choice in attempting to quantify oxygenation. Due to its ease of measurement arterial oxygen saturation is commonly used in clinical settings, but it gives at best an incomplete picture. It peaks when all the blood is diverted to the upper body, and the lower body is starved of oxygen, which is not a clinically desirable outcome. Total oxygen delivery to systemic tissues has also been considered as a variable to be maximised, yet for any given cardiac output, it is simply proportional to arterial saturation, and so this choice would also favour a complete diversion of blood to the upper body, regardless of the status of the lower body. Choosing one of the two parts of the systemic circulation individually offers no advantage: maximising upper body oxygen delivery would require diverting all the blood to the upper body; maximising lower body oxygen delivery, although plausible at low values of k, would require diverting almost all the blood to the lower body at higher values of k, which represent relatively less metabolism in the lower body. Since the two partial-body oxygen delivery parameters favour discordant and clinically unsuitable flow distributions, no oxygen delivery parameter is suitable for maximisation in seeking to optimise tissue oxygenation in a circulation with a cavopulmonary anastomosis.

Previous considerations of the functionally univentricular circulation (156) and its partially corrected states (140) have concentrated on oxygen delivery

as a useful parameter to be maximised. Francis et al. questioned this principle in this setting (156), since oxygen delivery takes no account of the quality of oxygen at the point of its transfer into tissues: it implicitly assumes that a halving of arterial oxygen saturation can be fully compensated for by a doubling of flow, a clinically unacceptable assumption.

Lessons from venous parameters of oxygenation

Venous blood has returned from tissues with which it has recently been in equilibrium: its oxygen content offers useful information about tissue oxygenation not available from arterial parameters. This study shows that upper and lower body venous saturations (and therefore tissue oxygenation states) are different. Neither is always lower, and thus neither can be used in isolation as a summary of tissue oxygenation. This modeling study suggests that attention should be concentrated on whichever of these two parts of the systemic circulation is suffering the greater hypoxia. The variable $SminO_2$ was constructed to reflect this.

A key finding of this modeling study is that in situations when the lower body is responsible for the majority of the oxygen consumption, an equal distribution of flow between upper and lower body optimises $SminO_2$. In contrast, when the majority of metabolism is in the upper body, a distribution of flow in proportion to metabolic rate is preferable.

Does matching oxygen delivery to oxygen consumption optimise oxygenation?

Although maximisation of oxygen delivery may be unwise, a possible alternative would be to distribute flow in proportion to the balance of oxygen uptake. Previous theoretical work has proposed this to be optimal (in the situation where k=0.6) (140). The results of the current study support this choice, but observes that it is valid only as long as k remains in excess of 0.5.

For values of k below 0.5, our study indicates that such a choice would cause unnecessarily poor oxygenation in both upper and lower circulations. Figure 4.4 illustrates the difference between a strategy of matching oxygen delivery to consumption (which equalises SVC and IVC saturation, shown as circles and crosses superimposed) and a modified strategy of holding the flow balance constant at 50:50 when k falls below 0.5 (resulting in different oxygen saturations in SVC (circles) and IVC (crosses)). There is a clear difference in the tissue oxygenation achieved which is even larger when cardiac output is higher (0.3 l/kg/min, shown in Figure 4.4.

Oxygen delivery or tissue oxygenation?

The concept of oxygen delivery was originally advanced in high risk adult surgical patients without congenital heart disease as a means of combining into a single parameter cardiac output, oxygen carrying capacity and oxygen saturation (simply by multiplying them together). Since increasing any one of them increases their product, and would intuitively be expected to be beneficial, oxygen delivery is attractive as a summary of overall oxygenation

(137). Survivors were found to have higher oxygen deliveries than non-survivors which has led to the hypothesis that elevating oxygen delivery to supranormal levels may confer survival benefit. Although randomised controlled trials results of this intervention have had conflicting results (138,157) a consensus seems to have developed that when oxygen delivery is low, interventions to raise it may be worthwhile, while in those with normal oxygen deliveries, the benefits of elevating it supranormally are less convincing (158,159).

In congenital heart disease with a single functioning ventricle, however, there can be an unavoidable trade-off between blood flow and arterial oxygen saturation. This means that it becomes important how the summary parameter values the relative merits of flow and saturation: oxygen delivery considers them to be interchangeable. In such circumstances, therefore, oxygen delivery can be a seriously misleading measure of oxygenation (156).

Impact of bidirectional cavopulmonary anastomosis on exercise capacity

The bidirectional cavopulmonary anastomosis is inherently inefficient, and becomes increasingly so with growth and maturation. Growth is associated with a relative increase in blood flow to the lower body. While in newborns the superior vena caval flow accounts for approx. 50% of the cardiac output, its contribution diminishes to 35% by the age of 6 years (160). During exercise lower body blood flow increases further (due to increased oxygen and metabolic demand). This requires augmented cardiac output and imposes additional strain on the univentricular heart. Maintaining an optimal

ratio between upper and lower body perfusion is – in theory - an alternative and may be most important in patients with limited cardiac output reserve. However, this may not be possible during exercise due to local tissue factors (such as lactate and acidosis) leading to local vasodilation and a disproportionate increase in lower body blood flow. Ultimately, therefore, the Glenn circulation becomes inadequate, and increasingly places limits on the level of exercise that can be achieved.

The only practical solution is definitive surgical completion of the Fontan circulation. These theoretical results are confirmed by previous studies showing that growth and maturation are inversely correlated with arterial oxygen saturation in this setting (161).

With a right-to-left shunt deoxygenated systemic venous blood returns directly to the systemic arterial circulation. The oxygen content of the systemic arterial blood is reduced in proportion to the volume of systemic venous blood mixing with the normal pulmonary venous return. With reduced oxygen content, even if cardiac output is normal, tissue oxygen delivery falls and the work capacity of the muscles is limited (162).

Studying patients with right-to-left shunt in the setting of fixed pulmonary blood flow this modelling study suggests that inducing right-to-left shunting in patients with fixed pulmonary blood flow (for example by creation of an atrial septostomy) increases raw systemic oxygen delivery for any given value of pulmonary blood flow. However, this is revealed to be an artefact of the way raw oxygen delivery is defined, which may not be appropriate for patients

with shunt lesions. In fact, mixed systemic oxygen saturation – arguably the most important index of tissue oxygenation – is not improved by right-to-left shunt on atrial level in this setting. These findings eliminate the possibility that globally improved oxygenation is a significant benefit of atrial septostomy. Rather, this study suggests that the clinical improvement reported in patients undergoing atrial septostomy must have another explanation, for example the greater leeway for flow redistribution available because of the increased systemic flow. In addition, our model suggests why patients with impaired ventricular function may be less able to derive clinical benefits from atrial septostomy.

Clinical studies have shown that atrial septostomy is safe and effective in patients with idiopathic pulmonary hypertension (145,146,151). It has been reported that syncope is abolished, functional class improves and right heart failure ameliorates in many patients. Furthermore, by transferring blood from right to left in patients with severe pulmonary hypertension it has the potential to attenuate right ventricular volume overload and improve left ventricular filling leading to improved biventricular function (146,150).
But beyond the immediate hemodynamic effect, whether oxygenation is improved is a difficult question to answer by qualitative reasoning alone. It is said that "oxygen transport to the tissues improves due to an increase in cardiac output, despite a modest reduction in systemic arterial oxygen saturation." as a result of atrial septostomy (146). This study does agree that atrial septostomy augments raw arterial oxygen delivery. However, the effect of simultaneously increasing systemic blood flow and reducing arterial

oxygen saturation on arterial oxygen delivery is not intuitively obvious and depends on the balance between these two factors. This study shows, that increased oxygen delivery does not coincide with improved tissue oxygenation in this setting. In fact, average tissue oxygenation (as quantified by venous oxygen status) is found to be entirely unaffected by the addition of the intracardiac shunt and in fact it is determined only by the ratio between total oxygen consumption and pulmonary blood flow.

One possible explanation for this is that therapeutic right-to-left shunting is effectively permitting blood that has recently left contact with body tissues to simply return for a second contact with body tissues. Unless there had been insufficient time for equilibrium to take place on the first visit, the second visit will, on average not result in any significant further transfer of blood gases. Since blood flow is only very rarely so fast that oxygen does not have time to equilibrate, and patients with this physiology are in fact at the opposite end of the blood flow velocity spectrum, it is very likely that the second circulation of blood in these patients does not assist oxygen transport.

It appears likely that the source of the clear clinical benefits of atrial septostomy must therefore lie elsewhere. Despite not improving tissue oxygenation, atrial septostomy augments systemic blood flow, potentially benefiting organs that lack the ability to autoregulate blood flow at low blood pressures. It has been reported that a mean arterial pressure of at least 60 mmHg is required to enable cerebral autoregulation (163). Interestingly, atrial septostomy has been demonstrated to be especially effective in abolishing

syncope. Atrial septostomy could protect against syncope by maintaining systemic cardiac output, particularly when the pulmonary arterial pressure rises acutely (146). In the situation of desperately poor systemic blood flow, autoregulation may not be able to successfully maintain flow to some vital territories - an increase in total flow could permit autoregulatory processes to restore perfusion to vital organs, even if the overall average tissue oxygen status is no better.

Clinical Implications

The concept of oxygen delivery is useful in patients without congenital heart disease as a simple means of combining cardiac output, oxygen carrying capacity and oxygen saturation into a single parameter (137). In patients with shunt lesions, however, there can be an unavoidable trade-off between blood flow and arterial oxygen saturation. In such circumstances, therefore, oxygen delivery can be a seriously misleading measure of oxygenation(156,164) and should be avoided.

Atrial septostomy does not improve average tissue oxygen status as defined by venous oxygenation. Rather, this study suggests that tissue oxygen status can be improved by increasing pulmonary blood flow (using advanced medical therapies), limiting maximal oxygen consumption (for example by discouraging extreme exertion), and improving blood oxygen carrying capacity (by maintaining adequate haemoglobin concentrations and avoiding iron-deficient anaemia in cyanotic patients) (28,165). This model also suggests that a critical pulmonary blood flow exists below which adequate

tissue oxygenation cannot be achieved. The exact value of this metabolically critical pulmonary blood flow depends on oxygen consumption, blood oxygen carrying capacity and the ability of tissue to extract oxygen - again emphasizing the need of adequate haemoglobin concentrations in these patients. Metabolically, critical pulmonary blood is found to be independent of right-to-left shunt volume.

The current study cannot evaluate the relationship between size of the interatrial communication and right-to-left shunt volume. In fact, this association is complex and confounded by numerous dynamic physiologic processes such as atrial wall distensibility, relative resistance to filling of the right and left ventricle, heart rate, and systemic venous return (166,167). The study suggests, however, that the empirically determined size of the interatrial communication is more critical in patients with low pulmonary blood flow. In these patients a small increase in right-to-left shunt volume will cause a greater decrement in arterial oxygen saturation compared to patients with higher values of pulmonary blood flow.

Right ventricular function is critical in maintaining adequate pulmonary blood flow. It has been reported that atrial septostomy attenuates right ventricular volume overload and has the potential to improve right ventricular function (150). If however despite atrial septostomy right ventricular function continues to deteriorate pulmonary blood flow decreases and tissue oxygen status worsens. As illustrated in Figure 4.7 mixed venous oxygen content is independent of shunt volume, therefore increased right-to-left shunting

cannot compensate for right ventricular failure in this setting. Our modeling study, therefore, emphasizes the importance of adequate biventricular function in patients with pulmonary arterial hypertension considered for atrial septostomy.

4.6. Conclusion

Investigating two "unphysiologic" circulatory conditions unique to patients with congenital heart disease these modelling studies shed light on the effects of blow flow distribution, right-to-left shunting and pulmonary perfusion on oxygen uptake and ultimately exercise capacity in patients with bidirectional cavopulmonary anastomosis or right-to-left shunt on atrial level and pulmonary hypertension. It was revealed that oxygen delivery (total, upper body or lower body) is not a suitable parameter to maximise in patients a bidirectional cavopulmonary anastomosis. Even matching oxygen delivery distribution to the oxygen consumption of the upper and lower body does not (in general) optimise tissue oxygenation. Because there is a trade-off between flow distribution and saturation, it is unwise to concentrate on maximizing oxygen delivery. Maximizing systemic venous saturations is conceptually different and physiologically preferable for tissue oxygenation. However, this study also revealed that the bidirectional cavopulmonary anastomosis is inherently inefficient and cannot be "optimized" in a real life scenario. It limits exercise tolerance by uncoupling oxygen consumption from oxygen uptake and by inducing cyanosis, thereby reducing arterio-venous oxygen difference. The only practical solution to improve exercise capacity in

this situation is definitive surgical completion of the Fontan circulation. Similarly, oxygen delivery was found to an inadequate parameter to describe tissue oxygen status in patients with atrial right-to-left shunt and fixed pulmonary blood flow. Interatrial shunting was revealed to have no effect on tissue oxygen status in this setting. Therefore, from a theoretical point of view, exercise capacity cannot be improved by creating a right-to-left shunt in patients with pulmonary hypertension. Rather, this study emphasizes the importance of adequate pulmonary blood flow and blood oxygen carrying capacity in maintaining tissue oxygenation thereby improving exercise capacity.

Chapter V Inflammation and Endothelial Progenitor Cells

5.1. Background of the study

Endothelial dysfunction is ubiquitous in patients with heart failure and adults with congenital heart disease and relates to exercise intolerance and symptoms (57-60). Recently endothelial dysfunction has been linked to reduced availability of vasoactive mediators, the presence of inflammation and reduced number of bone marrow derived endothelial progenitor cells (EPCs) (69). This study aimed to test the hypothesis that patients with congenital heart disease have reduced number and impaired function of EPCs, altered concentrations of vasoactive mediators, increased levels of asymmetric dimethylarginine (ADMA) and evidence of inflammation. This study focused on adult patients with Eisenmenger syndrome (pulmonary hypertension and cyanosis in the presence of congenital heart disease) known to have the worst exercise capacity among ACHD patients. Healthy individuals and patients with idiopathic pulmonary hypertension (IPAH) were included as control subjects.

5.2. Methods

Study Subjects

Subjects were recruited from the Adult Congenital Heart Centre and Centre for Pulmonary Hypertension, Royal Brompton Hospital and the Hammersmith Hospital, London between July 2006 and December 2007. Approval from the

local ethics committees was obtained, and all study subjects provided written informed consent.

Flow Cytometric Detection of Circulating Endothelial Progenitor Cells

Peripheral blood mononuclear cells (PBMNCs) were isolated by density centrifugation using Vacutainers CPT cell preparation tubes (BD Biosciences) or Ficoll-Paque (GE Healthcare Life Sciences, Little Chalfont, UK), according to the manufacturer's instructions. Endothelial progenitor cells were enumerated, using established criteria, as CD34+ cells co-expressing AC133 and VEGF receptor-2 (VEGFR2/fetal liver kinase 1/KDR) or cells co-expressing CD34 and KDR (168,169). 10^6 PBMCs were incubated with FITC-labeled monoclonal mouse anti-human CD34 (BD Biosciences, Oxford, UK), PE-labeled monoclonal mouse anti-human AC133 (Miltenyi Biotec, Surrey, UK) and allophycocyanin-labeled monoclonal mouse anti-human KDR (R&D Systems, Abingdon, UK) antibody for 30 min at 4°C, according to the manufacturers' instructions. In parallel experiments, appropriate isotype controls were used and at least 100,000 events acquired in the lymphomonocytic gate using a FACSCalibur cytometer (Becton Dickinson, Oxford, UK). The number of progenitor cells was expressed as a percentage of all lymphomonocytic cells.

Case and colleagues reported that PBMNCs co-expressing CD34+AC133+KDR+ may represent primitive hematopoietic progenitors and may not relate to colony-forming unit endothelial cells (170). In contrast, CD34+cells not expressing the common leukocyte antigen (CD45) were

found to yield colony-forming units endothelial cells in their study. We therefore quantified endothelial progenitor cells as lymphomonocytic cells expressing CD34 with low expression of CD45 (PE labeled monoclonal mouse anti-human CD45, BD Biosciences, Oxford, UK) as CD45lowCD34+-EPCs in accordance to previous studies (171,172). Furthermore, to account for the possibility of different levels of lymphomonocytic cells in the different subject groups, EPC levels were corrected for the number of lymphomonocytic cells and compared to the percentages of EPCs in the lymphomonocytic gate.

Enumeration of Colony Forming Units (CFU)

In addition to direct evaluation of circulating EPCs, the colony forming capacity and angiogenic potential of cells derived from PBMNCs in culture were assessed as these have been reported to correlate with systemic vascular endothelial dysfunction and prognosis in other cardiovascular diseases (173,174). Colony forming units were obtained from PBMNCs plated ($5x10^6$ PBMNCs/well) on fibronectin-coated six-well plates (BD Biosciences) in EndoCult™ medium (StemCell Technologies, London, UK) according to the manufacturer's instructions. After 48 h, non-adherent cells were collected and re-plated ($1x10^6$ cells/well) in duplicate in fibronectin-coated 24-well plates. Colonies were counted three days later by two investigators in a blinded fashion. Colonies were defined as a central core of "round" cells with radiating "spindle-like" cells at the periphery and referred to as early outgrowth CFU-endothelial cells (also referred to as CFU-Hill colonies)(173) .

Staining of Cultured Cells and Incorporation into Tube-Like Structures

Isolated PBMNCs (10^7 cells/well) were cultured in fibronectin-coated six well plates in endothelial-basal medium-2 (EBM2) supplemented with EGM SingleQuots (Cambrex Bio Science Ltd., Wokingham, UK) for 7 days. Adherent cells were identified, as previously described (175,176), by uptake of 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate-labelled acetylated low-density lipoprotein (5 µg/ml Dil-Ac-LDL for 1 hr at 37°C; Serotec Ltd., Kidlington, Oxford, UK.); binding of fluorescein isothiocyanate (FITC)-conjugated Ulex europeus agglutinin (10 µg/ml UEA-1 for 1 hr at 37°C; Sigma-Aldrich Co., Ltd., Gillingham, UK); and immunostaining for Von Willebrand factor (vWf), using a polyclonal rabbit anti-human vWf antibody (A0082; DakoCytomation Ltd., Ely, UK). After washing, cells were stained with Hoechst 33342 nuclear dye (0.5 µmol/L for 1 hr at room temperature) and observed by fluorescence microscopy. Adherent cells were trypsinized after 7 days in culture and the number derived from the 10^7 PBMNCs originally plated was quantified using a haemocytometer.

The angiogenic potential of cultured mononuclear cells was defined by the number of cells incorporated in to endothelial tube-like structures, as previously described (177). Briefly, adherent cells were labeled with Vybrant® CFDA SE cell tracer (Invitrogen, Paisley, UK) after 7 days in culture. Following treatment with trypsin, cells (5×10^3 cells/well) were mixed with human umbilical vein endothelial cells (2×10^4 cells/well) in 300 µl of EBM-2 medium with EGM SingleQuots and incubated in eight-well chamber glass slides (Lab-Tek™) pre-coated with 200 µl Matrigel (BD Bioscience, Oxford, UK). After eight hours of incubation in a 5% CO_2 humidified

atmosphere at 37°C, cells were examined by fluorescence microscopy. The number of labelled cells incorporated into endothelial tubes was determined in three randomly selected fields per well, by at least two investigators in a blinded fashion.

Detection of Circulating Inflammatory Mediators, Asymmetric Dimethylarginine, Nitric Oxide Metabolites, cGMP and Brain Natriuretic Peptide

Blood, obtained at the same time as the sample for PBMNC isolation, was drawn from the antecubital vein, collected in EDTA and citrate tubes and placed immediately on ice. Following centrifugation, plasma samples were stored at -80°C until further analysis. Levels of the chemokine (C-C motif) ligand-2 (CCL)-2 (formerly monocyte chemotactic protein-1, Quantikine® immunoassay DCP00, sensitivity < 5.0 pg/mL, R&D Systems, Abingdon, UK); tumour necrosis factor-α (TNF-α.Quantikine® HS immunoassay HSTA00D, mean minimum detectable dose 0.106 pg/mL, R&D Systems); interlukin-6 (IL-6, Quantikine® HS immunoassay HS600B, mean minimum detectable dose 0.039 pg/mL, R&D Systems); vascular endothelial growth factor (VEGF, (QuantiGlo® immunoassay DVE00, sensitivity < 9 pg/mL, R&D Systems, Abingdon, UK); cGMP (Enzyme-immunoassay Biotrak® System RPN226, sensitivity 161 pg/ml, GE Healthcare, Little Chalfont, UK); C-reactive protein (CRP, Quantikine® immunoassay DCRP00, mean minimum detectable dose 0.010 ng/mL, R&D Systems) and ADMA (DLD Diagnostica, Hamburg, Germany), were quantified using commercial ELISA assays according to the manufacturer's instructions.

The combined concentrations (NOx) of plasma nitrite (NO_2^-) and nitrate (NO_3^-), the stable oxidation products of nitric oxide (NO), were determined using a NO chemiluminescence detector (NOA 280, Sievers), as described previously (178). Plasma samples were deproteinated by centrifugation in 3KDa MW cut-off filter units (Uitracel Microcon YM-3). In addition, a full blood count, renal function tests and brain-type natriuretic peptide (BNP) levels were obtained.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). Statistical analysis was performed with the non-parametric Mann-Whitney U test and Spearman correlation, using R version 2.4.1. (179) and MedCalc 8.1 (MedCalc Software, Mariakerke, Belgium). Regression analyses were performed after variables were log-transformed to achieve normal distribution. For all analyses, a 2-sided P-value of less than 0.05 was considered statistically significant.

5.3. Results

Subjects

Fifty-five patients with idiopathic PAH (44 female, age 46±14 years), 41 Eisenmenger patients (27 female, age 37±12 years, 13 with Down syndrome) and 47 healthy controls (24 female, age 36±9 years) were recruited. To account for differences in gender distribution between the different subject groups, EPC numbers were examined separately in male

and female subjects. The Eisenmenger population was younger than the IPAH cohort and patients exhibited secondary erythrocytosis, with raised haematocrit and haemoglobin levels as well as cyanosis (Table 5.1).

Endothelial Progenitor Numbers

Circulating EPC numbers, as defined by the number of double-(CD34+/KDR+) or triple-labeled cells ($CD34^+/AC133^+/KDR^+$) cells, were significantly lower in patients with Eisenmenger syndrome and IPAH (Figure 5.1) in, both, the female and the male population. Within the Eisenmenger population, patients with Down syndrome had the lowest number of circulating EPCs irrespective of gender. Progenitor cell numbers, defined as single ($CD34^+$) or double-labeled cells ($CD34^+/AC133^+$) were also lower in the Eisenmenger population compared to control and IPAH subjects whereas the two latter groups had similar levels of progenitor cells (Figure 5.1). Stratifying subjects by decades of age revealed that differences in EPC numbers between patients and controls were maintained throughout all age groups. No significant difference in the lymphomonocyte cell counts were found between controls, Eisenmenger and IPAH subjects as shown in Table 5.1. Consistent with this observation, correcting EPC numbers (expressed as a % of lymphomonocytes) for actual lymphomonocyte cell counts in individual patients did not affect the results of the study. In addition, $CD45^{low}CD34^+$-cells were significantly lower in non-Down Eisenmenger syndrome patients (0.377 vs. 1.073 %; $P = 0.003$) and Down-Eisenmenger syndrome patients (0.430 vs. 1.073; $P = 0.02$) compared to control subjects.

Levels of $CD45^{low}CD34^{+}$-cells were not significantly different between controls and idiopathic PAH subjects.

Association between Endothelial Progenitor Numbers and Exercise Tolerance

Within the Eisenmenger population there was an inverse relationship between circulating EPC numbers and exercise tolerance as judged by NYHA functional class and six minute walk distance. EPC numbers were significantly higher in Eisenmenger patients in NYHA functional class 2 compared to those in NYHA class 3, as determined by the number of single- (CD34+: median 0.099 vs. 0.062, P =0.03), double- ($CD34^{+}/AC133^{+}$: median 0.032 vs. 0.013, P =0.009; CD34+KDR+: median 0.029 vs. 0.012, P=0.02) or triple-labeled cells ($CD34^{+}/AC133^{+}/VEGFR2^{+}$: median 0.007 vs. 0.002, P =0.008). The number of progenitor cells also correlated directly with six-minute walk test distance in the Eisenmenger population (r=0.64, P =0.008), as shown in Figure 5.2.

In contrast, no significant difference was found in the EPC numbers in IPAH patients assigned to NYHA class 2, 3 and 4.

Association between Treatment for PAH and Endothelial Progenitor Numbers

IPAH patients receiving treatment with the phosphodiesterase type 5 (PDE5) inhibitor sildenafil exhibited significantly higher circulating EPC levels than patients not receiving sildenafil (Figure 5.3). Furthermore, there was a dose-dependent relationship between sildenafil exposure and circulating EPC

numbers, irrespective of the surface labels used to identify the cells (Figure 5.3). In contrast, no such difference in EPC numbers was found in IPAH patients treated with bosentan or other targeted therapies compared to treatment naïve patients and control subjects (Figure 5.3). Similarly, no significant difference was found in the number of progenitor cells in Eisenmenger patients treated with bosentan compared to treatment naïve patients (data not shown). Eleven IPAH patients were also taking a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (atorvastatin, 20 mg od, n=1; simvastatin, 40-80 mg od, n=10). The circulating EPC numbers in this cohort did not differ significantly from controls, but they were nonetheless excluded from the analyses of patients receiving sildenafil.

Colony Forming Units-Endothelial Cells and Adherent Cells

Characteristic CFU-endothelial cells were observed following culture of PBMNCs in Endocult™ medium for 5 days (Figure 5.4). Consistent with the flow cytometric analysis of circulating progenitors, significantly fewer colony forming units were obtained with PBMNCs isolated from patients with Eisenmenger syndrome, compared to controls, whereas there was a trend towards increased numbers of colonies in patients with IPAH (Figure 5.4). No significant difference was found in the colony forming capacity of Eisenmenger patients with and without Down syndrome (Figure 5.4). Similarly, patients with Eisenmenger syndrome had significantly lower numbers of adherent cells derived from PBMNCs after 7 days in culture compared to control in IPAH subjects. In contrast, IPAH patients had

significantly higher numbers of adherent cells compared to control subjects (Figure 5.5).

Incorporation of Cultured Cells into Tube-Like Structures

After 7 days in culture, all adherent cells exhibited Vybrant® CFDA SE cell tracer labeling, Dil-Ac-LDL uptake and immunostaining for vWf and a major proportion also displayed UEA-1 binding (Figure 5.5). Cells derived from patients with Eisenmenger syndrome had a reduced capacity to incorporate into tube-like structures with human umbilical vein endothelial cells (median 14.8 cells/field [IQR 11.5-40.7]; n= 5) compared to cells derived from normal controls (51.3 cells/field [IQR 42.7-59.3]; n= 8 ; P = 0.028) and patients with IPAH (60.3 cells/field [IQR 44.5-71.0]; n= 8 ; P = 0.019), as illustrated in Figure 5.5. In contrast no significant difference was found between control subjects and patients with IPAH.

Circulating Inflammatory Mediators and Indices of Endothelial Dysfunction

The plasma levels of inflammatory mediators were significantly higher in Eisenmenger and IPAH patients compared to control subjects and, within the Eisenmenger population, patients with Down syndrome had higher levels of TNF-α, IL-6, CCL-2 and CRP compared to non-Down Eisenmenger patients (Figure 5.6). Levels of inflammatory mediators correlated with each other and the white blood cell count (WBC) (r-values between 0.25 – 0.62, for TNF-α vs IL-6, CRP and WBC, as shown in Figure 5.7. Furthermore, in the Eisenmenger population the concentration of TNF- α and CRP also correlated with combined plasma NO_{-2} and NO_{-3} levels (Figure 5.7).

Plasma ADMA levels were significantly higher in patients with Eisenmenger syndrome and IPAH, compared to healthy control subjects (Figure 5.6). Within the Eisenmenger population, those with Down syndrome had significantly higher ADMA levels (median 1.23 μmol/l [IQR 0.90-1.69]) compared to patients without Down syndrome (median 0.82 μmol/l [IQR 0.72-0.89]; *P*=0.009). Circulating levels of cGMP and NO metabolites were also both significantly elevated in patients with Eisenmenger syndrome or IPAH compared to control subjects, with markedly higher levels occurring in patients with Down syndrome (Figure 5.6). Plasma NOx levels comprised mainly NO_3^-, with no differences observed in the levels of NO_2^- among the groups. While no direct correlation was found between cGMP-levels and NOx levels, BNP and cGMP levels were positively associated in patients with Eisenmenger syndrome (r=0.74 , *P* =0.0001), as shown in Figure 5.8.

In contrast to the elevated cytokines, plasma VEGF levels were significantly lower in Eisenmenger patients compared to control subjects and IPAH patients, with no significant difference between Down and non-Down Eisenmenger patients (Figure 5.6).

Association between circulating EPC Numbers and Inflammatory Mediators, ADMA, BNP and cGMP

Overall, the number of circulating $CD34^+KDR^+$- EPCs was negatively correlated with levels of TNF-α, IL-6 and CRP and raised levels of ADMA were also associated with lower EPC numbers (Figure 5.7). A bimodal

relationship was found between cGMP levels and numbers of circulating EPCs. When subjects were stratified according to diagnosis a positive association was observed between cGMP levels and circulating EPC numbers in control subjects and to a lesser degree in IPAH patients (Figure 5.9). In contrast, a significant negative association was found between cGMP levels and circulating EPCs in Eisenmenger patients (Figure 5.9), who on average had the highest cGMP levels (Figure 5.6). In addition, BNP levels and circulating EPC numbers were also inversely correlated in the Eisenmenger population (Figure 5.8). Furthermore, a significant inverse correlation between BNP levels and plasma cGMP levels was found potentially confounding the relationship between plasma cGMP levels and EPC numbers in the Eisenmenger population.

Association between circulating EPC Numbers, cGMP levels and haemodynamic parameters

Higher circulating EPC numbers and cGMP levels were found to be associated with significantly lower mean pulmonary arterial pressures and higher cardiac index in patients with IPAH (Figure 5.10).

5.4. Tables

	Eisenmenger (N = 41)	IPAH (N=55)	Control subjects (N=47)
Age (years)	37±12 †	46±14 *	36±9
Male/female	14 / 27	11 / 44 *	24 / 23
Diabetic patients (%)	0	4	-
NYHA class 2 / 3 / 4 (%)	49 / 51 / 0	42 / 54 / 4	-
6MWT distance (m)	303±81 †	362±110	-
Arterial SpO_2 rest (%)	83±7 †	93±6	-
Time since diagnosis (years)	22.3±10.7 †	3.6±2.8	-
Medication			
Warfarin	15 % †	87 %	-
CCBs	3 %	9 %	-
Digitalis	8 %	24%	-
Diuretics	30 % †	52 %	-
Statins	5 %	20 %	-
ACE-Inhibitors	14 %	7 %	-
Organic nitrates	5 %	0 %	-
Bosentan	23 % †	56 %	-
Sildenafil	3 % †	45 %	-
Prostacyclin analogues	3 % †	28 %	-
Double therapy [1]	3 % †	35 %	-
Triple therapy [1]	0 %	4 %	-
Treatment naïve	74 %	11 %	
Hemodynamic Parameters [2]			
Mean PAP (mmHg)	-	57±12	-
Systolic PA pressure (mmHg)	103±20 †	87±22	-
PVR dyne · s · cm^{-5}	-	1034±478	-
Cardiac Index (L/min/m^2)	-	2.32±0.95	-
Laboratory parameters			
Hemoglobin (g/dl)	20.1±2.6 * †	15.0±2.3	14.6±1.3
Hematocrit (%)	61.7±8.3 * †	42.4±11.3	42.3±13.6
White blood cell count (10^9/L)	7.2±2.4 *	7.9±2.1 *	5.5±1.8
Lymphocyte count (10^9/L)	1.8±0.6	1.9±0.8	1.7±0.4
Monocyte count (10^9/L)	0.6±0.2	0.6±0.2	0.6±0.4
Lymphomonocyte count (10^9/L)	2.4±0.6	2.5±0.9	2.3±0.7

[1] Double and triple therapy, refers to combined use of endothelin receptor antagonists (bosentan or sitaxsentan), phosphodiesterase type 5 inhibitors (sildenafil) and prostacyclin analogues (epoprostenol or treprostinil). Mann-Whitney U tests and χ^2 tests were used for comparison as appropriate. *P<0.05 versus control subjects; † P<0.05 versus IPAH patients. 6MWT distance, six-minute walk test distance; ACE-Inhibitors, angiotensin converting enzyme inhibitors; CCB, calcium channel blockers; IPAH, idiopathic pulmonary arterial hypertension; NYHA, New York Heart Association functional class; PVR, pulmonary vascular resistance; SpO_2 transcutaneous resting arterial oxygen saturation (%); PAP, pulmonary arterial pressure.

Table 5.1: Patients' characteristics

5.5. Figures

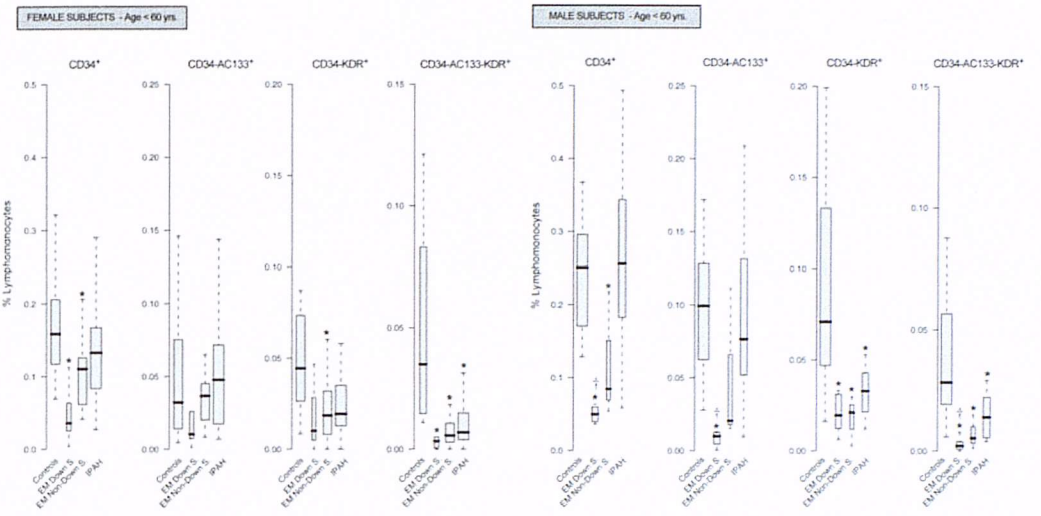


Figure 5.1: Circulating progenitor cells in female and male subjects. Proportion of $CD34^+$, $CD34^+/AC133^+$, $CD34^+/KDR^+$ and $CD34^+/AC133^+/KDR^+$ labeled cells, expressed as a percentage of lymphomonocytic cells, in controls and patients with Eisenmenger syndrome (EM) with and without Down syndrome or idiopathic pulmonary hypertension (IPAH). *, $P < 0.05$ versus controls; †, $P < 0.05$ versus IPAH patients.

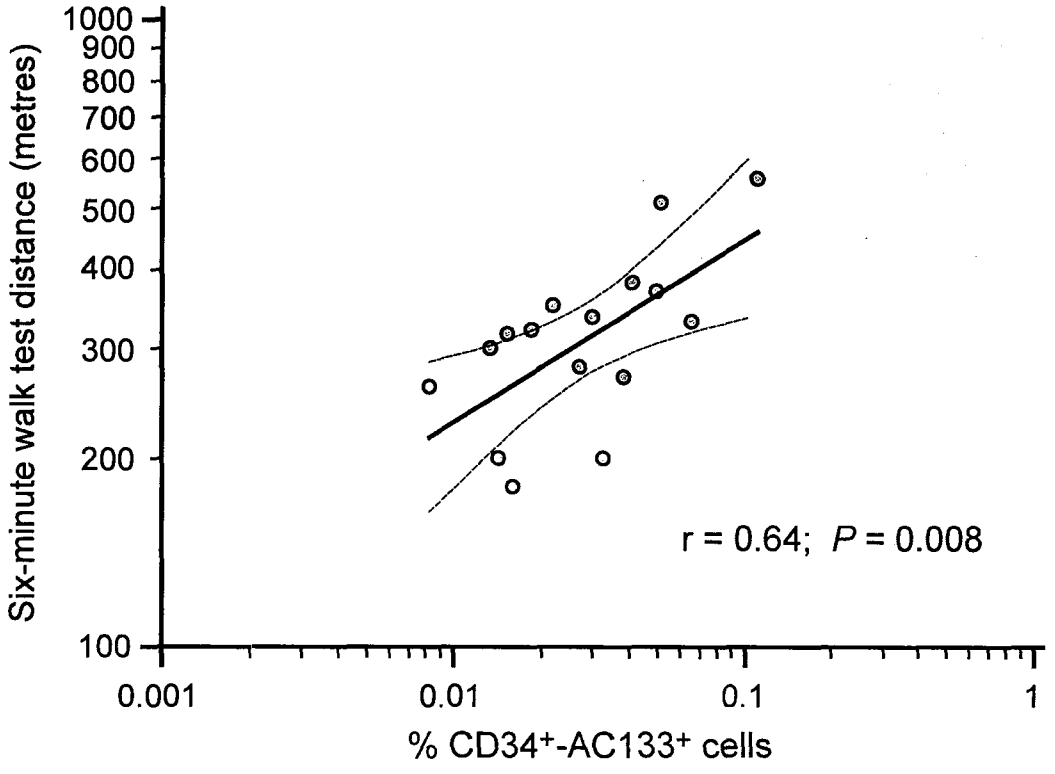


Figure 5.2: Correlation between progenitor cell numbers and six minute walk test distance in Eisenmenger patients without Down syndrome.

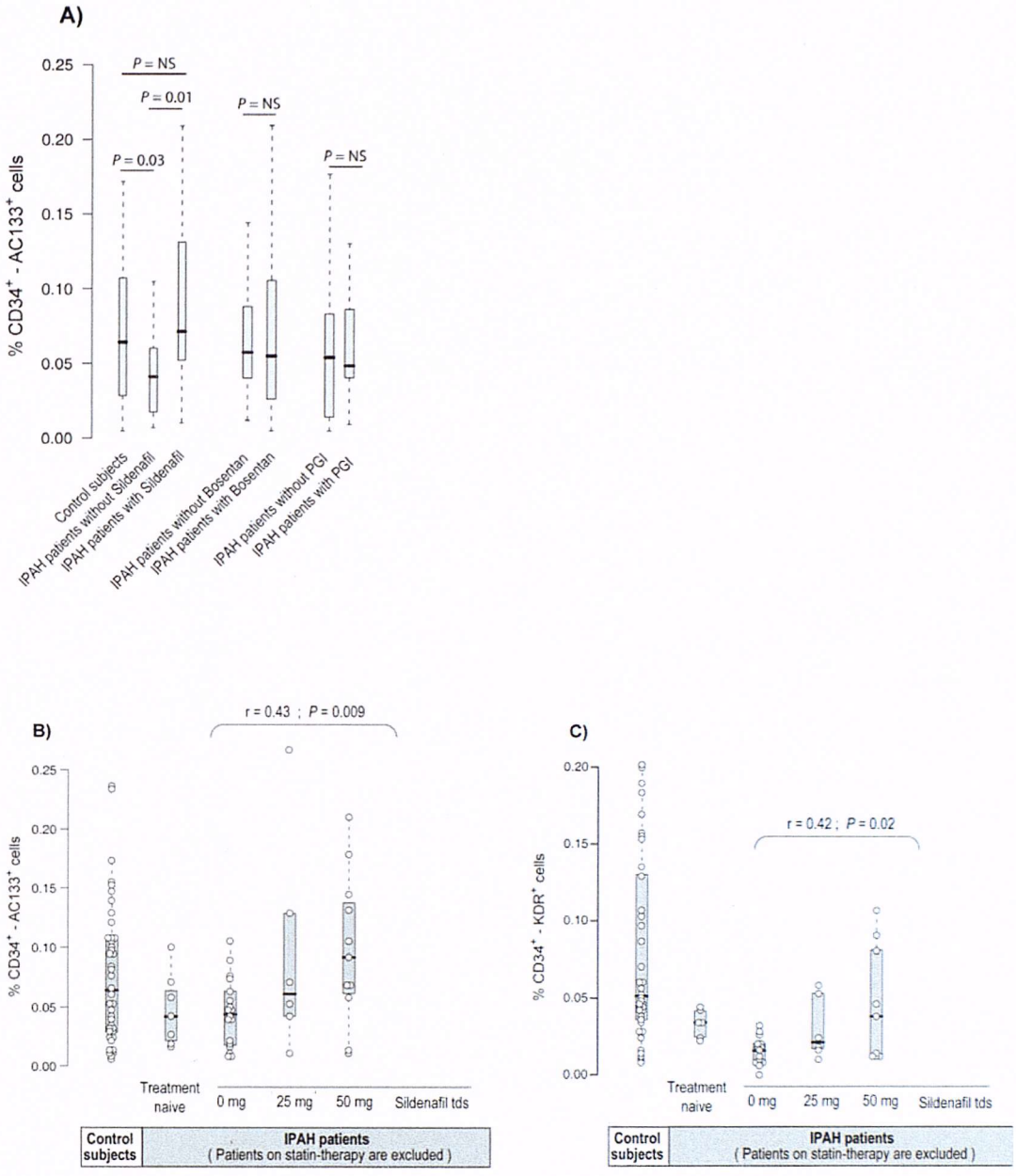


Figure 5.3: Relationship between sildenafil treatment and circulating progenitor cells. A, Comparison between circulating $CD34^{+}/AC133^{+}$ cells in controls and IPAH patients treated with sildenafil, bosentan or prostacyclin analogues. B-C, Dose-dependent correlation between sildenafil therapy and $CD34^{+}/AC133^{+}$ or $CD34^{+}/KDR^{+}$ cell numbers, compared to treatment naive patients and controls.

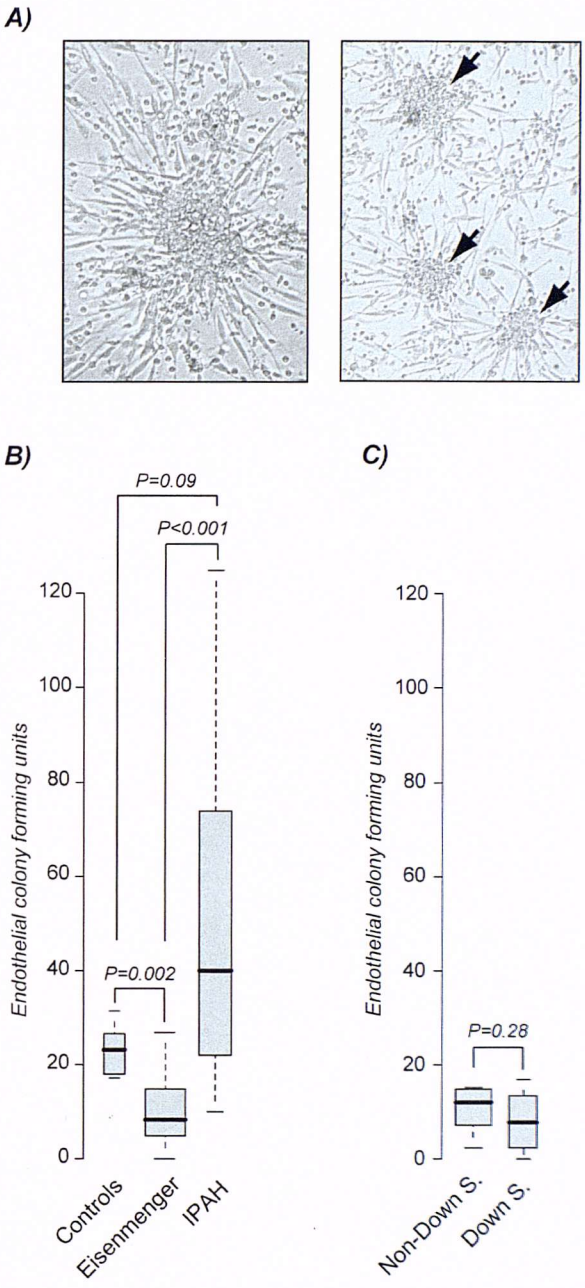


Figure 5.4: Colony forming units-endothelial cells. A, Examples of typical colony forming units. B, Comparison between control subjects, Eisenmenger and IPAH patients. C, Comparison between Down and non-Down subjects within the Eisenmenger population.

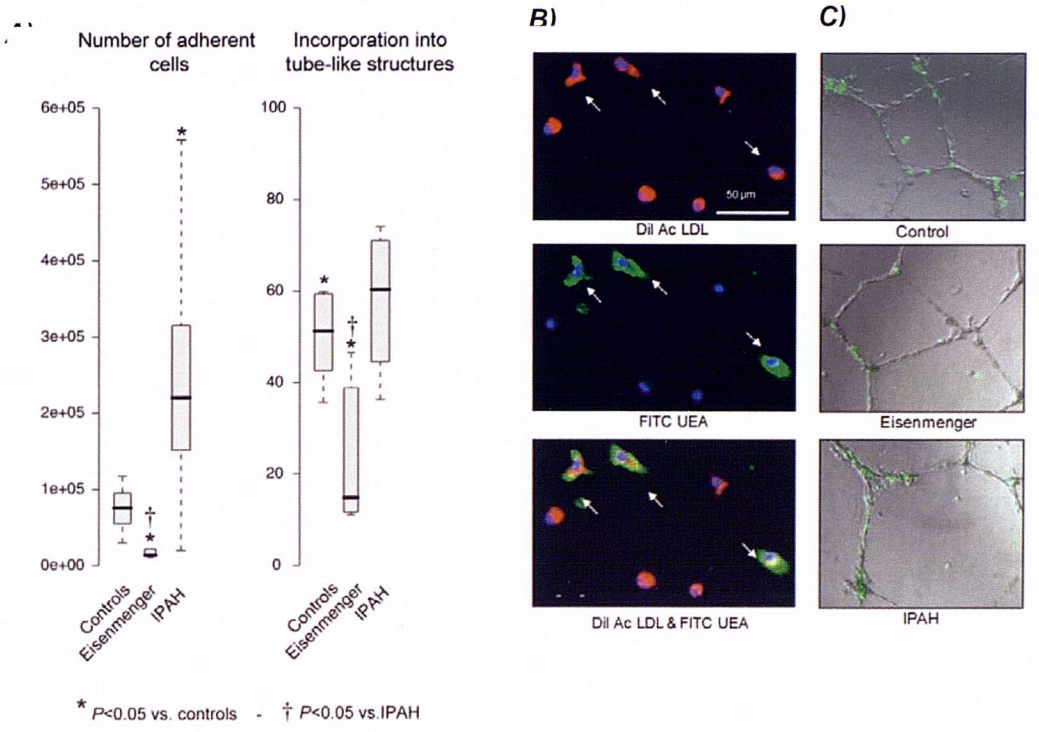


Figure 5.5: A) Number of adherent cells from control, Eisenmenger and IPAH subjects and number of cells incorporated in to HUVEC tube-like structures. B) Adherent cells, after 7 days culture displaying (arrows) Dil-Ac-LDL uptake, FITC-UEA-1 labeling and Hoechst counterstained nuclei; C) Incorporation of Vybrant-labeled cells (green fluorescence) in to HUVEC tube-like structures. *, $P < 0.05$ versus controls; †, $P < 0.05$ versus IPAH patients.

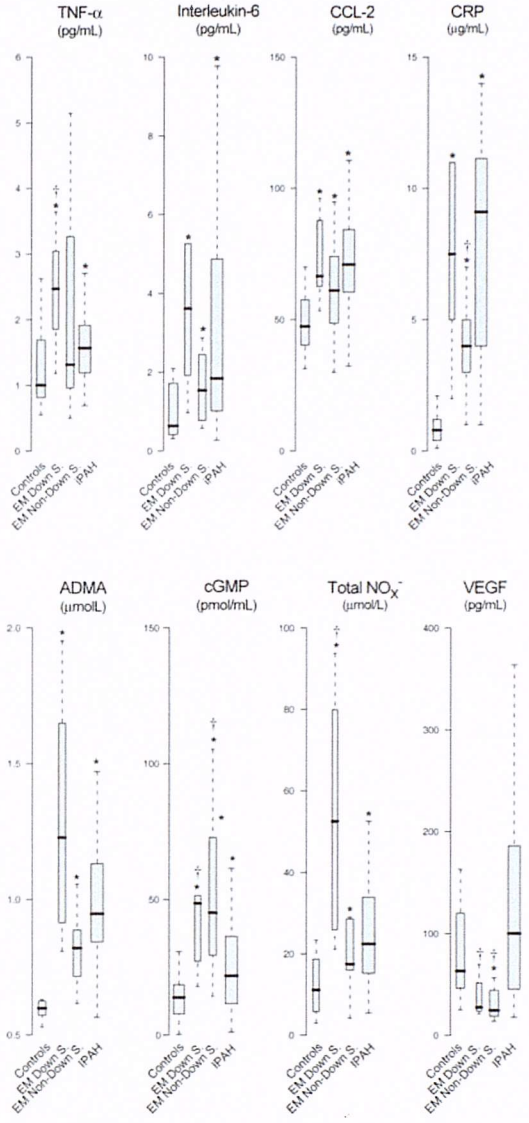


Figure 5.6: A) Plasma levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1/CCL-2) and C-reactive protein (CRP). B) Plasma levels of asymmetrical dimethylarginine (ADMA), cyclic GMP (cGMP), total plasma nitrate/nitrite levels (NOx^-) and vascular endothelial growth factor (VEGF) in control, Eisenmenger (EM) with and without Down syndrome and IPAH subjects. *, $P < 0.05$ versus controls; †, $P < 0.05$ versus IPAH patients.

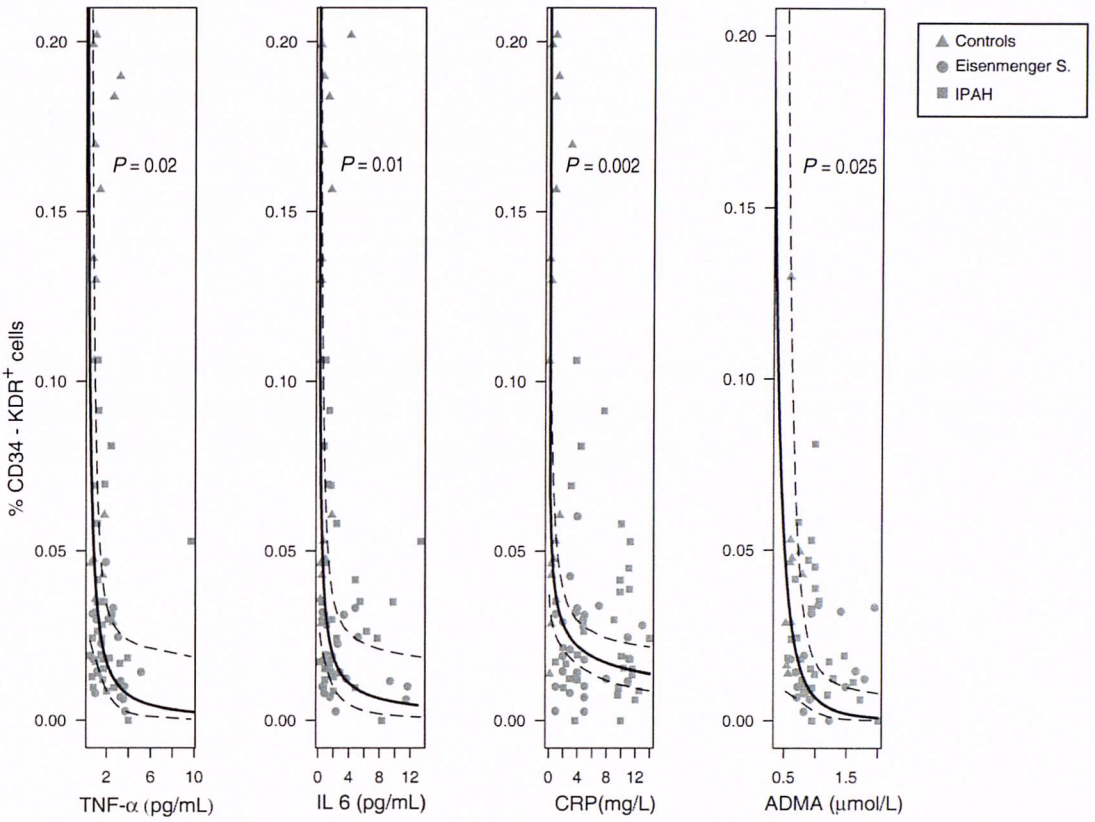


Figure 5.7: Correlation between inflammatory mediators (tumor necrosis factor-α [TNF-α], interleukin-6 [IL-6] and C-reactive protein [CRP]) or asymmetrical dimethylarginine (ADMA) and numbers of circulating $CD34^+/KDR^+$ cells. Regression analyses performed after log-transformation.

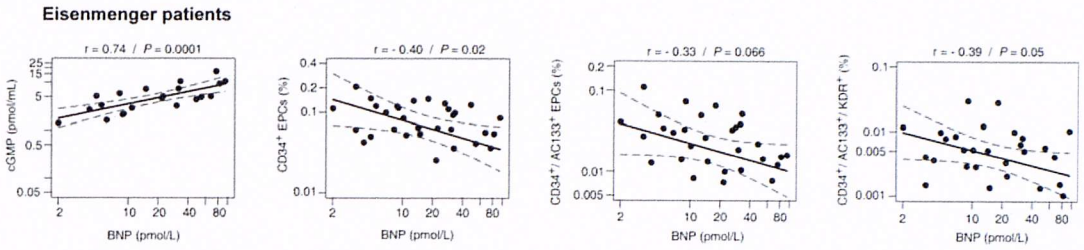


Figure 5.8: Correlation between brain-type natriuretic peptide (BNP) and cyclic GMP (cGMP) levels, $CD34^+$, $CD34^+$-$AC133^+$ or $CD34^+$-$AC133^+$-KDR^+ progenitor cells in patients with Eisenmenger syndrome.

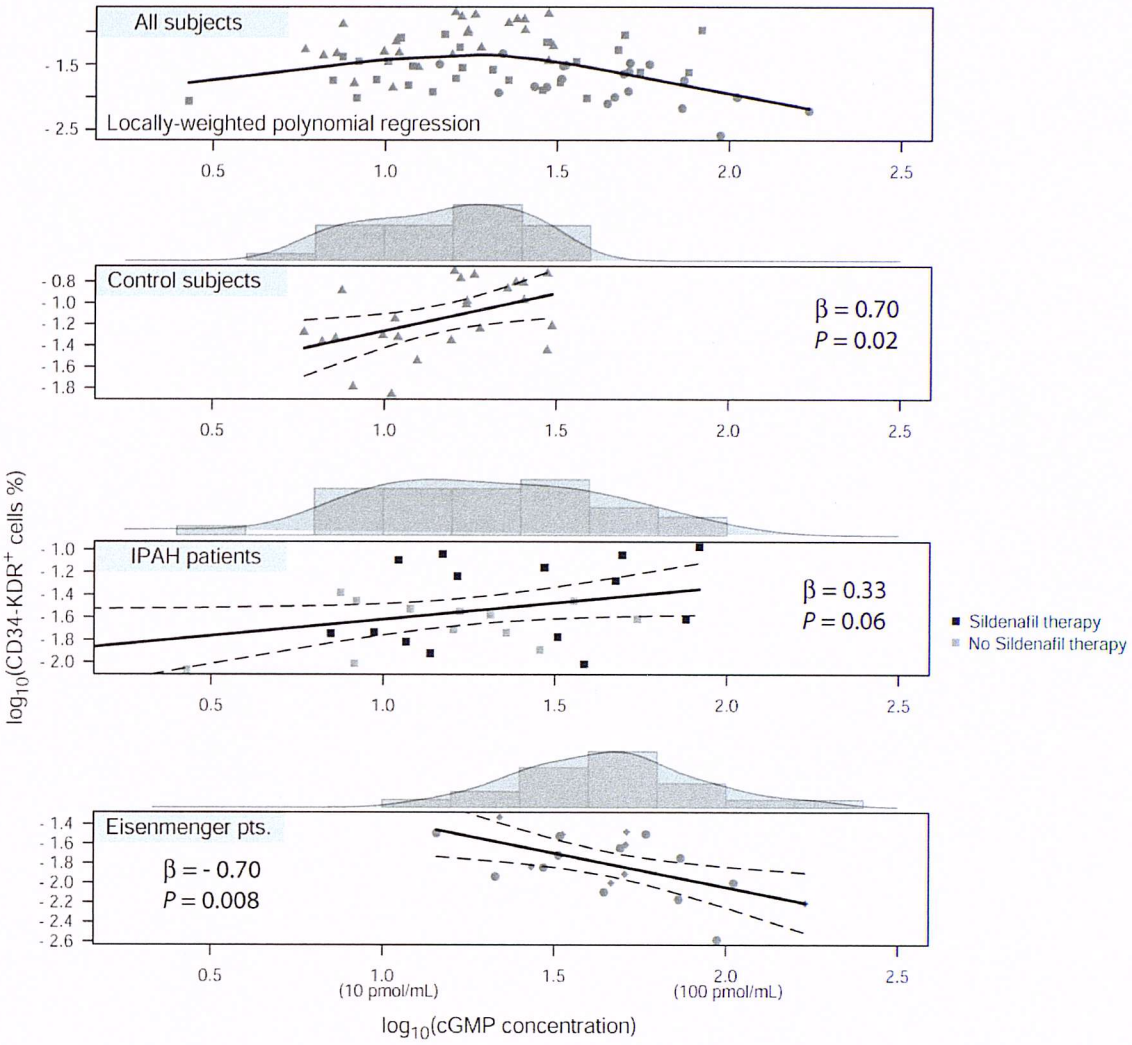


Figure 5.9: Relationship between plasma cGMP levels and circulating $CD34^+/KDR^+$ EPCs in control, Eisenmenger and IPAH subjects. Overall, locally weighted regression analysis indicates a bimodal relationship. Stratification into cohorts reveals positive association between cGMP levels and EPC numbers in control and IPAH subjects and a negative association in Eisenmenger subjects. Histograms with overlaid density estimates illustrate the higher levels of cGMP in Eisenmenger subjects compared to control and IPAH subjects (see also Figure 5B).

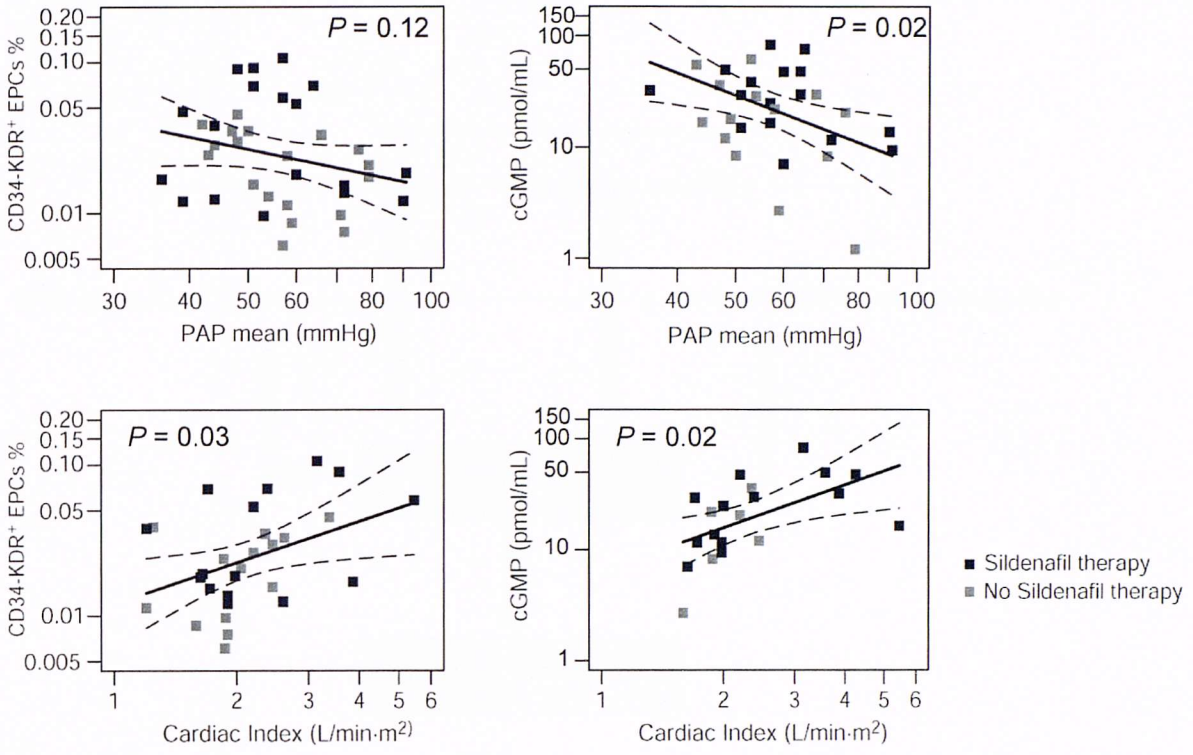


Figure 5.10: Correlation between haemodynamic parameters (mean pulmonary arterial pressure [PAP] or cardiac index) and cGMP levels or circulating $CD34^+/KDR^+$ cells in patients with IPAH. Parameters were log-transformed.

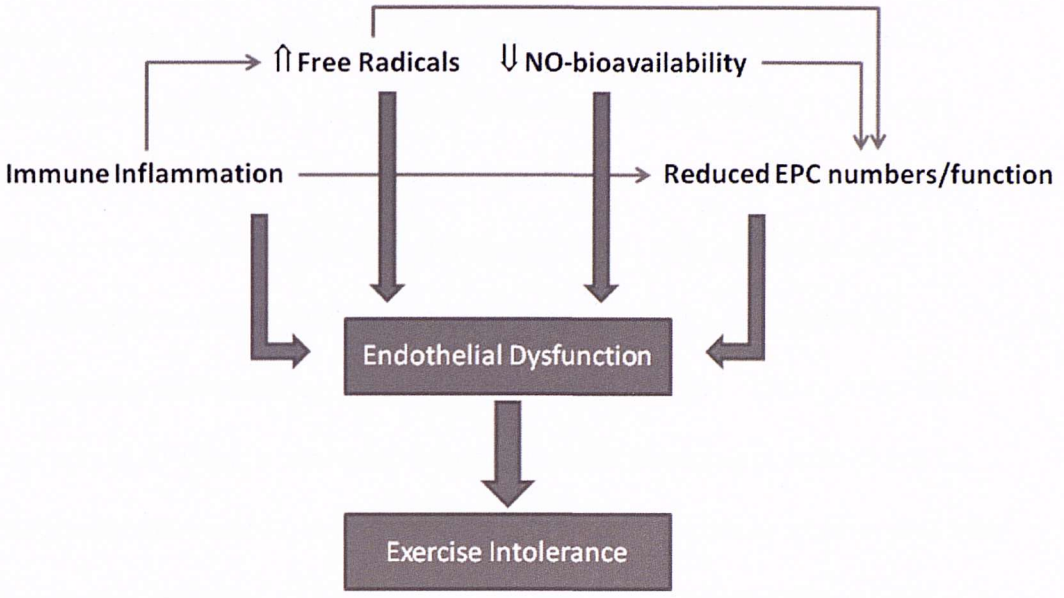


Figure 5.11: Interplay between immune inflammation, oxidative stress (↑ free radicals), reduced nitric oxide (NO)-bioavailability and endothelial progenitor cell (EPC) numbers and function, endothelial dysfunction and ultimately exercise intolerance.

5.6. Discussion

Endothelial dysfunction has been reported in adult patients with congenital heart disease and pulmonary hypertension and may be associated with exercise intolerance in this group of patients (70). Recently, it has been suggested that endothelial progenitor cells (EPCs) derived from the bone marrow have a role in ongoing endothelial repair and that impaired mobilization or depletion of these cells may contribute to endothelial dysfunction and peripheral vascular disease (173,180). The number and function of EPCs are altered in atherosclerosis, in high-risk individuals for cardiovascular events, in patients with stable and unstable angina, and after myocardial infarction (173,180-182). In addition, immune inflammation is prevalent in adult congenital heart disease and is linked to endothelial dysfunction and thereby reduced exercise capacity (80,183). The complex interplay between immune inflammation, oxidative stress, reduced nitric oxide bioavailability and EPC numbers and function is illustrated in Figure 5.11.

This study assessed the number of circulating EPCs and function of cultured mononuclear cells in patients with Eisenmenger syndrome and idiopathic pulmonary arterial hypertension (IPAH). The major findings were that the number of circulating EPCs ($CD34^+/KDR^+$ or $CD34^+/AC133^+/KDR^+$) was markedly reduced in patients with Eisenmenger syndrome or IPAH. This reduction was most profound in Eisenmenger patients with Down syndrome. The reduction in circulating EPCs was associated with reduced functional capacity of cultured PBMNCs in patients with Eisenmenger syndrome, but

not in patients with IPAH. Elevated levels of inflammatory mediators, indices of nitric oxide synthesis and ADMA were found in PAH patients and correlated directly with EPC numbers. In Eisenmenger patients, there was a close correlation between plasma levels of BNP and cGMP and BNP related inversely to EPC numbers in this cohort. Notably, EPC numbers were also related to pulmonary haemodynamic parameters in IPAH patients and chronic sildenafil treatment was associated with dose-dependent elevation of circulating EPC numbers in this cohort.

Both Eisenmenger syndrome and IPAH are associated with pulmonary and systemic vascular endothelial dysfunction (57,59,184). In the present study, the reduction in EPC numbers in Eisenmenger patients showed a correlation with disease severity, as measured by functional class and six minute walk distance, and numbers were lowest in patients with Down syndrome. As a subgroup, Eisenmenger patients with Down syndrome have particularly poor survival prospects (185) and show more rapid progression of symptomatic pulmonary vascular disease than other patients with Eisenmenger syndrome (30). These findings are in accordance with observations in various cardiovascular cohorts, suggesting that low EPC numbers are associated with a worse prognosis (169,173,174). Patients with IPAH also exhibited significantly fewer circulating EPCs compared with healthy controls and the number of cells correlated directly with cardiac index. In addition, there was a trend towards lower EPC numbers in IPAH patients with higher mean pulmonary arterial pressures, although this association was potentially confounded by the effect of sildenafil treatment. It is also possible that

mechanistic differences (e.g. involving chronic cyanosis, erythrocytosis, increased blood viscosity and associated shear stress) between Eisenmenger and IPAH patients have diverse effects on the number of circulating cells and function of cultured cells.

The mobilization and function of EPCs is thought to be critically dependent on nitric oxide (NO) (186). In the current study plasma concentration of ADMA was raised in both Eisenmenger and IPAH patients, which is in keeping with previous studies (187),(188). Although increased ADMA levels are associated with reduced NO bioavailability, elevated systemic levels of stable NO oxidation products were found in this study, which is consistent with earlier reports of raised nitrate levels in adults as well as children with congenital heart disease (189). A positive correlation was found between cGMP levels and EPC numbers in control subjects and IPAH patients, yet despite high NOx and cGMP plasma concentrations, circulating EPCs were not only deficient in Eisenmenger patients but also exhibited a negative association with plasma cGMP levels. There are several potential explanations for this phenomenon, but NO and the natriuretic peptides are the major factors stimulating cGMP synthesis in pulmonary vascular tissue (190). In experimental animals, increased pulmonary blood flow and associated pulmonary hypertension is accompanied by raised circulating levels of BNP and cGMP (191) .The levels of these two factors were also closely correlated in patients with Eisenmenger syndrome, potentially obscuring any direct association between NO and cGMP production. Increased circulating inflammatory mediators also accompanied raised

plasma NOx levels and cytokine inducible NO synthase expression has been described in pulmonary arteries and cardiac tissues of patients with flow-associated pulmonary hypertension and cyanotic congenital heart disease (192,193). This represents a potentially important source of "high-output" NO production that may be associated with oxidative stress and the production of reactive NO species that attenuate the mobilization, function and survival of EPCs (194,195).

Increasing evidence indicates that inflammation has a key role in the pathogenesis of PAH. As in earlier studies (196-198), evidence of chronic inflammation with raised plasma levels of the inflammatory mediators TNF-α, IL-6 and CCL-2 was found. Increased pulmonary production of CCL-2 in PAH is postulated to act as a chemoattractant for circulating monocytes (198), whereas both TNF-α and IL-6 have a negative effect on the number and function of EPCs (169,199,200). We also found that plasma CRP was elevated and, together with the inflammatory cytokines, negatively associated with the number of circulating EPCs. In this regard, CRP has been found to attenuate endothelial NO synthase (eNOS) expression and the mobilization, differentiation and survival of EPCs (201,202).

An important finding of this study was that IPAH patients treated with the PDE5 inhibitor sildenafil exhibited a selective and dose-dependent increase in circulating EPCs. Studies of erectile dysfunction have indicated that acute and chronic PDE5 inhibition, with vardenafil and tadalafil respectively, is associated with increased circulating $CD34^+/AC133^+$ progenitor cells

(203,204). This study, however, is the first report of the effects of a PDE5 inhibitor on EPC numbers in patients with PAH. Given that eNOS is expressed in cultured EPCs (205) and NO released from organic nitrates can augment circulating EPCs (186,194), it is possible that, unlike other targeted therapies for PAH, sildenafil stimulates an intrinsic NO-cGMP pathway, thereby augmenting EPC numbers. It is unclear whether the effect of sildenafil confers a therapeutic advantage, but there is active interest in using bone marrow-derived cells to treat IPAH (206-208). Data obtained from experimental models suggest that these cells may induce regeneration of pulmonary perfusion, leading to improved hemodynamics and survival (206,208). Furthermore, Wang et al., recently found that the intravenous infusion of cultured autologous cells in IPAH patients was associated with an augmented six-minute walk test distance and pulmonary haemodynamics 12 weeks later (209). Nonetheless, there remain a number of unanswered questions with this therapeutic approach and a simpler alternative strategy may be the pharmacological manipulation of EPCs in vivo.

In this study circulating progenitor cells were characterised using a variety of surface markers (168,169), (170), (171,172). Adherent PBMNCs, following short term culture, have been widely studied and previously also classified as EPCs, but are now considered to be mainly of monocyte origin (210). Despite their distinct phenotypes (170), the colony forming capacity and in vitro function of cultured PBMNCs has been related to endothelial dysfunction, cardiovascular symptoms and outcome (173,211). In the present study, in vitro analysis of PBMNCs revealed differences between

patients similar to those of circulating $CD34^+/AC133^+$ cells. Thus, while the number and in vitro function of cells from IPAH patients was similar or even greater than controls both were severely impaired in Eisenmenger patients. It is uncertain whether therapeutic intervention in PAH patients influences cultured as well as circulating cells, but components of the NO-cGMP pathway are present in PBMNCs (205),(212) and PDE5 inhibition for erectile dysfunction associated with augmented function of cultured cells (213). Several cytokines and growth factors are implicated in the mobilization and homing of EPCs. While levels of inflammatory mediators and ADMA were elevated in both forms of PAH studied, levels of the angioenic protein VEGF were selectively reduced in Eisenmenger patients. VEGF is a potent stimulus for mobilizing bone marrow-derived EPCs (214) and lower levels may be important in this patient group. Indeed, it may be interesting to investigate whether there is an imbalance between other angiogenic (e.g. IL-8) and anti-angiogenic factors (e.g. endostatin) in different forms of PAH.

Clinical Implications

Endothelial dysfunction is a hallmark of pulmonary hypertension and congenital heart disease and recent evidence suggests that bone marrow-derived cells participate in postnatal blood vessel repair and neovascularisation (206). The relative deficiency of circulating EPCs in PAH patients may contribute to the pulmonary vascular pathology and exercise intolerance in this cohort of patients. Chronic pharmacological augmentation with PDE5 inhibitors could offer a novel therapeutic strategy. On the other hand, resistance to apoptosis and proliferation of pulmonary vascular

endothelial cells are implicated in the progression of PAH(215) and incorporation of circulating progenitors may have adverse long-term consequences. Further studies are needed to understand the therapeutic implications of chronic PDE5 inhibition on circulating EPCs in PAH.

5.7. Limitations

One of the limitations of this study is that it did not investigate the dynamics of circulating EPCs and so cannot report on whether differences reflect alterations in EPC mobilization, survival or tissue uptake. This study provides no information on changes in EPCs with time in individual patients, particularly in relation to clinical status, measures of vascular endothelial function and the abundance of EPCs in the pulmonary vasculature.

5.8. Conclusion

Circulating EPCs are reduced in patients with Eisenmenger syndrome or IPAH. Circulating EPC numbers correlated with levels of inflammatory mediators, indices of NO synthesis and ADMA production and are related to exercise capacity and central haemodynamic markers. Treatment with sildenafil is associated with a dose-related increase in EPC numbers and may represent a novel pharmacological means of increasing circulating EPCs in PAH patients.

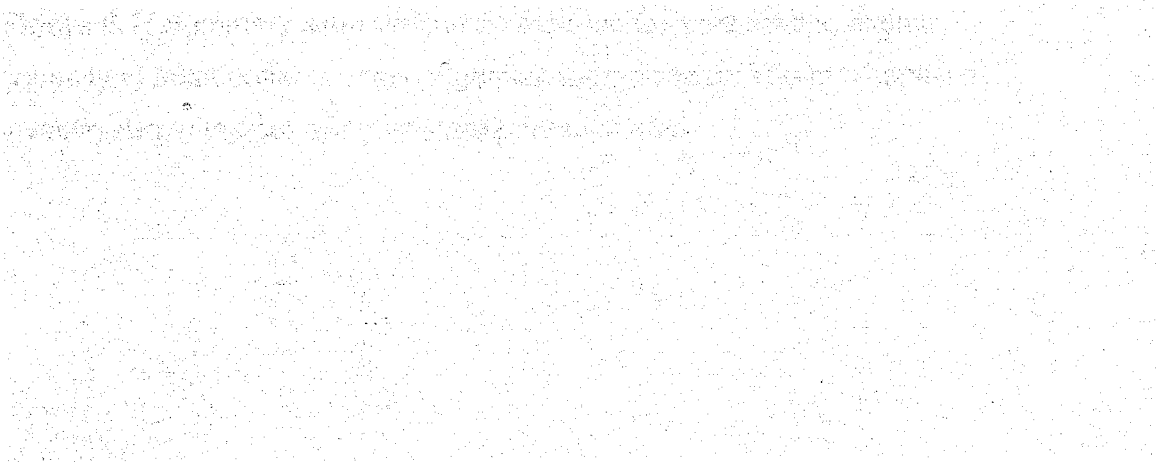
Chapter VI Conclusions

6.1. Conclusion

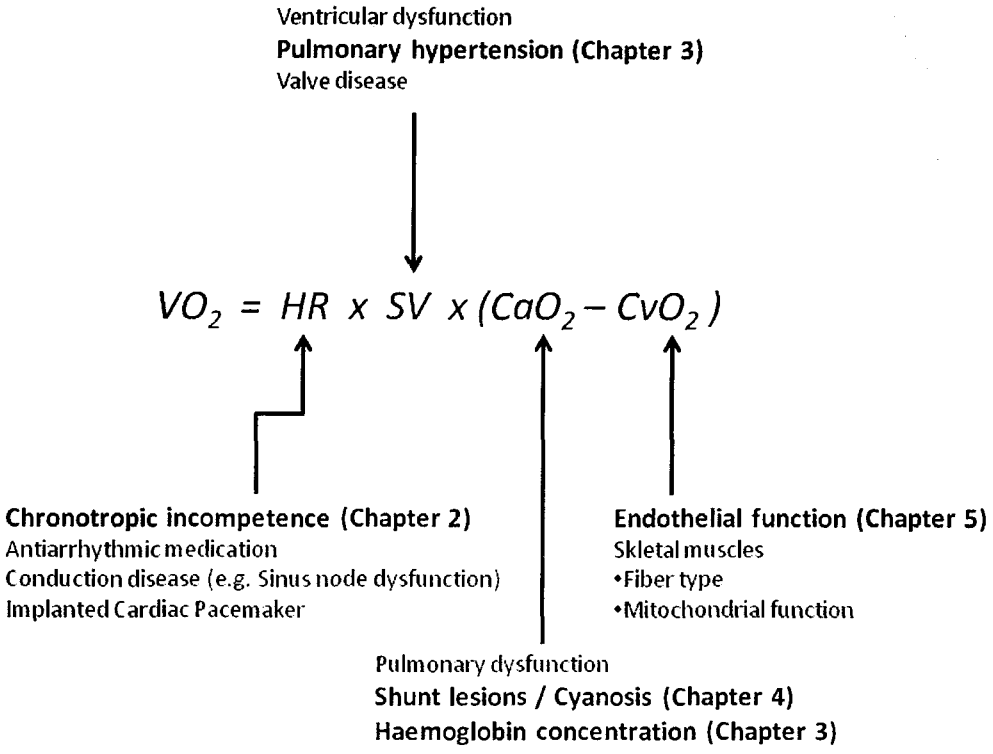
Adult congenital heart disease (ACHD) patients represent a heterogeneous patient population. Approximately one third of ACHD patient complain of symptoms of exercise intolerance and different mechanisms limit exercise capacity in different patients (7,19). To complicate things further, every element of the cardio-respiratory chain can limit exercise capacity in ACHD patients (see Figure 6.1) and it is not uncommon to have exercise limitation due to a combination of pathophysiologic factors in ACHD patients. Despite the heterogeneity, however, important similarities exist in the pathophysiology of exercise intolerance between different diagnostic groups and there are parallels to exercise limitation in patients with acquired heart failure. The aim of the present thesis was to investigate the mechanisms limiting exercise capacity in ACHD patients. Due to obvious limitations in time and resources the thesis had to focus on certain mechanisms of exercise capacity. Therefore, this thesis was focused on heart rate response to exercise (Chapter 2), pulmonary vascular disease (Chapter 3), cyanosis and shunt-lesions (Chapter 4) as well as the role of endothelial progenitor cells and factors affecting endothelial function such as immune inflammation and nitric oxide bioavailability (Chapter 5). Employing a combination of retrospective and prospective analyses this thesis emphasises the importance of an adequate heart rate increase with exercise for normal exercise capacity. However, it also shows that inadequate heart rate increase with exercise (chronotropic incompetence) is not causally related to

exercise intolerance in many ACHD patients and that artificially augmenting heart rate during exercise does not improve objective exercise capacity in the majority of patients with a systemic right ventricle and a pre-existing pacemaker. The results of this thesis rather indicate that higher heart rates may be detrimental in these patients by impairing diastolic filling and possibly myocardial perfusion at higher heart rates. Beyond the impact on exercise capacity chronotropic incompetence emerged as an important prognostic marker across the spectrum of ACHD. The results of this thesis also highlight the prevalence of exercise intolerance in ACHD patients with pulmonary arterial hypertension and cyanosis (Eisenmenger syndrome) and show that treatment with a pulmonary vasodilator (Bosentan) improves objective exercise capacity in this cohort. It also highlights the importance of adequate haemoglobin concentration (secondary erythrocytosis) and avoidance of iron deficiency in this patient population. Employing mathematical modelling this thesis shows how "unphysiologic" circulatory conditions (e.g. in patients with a bidirection cavopulmonary anastomosis) and right-to-left shunt lesions limit exercise capacity. It also emphasises the limitations of physiologic concepts such as the concept of arterial oxygen delivery in ACHD patients with complex conditions. Previous studies have underscored the importance of endothelial function for normal exercise capacity. Endothelial dysfunction is common in various cardiovascular cohorts including ACHD patients (59,60). Recent studies have highlighted the central role of endothelial progenitor cells in maintaining endothelial homeostasis. This study shows that patients with Eisenmenger syndrome – exhibiting severe exercise limitation as a group – have significantly lower EPC numbers and impaired EPC function

and that the number of EPCs correlates directly with symptoms and objective exercise capacity. In addition abnormal levels of immune inflammatory markers as well as asymmetric dimethylarginine – an inhibitor of nitric oxide synthesis – known to adversely affect EPC numbers and endothelial function were found in this patient population. Further studies are required for example to assess the impact of stroke volume limitations on exercise capacity and to investigate changes in skeletal muscle fibre composition in patients with ACHD. A better understanding of the mechanisms responsible for exercise intolerance in ACHD patients could facilitate development of novel therapeutic approaches or provide justification to use available treatment options developed for unrelated conditions in ACHD patients.



6.2. Figures



VO_2 = oxygen uptake; HR = heart rate; SV = stroke volume; CaO_2 = arterial oxygen content; CvO_2 = venous oxygen content.

Figure 6.1: Summary slide illustrating the mechanisms limiting exercise capacity in adult patients with congenital heart disease. The mechanisms investigated in the current thesis are printed in bold.

References

1. Diller GP, Dimopoulos K, Okonko D, et al. Exercise intolerance in adult congenital heart disease: comparative severity, correlates, and prognostic implication. Circulation 2005;112:828-35.

2. Fredriksen PM, Veldtman G, Hechter S, et al. Aerobic capacity in adults with various congenital heart diseases. Am J Cardiol 2001;87:310-4.

3. Fredriksen PM, Therrien J, Veldtman G, et al. Aerobic capacity in adults with tetralogy of Fallot. Cardiology in the young 2002;12:554-559.

4. Fredriksen PM, Therrien J, Veldtman G, et al. Lung function and aerobic capacity in adult patients following modified Fontan procedure. Heart 2001;85:295-299.

5. Fredriksen PM, Chen A, Veldtman G, Hechter S, Therrien J, Webb G. Exercise capacity in adult patients with congenitally corrected transposition of the great arteries. Heart 2001;85:191-195.

6. Dimopoulos K, Okonko DO, Diller GP, et al. Abnormal ventilatory response to exercise in adults with congenital heart disease relates to cyanosis and predicts survival. Circulation 2006;113:2796-802.

7. Dimopoulos K, Diller GP, Piepoli MF, Gatzoulis MA. Exercise intolerance in adults with congenital heart disease. Cardiol Clin 2006;24:641-60, vii.

8. Davlouros PA, Kilner PJ, Hornung TS, et al. Right ventricular function in adults with repaired tetralogy of Fallot assessed with cardiovascular magnetic resonance imaging: detrimental role of right ventricular outflow aneurysms or akinesia and adverse right-to-left ventricular interaction. J Am Coll Cardiol 2002;40:2044-52.

9. Brickner ME, Hillis LD, Lange RA. Congenital heart disease in adults. First of two parts. N Engl J Med 2000;342:256-63.

10. Hauser M, Bengel FM, Hager A, et al. Impaired myocardial blood flow and coronary flow reserve of the anatomical right systemic ventricle in patients with congenitally corrected transposition of the great arteries. Heart 2003;89:1231-5.

11. Azarbal B, Hayes SW, Lewin HC, Hachamovitch R, Cohen I, Berman DS. The incremental prognostic value of percentage of heart rate reserve achieved over myocardial perfusion single-photon emission computed tomography in the prediction of cardiac death and all-cause mortality: superiority over 85% of maximal age-predicted heart rate. J Am Coll Cardiol 2004;44:423-30.

12. Jouven X, Empana JP, Schwartz PJ, Desnos M, Courbon D, Ducimetiere P. Heart-rate profile during exercise as a predictor of sudden death. N Engl J Med 2005;352:1951-8.

13. Lauer MS, Francis GS, Okin PM, Pashkow FJ, Snader CE, Marwick TH. Impaired chronotropic response to exercise stress testing as a predictor of mortality. JAMA 1999;281:524-9.

14. Lauer MS, Okin PM, Larson MG, Evans JC, Levy D. Impaired heart rate response to graded exercise. Prognostic implications of chronotropic incompetence in the Framingham Heart Study. Circulation 1996;93:1520-6.

15. Katritsis D, Camm AJ. Chronotropic incompetence: a proposal for definition and diagnosis. Br Heart J 1993;70:400-2.

16. Astrand I. Aerobic work capacity in men and women with special reference to age. Acta Physiol Scand 1960;49(Suppl 169):1-92.

17. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. J Am Coll Cardiol 2001;37:153-6.

18. Clark AL, Coats AJ. Chronotropic incompetence in chronic heart failure. Int J Cardiol 1995;49:225-31.

19. Engelfriet P, Boersma E, Oechslin E, et al. The spectrum of adult congenital heart disease in Europe: morbidity and mortality in a 5 year follow-up period. The Euro Heart Survey on adult congenital heart disease. Eur Heart J 2005;26:2325-33.

20. Glaser S, Opitz CF, Bauer U, et al. Assessment of symptoms and exercise capacity in cyanotic patients with congenital heart disease. Chest 2004;125:368-76.

21. Fick A. Ueber die Messung des Blutquantum in den Herzventrikeln. Sb Phys Med Ges Wuerzburg 1870:16-17.

22. Komajda M, Anker SD, Charlesworth A, et al. The impact of new onset anaemia on morbidity and mortality in chronic heart failure: results from COMET. Eur Heart J 2006;27:1440-6.

23. Kalra PR, Bolger AP, Francis DP, et al. Effect of anemia on exercise tolerance in chronic heart failure in men. Am J Cardiol 2003;91:888-91.

24. Kalra PR, Collier T, Cowie MR, et al. Haemoglobin concentration and prognosis in new cases of heart failure. Lancet 2003;362:211-2.

25. Sharma R, Francis DP, Pitt B, Poole-Wilson PA, Coats AJ, Anker SD. Haemoglobin predicts survival in patients with chronic heart failure: a substudy of the ELITE II trial. Eur Heart J 2004;25:1021-8.

26. Okonko DO, Grzeslo A, Witkowski T, et al. Effect of intravenous iron sucrose on exercise tolerance in anemic and nonanemic patients with symptomatic chronic heart failure and iron deficiency FERRIC-HF: a randomized, controlled, observer-blinded trial. J Am Coll Cardiol 2008;51:103-12.

27. Oechslin E, . Eisenmenger's Syndrome. In: Gatzoulis MA, Webb GD, Daubeney PEF, editors. Adult Congenital Heart Disease. Philadelphia: Elsevier; 2003. p 363-378.

28. Broberg C, Jayaweera R, Diller G, et al. Abstract 2447: The Optimal Relationship between Oxygen Saturation and Hemoglobin in Adult Patients with Cyanotic

Congenital Heart Disease can be Determined and Correlates with Exercise Capacity. Circulation 2006;114:II_503-c-.

29. Kaemmerer H, Fratz S, Braun SL, et al. Erythrocyte indexes, iron metabolism, and hyperhomocysteinemia in adults with cyanotic congenital cardiac disease. Am J Cardiol 2004;94:825-8.

30. Diller GP, Gatzoulis MA. Pulmonary vascular disease in adults with congenital heart disease. Circulation 2007;115:1039-50.

31. Perloff JK, Marelli AJ, Miner PD. Risk of stroke in adults with cyanotic congenital heart disease. Circulation 1993;87:1954-9.

32. Mitchell JA, Ali F, Bailey L, Moreno L, Harrington LS. Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium. Exp Physiol 2008;93:141-7.

33. Haworth SG. Role of the endothelium in pulmonary arterial hypertension. Vascul Pharmacol 2006;45:317-25.

34. Furchgott RF, Jothianandan D. Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. Blood Vessels 1991;28:52-61.

35. Furchgott RF. 1988. Studies on relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that the acid activatable inhibitory factor from retractor penis is inorganic nitrite and the endothelium-derived relaxing factor is nitric oxide. In Vasodilatation: Vascular Smooth Muscle. Peptides, and Endothelium. ed. PM Vanhoutte, pp. 401-14. New York: Raven.

36. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 2006;113:1708-14.

37. Sessa WC. eNOS at a glance. J Cell Sci 2004;117:2427-9.

38. Galie N, Ghofrani HA, Torbicki A, et al. Sildenafil citrate therapy for pulmonary arterial hypertension. N Engl J Med 2005;353:2148-57.

39. Siroen MP, Teerlink T, Nijveldt RJ, Prins HA, Richir MC, van Leeuwen PA. The clinical significance of asymmetric dimethylarginine. Annu Rev Nutr 2006;26:203-28.

40. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411-5.

41. Clarke JG, Benjamin N, Larkin SW, Webb DJ, Davies GJ, Maseri A. Endothelin is a potent long-lasting vasoconstrictor in men. Am J Physiol 1989;257:H2033-5.

42. Schiffrin EL. Vascular endothelin in hypertension. Vascul Pharmacol 2005;43:19-29.

43. Luscher TF, Barton M. Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. Circulation 2000;102:2434-40.

44. Dupuis J, Hoeper MM. Endothelin receptor antagonists in pulmonary arterial hypertension. Eur Respir J 2008;31:407-15.

45. Farber HW, Loscalzo J. Pulmonary arterial hypertension. N Engl J Med 2004;351:1655-65.

46. Channick RN, Simonneau G, Sitbon O, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. Lancet 2001;358:1119-23.

47. Rubin LJ, Badesch DB, Barst RJ, et al. Bosentan therapy for pulmonary arterial hypertension. N Engl J Med 2002;346:896-903.

48. Barst RJ, Langleben D, Badesch D, et al. Treatment of pulmonary arterial hypertension with the selective endothelin-A receptor antagonist sitaxsentan. J Am Coll Cardiol 2006;47:2049-56.

49. Barst RJ, Langleben D, Frost A, et al. Sitaxsentan therapy for pulmonary arterial hypertension. Am J Respir Crit Care Med 2004;169:441-7.

50. Galie N, Badesch D, Oudiz R, et al. Ambrisentan therapy for pulmonary arterial hypertension. J Am Coll Cardiol 2005;46:529-35.

51. Galie N, Olschewski H, Oudiz RJ, et al. Ambrisentan for the treatment of pulmonary arterial hypertension: results of the ambrisentan in pulmonary arterial hypertension, randomized, double-blind, placebo-controlled, multicenter, efficacy (ARIES) study 1 and 2. Circulation 2008;117:3010-9.

52. Galie N, Rubin L, Hoeper M, et al. Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. Lancet 2008;371:2093-100.

53. McLaughlin VV, Sitbon O, Badesch DB, et al. Survival with first-line bosentan in patients with primary pulmonary hypertension. Eur Respir J 2005;25:244-9.

54. Galie N, Torbicki A, Barst R, et al. Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. Eur Heart J 2004;25:2243-78.

55. Simonneau G, Barst RJ, Galie N, et al. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med 2002;165:800-4.

56. Olschewski H, Simonneau G, Galie N, et al. Inhaled iloprost for severe pulmonary hypertension. N Engl J Med 2002;347:322-9.

57. Celermajer DS, Cullen S, Deanfield JE. Impairment of endothelium-dependent pulmonary artery relaxation in children with congenital heart disease and abnormal pulmonary hemodynamics. Circulation 1993;87:440-6.

58. Drexler H, Hayoz D, Munzel T, et al. Endothelial function in chronic congestive heart failure. Am J Cardiol 1992;69:1596-601.

59. Oechslin E, Kiowski W, Schindler R, Bernheim A, Julius B, Brunner-La Rocca HP. Systemic endothelial dysfunction in adults with cyanotic congenital heart disease. Circulation 2005;112:1106-12.

60. Mahle WT, Todd K, Fyfe DA. Endothelial function following the Fontan operation. Am J Cardiol 2003;91:1286-8.

61. Hambrecht R, Fiehn E, Weigl C, et al. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. Circulation 1998;98:2709-15.

62. Katz SD, Biasucci L, Sabba C, et al. Impaired endothelium-mediated vasodilation in the peripheral vasculature of patients with congestive heart failure. J Am Coll Cardiol 1992;19:918-25.

63. Sullivan MJ, Knight JD, Higginbotham MB, Cobb FR. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion pressure. Circulation 1989;80:769-81.

64. Fischer D, Rossa S, Landmesser U, et al. Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. Eur Heart J 2005;26:65-9.

65. Katz SD, Hryniewicz K, Hriljac I, et al. Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. Circulation 2005;111:310-4.

66. Clark AL, Poole-Wilson PA, Coats AJ. Exercise limitation in chronic heart failure: central role of the periphery. J Am Coll Cardiol 1996;28:1092-102.

67. Linke A, Recchia F, Zhang X, Hintze TH. Acute and chronic endothelial dysfunction: implications for the development of heart failure. Heart Fail Rev 2003;8:87-97.

68. Munzel T, Sinning C, Post F, Warnholtz A, Schulz E. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Ann Med 2008;40:180-96.

69. Le Brocq M, Leslie SJ, Milliken P, Megson IL. Endothelial dysfunction: from molecular mechanisms to measurement, clinical implications, and therapeutic opportunities. Antioxid Redox Signal 2008;10:1631-74.

70. Khakoo AY, Finkel T. Endothelial progenitor cells. Annu Rev Med 2005;56:79-101.

71. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. N Engl J Med 1999;341:1351-7.

72. Wilkoff BL, Corey J, Blackburn G. A mathematical model of cardiac chronotropic response to exercise. J Electrophysiol. 1989;3:176-180.

73. Bruce RA, Blackman JR, Jones JW. Exercise testing in adult normal subjects and cardiac patients. Pediatrics 1963;32:742-755.

74. Gatzoulis MA, Clark AL, Cullen S, Newman CG, Redington AN. Right ventricular diastolic function 15 to 35 years after repair of tetralogy of Fallot. Restrictive physiology predicts superior exercise performance. Circulation 1995;91:1775-81.

75. Porszasz J, Casaburi R, Somfay A, Woodhouse LJ, Whipp BJ. A treadmill ramp protocol using simultaneous changes in speed and grade. Med Sci Sports Exerc 2003;35:1596-603.

76. Jones NL, Summers E, Killian KJ. Influence of age and stature on exercise capacity during incremental cycle ergometry in men and women. Am Rev Respir Dis 1989;140:1373-80.

77. Kindermann M, Schwaab B, Finkler N, Schaller S, Bohm M, Frohlig G. Defining the optimum upper heart rate limit during exercise: a study in pacemaker patients with heart failure. Eur Heart J 2002;23:1301-8.

78. Li W, Hornung TS, Francis DP, et al. Relation of biventricular function quantified by stress echocardiography to cardiopulmonary exercise capacity in adults with Mustard (atrial switch) procedure for transposition of the great arteries. Circulation 2004;110:1380-6.

79. Lissin LW, Li W, Murphy DJ, Jr., et al. Comparison of transthoracic echocardiography versus cardiovascular magnetic resonance imaging for the assessment of ventricular function in adults after atrial switch procedures for complete transposition of the great arteries. Am J Cardiol 2004;93:654-7.

80. Bolger AP, Sharma R, Li W, et al. Neurohormonal activation and the chronic heart failure syndrome in adults with congenital heart disease. Circulation 2002;106:92-9.

81. Francis DP, Shamim W, Davies LC, et al. Cardiopulmonary exercise testing for prognosis in chronic heart failure: continuous and independent prognostic value from VE/VCO(2)slope and peak VO(2). Eur Heart J 2000;21:154-61.

82. Fox SM, 3rd, Naughton JP, Haskell WL. Physical activity and the prevention of coronary heart disease. Ann Clin Res 1971;3:404-32.

83. Astrand PO, Ryhming I. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during sub-maximal work. J Appl Physiol 1954;7:218-21.

84. Inbar O, Oren A, Scheinowitz M, Rotstein A, Dlin R, Casaburi R. Normal cardiopulmonary responses during incremental exercise in 20- to 70-yr-old men. Med Sci Sports Exerc 1994;26:538-46.

85. Londeree BR, Moeschberger ML. Effect of age and other factors on maximal heart rate. Res Q Exerc Sport. 1982;53:297-304.

86. Ellestad MH, Wan MK. Predictive implications of stress testing. Follow-up of 2700 subjects after maximum treadmill stress testing. Circulation 1975;51:363-9.

87. Ellestad MH. Chronotropic Incompetence : The Implications of Heart Rate Response to Exercise (Compensatory Parasympathetic Hyperactivity?). Circulation 1996;93:1485-1487.

88. Dreifus LS, Fisch C, Griffin JC, Gillette PC, Mason JW, Parsonnet V. Guidelines for implantation of cardiac pacemakers and antiarrhythmia devices. A report of the American College of Cardiology/American Heart Association Task Force on Assessment of Diagnostic and Therapeutic Cardiovascular Procedures. (Committee on Pacemaker Implantation). Circulation 1991;84:455-67.

89. Gregoratos G, Cheitlin MD, Conill A, et al. ACC/AHA guidelines for implantation of cardiac pacemakers and antiarrhythmia devices: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Pacemaker Implantation). J Am Coll Cardiol 1998;31:1175-209.

90. Robergs RA, Landwehr R. The surprising history of the "HRmax=220-age" equation. Journal of Exercise Physiology 2002;5:1-8.

91. Corbelli R, Masterson M, Wilkoff BL. Chronotropic response to exercise in patients with atrial fibrillation. Pacing Clin Electrophysiol 1990;13:179-87.

92. Duncan AM, O'Sullivan CA, Gibson DG, Henein MY. Electromechanical interrelations during dobutamine stress in normal subjects and patients with coronary artery disease: comparison of changes in activation and inotropic state. Heart 2001;85:411-6.

93. Duncan A, Francis D, Gibson D, Pepper J, Henein M. Electromechanical left ventricular resynchronisation by coronary artery bypass surgery. Eur J Cardiothorac Surg 2004;26:711-9.

94. Wasserman K, Hansen JE, Sue DY, Stringer WW, Whipp BJ. Principles of exercise testing and interpretation: Including pathophysiology and clinical applications. Philadelphia: Lippincott Williams & Wilkins 2004.

95. Derrick GP, Narang I, White PA, et al. Failure of stroke volume augmentation during exercise and dobutamine stress is unrelated to load-independent indexes of right ventricular performance after the Mustard operation. Circulation 2000;102:III154-9.

96. Colucci WS, Ribeiro JP, Rocco MB, et al. Impaired chronotropic response to exercise in patients with congestive heart failure. Role of postsynaptic beta-adrenergic desensitization. Circulation 1989;80:314-23.

97. Fei L, Keeling PJ, Sadoul N, et al. Decreased heart rate variability in patients with congestive heart failure and chronotropic incompetence. Pacing Clin Electrophysiol 1996;19:477-83.

98. Routledge HHC, Townend JJN. Why does the heart rate response to exercise predict adverse cardiac events? Heart 2006;92:577-578.

99. Sandvik L, Erikssen J, Ellestad M, et al. Heart rate increase and maximal heart rate during exercise as predictors of cardiovascular mortality: a 16-year follow-up study of 1960 healthy men. Coron Artery Dis 1995;6:667-79.

100. Barst RJ, McGoon M, Torbicki A, et al. Diagnosis and differential assessment of pulmonary arterial hypertension. J Am Coll Cardiol 2004;43:40S-47S.

101. Engelfriet PM, Duffels MG, Moller T, et al. Pulmonary arterial hypertension in adults born with a heart septal defect: the Euro Heart Survey on adult congenital heart disease. Heart 2007;93:682-7.

102. Kidd L, Driscoll DJ, Gersony WM, et al. Second natural history study of congenital heart defects. Results of treatment of patients with ventricular septal defects. Circulation 1993;87:I38-51.

103. Steele PM, Fuster V, Cohen M, Ritter DG, McGoon DC. Isolated atrial septal defect with pulmonary vascular obstructive disease--long-term follow-up and prediction of outcome after surgical correction. Circulation 1987;76:1037-42.

104. Eisenmenger V. Die angeborenen Defekte der Kammerscheidewände des Herzens. Zeitschr Klin Med. 1897;32:1-28.

105. Wood P. The Eisenmenger syndrome or pulmonary hypertension with reversed central shunt. Br Med J 1958;46:755-62.

106. Wood P. The Eisenmenger syndrome or pulmonary hypertension with reversed central shunt. I. Br Med J 1958;46:701-9.

107. Partin C. The evolution of Eisenmenger's eponymic enshrinement. Am J Cardiol 2003;92:1187-91.

108. Diller G-P, Dimopoulos K, Kafka H, Ho SY, Gatzoulis MA. Model of chronic adaptation: right ventricular function in Eisenmenger syndrome. Eur Heart J Suppl 2007;9:H54-60.

109. Finkelstein DM, Muzikansky A, Schoenfeld DA. Comparing survival of a sample to that of a standard population. J Natl Cancer Inst 2003;95:1434-9.

110. Niwa K, Perloff JK, Kaplan S, Child JS, Miner PD. Eisenmenger syndrome in adults: ventricular septal defect, truncus arteriosus, univentricular heart. J Am Coll Cardiol 1999;34:223-32.

111. Gatzoulis MA, Rogers P, Li W, et al. Safety and tolerability of bosentan in adults with Eisenmenger physiology. Int J Cardiol 2005;98:147-51.

112. Cantor WJ, Harrison DA, Moussadji JS, et al. Determinants of survival and length of survival in adults with Eisenmenger syndrome. Am J Cardiol 1999;84:677-81.

113. Daliento L, Somerville J, Presbitero P, et al. Eisenmenger syndrome. Factors relating to deterioration and death. Eur Heart J 1998;19:1845-55.

114. Sandoval J, Aguirre JS, Pulido T, et al. Nocturnal oxygen therapy in patients with the Eisenmenger syndrome. Am J Respir Crit Care Med 2001;164:1682-7.

115. Walker F, Mullen MJ, Woods SJ, Webb GD. Acute effects of 40% oxygen supplementation in adults with cyanotic congenital heart disease. Heart 2004;90:1073-4.

116. Miyamoto S, Nagaya N, Satoh T, et al. Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension. Comparison with cardiopulmonary exercise testing. Am J Respir Crit Care Med 2000;161:487-92.

117. Paciocco G, Martinez FJ, Bossone E, Pielsticker E, Gillespie B, Rubenfire M. Oxygen desaturation on the six-minute walk test and mortality in untreated primary pulmonary hypertension. Eur Respir J 2001;17:647-52.

118. Wensel R, Opitz CF, Anker SD, et al. Assessment of survival in patients with primary pulmonary hypertension: importance of cardiopulmonary exercise testing. Circulation 2002;106:319-24.

119. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-7.

120. Hoeper MM, Oudiz RJ, Peacock A, et al. End points and clinical trial designs in pulmonary arterial hypertension: clinical and regulatory perspectives. J Am Coll Cardiol 2004;43:48S-55S.

121. Oudiz RJ, Barst RJ, Hansen JE, et al. Cardiopulmonary exercise testing and six-minute walk correlations in pulmonary arterial hypertension. Am J Cardiol 2006;97:123-6.

122. Broberg C, Ujita M, Babu-Narayan S, et al. Massive pulmonary artery thrombosis with haemoptysis in adults with Eisenmenger's syndrome: a clinical dilemma. Heart 2004;90:e63.

123. Hopkins WE, Ochoa LL, Richardson GW, Trulock EP. Comparison of the hemodynamics and survival of adults with severe primary pulmonary hypertension or Eisenmenger syndrome. J Heart Lung Transplant 1996;15:100-5.

124. Ghofrani HA, Wiedemann R, Rose F, et al. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: a randomised controlled trial. Lancet 2002;360:895-900.

125. Sastry BK, Narasimhan C, Reddy NK, Raju BS. Clinical efficacy of sildenafil in primary pulmonary hypertension: a randomized, placebo-controlled, double-blind, crossover study. J Am Coll Cardiol 2004;43:1149-53.

126. Apostolopoulou SC, Manginas A, Cokkinos DV, Rammos S. Effect of the oral endothelin antagonist bosentan on the clinical, exercise, and haemodynamic status of patients with pulmonary arterial hypertension related to congenital heart disease. Heart 2005;91:1447-52.

127. Galie N, Beghetti M, Gatzoulis M, et al. BREATHE-5: Bosentan improves hemodynamics and exercise capacity in the first randomized placebo controlled trial in Eisenmenger physiology [Abstract]. Chest 2005;128:496S.

128. Galie N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. Cardiovasc Res 2004;61:227-37.

129. Cacoub P, Dorent R, Maistre G, et al. Endothelin-1 in primary pulmonary hypertension and the Eisenmenger syndrome. Am J Cardiol 1993;71:448-50.

130. Giaid A, Yanagisawa M, Langleben D, et al. Expression of Endothelin-1 in the Lungs of Patients with Pulmonary Hypertension. N Engl J Med 1993;328:1732-1739.

131. Motte S, McEntee K, Naeije R. Endothelin receptor antagonists. Pharmacol Ther 2006;110:386-414.

132. Galie N, Beghetti M, Gatzoulis MA, et al. Bosentan therapy in patients with Eisenmenger syndrome: a multicenter, double-blind, randomized, placebo-controlled study. Circulation 2006;114:48-54.

133. Schulze-Neick I, Gilbert N, Ewert R, et al. Adult patients with congenital heart disease and pulmonary arterial hypertension: first open prospective multicenter study of bosentan therapy. Am Heart J 2005;150:716.

134. Badesch DB, McLaughlin VV, Delcroix M, et al. Prostanoid therapy for pulmonary arterial hypertension. J Am Coll Cardiol 2004;43:56S-61S.

135. Sitbon O, Badesch DB, Channick RN, et al. Effects of the dual endothelin receptor antagonist bosentan in patients with pulmonary arterial hypertension: a 1-year follow-up study. Chest 2003;124:247-54.

136. Stessel H, Brunner F. Effect of endothelin antagonism on contractility, intracellular calcium regulation and calcium regulatory protein expression in right ventricular hypertrophy of the rat. Basic Clin Pharmacol Toxicol 2004;94:37-45.

137. Schumacker PT, Cain SM. The concept of a critical oxygen delivery. Intensive Care Med 1987;13:223-9.

138. Hayes MA, Timmins AC, Yau EH, Palazzo M, Hinds CJ, Watson D. Elevation of systemic oxygen delivery in the treatment of critically ill patients. The New England Journal Of Medicine 1994;330:1717-1722.

139. Rossi AF, Sommer RJ, Lotvin A, et al. Usefulness of intermittent monitoring of mixed venous oxygen saturation after stage I palliation for hypoplastic left heart syndrome. Am J Cardiol 1994;73:1118-23.

140. Santamore W, Barnea O, Riordan C, Ross M, Austin E. Theoretical optimisation of pulmonary-to-systemic flow ratio after a bidirectional cavopulmonary anastomosis. Am J Physiol. 1998;274:H694-H700.

141. Chang AC, Kulik TJ, Hickey PR, Wessel DL. Real-time gas-exchange measurement of oxygen consumption in neonates and infants after cardiac surgery. Crit Care Med 1993;21:1369-75.

142. Salim MA, Case CL, Sade RM, Watson DC, Alpert BS, DiSessa TG. Pulmonary/systemic flow ratio in children after cavopulmonary anastomosis. J Am Coll Cardiol 1995;25:735-8.

143. Sun X-G, Hansen JE, Oudiz RJ, Wasserman K. Exercise Pathophysiology in Patients With Primary Pulmonary Hypertension. Circulation 2001;104:429-435.

144. Webb G, Gatzoulis MA. Atrial septal defects in the adult: recent progress and overview. Circulation 2006;114:1645-53.

145. Kerstein D, Levy PS, Hsu DT, Hordof AJ, Gersony WM, Barst RJ. Blade Balloon Atrial Septostomy in Patients With Severe Primary Pulmonary Hypertension. Circulation 1995;91:2028-2035.

146. Micheletti A, Hislop AA, Lammers A, et al. Role of atrial septostomy in the treatment of children with pulmonary arterial hypertension. Heart 2006;92:969-972.

147. Klepetko W, Mayer E, Sandoval J, et al. Interventional and surgical modalities of treatment for pulmonary arterial hypertension. J Am Coll Cardiol 2004;43:73S-80S.

148. Rothman A, Sklansky MS, Lucas VW, et al. Atrial septostomy as a bridge to lung transplantation in patients with severe pulmonary hypertension. The American Journal of Cardiology 1999;84:682-686.

149. Rich MDS, Dodin MDE, McLaughlin MDVV. Usefulness of Atrial Septostomy as a Treatment for Primary Pulmonary Hypertension and Guidelines for its Application. The American Journal of Cardiology 1997;80:369-371.

150. Sandoval J, Gaspar J, Pulido T, et al. Graded balloon dilation atrial septostomy in severe primary pulmonary hypertension. A therapeutic alternative for patients nonresponsive to vasodilator treatment. J Am Coll Cardiol 1998;32:297-304.

151. Thanopoulos BD, Georgakopoulos D, Tsaousis GS, Simeunovic S. Percutaneous balloon dilatation of the atrial septum: immediate and midterm results. Heart 1996;76:502-6.

152. Law MA, Grifka RG, Mullins CE, Nihill MR. Atrial septostomy improves survival in select patients with pulmonary hypertension. Am Heart J 2007;153:779-84.

153. Berman EB, Barst RJ. Eisenmenger's syndrome: current management. Prog Cardiovasc Dis 2002;45:129-38.

154. Glenn WW. Circulatory bypass of the right side of the heart. IV. Shunt between superior vena cava and distal right pulmonary artery; report of clinical application. N Engl J Med 1958;259:117-20.

155. Freedom RM, Nykanen D, Benson LN. The physiology of the bidirectional cavopulmonary connection. Ann Thorac Surg 1998;66:664-667.

156. Francis DP, Willson K, Thorne SA, Davies LC, Coats AJ. Oxygenation in patients with a functionally univentricular circulation and complete mixing of blood: are saturation and flow interchangeable? Circulation 1999;100:2198-203.

157. Boyd O, Grounds RM, Bennett ED. A randomized clinical trial of the effect of deliberate perioperative increase of oxygen delivery on mortality in high-risk surgical patients. JAMA 1993;270:2699-707.

158. Heyland DK, Cook DJ, King D, Kernerman P, Brun-Buisson C. Maximizing oxygen delivery in critically ill patients: a methodologic appraisal of the evidence. Crit Care Med 1996;24:517-24.

159. Russell JA, Phang PT. The oxygen delivery/consumption controversy. Approaches to management of the critically ill. Am J Respir Crit Care Med 1994;149:533-7.

160. Salim MA, DiSessa TG, Arheart KL, Alpert BS. Contribution of superior vena caval flow to total cardiac output in children. A Doppler echocardiographic study. Circulation 1995;92:1860-5.

161. Gross GJ, Jonas RA, Castaneda AR, Hanley FL, Mayer JE, Jr., Bridges ND. Maturational and hemodynamic factors predictive of increased cyanosis after bidirectional cavopulmonary anastomosis. Am J Cardiol 1994;74:705-9.

162. Sommer RJ, Hijazi ZM, Rhodes JF, Jr. Pathophysiology of Congenital Heart Disease in the Adult: Part I: Shunt Lesions. Circulation 2008;117:1090-1099.

163. Mathew RJ. Postural syncope and autoregulation of cerebral blood flow. Biol Psychiatry 1996;40:923-6.

164. Diller GP, Uebing A, Willson K, et al. Analytical identification of ideal pulmonary-systemic flow balance in patients with bidirectional cavopulmonary shunt and univentricular circulation: oxygen delivery or tissue oxygenation? Circulation 2006;114:1243-50.

165. Diller GP, Dimopoulos K, Broberg CS, et al. Presentation, survival prospects, and predictors of death in Eisenmenger syndrome: a combined retrospective and case-control study. Eur Heart J 2006;27:1737-42.

166. Little RC, Opdyke DF, Hawley JG. Dynamics of experimental atrial septal defects. Am J Physiol 1949;158:241-250.

167. Mitsuru F, Junichiro F, Yoshiharu U, Kohji U, Kenji S. Effect of increase in heart rate on interatrial shunt in atrial septal defect. Pediatric Cardiology 1992;V13:146-151.

168. George J, Shmilovich H, Deutsch V, Miller H, Keren G, Roth A. Comparative analysis of methods for assessment of circulating endothelial progenitor cells. Tissue Eng 2006;12:331-5.

169. Valgimigli M, Rigolin GM, Fucili A, et al. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. Circulation 2004;110:1209-12.

170. Case J, Mead LE, Bessler WK, et al. Human CD34+AC133+VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. Exp Hematol 2007;35:1109-18.

171. Kondo T, Hayashi M, Takeshita K, et al. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. Arterioscler Thromb Vasc Biol 2004;24:1442-7.

172. Numaguchi Y, Sone T, Okumura K, et al. The Impact of the Capability of Circulating Progenitor Cell to Differentiate on Myocardial Salvage in Patients With Primary Acute Myocardial Infarction. Circulation 2006;114:I-114-119.

173. Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003;348:593-600.

174. Werner N, Kosiol S, Schiegl T, et al. Circulating Endothelial Progenitor Cells and Cardiovascular Outcomes. N Engl J Med 2005;353:999-1007.

175. Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 2000;95:952-8.

176. Vasa M, Fichtlscherer S, Adler K, et al. Increase in Circulating Endothelial Progenitor Cells by Statin Therapy in Patients With Stable Coronary Artery Disease. Circulation 2001;103:2885-2890.

177. Thum T, Tsikas D, Stein S, et al. Suppression of Endothelial Progenitor Cells in Human Coronary Artery Disease by the Endogenous Nitric Oxide Synthase Inhibitor Asymmetric Dimethylarginine. Journal of the American College of Cardiology 2005;46:1693-1701.

178. Connelly L, Madhani M, Hobbs AJ. Resistance to endotoxic shock in endothelial nitric-oxide synthase (eNOS) knock-out mice: a pro-inflammatory role for eNOS-derived no in vivo. J Biol Chem 2005;280:10040-6.

179. R Development Core Team . R: A Language and Environment for Statistical Computing, Vienna, Austria, 2006 ISBN 3-900051-07-0, http://www.Rproject.org.

180. Fadini GP, Miorin M, Facco M, et al. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. J Am Coll Cardiol 2005;45:1449-57.

181. Fadini GP, Agostini C, Avogaro A. Endothelial progenitor cells in cerebrovascular disease. Stroke 2005;36:1112-3; author reply 1113.

182. Shantsila E, Watson T, Lip GY. Endothelial progenitor cells in cardiovascular disorders. J Am Coll Cardiol 2007;49:741-52.

183. Yndestad A, Damas JK, Oie E, Ueland T, Gullestad L, Aukrust P. Systemic inflammation in heart failure--the whys and wherefores. Heart Fail Rev 2006;11:83-92.

184. Hughes R, Tong J, Oates C, Lordan J, Corris PA. Evidence for systemic endothelial dysfunction in patients and first-order relatives with pulmonary arterial hypertension. Chest 2005;128:617S.

185. Yang Q, Rasmussen SA, Friedman JM. Mortality associated with Down's syndrome in the USA from 1983 to 1997: a population-based study. Lancet 2002;359:1019-25.

186. Aicher A, Heeschen C, Mildner-Rihm C, et al. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med 2003;9:1370-6.

187. Kielstein JT, Bode-Boger SM, Hesse G, et al. Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension. Arterioscler Thromb Vasc Biol 2005;25:1414-8.

188. Gorenflo M, Zheng C, Werle E, Fiehn W, Ulmer HE. Plasma levels of asymmetrical dimethyl-L-arginine in patients with congenital heart disease and pulmonary hypertension. J Cardiovasc Pharmacol 2001;37:489-92.

189. Han TH, Perloff JK, Liao JC. Nitric oxide metabolism in adults with cyanotic congenital heart disease. Am J Cardiol 2007;99:691-5.

190. Zhao L, Mason NA, Strange JW, Walker H, Wilkins MR. Beneficial Effects of Phosphodiesterase 5 Inhibition in Pulmonary Hypertension Are Influenced by Natriuretic Peptide Activity. Circulation 2003;107:234-237.

191. Oishi P, Sharma S, Grobe A, et al. Alterations in cGMP, soluble guanylate cyclase, phosphodiesterase 5, and B-type natriuretic peptide induced by chronic increased pulmonary blood flow in lambs. Pediatr Pulmonol 2007;42:1057-71.

192. Ferreiro CR, Chagas AC, Carvalho MH, et al. Influence of hypoxia on nitric oxide synthase activity and gene expression in children with congenital heart disease: a novel pathophysiological adaptive mechanism. Circulation 2001;103:2272-6.

193. Berger RM, Geiger R, Hess J, Bogers AJ, Mooi WJ. Altered arterial expression patterns of inducible and endothelial nitric oxide synthase in pulmonary plexogenic arteriopathy caused by congenital heart disease. Am J Respir Crit Care Med 2001;163:1493-9.

194. Thum T, Fraccarollo D, Thum S, et al. Differential Effects of Organic Nitrates on Endothelial Progenitor Cells Are Determined by Oxidative Stress. Arterioscler Thromb Vasc Biol 2007;27:748-754.

195. DiFabio JM, Thomas GR, Zucco L, et al. Nitroglycerin attenuates human endothelial progenitor cell differentiation, function, and survival. J Pharmacol Exp Ther 2006;318:117-23.

196. Humbert M, Monti G, Brenot F, et al. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. Am J Respir Crit Care Med 1995;151:1628-31.

197. Itoh T, Nagaya N, Ishibashi-Ueda H, et al. Increased plasma monocyte chemoattractant protein-1 level in idiopathic pulmonary arterial hypertension. Respirology 2006;11:158-63.

198. Sanchez O, Marcos E, Perros F, et al. Role of endothelium-derived CC chemokine ligand 2 in idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med 2007;176:1041-7.

199. Herbrig K, Haensel S, Oelschlaegel U, Pistrosch F, Foerster S, Passauer J. Endothelial dysfunction in patients with rheumatoid arthritis is associated with a reduced number and impaired function of endothelial progenitor cells. Ann Rheum Dis 2006;65:157-63.

200. Seeger FH, Haendeler J, Walter DH, et al. p38 mitogen-activated protein kinase downregulates endothelial progenitor cells. Circulation 2005;111:1184-91.

201. Schwartz R, Osborne-Lawrence S, Hahner L, et al. C-Reactive Protein Downregulates Endothelial NO Synthase and Attenuates Reendothelialization In Vivo in Mice. Circ Res 2007;100:1452-1459.

202. Verma S, Kuliszewski MA, Li S-H, et al. C-Reactive Protein Attenuates Endothelial Progenitor Cell Survival, Differentiation, and Function: Further Evidence of a Mechanistic Link Between C-Reactive Protein and Cardiovascular Disease. Circulation 2004;109:2058-2067.

203. Foresta C, Caretta N, Lana A, et al. Relationship between vascular damage degrees and endothelial progenitor cells in patients with erectile dysfunction: effect of vardenafil administration and PDE5 expression in the bone marrow. Eur Urol 2007;51:1411-7; discussion 1417-9.

204. Foresta C, Ferlin A, De Toni L, et al. Circulating endothelial progenitor cells and endothelial function after chronic Tadalafil treatment in subjects with erectile dysfunction. Int J Impot Res 2006;18:484-8.

205. Hur J, Yoon CH, Kim HS, et al. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. Arterioscler Thromb Vasc Biol 2004;24:288-93.

206. Zhao YD, Courtman DW, Deng Y, Kugathasan L, Zhang Q, Stewart DJ. Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. Circ Res 2005;96:442-50.

207. Stewart DJ, Zhao YD, Courtman DW, et al. Cell Therapy for Pulmonary Hypertension: What Is the True Potential of Endothelial Progenitor Cells? * Response. Circulation 2004;109:e172-173.

208. Nagaya N, Kangawa K, Kanda M, et al. Hybrid Cell-Gene Therapy for Pulmonary Hypertension Based on Phagocytosing Action of Endothelial Progenitor Cells. Circulation 2003;108:889-895.

209. Wang XX, Zhang FR, Shang YP, et al. Transplantation of autologous endothelial progenitor cells may be beneficial in patients with idiopathic pulmonary arterial hypertension: a pilot randomized controlled trial. J Am Coll Cardiol 2007;49:1566-71.

210. Prater DN, Case J, Ingram DA, Yoder MC. Working hypothesis to redefine endothelial progenitor cells. Leukemia 2007.

211. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-7.

212. Mateos-Caceres PJ, Garcia-Cardoso J, Lapuente L, et al. Soluble guanylate cyclase beta1-subunit expression is increased in mononuclear cells from patients with erectile dysfunction. Int J Impot Res 2006;18:432-7.

213. Bocchio M, Pelliccione F, Passaquale G, et al. Inhibition of phosphodiesterase type 5 with tadalafil is associated to an improved activity of circulating angiogenic cells in men with cardiovascular risk factors and erectile dysfunction. Atherosclerosis 2006.

214. Asahara T, Takahashi T, Masuda H, et al. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. Embo J 1999;18:3964-72.

215. Levy M, Maurey C, Celermajer DS, et al. Impaired apoptosis of pulmonary endothelial cells is associated with intimal proliferation and irreversibility of pulmonary hypertension in congenital heart disease. J Am Coll Cardiol 2007;49:803-10.

Publications arising from the work in this thesis

Original publications

Chapter 1

Diller GP, Dimopoulos K, Okonko D, Li W, Babu-Narayan SV, Broberg CS, Johansson B, Bouzas B, Mullen MJ, Poole-Wilson PA, Francis DP, Gatzoulis MA. Exercise intolerance in adult congenital heart disease: comparative severity, correlates, and prognostic implication. *Circulation*. 2005 Aug 9;112(6):828-35.

Chapter 2

Diller GP, Okonko DO, Uebing A, Dimopoulos K, Bayne S, Sutton R, Francis DP, Gatzoulis MA. Impaired heart rate response to exercise in adult patients with a systemic right ventricle or univentricular circulation: Prevalence, relation to exercise, and potential therapeutic implications. *Int J Cardiol.* 2008. *Epub ahead of print*

Diller GP, Dimopoulos K, Okonko D, Uebing A, Broberg CS, Babu-Narayan S, Bayne S, Poole-Wilson PA, Sutton R, Francis DP, Gatzoulis MA. Heart rate response during exercise predicts survival in adults with congenital heart disease. *J Am Coll Cardiol.* 2006 Sep 19;48(6):1250-6.

Chapter 3

Diller GP, Dimopoulos K, Broberg CS, Kaya MG, Naghotra US, Uebing A, Harries C, Goktekin O, Gibbs JS, Gatzoulis MA. Presentation, survival prospects, and predictors of death in Eisenmenger syndrome: a combined retrospective and case-control study. *Eur Heart J.* 2006 Jul;27(14):1737-42.

Diller GP, Dimopoulos K, Kaya MG, Harries C, Uebing A, Gibbs JSR, Gatzoulis MA. Long-term Safety, Tolerability and Efficacy of Bosentan in Adults with Pulmonary Arterial Hypertension Associated with Congenital Heart Disease. *Heart* 2007 Aug;93(8):974-6.

Chapter 4

Diller GP, Uebing A, Willson K, Davies LC, Dimopoulos K, Thorne SA, Gatzoulis MA, Francis DP. Analytical identification of ideal pulmonary-systemic flow balance in patients with bidirectional cavopulmonary shunt and univentricular circulation: oxygen delivery or tissue oxygenation? *Circulation.* 2006 Sep 19;114(12):1243-50.

Chapter 5

Diller GP, van Eijl S, Okonko DO, Howard LS, Ali O, Thum T, Wort SJ, Bedard E, Gibbs JSR, Bauersachs J, Hobbs AJ, Wilkins MR, Gatzoulis MA, Wharton J. Circulating endothelial progenitor cells in patients with Eisenmenger syndrome and idiopathic pulmonary arterial hypertension. *Circulation*. 2008;117:3020-3030.

Reviews

Chapter 3

Diller GP, Gatzoulis MA. Pulmonary vascular disease in adults with congenital heart disease. *Circulation*. 2007;115(8):1039-50.

Diller GP, Dimopoulos K, Kafka H, Ho SY, Gatzoulis MA. Model of chronic adaptation: right ventricular function in Eisenmenger syndrome. Eur Heart J Suppl 9: H54-H60.